Genetic determinants of cancer drug efficacy and toxicity: practical considerations and perspectives

Myrna Candelaria[a], Lucia Taja-Chayeb[b], Claudia Arce-Salinas[a], Silvia Vidal-Millan[b], Alberto Serrano-Olvera[a] and Alfonso Dueñas-Gonzalez[c]

Drug-metabolizing enzymes are responsible for the activation or detoxification of cytotoxic drugs. Allelic variants are present with a variable frequency in different populations around the world and have an important role in the therapeutic index of such drugs. It is known that polymorphisms in thiopurine methyltransferase and dihydropyrimidine dehydrogenase have been associated with altered drug metabolism and increased risk of severe toxicity from 6-mercaptopurine and 5-fluorouracil, respectively. Additionally, a variant number of dinucleotide-repeat sequences in the promotor for uridine 5′-diphosphate glucuronosyltransferase 1A1 influences the glucuronidation of SN-38, the active metabolite of irinotecan, which is associated with severe toxicity, including diarrhea and neutropenia. In the same way, polymorphisms in thymidylate synthase have been associated with pyrimidine-associated toxicity and also with response to chemotherapy. The examples shown in this review demonstrate the usefulness of pre-screening patients for well-characterized polymorphism to identify the best-tolerated and most-effective treatment. ***Anti-Cancer Drugs*** **16:923–933**

Anti-Cancer Drugs 2005, **16**:923–933

Keywords: adverse drug reactions, pharmacogenomics, single-nucleotide polymorphism

Divisions of [a]Clinical Research, [b]Basic Research, Instituto Nacional de Cancerología and [c]Unidad de Investigación Biomédica en Cancer, Instituto Nacional de Cancerologia/Instituto de Investigaciones Biomédicas, UNAM, Tlalpan, Mexico.

Correspondence to M. Candelaria, Instituto Nacional de Cancerología. Ave. San Fernando 22, Col. Sección XVI, CP 14080, Tlalpan, Mexico, DF Mexico. Tel: +52 55 5628 04 79; fax: +52 55 5573 46 62; e-mail: myrnac@prodigy.net.mx

Received 9 May 2005 **Revised form accepted** 8 July 2005

Introduction

Inherited genetic variations in genes related to drug metabolism and the mechanism of action of anti-cancer drugs can influence the toxicity and response to chemotherapy in cancer patients [1]. Pharmacogenetics, the science of incorporating information on inherited genetic variability into predicting treatment response, has been developed in recent years as new information derived from the Human Genome Project has become available. The field of pharmacogenomics encompasses a wide range of efforts designed to elucidate genetic determinants of drug toxicity and efficacy [2].

Response to chemotherapeutic agents is primarily determined by the tumor genome and the individual's genome, but toxicity depends almost exclusively on the genotype of the non-tumor tissue [3]. Since the therapeutic index of such agents is narrow in most cases, research has been focused on single polymorphisms to get a more comprehensive approach, based on genetic variation in biological and pharmacological pathways, which may prescribe an optimal dose with a lower risk of toxicity and probably a higher response rate [4,5]. Polymorphisms have only a small variation from wild-type enzymes, but they constitute part of the polygenic metabolic or pharmacologic pathways.

In the UK, it has been estimated that adverse drug reactions (ADRs) due to cancer chemotherapy increase the overall hospital costs by 1.9% and drug costs by 15% [6,7]. Clearly, the current regimen of 'one dose fits all' for chemotherapy treatment is not ideal for all the patients and is not cost-effective for the health services. In the US, more than 2 million hospitalized patients experienced serious ADRs and more than 100 000 fatalities were registered, ranking ADRs as the fifth leading cause of death [8,9].

In addition to environmental influences, variation in the genetic constitution between individuals can have a major impact on drug activity. Allelic variants that occur with a frequency greater than 1% are called polymorphisms, whereas mutations are present at a frequency less than 1% [10]. Single-nucleotide polymorphisms (SNPs) account for over 90% of the genetic variation in the human genome. The remainder of the variation is caused by insertions and deletions (indels), tandem repeats and microsatellites [7].

This review summarizes the known polymorphisms which influence the metabolic pathways of cytotoxic drugs, as well as the importance of this genetic variation on the patient's toxicity and therapeutic outcome.

0959-4973

Single gene/single polymorphism

Genetic polymorphisms include nucleotide repeats, deletions, insertions and single-nucleotide substitutions that influence gene expression and/or function [11,12]. They have been identified in 93% of all known genes, with two coding-region SNPs observed in most genes evaluated, and have been linked to interindividual differences in the efficacy and toxicity of many medications [7].

For instance, polymorphisms in thiopurine methyltransferase (TPMT) and dihydropyrimidine dehydrogenase (DPYD) have been associated with altered drug metabolism and increased risk of severe toxicity from 6-mercaptopurine (6-MP) and 5-fluorouracil (5-FU), respectively [13–15]. Additionally, a variant number of dinucleotide-repeat sequences in the promotor for uridine 5′-diphosphate glucuronosyltransferase 1A1 (UGT1A1) influences the glucuronidation of SN-38, the active metabolite of irinotecan [16], and is associated with severe toxicity, including diarrhea and neutropenia.

Table 1 summarizes the polymorphisms described in metabolic pathways related to cytotoxic drugs.

Specific gene polymorphisms

TPMT

One of the most developed examples of clinical pharmacogenomics involves the genetic polymorphism of TPMT. TPMT is a cytoplasmic transmethylase present in prokaryotes and eukaryotes. It was originally found in the kidney and liver of rats and mice, and subsequently shown to be present in most human tissues. Human TPTM has a molecular mass of 28 kDa, comprises 245 amino acids and is not metal dependent. TPMT catalyzes the *S*-methylation of the thiopurine agents, azathioprine, 6-MP and thioguanine, which are used for a range of medical indications, including acute lymphoblastic leukemia, as well as immunosuppression for inflammatory diseases, severe rheumatic diseases or following organ transplantation [17,18]. The natural substrate for TPMT is not known.

TPMT activity exhibits genetic polymorphisms and mutant alleles with a codominant inheritance: approximately 90% of individuals inherit high activity (wild-type), 10% have intermediate activity because of polymorphism heterozygosity and 0.3% have low or no detectable enzyme activity, because they inherit two non-functional alleles [19].

Currently, 13 polymorphisms have been described [20,21], and most of them are located either on exon 7, 5 and/or 10 (Fig. 1). TPMT*1 has been assigned to the wild-type form. The first identified variant allele, TPMT*2, contains a 238 G→C transversion, leading to the substitution of a rigid proline for a more flexible alanine residue (Ala80Pro). As a result, the tertiary structure of the TPMT protein changes, leading to protein instability and decreased catalytic activity [22].

The second variant allele isolated, TPMT*3A, contains two transition polymorphisms [23], one in exon 7 (460 G→A) and the other one in exon 10 (719 A→G), each causing an amino acid change, whereas TPMT*3C contains only the single change in exon 10 (719 A→G) [24].

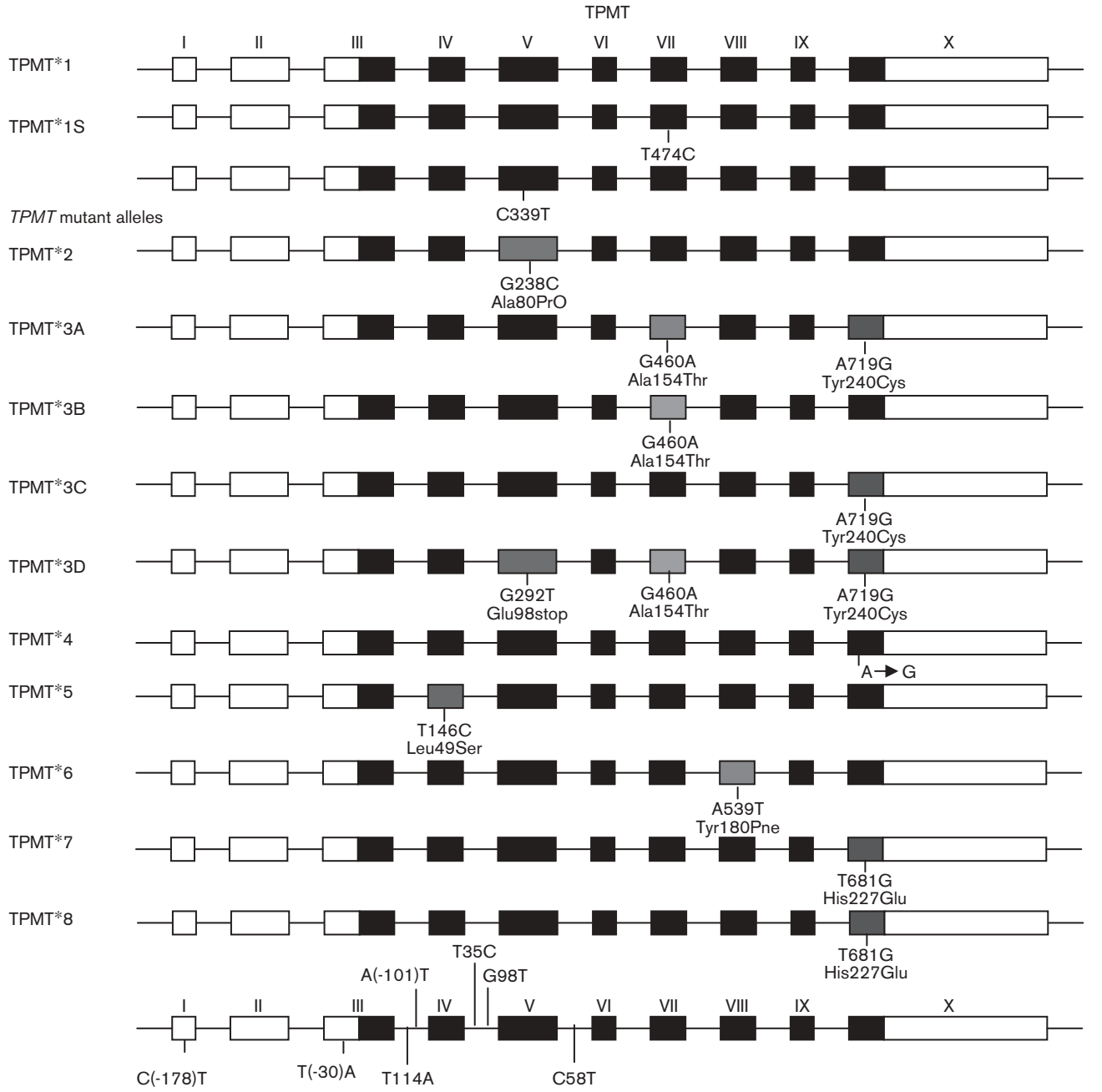
Most SNPs described for TPMT are functional – they either lead to amino acid substitutions (TPMT*2, *3A, *3B, *3C, *3D, *5, *6, *7 and *8), formation of a premature stop codon (TPMT*3D) or destruction of a splice site (TPMT*4). Additionally, SNPs for TPMT that do not alter encoded amino acids or are located within introns have been identified within exon 3, and two single polymorphisms within exons 5 and 12.

The frequency and type of TPMT polymorphisms have been documented in different populations. TPMT*3A is the most prevalent mutated allele in Caucasian, Argentinean and European populations. In contrast, TPMT*3C has been found only in African and Japanese populations (Table 2). Some authors have suggested that these polymorphisms may be used to study the migration of populations.

Table 1 **Polymorphisms in metabolism and response to cancer drugs**

Enzyme/metabolic pathway	Polymorphism	Drug involved	Findings and clinical significance
DPD	punctual mutation	fluoropyrimidines	higher toxicity: neurotoxicity myelosuppression
TPMT	protein instability	6-MP; azathioprine; thioguanine	acute: myelosuppression; chronic: second neoplasms
Glutathione transferase	deletions; punctual mutations	topoisomerase II inhibitors; alkylating agents	higher toxicity and anti-tumor effects
Glucuronosyl transferase	TA repetitions on promoter	irinotecan	increased diarrhea, myelosuppression
CYP 17	punctual mutation on promoter	estrogens	higher risk of secondary neoplasms
5,10-MTHFR	punctual mutation allowing an unstable protein	methotrexate	higher risk of mucositis
CYP3A4	polymorphism on promoter region	epidophyllotoxins	higher risk on developing secondary leukemia in wild-type

Fig. 1



Polymorphisms on TPMT. TPMT*1 has been assigned to the wild-type form. Most polymorphisms are located on exons 5, 7 and 10 or a combination of them.

Based on studies in different world populations [25–33], TPMT*3A, *3C and *2 are the predominant variant alleles, and genotyping has provided a molecular diagnostic with more than 95% concordance between genotype and phenotype [19,34–36].

Practical implications

Evans *et al.* [37] reported that patients with bone marrow intolerance to 6-MP were phenotypically TPMT deficient. A concordance has been found in patients with decreased activity and dose adjustment strategy: homozygous mutant patients required a median of 91% of dose reductions (50–94%) to tolerate full doses of all other chemotherapy treatments. Other authors recommend beginning with 6–10% of the standard dose of thiopurines. However, heterozygous patients had less concordance, with dose reductions ranging from 20 to 50% [38], and most of authors agree that heterozygous patients can

Table 2 Frequency and type of TPMT polymorphism in different populations

Reference	Population	Frequency (%)	Type of polymorphism
[25–33]	All	5–8	
[25,26]	Caucasian	Total: 10.1	TPMT2*A
		3.2–5.7	TPMT*2
		0.2–0.8	TPMT*3C
[27]	African	5.4–7.6	TPMT*3C
		not described	TPMT*3[a]
[28]	Bulgarians	7.4	TPMT*3A, *2, *3C
[29]	Argentina	8.2	TPMT*3A, *2, *4
[30]	Swedish	3.75	TPMT*3A
		0.44	TPMT*3C
		0.13	TPMT*3B
[31]	Italian	10.6 (total)	
		7.7	TPMT*3A
		1.9	TPMT*3C
		1.0	TPMT*2
[32]	south-west Asians; Chinese	2.0	TPMT*3A
		4.7	TPMT*3C (all)
[33]	Japan	0.8	TPMT*3C

begin with full doses, but have a significantly higher probability of requiring a dose reduction to avoid toxicity [18].

It is now well known that interindividual variation in sensitivity to thiopurines can be due to the common genetic polymorphisms affecting the TPMT gene. Detection of such SNPs is useful in patients before administration of thiopurines [39,40].

DPYD

Fluoropyrimidines are prescribed for the treatment of solid tumors, such as breast and colorectal cancer. In the liver, more than 80% of 5-FU is inactivated by DPYD. Approximately 3% of the population are thought to carry heterozygous mutations that inactivate DPYD and 0.1% is homozygous for inactivating mutations [41,42]. Patients with partial deficiency, who were thought to be heterozygotes for this disorder, also develop toxicity, since DPYD inheritance is autosomal codominant.

So far, at least 30 mutations have been described [43–57]; approximately 20 are functional in the DPYD gene and some of them have controversial results on DPYD activity [58] (Table 3). However, many of these polymorphisms have not been definitively associated with altered DPYD activity and not all toxicity to 5-FU can be explained from reduced DPYD activity. The most common consistent data are for allele DPYD*2A, which is a splice site mutation (G→A) that causes a skipping of exon 14, resulting in the production of a truncated mRNA, which leads to the formation of a defective protein. This defective DPYD is rapidly degraded, resulting in decreased detectable DPYD enzyme activity [59,60].

Practical implications

DPYD*2A has been associated with lethal outcomes in different trials [61,62]. Total DPYD deficiency results in

Table 3 Reported variations in the coding region of the DPYD gene

Reference	DPD enzyme activity	DPYD	Genotype	Exon
[43]	normal	*12	62G→A	2
[44]	uncertain	*12	74G→A	2
[45–48]	normal/deficient	*9A	85 T→C	2
[49]	deficient	*9A	257 C→T	4
[50]	deficient	*7	del TCAT 295–298	4
[51]	normal	*7	496 A→G	6
[49]	deficient	*7	601A→C	6
[49]	deficient	*7	632 A→G	6
[49]	deficient	*8	703C→T	7
[44]	uncertain	*8	703C→T	8
[52]	normal	*8	775A→G	8
[43]	deficient	*11	1003G→T	10
[49]	deficient	*11	1108A→G	10
[49]	deficient	*11	del TG 1039–1042	10
[43]	deficient	*12	1156G→T	11
[44]	normal	*12	1217T→C	11
[49]	deficient	*12	1475C→T	12
[46,53,54]	normal/uncertain	*4	1601G→A	13
[54]	normal/deficient	*5	1627 A→G	13
[45,46]	deficient	*13	1679T→G	13
[44]	uncertain	*13	1714C→G	13
[47]	normal/deficient	*2A	IVS14+1G→A	14
[44]	uncertain	*2A	T1896C	14
[55]	deficient	*3	1897delC	14
[43,46,47]	normal/deficient	*6	2194G→A	18
[48]	uncertain	*6	2657G→A	21
[47]	deficient	*6	2846A→T	22
[56]	uncertain	*6	2921A→T	23
[49]	deficient	*6	2933A→G	23
[57]	deficient	*10	2983G→T	23
[57]	normal	*10	3067C→T	23

defective pyrimidine metabolism and is associated with neurological disorders [63], whereas decreased activity is associated with more than 4-fold risk of severe or fatal toxicity from standard doses of 5-FU and is often accompanied by febrile neutropenia or agranulocytosis [64].

For patients who have received 5-FU and may have a DPYD deficiency, one may attempt to remove this fluoropyrimidine by hemodialysis and hemoperfusion. Other approaches to manage toxicity include administration of the pyrimidine nucleosides thymidine or uridine as soon as possible. Unfortunately, most of cases with DPYD deficiency are suspected following the development of severe toxicity, such as agranulocytosis, where intensive supportive care including colony-stimulating growth factors, appropriate antibiotic coverage for infections as well as appropriate fluid and electrolyte support with hospitalization in the intensive care unit is indicated.

Due to the DPYD*2A prevalence among patients with 5-FU severe toxicity, compared with normal individuals, it has been proposed that patients should be screened for this mutation [65]. The routine application of DPYD pharmacogenetic information to clinical practice has yet to be achieved. To date, the most reliable method for determining phenotype uses a radioassay for DPYD activity in human peripheral lymphocytes. Recently, a so-called 'uracil breath test' has been described which uses oral administration of [2–13C]uracil, resulting in the

release of the 2-carbon of uracil as $^{13}CO_2$ in the presence of active DPYD [66].

Thymidylate synthase (TS)

TS plays an important role in pyrimidine metabolism. This enzyme is required for *de novo* pyrimidine synthesis, together with a methyl cofactor, and it catalyses the methylation of dUMP to dTMP. As a pyrimidine analog, the 5-FU metabolite FdUMP forms a stable ternary complex with TS and the methyl cofactor, blocking the production dTMP and ultimately inhibiting DNA synthesis (Fig. 2). Three polymorphisms have been described in the TS gene (TYMS):

- A polymorphic 28-bp tandem repeat in the promoter enhancer region (TSER), which varies from two (TSER*2) to nine (TSER*9) copies of the tandem repeat. TSER*2 and TSER*3 are the most common alleles [67,68]. The higher numbers of repeats are found in African populations and their roles in TS expression are not known.
- A SNP has been described in the second repeat of the TSER*3 allele, which may also affect the level of TS expression in patients by abolishing a USF1-binding site. Preliminary results show a common allele (denoted 3RG) in 56% of Caucasian populations [69].
- A third polymorphism in the TYMS gene is a 6-bp deletion located in the 3′ untranslated region (UTR), 447 bp downstream from the stop codon. This deletion is present at an allele frequency of 27% in Caucasian populations [70,71]. Its presence is associated with a decreased response to 5-FU chemotherapy.

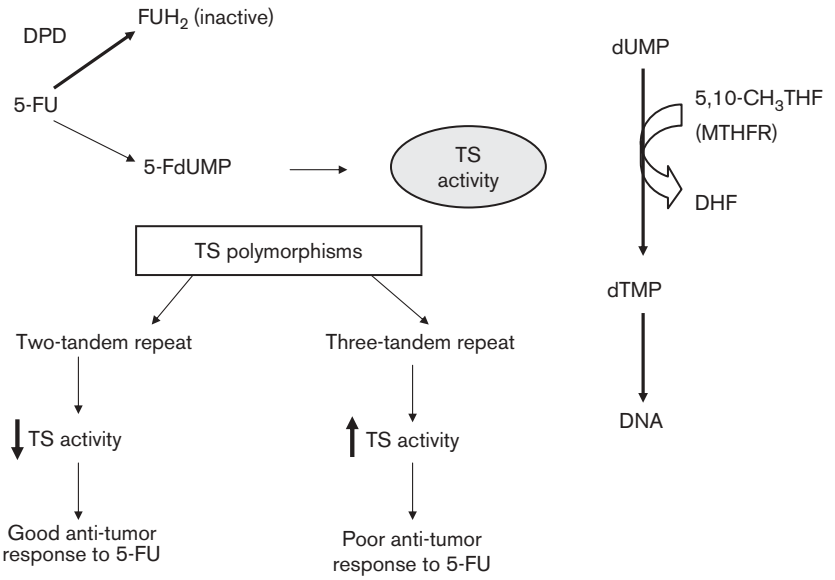
Practical implications

The overexpression of TS has been linked to 5-FU resistance and other TS inhibitors. In 2001, Villafranca [72] studied the TSER in 65 rectal cancer patients treated with chemoradiation. Over 60% of patients with at least one TSER*2 allele responded to treatment, whereas only 22% of homozygous patients for TSER*3 had tumor downstaging ($P = 0.002$). Larger-scale trials are required to better define the role of TSER polymorphism on the outcome of fluoropyrimidines chemotherapy.

Additionally, a functional role of the 3RG allele has been suggested in a study including 208 colorectal cancer patients and 675 controls, where a 1.3-fold increased risk of colorectal cancer was documented for patients with the 3RG allele [73].

Recently, McLeod *et al.* [74] demonstrated that homozygous patients for the 6-bp sequence had an odds ratio of 2.0 for response to 5-FU containing combination chemotherapy and also a significant association of this deletion allele with a reduced response to 5-FU chemotherapy.

Fig. 2



TS activity and polymorphisms. TS is required for *de novo* synthesis of pyrimidines. As a pyrimidine analog, the 5-FU metabolite FdUMP forms a stable ternary complex with TS and the methyl cofactor, blocking the production dTMP and ultimately inhibiting DNA synthesis. 5-FU is inactivated by DPD. Three types of polymorphisms have been described, including a polymorphic 28-bp tandem repeat in the promoter enhancer region, which translates the most common alleles TSER*2 and *3 (with two and three copies, respectively) and influence on tumor response to 5-FU after regulating TS activity. TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; FUH_2, dihydro-5-fluorouracil; 5-FdUMP, 5-fluoro-2-deoxyuridine monophosphate; dUMP, deoxyuridine monophosphate; DHF, dihydrofolate; 5,10-MTHFR, 5,10-methylenetetrahydrofolate.

A large-scale assessment of the role of each TS polymorphism, individually and as a haplotype, is now required to determine whether prospective assessment is warranted in patients prior to 5-FU-containing chemotherapy regimens.

Methylenetetrahydrofolate reductase (MTHFR)

MTHFR regulates the pool of intracellular folates for nucleic acid and protein synthesis. MTHFR converts 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a methyl donor in the conversion of homocysteine to methionine during protein synthesis [75] (Fig. 3). This enzyme maintains normal levels of reduced folates and homocysteine, and deficiencies of MTHFR have been implicated in neurological and vascular diseases.

Two polymorphism have been described in the MTHFR gene, the first one is a common genetic polymorphism, with a C→T transition at nucleotide 677, allowing substitution of alanine by valine at this position; this MTHFR variant has *in vitro* thermolability and 35% of residual activity. This C677T polymorphism alters the distribution of intracellular folates, creating retention of folates destined for purine and pyrimidine synthesis [76]. The second polymorphism occurs at 1298C;Glu429Ala, and also results in decreased of MTHFR activity.

Practical implications

Individuals with a homozygous mutant TT or heterozygous CT genotype have reduced MTHFR activity and generally have lower folate levels than those with a CC genotype [77]. The clinical significance of this polymorphism is likely to be dependent on dietary folate intake, which may differ substantially in countries that supplement cereal products with folate versus those that do not. The relevance of the C677T mutation has been shown in a phase I trial, including patients treated with CPT-11/raltritexed. Patients with a TT genotype seemed to be protected from raltritexed associated toxicity, compared with CC/CT patients [78].

A reduced risk of certain forms of acute adult and pediatric leukemias [79,80] and colon cancer [81] has been described in individuals with the second polymorphism (at 1298C;Glu429Ala).

Irinotecan and UGT1A1

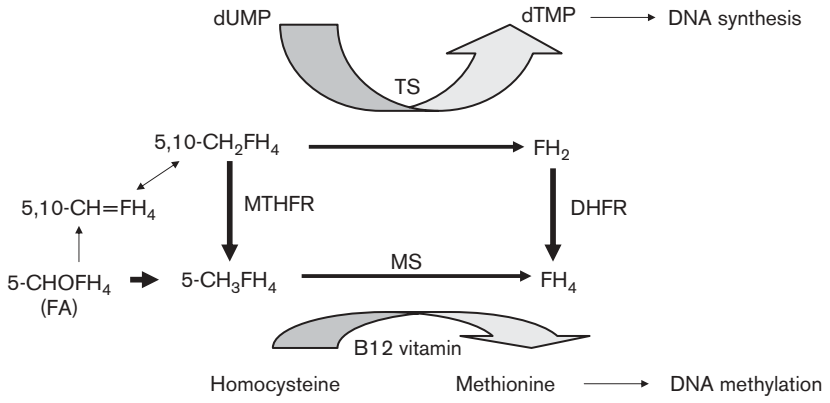
Irinotecan, a camptothecin analog, is used to treat colorectal, lung and other solid tumors. The active form of irinotecan, SN-38, can be inactivated through glucuronidation by a member of the UDP-glucuronosyltransferase family and the clinical pharmacogenetics of irinotecan (CPT-11) is mainly focused on this pathway. Hepatic UGT1A1, which has bilirubin as its endogenous substrate [82], inactivates SN-38 into the more polar SN-38 glucuronide, which is then eliminated in bile and urine [83].

A dinucleotide repeat in a TATA box in the UGT1A1 promoter results in altered UGT1A1 activity [84]. The variable number of TA repeats range from five to eight copies, six TA repeats represent the most common allele, with up to 33% in Caucasians having a variant allele containing seven repeats (UGT1A1*28) [85].

Practical implications

The dose-limiting toxicities of irinotecan consist of diarrhea and leukopenia [83], and these toxic effects are associated with excessive formation of SN-38. Significant associations between patients with the UGT1A1*28 allele and reduced UGT1A1 expression,

Fig. 3



Metabolic pathways related to MTHFR. 5,10-CH=FH_4, 5,10-methenyltetrahydrofolate; 5,10-CH_2FH_4, 5,10-methylenetetrahydrofolate; 5-CH_3FH_4, 5-methylentetrahydrolate; 5-$CHOFH_4$ (FA), 5-formyltetrahydrofolate; DHFR, dihydrofolate reductase; dTMP, deoxythymine 5′-monophosphate; dUMP, deoxyuridine 5′-monophosphate; FdUMP, 5-fluorodeoxyuridine 5′-monophosphate; FH_2, dihydrofolate; FH_4, tetrahydrofolate; MS, methionine synthase; MTHFR, methylenetetrahydrofolate reductase; TS, thymidylate synthase.

and, consequently, reduced SN-38 glucuronidation, have been shown in several studies [86,87]. Reduced UGT1A1 is linked to a high risk (approximately 4-fold) of severe toxicity from irinotecan treatment, including dose limiting diarrhea and neutropenia [88]. A prospective trial of UGT1A1 pharmacogenetics in cancer patients receiving 300 mg/m^2 CPT-11 found that the presence of the UGT1A1*28 allele altered SN-38 disposition. Highly significant lower SN-38G:SN-38 area under curve (AUC) ratios, and a trend toward more severe neutropenia and diarrhea were found in UGT1A1*28 carriers, compared with wild-type [85]. Recently, pharmacogenetic data from the North Central Cancer Treatment Group N9741 study showed that patients receiving CPT-11-based chemotherapy who were homozygous for the most common allele (six TA repeats) had significantly lower rates of grade 4 neutropenia (8.6%) than those who carried the 7/7 genotype (36%) [89].

Assessment of the presence of the UGT1A1*28 allele in patients prior to irinotecan treatment may predict individuals at risk for severe toxicity from irinotecan, allowing the selection of lower doses or alternative therapies.

ERCC1 gene and platinum chemotherapy

The platinum analogs (cisplatin, carboplatin and oxaliplatin) are widely used in the treatment of a variety of solid tumors, including testicular, lung, ovarian, breast and gastrointestinal cancer. It is known that the success of these drugs depends on their ability to form various types of DNA adducts and genetic variations in genes involved in the nucleotide excision repair pathway [xeroderma pigmentosum (XPD), ERCC1] may influence the response to platinum chemotherapy [90].

Practical implications

Recently, several studies suggested that common variants within the XPD and/or XRCC1 genes may predict clinical outcome after the administration of platinum-based chemotherapy. In a retrospective study in which patients with colorectal cancer received oxaliplatin plus 5-FU, those who carried the *XPDLys751Gln* polymorphism (either heterozygous or homozygous) had significantly decreased response rates and survival compared with those homozygous for the *Lys751/Lys751* genotype [91].

A prospective, phase II study including patients with metastatic breast cancer examined the impact of the common *XPD (C156A, Asp312Asn* and *Lys751Gln*) and SRCC1 (*Arg399Gln*) polymorphisms in women receiving docetaxel and carboplatin as first-line treatment. Patients with the *XPD 312 Asp/Asp* genotype (wild-type) had lower response rates (50%) and clinical benefit (50%) than did patients with one *Asn* allele (response rate 56%) and clinical benefit 67%). Patients homozygous for the XPD 312 *Asn/Asn* genotype had the highest response and clinical benefit rate (88%, $P = 0.07$). No correlation with either response rates or clinical benefit was seen with the *XRCC1 Arg399Gln* polymorphism.

Cytidine deaminase (CDA) and gemcitabine chemotherapy

Gemcitabine (*2′,2′*-difluorodeoxycytidine) is an antineoplastic agent with activity against a variety of solid tumors either alone or in combination with other agents and radiation. Its major adverse effects are leukopenia, anemia, thrombocytopenia, weakness and emesis. Single-agent and platinum combination gemcitabine therapy is relatively well tolerated, but hospitalization is occasionally required due to significant hematologic toxicity [92], which has been difficult to predict. Gemcitabine has a complex metabolic pathway. It is transported into the cell by nucleoside transporters to then be phosphorylated to the active metabolite *2′,2′*-difluorodeoxycytidine triphosphate (dFdCTP) by the deoxycytidine kinase (DCK). Increased intracellular concentrations of dFdCTP can inhibit dCMP deaminase and decrease the catabolism of dFdCMP, allowing for the self-potentiation of dFdC cytotoxic activity [93]. On the other hand, increased activity of CDA can increase the breakdown of gemcitabine before it enters the cell [94,95].

A total of 14 genetic variants were discovered among the 13 gemcitabine metabolic pathway genes [96]. Three SNPs were identified in the *CDA* gene. One SNP, *CDA* 435C→T, did not encode for an amino acid change. *CDA* 79 A→C encoded for a lysine to glutamine amino acid change (K27Q) and the SNP *CDA* 208G→A encodes for an alanine to threonine amino acid change (A70 T). The two SNPs identified in the *DCK* gene, *DCK* IVS1-1110T→C and *DCK* 2190A→T, did not encode for an amino acid substitution. A (T→C) SNP at nucleotide 315 was reported in the *DCTD* gene. A SNP was identified in *POLA2*, which encoded for a glycine to arginine (G583R) amino acid change. Five SNPs were identified in the nucleotide transporter genes. *SLC28A1* 1383C→T and *SLC29A1.* A 3′-UTR deletion and an enhancer region repeat in the *TYMS* gene has been identified. Of the 14 gemcitabine pathway variants, 12 were observed in the general population samples. All genotype frequencies were in Hardy–Weinberg equilibrium. Among them, four genes had more than one genetic variant – *CDA*, *DCK*, *SLC28A1* and *TYMS* [97].

Among six SNPs that encode for an amino acid change, *CDA* 208G→A encodes for a hydrophobic amino acid (alanine) to a hydrophilic amino acid substitution (threonine). Using the SPDB to evaluate *CDA* 208G→A, the proximity of this amino acid substitution to the active site of this protein may affect the activity of CDA by influencing the packing of the helix in which it resides [98].

Table 4 Frequency of poor metabolizers and ultrarapid metabolizers in different populations (evaluated for CYP2D6 enzyme)

Reference	Population	Poor metabolizers (%)		Ultrarapid metabolizers (%)
		*CYP2D6*4*	*CYP2D6*5*	CYP2D6*2×*N*
[107]	Caucasian	12–21	2–7	1–5
[108]	Mediterranean area	1	5	7–12
[108]	Asians	1	6	0–2
[109]	black Africans	2	4	29
[108,109]	Saudi Arabia	1–4	1–3	10–21

*CYP2D6*4* has a defective splicing, which is translated to an inactive enzyme.
*CYP2D6*5* has a gene deletion that causes no enzyme synthesis.
*CYP2D6*2×N* has a gene duplication or multiplication, which increased enzyme activity.

Practical implications

In an effort to find tools for prospective identification of patients at risk for gemcitabine toxicity, several SNPs in genes involved in gemcitabine metabolic pathway genes were described [97]. Remarkably, the CDA 208G→A polymorphism coding for an amino acid change near the active site of this protein was demonstrated to affect the activity of CDA [99]. These data were confirmed by the finding of a patient with pancreatic cancer who showed a severe hematological and non-hematological toxicity during the first course of gemcitabine-based chemotherapy. The AUC value of gemcitabine for this patient (54.54 μg·h/ml) was 5 times higher than the average and the AUC of 2′,2′-difluorodeoxyuridine (41.58 μg·h/ml) was less than half of average value. Interestingly, this patient proven to be homozygous for 208A[Thr(70)] in the *CDA* gene. Most likely, the homozygous 208G→A alteration in CDA might have caused the severe drug toxicity experienced by the cancer patient [99], which opens the way for further study and confirms the predictive role of this polymorphism in gemcitabine toxicity.

5-Hydroxytryptamine type 3 (5-HT_3) receptor antagonists and P450 enzymes

Antagonists of serotonin 5-HT_3 receptor have reduced the incidence of nausea and vomiting in patients receiving chemotherapy [100]. Cytochrome P450 enzymes metabolize different drugs; most of them are primarily cleared by CYP3A4, followed by CYP2D6 (20%), 2C9, 2C19, 2E1, 2A6 and 1A2, including antagonists of serotonin 5-HT_3 receptor: tropisetron and dolasetron predominantly by CYP2D6, ondansetron partially by CYP2D6, but also by CY3A4, 2E1 or 1A2, and granisetron mainly by CYP3A4 [101–106]. All of these enzymes are inducible, except for CYP2D6.

Practical implications

Approximately 20–30% of patients do not respond satisfactorily to 5-HT_3 receptor antagonists [100]. It is known that ultrarapid metabolizers, which result from more than two active genes duplicated or even a several-fold amplification of the *CYP2D6* gene, show an increased clearance of these drugs. On the other hand, *CYP2D6* mutations or deletions occur in approximately 10% of the general population and have been associated with a poor metabolizer phenotype. The proportion of poor and ultrarapid metabolizers varies between different populations, as shown in Table 4 [107–110]. Candiotty *et al.* [111] have evaluated the incidence of post-operative nausea and vomiting in patients treated with ondansetron. Patients with one, two or three DYP2D6 copies have an incidence of vomiting of approximately 27, 14 and 30%. However, when they are analyzed by phenotype, the incidence of vomiting in poor, intermediate, extensive and ultrarapid metabolizers was 8, 17, 15 and 45%, respectively. These results demonstrate that anti-emetic treatment could be improved by the identification of none, low, and high responders on a pharmacogenetic basis, thus allowing the clinician to predict who will require a specific and individual anti-emetic dose adaptation [111].

Polygenic pathways

The examples shown in this review demonstrate the usefulness of pre-screening patients for well-characterized polymorphisms to identify the best-tolerated and most-effective treatment. Unfortunately, genes do not act in isolation and drugs are often involved in complex metabolic pathways in the cell before they are converted to active or inactive forms. 5-FU is an example; this drug uses the cell's pyrimidine metabolic pathway, and variations in DPYP as well as in the TYMS gene may affect the systemic toxicity and/or tumor response. It is known that there is marked variability in response to therapy, as well as frequency of severity of toxicities. The integration of drug pathway analysis into clinical trials to predict chemotherapy activity and response is mandatory [112].

Conclusions

Considering the narrow therapeutic index for many cancer chemotherapeutic agents, the ability to better predict response and potentially life-threatening toxic effects is highly important for the clinician. The convergence of pharmacogenetics with rapid advances in human genomics has resulted in the ongoing transformation of pharmaco*genetics* into pharmaco*genomics*. Genetic insights should also lead to mechanism-based

approaches to the discovery and development of new medications.

References

1 Ulrich CM, Robien K, McLeod H. Cancer Pharmacogenetics: polymorphisms, pathways and beyond. *Nat Rev Cancer* 2003; **3**:912–920.
2 Evans WE, Relling MV. Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999; **286**:487–491.
3 Relling MV, Cervieux T. Pharmacogenetics and cancer therapy. *Nat Rev. Cancer* 2001; **1**:99–108.
4 Ulrich CM, Robien K, Sparks R. Pharmacogenetics and folate metabolism: a promising direction. *Pharmacogenomics* 2002; **3**:299–313.
5 Kallioniemi O. Profile of a tumour. *Nature* 2004; **428**:379–382.
6 Wiffen P, Gill M, Edwards J, Moore A. Adverse drug reactions on hospital patients: a systematic review of the prospective and retrospective studies. *Badolier Extra* 2002; http//www.jr2.ox.ac.uk/bandolier/extra.htmal.
7 Marsh S, McLeod HL. Cancer pharmacogenetics. *Br J Cancer* 2004; **90**:8–11.
8 Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *J Am Med Ass* 1998; **279**:1200–1205.
9 Nebert D, Jorge-Neber L, Vessell ES. Pharmacogenomics and 'individualized drug therapy'. *Am J Pharmacogenomics* 2003; **3**:361–370.
10 Donnelly JG. Pharmacogenetics in cancer chemotherapy. Balancing toxicity and response. *Ther Drug Monit* 2004; **26**:231– 235.
11 Watters JW, McLeod HL. Cancer pharmacogenomics: current and future applications. *Biochim Biophys* 2003; **2**:99–111.
12 McLeod H, Jinsheng Y. Cancer Pharmacogenetics: SNPs, chips, and the Individual Patient. *Cancer Invest* 2003; **21**:630–640.
13 Relling MV, Hancock ML, Rivera GK, Sandlund JT, Ribeiro RC, Krynetski EY, *et al.* Mercaptopurine therapy intolerance and heterozygosity at the thiopurine *S*-methyltransferase gene locus. *J Natl Cancer Inst* 1999; **91**:2001–2008.
14 Marshall E. Preventing toxicity with a genetic test. *Science* 2003; **302**:588–590.
15 Milano G, McLeod HL. Can dihydropyrimidine dehydrogenase impact 5-flurouracil-based treatment? *Eur J Cancer* 2000; **36**:37–42.
16 Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S, *et al.* Phenotype–genotype correlation of *in vitro* SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism *Clin Pharm Ther* 1999; **65**:576–582.
17 Evans WE. Pharmacogenetics of thiopurine *S*-methyltransferase and thiopurine therapy. *Ther Drug Monit* 2004; **26**:186–191.
18 Krynetski E, Evans WE. Drug methylation in cancer therapy: lessons from the TPMT polymorphism. *Oncogene* 2003; **22**:7403–7413.
19 Yates Cr, Krynetsi EY, Loennechen T, Fessing MY, Tai HL, Pui CH, *et al.* Molecular diagnosis of thiopurine *S*-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. *Ann Intern Med* 1997; **126**:608–614.
20 Schaeffeler E, Lang T, Zanger UM, Eichelbaum M, Schwab M. High-throughput genotyping of thiopurine *S*-methyltransferase by denaturating HPLC. *Clin Chem* 2001; **47**:548–555.
21 Lindqvist M, Haglund S, Almer S, Peterson C, Taipalensu J, Hertervig E, *et al.* Identification of two novel sequence variants affecting thiopurine methyltransferase enzyme activity. *Pharmacogenetics* 2004; **14**:261–265.
22 Krynetski EY, Schuetz JD, Galpin AJ, Pui CH, Relling MV, Evans WE. A single point mutation leading to loss of catalytic activity in human thiopurine *S*-methyltransferase. *Proc Natl Acad Sci USA* 1995; **92**:949–953.
23 Tai HL, Krynetski EY, Yates CR, Loennechen T, Fessing MY, Krynetskaia NF, *et al.* Thiopurine *S*-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am J Hum Genet* 1996; **58**:694–702.
24 Loennechen T, Yates CR, Fessing MY, Relling MV, Krynetski EY, Evans WE. Isolation of a human thiopurine *S*-methyltransferase (TPMT) complementary DNA with a single nucleotide transition A719G (TPMT*3C) and its association with loss of TPMT protein and catalytic activity in humans. *Clin Pharm Ther* 1998; **64**:46–51.
25 McLeod HL, Coulthard S, Thomas AE, Pritchard SC, King DJ, Richards SM, *et al.* Analysis of thiopurine methyltransferase variant alleles in childhood acute lymphoblastic leukaemia. *Br J Haematol* 1999; **105**:696–700.
26 Haglund S, Lindqvist M, Almer S, Peterson C, Taipalensu J. Pyrosequencing of TPMT alleles in a general Swedish population and in patients with inflammatory bowel disease. *Clin Chem* 2004; **50**: 288–295.
27 Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine *S*-methyltransferase gene in African-Americans. *Hum Mol Genet* 1999; **8**:371–376.
28 Indjova I, Atanasova S, Shipkova M, Armstrong VW, Oellerich M, Svinarov D. Phenotypic and genotypic analysis of thiopurine *S*-methyltransferase polymorphism in the Bulgarian population. *Ther Drug Monit* 2003; **25**: 631–636.
29 Larove LE, de Kremer RD, Lambooy LH, De Abreu RA. Genetic polymorphism of thiopurine *S*-methyl transferase in Argentina. *Ann Clin Biochem* 2003; **40**:388–393.
30 Lennard L, Van Loon JA, Weinshilboum RM. Pharmacogenetics of acute azathioprine toxicity: relationship to thiopurine methyltransferase genetic polymorphism. *Clin Pharmacol Ther* 1989; **46**:149–154.
31 Rossi AM, Bianchi M, Guarnieri C, Barale R, Pacifici GM. Genotype–phenotype correlation for thiopurine *S*-methyltransferase in healthy Italian subjects. *Eur J Clin Pharmacol* 2001; **57**:51–54.
32 Collie-Duguid ES, Pritchard SC, Powrie RH, Sludden J, Collier DA, Li T, *et al.* The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. *Pharmacogenetics* 1999; **9**:37–42.
33 Hiratsuka M, Inoue T, Omori F, Agatsuma Y, Mizugaki M. Genetic analysis of thiopurine methyltransferase polymorphism in a Japanese population. *Mutat Res* 2000; **448**:91–95.
34 Coulthard SA, Howell C, Robson J, Hall AG. The relationship between thiopurine methyltransferase activity and genotype in blasts from patients with acute leukemia. *Blood* 1998; **92**:2856–2862.
35 Evans WE, Horner M, Chu YQ, Kalwinsky D, Roberts WM. Altered mercaptopurine metabolism, toxic effects, and dosage requirement in a thiopurine methyltransferase-deficient child with acute lymphocytic leukemia. *J Pediatr* 1991; **119**:985–989.
36 Otterness D, Szumlanski C, Lennard L, Klemetsdal B, Aarbakke J, Park-Hah JO, *et al.* Human thiopurine methyltransferase pharmacogenetics: gene sequence polymorphisms. *Clin Pharmacol Ther* 1997; **62**:60–73.
37 Evans WE, Hon YY, Bomgaars L. Preponderance of thiopurine *S*-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J Clin Oncol* 2001; **19**:2293–2301.
38 Innocenti F, Ratain MJ. Update on pharmacogenetics in cancer chemotherapy. *Eur J Cancer* 2002; **38**:639–644.
39 Ma XL, Zhu P, Wu My, Li ZG, Hu YM. Relationship between single nucleotide polymorphisms in thiopurine methyltransferase gene and tolerance to thiopurines in acute leukemia. *Zonghua Er ke Za Zhi* 2003; **41**:929–933.
40 Dubinsky MC. Optimizing immunomodulator therapy for inflammatory bowel disease. *Curr Gastroenterol Rep* 2003; **5**:506–511.
41 Relling M, Dervieux T. Pharmacogenetics and cancer therapy. *Nat Rev Cancer* 2001; **1**:99–108.
42 Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renee N, *et al.* Population study of dihydropyrimidine dehydrogenase in cancer patients. *J Clin Oncol* 1994; **12**:2248–2253.
43 Kouwaki M, Hamajima N, Sumi S, Nonaka M, Sasaki M, Dobashi K, *et al.* Identification of novel mutations in the dihydropyrimidine dehydrogenase gene in a Japanese patient with 5-fluorouracil toxicity. *Clin Cancer Res* 1998; **4**:2999–3004.
44 Yamaguchi K, Arai K, Kanda Y, Akagi K. Germline mutations of dihydropyrimidine dehydrogenase gene among a Japanese population in relation to toxicity to 5-fluorouracil. *Jpn J Cancer Res* 2001; **92**:337–342.
45 Johnson WR, Wang K, Diasio RB. Profound dihydropyrimidine dehydrogenase (DPD) deficiency resulting from a novel compound heterozygote genotype. *Clin Cancer Res* 2002; **8**:768–774.
46 Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics* 2000; **10**:217–223.
47 van Kuilenburg AB, Haasjes J, Richel DJ, Zoetekouw L, Van Lenthe H, De Abreu RA, *et al.* Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. *Clin Cancer Res* 2000; **6**:4705–4712.
48 Vreken P, van Luilenburg A, Meinnsma R, Van Gennip A. Dihydropyrimidine dehydrogenase (DPD) deficiency: identification and expression of missense mutations C29R, R886 H and R235W. *Hum Genet* 1997; **101**:333–338.
49 van Kuilenburg AB, Dobritzsch D, Meinsma R, Haasjes J, Waterham HR, Nowaczyk MJ, *et al.* Novel disease-causing mutations in the dihydropyrimidine dehydrogenase gene interpreted by analysis of the three-dimensional protein structure. *Biochem J* 2002; **364**:157–163.
50 Vreken P, van Küllenburg AB, Meinsma R, van Gennip AH. Dihydropyrimidine dehydrogenase deficiency. Identification of two novel

mutations and expression of missense mutations in *E. coli*. *Adv Exp Med Biol* 1998; **431**:341–346.
51 Meinsma R, Fernandez-Salguero P, Van Kuilenburg AB, Van Gennip AH, Gonzalez FJ. Human polymorphism in drug metabolism: mutation in the dihydropyrimidine dehydrogenase gene results in exon skipping and thymine uraciluria. *DNA Cell Biol* 1995; **14**:1–6.
52 Gross E, Neubauer S, Seck K. High throughput mutation screening of the DPYD gene by denaturating HPLC. *Proc Am Ass Cancer Res* 2002; **43**:322a.
53 Ridge SA, Sludden J, Wei X, Sapone A, Brown O, Hardy S, *et al.* Dihydropyrimidine dehydrogenase pharmacogenetics in patients with colorectal cancer. *Br J Cancer* 1998; **77**:497–500.
54 Ridge SA, Sludden J, Brown O, Robertson L, Wei X, Sapone A, *et al.* Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *Br J Clin Pharmacol* 1998; **2**:151–156.
55 Vreken P, Van Kuilenburg A, Meinsma R, van Gennip A. Identification of novel point mutations in the dihydropyrimidine dehydrogenase gene. *J Inherit Metab Dis* 1997; **20**:335–338.
56 Albin N, Johnson MR, Shahinian H. Initial characterization of the molecular defect in human dihydropyrimidine dehydrogenase deficiency. *Proc Am Ass Cancer Res* 1995; **36**:211a.
57 Vreken P, van Kuilenburg AB, Meinsma R, Beemer FA, Duran M, van Gennip AH. Dihydropyrimidine dehydrogenase deficiency: a novel mutation and expression of missense mutation in *E. coli J Inherit Metab Dis* 1998; **21**:276–279.
58 McLeod HL, Collie-Duguid ES, Vreken P, Johnson MR, Wei X, Sapone A, *et al.* Nomenclature for human DPYD alleles. *Pharmacogenetics* 1998; **8**:455–459.
59 Ezzeldin H, Okamoto Y, Johnson MR, Diasio RB. A High-throughput denaturing high-performance liquid chromatography method for the identification of variant alleles associated with dihydropyrimidine dehydrogenase deficiency. *Anal Biochem* 2002; **306**:63–73.
60 Ezzeldin H, Johnson MR, Okamoto Y, Diaisio R. Denaturing high performance liquid chromatography analysis of the DPYD gene in patients with lethal 5-fluorouracil toxicity. *Clin Cancer Res* 2003; **9**:3021–3028.
61 van Kuilenburg AB, Muller EW, Haasjes J, Meinsma R, Zoetekouw L, Waterham HR, *et al.* Letal outcome of a patient with a complete dihydropyrimidine dehydrogenase (DPD) deficiency alter administration of 5-fluorouracil:frequency of the common IVS14+IG>A mutation causing DPD deficiency. *Clin Cancer Res* 2001; **7**:1149–1153.
62 Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. *Pharmacogenetics* 2000; **10**:217–233.
63 Berger R, Stoker-de Vries SA, Wadman SK, Duran M, Beemer FA, de Bree PK, *et al.* Dihydropyrimidine dehydrogenase deficiency leading to thymidine-uraciluria. An inborn error of pyrimidine metabolism. *Clin Chim Acta* 1984; **141**:227–234.
64 Diario RB. *Dihydropyrimidine dehydrogenase (DPD) deficiency*: a 5-fluorouracil-associated pharmacogenomic syndrome. Horizons in cancer therapeutics: from bench to bedside. West Conshohocken, Philadelphia: Meniscus; 2003, pp. 9–21.
65 Raida M, Schwabe W, Hausler P, Van Kuilenburg AB, Van Gennip AH, Behnke D, *et al.* Prevalence of a common point mutation in the dihydropyrimidine dehydrogenase (DPD) gene within the 5′ splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)-related toxicity compared with controls. *Clin Cancer Res* 2001; **7**:2832–2839.
66 Goetz MP, Ames MM, Weinshilboum RM. Primer of medical genomics. Part XUU: Pharmacogenomics – general principles with cancer as a model. *Mayo Clin Proc* 2004; **79**:376–384.
67 Marsh S, Ameyaw MM, Githan'a J, Indalo A, Ofori-Adjei D, McLeod HL. Novel thymidylate synthase enhancer region alleles in African population. *Hum Mutat* 2000; **20**:174–179.
68 Marsh S, McKay JA, Casidy J, McLeod HL. Polymorphism in the thymidylate synthase prometor enhancer region in colorectal cancer. *Int J Oncol* 2001; **19**:383–386.
69 Mandola MV, Stoehlmacher J, Muller-Weeks S, Cesarone G, Groshen S, Tsao-Wet DD, *et al.* A novel SNP within the tandem repeat polymorphism of the thymidylate synthase gene abolishes USF-1 binding and predicts outcome of metastatic colorectal carcinoma treated with 5-fluorouracil. *Proc Am Ass Cancer Res* 2003; **44**:597.
70 Ulrich CM, Bigler J, Velicer CM, Greene EA, Farin FM, Potter JD. Searching expressed sequence tag databases: discovery and confirmation of a common polymorphism in the thymidylate synthase gene. *Cancer Epidemiol Biomarkers Prev* 2000; **9**:1381–1385.
71 Lenz HJ, Zhang W, Zahedy S, Gil J, Yu M, Stoehlmacher J. A 6 base-pair deletion in the 3′ UTR of the thymidylate synthase (TS) gene predicts TS mRNA expression in colorectal tumors. A possible candidate gene for colorectal cancer risk. *Proc Am Assoc Cancer Res* 2002; **43**:660.
72 Villafranca E, Okruzhnov Y, Domínguez MA, Garcia-Foncillas J, Azinovic L, Martinez E, *et al.* Polymorphisms of the repeated sequences in the enhancer region of the thymidylate synthase gene promoter may predict downstaging after preoperative chemoradiation in rectal cancer. *J Clin Oncol* 2001; **19**:1779–1786.
73 Stoehlmacher J, Mandola MV, Yun J, Sones E, Zhang W, Gil J, *et al.* Alterations of the thymidylate synthase (TS) pathway and colorectal cancer risk- the impact of three TS polymorphisms. *Proc Am Assoc Cancer Res* 2003; **44**:597.
74 McLeod HL, Sargent DJ, Marsh S, Fuchs CS, Ramnathan RK, Williamson SK, *et al.* Pharmacogenetic analysis of systemic toxicity and response after 5-fluorouracil (5FU)/CPT-11, 5FU/oxaliplatin (oxal), or CPT-11/oxal therapy for advanced colorectal cancer. *Proc Am Ass Clin Oncol* 2003; **2**:252.
75 Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications for the MTHFR C677 polymorphism. *Trends Pharmacol Sci* 2001; **22**:195–201.
76 Gollete P. Human methylenetetrahydrofolate reductase: isolation of cDNA, mapping and mutation identification. *Nat Genet* 1994; **7**:195–200.
77 Molloy AM. Thermolabile variant of 5,10-methylenetetrahydrofolate reductase associated with low red cell-folates: implications for folate intake recommendations. *Lancet* 1997; **349**:1591–1593.
78 Stevenson JP, Redlinger M, Kluijtmans LA, Sun W, Algazy K, Giantonio B, *et al.* Phase I clinical and pharmacogenetic trial of irinotecan and raltitrexed administered every 21 days to patients with cancer. *J Clin Oncol* 2001; **19**:4081–4087.
79 Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, *et al.* Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci USA* 1999; **96**:12810–12815.
80 Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF. United Kingdom Childhood Cancer Study investigators. Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk of molecularly defined subtypes of childhood acute leukemia. *Proc Natl Acad Sci USA* 2001; **98**:4004–4009.
81 Keku T, Millikan R, Worley K, Winkel S, Eaton A, Biscocho L, *et al.* 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. *Cancer Epidemiol Biomarkers Prev* 2002; **11**:1611–1621.
82 Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, *et al.* The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 1995; **333**:1171–1175.
83 Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ. Metabolic rate of irinotecan in humans: correlation of glucuronidation with diarrhea. *Cancer Res* 1994; **54**:3723–3725.
84 Monaghan G, Ryan M, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UPD-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* 1996; **347**:578–581.
85 Iyer L, Das S, Janish L, Wen M, Ramirez J, Karrison T, *et al.* UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *Pharmacogenomics J* 2002; **2**:43–47.
86 Ando Y, Saka H, Asai G, Sugiura S, Shimokata K, Kamataki T. UGT1A1 genotypes and glucuronidation of SN-38, the active metabolite of irinotecan. *Ann Oncol* 1998; **9**:845–847.
87 Iyer L, Hall D, Das S, Mortell MA, Ramirez J, Kim S, *et al.* Phenotype-genotype correlation of *in vitro* SN-38 (active metabolite of irinotecan) and bilirubin glucuronidation in human liver tissue with UGT1A1 promoter polymorphism. *Clin Pharmacol Ther* 1999; **65**:576–582.
88 Wasserman E, Myara A, Lokiec F, Goldwasser F, Trivin F, Mahjoubi M, *et al.* Severe CPT-11 toxicity in patients with Gilbert's syndrome: two case reports. *Ann Oncol* 1997; **8**:1049–1051.
89 Yu J, Shannon WD, Watson MA, McLeod HL. Gene expression profiling of the irinotecan pathway in colorectal cancer. *Clin Cancer Res* 2005; **11**:2053–2062.
90 Stoehlmacher J, Ghaderi V, Lobal S, Groshen S, Tsao-Wei D, Park D, *et al.* Polymorphism of the XRCC1 gene predicts for response to platinum based treatment in advanced colorectal cancer. *Anticancer Res* 2001; **21** (**4B**):3075–3079.
91 Park DJ, Stoehlmacher J, Zhang W, Tsao-Wei DD, Groshen S, Lenz HJ. A xeroderma pigmentosum group D gene polymorphism predicts clinical outcome to platinum-based chemotherapy in patients with advanced colorectal cancer. *Cancer Res*. 2001; **61**:8654–8658.

92 Cortes-Funes H, Martin C, Abratt R, Lund B. Safety profile of gemcitabine, a novel anticancer agent, in non-small cell lung cancer. *Anticancer Drugs* 1997; **8**:582–587.
93 Heinemann V, Schulz L, Issels RD, Plunkett W. Gemcitabine: a modulator of intracellular nucleotide and deoxynucleotide metabolism. *Semin Oncol* 1995; **22**(**4 Suppl 11**):11–18.
94 Neff T, Blau CA. Forced expression of cytidine deaminase confers resistance to cytosine arabinoside and gemcitabine. *Exp Hematol* 1996; **24**:1340–1346.
95 Miwa M, Eda H, Ura M, Ouchi KF, Keith DD, Foley LH, *et al.* High susceptibility of human cancer xenografts with higher levels of cytidine deaminase to a 2′-deoxy cytidine antimetabolite, 2′-deoxy-2′-methylidenecytidine. *Clin Cancer Res* 1998; **4**:493–497.
96 Gridelli C, Perrone F, Gallo C, Cigolari S, Rossi A, Piantedosi F, *et al.* Chemotherapy for elderly patients with advanced non-small-cell lung cancer: the Multicenter Italian Lung Cancer in the Elderly Study (MILES) phase III randomized trial. *J Natl Cancer Inst* 2003; **95**:362–372.
97 Fukunaga AK, Marsh S, Murry DJ, Hurley TD, McLeod HL. Identification and analysis of single-nucleotide polymorphisms in the gemcitabine pharmacologic pathway. *Pharmacogenomics J* 2004; **4**:307–314.
98 Guex N, Peitsch MC. SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling. *Electrophoresis* 1997; **18**:2714–2723.
99 Yonemori K, Ueno H, Okusaka T, Yamamoto N, Ikeda M, Saijo N, *et al.* Severe drug toxicity associated with a single-nucleotide polymorphism of the cytidine deaminase gene in a Japanese cancer patient treated with gemcitabine plus cisplatin. *Clin Cancer Res* 2005; **11**:2620–2624.
100 Gregory RE, Ettinger DS: 5-HT_3 receptor antagonists for the prevention of chemotherapy-induced nausea and vomiting: a comparison of their pharmacology and clinical efficacy. *Drugs* 1998; **55**:173–189.
101 Fischer V, Vickers AE, Hetz F. The polymorphic cytochrome P-450D6 is involved in the metabolism of both 5-hydroxytryptamine antagonists, tropisetron and ondansetron. *Drug Metab Dispos* 1994; **22**:269–274.
102 Sanwald P, David M, Dow J. Characterization of the cytochrome p450 enzymes involved in the *in vitro* metabolism of dolasetron. Comparison with other indole-containing 5-HT_3 antagonists. *Drug Metab Dispos* 1996; **24**:602–609.
103 Corriga BW, Nicholls B, Tharkar B. Heterogeneity in systemic availability of ondansetron and granisetron following oral administration. *Drug Metab Dispos* 1999; **27**:110–112.
104 Dixon CM, Cothup PV, Serabjit-Singh CJ. Multiple forms of cytochrome p450 are involved in the metabolism of ondansetron in humans. *Drug Metab Dispos* 1995; **23**:1225–1230.
105 Firkusny L, Kroemer HK, Eichelbaum M. *In vitro* characterization of cytochrome P450 catalysed metabolism of the antiemetic tropisetron. *Biochem Pharmacol* 1995; **49**:1777–1784.
106 Bloomer JC, Baldwin SJ, Smith GJ. *In vitro* characterization of cytochrome P450 enzymes involved in the *in vitro* metabolism of granisetron. *Br J Clin Pharmacol* 1994; **38**:557–566.
107 Johansson I, Lundqvist E, Bertilsson L. Inherited amplification of an active gene in the cytochrome p450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci USA* 1993; **90**: 11825–11829.
108 Ingelman-Sundberg M, Oscarson M, McLellan RA. Polymorphic human cytochrome p450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol Sci* 1999; **20**:342–349.
109 Hesketh PJ, Kris MG, Grunberg SM. Proposal for classifying the acute emetogenicity of cancer chemotherapy. *J Clin Oncol* 1997; **15**: 103–109.
110 Kaiser R, Sezer O, Papies A, Bauer S, Schelenz C, Pierre-Benoit T, *et al.* Patient- tailored antiemetic treatment with 5-hydroxytryptamine type 3 receptor antagonists according to cytochrome p-450 2D6 genotypes. *J Clin Oncol* 2002; **20**:2805–2811.
111 Candiotti KA, Birnbach DJ, Lubarsky DA, Nhuch F, Kamat A, Koch WH, *et al.* The impact of pharmacogenomics on postoperative nausea and vomiting: do YCP2D6 allele copy number and polymorphisms affect the success or failure of ondansetron prophylaxis? *Anesthesiology* 2005; **102**:543–549.
112 Tan BR, McLeod HL. Pharmacogenetic influences on treatment response and toxicity in colorectal cancer. *Semin Oncol* 2005; **31**:113–119.