The role of pharmacogenetics in capecitabine efficacy and toxicity

SW Lam
HJ Guchelaar
E Boven

Cancer Treat Rev 2016 (in press)
Abstract

Capecitabine is an oral prodrug of 5-fluorouracil (5-FU) and approved for treatment of various malignancies. Hereditary genetic variants may affect a drug’s pharmacokinetics or pharmacodynamics and account for differences in treatment response and adverse events among patients. In this review we present the current knowledge on genetic variants, commonly single-nucleotide polymorphisms (SNPs), tested in cohorts of cancer patients and possibly useful for prediction of capecitabine efficacy or toxicity. Capecitabine is activated to 5-FU by CES, CDA and TYMP, of which SNPs in CDA and CES2 were found to be associated with efficacy and toxicity. In addition, variants in genes of the 5-FU metabolic pathway, including TYMS, MTHFR and DPYD also influenced capecitabine efficacy and toxicity. In particular, well-known SNPs in TYMS and DPYD as well as putative DPYD SNPs had an association with clinical outcome as well as adverse events. Inconsistent findings may be attributable to factors related to ethnic differences, sample size, study design, study endpoints, dosing schedule and the use of multiple agents. Of the SNPs described in this review, dose reduction of fluoropyrimidines based on the presence of DPYD variants *2A (rs3918290), *13 (rs55886062), -2846A>T (rs67376798) and -1236G>A/HapB3 (rs56038477) has already been recommended. Other variants merit further validation to establish their definite role in explanation of interindividual differences in the outcome of capecitabine-based therapy.
A REVIEW ON SNPS AND CAPECITABINE

Introduction

Capecitabine, a prodrug of the antimetabolite 5-fluorouracil (5-FU), has been registered for treatment of colon cancer in the adjuvant setting as well as for treatment of advanced colon, breast and gastric cancer. The drug is active as single agent, but can also be combined with other cytotoxic agents, such as oxaliplatin, irinotecan, a taxane or cisplatin. In colon cancer, a pooled analysis of randomized trials has shown equivalence in efficacy between infusional 5-FU- and capecitabine-containing regimens. In advanced esophageo-gastric cancer, meta-analysis of two randomized trials in which patients received infusional 5-FU or capecitabine combinations, overall survival (OS) was even superior for the latter treatment regimen. The convenience of an oral formulation given daily for a particular period mimicking continuous 5-FU infusion makes capecitabine an attractive treatment option, although regular monitoring of patient’s adherence to oral anticancer medication balanced by tolerability is important to ensure optimal drug exposure. Of interest, some tumors express high levels of thymidine phosphorylase (TYMP), the rate-limiting enzyme activating capecitabine to 5-FU, enabling high and sustained intratumoral levels of active drug.

Although the efficacy of capecitabine is considered to be equivalent to 5-FU, their toxicity profiles vary. Both drugs induce gastrointestinal adverse events (AEs), of which the incidence of nausea is not different among comparative treatment groups. In case of capecitabine, the incidence of stomatitis is significantly lower, while that of diarrhea is significantly increased especially when combined with irinotecan. In comparison with intermittent 5-FU, capecitabine is associated with a lower rate of neutropenia, but hand-foot syndrome (HFS) occurs far more frequently. Both drugs are known for a low prevalence of cardiovascular toxicity.

The incidence and severity of AEs of capecitabine depend on therapy-related factors, such as dosing schedule, duration, previous treatment and overlapping toxicity when combined with cytotoxic agents. Dosing usually consists of administration twice daily for two weeks followed by a rest period of one week in a three-week cycle. The starting dose is 1,250 mg/m² twice daily when given as single agent, but dose reductions are frequently required to improve tolerability. In breast cancer, a lower starting dose of 1,000 mg/m² or dose-adjusting capecitabine during treatment does not seem to compromise efficacy. In combination regimens, initial doses vary between 825–1,000 mg/m² twice daily.

Host-related factors of influence on capecitabine-induced AEs are dihydropyrimidine dehydrogenase (DPD) enzymatic activity, renal dysfunction, gender and age, body weight, regional differences, and drug-drug interactions. The DPD enzyme is required to convert 5-FU to 5-fluorodihydrouracil. Deficient or low DPD activity due to alterations in the DYPD gene is estimated to occur in 3–5% of individuals, which may lead to increased
toxicity from 5-FU as well as capecitabine. Another important factor of influence on interindividual differences in AEs is renal function. A 50% decrease in creatinine clearance is associated with a 50% reduction in clearance of the toxic catabolite fluoro-beta-alanine (FBAL). Concentration-effect analyses have shown a positive relationship between the area under the curve (AUC) of FBAL and treatment-related grade ≥3 diarrhea. For that reason, tailored doses of capecitabine are recommended in case of reduced creatinine clearance, while therapy is withheld if clearance is less than 30 mL/min. For gender, the clearance of FBAL is less in women. The age-related increase in concentration of FBAL might be explained by a physiological decrease in renal function in the elderly. A high body weight results in a high body surface area, which is associated with a high volume of distribution and a decreased clearance of FBAL. Regional variations in the tolerability of capecitabine as well as 5-FU have been reported in studies in which patients were included from US and East-Asia, but underlying reasons for the differences are not clear. For drug-drug interactions, some drugs are mentioned to be of influence on metabolism, while caution is required with concomitant use of nephrotoxic agents.

Research in pharmacogenetics has gained interest with respect to its contribution to our understanding of the interindividual variation in drug effects. Genetic polymorphisms, primarily single nucleotide polymorphisms (SNPs), may affect expression and/or activity of various proteins including drug-metabolizing enzymes, drug transporters and targets, or transcription factor binding sites resulting in altered gene expression, i.e. encoding for proteins involved in detoxification or excretion. Extensive studies have been carried out on SNPs linked to the 5-FU metabolic pathway for prediction of treatment response and/or toxicity. The well-known example is DPYD of which the DPYD*2A variant results in a catalytic inactive form of the enzyme leading to excessive toxicity. Given similarities between capecitabine and 5-FU in terms of their mechanism of action and elimination, these genetic variations also affect the outcome of capecitabine. Moreover, novel genetic variants might be identified in the key enzymes of capecitabine activation to 5-FU. In this comprehensive review, we summarized the information available on SNPs in the capecitabine-activating pathway as well as 5-FU-metabolizing genes in order to determine, whether these genetic variants play a role in the differential efficacy and toxicity from capecitabine among individuals.

**Capecitabine metabolic pathway**

Capecitabine is activated to 5-FU through a three-step enzymatic process consecutively requiring carboxylesterase (CES), cytidine deaminase (CDA) and TYMP (Figure 1). After rapid intestinal absorption, the first step of activation primarily occurs in the liver and involves enzymatic hydrolysis by CES producing S'-deoxy-5-fluorocytidine (S'-DFCR). Among three 60-kDa CES isoenzymes, CES1A2 and CES2 exert highest catalytic efficiencies in the
hydrolysis of capecitabine *in vitro* 16. 5'-DFCR is converted to 5'-deoxy-5-fluorouridine (5'-DFUR) by CDA, which is a ubiquitous enzyme mainly expressed in the liver. High CDA activity in cancer cells has been associated with increased sensitivity to capecitabine 17,18. Moreover, a potential role of CDA in capecitabine toxicity has been suggested in patients that developed severe life-threatening AEs in the presence of high serum activity of CDA 19,20. It is of note that while CDA is involved in the activation of capecitabine, it functions as a major detoxifying enzyme for other antimetabolites, such as gemcitabine and cytarabine 17,18. The final conversion of 5'-DFUR to 5-FU is mediated by TYMP. Given the relatively higher TYMP expression in some tumors compared to healthy tissue, preferential activation of capecitabine to 5-FU might lead to tumor selectivity 6,21,22. *TYMP* expression is elevated in the palm compared with the back of the hand, which was hypothesized to be a major causative mechanism for capecitabine-related HFS 23.
The mechanism of action of 5-FU has been described elsewhere \(^2^4\) and entails, briefly, misincorporation of 5-FU metabolites into RNA and DNA and inhibition of thymidylate synthase (TYMS). In particular, TYMS inhibition by 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) triggers a cascade of molecular alterations that lead to misincorporation of 5-FU metabolites into DNA, impaired DNA replication, synthesis and repair, which eventually leads to DNA breaks. Preclinical findings in human cancer cell lines have demonstrated that high TYMS activity was associated with 5-FU resistance \(^2^5\). Methylenetetrahydrofolate reductase (MTHFR) is one of the many enzymes that play a role in the metabolism of folates, their primary source is diet. MTHFR carries out a central reaction by irreversibly catalyzing the conversion of 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate, the primary circulating form of folate, which serves as a methyl-group for DNA methylation reactions \(^2^6\). An elevated level of 5,10-MTHF, such as in low MTHFR activity, might theoretically lead to greater inhibition of TYMS and enhanced cytotoxicity of 5-FU.

The catabolism of 5-FU is mainly controlled by DPD, which is a rate-limiting enzyme in the liver responsible for conversion of 80% of 5-FU into dihydrofluorouracil (DHFU) \(^1^5\). DPD levels vary considerably among individuals with consequences for efficacy and toxicity during 5-FU therapy \(^1^1,1^4\). Low DPD activity results into severe AEs due to accumulation of active 5-FU metabolites \(^1^1,1^4\). DHFU is then converted to fluoro-β-ureidopropionate (FUPA) and subsequently to FBAL by dihydropyrimidinase and β-ureidopropionase, respectively \(^1^5\). Excretion of the metabolites occurs by the kidney \(^2^2\). Mean urinary recovery of the administered dose amounts to 71–87% and mainly consists of FBAL (51–62%), followed by 5'-DFUR (7–11%) and 5'-DFCR (6–7%) and small percentages of other compounds.

**Genetic polymorphisms and functionality**

Several candidate SNPs involved in capecitabine efficacy and/or toxicity have been investigated for functionality in the past. A brief overview is provided here for better interpretation of pharmacogenetic results.

*TYMS* genetic variants located in the regulatory regions have shown to influence the transcription rate. Higher intratumoral TYMS levels may translate into relative resistance to 5-FU \(^2^7-2^9\). Of particular interest is *TYMS* 2R or 3R (rs45445694) constituting double or triple tandem repeats of 28 base pairs (bp) in the 5’untranslated region (UTR). An enhancer box (E-box) sequence containing a binding site for upstream stimulating factors (USFs) is located in the first of the double tandem repeats of the 2R allele and the two first of the triple tandem repeats of the 3R allele. Binding of USFs to the E-box enhances the *TYMS* transcription rate and, consequently, 3R compared to 2R will result in greater enzyme activity as demonstrated *in vitro* \(^2^7,2^9\). Furthermore, a glycine to cysteine substitution in the second of the triple tandem repeats of the 3R allele is denoted as *TYMS* 3RC or 3RG (rs2853542). *TYMS* 3RG is associated with a reduced transcription rate *in vitro* presumably due to the
loss of the second E-box binding site. In few studies, patients were grouped in a low activity (2R/2R, 2R/3RC or 3RC/3RC), intermediate activity (2R/3RG or 3RC/3RG), and high activity class (3RG/3RG). Another putative SNP (rs183205964) is located in the 5’UTR of TYMS constituting a glycine to cysteine substitution in the first repeat of 2R (denoted as 2RC), which affects the functional E-box resulting in reduced TYMS expression. Lastly, a SNP constituting an insertion or deletion of 6 bp in the 3’ UTR, TYMS 3’UTR ins6 or del6 (rs16430), in which TYMS 3’UTR del6 conferred reduced transcription.

Two SNPs related to MTHFR activity are located in exon 4 (MTHFR -677C>T, rs1801133) and in exon 7 (MTHFR -1298C>A, rs1801131), of which the MTHFR -667T and -1298C alleles and the haplotype of both risk alleles led to lower enzymatic activity in vitro. Reduced enzyme activity may result in enhanced cytotoxicity of fluoropyrimidines, but unequivocal evidence is lacking. Moreover, high intracellular folate appears to stabilize the protein structure of MTHFR, thereby counteracting the detrimental effect of MTHFR -667T and -1298A alleles on enzyme activity. Folate status, which is dependent on dietary habit and intake of folate supplements, is an important confounding factor, thereby potentially obscuring the effects of MTHFR SNPs.

DPYD is a large and highly polymorphic gene with several hundreds of reported genetic variants. SNPs in DPYD may cause enzyme deficiency resulting in toxicity from fluoropyrimidine treatment. It is estimated that up to 5% of the population is deficient in DPD enzyme activity. The rare DPYD IVS14+1G>A (*2A, rs3918290) entails a glycine to alanine substitution at the conserved splice donor site of intron 14. This causes exon 14 skipping resulting in a nonfunctional DPD protein, which has repeatedly been shown to induce severe toxicity. Carriers of the *2A allele had an approximately two-fold higher exposure to 5-FU, as apparent from dose-normalized AUC, than wild-type individuals. More frequently observed genetic variants are -1627A>G (*5, rs1801159), -2194G>A (*6, rs1801160) and -85T>C (*7, rs1801165), but their association with DPD activity has been inconsistent. Other rare functional variants include *13 (rs55886062), -2846A>T (rs67376798) and -1236G>A/HapB3 (rs56038477). A genome-wide association study (GWAS) has pointed towards putative DYPD SNPs associated with toxicity, but their functional impact remains to be elucidated.

Since detoxification of 5-FU by DPD is a rate-limiting process, increased activation of capecitabine might augment the likelihood of AEs. To date, functional evidence regarding TYMP and CES SNPs is lacking. With respect to capecitabine and metabolites, CES2 -823C>G (rs11075646) was not associated with the AUC of 5-FU. CDA SNPs may explain highly variable enzyme activity among individuals. An ultra-metabolizer status was found to be associated with increased efficacy and severe toxicity from capecitabine. Mostly investigated CDA SNPs, such as CDA 208G>A (*3, rs60369023; occurring in Japanese and Korean subjects), CDA -451C>T (rs532545), -943del/insC (rs3215400) and -79A>C (*2, rs2072671), have shown to affect exposure to CDA-metabolized drugs or to be associated
with altered enzyme activity \(^{18,28,39}\), but data on capecitabine pharmacokinetics are lacking.

**Genetic polymorphisms possibly associated with efficacy from capecitabine**

**Thymidylate synthase**

Pharmacogenetic research on capecitabine efficacy has mostly been carried out with focus on *TYMS*, because of its role as the key therapeutic target (Table 1). In two out of seven studies on capecitabine monotherapy, a possible role for *TYMS* SNPs was suggested to explain differences in efficacy among individuals. *TYMS* 5’ 3RG/3RG was associated with shorter progression-free survival (PFS) in 105 advanced breast cancer patients \(^{30}\), whereas *TYMS* 5’ 2R/2R was associated with a higher response rate in a small cohort of patients with metastatic colorectal cancer \(^{40}\). In most studies (n = 10), however, treatment was capecitabine based including other cytotoxic agents. An association between *TYMS* SNPs and clinical outcome has been mentioned in four reports. In 58 metastatic colorectal cancer patients, it appeared that both *TYMS* 5’ 2R/2R and *TYMS* 3’UTR ins6/ins6 were preferentially present in the group with a good response on capecitabine and raltitrexed \(^{41}\). In 125 patients with metastatic gastric cancer receiving a capecitabine-based regimen \(^{47}\), carriers of a *TYMS* 3’UTR del6 allele had a significantly longer median OS than those harboring the *TYMS* 3’UTR ins6/ins6 genotype (11.4 vs 6.8 months, \(p = 0.014\)). The *TYMS* 3’UTR ins6/ins6 genotype appeared to be an independent prognostic factor for short PFS and OS. LaBonte et al. \(^{31}\) reported no association of *TYMS* 5’UTR SNPs (2R/3R, 3RC/3RG) or 3’UTR ins6/del6 with treatment response or time to tumor progression (TTP) in 240 patients with HER2-positive metastatic breast cancer receiving capecitabine with or without lapatinib. However, when considering the group treated with capecitabine monotherapy (n = 125), patients carrying *TYMS* 5’UTR variations (2R/3RG, 3RC/3RG and 3RG/3RG) demonstrated a longer TTP of 7.1 months compared to those carrying alternate genotypes (2R/2R, 2R/3RC or 3RC/3RC). In that study, OS was not an endpoint. Joerger et al. \(^{43}\) recently reported that the presence of 3RG, denoted as *TYMS* high-expression genotype, was associated with shorter PFS in advanced colorectal cancer patients (Hazard ratio [HR] = 2.03, \(p = 0.006\)) and in advanced gastroesophageal cancer patients (HR = 5.4, \(p <0.001\)) as well as with shorter OS in the advanced gastroesophageal cancer group (HR = 4.74, \(p <0.001\)). When correcting for prognostic factors, the *TYMS* high-expression genotype predicted for worse OS in advanced gastroesophageal cancer patients (HR = 5.44, \(p <0.001\)).

Of particular interest is the study of Pander et al. \(^{44}\) that was performed in 279 metastatic colorectal cancer patients treated with capecitabine, oxaliplatin and bevacizumab. None of the 17 SNPs involved in pathways of each of the three agents was associated with PFS. However, a genetic interaction profile consisting of polymorphisms in the capecitabine and bevacizumab pathways (*TYMS* 3RG and *VEGF* -405G>C) could stratify patients into groups
with different PFS. Patients allocated to the beneficial profile group had a significantly longer PFS than those in the unfavorable profile group (13.3 vs 9.7 months, \( p < 0.001 \)). Although the presence of a real interaction was not examined, these findings show that analysis of SNPs representing different therapeutic pathways may provide more comprehensive predictive information.

**Methylenetetrahydrofolate reductase**

In all eight pharmacogenetic studies on *MTHFR* and capecitabine included in this review, *MTHFR* -677C>T or -1298C>A were not associated with treatment outcome (Table 1). Among them were two studies on capecitabine monotherapy.30,45

**Dihydropyrimidine dehydrogenase**

The rare variant *DPYD* IVS14+1G>A has been investigated in five studies, but an association with capecitabine efficacy has not been reported (Table 1). Since *DPYD* is a polymorphic gene with multiple variants, Deenen et al. 46 sequenced the coding region to identify novel associations of putative SNPs with capecitabine efficacy. Although the investigators primarily focused on capecitabine-related toxicity, eight SNPs were tested for their association with PFS and OS in 568 patients with advanced colorectal cancer. None of these was individually related to clinical outcome, but patients carrying a haplotype consisting of six SNPs (*DPYD* -85T, -496A, -1236G, -1601G, -1627A and -2194G) experienced a longer OS (HR = 0.57, \( p = 0.03 \)). The frequency of this haplotype was rather low (2.7%).

**Cytidine deaminase and carboxylesterase**

To date, few investigators have assessed SNPs of enzymes for capecitabine activation, such as CDA and CES, in relation with capecitabine efficacy. Ribelles et al. 47 were the first to report on *CES2* 5’UTR -823C>G (rs11075646) and capecitabine efficacy in 136 patients with advanced breast or colorectal cancer. Carriers of a *CES2* 5’UTR -823 G-allele had a significantly higher response rate (59 vs 32%, \( p = 0.015 \)) and longer TTP (8.7 vs 5.3 months, \( p = 0.014 \)) than wild-type carriers. The prognostic potential of *CES2* 5’UTR -823CG remained significant for longer TTP after adjustment for clinical confounders (HR = 0.56, \( p = 0.036 \)).

*CDA* SNPs were not associated with outcome in that study. In 111 patients with metastatic breast cancer on capecitabine monotherapy, Martin et al. 48 reported that *CDA* rs602950 was associated with PFS (HR per allele 1.44, \( p = 0.038 \)), while *CDA* rs2072671 was associated with PFS (HR = 1.77, \( p = 0.0031 \)) and OS (HR = 1.55, \( p = 0.032 \)). Interestingly, two SNPS in *TYMP*, namely rs11479 and rs470119, were associated with OS (HR = 2.36, \( p = 0.010 \), and HR = 1.46, \( p = 0.034 \), respectively).

**Other genetic polymorphisms possibly associated with capecitabine efficacy**

Molecular pathways not apparently related to capecitabine metabolism or mechanism of action have been evaluated in search for putative genetic markers potentially useful to predict capecitabine efficacy. SNPs in apoptosis-related genes might be associated with
decreased cell death and, therefore, indicate therapy resistance \(^49\). In 76 metastatic colorectal cancer patients treated with capecitabine and oxaliplatin, 17 variants in genes regulating the apoptotic process were investigated for an association with response, PFS or OS \(^49\). Only the TT genotype of PTGS2 8473T>C (rs5275), a gene encoding prostaglandin synthase 2 as an enzyme involved in prostaglandin synthesis, was associated with poor PFS (HR = 0.47, \(p = 0.046\)) and OS (HR = 0.16, \(p = 0.013\)) independent of clinically prognostic factors. However, since many anticancer agents can induce apoptosis in tumor cells, PTGS2 8473T>C may not specifically be associated with capecitabine efficacy.

In another study on capecitabine combined with docetaxel for advanced breast cancer, the Drug Metabolizing Enzymes and Transporters (DMET) genotyping platform was employed to assay 79 genetic variations in cytochrome P450 (CYP) enzymes \(^50\). From the analysis, CYP1A1 rs1048943 A>G was associated with longer PFS for carriers of a G-allele compared with wild-type carriers (8.3 vs 5.3 months, \(p = 0.0003\)). CYP1A1 rs1048943 A>G remained prognostic for PFS after adjusting for hormone receptor and menstruation status. Since the role of CYP1A1 in either the taxane or the capecitabine pathway or even in breast cancer is not known, further confirmation of this finding is needed.

**Genome-wide association study**

Recent advances in high-throughput technologies enable simultaneous profiling of thousands of genetic variants and may lead to the identification of novel genetic associations, which cannot be detected by the traditional gene-based approach. Recently, O’Donnell et al. \(^51\) used the publicly available, genome-wide SNP data from the International Haplo-type Map project, which have previously been generated from human lymphoblastoid cell lines from different ethnic individuals. Capecitabine sensitivity was determined for the same cell lines by a cell growth inhibition assay and was correlated with GWAS data. This analysis showed that cell lines from the Caucasian population were least sensitive to capecitabine, whereas cell lines from the population of Yoruba individuals from Ibadan, Nigeria were the most sensitive. From the independent analysis of each population, adenylate cyclase 2 (ADCY2 rs4702484) was associated with capecitabine sensitivity at a near genome-wide significant level for the Caucasian population (\(p = 5.2 \times 10^{-6}\)). Meta-analysis of all populations revealed several SNPs, including ADCY2 rs4702484, although none reached genome-wide statistical significance. This study illustrates the opportunity of integrating \textit{in vitro} data and high-throughput genotyping data for discovery of novel genetic markers associated with drug sensitivity. However, the predictive value of ADCY2 rs4702484 as well as another two SNPs for PFS, RR, clinical benefit and OS could not be confirmed in 268 metastatic colorectal cancer patients randomized for capecitabine without or with oxaliplatin \(^52\). It has to be stressed, however, that the investigators corrected for multiple testing requiring lower significance values.
Table 1. Genetic polymorphisms possibly associated with efficacy from capecitabine-containing therapy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study</th>
<th>Tumor type</th>
<th>Stage</th>
<th>No of patients</th>
<th>Efficacy end-points</th>
<th>Capcitabine relevant genes</th>
<th>No of relevant SNPs</th>
<th>Main findings with respect to capcitabine relevant genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capecitabine</td>
<td>Largillier et al. [38]</td>
<td>Breast</td>
<td>Advanced</td>
<td>105</td>
<td>PFS</td>
<td>TYMS; MTHFR; DPYD</td>
<td>6</td>
<td>TYMS 5’ genotype class was associated with shorter PFS with sequence class 2 &gt; class 3 &gt; class 4 (p = 0.037)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Park et al. [40]</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>24</td>
<td>Tumor response (≥50% decrease for at least 6 weeks)</td>
<td>TYMS</td>
<td>1</td>
<td>TYMS 5’ 2R/2R was associated with higher RR (p = 0.036)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Garcia et al. [42]</td>
<td>Cervix</td>
<td>Advanced/recurrent</td>
<td>25</td>
<td>RR</td>
<td>TYMS</td>
<td>2</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Sharma et al. [46]</td>
<td>Colorectal</td>
<td>Advanced/metastatic</td>
<td>56</td>
<td>RR, OS</td>
<td>TYMS; MTHFR</td>
<td>4</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Ribelles et al. [47]</td>
<td>Breast (n = 76) and colorectal (n = 60)</td>
<td>Metastatic</td>
<td>136</td>
<td>RR, TTP</td>
<td>TYMS; DPYD; CDA; CES2</td>
<td>14</td>
<td>CES2 5’UTR -823CC was associated with low ORR (p = 0.015) and shorter TTP (p = 0.014) in patients with liver metastases (n = 48) CES2 5’UTR -823CG plus presence of liver metastases was predictive for high RR (OR = 3.6, p = 0.0096) CES2 5’UTR -823CG was prognostic for longer TTP in multivariate analysis (HR = 0.56, p = 0.036)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Martin et al. [48]</td>
<td>Breast</td>
<td>Metastatic</td>
<td>111</td>
<td>PFS, OS</td>
<td>TYMS; TYPYMP; DPYD; CES2; CDA</td>
<td>16</td>
<td>CDA -92A&gt;G was associated with PFS (HR = 1.44, p = 0.038) CDA -79A&gt;G was associated with PFS (HR = 1.77, p = 0.031) and OS (HR = 1.55, p = 0.032) TYPYMP rs11479 (HR = 2.36, p = 0.010) and rs470119 (HR = 1.46, p = 0.034) were associated with OS</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin</td>
<td>Martinez-Balibrea et al. [49]</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>47</td>
<td>RR, disease-control rate, PFS</td>
<td>TYMS</td>
<td>3</td>
<td>No significant association</td>
</tr>
<tr>
<td>Treatment</td>
<td>Study</td>
<td>Tumor type</td>
<td>Stage</td>
<td>No of patients</td>
<td>Efficacy endpoints</td>
<td>Capcitabine relevant genes</td>
<td>No of relevant SNPs</td>
<td>Main findings with respect to capcitabine relevant genes</td>
</tr>
<tr>
<td>-----------</td>
<td>-------</td>
<td>------------</td>
<td>------------</td>
<td>----------------</td>
<td>--------------------</td>
<td>---------------------------</td>
<td>---------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin</td>
<td>Spindler et al.</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>68</td>
<td>RR, PFS, OS</td>
<td>TYMS</td>
<td>1</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin</td>
<td>Kim et al.</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>76</td>
<td>RR, PFS, OS</td>
<td>Cell death related SNPs: AKT1; BCL2; BID; CASP3; CASP6; CASP7; CASP8; CASP9; CASP10; FAS; FASLG; RIPK1; TN-FRSF10B; TPS3; PTGS2</td>
<td>16</td>
<td>PTGS2 -8473TT was associated with longer PFS (HR = 0.47, p = 0.046) and OS (HR = 0.16, p = 0.013)</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin</td>
<td>Van Huis-Tanja et al.</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>268</td>
<td>PFS, OS, RR, clinical benefit</td>
<td>MTRR; MTHFR; ADCY2; SMARCAD1; intergenic SNPs</td>
<td>13</td>
<td>ADCY2 wild-type tended to have shorter PFS in capcitabine alone group (p = 0.018, multivariate p = 0.029) MTRR rs1533268 variant tended to have more clinical benefit (p = 0.054) MTRR rs162036 wild-type tended to have more clinical benefit (p = 0.039)</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin + bevacizu-mab</td>
<td>Pander et al.</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>279</td>
<td>PFS</td>
<td>TYMS; MTHFR</td>
<td>4</td>
<td>No genotypes or haplotypes were individually associated with PFS Patients with 'beneficial profile' for PFS had VEGF +405C allele and TYMS 5'UTR no 3RG allele or VEGF +405GG genotype and TYMS 5'UTR 3RG allele, while patients with other combinations had 'unfavorable profile' for PFS (p &lt;0.001)</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin + bevaci-zumab ± cetuximab</td>
<td>Deenen et al.</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>568</td>
<td>PFS, OS</td>
<td>Sequencing of entire DYPD coding regions and 3' UTR</td>
<td>NA</td>
<td>One haplotype block consisting of six DYPD SNPs (-85T,-496A,-1236G,-1601G,-1627A,-2194G) was associated with increased OS (HR = 0.57, p = 0.03)</td>
</tr>
<tr>
<td>Capecitabine + docetaxel + oxaliplatin</td>
<td>Deenen et al.</td>
<td>Stomach or gastro-epiophageal</td>
<td>Advanced</td>
<td>34</td>
<td>PFS, OS</td>
<td>TYMS; MTHFR; DPYD; CDA; ENOSF1</td>
<td>7</td>
<td>No significant association</td>
</tr>
<tr>
<td>Treatment</td>
<td>Authors</td>
<td>Tumor Type</td>
<td>Stage</td>
<td>Sample Size</td>
<td>Response Rate</td>
<td>PFS, OS</td>
<td>Genes</td>
<td>Clinical Benefit</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-------------------------------</td>
<td>------------------</td>
<td>------------------</td>
<td>-------------</td>
<td>---------------</td>
<td>---------</td>
<td>-------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Capecitabine + oxaliplatin or</td>
<td>Joerger et al. (^{41})</td>
<td>Colorectal</td>
<td>Advanced/metastatic</td>
<td>140</td>
<td>RR</td>
<td>PFS, OS</td>
<td>TYMS, MTHFR, DPYD</td>
<td>TYPMS 5'UTR 2R/3RG, 3RC/3RG or 3RG/3RG variants (high-expression genotype) were associated with worse PFS in both patient groups (both p &lt;0.01) and worse OS in the gastroesophageal cancer group (p &lt;0.01)</td>
</tr>
<tr>
<td>capecitabine + epirubicin +</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TYMS, MTHFR</td>
<td>No significant association</td>
</tr>
<tr>
<td>cisplatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TYMS, MTHFR</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine + irinotecan</td>
<td>Carlini et al. (^{40})</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>66</td>
<td>RR</td>
<td>PFS, OS</td>
<td>TYMS</td>
<td>For capecitabine alone group, TYMS 5'UTR 2R/2R, 2R/3RC, 3RC/3RC was associated with decreased TTP (HR &gt;1)</td>
</tr>
<tr>
<td>Capecitabine + irinotecan +</td>
<td>Zarate et al. (^{39})</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>60</td>
<td>RR, PFS, OS</td>
<td></td>
<td>TYMS, MTHFR</td>
<td>No significant association</td>
</tr>
<tr>
<td>oxaliplatin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>TYMS, MTHFR</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine ± lapatinib</td>
<td>LaBonte et al. (^{31})</td>
<td>Breast</td>
<td>Metastatic</td>
<td>240</td>
<td>RR, clinical</td>
<td></td>
<td>TYMS, MTHFR</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>benefit</td>
<td></td>
<td>TTP</td>
<td></td>
</tr>
<tr>
<td>Capecitabine + raltitrexed</td>
<td>Salgado et al. (^{41})</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>58</td>
<td>RR (WHO</td>
<td></td>
<td>TYMS, DPYD</td>
<td>TYPMS 5' 3R/3R preferentially found in poor response group (p &lt;0.01) TYPMS 3'UTR del6/del6 was only found in poor response group (p &lt;0.05)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>criteria</td>
<td></td>
<td>TYMS, DPYD</td>
<td></td>
</tr>
<tr>
<td>Capecitabine + paclitaxel</td>
<td>Gao et al. (^{62})</td>
<td>Stomach</td>
<td>Metastatic</td>
<td>125</td>
<td>RR, PFS, OS</td>
<td></td>
<td>TYMS, MTHFR</td>
<td>TYPMS 3'UTR ins6/ins6 was associated with shorter OS (p = 0.014) TYPMS 3'UTR ins6/ins6 was an independent predictor of short PFS (HR = 2.251; p = 0.013) and OS (HR = 3.182; p = 0.001) in multivariate analysis</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DPYD</td>
<td></td>
</tr>
<tr>
<td>Capecitabine + docetaxel</td>
<td>Dong et al. (^{50})</td>
<td>Breast</td>
<td>Metastatic</td>
<td>69</td>
<td>RR, PFS, OS</td>
<td></td>
<td>79 SNPs in CYP450</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CYP1A1 G allele was associated with longer PFS (p = 0.0003) CYP1A1 G allele was an independent predictor of longer PFS (p = 0.004)</td>
</tr>
<tr>
<td>NA</td>
<td>O'Donnell et al. (^{51})</td>
<td>Lymphoblastoid cell lines</td>
<td>NA</td>
<td>503</td>
<td>In vitro sensitivity</td>
<td></td>
<td>GWAS</td>
<td>One SNP (rs4702484) in ADCY2 was associated at a near significant level with capecitabine sensitivity in Caucasian population Four SNPs (rs4702484, rs8101143, rs576523, rs361433) showed a trend of association with capecitabine sensitivity in the total cohort</td>
</tr>
</tbody>
</table>

\(^{a}\)see Supplementary Table 1 for individual SNPs

Abbreviations: CR, complete response; GWAS, genome-wide association study; NA, not applicable; OR, odds ratio; OS, overall survival; PFS, progression-free survival; PR, partial response; RR, response rate; SNP, single nucleotide polymorphism; TTP, time to tumor progression; WHO, World Health Organization
Genetic polymorphisms possibly associated with toxicity from capecitabine

Thymidylate synthase

TYMS SNPs were generally not associated with overall toxicity of capecitabine or specific AEs, including gastrointestinal symptoms, neutropenia and HFS (Table 2). In all capcitabine monotherapy studies (n = 6), a clear association between TYMS variants and capcitabine-related toxicity was not evident.

In 239 patients with different stages of colorectal cancer, TYMS 2R/3R was univariately associated with dose delay/reduction/discontinuation of capecitabine as well as with grade >1 HFS. In the multivariate analysis, carriers of 2R/2R had an increased risk of capecitabine dose delay/reduction/discontinuation (odds ratio [OR] 3.07, p = 0.016), grade >1 HFS (OR 3.78, p < 0.001), and grade >2 HFS (OR 3.63, p = 0.025). In the same study, univariate analysis pointed towards TYMS 3’UTR ins6/del6 of which the percentage of nausea/vomiting grade >2 was higher in del6/del6 carriers, while the percentage of HFS grade >1, HFS grade >2 and that of asthenia grade >2 was higher in ins6/ins6 carriers. In the multivariate analysis, however, TYMS 3’UTR ins6/del6 was not a significant risk factor. In the large QUASAR2 trial of adjuvant capcitabine with or without bevacinumab for colorectal cancer, both TYMS 2R and TYMS 3’UTR ins6 were significantly associated with an increased risk of overall grade ≥3 toxicity (respectively, OR = 1.48, p = 0.000079 and OR = 1.67, p = 0.00084) and grade ≥3 HFS (respectively, OR = 1.44, p = 0.0013 and OR = 1.47, p = 0.021) when combined into a TYMS risk score based on the number of high-risk alleles, TYMS 2R and TYMS 3’UTR ins6 were predictive for overall toxicity (OR = 1.38, p = 0.00031) as well as HFS (OR = 1.31, p = 0.0063). Of interest, a meta-analysis combining current study data with data from other pharmacogenetic studies on capcitabine monotherapy, TYMS 2R or TYMS 3’UTR ins6 remained a significant risk factor for developing overall grade ≥3 toxicity (respectively, OR = 1.36, p = 0.00028 and OR = 1.35, p = 0.012) as well as grade ≥3 HFS (respectively, OR = 1.33, p = 0.0029 and OR = 1.43, p = 0.0091). In a recent report on 1,605 patients treated with fluoropyrimidines (89% capcitabine-based, 11% 5-FU-based), genotype analysis was carried out for TYMS 3RC and 2RC. Patients carrying 3RC/2RC, 2RG/2RC or 2RC/2RC were considered to have higher TYMS enzymatic activity. Indeed, the 20 patients with these genotypes had a higher risk of global severe toxicity (OR = 3.0, p = 0.039), treatment discontinuation (OR 3.6, p = 0.025) and hospitalization for toxicity (OR = 3.8, p = 0.018). In the multivariate analysis, the association remained significant for global severe toxicity (OR = 3.0, p = 0.043) and hospitalization for toxicity (OR 3.8, p = 0.024). Lastly, TYMS -1053C>T was associated with overall grade ≥3 toxicity (p = 0.004), in which a higher rate was observed in the group CT + TT carriers.

In the QUASAR2 trial cohort GWAS has been carried out in search for novel genetic markers which can complement current SNP markers. A total of 1,456 genetic variants in the 5-FU metabolic pathway were determined. Interestingly, an intronic SNP (rs2612091) of
ENOSF1, located downstream of TYMS, was associated with overall grade ≥3 toxicity (OR = 1.59, p = 5.28×10⁻⁶) and grade ≥3 HFS (OR = 1.57, p = 2.94×10⁻⁶). Further analysis was performed to explore the relationship of ENOSF1 rs2612091 and two 5-FU toxicity variants in TYMS (TYMS 2R/3R or TYMS 3’UTR ins6/del6). Interestingly, the G-allele of ENOSF1 rs2612091 alone predicted HFS irrespective of the two TYMS genotypes (p = 0.0021). It thus appears, that ENOSF1 rs2612091 may account for the association between TYMS genetic polymorphisms and capecitabine-induced AEs. Recently, ENOSF1 rs2612091 as a candidate marker of toxicity was confirmed in two studies reporting an association with grade>1 HFS (OR 2.28, p = 0.027) as well as overall grade ≥3 toxicity (p = 0.027). The function of ENOSF1 is not fully characterized, although it has been suggested to regulate TYMS mRNA expression or protein levels.

Methylenetetrahydrofolate reductase

Findings from three relatively small studies have shown an association of MTHFR -677C>T and -1298A>C with capecitabine-related AEs, whereas another six were negative. Sharma et al. reported in 54 advanced colorectal cancer patients on capecitabine monotherapy that patients with the MTHFR -677TT genotype experienced less overall grade 2–3 toxicity (OR = 0.1, p <0.05), while CT and TT individuals experienced less grade 2–3 fatigue (OR = 0.08, p <0.05). Carriers of a T-allele of MTHFR -677C>T tended to have a higher risk of grade 2–3 HFS (OR = 10.8, p = 0.05). Furthermore, the MTHFR -1298 C-allele was associated with more overall grade 2–3 toxicity (OR = 5.6, p <0.01) and grade 2–3 fatigue (OR = 10.8, p <0.05). In another study on 244 patients with different solid tumors receiving a capecitabine-based regimen, the MTHFR-1298 CC genotype indicated a higher risk of grade 2–3 HFS than the AA or AC genotypes (OR = 9.99, p = 4.1 x 10⁻⁶). Zarate et al. investigated both SNPs in 60 colorectal cancer patients treated with capecitabine, irinotecan and oxaliplatin, although HFS was not a specific endpoint. Carriers of the MTHFR -1298AA genotype experienced more AEs, including grade 3–4 neutropenia (p = 0.035), hematological (p = 0.05) and gastrointestinal toxicity (p = 0.023). However, the recent analysis of 927 colorectal cancer patients participating in the QUASAR2 trial could not confirm the predictive value of either MTHFR -677C>T or -1298A>C for overall grade ≥3 toxicity, grade ≥3 diarrhea or grade ≥3 HFS.

Dihydropyrimidine dehydrogenase

The predictive value of DPYD IVS14+1G>A (⁎2A) for capecitabine-related AEs has clearly been assessed in several studies, but most investigators could not report an association possibly due to its low frequency. Of interest, the rare patients with a IVS14+1G>A mutation experienced excessive or even life-threatening toxicity or mutation carriers were not present in the cohort.

Several studies have been done in search for putative DPYD polymorphisms demonstrating novel associations with capecitabine-related AEs. Through sequencing the coding region of DPYD, Deenen et al. identified eight candidate SNPs discriminating between metastatic
colorectal cancer patients experiencing grade ≥3 capecitabine-related toxicities (n = 45) and those without such toxicities (n = 100). These SNPs were validated in the total cohort (n = 568) for their association with diarrhea, HFS and overall toxicity. Five DPYD SNPs (-496A>G, -1236G>A/HapB3, IVS14+1G>A, -2194G>A, -2846A>T) were associated with grade 3–4 diarrhea (p ≤0.04), but their positive predictive values were low to moderate (33–71%). Only the DPYD -496 G-allele indicated the development of grade 2–3 HFS (p = 0.03). Of note, capecitabine dose reduction was more often observed in heterozygous carriers of DPYD IVS14+1G>A (p <0.001) and -2846A>T (p = 0.005). Haploblocks on the basis of six SNPs were formed and the haploblock consisting of five wild-type loci and one SNP heterozygous for -85C>T was associated with a decreased risk of grade 3–4 diarrhea (p <0.05). This finding points in a similar direction to that in another study, in which the DPYD -85 C-allele and the -2846 T-allele was associated with diarrhea (respectively, p = 0.023 and p = 0.028) and the DPYD -85 C-allele was also associated with HFS (p = 0.033). The DPYD -1896 C-allele was associated with stomatitis in that study (p = 0.021). In the QUASAR2 trial, an increased risk of overall grade ≥3 toxicity was found for carriers of the A-allele of DPYD -2846T>A (OR = 9.35, p = 0.0043). In addition, carrying either a DPYD IVS14+1 A-allele or -2846 A-allele was significantly associated with an increased risk of overall grade ≥3 toxicity (OR = 5.51, p = 0.0013). This prompted the same investigators to perform GWAS in the QUASAR2 trial cohort in search for additional genetic markers which can complement current SNP markers. A total of 1,456 genetic variants in the 5-FU metabolic pathway were determined. Several putative SNPs were predictive for capecitabine-related toxicities including the intergenic SNP (rs12132152) located 22 kb downstream of DYPD, which was associated with overall grade ≥3 toxicity (OR = 3.83, p = 4.31×10^-6) and grade ≥3 HFS (OR = 6.12, p = 3.29×10^-8). Another putative intronic SNP in DYPD (rs7548189), occurring at a high frequency (20%), indicated an increased risk of overall grade ≥3 toxicity (OR = 1.23, p = 6.82×10^-6) and grade ≥3 diarrhea (OR = 1.18, p = 1.54×10^-5) for variant carriers.

Capecitabine-activating enzymes
Several case reports have emerged documenting life-threatening toxicities following capecitabine administration to patients with high CDA activity, but normal DPD activity, who were previously treated uneventfully with 5-FU. These findings point towards the importance of the activation cascade of capecitabine involving CES, CDA and TYMP and the occurrence of AEs. Information on CDA SNPs and possible toxicity from capecitabine is most extensive.

The frequently assessed SNPs in CDA are -451C>T (rs532545), -943insC (rs3215400) and -79A>C (rs2072671). The presence of a T-allele of CDA -451C>T indicated a higher risk of grade 3 HFS (OR = 2.02, p = 0.039) in 130 patients with breast or colorectal cancer receiving capecitabine monotherapy. Functional analysis, however, showed no association between CDA -451C>T and mRNA expression, which suggested that another, co-inherited variation in the CDA promoter would be of more importance. CDA -943insC, in linkage disequilibrium with CDA -451C>T, appeared to affect CDA mRNA expression and might better
discriminate the HFS phenotype. Carriers of CDA -943insC had a lower risk of grade 3 HFS (OR = 0.51, p = 0.028). The predictive value of CDA -943insC for HFS could not be replicated in several other studies.\textsuperscript{47,48,58} In 244 patients with different cancer types, Loganayagam et al.\textsuperscript{58} also investigated CDA -451C>T and reported its association with grade 2–4 diarrhea in the first four cycles of capecitabine-based therapy (OR = 2.3, p = 0.0082). In that study, CDA -92A>G was associated with grade 2–4 diarrhea and grade 2–4 dehydration. Regarding CDA -79A>C, no significant association with capecitabine-related toxicities was reported in five studies, whereas in two studies CDA -79A>C was indicative of overall grade≥3 toxicity (OR = 1.84, p = 0.029)\textsuperscript{53} as well as grade≥3 hematological toxicity\textsuperscript{56}. Particularly, in the analysis of 927 colorectal cancer patients in the QUASAR2 study\textsuperscript{54}, CDA -451C>T or -79A>C appeared not to be predictive for capecitabine-related toxicities \textit{i.e.} overall toxicity, HFS and diarrhea. García-González et al.\textsuperscript{53} reported that apart from CDA -79A>C, also ABCB1*1 (rs1128503, rs2032582, rs1045642) was associated with overall toxicity (p <0.001), and calculated a CDA-ABCB1 risk score based on the number of risk alleles (from 0–8). A CDA-ABCB1 score >5 predicted overall toxicity with a sensitivity of 43.5%, a specificity of 76.9% and the positive predictive value was 54.1%.

Five studies on CES2 SNPs have been performed, in which -823C>G has primarily been investigated. Only Martin et al.\textsuperscript{48} described an increased risk of grade ≥3 HFS for carriers of the G-allele of CES2 5'UTR -823C>G (OR = 4.49, p = 0.01) in 99 advanced breast cancer patients on capecitabine monotherapy. In the few studies on SNPs in CES1\textsuperscript{36} as well as in TYMP\textsuperscript{36,54,55} no associations with capecitabine-related AEs were reported.
### Table 2. Genetic polymorphisms possibly associated with toxicity from capecitabine-containing therapy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study</th>
<th>Tumor type</th>
<th>Stage</th>
<th>No of patients</th>
<th>Definition of toxicity</th>
<th>Capesitabine relevant genes</th>
<th>No of relevant SNPs</th>
<th>Main findings with respect to capecitabine relevant genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capecitabine</td>
<td>Largillier et al. 30</td>
<td>Breast</td>
<td>Advanced</td>
<td>105</td>
<td>Overall grade 3–4 toxicity at 1st and 3rd cycle</td>
<td>TYMS; MTHFR; DPYD</td>
<td>6</td>
<td>TYMS 3RG/3RG tended for more overall toxicity at 1st cycle ($p = 0.064$)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Park et al. 40</td>
<td>Colorectal</td>
<td>Metastatic</td>
<td>23</td>
<td>Grade ≥3 toxicity</td>
<td>TYMS</td>
<td>1</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Garcia et al. 67</td>
<td>Cervix</td>
<td>Advanced/recurrent</td>
<td>25</td>
<td>Grade 3–4 anemia, gastrointestinal and dermatological toxicity</td>
<td>TYMS</td>
<td>2</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Sharma et al. 43</td>
<td>Colorectal</td>
<td>Advanced/metastatic</td>
<td>54</td>
<td>Overall grade 2–3 toxicity, grade 2–3 fatigue, grade 2–3 HFS</td>
<td>TYMS; MTHFR</td>
<td>5</td>
<td>MTHFR -677TT was associated with lower incidence of overall toxicity (OR = 0.1, $p &lt; 0.05$) MTHFR -677CT and TT was associated with less fatigue (OR = 0.8, $p &lt; 0.05$), but tended to more HFS (OR = 9.1, $p = 0.05$) MTHFR -1298AC+ CC was associated with higher incidence of overall toxicity (OR = 5.6, $p &lt; 0.01$) and fatigue (OR = 10.8, $p &lt; 0.05$) MTHFR -677TT plus -1298AA was associated with lower incidence of overall toxicity than alternate combinations (OR = 0.09, $p ≤0.05$) Patients with one or two MTHFR CC haplotypes experienced more overall toxicity than those without CC haplotypes of MTHFR -677C&gt;T and -1298A&gt;C (OR = 6.8, $p &lt; 0.01$)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Ribelles et al. 47</td>
<td>Breast &amp; Colon</td>
<td>Metastatic</td>
<td>136</td>
<td>Overall grade 3–4 toxicity</td>
<td>TYMS; DPYD; CES2; CDA</td>
<td>14</td>
<td>No significant association</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Martín et al. 48</td>
<td>Breast</td>
<td>Metastatic</td>
<td>99</td>
<td>Grade ≥3 HFS</td>
<td>TYMS; TYMP; DPYD; CES2; CDA</td>
<td>16</td>
<td>CES2 5’UTR 823 G-allele was associated with increased risk of HFS (OR = 4.49, $p = 0.01$)</td>
</tr>
<tr>
<td>Capecitabine</td>
<td>Caronia et al. 55</td>
<td>Breast and colorectal</td>
<td>Localized/advanced</td>
<td>130</td>
<td>Grade 3 HFS</td>
<td>TYMS; TYMP; DPYD; CES2; CDA</td>
<td>16</td>
<td>CDA -451 T-allele was associated with more HFS (OR = 2.02, $p = 0.039$) CDA -943insC was associated with less HFS (OR = 0.51, $p = 0.028$)</td>
</tr>
</tbody>
</table>
| Capecitabine ± bevacizumab | Rosmarin et al. | Colorectal Localized | 927 | Overall grade ≥3 toxicity, grade ≥3 diarrhea, grade ≥3 HFS | TYMS; MTHFR; DYPD; CES2; CDA; TYMP; UMPs | TYMS 2R was associated with overall toxicity (OR = 1.48, \(p < 0.001\)) and HFS (OR = 1.44, \(p = 0.0013\))
| Capecitabine ± bevacizumab | Rosmarin et al. | Colorectal Localized | 968 | Binary comparison (grade 0–2 vs 3–4); overall toxicity, HFS, diarrhea | TYMS; MTHFR; DYPD; CES1; CES2; CDA; TYMP | TYMS 3’UTR ins6 was associated with overall toxicity (OR = 1.67, \(p < 0.001\)) and HFS (OR = 1.47, \(p = 0.021\))
| Capecitabine + oxaliplatin + bevacizumab + cetuximab | Deenen et al. | Colorectal Metastatic | 568 | Overall grade 3–4 toxicity, grade 3–4 diarrhea, grade 2–3 HFS | DYPD | DYPD -2846 A allele was associated with overall toxicity (OR = 9.35, \(p = 0.0043\))
| | | | | | | The combination of DYPD IVS14+1 A-allele and -2846 A-allele was associated with overall toxicity (OR = 5.51, \(p = 0.0013\))
| | | | | | | DYPD -496 G-allele was associated with HFS (\(p = 0.03\))
| | | | | | | Five DYPD SNPs (-496 G-allele, -1236 A/HapB3-allele, IVS14+1 A-allele, -2194 A-allele, -2846 T-allele) were associated with more diarrhea (\(p \leq 0.04\))
| | | | | | | Haplotype block DYPD (wild-type for 5 SNPs and -85 C allele) was associated with decreased risk of diarrhea (\(p = 0.002\))
| | | | | | | Haplotype block DYPD (wild-type for 5 SNPs and -2194 A-allele) was associated with increased risk of diarrhea (\(p = 0.01\))
| | | | | | | Haplotype block DYPD (wild-type for 4 SNPs, -1601 A-allele and other variant haplotype allele) was associated with increased risk of diarrhea (\(p = 0.01\))
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Study</th>
<th>Tumor type</th>
<th>Stage</th>
<th>No of patients</th>
<th>Definition of toxicity</th>
<th>Capecitabine relevant genes*</th>
<th>No of relevant SNPs</th>
<th>Main findings with respect to capecitabine relevant genes</th>
</tr>
</thead>
</table>
| Capecitabine + docetaxel + oxaliplatin | Deenen et al. 56           | Stomach or gastroesophageal         | Advanced              | 34             | Grade 2–3 gastrointestinal toxicity, grade 3-4 hematological toxicity, overall grade ≥3 toxicity | TYMS; MTHFR; DPYD; CDA; ENOSF1 | 7                   | CDA -79AC+CC associated with hematological toxicity ($p = 0.038$)  
TYMS -1053CT+TT associated with overall toxicity ($p = 0.004$)  
ENOSF1 rs2612091 AA associated with overall toxicity ($p = 0.027$) |
| Capecitabine + Oxaliplatin or capecitabine + epirubicin + cisplatin | Joerger et al. 43          | Colorectal (n = 64) and gastroesophageal (n = 74) | Advanced/metastatic  | 140            | Grade ≥1 HFS, grade ≥1 nausea, grade ≥1 diarrhea, grade ≥1 stomatitis                     | TYMS, MTHFR, DPYD             | 44                  | Colorectal cancer group:  
DPYD -85 C-allele was associated with HFS ($p = 0.033$)  
MTHFR -677 T-allele was associated with nausea ($p = 0.036$)  
Gastroesophageal cancer group:  
DPYD -85 C-allele was associated with diarrhea ($p = 0.023$)  
DPYD -1896 C-allele was associated with stomatitis ($p = 0.021$)  
DPYD -2846 T-allele was associated with diarrhea ($p = 0.028$) |
| Capecitabine + irinotecan         | Carlini et al. 70          | Colorectal                           | Metastatic            | 66             | Grade 3–4 diarrhea or neutropenia during first two cycles                               | TYMS                          | 2                   | No significant association                                                                                               |
| Capecitabine + irinotecan + oxaliplatin | Zarate et al. 59          | Colorectal                           | Metastatic            | 60             | Each individual grade 3–4 toxicity or grouped into hematological, gastrointestinal, other toxicity | TYMS; MTHFR                   | 5                   | MTHFR -1298AA associated with more neutropenia ($p = 0.035$)  
MTHFR -1298AA associated with more hematological toxicity ($p = 0.05$) and with more gastrointestinal toxicity ($p = 0.023$) |
| Capecitabine-based therapy        | Logana-yagam et al. 58     | Different cancer types               | Different stages      | 244            | Grade 3–4 diarrhea, neutropenia, mucositis in the first four cycles of treatment; grade 2–3 HFS | TYMS; MTHFR; DPYD; CDA; DPYS   | 26                  | CDA -92A>G was associated with diarrhea ($p = 0.002$) and dehydration ($p = 0.042$)  
CDA -451C>T was associated with diarrhea (OR = 2.3, $p = 0.0082$)  
MTHFR -1298CC was associated with HFS (OR = 9.99, $p < 0.001$) |

**Table 2.** (continued)
| Capecitabine-based therapy | Meulen-dijks et al. | Different cancer types | Different stages | 1,605 | TYMS | Univariate analysis: TYMS [2RG/2RC, 3RC/2RC, 2RC/2RC] higher risk of global severe toxicity (OR = 3.0, \( p = 0.039 \)), treatment discontinuation (OR = 3.6, \( p = 0.025 \)) and hospitalization for toxicity (OR = 3.8, \( p = 0.018 \)) in patients with risk genotypes [3RC/2RC, 2RG/2RC, and 2RC/2RC] 
Multivariate analysis: TYMS [2RG/2RC, 3RC/2RC, 2RC/2RC] higher risk of global severe toxicity (OR = 3.0, \( p = 0.043 \)) and hospitalization for toxicity (OR = 3.8, \( p = 0.024 \)) in patients with risk genotypes [3RC/2RC, 2RG/2RC, and 2RC/2RC] |
| Capecitabine-based therapy | García-González et al. | Colorectal | Different stages | 239 | MTHFR; CDA; ENOSF1; TYMS | Univariate analysis revealed significant associations for: CDA -79A>C with overall toxicity (\( p = 0.008 \)) TYMS 2R/3R with dose delay/reduction/discontinuation (\( p = 0.034 \)) and grade >1 HFS (\( p = 0.001 \)) TYMS ins6/del6 with nausea/vomiting (\( p = 0.03 \)), grade >1 HFS (\( p = 0.011 \)), grade >2 HFS (\( p = 0.003 \)), asthenia (\( p = 0.02 \)) ENOSF1 rs2612091 grade >1 HFS (\( p = 0.041 \)), asthenia (\( p = 0.02 \)) ABCB1*1 with dose delay/reduction/discontinuation (\( p = 0.002 \)), diarrhea (\( p = 0.018 \)), overall toxicity (\( p < 0.001 \)) 
Multivariate analysis: CDA -79AA associated with overall toxicity (OR = 1.84, \( p = 0.029 \)) TYMS 2R/2R associated with dose delay/reduction/discontinuation (OR = 3.07, \( p = 0.016 \)), grade >2 HFS (OR = 3.78, \( p < 0.001 \)), grade >2 HFS (OR = 3.63, \( p = 0.025 \)) ENOSF1 rs2612091 GG associated with grade >1 HFS (OR = 2.28, \( p = 0.027 \)) ABCB1*1 with dose delay/reduction/discontinuation (OR = 4.49, \( p = 0.006 \)), diarrhea (OR = 3.16, \( p = 0.012 \)), overall toxicity (OR = 4.06, \( p < 0.001 \)) |

*see Supplementary Table 1 for individual SNPs

Abbreviations: GWAS, genome-wide association study; HFS, hand-foot syndrome; NA, not applicable; OR, odds ratio; ORbin, odds ratio from binary comparison; ORcont, odds ratio from continuous comparison
Discussion

In this comprehensive review we summarize findings derived from pharmacogenetic reports on capecitabine. Currently available evidence indicates several genetic variants in 5-FU-metabolizing enzymes *TYMS*, *DYPD*, as well as in capecitabine-activating enzymes *CDA*, *CES2*, having an impact on efficacy or toxicity, although reported associations are somewhat inconsistent. Factors such as patients’ characteristics, population differences in allele frequency, sample size, study design (case-control, randomized trial), definition and assessment of study endpoints, schedule of administration, drug dosing, combination therapy, differ across studies rendering inconclusive results.

In most studies in this review 5-FU-metabolizing genes have been assessed including *TYMS*, *MTHFR* and *DYPD*, of which *TYMS* was the most frequently investigated candidate gene. Although the majority of investigators did not find an association, poor clinical outcome has been reported in patients carrying *TYMS* 3’ 3R/3R, *TYMS* 3’UTR del6/del6, *TYMS* 3’UTR ins6/ins6 as well as a combination of several *TYMS* variants. This is in line with extensive data from 5-FU pharmacogenetic reports, because of which the role of *TYMS* variants as indicator of clinical outcome remains undetermined. Regarding toxicity, a recent large-scale study has pointed towards a potential role for *TYMS* 2R, 3’UTR ins6 or the combination of both SNPs for the prediction of overall toxicity as well as HFS, but these findings warrant further confirmation. Of interest is the finding that *ENOSF1* rs2612091 may reflect the presence of *TYMS* genetic polymorphisms associated with a higher risk of HFS.

The impact of *DYPD*, a major detoxifying enzyme of 5-FU, in the development of severe 5-FU-related toxicity has been well acknowledged. Genotyping of *DPYD* IVS14+1G>A (*2) and other risk variants [-1679T>G (*13) and -2846A>T] is generally accepted to screen individuals at risk of developing severe and potentially life-threatening toxicities from fluoropyrimidine treatment. For patients carrying risk alleles, dose reduction is recommended according to the Clinical Pharmacogenetics Implementation Consortium guideline. For capecitabine, one study has been reported in which the combination of a *DPYD* IVS14+1 A-allele and a *DPYD* -2846 A-allele was associated with overall toxicity. Other investigators have described *DPYD* -85T>C, *DPYD* -1896T>C and *DPYD* -2846A>T to be associated with gastrointestinal toxicity and *DPYD* -85T>C with HFS. Meulendijks et al. have reviewed eight pharmacogenetic studies on *DPYD* variants and toxicity from fluoropyrimidines, in which -1679T>G (*13) and -1236G>A/HapB3, but not -1601G>A (rs1801158), were found to be clinically relevant predictors. Of interest are several putative genetic variants in *DPYD* detected by GWAS as possible markers for capecitabine-induced AEs, although their functional impact on 5-FU metabolism remains to be elucidated.

Of variants in genes encoding enzymes responsible for capecitabine activation (*CDA,
CES and TYMP), CDA -92A>G and 79A>C 48, CES2 -823C>T 47 and TYMP Ser741Leu 48 have shown an association with outcome of patients treated with capecitabine monotherapy. Regarding AEs, SNPs in CDA (-92A>G, -451C>T, -943delC) and in CES2 (-823C>T) have been associated with gastrointestinal toxicity as well as HFS 48,55,58. Although further confirmation is needed, these findings indicate the importance of capecitabine-activating enzymes as putative biomarkers specifically useful for the prediction of capecitabine efficacy and toxicity.

Advancements in array technology have enabled near-genome wide and high-throughput analysis of several hundreds to thousands of genetic variations. The potential of this technology is exemplified by one recent GWAS in which several novel SNPs well as a common variant of DYPD have been selected to be associated with capecitabine AEs 36. Although in most studies on capecitabine a traditional candidate gene approach has been employed, it is expected that the GWAS approach using a SNP array will be increasingly conducted for the identification of novel variants of clinical relevance.

Genetic polymorphisms associated with increased or decreased enzyme activity may likely affect drug pharmacokinetics and, thereby, be useful as biomarkers. However, even carriers of a dysfunctional DYPD variant do not always experience AEs suggesting that the effect of one single genetic variant on enzyme activity may be modest. Haplotype analysis considering multiple functional variants within one gene or in multiple genes has been advocated to provide a more powerful approach to detect a more realistic association than one single genetic variant 63, such as used by Deenen et al. 46. Moreover, given the complexity of drug metabolism involving various steps, assessment of multiple genetic polymorphisms of enzymes in the activation or detoxification pathways may be preferred over a single genetic marker. Lastly, apart from genetic polymorphisms, other mechanisms including miRNA, methylation and copy number variations are able to regulate gene expression inducing changes in enzyme synthesis.

Currently, few clinically valid pharmacogenetic markers are available that may help to individualize initial dosing of capecitabine-based therapy. Of DPD, *2A, -1679T>G(*13), -2846A>T and -1236G>A/HapB3, are convincingly associated with fluoropyrimidine-associated severe AEs 34. Some groups have already incorporated DYPD SNPs into clinical practice to select the initial drug dose 34,35,64. Of interest, a prospective DYPD genotyping study of the aforementioned four SNPs is running in which heterozygous carriers receive reduced starting doses followed by further dose adjustment based on tolerability 65. Individual drug dosing might also be considered on the basis of DPD functional activity measurements prior to treatment 66. Genotype screening technology, however, is within reach at decreasing costs enabling clinicians to have easy access to this life-saving strategy in the near future 35.

In conclusion, pharmacogenetic studies have accumulated valuable data supporting the
use of genetic polymorphisms to differentiate efficacy and toxicity from capecitabine therapy. Evidence points towards particular variants in \textit{DPYD} with respect to toxicity from fluoropyrimidines, because of which upfront screening with use of an extended panel for safety reasons is recommended \cite{14,62}. Further, novel variants in genes encoding enzymes activating the capecitabine-activation pathway as well as several putative SNPs identified by GWAS deserve further research.

Supplementary material is available at http://dx.doi.org/10.1016/j.ctrv.2016.08.001
REFERENCE


A REVIEW ON SNPS AND CAPECITABINE


