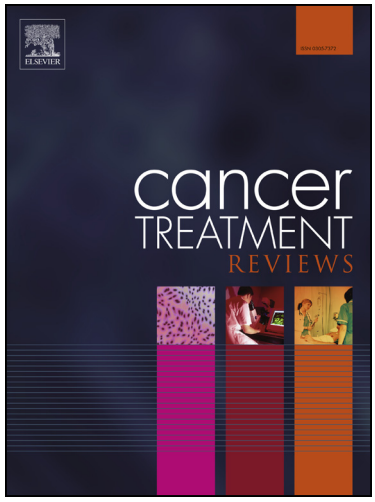

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The role of pharmacogenetics in capecitabine efficacy and toxicity

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The role of pharmacogenetics in capecitabine efficacy and toxicity

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The role of pharmacogenetics in capecitabine efficacy and toxicity

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 influence 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 *DPYP* 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.

Key words: Genetic polymorphisms, capecitabine, toxicity, efficacy

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 [1,2], irinotecan [2], a taxane [3] or cisplatin [1] . In colon cancer, a pooled analysis of randomized trials has shown equivalence in efficacy between infusional 5-FU- and capecitabine-containing regimens [4]. In advanced esophago-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 [5]. 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 [6].

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 [4]. In case of capecitabine, the incidence of stomatitis is significantly lower [4], while that of diarrhea is significantly increased especially when combined with irinotecan [7]. In comparison with intermittent 5-FU, capecitabine is associated with a lower rate of neutropenia, but hand-foot syndrome (HFS) occurs far more frequently [4]. Both drugs are known for a low prevalence of cardiovascular toxicity [8].

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/m^2 twice daily when given as single agent, but dose reductions are frequently required to improve tolerability [2,3]. In breast cancer, a lower starting dose of 1,000 mg/m^2 or dose-adjusting capecitabine during treatment does not seem to compromise efficacy [9]. In combination regimens, initial doses vary between 825 – 1,000 mg/m^2 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 [2,10-12]. The DPD enzyme is required to convert 5-FU to 5-fluorodihydrouracil. Deficient or low DPD activity due to alterations in the *DPYD* gene is estimated to occur in 3-5% of individuals, which may lead to increased toxicity from 5-FU as well as capecitabine [11]. 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) [12]. Concentration-effect analyses have shown a positive relationship between the area under the curve (AUC) of FBAL and treatment-related grade ≥3 diarrhea [13]. 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 [12]. For gender, the clearance of FBAL is less in women [12]. The age-related increase in concentration of FBAL might be explained by a physiological decrease in renal function in the elderly [2,12]. 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 [12]. 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 [2], 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 [2,12].

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 DPD of which the *DPYD**2A variant results in a catalytic inactive form of the enzyme leading to excessive toxicity [14]. 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) [15]. After rapid intestinal absorption, the first step of activation primarily occurs in the liver and involves enzymatic hydrolysis by CES producing 5'-deoxy-5-fluorocytidine (5'-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 [24] 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 [25]. Methylene tetrahydrofolate 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-methylene tetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate, the primary circulating form of folate, which serves as a methyl-group for DNA methylation reactions [26]. 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) [15]. DPD levels vary considerably among individuals with consequences for efficacy and toxicity during 5-FU therapy [11,14]. Low DPD activity results into severe AEs due to accumulation of active 5-FU metabolites [11,14]. DHFU is then converted to fluoro-β-ureidopropionate (FUPA) and subsequently to FBAL by dihydropyrimidinase and β-ureidopropionase, respectively [15]. Excretion of the metabolites occurs by

the kidney [22]. 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 [27-29]. 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* [27,29]. 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 [27,29]. In few studies [30,31], 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 [32]. 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 [27,29].

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* [33]. Reduced enzyme activity may result in enhanced cytotoxicity of fluoropyrimidines [28], but unequivocal evidence is lacking [33]. 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 [33]. 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 [11,14,34]. 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 [14]. 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 [35]. More frequently observed genetic variants are -1627A>G (*5, rs1801159), -2194G>A (*6, rs1801160) and -85T>C (*9A, rs1801265), but their association with DPD activity has been inconsistent [14]. 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 [36].

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 [37,38]. With respect to capecitabine and metabolites, *CES2* -823C>G (rs11075646) was not associated with the AUC of 5-FU [38]. *CDA* SNPs may explain highly variable enzyme activity among individuals [18]. An ultra-metabolizer status was found to be associated with increased efficacy [17] and severe toxicity from capecitabine [18,19]. 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 [42], carriers of a *TYMS* 3'UTR del6 allele had a significantly longer median overall survival (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 Haplotype 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 ($p=5.2 \times 10^{-8}$). 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 *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.

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 capecitabine monotherapy studies (n=6), a clear association between *TYMS* variants and capecitabine-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 [53]. 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 capecitabine with or without bevacizumab 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) [54]. 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 capecitabine monotherapy [30,45,47,55], *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 fluoropryimidines (89% capecitabine-based, 11% 5-FU-based), genotype analysis was carried out for *TYMS* 3RC and 2RC [32]. 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 [56].

In the QUASAR2 trial cohort GWAS has been carried out in search for novel genetic markers which can complement current SNP markers [36]. 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, $p=5.28\times10^{-6}$) and grade ≥3 HFS (OR = 1.57, $p=2.94\times10^{-6}$). 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) [53] as well as overall grade ≥3 toxicity (p=0.027) [56]. The function of *ENOSF1* is not fully characterized, although it has been suggested to regulate *TYMS* mRNA expression or protein levels [57].

Methylenetetrahydrofolate reductase

Findings from three relatively small studies [30,45,58] have shown an association of *MTHFR* -677C>T and -1298A>C with capecitabine-related AEs, whereas another six were negative. Sharma et al. [45] 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, $p=4.1 \times 10^{-6}$) [58]. Zarate et al. [59] 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 [54].

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 [30,43,55,58] or mutation carriers were not present in the cohort [47].

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. [46] 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<0.0001) 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 were 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) [43]. 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) [54]. 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 [36]. 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, $p=4.31\times10^{-6}$) and grade ≥3 HFS (OR = 6.12, $p=3.29\times10^{-8}$). 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, $p=6.82\times10^{-6}$) and grade ≥3 diarrhea (OR = 1.18, $p=1.54\times10^{-5}$) 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 [19,20]. 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 [55]. 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 [47,48,58]. In 244 patients with different cancer types, Loganayagam et al. [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) [53] as well as grade≥3 hematological toxicity [56]. Particularly, in the analysis of 927 colorectal cancer patients in the QUASAR2 study [54], *CDA* −451C>T or -79A>C appeared not to be predictive for capecitabine-related toxicities i.e. overall toxicity, HFS and diarrhea. García-González et al. [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 [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* [36] as well as in *TYMP* [36,54,55] no associations with capecitabine-related AEs were reported.

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 [60].

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* 5' 3R/3R, *TYMS* 3'UTR del6/del6 [41], *TYMS* 3'UTR ins6/ins6 [42] as well as a combination of several *TYMS* variants [31,43]. This is in line with extensive data from 5-FU pharmacogenetic reports [61], 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 [54], 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 [36].

The impact of *DYPD*, a major detoxifying enzyme of 5-FU, in the development of severe 5-FU-related toxicity has been well acknowledged [11]. 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 [14]. 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 [54]. 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 [43]. Meulendijks et al. [62] 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 [36], 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 identified 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 microRNA, 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 *DPYD*, *2A, -1679T>G(*13), -2846A>T and -1236G>A/HapB3, are convincingly associated with fluoropyrimidine-associated severe AEs [34]. Some groups have already incorporated *DPYD* SNPs into clinical practice to select the initial drug dose [34,35,64]. Of interest, a prospective *DPYD* 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 [65].

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 *DPYD* with respect to toxicity from fluoropyrimidines, because of which upfront screening with use of an extended panel for safety reasons is recommended [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.

Conflict of Interest statement

The authors report no conflict of interest.

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Table 1. Genetic polymorphisms possibly associated with efficacy from capecitabine-containing therapy

Treatment	Study	Tumor type	Stage	Number of patients assessed	Efficacy endpoints	Capecitabine relevant genes[a]	Number of relevant SNPs assessed	Main findings with respect
Capecitabine	Largillier et al. [30]	Breast	Advanced	105	PFS	*TYMS; MTHFR; DPYD*	6	*TYMS* 5' genotype class was sequence class 2 > class 3 >
Capecitabine	Park et al. [40]	Colorectal	Metastatic	24	Tumor response (≥50% decrease for at least 6 weeks)	*TYMS*	1	*TYMS* 5' 2R/2R was associat
Capecitabine	Garcia et al. [67]	Cervix	Advanced/recurrent	25	RR	*TYMS*	2	No significant association
Capecitabine	Sharma et al. [45]	Colorectal	Advanced/metastatic	56	RR, OS	*TYMS; MTHFR*	4	No significant association *MTHFR* -677TT tended to ha
Capecitabine	Ribelles et al. [47]	Breast (n=76) and colorectal (n=60)	Metastatic	136	RR, TTP	*TYMS; DPYD; CDA; CES2*	14	*CES2* 5'UTR -823CC was asso shorter TTP (p=0.014) *CES2* 5'UTR -823CC was asso patients with liver metastas *CES2* 5'UTR -823CG plus pre predictive for high RR (OR=3 *CES2* 5'UTR -823CG was pro multivariate analysis (HR=0.
Capecitabine	Martín et al. [48]	Breast	Metastatic	111	PFS, OS	*TYMS; TYMP; DPYD; CES2; CDA*	16	*CDA* -92A>G was associated *CDA* -79A>G was associated (HR=1.55, p=0.032) *TYMP* rs11479 (HR=2.36, p= p=0.034) were associated w
Capecitabine + oxaliplatin	Martinez-Balibrea et al. [68]	Colorectal	Metastatic	47	RR, disease-control rate, PFS	*TYMS*	3	No significant association
Capecitabine + oxaliplatin	Spindler et al. [69]	Colorectal	Metastatic	68	RR, PFS, OS	*TYMS*	1	No significant association
Capecitabine + oxaliplatin	Kim et al. [49]	Colorectal	Metastatic	76	RR, PFS, OS	Cell death related SNPs: *AKT1; BCL2; BID; CASP3; CASP6; CASP7; CASP8; CASP9; CASP10; FAS; FASLG; RIPK1; TNFRSF10B; TP53; PTGS2*	16	*PTGS2* -8473TT was associat p=0.046) and OS (HR=0.16,
Capecitabine + oxaliplatin	Van Huis-Tanja et al. [52]	Colorectal	Metastatic	268	PFS, OS, RR, clinical benefit	*MTRR; MTHFR; ADCY2; SMARCAD1;* intergenic SNPs	13	*ADCY2* wild-type tended to alone group (p=0.018, multi *MTRR* rs1533268 variant te (p=0.054) *MTRR* rs162036 wild-type te (p=0.039)
Capecitabine + oxaliplatin + bevacizumab	Pander et al. [44]	Colorectal	Metastatic	279	PFS	*TYMS; MTHFR*	4	No genotypes or haplotypes PFS Patients with 'beneficial pro and *TYMS* 5'UTR no 3RG alle *TYMS* 5'UTR 3RG allele, whi had 'unfavorable profile' fo
Capecitabine + oxaliplatin + bevacizumab ± cetuximab	Deenen et al. [46]	Colorectal	Metastatic	568	PFS, OS	Sequencing of entire DYPD coding regions and 3' UTR	NA	One haplotype block consis 1236G, -1601G, -1627A, -21 OS (HR=0.57, p=0.03)
Capecitabine + docetaxel + oxaliplatin	Deenen et al. [56]	Stomach or gastro-esophageal	Advanced	34	PFS, OS	*TYMS; MTHFR; DPYD; CDA; ENOSF1*	7	No significant association
Capecitabine + Oxaliplatin or capecitabine + epirubicin + cisplatin	Joerger et al.[43]	Colorectal (n=64) and gastro-esophageal (n=76)	Advanced/metastatic	140	PFS, OS, RR	*TYMS, MTHFR, DPYD*	44	*TYMS* 5'UTR 2R/3RG, 3RC/3 expression genotype) were patient groups (both p<0.01 gastroesophageal cancer gr
Capecitabine + irinotecan	Carlini et al. [70]	Colorectal	Metastatic	66	RR	*TYMS*	3	No significant association
Capecitabine + irinotecan + oxaliplatin	Zarate et al. [59]	Colorectal	Metastatic	60	RR, PFS, OS	*TYMS; MTHFR*	5	No significant association
Capecitabine	LaBonte et al.	Breast	Metastatic	240	RR, clinical benefit, TTP	*TYMS; MTHFR*	4	For capecitabine alone grou

± lapatinib	[31]							3RC/3RC was associated wit
Capecitabine + raltitrexed	Salgado et al. [41]	Colorectal	Metastatic	58	RR (WHO criteria)	*TYMS; DPYD*	3	*TYMS* 5' 3R/3R preferentiall (p<0.01) *TYMS* 3'UTR del6/del6 was (p<0.05)
Capecitabine + paclitaxel	Gao et al. [42]	Stomach	Metastatic	125	RR, PFS, OS	*TYMS; MTHFR; DPYD*	4	*TYMS* 3'UTR ins6/ins6 was a *TYMS* 3'UTR ins6/ins6 was a PFS (HR=2.251; p=0.013) an multivariate analysis
Capecitabine + docetaxel	Dong et al. [50]	Breast	Metastatic	69	RR, PFS, OS	79 SNPs in *CYP450*	79	*CYP1A1* G allele was associa *CYP1A1* G allele was an inde (p=0.004)
NA	O'Donnell et al. [51]	Lymphoblastoid cell lines	NA	503	In vitro sensitivity	GWAS	NA	One SNP (rs4702484) in *AD* significant level with capecit population Four SNPs (rs4702484, rs81 a trend of association with cohort

[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

Table 2. Genetic polymorphisms possibly associated with toxicity from capecitabine-containing therapy

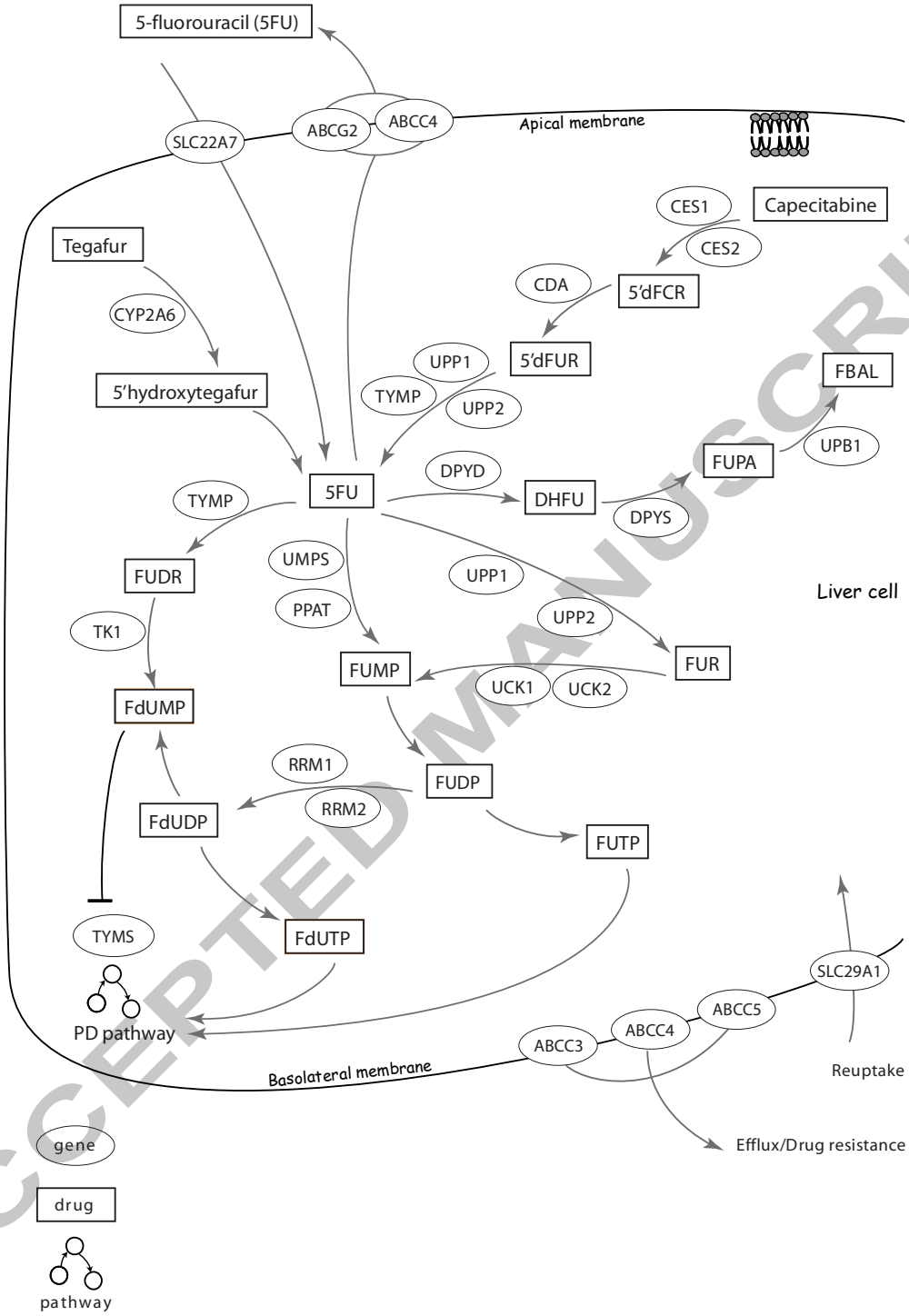
Treatment	Study	Tumor type	Stage	Number of patients assessed	Definition of toxicity	Capecitabine relevant genes[a]	Number of relevant SNPs assessed	Main findings with resp
Capecitabine	Largillier et al. [30]	Breast	Advanced	105	Overall grade 3–4 toxicity at 1st and 3rd cycle	*TYMS; MTHFR; DPYD*	6	*TYMS* 3RG/3RG tended f (p=0.064)
Capecitabine	Park et al. [40]	Colorectal	Metastatic	23	Grade ≥3 toxicity	*TYMS*	1	No significant associatio
Capecitabine	Garcia et al. [67]	Cervix	Advanced/recurren t	25	Grade 3–4 anemia, gastrointestinal and dermatological toxicity	*TYMS*	2	No significant associatio
Capecitabine	Sharma et al. [45]	Colorectal	Advanced/metastat ic	54	Overall grade 2–3 toxicity, grade 2-3 fatigue, grade 2-3 HFS	*TYMS; MTHFR*	5	*MTHFR* -677TT was asso toxicity (OR=0.1, p<0.05) *MTHFR* -677CT and TT w p<0.05), but tended to n *MTHFR* -1298AC+ CC wa overall toxicity (OR=5.6, *MTHFR* -677TT plus -129 of overall toxicity than a Patients with one or two overall toxicity than thos 677C>T and -1298A>C (O
Capecitabine	Ribelles et al. [47]	Breast & Colon	Metastatic	136	Overall grade 3–4 toxicity	*TYMS; DPYD; CES2; CDA*	14	No significant associatio
Capecitabine	Martín et al. [48]	Breast	Metastatic	99	Grade ≥3 HFS	*TYMS; TYMP; DPYD; CES2; CDA*	16	*CES2* 5'UTR 823 G-allele (OR=4.49, p=0.01)
Capecitabine	Caronia et al. [55]	Breast and colorectal	Localized/advanced	130	Grade 3 HFS	*TYMS; TYMP; DPYD; CES2; CDA*	16	*CDA* -451 T-allele was as *CDA* -943insC was associ
Capecitabine ± bevacizumab	Rosmarin et al. [54]	Colorectal	Localized	927	Overall grade ≥3 toxicity, grade ≥3 diarrhea, grade ≥3 HFS	*TYMS; MTHFR; DYPD; CES2; CDA; TYMP; UMPS*	21	*TYMS* 2R was associated and HFS (OR=1.44, p=0.0 *TYMS* 3'UTR ins6 was ass p<0.001) and HFS (OR=1 *TYMS* risk score, a combi was associated with ove (OR=1.31, p=0.0063) *DPYD* -2846 A allele was p=0.0043) The combination of *DPY* associated with overall t
Capecitabine ± bevacizumab	Rosmarin et al. [36]	Colorectal	Localized	968	Binary comparison (grade 0–2 vs 3–4): overall toxicity, HFS, diarrhea Continuous comparison (grade 0–1 vs 2 vs 3–4): overall toxicity, HFS, diarrhea	*TYMS; MTHFR; DPYD; CES1; CES2; CDA; TYMP* *ABCB1; ABCC3; ABCC4; ABCC5; ABCG2; DPYS; PPAT; RRM1; RRM2; SLC22A7; SLC29A1; TK1; UCK1; UCK2; UMPS; UPB1; UPP1; UPP2*	1,456 (GWAS)	*DPYD* rs7548189 was ass and diarrhea (ORcont=1. *DPYD* rs12132152 was a and ORcont=1.61) and H *TYMS/ENOSF1* rs261209 (ORbin=1.59 and ORcont ORcont=1.21) Imputed SNPs: *DPYD* rs76387818 was a and OR_{cont}=1.66) and HF *DPYD* rs12022243 was a and OR_{cont}=1.23) and dia *TYMS/ENOSF1* rs274117 (OR_{bin}=1.60 and OR_{cont}=1
Capecitabine + oxaliplatin + bevacizumab ± cetuximab	Deenen et al.[46]	Colorectal	Metastatic	568	Overall grade 3–4 toxicity, grade 3–4 diarrhea, grade 2–3 HFS	*DYPD*	8	*DPYD* -496 G-allele was a Five *DPYD* SNPs (-496 G- allele, -2194 A-allele, -28 diarrhea (p≤0.04) Haplotype block *DPYD* (v associated with decrease Haplotype block *DPYD* (v associated with increase Haplotype block *DPYD* (v other variant haplotype diarrhea (p=0.01)

Capecitabine + docetaxel + oxaliplatin	Deenen et al. [56]	Stomach or gastro-esophageal	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 associate *TYMS* -1053CT+TT assoc *ENOSF1* rs2612091 AA a
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 as *MTFR* -677 T-allele was a Gastroesophageal cance *DPYD* -85 C-allele was as *DPYD* -1896 C-allele was *DPYD* -2846 T-allele was
Capecitabine + irinotecan	Carlini et al. [70]	Colorectal	Metastatic	66	Grade 3–4 diarrhea or neutropenia during first two cycles	*TYMS*	2	No significant associatio
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 associa *MTHFR* -1298AA associa (p=0.05) and with more
Capecitabine-based therapy	Loganayagam 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 associa dehydration (p=0.042) *CDA* -451C>T was associa *MTHFR* -1298CC was ass
Capecitabine-based therapy	Meulendijks et al. [32]	Different cancer types	Different stages	1,605	During the first cycle: overall grade ≥3 toxicity, grade ≥3 gastrointestinal toxicity, grade ≥3 hematological toxicity, grade ≥3 HFS; treatment discontinuation yes/no; hospitalization for toxicity yes/no	*TYMS*	3	Univariate analysis: *TYMS* (2RG/2RC, 3RC/2R toxicity (OR=3.0, p=0.03 p=0.025) and hospitaliza patients with risk genoty Multivariate analysis: *TYMS* (2RG/2RC, 3RC/2R toxicity (OR=3.0, p=0.04 p=0.024) in patients wit 2RC/2RC)
Capecitabine-based therapy	García-González et al. [53]	Colorectal	Different stages	239	Overall grade ≥3 toxicity, grade >1 HFS, grade >2 HFS, grade >2 diarrhea, grade >2 nausea/vomiting, grade >2 hematological toxicity, grade >2 asthenia; dose delay/reduction/discontinuation yes/no	*MTHFR; CDA; ENOSF1; TYMS*	9	Univariate analysis revea *CDA* -79A>C with overall *TYMS* 2R/3R with dose d and grade >1 HFS (p=0.0 *TYMS* ins6/del6 with nau (p=0.011), grade>2 HFS (*ENOSF1* rs2612091 grad *ABCB1*1* with dose dela diarrhea (p=0.018), over Multivariate analysis: *CDA* -79AA associated w *TYMS* 2R/2R associated (OR=3.07, p=0.016), grad (OR=3.63, p=0.025) *ENOSF1* rs2612091 GG a p=0.027) *ABCB1*1* associated wit (OR=4.49, p=0.006), diar (OR=4.06, p<0.001)

[a]see Supplementary Table 1 for individual SNPs

Abbreviations: GWAS, genome-wide association study; HFS, hand-foot syndrome; NA, not applicable; OR, odds ratio; OR_{bin}, odds ratio from binary comparison; OR_{cont}, odds ratio from continuous comparison

Figure 1. Pharmacokinetic pathway of fluoropyrimidines



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Highlights 'The role of pharmacogenetics in capecitabine efficacy and toxicity'

- Review on pharmacogenetic research to elucidate interpatient variations in capecitabine efficacy or toxicities
- Current research has primarily focused on well-known 5-FU-metabolizing enzymes
- Emerging data are available on genetic variants of capecitabine-activating enzymes displaying novel associations with efficacy or toxicities from capecitabine