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REVIEW

Pharmacogenomics of drug metabolizing enzymes and transporters: implications for cancer therapy

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Abstract: The new era of personalized medicine, which integrates the uniqueness of an individual with respect to the pharmacokinetics and pharmacodynamics of a drug, holds promise as a means to provide greater safety and efficacy in drug design and development. Personalized medicine is particularly important in oncology, whereby most clinically used anticancer drugs have a narrow therapeutic window and exhibit a large interindividual pharmacokinetic and pharmacodynamic variability. This variability can be explained, at least in part, by genetic variations in the genes encoding drug metabolizing enzymes, transporters, or drug targets. Understanding of how genetic variations influence drug disposition and action could help in tailoring cancer therapy based on individual's genetic makeup. This review focuses on the pharmacogenomics of drug metabolizing enzymes and drug transporters, with a particular highlight of examples whereby genetic variations in the metabolizing enzymes and transporters influence the pharmacokinetics and/or response of chemotherapeutic agents.

Keywords: polymorphisms, personalized medicine, oncology, enzymes, transporters, drug

Introduction

The new era of personalized medicine, which integrates the uniqueness of an individual with respect to the pharmacokinetics and pharmacodynamics of a drug, holds promise as a means to provide greater safety and efficacy in drug design and development. Personalized medicine is particularly important in oncology whereby most clinically used anticancer drugs have a narrow therapeutic window and exhibit a large interindividual pharmacokinetic and pharmacodynamic variability. This variability can lead to therapeutic failure or severe toxicity. Understanding of how genetic variations influence drug disposition and action could help in tailoring cancer therapy based on individual's genetic makeup. Pharmacogenomics is the study of how variations in the human genome affect the response to medications. Each drug, after it enters the body, interacts with numerous proteins, such as carrier proteins, transporters, metabolizing enzymes, and multiple types of receptors. These protein interactions determine drug pharmacokinetics (ie, drug absorption, distribution, metabolism, and excretion) and pharmacodynamics (ie, target site of action, pharmacological and toxicological effects). Moreover, drugs trigger downstream secondary events which may impact additional gene or protein expression responses and can also vary among patients. As a result, the overall response to a drug is determined by the interplay of multiple genes that are involved in the pharmacokinetic and pharmacodynamic pathways of a drug. In general, important genetic variation in drug effect can be envisioned at the level of drug metabolizing enzymes, drug transporters, and drug targets. This review provides an overview

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on the commonly occurring, functionally and/or clinically relevant genetic polymorphisms within the genes encoding important drug metabolizing enzymes and drug transporters, with a particular highlight of examples whereby genetic variations in these genes influence the pharmacokinetics and/or response of chemotherapeutic agents.

Drug metabolizing-enzyme pharmacogenomics

Drug metabolizing enzymes are proteins that catalyze the biochemical modifications of xenobiotics (eg, drugs) and endogenous chemicals (eg, hormones, neurotransmitters). Broadly, drug metabolizing enzymes are divided into two categories: Phase I (functionalizing) enzymes that introduce or remove functional groups in a substrate through oxidation, reduction, or hydrolysis; and Phase II (conjugating) enzymes that transfer moieties from a cofactor to a substrate. Essentially all of the major human metabolizing enzymes exhibit genetic polymorphisms at the genomic level, and many of these enzymes have clinically relevant genetic polymorphisms.[1] A gene is considered to be polymorphic when the frequency of a variant allele in a specific population is at least 1%.

Phase I enzymes

Phase I metabolizing enzymes include those involved in:

- Oxidation – cytochrome P450, alcohol dehydrogenase, aldehyde dehydrogenase, dihydropyrimidine dehydrogenase, monoamine oxidase, and flavin-containing monooxygenase;
- Reduction – nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase and reduced cytochrome P450;
- Hydrolysis – epoxide hydrolase, esterases, and amidases.

The most important Phase I enzymes that exhibit clinical relevant genetic polymorphisms are the cytochrome P450 (CYP) superfamily. The human CYP superfamily represents the most important system responsible for catalyzing the oxidation of a large number of endogenous and exogenous compounds including drugs, toxins, and carcinogens. In this superfamily, 57 genes and 58 pseudogenes have been identified, which are divided into 18 families and 43 subfamilies (http://drnelson.utmem.edu/cytochromeP450.html). Among them, three subfamilies of CYPs, including CYP1, CYP2, and CYP3, contribute to the oxidative metabolism of more than 90% of clinically used drugs.

The human CYP genes are highly polymorphic. The polymorphisms within the CYP genes, which include gene deletions, missense mutations, deleterious mutations creating splicing defects or premature stop codon, and gene duplications, could produce abolished, reduced, normal, or enhanced enzyme activity. As a result, patients can be classified into four phenotypes based on the level of a CYP enzyme activity: poor metabolizer (abolished activity), intermediate metabolizer (reduced activity), extensive metabolizer (normal activity), and ultrarapid metabolizer (enhanced activity). It is expected that poor metabolizers would have higher concentrations of a drug that is inactivated by that enzyme pathway and therefore require a lower dose to avoid adverse reactions, whereas ultrarapid metabolizers would require a higher dose to achieve therapeutic effective drug concentrations. The opposite pattern of reactions is expected for a prodrug that undergoes metabolic activation. A prodrug may have little therapeutic effect in poor metabolizers, while producing a toxic level of active form in ultrarapid metabolizers. Substantial evidence suggests that genetic polymorphisms within the CYP genes have significant impact on drug disposition and/or response. The common functional polymorphisms in the major human CYP genes and their clinical relevance are summarized in Table 1. Notably, the most pharmacologically and clinically relevant CYP polymorphisms are found in *CYP2D6*, *CYP2C9*, and *CYP2C19*. Of the Food and Drug Administration (FDA)-approved drug labels referring human genomic biomarkers, 62% are pertained to polymorphisms in the CYP enzymes, with CYP2D6 (35%), CYP2C19 (17%), and CYP2C9 (7%) being the most common.[2]

CYP2D6 is not inducible and therefore, the variations in the enzyme expression and activity are largely attributable to genetic polymorphisms. The *CYP2D6* gene is highly polymorphic with more than 63 functional variants identified to date (http://www.cypalleles.ki.se). These alleles result in abolished, decreased, normal, or ultrarapid CYP2D6 enzyme activity. The most important null alleles are *CYP2D6*4* (splicing defect) and *CYP2D6*5* (gene deletion); the common alleles with severely reduced enzyme activity are represented by *CYP2D6*10*, **17*, and **41*; duplication or multiduplications of active *CYP2D6* genes (eg, *CYP2D6*1* × N (N ≥ 2)) result in ultrarapid enzyme activity (Table 1). The distributions of *CYP2D6* alleles exhibit notable interethnic differences. The nonfunctional allele *CYP2D6*4* is prevalent in Caucasians (allelic frequency, ~25%), while the reduced function allele *CYP2D6*10* and *CYP2D6*17* is common in Asians (allelic frequency, ~40%) and Africans (allelic frequency, ~34%).[3] As a result, poor metabolizers of CYP2D6, mainly resulted from null allele *CYP2D6*4*, has

Table 1 Most common naturally occurring functional polymorphisms in the major human CYP genes: allele frequency, functional effect, and highlights of clinical relevance

Common allelic variants	Polymorphism/substitution	Allele frequency (%)[a]			Functional effect[b]	Highlights of clinical relevance[c]
		Caucasian	Asian	African		
CYP1A1						• Mainly expressed in extrahepatic tissues.
*CYP1A1*2A*	3698T>C(MspI)	6.6–19.0	33–54	22–28	↑ Inducibility	• CYP1A1, 1A2, and 1B1 play important role in the bioactivation of a variety of carcinogens.
*CYP1A1*2B*	I462V; 3698T>C(MspI)	–	–	–	↑ Inducibility	• ↑Lung cancer risk generally associated with highly inducible or active CYP1A1 polymorphisms such as CYP1A1*2C.
*CYP1A1*2C*	I462V	2.2–8.9	28–31	0.0–2.7	↑ Activity	• CYP1A1 genotypes also associated with risk to breast, prostate, and ovarian cancers, which are possibly related to estrogen activation.
*CYP1A1*3*	3204T>C	0	0	7.6–14.0	Normal	
*CYP1A1*4*	T461N	2.0–5.7	–	–	Normal	
CYP1A2						• CYP1A2 accounts for ~13% of total hepatic CYP content.
*CYP1A2*1C*	–3860G>A				↓ Inducibility	• High inducible *1F genotype associated with ↑clearance of CYP1A2 substrates (eg, caffeine) after smoking or omeprazole treatment.
*CYP1A2*1F*	–163C>A	33	68		↑ Inducibility	• *1K associated with ↓ in-vivo caffeine metabolism.
*CYP1A2*1K*	Haplotype (–63C>A, –739T>G, –729C>T)	0.5			↓ Inducibility ↓ Activity	• CYP1A2 genotypes are associated with cancer risk.
CYP2A6						• CYP2A6 accounts for 1%–10% of total hepatic CYPs.
*CYP2A6*1 × 2*	Gene duplication	1.7		0.4	↑ Activity	• The frequency of CYP2A6 alleles has marked ethnic difference. CYP2A6*4 accounts for the majority of PMs in Asians.
*CYP2A6*2*	L160H	1–3		<1	↓ Activity	• Because nicotine is converted to cotinine by CYP2A6, a high expression/activity of CYP2A6 is proposed to increase the susceptibility to nicotine addiction and the risk of tobacco-related cancers. Therefore, CYP2A6 genetic variation could play a role in nicotine addition and tobacco-related cancer risks.
*CYP2A6*4*	Gene deletion	0.5–1.0	7–22	15–20	Abolished activity	
CYP2B6						• CYP2B6 is mainly expressed in the liver, accounting for 6% of total CYPs.
*CYP2B6*4*	K262R	5			↑ Activity	• The anticancer drug CPA is bioactivated by CYP2B6. CYP2B6 polymorphisms would likely affect the PK and/or PD of CPA. For example, CYP2B6*6 carriers exhibited ↑ CPA clearance and CPA 4-hydroxylation activity.
*CYP2B6*5*	R487C	11–14	1		↓ Expression	
*CYP2B6*6*	Q172H; K262R	16–26	16		↑ Activity	
*CYP2B6*7*	Q172H; K262R; R487C	13	0		↑ Activity	• CYP2B6 polymorphisms may affect the PK and therapeutic outcome of anti-HIV agents such as efavirenz and nevirapine. For example, CYP2B6 Q172H variant is associated with ↑ plasma concentrations of efavirenz and nevirapine.
CYP2C8						• CYP2C8 accounts for ~7% of total hepatic CYP contents.
*CYP2C8*2*	I269F	0.4		18	↓ Activity	• CYP2C8*3 is associated with ↓ clearance of both R- and S-ibuprofen.
*CYP2C8*3*	R139K; K399R	13	0	2	↓ Activity	
*CYP2C8*4*	I264M	7.5			↓ Activity	
CYP2C9						• CYP2C9 accounts for ~20% of total hepatic CYP contents.
*CYP2C9*2*	R144C	13–22	0	3	↓ Activity	• CYP2C9*2 and *3 have been shown to affect the oral clearance of at least 17 different CYP2C9 substrate drugs, eg, S-warfarin, celecoxib, ibuprofen, and phenytoin.
*CYP2C9*3*	I359L	3–16	3	1.3	↓ Activity	
*CYP2C9*5*	D360E	0	2	0	↓ Activity	

(*Continued*)

Table 1 (*Continued*)

Common allelic variants	Polymorphism/substitution	Allele frequency (%)[a]			Functional effect[b]	Highlights of clinical relevance[c]
		Caucasian	Asian	African		
CYP2C19						• The PM phenotype of CYP2C19 occurs in 12%–23% of Asian population, while in 1%–6% of Caucasians and 1.0%–7.5% of black Africans. • Polymorphisms in the CYP2C19 gene are known to affect the PK and/or response of several classes of drugs, including proton pump inhibitors (eg, omeprazole) and barbiturates.
*CYP2C19*2*	Splicing defect; I331V	15	30	17	Abolished activity	
*CYP2C19*3*	W212X; I331V	0.04	5	0.4	Abolished activity	
*CYP2C19*17*	I331V	18	4		↑ Transcription	
CYP2D6						• CYP2D6 accounts for ~2% of total hepatic CYP contents. However, it is involved in the metabolism of ~25% of all drugs in clinical use. • Unlike other CYPs, CYP2D6 is not inducible, and thus genetic polymorphisms are largely responsible for the variation in enzyme expression and activity. • CYP2D6 genotypes exhibit large interethnic differences: low frequency of PM in Asian (~1%) and African (0%–5%) population, compared with Caucasian (5%–14%). • CYP2D6 genotype is of great importance for the PK and response of many drugs, including tricyclic antidepressants, antiarrhytmics, neuroleptics, analgesics, antiemetics, and anticancer drugs.
*CYP2D6*3*	Frameshift	1–2	<1		Abolished activity (PM)	
*CYP2D6*4*	Splicing defect	20–25	1	6–7	Abolished activity (PM)	
*CYP2D6*5*	Gene deletion	4–6	4–6	4–6	Abolished activity (PM)	
*CYP2D6*10*	P34S; S486T	<2	50	3–9	↓ Activity (IM)	
*CYP2D6*17*	T107I; R296C; S486T	<1		20–34	↓ Activity (IM)	
*CYP2D6*41*	R296C; splicing defect; S486T	1.3	2	5.8	↓ Activity (IM)	
*CYP2D6*1* × *N*, *N* ≥ 2	Gene duplication				↑ Activity (UM)	
*CYP2D6*2* × *N*, *N* ≥ 2	Gene duplication				↑ Activity (UM)	
CYP3A4						• CYP3A4 has the highest abundance in the human liver (~40%) and metabolizes over 50% of all currently used drugs. • Genetic polymorphisms in CYP3A4 appear to be more prevalent in Caucasians than in Asians. • There is no consensus on a direct functional or clinical association of CYP3A4 polymorphism. CYP3A4 polymorphism may have minor or moderate clinical relevance.
*CYP3A4*1B*	5′ flanking region	2–9	0	35–67	Altered expression	
*CYP3A4*2*	S222P	2.7–4.5	0	0	Substrate-dependent altered activity	
*CYP3A4*3*	M445T	1.1			↓ Activity	
*CYP3A4*17*	F189S	2.1			↓ Activity	
*CYP3A4*18*	L293P	0		1	↑ Activity	
CYP3A5						• The clinical relevance of the CYP3A5 polymorphism is demonstrated by the fact that the PK of the immunosuppressive drug tacrolimus is associated with CYP3A5 genotype.
*CYP3A5*3*	Splicing defect	90	75	50	Abolished activity	
*CYP3A5*6*	Splicing defect	0	0	7.5	Severely ↓ activity	
*CYP3A5*7*	346Frameshift	0	0	8	Severely ↓ activity	
CYP3A7						• CYP3A7 is a predominantly fetal enzyme. • The in-vivo functional effect of CYP3A7 polymorphism is demonstrated by the fact that carriers of CYP3A7*1C allele had significantly decreased endogenous level of DHEAS, a specific substrate of CYP3A7.
*CYP3A7*1C*	Promoter	3		6	↑ Expression	
*CYP3A7*2*	T409R	8	28	62	↑ Activity	

Notes: [a]Allele frequency data are obtained from seven studies;[3,131–136] [b]Functional effect data are obtained from the Human Cytochrome P450 (*CYP*) Allele Nomenclature Committee website (http://www.cypalleles.ki.se/); [c]Comprehensive reviews were written by Zhou et al,[4] Bozina et al,[137] and Ingelman-Sundberg.[138]

Abbreviations: CPA, cyclophosphamide; CYP, cytochrome P450; DHEAS, dehydroepiandrosterone sulfate; IM, intermediate metabolizer; PD, pharmacodynamics; PK, pharmacokinetics; PM, poor metabolizer; UM, ultra rapid metabolizer.

a higher frequency in Caucasians (5%–14%) compared with Africans (0%–5%) and Asians (0%–1%). Ultrarapid metabolizers of CYP2D6, resulted from gene duplication or multiduplications, have a higher frequency in Saudi Arabians (20%) and black Ethiopians (29%) compared with Caucasians (1%–10%).[3] The inter-ethnic difference in the *CYP2D6* genotypes may contribute to the inter-ethnic variations in the disposition and response of substrate drugs. CYP2D6 is involved in the metabolism of ~25% of all drugs in clinical use, although it accounts for ~2% of total hepatic CYP content. *CYP2D6* genotype is of great importance for the pharmacokinetics and response of many drugs, including tricyclic antidepressants, antiarrhytmics, neuroleptics, analgesics, antiemetics, and anticancer drugs.[4]

The human *CYP2C9* and *CYP2C19* genes are highly homologous at the nucleotide level. The most common nonsynonymous *CYP2C9* polymorphisms, *CYP2C9*2* and *CYP2C9*3*, produce enzyme with differing affinity or intrinsic clearance for different substrates. While *CYP2C9*2* effects appear to be more substrate specific, *CYP2C9*3* variant exhibits reduced catalytic activity towards the majority of CYP2C9 substrates. The clinical importance of *CYP2C9* polymorphisms is exemplified by the dose adjustment of an oral anticoagulant warfarin based on CYP2C9 genotype. The patients carrying either *CYP2C9*2* or *CYP2C9*3* require a significantly smaller daily dose of warfarin to maintain desired therapeutic effects while avoiding severe toxicity, compared with patients carrying the wild-type *CYP2C9*.[5] With respect to *CYP2C19*, a splice site mutation in exon 4 (*CYP2C19*2*) and a premature stop codon in exon 4 (*CYP2C19*3*) represent the two most predominant null alleles. By genotyping for *CYP2C19*2* and **3*, one could detect ~84%, ~100%, and >90% of poor CYP2C19 metabolizers in Caucasians, Asians, and Africans, respectively. By also including *CYP2C19*4* and **6* alleles, ~92% of poor metabolizers in Caucasians can be detected. Generally, the poor metabolizer phenotype of *CYP2C19* occurs in 12%–23% of the Asian population, in 1%–6% of Caucasians, and in 1%–7.5% of black Africans. Polymorphisms in *CYP2C19* are known to affect the pharmacokinetics and/or response of several classes of drugs, including proton pump inhibitors (eg, omeprazole), barbiturates, and anticancer drugs.[4]

Phase II enzymes

The most important Phase II enzymes that exhibit functional and clinical relevant genetic polymorphisms are uridine diphosphate glucuronosyltransferase (UGT), sulfotransferase (SULT), glutathione S-transferases (GST), N-acetyltransferase (NAT), and thiopurine methyltransferase (TPMT).[1] Table 2 summarizes the most common functional polymorphisms in these Phase II enzymes and highlights their clinical significance.

Uridine diphosphate glucuronosyltransferases (UGTs)

The human UGT superfamily is a group of conjugating enzymes that catalyze the transfer of the glucuronic acid group of uridine diphosphoglucuronic acid to the functional group (eg, hydroxyl, carboxyl, amino, sulfur) of a specific substrate.[6] Glucuronidation increases the polarity of the substrates and facilitates their excretion in bile or urine. UGTs are membrane-bound enzymes localized in the endoplasmic reticulum of liver and many other extrahepatic tissues. Seventeen human UGT genes have been identified thus far, and classified into two subfamilies (ie, UGT1 and UGT2). Genetic polymorphisms have been identified for almost all the UGT family members. Genetic variations in the UGT genes could alter the function or expression of the protein, and potentially modify the glucuronidation capacity of the enzyme towards a given drug, carcinogen or endogenous compounds. It is evident that genetic variations in the UGT genes contribute to differential susceptibility to diseases (eg, cancer) as well as influence the pharmacokinetics and clinical outcome of substrate drugs.[6,7] The most common functional polymorphisms within the major UGT enzymes and their clinical relevance are summarized in Table 2. A representative example is that the *UGT1A1* low promoter activity alleles (ie, *UGT1A1*28*) is associated with decreased glucuronidation of SN-38 (an active metabolite of irinotecan), thereby resulting in increased risk for irinotecan-induced toxicity.

Sulfotransferases (SULTs)

Cytosolic SULTs are Phase II enzymes that catalyze the transfer of the sulfonyl group from the cofactor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the nucleophilic sites of a variety of substrates including hormones and xenobiotics. Sulfo conjugation of xenobiotics can lead to the formation of polar, excretable products as well as reactive, potentially mutagenic and carcinogenic metabolites.[8] A total of 11 SULT proteins encoded by 10 genes have been identified in humans. They differ in substrate specificity and tissue distribution. Single nucleotide polymorphisms (SNPs) have been identified in most of the human SULT genes. Functional SNPs in SULTs that are associated with altered enzymatic activity have potential to influence therapeutic response and to modify cancer susceptibility.[8,9] One widely studied

Table 2 Most common naturally occurring functional polymorphisms in major human Phase II drug-metabolizing enzymes: allele frequency, functional effect, and highlights of clinical relevance

Allelic variants	Polymorphism/ substitution	Allele frequency (%)			Functional effect	Highlights of clinical relevance
		Caucasian	Asian	African		
UGT1A1[a]						• UGT1A1 low promoter activity alleles (eg, UGT1A1*28) is significantly associated with ↓ glucuronidation of SN-38 (the active metabolite of irinotecan), thereby resulting in ↑ risk for irinotecan-induced toxicity. • Genetic variations in UGT1A1 may modify susceptibility to steroid-related cancers including breast, ovarian, endometrial and prostate cancers.
*UGT1A1*6*	G71R	0	13–23	–	↓ Activity	
*UGT1A1*28*	(TA)6>(TA)7 in promoter	29–40	13–16	36–43	↓ Expression	
*UGT1A1*33*						
*UGT1A1*34*	(TA)6>(TA)5 in promoter	0.0–0.7	0	3–8	↑ Expression	
	(TA)6>(TA)8 in promoter	0–0.7	0	0.9–7.0	↓ Expression	
UGT1A6[a]						• UGT1A6 catalyses the glucuronidation of aspirin and acetaminophen. • "Low activity" UGT1A6 variants, leading to increased salicylate levels in aspirin users, are associated with a lower risk of colon cancer.
*UGT1A6*2*	T181A, R184S	30	23		↓ Activity	
*UGT1A6*3*	R184S	1–2	1.6		Unknown	
*UGT1A6*4*	T181A	2.4			Unknown	
UGT1A7[a]						• UGT1A7 is an important extrahepatic UGT that inactivates a variety of carcinogenes. • Low-activity UGT1A7 variants increases the risk of developing tobacco-related cancers, specifically orolaryngeal cancer.
*UGT1A7*2*	N129K, R131K	24–34	15	39	Similar activity	
*UGT1A7*3*	N129K, R131K, W208R	23–36	26	23	↓ Activity	
*UGT1A7*4*	W208R	1–1.7	0	1	↓ Activity	
UGT2B7[a]						• UGT2B7 is of major significance for the glucuronidation of a number of clinically important drugs (eg, morphinan derivatives, epiribicin, and zidovudine. • Further studies are needed to elucidate the clinical impact of the UGT2B7 polymorphism.
*UGT2B7*2*	H268Y	49–54	27		Similar or decreased activity	
UGT2B15[a]						• UGT2B15 is the most efficient UGT2B involved in the inactivation of steroid hormones, mainly androgens. • UGT2B15 polymorphisms have a potential role in a modified risk of prostate cancer.
*UGT2B15*2*	D85Y	52–55	36–49	39	↑ Activity	
SULT1A1[b]						• SULT1A1 is the most highly expressed hepatic SULT. • SULT1A1 plays an important role in the sulfation of the metabolites of tamoxifen, 4-hydroxy-tamoxifen and endoxifen. SULT1A1*2 is associated with decreased survival of breast cancer patients treated with tamoxifen.
*SULT1A1*2*	R213H	25–36	4.5–17.0	27–29	↓ Activity and ↓ thermal stability	
*SULT1A1*3*	M223V	1.2	0.6	23	Similar activity	
GST[c]						• GSTA1 is involved in glutathione conjugation of the active metabolites of CPA. GSTA1*B allele is associated with higher survival rate of breast cancer patients treated with CPA-containing chemotherapy. • The GSTM null genotype is associated with an increased risk of the lung, colon, and bladder cancer. • AML patients carrying GSTM*0 appears to have a better response to adriamycin and cyclophosphamide treatment.
*GSTA1*B*	Promoter point mutation (T-631G, T-567G, C-69T, G-52A)	40		41	↓ Expression	
*GSTM1*0*	Gene deletion	42–58		27–41	Abolished activity	

*GSTP1*B*	I105V	6–40		54	↓ Activity	• The GSTP1*B allele is associated with lower clearance of etoposide and reduced risk of relapse in childhood ALL patients. • The GSTP1*B allele is associated with increased survival rate in patients with advance colorectal cancer or breast cancer. • The GSTT1 deletion is associated with reduced risk of relapse in childhood ALL patients. • The GSTT1 deletion is a poor prognostic factor for survival in adult ALL.
*GSTT1*0*	Gene deletion	2–42			Abolished activity	
***NAT*[d]**						• NAT1*14 and *17 are associated with slow acetylator phenotype. • NAT2*5, *6, *7, *10, *14, and *19 lead to slow acetylator phenotype. • NAT2 slow acetylator phenotype is associated with increased susceptibility to hydrolazine- and isoniazid-induced toxicity. • NAT2 slow acetylator phenotype is associated with increased risk of bladder cancer.
*NAT1*4*	Wild-type				Normal	
*NAT1*14*	R187Q	1.3–3.7			↓ Activity	
*NAT1*14*	R187Stop				↓ Activity	
*NAT1*17*	R64W				↓ Activity	
*NAT1*19*	R33Stop				↓ Activity	
*NAT1*22*	D251V				↓ Activity	
*NAT2*4*	Wild-type				Normal	
*NAT2*5*	I114T				↓ Activity	
*NAT2*6*	R197Q				↓ Activity	
*NAT2*7*	G286E				↓ Activity	
*NAT2*10*	E167K				↓ Activity	
*NAT2*14*	R64Q				↓ Activity	
*NAT2*17*	Q145P				↓ Activity	
*NAT2*19*	R64W				↓ Activity	
***TPMT*[e]**						• TPMT is involved in the methylation reaction of mercaptopurine, an anticancer drug used in the treatment of childhood ALL. • The TPMT genotype correlated well with in-vivo enzyme activity and is clearly associated with a risk of mercaptopurine-induced toxicity. Patients with poor or intermediate TMPT activity may tolerate only one-tenth to half of the average mercaptopurine dose.
*TPMT*2*	A80P	0.0–0.5	0	0.0–0.4	↓ Activity	
*TPMT*3A*	A154Y, Y240C	0.0–0.6	0–1	0.0–0.8	Abolished activity	
*TPMT*3B*	Y240C	–	0	–	9-fold ↓ activity	
*TPMT*3C*	A154Y	0.2–3.3	0.0–0.2	2.4–7.6	1.4-fold ↓ activity	

Notes: [a]Data on UGT SNP allele frequencies, function effect, and clinical relevance are summarized from Guillemette[6] and Nagar and Remmel;[7] [b]Data on *SULT1A1* SNP allele frequencies, function effect, and clinical relevance are summarized from Glatt and Meinl[8] and Nowell and Falany;[9] [c]Data on GST SNP allele frequencies, function effect, and clinical relevance are summarized from Lo and Ali-Osman[10] and McIlwain;[11] [d]Data on NAT SNP allele frequencies, function effect, and clinical relevance are summarized from Sim et al,[13] Hein,[15] and Agundez;[139] [e]Data on TPMT SNP allele frequencies, function effects, and clinical relevance are summarized from Zhou[18] and Hamdy et al.[140]
Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CPA, cyclophosphamide.

functional SNP is *SULT1A1*2* (Arg213His) that exhibits reduced enzymatic activity and thermal stability (Table 2).

Glutathione S-transferases (GST)

The super family of human GST catalyzes the conjugation of glutathione (GSH) to a wide range of endogenous metabolites and xenobiotics including alkylating and free radical generating anticancer drugs.[10] Human GSTs are categorized into three main families: cytosolic/nuclear, mitochondrial, and microsomal. The cytosolic GSTs are further divided into seven classes: alpha, mu, omega, pi, sigma, theta, and zeta. Besides their enzymatic function, GSTs also possess nonenzymatic functions, in which they act as regulators of cell signaling and post-translational modification pathway in response to stress, growth factors, and DNA damage, and in cell proliferation, cell death, and other processes that ultimately lead to tumor growth and drug resistance. These multiple functionalities establish the importance of GSTs as determinants of cancer susceptibility, therapeutic response, and prognosis.[10,11] Most human GSTs have SNPs and, less frequently, deletions. The association of GST polymorphisms with cancer incidence, cancer treatment, and prognosis is highlighted in Table 2.

N-acetyltransferase (NAT)

The human NATs catalyze the transfer of an acetyl group from acetyl coenzyme A to arylamines, arylhydroxylamines, and arylhydrazines.[12] Two human NAT genes, *NAT1* and *NAT2*, carry functional polymorphisms that influence the enzyme activity. Based on the level of NAT activity, patients can be classified into two phenotypes: fast acetylator (wild-type NAT acetylation activity) and slow acetylator (reduced NAT enzyme activity). For example, polymorphisms or haplotype in the *NAT1* (ie, *NAT1*14*, **15*, **17*, **19*, and **22*) and *NAT2* (eg, *NAT2*5*, **6*, **7*, **10*, **14*, and **17*) lead to slow acetylation phenotype.[13] A comprehensive list of the *NAT1/2* alleles is presented on the website http://louisville.edu/medschool/pharmacology/NAT.htlm. NAT2 plays an important role in the activation and/or deactivation of a large and diverse number of aromatic amine and hydrazine drugs used in clinic, and therefore the *NAT2* genotype is particular relevant to the response to these drugs. One representative example is the association of the slow-acetylator *NAT2* phenotype with increased risk for an antituberculosis drug (isoniazid)-induced hepatitis.[14] In addition, because NAT1 and NAT2 catalyze the bioactivation (via O-acetylation) of aromatic and hetercyclic amine carcinogens, genetic variations in the *NAT1/2* genes may modify the cancer risk related to exposure to these carcinogens.[15] For instance, the slow-acetylator *NAT2* phenotype is known to relate to a higher risk for bladder cancer.[16,17]

Thiopurine S-methyltransferase (TPMT)

TPMT is best known for its key role in the metabolism of the thiopurine drugs (eg, 6-mercaptopurine, azathiopurine, and 6-thioguanine) by catalyzing the S-methylation of thiopurine drugs via S-adenosyl-L-methionine as the S-methyl donor. These drugs are clinically used to treat cancers or as immunosuppressants. The *TPMT* gene exhibits significant genetic polymorphisms across all ethnic groups studied, with 18 *TMPT* alleles identified to date. Three main *TPMT* alleles, namely *TPMT*2* (reduced activity), **3A* (abolished activity), and **3C* (reduced activity), account for 80%–95% of the intermediate and poor metabolizers.[18] Patients who inherit defect *TPMT* alleles or *TPMT* deficiency (ie, two nonfunctional alleles) are at significantly increased risk for thiopurine-induced toxicity (eg, myelosuppression). Indeed, patients with absent TMPT activity (~0.3% prevalence) or low activity (~10% prevalence) may tolerate only 5%–50% of the average mercaptopurine dose. Clinical diagnostic tests are now available for the detection of the SNPs in the human *TPMT* gene that lead to decreased or abolished enzyme activity. On the FDA-approved drug labels, *TPMT* variant pharmacogenetic test is recommended before treating patients with azathiopurine, mercaptopurine, and thioguanine.[19]

Drug-transporter pharmacogenomics

In addition to drug metabolizing enzymes, uptake and efflux transporters that facilitate the movement of drugs in or out of the cell are important determinants of drug disposition and response. Broadly, drug transporters are classified into two families, namely efflux transporters of the adenosine triphosphate (ATP)-binding cassette (ABC) family and uptake transporters of the solute carrier (SLC) family. In the ABC transporter family, 49 genes have been identified and classified into seven subfamilies from ABCA through ABCG based on the sequence homology (http://nutrigene.4t.com/humanabc.htm). The ABC transporters are responsible for transport of diverse substrates out of the cell using ATP as an energy source. Among these, ABCB1, ABCC1/2, and ABCG2 have been well characterized for their roles in drug disposition and response. In the SLC family, 360 genes have been identified and classified into 46 subfamilies

(http://www.bioparadigms.org/slc/menu.asp). Of particular relevance to drug disposition are members of the organic anion transporting polypeptides (OATP), organic cation transporter (OCT), and organic anion transporter (OAT) subfamilies.

Table 3 summarizes the pharmacologically most important efflux ABC transporters (including ABCB1, ABCC1/2, and ABCG2) and uptake SLC transporters (including OATP, OCT, and OAT families), their tissue distributions, and representative drug substrates. These transporters play crucial roles in the intestinal absorption, biliary excretion, renal excretion, and tissue/cellular penetration of a wide variety of therapeutic drugs, and therefore they are important determinants of drug exposure in the system and at the site of action.[20] Genetic polymorphisms may influence the expression, subcellular localization, substrate specificity, and/or intrinsic transport activity of the transporter proteins and therefore, influence the disposition and response of drug substrates. The following sections serve to highlight the functional and clinical significance of the most commonly naturally occurring genetic polymorphisms within the pharmacologically most important ABC and SLC transporters with respect to drug disposition and response. A comprehensive list of genetic variants in the ABC and SLC transporters and related information are

Table 3 The pharmacologically most important efflux and uptake drug transporters, tissue distribution, and representative substrate drugs[a]

Gene	Protein	Tissue distribution	Polarity	Representative drug substrates
ABC transporters				
ABCB1	MDR1 (P-gp)	Liver, intestine, kidney, blood–brain barrier, lymphocytes, placenta	AP	Anthracyclines, taxanes, vinca alkaloids, imatinib, etoposide, levofloxacin, erythromycin, cyclosporine, tacrolimus, digoxin, quinidine, verapamil, diltiazem, ritonavir, saquinavir, talinolol, phenytoin, cimetidine, simvastatin, morphine, hydrocortisone
ABCC1	MRP1 (GS-X)	Ubiquitous	BL	Anthracyclines, vinca alkaloids, irinotecan, SN-38, methotrexate, camptothecins, saquinavir, ritonavir, difloxacin, drug-glucuronate/-glutathione/-sulfate conjugates
ABCC2	MRP2 (cMOAT)	Liver, kidney, intestine	AP	Anthracyclines, vinca alkaloids, methotrexate, camptothecins, rifampin, pravastatin, and drug-glucuronate/-glutathione/-sulfate conjugates
ABCG2	BCRP	Liver, intestine, placenta, breast	AP	Anthracyclines, irinotecan, SN38, SN38G, imatinib, tamoxifen
SLC transporters				
OATP family				
SLC21A3	OATP1A2 (OATP-A)	Ubiquitous, with highest expression in brain and testis	BL	Rosuvastatin, methotrexate, ouabain, D-penicillamine
SLC21A6	OATP1B1 (OATP-C)	Liver	BL	Statin, pravastatin, fexofenadine, and repaglinide, rosuvastatin, ouabain, D-penicillamine, rifampin
SLC21A8	OATP1B3 (OATP8)	Liver	BL	Digoxin, rifampin, ouabain, methotrexate, D-penicillamine, rosuvastatin, cyclosporin
SLC21A9	OATP2B1 (OATP-B)	Ubiquitous	BL	Benzylpenicillin, rosuvastatin
OCT family				
SLC22A1	OCT1	Liver	BL	Metformin, cisplatin, oxaliplatin, imatinib, procainamide, citalopram, cimetidine, quinidine, verapamil, acyclovir
SLC22A2	OCT2	Kidney	BL	Metformin, cisplatin, oxaliplatin, imatinib, procainamide, citalopram, cimetidine, quinidine, amantadine
SLC22A3	OCT3	Brain, liver, kidney, heart, muscle, placenta, and blood vessels	BL	Cimetidine, agmatine, adefovir, catecholamines
OAT family				
SLC22A6	OAT1	Kidney, brain	BL	Methotrexate, salicylate, antiviral agents (eg, acyclovir)
SLC22A7	OAT2	Liver, kidney	BL	Methotrexate, salicylate, tetracyclines
SLC22A8	OAT3	Kidney, brain, muscle	BL	Methotrexate, antiviral agents (eg, acyclovir), cimetidine, pravastatin, salicylate
SLC22A11	OAT4	Kidney, placenta	AP	Methotrexate, cimetidine, salicylate, tetracyclines

Notes: [a]Comprehensive information on tissue distribution, substrates, and other transporter-related information can be found at http://www.tp-search.jp, http://www.bioparadigms.org/slc/menu.asp, and http://nutrigene.4t.com/humanabc.htm.

Abbreviations: AP, apical; BL, basolateral; BCRP, breast cancer resistance protein; GS-X, glutathione S-conjugate pump; MDR1, multidrug resistance 1; MOAT, multispecific organic anion transporter; MRP, multidrug resistance-related protein; OATP, organic anion transporting peptides; OCT, organic cation transporter; OAT, organic anion transporter; P-gp, P-glycoprotein.

available in Pharmacogenetics Research Network databases at http://www.pharmGKB.org.

ABCB1, ABCC1/2, and ABCG2 efflux transporters

ABCB1 gene

The *ABCB1* gene, also named as the multidrug resistance 1 (*MDR1*) gene, encodes a polypeptide (P-glycoprotein) that has two halves, each containing six hydrophobic transmembrane domains and an ATP-binding domain. ABCB1, located on the apical or luminal surface of the epithelial cells, functions as an efflux transporter in restricting intestinal absorption, facilitating hepatobiliary excretion and renal excretion, and protecting the brain and fetus from xenobiotics. In addition, ABCB1 overexpression in cancer cells is implicated in multidrug resistance to chemotherapeutic agents.[21] ABCB1 transports a broad spectrum of structurally and functionally diverse drugs, including anticancer agents, antibiotics, immunosuppresants, cardiac drugs, calcium channel antagonists, and HIV protease inhibitors (Table 3). Of note, there is a strong overlap in substrate specificity and tissue distribution for ABCB1 and CYP3A4/5.[22]

More than 50 SNPs have been identified in the human *ABCB1* coding region. The most common SNPs are the synonymous 1236C>T and 3435C>T and the nonsynonymous 2677G>T (Ala899Ser). The allele frequencies of these three SNPs vary in different ethnic populations (Table 4). The 3435C>T SNP has strong linkage disequilibrium with other SNPs in the *ABCB1* gene, creating common haplotypes consisting of 3435C>T combined with 2677G>T and/or 1236C>T.

Given the important role of ABCB1 in drug absorption and disposition, genetic polymorphisms in the *ABCB1* gene may influence the outcome of pharmacotherapy. The first investigation of the functional and clinical effect of ABCB1 polymorphism was reported for a silent SNP 3435C>T, which was found to be associated with decreased duodenal expression of ABCB1 and thereby increased plasma concentration of digoxin after oral administration in humans.[23] A recent study demonstrates that the 3435C>T SNP affects the timing of cotranslational folding and insertion of ABCB1 into the membrane, thereby altering substrate specificity.[24] In the past decade, a number of preclinical and clinical studies have been conducted investigating the

Table 4 Most common functional polymorphisms in human ABCB1, ABCC1/2, and ABCG2: allele frequency and functional effects

Allele variants	Polymorphism/ substitution	Allele frequency (%)[a]			Functional effects
		Caucasian	Asian	African	
ABCB1					
1236C>T	Silent	34–42	60–72	15–21	Affects co-translational folding in nearby amino acids that are essential for ATP-binding and ATP hydrolysis[141]
2677G>T /A	A893S /T	38–47 /1–10	32–62 /3–22	15/ND	Affects ABCB1 expression or function, but data are inconsistent[27]
3435C>T	Silent	48–59	37–66	10–27	Affects co-translational folding in nearby amino acids, thereby altering substrate specificity[24]
ABCB1*13	1236C>T/2677G>T/3435C>T haplotype	23–42	28–56	4.5–8.7	Affects the inhibition of ABCB1 by a small subset of modulators[24]
ABCC1					
128G>C	C43S		1		Reduced plasma membrane localization, ↓vincristine resistance in transfected cells[142]
1299G>T	R433S	1.4			Changes in transport and resistance[143]
2012G>T	G671V	2.8			Associated with anthracycline-induced cardiotoxicity[32]
ABCC2					
1271A>G	R412G				DJS; ↓ in methotrexate elimination[144]
1249G>A	V417I	22–26	13–19	14	Changes in ABCC2 expression and localization[33,36,43]
3563T>A	V1188E	4–7	1		Associated with anthracycline-induced cardiotoxicity[32]
4544G>A	C1515Y	4–9			Associated with anthracycline-induced cardiotoxicity[32]
ABCG2					
34G>A	V12M	2–10	15–18	4–6	Changes in transport and resistance[145,146]
376C>T	Q126stop	0	0.9–1.7	0	Loss of transport activity[147]
421C>A	Q141K	9–14	27–35	1–5	Affects the ATP-binding domain, thereby leading to reduced transport activity[145,146]

Note: [a]Data of allele frequencies are obtained from Marzolini et al[27] and Gradhand and Kim.[31]
Abbreviations: ATP, adenosine triphosphate; DJS, Dubin-Johnson Syndrome.

association of *ABCB1* genotype with its tissue expression and function, and with pharmacokinetics and pharmacodynamics of substrates drugs (Table 4).[25] However, data reported on the functional and clinical impacts of *ABCB1* polymorphisms are often inconsistent.[20,25–28] The desrepancies may be partly explained by lack of standardized methodology and assays among different studies. In addition, SNPs of ABCB1 may often result in very subtle functional outcomes. For example, a recent study demostrates that the haplotype 1236C>T/2677G>T/3435C>T does not change the substrate transport per se but instead affects the inhibition of transport by a small subset of modulators.[24] Conflicting results on the clinical impact of *ABCB1* polymorphisms may reflect the complex disposition pathway of the substrate drugs. For example, the commonly used in-vivo ABCB1 probe drugs such as digoxin, fexofenadine, and talinolol were found to be the dual substrates for both ABCB1 and OATP transporters; cyclosporine is not only transported by ABCB1 but also metabolized by CYP3A4. This means that potential ABCB1 effect may be marked by the activity of OATP transporters or CYP3A4. Hence, a systemic analysis of polymorphisms in multiple genes known or suspected to contribute to drug disposition and response would provide better insights on the genetic impact on pharmacotherapy. In addition, the *ABCB1* has multiple polymorphisms, some of which are in linkage disequilibrium, and therefore a haplotype approach would allow a more accurate prediction of clinical phenotypes.

ABCC1 and ABCC2

ABCC1/2, also called multidrug resistance-related proteins (MRP1/2), plays an essential role in transport and excretion of organic anions including physiological metabolites, carcinogens, and drugs. They are also believed to confer multidrug resistance to chemotherapeutic agents.[29] ABCC1 and ABCC2 have overlapping substrate specificities, typically glutathione, glucuronate, or sulfate conjugated and unconjugated drugs, including many anticancer agents (eg, vincristine and doxorubicin), HIV protease inhibitors (eg, ritonavir and saquinavir), and antibiotics (eg, difloxacin and grepafloxacin) (Table 3). Both ABCC1 and ABCC2 require co-transport of reduced glutathione (GSH) to transport some of their substrates.[30] ABCC1 is located in basolateral membranes of polarized cells, whereas ABCC2 is located to the apical domain. While ABCC1 is ubiquitously expressed, ABCC2 is mainly expressed in hepatocytes, renal proximal tubule cells, intestine, and brain (Table 3).

The human *ABCC1* appears to be a conserved gene because many of the naturally occurring genetic variants in *ABCC1* are relatively rare. Of the identified SNPs in the non-oding and coding region of *ABCC1*, 16 are known to result in amino acid changes, and some of them exhibit functional effects on either expression or function of the protein (Table 4).[31] Data on the role of *ABCC1* polymorphisms in terms of in-vivo physiology and clinical drug resistance or toxicity are rather limited. Interestingly, one study has identified significant associations of *ABCC1* 2012G>T (Gly671Val) and a haplotype of *ABCC2* with anthracycline-induced cardiotoxicity among non-Hodgkin lymphoma patients treated with doxorubicin.[32]

Mutations in the ABCC2 gene have been initially identified in Dubin–Johnson Syndrome (DJS), a relatively rare recessive disorder characterized by conjugated hyperbilirubinemia resulting from loss of expression and function of ABCC2 in the liver. However, the impact of this loss of hepatic ABCC2-medicated transport on the pharmacokinetics of drug substrates in humans is unknown. Of the more commonly occurring *ABCC2* SNPs, 1249G>A (Val417Ile) has been extensively studied. The effect of this SNP on ABCC2 expression varies depending on the tissue examined. For example, 1249G>A SNP was associated with lower ABCC2 mRNA and protein levels in preterm placenta, but not in duodenum and liver.[33,34] One study demonstrated a possible association of 1249G>A variant with tenofovir-induced renal proximal tubulopathy, suggesting this SNP may influence renal excretion of some ABCC2 substrates.[35] In addition, 1249G>A SNP has been associated with changes in the ABCC2 localization in neuroepithelial tumors.[36] A number of other nonsynonymous and synonymous SNPs have been studied for their potential functional influence on the ABCC2 expression and transport activity (Table 4). It appears that *ABCC2* SNPs have varied functional influence on different organs, or different substrates, or between in vitro and in vivo studies.[31]

ABCG2

The ABCG2 (also known as BCRP, ABCP, or MXR) protein is an ABC half-transporter that bears six transmembrane domains and one ATP-binding domain. The protein actively extrudes a wide variety of chemically unrelated hydrophobic or partially hydrophobic compounds from the cells, including cytotoxic compounds (eg, mitoxantrone, topotecan, SN-38, flavopiridol, and methotrexate), fluorescent dyes (eg, Hoechst 33342), and toxic compounds found in normal food (eg, pheophorbide A) (Table 3). ABCG2 is expressed in the canalicular

membrane of hepatocytes, in the epithelia of small intestine, colon, placenta, lung, kidney, adrenal and sweat glands, as well as in the endothelia of the central nerve system vasculature. It is responsible for host detoxification and protection against potentially toxic xenobiotics.[37–39] ABCG2 transporter-mediated efflux has been increasingly recognized to not only confer drug resistance but also significantly modulate drug absorption, distribution, metabolism, and excretion.[40–44]

More than 80 polymorphisms in the *ABCG2* gene have been identified in different ethnic populations.[45–48] Several naturally occurring SNPs in ABCG2 have been found to affect the function and/or expression of its encoded protein.[46,49–51] In particular, a functional SNP in exon 5 of the *ABCG2* gene, in which a C→A nucleotide transition at position 421 (*ABCG2* 421C>A), results in a nonsynonymous variant protein with a glutamine to lysine amino acid substitution in codon 141 (Q141K).[46] The *ABCG2* 421C>A variant has been associated with low ABCG2 expression levels and altered substrate specificity,[46] and has been found to alter the pharmacokinetics of diflomotecan and topotecan.[40,52] In addition, recent studies have demonstrated that the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors such as gefitinib and erlotinib are ABCG2 substrates, and the *ABCG2* 421C>A variant is associated with greater gefitinib accumulation at steady-state and related to higher incidence of gefitinib-induced grade 1 or 2 diarrhea in cancer patients compared with the wild-type ABCG2.[53,54]

OATP, OCT, and OAT uptake transporters

Organic anion transporting polypeptides (OATPs)

OATPs are membrane influx transporters that facilitate cellular uptake of a wide range of endogenous compounds (eg, bile salts, hormones, and steroid conjugates) and clinically important drugs (eg, HMG-CoA-reductase inhibitors, cardiac glycosides, anticancer agents, and antibiotics) (Table 3). Of the 11 human OATP transporters, OATP1A2, OATP1B1, OATP1B3, and OATP2B1 are best characterized for their roles in drug pharmacokinetics. OATP1A2 is expressed on the luminal membrane of small intestinal enterocytes and at the blood–brain barrier and may facilitate the intestinal absorption and brain penetration of its substrates. OATP1B1, OATP1B3, and OATP2B1 are mainly expressed on the sinusoidal membrane of hepatocytes and can facilitate the hepatic uptake of their substrate drugs for further metabolism or biliary excretion.[55]

A number of SNPs and other genetic variations have been identified in the *SLCO1B1* gene (encoding OATP1B1), and their allele frequencies vary markedly across different populations (Table 5).[56] Some of *SLCO1B1* SNPs and haplotypes have been associated with impaired transport activity in vitro towards different substrates.[57–59] These functional impaired OATP1B1 variants may limit the uptake of the substrate drugs into the hepatocytes, thereby resulting in decreased biliary excretion or hepatic metabolism and thus increased systemic exposure. For example, a common variant allele, 521T>C, is associated with increased systemic exposure (eg, AUC) of several OATP1B1 drug substrates, including repaglinide and statins such as pravastatin.[60,61] *OATP1B1*15* (a haplotype of 388A>G and 512T>C) is associated with increased plasma concentrations of pravastatin[62] and increased concentrations of SN-38.[63,64] *OATP1B1*17* (a haplotype of -11187G>A, 388A>G and 512T>C) is associated with increased effect of pravastatin on rate of cholesterol synthesis. A recent genome-wide association study has demonstrated that a noncoding rs4363657 SNP, which is in nearly complete linkage disequilibrium with the *SLCO1B1* 521T>C SNP, is the only strong marker associated with simvastatin-induced myopathy.[65]

With respect to the *SLCO1A2* gene (encoding OATP1A2), several nonsynonymous polymorphisms have been identified, some of which demonstrate decreased in-vitro transport activity towards OATP1A2 substrates (Table 5).[66] The impacts of these functional SNPs on the pharmacokinetics and clinical outcome of clinical used drugs need further studies. With respect to OATP1B1 and OATP2B1, there are few data on the clinical relevance of *SLCO1B3* and *SLCO2B1* polymorphisms, although some genetic variations within these two genes have been associated with altered in-vitro transport activity of the protein (Table 5).[67,68]

Organic cation transporter (OCT)

The OCTs belong to the solute carrier SLC22A family that mediate intracellular uptake of a broad range of structurally diverse small organic cations (molecular weight < 400). Three isoforms, OCT1, OCT2, and OCT3, with partially overlapping substrate spectrum, are identified in humans (Table 3). OCT1 is primarily expressed in the sinusoidal membrane of hepatocytes, whereas OCT2 is predominantly expressed in the basolateral membrane of the kidney proximal tubules; OCT3 is expressed in many tissues including placenta, heart, liver, and skeletal muscle (Table 3). The expression of OCTs was also detected in several cancer cell lines and tumor tissue samples.[69,70]

A number of nonsynonymous SNPs have been identified in the *SLC22A1* (encoding OCT1) and *SLC22A2* (encoding

Table 5 Most commonly naturally occurring nonsynonymous SNPs in genes encoding human OATP, OCT, and OAT transporters: allele frequency and functional effects

Allele variant	Polymorphism/ substitution	Allele frequency (%)[a]			Functional effects[b]
		Caucasian	Asian	African	
OATP					
***SLCO1A2* (OATP1A2)**					
38T>C	I3T	11.1	0	2.1	↑Transport activity
516A>C	E172D	5.3	0	2.1	↓Transport activity
833A	N278del	0	0	0.6	↓Transport activity
***SLCO1B1* (OATP1B1)**					
217T>C	F73L	2	0	0	↓Transport activity
388A>G	N130D	30	54	74	↓Transport activity
463C>A	P155T	16	0	2	No alteration
521T>C	V174A	14	0.7	2	↓Transport activity
1463G>C	G488A	0		9	↓Transport activity
2000A>G	E667G	2		34	↓Transport activity
***SLCO1B3* (OATP1B3)**					
334T>G	S112A	74			Unknown
699G>A	M233I	71			Unknown
1564G>T	G522C	1.9			Affect localization and ↓Transport activity
***SLCO2B1* (OATP2B1)**					
1457C>T	S486F	1.2	30.9		↓Transport activity
OCT					
SLC22A1 *(OCT1)*					
41C>T	S14F	0	0	3.1	↓Transport of metformin but ↑transport of MPP
480C>G	G160L	0.65	8.6–13.0	0.5	No alteration
1022C>T	P341L	0	16	8.2	↓Transport of MPP but not metformin
1201G>A	G401S	1.1	0	0.7	↓Transport activity
1222A>G	M408V	60	74–81	74	No alteration
1256delATG	M420del	18	0	2.9	↓Transport of metformin but not MPP
1393G>A	G465R	4	0	0	↓Transport activity
***SLC22A2* (OCT2)**					
596C>T	T199I	0	1	0	↓Transport activity
602C>T	T201M	0	1.3–2.0	0	↓Transport activity
808G>T	A270S	16	14–17	11	↓Transport activity
1198C>T	R400C	0	0	1.5	↓Transport activity
1294A>C	K432Q	0	0	1	↓Transport activity
OAT					
***SLC22A6* (OAT1)**					
20T>C	L7P	1	<1	1	
149G>A	R50H	1	1	1	↑Transport activity
1361G>A	R454Q	0	0	<1	↓Transport activity
***SLC22A7* (OAT2)**					
329C>T	T110I	1	1	1	Unknown
571G>A	V192I	1	1	1	Unknown
1520G>A	G507D	1	1	1	Unknown
***SLC22A8* (OAT3)**					
523A>G	I175V	1	1	1	Unknown
829C>T	R277W				↓Transport activity
***SLC22A11* (OAT4)**					
37G>A	V13M	1	1	1	Unknown
142C>T	R48Ter	1	1	1	Unknown
185C>G	T62R	1	1	1	Unknown
463G>A	V155M	1	1	1	Unknown
732C>T	A244V	1	1	1	Unknown
832G>A	E278K	1	1	1	Unknown
1015G>A	V339M	1	1	1	Unknown
1175C>T	T392I	1	1	1	Unknown

Notes: [a]Allele frequency data are obtained from four studies;[20,71,77,148] [b]Functional effect data are summarized from five studies.[66,71,149–151]
Abbreviations: OAT, organic anion transporter; OATP, organic anion transporting polypeptide; OCT, organic cation transporter; SNP, single nucleotide polymorphism.

OCT2) from different ethnic groups; some of them have demonstrated altered (mostly impaired) transport function in vitro (Table 5).[71] With respect to the *SLC22A3* (encoding OCT3), several synonymous SNPs have been identified, but their functional consequence is unknown. The functional polymorphisms in the *OCT* genes may influence the clinical pharmacokinetics and response of drug substrates. For example, functional polymorphisms of *OCT1* and *OCT2* impact the clinical effects and pharmacokinetics of metaformin, a drug used as a primary therapy for type 2 diabetes mellitus. Metaformin is eliminated predominantly by renal excretion in humans.[72] Because of the high expression of OCT2 in the kidney and the active renal secretion of metaformin via OCT2, defect function in OCT2 transport would result in decreased renal clearance of this drug. There is evidence that carriers of homozygous for low activity OCT2 variant 270S have a significant lower renal clearance and higher plasma concentration of metformin than those homozygous for the active variant (270A).[73,74] On the other hand, low-function OCT1 variants including R61C, G401S, M420del, and G465R have been associated with significantly higher renal clearance of metaformin.[75] In addition to the effect on metformin pharmacokinetics, low-function OCT1 variants (R61C, G401S, M420del, and G465R) have been also associated with significantly decreased glucose-lowering response of metaformin in healthy volunteers probably by reducing the metaformin uptake in hepatocytes, which is the major target site of metformin's action.[76]

Organic anion transporter (OAT)

The OATs belong to the SLC22 family of solute carriers that mediate cellular uptake of a broad range of structurally diverse small hydrophilic organic anions. OAT substrates include many clinically important anionic drugs, such as β-lactam antibiotics, diuretics, nonsterioidal anti-inflammatory drugs, nucleoside/nucleotide antiviral drugs, and anticancer agents (Table 3). There are at least six OAT members (OAT1–6). OAT1–3 localize to the basolateral membrane of the renal proximal tubule mediating the uptake of drug substrates from blood into the proximal tubule cells, whereas OAT4 localizes to the apical side of the renal proximal tubule functioning in the secretion of drug substrates into urine. Collectively, these transporters are responsible for the movement of drug substrates from the blood to the urine. Thus, genetic variations in the genes encoding OATs may contribute to interindividual variability in the renal clearance of drug substrates. To date, a number of polymorphisms have been reported in the coding region and 5′ regulatory region of human *SLC22A6* (encoding OAT1), *SLC22A7* (encoding OAT2), *SLC22A8* (encoding OAT3), and *SLC22A11* gene (encoding OAT4) (Table 5); some of them resulted in altered in vitro transport activity of the protein.[77,78] Nevertheless, the coding region polymorphisms in these genes are infrequent (~1%). The regulatory region polymorphisms of these genes, particularly *SLC22A8* (encoding OAT3), may be especially important in accounting for variation in the renal clearance of drug substrates.[78] The functional and clinical relevance of these coding and regulatory region polymorphisms need further study.

Pharmacogenomics in cancer therapy

5-fluorouracil (5-FU)

Since its introduction more than 50 years ago,[79] 5-FU has remained the most frequently prescribed anticancer drug for the treatment of malignancies of the gastrointestinal tract including colorectal and gastric cancer. 5-FU, a fluoropyrimidine analog, is a prodrug that is converted to the active metabolite, 5-fluoro-2-deoxyuridine monophosphate (FdUMP) that leads to inhibition of thymidylate synthase (TS) and subsequently inhibition of DNA synthesis. 5-FU is converted to FdUMP through three pathways: oratate phosphoribosyltransferase (OPRT) (pathway 1), uridine phosphorylase (UP) (pathway 2), and thymidine phosphorylase (TP) (pathway 3) (Figure 1).[80] The vast majority (~80%–85%) of administered 5-FU is metabolized by the enzyme dihydropyrimidine dehydrogenase (DPD) in the liver into the inactive form, dihydrofluorouracil (FDHU), and excreted as a fluoro-β-alanine.

The Phase I metabolizing enzyme DPD plays the most important role in detoxification of 5-FU. Expression of DPD has been related to tolerance and response to 5-FU-based chemotherapy. Specifically, low expression or absence of DPD has been associated with accumulation of 5-FU, thereby exposing patients to increased risk of severe toxicities, while high expression of DPD has been associated with poor response to 5-FU.[81,82] Patients lacking the DPD enzyme may experience severe to lethal toxicities when receiving 5-FU-based chemotherapy. Genetic aberration in the dihydropyrimidine dehydrogenase (*DPYD*) gene, such as exon skipping, deletion, and missense mutation, contributes to a DPD-deficiency phenotype. Approximately 3%–5% of the population is partially or completely deficient in DPD enzyme activity.[83] The most known *DPYD* SNPs associated with grade 3 and 4 toxicities are IVS14 + 1G>A, 2846A>T, 1679T>G, and 85T>C.[84] In particular, the exon 14-skipping

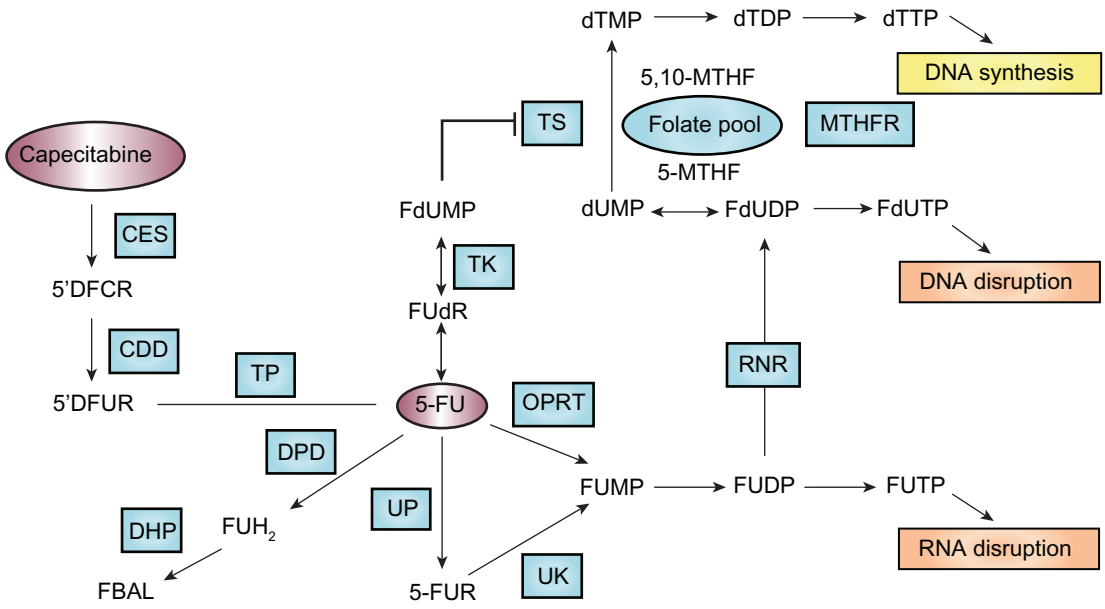

Figure 1 Pathways that affect 5-FU efficacy. Genetic polymorphisms within the genes that are involved in 5-FU metabolic activation (eg, OPRT), detoxification (eg, DPD), and target interaction (eg, TS) are important determinants of the efficacy and safety of 5-FU treatment.

Abbreviations: 5′DFCR, 5′deoxy-5-fluorocytidine; 5′DFUR, 3′deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; 5-FUR, 5-fluorouridine; CDD, cytosine deaminase; CES, carboxylesterase; DHP, dihydropyrimidinase; DPD, dihydropyrimidine dehydrogenase; FBAL, fluoro-b-alanine; FUH2, dihydro-5-fluorouracil; MTHFR, methylenetetrahydrofolate reductase; OPRT, orotate phosphoribosyltransferase; RNR, ribonucleotide reductase; TK, thymidine kinase; TP, thymidine phosphorylase; TS, thymidylate synthase; UK, uridine-cytidine kinase 2; UP, uridine phosphorylase 1.

mutation IVS14 + 1G>A, a G-to-A point mutation within the 5′-splicing site of intron 14, leads to a mutant DPD that lacks amino acids 581–635 and consequently lacks catalytic activity. The allele frequency of this mutation was 0.91% in a Dutch Caucasian population.[85] In patients heterozygous for the IVS14 + 1G>A allele, half of the mean normal activity of DPD is found, which is sufficient to lead to severe 5-FU toxicities. In patients homozygous for the IVS14 + 1G>A allele, DPD activity is completely lacking, and 5-FU toxicities become life-threatening and sometimes fatal.[86,87]

Orotate phosphoribosyltransferase (OPRT), an enzyme contributing to phosphorylation and activation of 5-FU (Figure 1), may serve as a predictor of response to 5-FU-based chemotherapy. Overexpression and high levels of OPRT mRNA, as well as a high OPRT/DPD ratio have been associated with improved response to 5-FU-based chemotherapy in patients with metastatic colorectal cancer.[88] In a retrospective study examining the association of TS and OPRT genotypes with 5-FU related toxicity, co-presence of the OPRT Gly213Ala variant allele and TS 2R/2R genotype was related to grade 3 and 4 neutropenia and diarrhea.[89]

In addition to pharmacogenetic influence of genes involved in 5-FU pharmacokinetics, genetic polymorphisms in the drug target also impact the clinical outcome of patients receiving 5-FU. 5-FU acts by inhibition of TS. TS expression varies considerably among tumors. The mechanism of the variability in TS expression is not fully understood; however, there is evidence that TS expression is modulated by three functional significant gemeline polymorphisms in the 5′ and 3′ untranslated regions (5′UTR and 3′UTR) of the TS gene. These include a polymorphic tandem repeat of a 28-base pair (bp) sequence that is present in either duplicate (2R) or in triplicate (3R) in the TS promoter enhancer region (TS 2R>3R polymorphism), a SNP (G>C) in the second repeat when three repeats are present (TS 3R G>C SNP), and a 6-bp deletion in the 3′UTR of the TS gene (TS 1494del6bp). The TS 2R>3R polymorphism is of clinical significance as patients with metastatic colorectal cancer homozygous for the triple repeat (TS 3R/3R) had significantly higher intratumoral TS gene expression.[90,91] The 28-bp TS tandem repeats contain elements that bind upstream stimulating factor (USF) and thus act to enhance transcriptional activity of the TS gene. The presence of a G>C SNP within the second repeat of the 3R allele results in decreased transcriptional activity by abolishing the binding of USF within the repeat.[92] The 6-bp deletion in the 3′UTR of the TS gene could decrease TS mRNA stability, and has been associated with decreased intratumoral TS mRNA level in patients.[93] TS polymorphisms are not only prognostic factors of disease-free survival and overall survival but also predictors of chemotherapeutic

benefit from 5-FU-based chemotherapy. In general, most studies support that patients with colorectal cancer who have a high-expression genotype (ie, TS-2R/3G, -3C/3G, -3G/3G, 3′-UTR +6bp/+6bp) showed a trend toward poor prognosis and worse response to 5-FU-based chemotherapy, but possibly less severe toxicities, compared with the low-expression group (ie, TS-2R/2R, -2R/3C, -3C/3C, 3′-UTR + 6bp/-6bp, -6bp/-bp).[94]

In summary, genetic polymorphisms within the genes that are involved in 5-FU metabolic activation (eg, OPRT), detoxification (eg, DPD), and target interaction (eg, TS) are important determinants of the efficacy and safety of 5-FU treatment. Systemic assessment of the genotypes of these genes would allow tailoring 5-FU-based chemotherapy for individual patients.

Irinotecan

The topoisomerase I inhibitor irinotecan (CPT-11) is a water-soluble, semisynthetic derivative of camptothecin, a plant alkaloid isolated from *Camptotheca acuminata* (family Nyssaceae). It is widely used in the treatment of metastatic colorectal cancer, either in combination with 5-fluorouracil in the first-line treatment setting or as monotherapy in the second-line setting.[95] Irinotecan acts as a prodrug and undergoes complex disposition pathways in vivo (Figure 2). Specifically, irinotecan is activated to 7-ethyl-10-hydroxycamptothecin (SN-38) by human carboxylesterase 1 and 2 (hCE1 and hCE2). SN-38 is subsequently detoxified by UGT1A1 to a β-glucuronide derivative, SN-38G.[96] In addition, irinotecan undergoes CYP3A4-mediated oxidation to form two inactive metabolites, 7-ethyl-10-(4-N-(5-aminopentanoic acid)-1-piperidino) carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino) carbonyloxycamptothecin (NPC), the latter of which also undergo a subsequent conversion by CES2 to SN-38.[97,98] The pharmacological behavior of irinotecan can be additionally complicated by the substrate affinity of irinotecan and its metabolites (ie, SN-38 and SN-38G) for the ABC transporters including ABCB1, ABCC1/2, and ABCG2[99,100] (Figure 2).

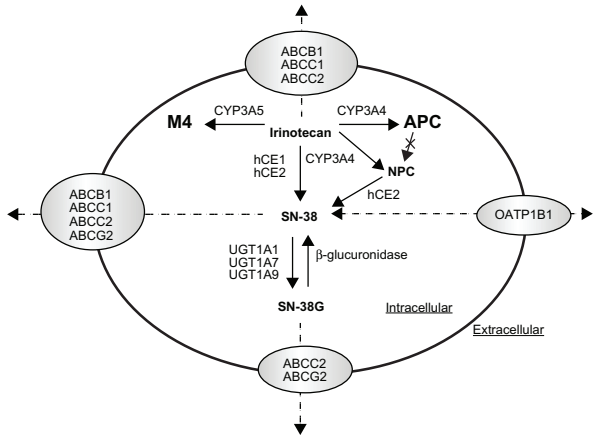

Figure 2 Schematic illustration of irinotecan disposition pathway. Irinotecan is activated to 7-ethyl-10-hydroxycamptothecin (SN-38) by human carboxylesterase 1 and 2 (hCE1 and hCE2), and SN-38 is subsequently detoxified by UGT1A1 to a β-glucuronide derivative, SN-38G. In addition, irinotecan undergoes CYP3A4-mediated oxidation to form the inactive metabolites 7-ethyl-10-(4-N-(5-aminopentanoic acid)-1-peperidino) carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-peperidino) carbonyloxycamptothecin (NPC), and NPC also undergo a subsequent conversion by hCE2 to SN-38. Irinotecan and its metabolites (ie, SN-38 and SN-38G) are also transported by the ABC transporters including ABCB1, ABCC1/2, or ABCG2 or the organic anion transporting polypeptide 1B1 (OATP1B1).
Adapted and reprinted by permission from the American Association for Cancer Research: van Erp NP, Baker SD, Zhao M, et al. Effect of milk thistle (*Silybum marianum*) on the pharmacokinetics of irinotecan. *Clin Cancer Res*. 2005;11:7800–7806.

In clinical use, irinotecan exhibits substantial interindividual variability in its pharmacokinetics, efficacy, and toxicity profiles,[101] which is, in part, related to genetic polymorphisms in the metabolic enzymes and transporters involved in irinotecan disposition. It is evident that the genetic variant *UGT1A1*28*, characterized by the presence of an additional TA repeat in the TATA sequence of the *UGT1A1* promoter ($(TA)_7TAA$ instead of $(TA)_6TAA$), is associated with reduced SN-38 glucuronidation and greater susceptibility to irinotecan induced gastrointestinal and hematological toxicity.[102–104] In addition, other polymorphisms in the *UGT1A1* gene have also been associated with irinotecan-related toxicity. Of particular importance to East Asian population is *UGT1A1*6* (Gly71Arg) with an allele frequency of ~12%, which reduces UGT1A1 catalytic activity by 60% in homozygotes.[105] This variant allele has been related to a higher incidence of toxicity in a population of Korean patients treated with irinotecan and cisplatin for advanced nonsmall cell lung cancer.[106] Additionally, two promoter variants (ie, *UGT1A1 −3263T>G* and *−3156G>A*), which are in strong linkage disequilibrium with *UGT1A1*28* in Caucasians while less apparent in African-Americans and Asians, have been associated with higher incidence of irinotecan-induced grade 4 neutropenia or diarrhea.[104,107]

Besides UGT1A1, associations between genetic polymorphisms within the genes encoding the drug transporters such as *ABCB1*, *ABCC1*, *ABCG2*, and *OATP* and irinotecan pharmacokinetics and/or toxicity have been reported, though the data is limited.[108] For *ABCB1*, *ABCB1 3435C>T* variant allele was related to higher irinotecan plasma concentration in Chinese,[109] and *ABCB1 1236C>T* variant allele resulted in a higher area under the plasma concentration-time curve (AUC) of irinotecan and SN-38 but lower AUC of SN-38G in a Caucasian population.[110] However, the true clinical relevance of a single SNP on *ABCB1* to irinotecan treatment

remains to be clarified. A haplotype approach may be more useful to estimate true genetic effects. A haplotype analysis in 49 Japanese patients indicated that the patients carrying the *ABCB1*2* haplotype (containing 1236C>T, 2677G>T, and 3435C>T) exhibit significantly lower renal clearance of irinotecan, SN-38, and APC.[111] With respect to ABCC2, which is involved in bile excretion of irinotecan, a functional SNP *ABCC2 3972C>T* was found to have significant effect on the AUC of irinotecan, APC, and SN-38G, all being higher in patients carrying homozygous 3972T.[112] With respect to ABCG2, though irinotecan and SN-38 are good substrates of this transporter, no significant associations between ABCG2 polymorphisms and irinotecan pharmacokinetics or toxicity have been demonstrated up to now. With respect to OATP1B1 that is involved in the transport of SN-38 but not SN-38G, variants of this transporter including 521T>C, −11187G>A, 388A>G, and *OATP1B1*15* haplotype have been associated with a lower clearance and higher systemic exposure of SN-38 and irinotecan.[63,64]

In addition to pharmacogenetic influence of genes involved in irinotecan pharmacokinetics, polymorphisms in the drug target of SN-38, topoisomerase I (TOP1), and cellular downstream effectors leading to DNA repair or cell death may influence patient outcomes to irinotecan treatment. A recent study in 107 advanced colorectal cancer patients showed that *TOP1* and *TDP1* haplotype tagging SNPs (htSNPs) were related to grade 3/4 neutropenia and response, respectively; and a DNA repair gene *XRCC1* haplotype was associated with response.[113]

Collectively, it is clear that *UGT1A1*28* is associated with greater susceptibility to irinotecan induced gastrointestinal and hematological toxicity. This risk was emphasized by a warning added to the package insert of irinotecan, where a reduced initial dose is recommended for patients homozygous for the *UGT1A1*28* allele. The true clinical significance of polymorphisms within other genes involved in the pharmacokinetics and pharmacodynamics of irinotecan with respect to patient outcome treated with this drug remains to be validated.

Tamoxifen

Tamoxifen, a selective estrogen receptor (ER) modulator, is a standard endocrine therapy for the treatment and prevention of ER-positive breast cancer. ER-positive breast cancers are often dependent on estrogen for growth. Selective ER modulators bind to the ligand-binding domain of an ER and block the binding of estrogen. This prevents conformational changes of the ER that it requires for its association with co-activators, thus blocking transcriptional activation functions of the ER and subsequently reducing or eliminating estrogen-driven proliferation of ER-postitive tumors.

Tamoxifen can be considered as a prodrug, which requires metabolic activation to exert its pharmacological activity. The metabolism of tamoxifen is complex and involves hepatic Phase I enzymes (including CYP3A4, CYP3A5, CYP2C9, CYP2C19, CYP1A2, CYP2B6, and CYP2D6, as well as flavin-containing monooxygenase 1 and 3) and Phase II enzymes (including SULT1A1 and UGTs) (Figure 3).[114–116] Specifically, tamoxifen is metabolized by hepatic CYP enzymes (mainly by CYP2D6 and CYP3A4/5) to form two main primary metabolites, 4-hydroxytamoxifen and N-desmethyltamoxifen. The formation of these metabolites accounts for ~92% and ~7% of primary tamoxifen oxidation, respectively.[115] Both of these metabolites are further converted to abundant and pharmacologically active 4-hydroxy-N-desmethyltamoxifen (endoxifen). Endoxifen formation from N-desmethyltamoxifen is almost exclusively catalysed by CYP2D6, and formation from 4-hydroxytamoxifen by CYP3A4/5.[115] In addition, tamoxifen and its metabolites undergo Phase II metabolism including sulphation and glucuronidation. Endoxifen and 4-hydroxytamoxifen show much greater affinity for the estrogen receptor than tamoxifen.[115–117] While 4-hydroxytamoxifen and endoxifen have a similar anti-estrogen activity, endoxifen plasma concentrations are 6- to 12-fold higher than those of 4-hydroxytamoxifen,[118] suggesting endoxifen is the predominant and crucial active metabolite responsible for the in vivo pharmacological activity of tamoxifen.

Given the key role of CYP2D6 in catalyzing the conversion of tamoxifen to its abundant active metabolite endoxifen, altered CYP2D6 activity due to either genetic or environmental (drug-induced or drug-inhibited) factor could directly affect endoxifen concentrations and likely the clinical outcome of patients treated with tamoxifen. As mentioned earlier, the functional alleles of CYP2D6 result in abolished, decreased, normal, or ultrarapid CYP2D6 enzyme activity (Table 1). As a result, patients can be classified as four phenotypes: poor metabolizer (PM) (abolished activity), intermediate metabolizer (IM) (reduced activity), extensive metabolizer (EM) (normal activity), and ultrarapid metabolizer (UM) (enhanced activity).

There is evidence that women with nonfunctional and reduced-function *CYP2D6* alleles appear to have significantly lower circulating endoxifen concentrations than those with wild-type *CYP2D6*.[119,120] Similarly, concomitant CYP2D6 inhibitors, such as certain selective serotonin

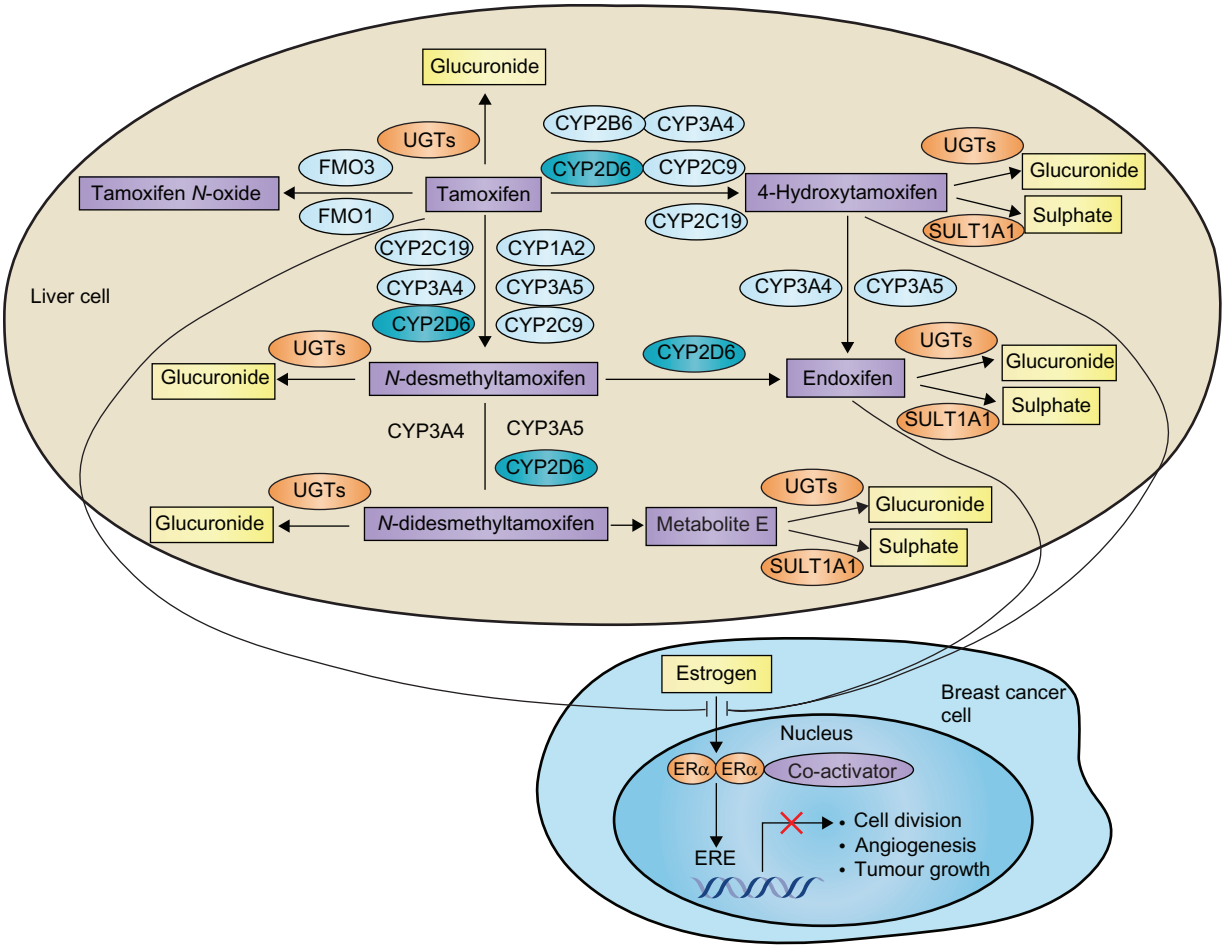

Figure 3 Metabolism pathway of tamoxifen and its interaction with estrogen receptors.
 Hoskins JM, Carey LA, McLeod HL. CYP2D6 and tamoxifen: DNA matters in breast cancer. *Nat Rev Cancer* 2009;9:576–586.[131]
Abbreviations: CYP, cytochrome P450; ER, estrogen receptor; FMO, flavin-containing monooxygenases; SULT1A1, sulphotransferase 1A1; UGT, uridine diphosphate glucuronosyltransferase.

reuptake inhibitors (SSRIs) (fluoretine and paroxetine) or selective noradrenaline reuptake inhibitors (SNRIs), have been shown to reduce the plasma concentrations of endoxifen.[118,119] It has also been shown that use of CYP2D6 inhibitors such as SSRIs and SNRIs has a negative impact on the efficacy of tamoxifen.[119] Collectively, these data support the notion that low CYP2D6 activity, caused by genetic polymorphisms or drug interactions, leads to low levels of the active tamoxifen metabolite.

The effect of CYP2D6 activity on tamoxifen pharmacokinetics also translates into an effect on clinical outcome. Despite conflicting data in some instance, the majority of retrospective studies suggest that the presence of nonfunctional or reduced-function alleles of CYP2D6 is associated with worse outcome of patients receiving tamoxifen.[121,122] Notably, a recent large retrospective analysis of 1325 patients with early-stage breast cancer treated with adjuvant tamoxifen suggests that compared with extensive metabolizer, those with decreased CYP2D6 activity (heterozygous extensive/intermedidate and poor metabolizers) have significantly increased risk of recurrence as well as worse event-free survival and disease-free survival.[123] In addition, *CYP2D6* genotype has also been shown to influence the efficacy of tamoxifen as a chemopreventive agent, whereby tamoxifen-treated women with poor metabolizer phenotype was associated with a significantly higher incidence of breast cancer compared with controls.[124] Findings from these studies support a role for the *CYP2D6* genotype in the activation of tamoxifen and likelihood of therapeutic benefit from testing for *CYP2D6* genoptype.

Besides *CYP2D6*, genetic variations in genes encoding for other enzymes involved in tamoxifen metabolism as well as genes encoding for the drug target (ie, estrogen receptor) may influence the efficacy and toxicicity of tamoxifen. This may

explain, at least in part, the descrepancies of results from different studies with respect to the role of CYP2D6 genotype in the clinical outcome of tamoxifen. Tamoxifen and its metabolites undergo Phase II metabolism by SULT1A1 and UGTs. Interindividual variation in the activity of Phase II enzymes may contribute to variability in circulating endoxifen levels and patient response to tamoxifen. It has been reported that women with high activity *UGT2B15* genotype (*UGT2B15*2/*2*) had a worse recurrence-free survival than those with wild-type alleles.[125] Interestingly, a retrospective analysis of 337 tamoxifen-treated women with breast cancer found that those with low-activity *SULT1A1* genotype (*SULT1A1*2/*2*) had approximately three times the risk of death as controls.[126] One possible explanation for this observation is that sulfation of tamoxifen metabolites (4-hydroxy-tamoxifen and endoxifen) by SULT1A1 forms highly reactive products leading to DNA adducts,[127,128] and therefore, individuals with low-activity *SULT1A1* genotype have poor clinical outcomes. In addition, genetic polymorphisms within the target genes (*ESR1* and *ESR2*) have been associated with altered susceptibility to tamoxifen-induced hot flashes and hormone-resistance.[129,130]

In conclusion, tamoxifen can be considered as a prodrug. Its metabolism is complex and involves multiple Phase I and II enzymes. Genetic variations in these metabolizing enzymes likely contribute to the variability in tamoxifen active metabolite concentrations and patient outcome. It is generally agreed that women with reduced CYP2D6 activity genotype appear to have lower circulating endoxifen concentration and are less likely to derive therapeutic benefit from tamoxifen compared with those with normal CYP2D6 activity. However, because of the lack of concordant data, mandatory *CYP2D6* genotyping test to guide the selection and dose of tamoxifen is premature. Idealy, large, prospective clinical studies should be conducted to systematically assess the impact of multiple genetic polymorphisms within multiple genes involved in the disposition and action of tamoxifen on the clinical outcome of patients receiving tamoxifen.

Conclusion

Pharmacogenomics provides a unique approach toward investigating, appreciating, and therapeutically serving the individual cancer patient. Continued investigation and adaptation of pharmacogenomics with respect to malignancy should likely provide improved risk versus benefit ratios with respect to therapeutic efficacy versus side-effect profiles. Further, effective re-evaluation of drug design toward the generation of novel and specific therapies focused on enzyme and transporter biology pertaining to malignancy may eventually be personalized and individualized to the patient for maximum efficacy.

Disclosure

The authors report no conflicts of interest in this work.

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