Current insights in the role of heritable genetic variation in the pharmacokinetics and pharmacodynamics of anti-cancer drugs

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Abstract
Pharmacogenetics in oncology ideally will allow oncologists to individualise therapy based upon a genetic test result. Severe toxicity and clinically significant under-dosing may be avoided, whereas predicted non-responders can be offered alternative therapy. This manuscript gives an overview of heritable variants in the genes of nine enzymes or pathways that have been studied most extensively in anti-cancer chemotherapy. Even though many pharmacogenetic association studies have been published, there is need for more research. In particular, there is need for replication of data and development of predictive models. Prospective trials are required to establish clinical value and cost-effectiveness of pharmacogenetic testing in oncology.

Introduction
Pharmacogenetics studies the association between heritable functional variants in DNA (genotype) with outcome of therapy (phenotype). In the recent years, pharmacogenetics in oncology has become an increasing field of research. Ideally, pharmacogenetic testing will allow oncologists to individualise therapy, with respect to the choice of a drug and the dose of the drug administered, based upon a genetic test result. Severe toxicity may be avoided, whereas predicted non-responders can be offered alternative therapy.

A polymorphism is an inheritable variant that occurs within at least 1% of the population. Moreover, a polymorphism is a neutral variant: the variant may have functional consequences on the protein level, without influencing existence of the individual. Variants in DNA can be single nucleotide polymorphisms (SNPs), deletions or insertions of a number of base pairs (bp) or variable number of tandem repeats resulting in changes in exons, introns or in untranslated regions (UTR), such as the promoter region of the gene. When DNA is transcripted into mRNA, some of these variants may result in altered mRNA stability. Some variants result in different amino acid composition of proteins or truncated proteins which may lead to altered enzyme activity and thus functionality (non-synonymous variants), whereas other variants do not result in amino acid change (synonymous or silent variants). Finally, variants in a UTR of a gene can alter the transcriptional activity of a gene and thus change the expression of an enzyme. As specific regions in DNA are conserved through generations, variants are often inherited as so called haplotypes, which can be measured by assessing linkage disequilibrium (LD).

In contrast to somatic variants, heritable (germ-line) variants in DNA are inherited from parents, and the presence of a variant can be either heterozygous (carrier of one normal and one variant allele) or homozygous (carrier of two variant alleles) as compared to wild-type (two normal alleles). The determination of germ-line variants in, for example DNA isolated from peripheral blood is much more feasible than determination of variants in DNA isolated from tumour samples. Interestingly, in a recent paper it was shown that there is a high degree of concordance between germ-line and somatic variants for a number of SNPs. However, genetic mutations related to the origin of the malignant phenotype are by definition in discordance to the germ-line phenotype.

In this manuscript, we give an overview of heritable variants in the genes encoding nine enzymes or pathways that have been studied most extensively in patients...
Medline was systematically searched (from July 1st to September 30th 2006) with the following set of keywords: pharmacogenetics, pharmacogenomics, polymorphism, SNP, genotype, phenotype, antineoplastic (protocols), chemotherapy, combined with the names of genes and enzymes, limiting results to human research published in English.

Figure 1 Schematic overview of enzymes involved in cellular response and metabolism to anti-cancer drugs

Abbreviations: CYP2D6: cytochrome P450 2D6; 4-OH-tam: 4-hydroxy-tamoxifen; DPD: dihydropyrimidine dehydrogenase; SUF: 5-fluorouracil; MTHFR: glutathione S-transferase; GST: glutathione S-transferase; ABCB1: ATP-binding cassette B1; MDR1: multi drug resistance 1; BCRP: breast cancer resistance protein; ABCG2: ATP-binding cassette G2; ERCC1: excision repair cross complementing group 1; XPD: xeroderma pigmentosum group D1; XRCC1: X-ray repair cross complementing group 1; CYP2D6: cytochrome P450 2D6; MTHFR: methylene tetrahydrofolate dehydrogenase; TS/TYMS: thymidylate synthase; TSER: thymidylate synthase enhancer region; UTR: untranslated region; bp: base pair; indel: insertion/deletion

Table 5 Enzymes involved in response to anti-cancer drugs and their common polymorphisms

<table>
<thead>
<tr>
<th>enzyme</th>
<th>variant allele</th>
<th>polymorphism</th>
<th>phenotype</th>
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<td>Ile105Val</td>
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<td>G&gt;C at bp 12 in T5ER-3</td>
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<td></td>
<td>T5MS 3’UTR</td>
<td>1494 6bp indel</td>
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TPMT: thiopurine S-methyltransferase; DPD/DPYD: dihydropyrimidine dehydrogenase; UGT: uridine diphosphate glucuronosyl transferase; GST: glutathione S-transferase; ABCB1: ATP-binding cassette B1; MDR1: multi drug resistance 1; BCRP: breast cancer resistance protein; ABCG2: ATP-binding cassette G2; ERCC1: excision repair cross complementing group 1; XPD: xeroderma pigmentosum group D1; ERCC2: excision repair cross complementing group 2; XRCC1: X-ray repair cross complementing group 1; CYP2D6: cytochrome P450 2D6; MTHFR: methylene tetrahydrofolate dehydrogenase; TS/TYMS: thymidylate synthase; TSER: thymidylate synthase enhancer region; UTR: untranslated region; bp: base pair; indel: insertion/deletion

Pharmacogenetic association studies

Thiopurine S-methyltransferase
An alkylation agent commonly used in maintenance treatment of acute lymphoblastic leukaemia (ALL) 6-mercaptopurine (6MP), which is deactivated by the enzyme thiopurine S-methyltransferase (TPMT). Approximately 0.3% and 10% of the population has undetectable and intermediate TPMT enzyme activity respectively.1,2 TPMT activity is inversely associated with exposure to the cytotoxic metabolite of 6MP, 6-thioguanine (6TGN), in red blood cells4 and in ALL blasts.5 Because of severe haematological
toxicity, 6MP dose must be reduced\textsuperscript{2}, with as much as 90% and 50-66% for the respective phenotypes.\textsuperscript{2} Because dose intensity proved to be a prognostic marker for outcome in ALL patients treated with 6MP, it is important to administer the right dose with regard to toxicity\textsuperscript{8} and efficacy.\textsuperscript{9}

Therefore, TPMT activity is a determinant for predicting the occurrence of toxicity. However, it must be noticed that TPMT activity is influenced by several common factors in ALL, such as methotrexate (MTX)/trimethoprim treatment\textsuperscript{10} or administration of red blood cells transfusions.\textsuperscript{11}

The molecular basis of decreased TPMT activity was found in 1995. A 238G>C SNP resulting in amino acid change of alanine to proline in codon 80 (Ala80Pro), and in 100 fold decrease in enzyme activity was found in a patient who experienced severe toxicity to 6MP.\textsuperscript{12} This allele is referred to as TPMT\textsuperscript{*2}.

The variant allele TPMT\textsuperscript{*3A} was found a year later (460G>A; Ala154Thr + 719A>G; Tyr240Cys) in a patient with almost absent TPMT activity.\textsuperscript{13} To date, at least 25 variant alleles have been found, and their functional significance has been described.\textsuperscript{14-16} However, approximately 85-95% of all variant alleles in Caucasians is TPMT\textsuperscript{*2}, TPMT\textsuperscript{*3A} and TPMT\textsuperscript{*3C}.\textsuperscript{17} The TPMT\textsuperscript{*3A} has an allele frequency of 4% but is absent in African and Asian populations.\textsuperscript{18,19} In these populations, the TPMT\textsuperscript{*3C} allele (719A>G; Tyr240Cys) is the most frequent variant allele\textsuperscript{20,21} and its functional impact has been demonstrated in Japanese children with ALL.\textsuperscript{22}

A strong relationship between genotype and phenotype has been demonstrated, resulting in 90% sensitivity and 99% specificity (variants TPMT\textsuperscript{*2}, TPMT\textsuperscript{*3A} and TPMT\textsuperscript{*3C}).\textsuperscript{23,24} In another report no TPMT\textsuperscript{*2}, TPMT\textsuperscript{*3A} and TPMT\textsuperscript{*3C} allele was detected in 5 of 9 patients with intermediate TPMT activity.\textsuperscript{25} Even though cost effectiveness models of TPMT genotyping have been reported recently,\textsuperscript{26} and the Food and Drug Administration (FDA) has included more information on inherited TPMT deficiency in the 6MP label [201], only few institutions commonly genotype patients prior to 6MP treatment.\textsuperscript{27}

Interestingly, exposure to the cytotoxic metabolite 6TGN is not only related to toxicity, but also to efficacy of 6MP therapy as shown by Stanulla et al. They found a 2.9 fold lower occurrence of residual disease in ALL patients who were heterozygous for any TPMT\textsuperscript{*2}, TPMT\textsuperscript{*3A}, TPMT\textsuperscript{*3C} or TPMT\textsuperscript{*9} (356A>C; Lys119Thr) allele and treated with a similar 6MP dose. They did not find a difference in the occurrence of toxicity.\textsuperscript{28}

Dihydropyrimidine dehydrogenase

An important anti-metabolite used for a vast number of different types of solid tumours is 5-Fluorouracil (SFU). Over 80% of SFU is inactivated in the liver by the enzyme dihydropyrimidine dehydrogenase (DPD), encoded by the gene \textit{DPYD}. Since a DPD deficient patient experiencing severe haematological toxicity to SFU was described in 1988\textsuperscript{29} many mutations in the \textit{DPYD} gene that result in decreased DPD activity have been identified. Apart from association studies between DPD enzyme activity and SFU toxicity, genetic associations have also been described. It must be noted that not all SFU related toxicity can be attributed to decreased DPD activity though. Despite having an allele frequency of <1% in Caucasians\textsuperscript{30-32}, a SNP in the 5’ invariant splice donor sequence in intron 14 of the \textit{DPYD} gene (IVS14+1G>A; deletion of exon 14, \textit{DPYD*2A}) seems to be one of the key mutations resulting in low DPD activity and increased incidence of SFU toxicity.\textsuperscript{33} Two studies have shown considerable effect of this polymorphism on the incidence of SFU toxicity. In 60 cancer patients who experienced grade 3-4 toxicity (according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC) [202]) to SFU containing chemotherapy, the frequency of the \textit{DPYD*2A} allele was 15%, which was significantly higher than 0.91% in the control population (P=0.001).\textsuperscript{34} Also, 50% of patients who experienced NCI-CTC grade 4 neutropenia carried the \textit{DPYD*2A} allele.\textsuperscript{35} The \textit{DPYD*2A} allele has not been detected in Asian populations.\textsuperscript{36,37}

Other variants of the \textit{DPYD} gene that have been linked to SFU toxicity include the \textit{DPYD*M} (1601G>A; Ser534Asn), \textit{DPYD*11} (1003G>T; Val335Leu), \textit{DPYD*12} (62G>A; Arg21GlN + 1156G>T; Glu386Ter) and \textit{DPYD*13} (1679T>G; Ile560Ser) alleles.\textsuperscript{38} On the other hand, methylation of the promoter region of the \textit{DPYD} gene also seems to reduce DPD activity due to a decrease of transcription.\textsuperscript{39} Homozygous patients with two low DPD activity alleles are rare, but multiple cases have been described of lethal outcome to SFU treatment in these patients.\textsuperscript{40,41}

Uridine diphosphoglucuronosyl transferase

Uridine diphosphoglucuronosyl transferase (UGT) is a phase II metabolic enzyme responsible for glucuronidation of several endogenous (such as bilirubin) and exogenous compounds. SN-38, the active metabolite of the topo-isomerase I inhibitor irinotecan, is predominantly inactivated by the isoforms UGT1A1 and UGT1A9 in the liver and by UGT1A7 in the upper gastro-intestinal tract.\textsuperscript{42} A TA insert polymorphism in the TATA box of the promoter region of the UGT1A1 gene has been studied extensively. The UGT1A1*28 allele has 7 TA repeats, whereas wild-type has 6 TA repeats. This polymorphism is associated with Gilbert’s syndrome, a condition of reduced bilirubin glucuronidation\textsuperscript{43} and has also been associated with decreased SN-38 glucuronidation \textit{in vitro}\textsuperscript{44} and \textit{in vivo}.\textsuperscript{45} The allele frequency of UGT1A1*28 is higher in Caucasians (22-39%)\textsuperscript{46-48} than in Asians (7-17%)\textsuperscript{44,46} and even higher in Blacks (~45%).\textsuperscript{48} Patients with metastatic colorectal cancer (mCRC) or other solid tumours who were treated with irinotecan, and who were homozygous for the UGT1A1*28 allele had
significant higher occurrence of grade 3 or 4 diarrhoea (according to criteria of the World Health Organization (WHO)\(^{31}\) and NCI-CTC grade 3 or 4 neutropenia\(^{49,50}\) compared with patients who carried at least one wild-type allele. Other studies have shown higher incidence of NCI-CTC grade 3 or 4 diarrhoea in mCRC patients\(^{51}\) and higher incidence of grade 3 or 4 diarrhoea and/or grade 4 leucopenia (according to criteria of the Japan Society for Cancer Therapy\(^{52}\)) in patients with solid tumours\(^{47}\) when carriers of the variant allele were compared with homozygote wild-type patients. In a recent report of 250 mCRC patients, the odds ratio for the incidence of NCI-CTC grade 3 or 4 haematological toxicity was 8.63 for patients homozygous for the variant allele compared with patients who were homozygote wild-type. However, this was only significant after the first cycle of treatment.\(^{48}\) Interestingly, response to irinotecan therapy was also improved for homozygote individuals for the variant allele compared with homozygote wild-type patients. In addition, survival of patients who were treated with a combination of 5FU and oxaliplatin\(^{61}\) was found to be in linkage disequilibrium (LD).\(^{45,49,50}\) The functional and clinical relevance of these haplotypes has not yet been established.

From these studies it is clear that the UGT1A*28 allele is associated with increased risk for neutropenia in patients receiving irinotecan. Due to increased exposure to SN-38, the active metabolite of irinotecan, it may also be expected that carriers of this allele experience increased efficacy but this has not yet been proven. A SNP in the phenobarbital responsive enhancer module (PBREM) of the UGT1A1 gene (-3279T>G; UGT1A1*60) has been associated with severe toxicity (grade 4 leucopenia and grade 3 or 4 diarrhoea; Japanese criteria) in Japanese cancer patients treated with irinotecan.\(^{53}\) However, the UGT1A1*60 variant allele was linked to the UGT1A1*28 variant.\(^{39}\) Several SNPs in various regions of the UGT1A1, UGT1A7 and UGT1A9 genes have been found to be in linkage disequilibrium (LD).\(^{45,49,50}\) The functional and clinical relevance of these haplotypes has not yet been established.

In 2005, the FDA approved the Invader® UGT1A1 molecular assay, a test for the UGT1A1*28 variant allele \(203\). Also, the package insert of irinotecan was modified in 2005 by the FDA, to include information on UGT1A1 variability \[204\]. Unfortunately, because no studies have determined the optimal dose per genotype, no advice for dose adjustment is made.

Glutathione S-transferase

Glutathione S-transferases (GSTs) make up a family of phase II enzymes that catalyze the conjugation of reduced glutathione to toxic substances. Members of this family are GST \(\pi\), GST \(\mu\) and GST \(\theta\), which are products of distinct loci in the genome. Among substrates for GSTs are cyclophosphamide, etoposide, doxorubicin, cisplatin, carboplatin and oxaliplatin and their metabolites. Theoretically, reduced activity of these enzymes would result in increased exposure to these drugs, possibly resulting in increased efficacy and toxicity.

The gene for GST \(\pi\), GSTP1, is known to be polymorphic. One polymorphism resulting in a non-synonymous SNP at codon 105 (313A>G; Ile105Val) in exon 5 causes decreased GST \(\pi\) activity.\(^{17}\) The allele frequency is approximately 20%, 30% and 40% in Asian, Caucasian and African American populations respectively.\(^{57-60}\)

A significant association toward better survival after cyclophosphamide containing chemotherapy was found for breast cancer patients carrying the variant 105Val allele \(^{14,53}\). The variant allele was also associated with increased survival in 107 mCRC patients who were treated with a combination of 5FU and oxaliplatin.\(^{61}\) Survival was 24.9, 13.3 and 7.9 months for Val/Val, Val/ile and ile/ile genotypes respectively (\(P=0.001\)).\(^{60}\) The variant allele was also associated with better response and longer survival in gastric cancer patients who were treated with 5FU and cisplatin.\(^{61}\) Colorectal, gastric and pancreatic cancer patients carrying at least one GSTP1-105Val allele experienced less toxicity to oxaliplatin containing chemotherapy.\(^{63}\)

The genes for subclasses GST \(\mu\) (GSTM1) and GST \(\theta\) (GSTT1) both have ‘null’ polymorphisms, where the total gene is deleted on both alleles. Both null genotypes were associated with increased survival among breast cancer patients, irrespective of treatment (either chemotherapy or radiation).\(^{64}\) However, this association was not found in another cohort of breast cancer patients\(^{60}\), nor in a cohort of colorectal cancer (CRC) patients.\(^{60}\) Survival of ovarian cancer patients treated with platinum containing chemotherapy was also better for GSTM1 null patients\(^{57,58}\).

These studies demonstrate that, because of decreased inactivation of the respective anti-cancer agents, carriers of the less active variant GST alleles have increased response and survival to chemotherapy.

Drug transporters

ABC\(B1\)

The ATP-binding cassette (ABC) B1 gene (ABC\(B1\)), formerly known as multi-drug resistance (MDR1) gene, encodes the P-glycoprotein (PGP), an ATP-dependent efflux pump that exports exogenous substances across the cell membrane. Through this mechanism, substances such as cytostatics are unable to retain sufficient intracellular concentrations to exert their anti-tumour activity. Two synonymous SNPs in exons 12 and 26 (1236C>T and 3435C>T respectively) and a non-synonymous SNP in exon 21 (2677G>T; Ala893Ser/Thr) have been studied extensively. These variant alleles occur together in a common haplotype (MDR1\(^*2\)), with a frequency of 27%, 31-49% and 6.5% in Caucasians, Asians and Blacks respectively.\(^{62-64}\) The MDR1\(^*2\) haplotype was associated with lower irinotecan and SN-38 clearance\(^{65}\) and with lower \(C_{\text{max}}\) for glucuronidated SN-38 (SN-38G).\(^{70}\)

The individual polymorphisms have been associated with decreased PGP function in vivo.\(^{66,72}\) Cancer patients homozygous for the 1236C>T variant allele had higher
exposure to both irinotecan and SN-38. In 58 patients with solid tumours, all patients homozygous for the 3435T variant allele experienced grade 3-4 neutropenia (specified as neutrophil count between 0.5 and 1.0 x 10^9/L and less than 0.5 x 10^9/L respectively) to docetaxel, compared with 77% and 54% for heterozygote and wild-type individuals respectively. As exposure to docetaxel is increased in carriers of the variant allele, this finding would be expected.

**ABCG2**

Another ATP binding cassette, formerly known as the breast cancer resistance protein (BCRP) is coded by the gene ABCG2. Overexpression of this enzyme is related to the occurrence of resistance to several anticancer agents such as SN-38, mitoxantrone, topotecan, daunorubicin and etoposide. A SNP in exon 5 (421C>A; Gln141Lys) has an allele frequency of 34%, 12% and 1-5% in Han Chinese, Caucasians and Blacks respectively, and results in lower BCRP expression and higher SN-38 and topotecan sensitivity in vitro. However, this SNP has found not to be associated with pharmacokinetic parameters of irinotecan and its metabolites in a cohort of cancer patients.

**DNA repair**

**Excision repair cross complementing group 1**

As part of the nucleotide excision (NER) pathway, excision repair cross complementing group 1 (ERCC1) is involved in DNA damage repair caused by platinum containing compounds such as cisplatin, carboplatin and oxaliplatin. Increased ERCC1 expression has been shown to lead to cisplatin resistance in vitro and to lower response in cisplatin treated bladder cancer patients in vivo. A prospective ERCC1 mRNA expression guided phase III study is ongoing. A silent SNP has been identified in exon 4 at codon 118 in the ERCC1 gene (496C>T; Asn118Asn). The allele frequency of the 118Asn allele was associated with increased incidence of WHO grade 2 or higher neutropenia. In stage III-IV NSCLC patients treated with platinum containing chemotherapy, homozygote individuals for the variant allele had worse survival compared to carriers of a wild-type allele.

**X-ray repair cross complementing group 1**

The X-ray repair cross complementing group 1 (XRCC1) enzyme is involved in repair of single-strand breaks in DNA. A SNP in the XRCC1 gene (1301G>A, Arg399Gln, allele frequency of 0.35 in Caucasians, 0.36 in Blacks and 0.22 in Asians) has been associated with worse response to oxaliplatin and SFU in mCRC patients, but there was no difference in time to progression or survival. In stage III NSCLC patients, survival was shorter for individuals homozygote for the variant allele compared with carriers of the wild-type allele.

Ovarian cancer patients who carried the variant allele had reduced risk of platinum resistance, but survival was not affected by genotype. One would expect that the SNP leading to reduced ERCC1 expression and hence to decreased DNA repair of DNA-platinum adducts, would result in increased platinum sensitivity and consequently to increased response and survival. However, most studies that are presented show the opposite result. The occurrence of linkage disequilibrium of the evaluated variant with other variants with opposing effects on enzyme function as well as that of other enzymes and pathways with a role in the drug’s pharmacokinetics and clinical variables could be of importance and explain this discrepancy.

**Excision repair cross complementing group 2**

The enzyme xeroderma pigmentosum group D (XPD) is coded by the excision repair cross complementing group 2 (ERCC2) gene, and is also involved in the NER pathway. Two SNPs in this gene (965G>A, Asp321Asn and 2251A>C, Lys751Gln) are associated with reduced DNA repair capacity. The allele frequency of the XPD-321 variant allele was 0.32 in a general Western population. The allele frequency of the XPD-751 variant allele is 0.44 in Caucasians, 0.16 in Blacks and 0.09 in Asians. Patients with mCRC who were homozygous for the variant ERCC2-751 allele and who were treated with oxaliplatin based chemotherapy had higher mortality compared with patients carrying the wild-type allele. The two SNPs in the ERCC2 gene were not associated with response and survival in cisplatin treated NSCLC patients, but the wild-type ERCC2-751 allele was associated with increased incidence of WHO grade 2 or higher neutropenia. In stage III-IV NSCLC patients treated with platinum containing chemotherapy, homozygote individuals for the ERCC2-321 variant had worse survival compared to carriers of a wild-type allele.
the 3'UTR) was associated with increased survival.65 Moreover, cisplatin treated patients with muscle invasive bladder cancer who carried one or more variant ERCC2-75 or XRCC1-399 allele had better survival compared to wild-type patients.66 These inconsistent and non-intuitive findings could in part be explained by the reasons that are given for conflicting results for ERCC1.

CYP2D6
Tamofoxifen is a widely used agent in treatment of breast cancer and is hydroxylated into the 100 times more active metabolites 4-hydroxy-tamoxifen and endoxifen by the cytochrome P450 iso-enzyme CYP2D6.97-99 The CYP2D6*4 (1846G>A) allele results in gene deletion and thus in absent CYP2D6 activity and has a frequency of 15-20% in the general population in Western countries.100-101 Other alleles resulting in lower CYP2D6 activity are CYP2D6*2 (Δ1707T), CYP2D6*5 (deletion of entire CYP2D6 gene) and CYP2D6*6 (Δ1707T). The CYP2D6*4 allele has been linked to reduced conversion of tamoxifen into 4-hydroxy-tamoxifen96 and the CYP2D6*3-6 genotypes have been related to lower endoxifen formation.103,104 As expected, breast cancer patients treated with adjuvant tamoxifen who were homozygous for the CYP2D6*4 allele had significant worse relapse-free time and shorter disease free survival compared with carriers of the wild-type allele. However, significance was not retained in multivariate analysis. Homozygote CYP2D6*4 patients did not experience moderate to severe flashes, which is a side effect of (the active metabolite of) tamoxifen.105 A similar finding was reported in a case control study for prevention of breast cancer with tamoxifen in hysterectomised women. The frequency of the CYP2D6*4/*4 genotype was higher in women who developed breast cancer during follow up than in women free of cancer.107 Contradictory to this, another study found that oestrogen receptor positive (ER+) breast cancer patients who carried the CYP2D6*4 allele had significant longer recurrence free survival when treated with adjuvant tamoxifen compared with CYP2D6*4 carriers who were not treated with tamoxifen. This difference was not observed for wild-type patients.106 Selection bias in this study may have influenced the outcome of this study.108

Methylene tetrahydrofolate reductase (MTHFR)
The enzyme MTHFR is one of the key enzymes in the folate pathway (see figure 1). Reduced MTHFR expression results in reduced sensitivity to the MTHFR inhibitor MTX. On the other hand, abundance of the MTHFR substrate 5,10-methylene tetrahydrofolate (5,10-MTHF) facilitates the inhibition of thymidylate synthase (TS) by SFU, therefore increasing sensitivity to this agent. A SNP in the MTHFR gene, 677C>T results in an amino-acid change of alanine to valine at codon 222 (Ala222Val). The allele frequency in all populations is 0.27-0.57.62,100-104 MTHFR activity is 70% and 35% for heterozygote and homozygote individuals respectively. In vitro assays showed that cell lines with the variant allele are more sensitive to SFU and less sensitive to MTX.105

Of six patients who experienced severe toxicity to adjuvant CMF (cyclophosphamide, MTX, SFU) for breast cancer, five were homozygous for the 677T allele.110 In a cohort of cancer patients who were treated with the SFU analogue raltitrexed, patients with 677TT genotype had significant more therapy related toxicity.111 Leukaemia patients homozygous for the 677T allele experienced more MTX related toxicity compared with patients who carried at least one wild-type allele.112 Also, ovarian cancer patients who were treated with MTX and were homozygous for the 677T allele experienced significant more WHO grade 3/4 side effects.112 In mCRC patients, the 677T allele has been associated with improved response to SFU based chemotherapy in several studies119-121, whereas another study did not find a significant association.122 No association was found in a cohort of advanced gastric cancer patients treated with SFU and cisplatin.62

Thymidylate synthase (TS)
TS is the central enzyme in the de-novo thymidine synthesis. In vitro resistance to SFU is associated with increased TS activity, which is also induced by SFU itself.123 The TS promoter enhancer region (TSER) of the gene encoding TS (TYMS) has been shown to contain either two or three tandem repeats designated as TSER*2 and TSER*3 respectively. The TSER*3 genotype results in increased TS expression, either through higher mRNA levels or increase in efficiency of mRNA translation.124,125 The allele frequency of the TSER*2 allele is 0.40-0.46 in Caucasians and Black126,127, compared to 0.18-0.21 in Asian populations.128,129,130

The TSER*3 allele was associated with increased response in CRC patients treated with SFU.120 On the other hand, the TSER*2 allele was associated with improved response to capecitabine in CRC patients.131

These conflicting results could in part be explained by a G>C SNP in the 12 th base pair of the TSER*3 allele132 that results in TS activity similar to that of the TSER*2 allele.133 This TSER*3C allele is found in 29%-57% of all TSER*3 alleles.132,134,135 Carriers of the TSER*3G allele had significantly worse response, disease free survival and overall survival in a cohort of mCRC patients treated with SFU.135 The TSER*3G allele was also associated with worse survival in advanced gastric cancer patients who were treated with SFU.62 As expected, most studies show that the TSER*3 allele, and especially the TSER*3G allele, is associated with lower response and survival to fluoropyrimidine therapy. Conflicting results could be explained by the G>C SNP in the TSER*3 allele.
A six base pair deletion (-6bp) in the 3’ UTR of the TYMS gene results in decreased mRNA stability and lower TS expression. The -6bp mutation is in linkage disequilibrium (LD) with the TSER*3 allele, and the +6bp allele is in LD with TSER*2. The -6bp variant allele is associated with decreased survival in mCRC patients treated with 5FU and oxaliplatin and with decreased response to 5FU based chemotherapy in advanced gastric cancer patients.

CRC patients treated with 5FU who were either homozygous for the TSER*3 allele (regardless of 3’ UTR genotype) or heterozygous for the TSER allele combined with homozygous for the +6bp genotype had significant better disease free survival (DFS) and overall survival (OS) compared with the other genotypes. In a cohort of gastric cancer patients, non-carriers of the TSER*3G allele together with one or two -6bp alleles had significant better DFS and OS compared to carriers of TSER*3G and two copies of +6bp. The haplotype TSER*3C and -6bp was associated with significant better OS compared with the haplotype TSER*2 and +6bp in CRC patients treated with 5FU.

Multiple gene studies

Drug response is a complex phenotype, especially in anti-cancer therapy, where multiple drug regimens are often applied. Only few studies have explored the influence of polymorphisms of multiple genes that are involved in the pathway of the drug. The role of polymorphisms in genes involved in response to 5FU (TYMS) and metabolism of cisplatin (GSTP1) was investigated by Ruzzo et al. Advanced gastric cancer patients treated with 5FU and cisplatin who were both homozygous for the GSTP1-105Ile allele and carrier of the TSER*3G allele had significant shorter progression free survival and over all survival compared with patients who were carriers of the GSTP1-105Val allele or patients who did not carry the TSER*3G allele. Stoehlmacher et al. looked at genes involved in response and metabolism of oxaliplatin (ERCC1, ERCC2 and GSTP1) and 5FU (TYMS). Favourable genotypes in mCRC patients were ERCC2-751 Lys/Lys, ERCC1-496 C/C, GSTP1-105Val/Val and TYMS-3’UTR +6bp/+6bp. Patients who carried none of these genotypes had median survival of 5.4 months, compared with 10.2 and 17.4 months for patients with one or ≥ two favourable genotypes (P<0.001).

When multiple variants in genes or variants in multiple genes are surveyed for example in combination chemotherapy regimens, both sample size and power are of great importance since opposite effects of different genetic variants can obliterate each other in small samples, whereas multiple testing may reveal false-positive associations.

Conclusion

There is ample evidence that pharmacogenetic traits are able to predict pharmacodynamics of several anti-cancer drugs. Polymorphisms that result in decreased metabolic enzyme levels or activity have shown to result in either increased toxicity or increased efficacy or both. Other polymorphisms lead to increased exposure to chemotherapy through decreased expression of membrane efflux pumps, whereas others lead to decreased capability to repair DNA damage caused by chemotherapy. Variants in genes that code for enzymes involved in the mode of action of anti-cancer drugs give altered response to chemotherapy. Despite emerging evidence, pharmacogenetic testing has not yet found its way to routine patient care.

Expert Opinion

Many pharmacogenetic studies that point towards association of heritable genetic variants and cytotoxic drug response have been presented in this paper. These genes and variations have been studied most extensively until now. This does not necessarily imply that these genes hold most promise for implementation in the standard of oncology care in the near future. Possibly, other genes and variations may emerge as potential predictors of response or toxicity. Consequently, there is a need for additional, but also for other types of research in pharmacogenetics to find its way to routine patient care. Obviously, there is need for replication of apparent conflicting findings, such as for the TSER polymorphism in the TYMS gene, or polymorphisms in the DNA repair genes, in larger cohorts in routine care environment. Also, cost-effectiveness of testing needs to be determined for pharmacogenetic tests. In this light, it is important to develop tests that are sensitive and specific, as well as simple and cheap.

Genetic variability is only one of the determinants of drug response. Therefore, another type of research that holds promise for the future is the development of prediction models that not only include pharmacogenetic data, but also non-genetic traits such as WHO performance status and organ function. Such models are only starting being developed, for instance regarding MTX response in rheumatoid arthritis.

Until now, genes are selected mainly through the candidate pathway gene approach. Obviously, this mechanistic approach seems logical. However, the disadvantage of this approach is that it is limited by current knowledge of pathophysiology and the mechanism of action of a drug. Therefore, future research will use hypothesis-free whole genome approach such as SNP arrays.
Finally, it is important to perform prospective studies on applying pharmacogenetics in patient care and to assess optimal dose and drug per genotype upon a predictive model including a pharmacogenetic test result. After all it is not possible to predict necessary dose adjustment based upon current knowledge. This is illustrated by the phrase in the irinotecan label that has recently been modified by the FDA: “A reduced initial dose should be considered for patients known to be homozygous for the UGT1A1*28 allele” [204]. Therefore, studies are necessary that prospectively investigate an adjusted dose for a certain genotype compared with normal dose for wild-type patients.

The above mentioned future pharmacogenetic research will enable oncologists to implement pharmacogenetics and to optimize individual cancer treatment.

References

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