A *Carboxylesterase 2* Gene Polymorphism as Predictor of Capecitabine on Response and Time to Progression

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Abstract: Capecitabine is a drug that requires the consecutive action of three enzymes: carboxylesterase 2 (CES 2), cytidine deaminase (CDD), and thymidine phosphorylase (TP) for transformation into 5-fluorouracil (5FU). The metabolism of 5FU requires the activity of thymidylate synthase (TS) and dihydropyrimidine dehydrogenase (DPD) among other enzymes. The present study prospectively examined the possible relationship between the toxicity and efficacy of capecitabine and 14 different polymorphisms in *CES 2*, *CDD*, *TS* and *DPD*. Between 2003 and 2005, a total of 136 patients with advanced breast or colorectal cancer treated with capecitabine were prospectively enrolled. The presence of two polymorphisms (CDD 943insC and CES 2 Exon3 6046 G/A) were associated with a non-statistically significant higher incidence of grade 3 hand-foot syndrome (HFS) (p=0.07) and grade 3-4 diarrhoea (p=0.09), respectively. Patients heterozygous or homozygous for the polymorphism CES 2 5'UTR 823 C/G exhibited a significantly greater response rate to capecitabine, and time to progression of disease (59%, 8.7 months) than patients with the wild type gene sequence (32%, p=0.015; 5.3 months, p=0.014). For the first time, an association between a polymorphism in the *CES2* gene and the efficacy of capecitabine has been described, providing preliminary evidence of its predictive and prognostic value.

Keywords: Carboxylesterase, cytidine deaminase, thymidine phosphorylase, pharmacogenomics, capecitabine, breast cancer, colorectal cancer, polymorphism.

INTRODUCTION

Capecitabine is a prodrug which was designed to produce 5-fluorouracil (5FU) inside tumour cells [1]. The first of the three enzymes that comprise the route of activation for capecitabine is liver carboxylesterase 2 (CES 2), which transforms it into 5-deoxyfluorocytidine. This metabolite is converted into 5-deoxyfluorouridine by cytidine deaminase (CDD), a ubiquitous enzyme which is found in high concentrations in liver, intestine, plasma and tumour tissue. Active 5FU is finally produced by the thymidine phosphorylase (TP) enzyme. In the liver, activity of this enzyme is higher than in other normal tissues [1-3], and in addition levels of this enzyme are 3 to 10 times higher in tumour tissue than in normal tissue. In the cell, 5FU is converted into various metabolites that act by altering RNA synthesis and the level of activity of the thymidylate synthase (TS) enzyme. Up to 85% of the 5FU administered is catabolised in the liver by dihydropyrimidine dehydrogenase (DPD) [4].

The heterogeneity of the efficacy and toxicity of different cytostatic compounds is well known [5]. This variability is due to genetic and non-genetic factors related to the patient (age, sex, main body function status and concomitant medication) and to the nature of the tumour. Genetic factors are responsible for 20-95% of the variability in the pharmacokinetics and pharmacodynamics of a number of drugs [6]. The majority of target or metabolic enzymes of cytotoxic agents contain genetic polymorphisms, and this could account for some of these differences [7].

In the case of 5FU, the two enzymes most commonly studied from the pharmacogenomic point of view are TS and DPD [8]. One of the polymorphisms in the *TS* gene consists of the variable repetition of a 28-bp tandem repeat in the TS enhancer region (TSER) [9]. The number of repetitions varies from 2 to 9 copies, the most frequent being alleles with TSER2 and TSER3. Studies into the relationship of these alleles with cytotoxic drug treatment have yielded conflicting results. *In vitro* studies have related the TSER3 variant to greater TS expression [10], and to higher levels of free TS in patients with cancer [11]. In studies of patients suffering from colorectal carcinoma, the presence of the TSER3/3 genotype in tumour cells has been related to a reduction in the efficacy of treatment with 5FU [9, 12-17] or with capecitabine [18]. Other studies have not confirmed these findings [19-22], and in still other studies, the TSER3/3 genotype [23], or an increase in TS-mRNA levels [24], has been related paradoxically to an increase in the efficacy of 5FU. A similar situation has been found in studies of patients with breast cancer. A significant correlation has been described between the increased expression of the TS protein and a reduction in the efficacy of 5FU [25-28]; however, other studies have conflicting results in patients treated with 5FU [29, 30] or capecitabine [31].

Individuals with a deficiency of the DPD enzyme have been shown to have a higher risk of suffering from severe toxicity with 5FU [32, 33]. A total of 39 mutations has been described in the gene that encodes *DPD* [34], yet cases of severe toxicity have been observed where no mutation could be detected, and, vice versa, where less toxicity has been seen in patients bearing some of these mutations [35]. In addition, some patients who suffer from this type of toxicity have been shown to have normal DPD enzyme activity [36].

From the pharmacogenomic point of view, it is also necessary to consider other enzymes that have been implicated in the capecitabine activation route. No data are available about the possible relationship between genetic polymorphisms in the *CES 2* and *CDD* genes, and the efficacy and toxicity of capecitabine, although they have been implicated in the metabolism and activation of other cytostatic agents [37, 38].

Capecitabine has been proven not only to be effective in the treatment of certain disseminated neoplasias [39], but also in the adjuvant setting [40]. In order to further our understanding into the mechanism of action of this efficacious cytotoxic agent, it is necessary to throw light onto the way in which the genetic variants of its metabolic and target enzymes influence its efficacy and toxicity. The purpose of this pilot study was to investigate prospectively any

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possible correlation between genetic polymorphic variants of *CES 2*, *CDD*, *DPD* and *TS*, and the efficacy and toxicity of capecitabine treatment in patients with metastatic colorectal cancer or breast cancer.

MATERIALS AND METHODS

Study Procedures

Between July 2003 and June 2005, patients with a histological diagnosis of metastatic colorectal or breast cancer who were being treated with capecitabine were prospectively enrolled in five Spanish hospitals. Patients had to be over the age of 18 years, with suitable status performance (ECOG ≤3), conserved bone marrow (neutrophils <1.5 x 10^9/L, platelets <100 x 10^9/L), kidney (serum creatinine <1.5 x ULN) and liver functions (total bilirubin ≤1.5 x ULN; ASAT/ALAT ≤ 2.5 x ULN), and without prior history of dermatological disease. Patients were not excluded if they had had prior cancer treatment in the form of chemotherapy or hormone therapy.

All patients provided informed consent, and the study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Clinical Research Forms were specifically designed to record the study data. Source data verification was performed on 80% of study information.

Patients enrolling in the study received capecitabine at 1250 mg per square meter of body-surface area, twice daily on days 1 through 14 every 21 days, unless toxicity became intolerable or there was progression of the disease. All patients who received at least one cycle of treatment were considered eligible for assessment of toxicity. The toxicity assessment was made before the beginning of each dosing cycle and also at 3 weeks following completion of the treatment. The severity of toxicity events was determined in accordance with the National Cancer Institute Common Toxicity Criteria (NCI CTC) grading system [41]. Only events graded 3-4 were considered for analysis of the association between toxicity and genetic variants, as they were considered the only ones relevant in the clinical setting.

Only those patients who received at least 3 cycles of chemotherapy were considered eligible for assessment of response to treatment. Response was evaluated according to Response Evaluation Criteria in Solid Tumors (RECIST) criteria [42] at the beginning of treatment and then at 3-monthly intervals until completion of the study.

Pharmacogenomic Analyses

Genomic DNA was extracted from 200 µl of whole blood using standard methods. Briefly, after overnight incubation in a solution of 100 ng/ml Proteinase K (Promega, Southampton, UK), 50 mmol/L TrisHCl (pH 7.5) 5 mmol/L $CaCl_2$ at 37°C, DNA was extracted using the phenol/chloroform method, and sequences were amplified using the polymerase chain reaction (PCR). All oligonucleotide primers used for PCR and sequencing are shown in Table **1**.

Polymorphisms were selected for further investigation according to whether they were associated with changes in amino acids or if they were located in regulatory regions of the genes.

CES 2 Genotyping

Two fragments of the *CES 2* gene, designated CES 2 UTR and CES 2 R27Q, were amplified. The CES 2 UTR fragment contains the 5'UTR region of the gene, and the CES 2 R27Q fragment contains exon 3 of the gene. PCRs were carried out under standard conditions: 200 µM dNTPs, 10 mM Tris-HCl (pH 9.0), 50 mm KCl, 1.5 mm $MgCl_2$, and PureTaq Ready-To-Go PCR Beads (Amersham BioSciences, Freiberg, Germany). For amplification of the CES 2 UTR fragment, which comprised 731-1035 nucleotides, CES 2 UTRF and CES 2 UTRR oligonucleotides primers were used at a concentration of 0.4µM. For amplification of the CES 2 R27Q fragment (nucleotides 5624-6554), R27QHF and R27QHR primers were used at 1 µM concentration. Primers were synthesised by Proligo (Paris, France).

Table 1. Oligonucleotide Primers Used in PCR and Sequencing

Oligonucleotide	Sequence
CES 2 UTRF	5′-CATTTCTCCATCTGGGGGAT -3′
CES 2 UTRR	5′- GGGAAAGGTGGGTGTGGTA-3′
R27QHF	5′-GTGCCTAACCATTTGCCCATG-3′
R27QHR	5′-GCAGTTCTGACACTGTTCCAA-3′
R27Q SEQ	5′-ATCCCTGTCCCTGCTACTT-3′
CDD Q27KF	5′-AACTCCACCCTCCAATTGAGATAC -3′
CDD Q27KR	5′- TGCGCCTCTTCCTGTACAC-3′
CDD 1SEQ	5′-AGCTCTCAGGTACGCAGCTTT-3′
CDD SEQ	5′-AAAGCTGCGTACCTGAGAGCT-3′
TSbjcF	5′-GTGGCTCCTGCGTTTCCCCC -3′
TSbjcR	5′-GCTCCGAGCCGGCCACAGGCA -3′

In the case of the CES 2 UTR fragment, the amplification reaction consisted of an initial denaturation step at 95ºC for 4 min followed by 8 cycles of amplification comprising a denaturation step at 94ºC (20 seconds), a second step of 20 seconds which began at 68ºC in the first cycle and was reduced by 0.8ºC in each subsequent cycle, and a final elongation step at 72ºC (20 seconds). This was followed by 28 cycles of 94ºC (20 seconds), 62ºC (20 seconds) and 72ºC (20 seconds). The reaction was completed with a final elongation step at 72ºC for 5 minutes.

In the case of the CES 2 R27Q fragment, the amplification reaction consisted of an initial denaturation at 95ºC for 4 min followed by 35 cycles of amplification of 94ºC (30 seconds), 60ºC (30 seconds) and 72ºC (3 minutes). The reaction was completed with a final elongation step at 72ºC for 5 minutes.

The amplified products (CES 2 UTR, 300 bp and CES 2 R27Q, 929 bp) were sequenced by standard techniques on an automatic ABI 310 sequencer (Applied Biosystems, Foster City, USA), using the CES 2 UTRF oligonucleotide for the CES 2 UTR product, and the R27QSEQ primer for the CES 2 R27Q product. The sequences obtained were analysed for the presence of 6 polymorphisms: 823 C/G and 854 G/C in the CES 2 UTR fragment (CES 2 5'UTR) and 5841 G/A, 6046 G/A, 6174 G/A and 6320 G/A in the CES 2 R27Q fragment (CES 2 Exon3).

CDD Genotyping

A fragment of the *CDD* gene, corresponding to nucleotides 254-1205, was amplified under standard conditions as above using the oligonucleotides CDD Q27KF and CDDQ27KR at 1 µM.

The amplification reaction consisted of an initial denaturation at 95ºC for 4 min followed by 35 cycles of 94ºC (30 seconds), 60ºC (30 seconds) and 72ºC (3 minutes). The reaction was completed with a final elongation step at 72ºC for 5 minutes.

The amplified product was sequenced as above using oligonucleotides CDD 1SEQ and CDD SEQ, to determine the presence of 6 polymorphisms: 575 C/T, 771 C/G, 794 G/A, 942 C/G, 943insC and 1052 A/C.

DPD Genotyping

Screening for the presence of the DPYD *2A mutation was performed as described previously [43].

TS Genotyping

PCR amplification was performed as described previously [44]. The amplified products were analysed using electrophoresis in polyacrylamide gels. The size of the products varies depending on the number of repetitions of 28 bp present in the TSER area. A

single band at 248 bp indicates a TSER3R/3R genotype, a single band at 220 bp a TSER2R/2R genotype and the presence of two bands at 248 and 220 bp, respectively, indicates a TSER2R/3R genotype.

Statistical Analysis

Each of the 14 polymorphisms studied was analysed separately. The purpose of each analysis was to evaluate the association between each polymorphism and toxicity, response to chemotherapy and time to progression. Contingency tables and Fisher's exact test were used to determine the relationship between each categorical variable and each of the 14 polymorphisms. The results for toxicity and response were summarized in groups.

The time to progression was calculated from the beginning of treatment with capecitabine until the first evidence of progression of the disease using the Kaplan-Meier method. The differences in time to progression were analysed using the log-rank test. A multivariate analysis was done for the rate of response and the time to progression using the Cox model.

All the statistical tests were carried out using Statistical Analysis System (SAS), version 9.0 and SAS Enterprise Guide, version 3.0 (SAS Institute, Cary, NC).

RESULTS

Patients and Gene Variants Incidence

One hundred and thirty six patients were enrolled, of whom 76 (56%) were suffering from breast cancer and 60 (44%) from colorectal carcinoma. All patients were Caucasian and their main baseline characteristics are shown in Table **2**.

Polymorphisms were adequately determined in 123 (90%) patients included in the study (Table **3**). In the remaining 13 cases, PCR was not possible because of insufficient blood sample or degradation of the DNA. Mutations in the *DPD* gene were not detected in any of the samples. In another 6 polymorphisms studied in the *CES 2* gene — both CES 2 5'UTR or CES 2 Exon3 — and *CDD* gene, the incidence of genetic variants was rare or absent.

Analysis of Grade 3-4 Toxicity and Correlation with Polymorphisms

All patients were considered evaluable for toxicity. Fifty-three (39%) patients experienced some type of grade 3-4 toxicity (94 episodes). The most frequent severe adverse effects were hand-foot syndrome (HFS) in 24 (18%) of cases, asthenia in 11 (8%), diarrhoea in 9 (7%), mucositis in 5 (4%) and nausea/vomiting in 4 (3%).

Only 2 out of the 14 polymorphisms studied showed a possible association with a greater incidence of grade 3-4 toxicity, but the trend did not reached statistical significance (HFS/CDD 943 insC and diarrhea/CES 2 Exon3 6046 G/A). Of patients who were heterozygous or homozygous for a polymorphism consisting of the insertion of a cytosine in position 943 of the *CDD* gene, 21% experienced an episode of grade 3 HFS compared to 8% of patients with the wild type sequence (p=0.07). There was an association which tended towards significance between the incidence of grade 3-4 diarrhea and polymorphism CES 2 Exon3 6046 G/A (50% of the heterozygous or homozygous population who experienced at least one episode compared to 4% of patients with the wild type sequence (p=0.09).

Analysis of Efficacy and Correlation with Polymorphisms

Response Rate

Response rate could be assessed in 113 patients (83%). The remaining 23 (17%) did not meet RECIST criteria, and were therefore excluded from the efficacy analysis. Forty-one patients (36%, 95% CI: 28-45) experienced an objective response, which was complete in 5 cases (4%, 95% CI: 2-10) and partial in 36 (32%, 95% CI: 24-41). The disease was stabilised in 36 patients (32%, 95% CI: 24-41) and disease progression was seen in 29 (26%, 95% CI: 18-34). Seven patients (6%) were considered not evaluable for response because they did not receive the required 3 treatment cycles (Table **4**). The group of patients with hepatic metastases responded better to capecitabine treatment than those without (50%, 95% CI: 36-63 vs 29%, 95% CI: 18-41; p=0.02).

All genetic variants were analysed, but only the presence of a polymorphism in position 823 C/G of 5'UTR CES 2 showed a statistically significant association with objective response rate (Table **5**). Hence, patients who were either heterozygous or homozygous for this genetic variant responded better to capecitabine treatment than patients with the wild type gene sequence (59%, 95% CI: 40-75 vs 32%, 95% CI: 22-44; p=0.015). The association between CES 2 5'UTR 823 C/G polymorphism and response rate was also ob-

Table 2. Patient Baseline Characteristics

Baseline Characteristics (n=136)	N	%
Age, median (range)	66 (32-90)	
Breast/Colorectal Cancer	76/60	56/44
ECOG		
0-1	113	83
2-3	19	14
Unknown	4	3
Male/Female	35/101	26/74
No. of prior treatments		
0	42	31
1	52	38
2	30	22
≥3	12	9
Hepatic metastases		
Yes	62	46
No	72	53
Unknown	2	1

Table 3. Gene Variants Incidence (n=123)

	WT	HT	HM	SNP Database
	N (%)	N (%)	N (%)	
CES 2 5'UTR				
823 C/G	90 (73)	31 (25)	2 (2)	rs11075646[†]
854 G/C	123 (100)	____	____	rs1200937[†]
CES 2 Exon 3				
5841 G/A	123 (100)	____	____	rs11568312[†]
6046 G/A*	120 (97)	1 (1)	1 (1)	rs8192924[†]
6174 G/A*	122 (99)	____	____	rs10852434[†]
6320 G/A*	120 (97)	2 (2)	____	NR[§]
CDD				
575 C/T	42 (34)	63 (51)	18 (15)	IMS-JST008767[‡]
771 C/G	35 (29)	63 (51)	25 (20)	NR[§]
794 G/A	109 (89)	14 (11)	____	NR[§]
942 C/G*	122 (99)	____	____	rs3215400[†]
943 insC*	37 (30)	68 (55)	17 (14)	rs3215400[†]
1052 A/C*	41 (33)	66 (54)	14 (11)	rs2072671[†]
DPD				
DPYD *2A	122 (99)	____	____	____
	2R/2R	**2R/3R**	**3R/3R**	
	N (%)	**N (%)**	**N (%)**	
TS	**34 (28)**	**61 (50)**	**28 (22)**	____

WT: wild type, HT: heterozygous, HM: homozygous.
*Gene variants could only be determined in 122 or 121 patients.
[†]NCBI SNP database (www.ncbi.nlm.nih.gov/projects/SNP).
[‡]Japanese SNP database (http://snp.ims.u-tokyo.ac.jp).
[§]NR: no registered.

Table 4. Patient Response Rate

Response Rate (n=113)	N	%
Complete Response	5	4
Partial Response	36	32
Stable Disease	36	32
Progression Disease	29	26
Not evaluable	7	6

served in the group of patients with hepatic metastasis (78% of patients heterozygous or homozygous for this mutation, 95% CI: 52-92 compared to 41% of patients with the wild type gene sequence, 95% CI: 26-57; p=0.02) (see Table **5**).

There was no significant association between genotype TS and response rate. Forty-two percent of patients heterozygous or homozygous for the TSER2R allele (95% CI: 31-53) and 32% of patients homozygous for the TSER3R allele (95% CI: 16-53) showed a response to capecitabine treatment (p=0.7).

Time to Progression

After a median follow-up period of 22.6 months (range 0.7-37.8 months), the median time to progression for all patients was 7.2 months. Again, only the presence of the genetic variant CES 2 5'UTR 823 C/G showed a statistically significant association with time to progression (8.7 months compared to 5.3 months for the heterozygous or homozygous patients, and patients with wild type sequence, respectively, p=0.014) (Fig. **1**). This difference was also confirmed in patients with hepatic metastasis (8.7 months and 5.1 months for the heterozygous or homozygous patients, and patients with wild type sequence, respectively, p=0.049) (Fig. **2**).

When a possible influence of genetic variants of *TS* on time to progression was analyzed, the absolute difference observed did not reach statistical significance (patients heterozygous or homozygous for the TSER2R allele, 7.3 months; and patients homozygous for the TSER3R allele, 4.8 months; p=0.13).

Multivariate Analysis

In the multivariate analysis for response rate and time to progression, all variables considered potentially relevant and intrinsic to the patients themselves were included: age (<60 vs ≥ 60 years), sex, ECOG (0-1 vs 2-3), type of neoplasia (colorectal vs breast), hepatic metastasis, and carrier of the genetic variant CES 2 5'UTR 823 C/G.

Among all variables, only the presence of hepatic metastasis (Odds ratio: 3.5, 95% CI=1.4-8.8, p=0.0057) together with being a carrier of polymorphism CES 2 5'UTR 823 C/G (Odds ratio 3.6,

Table 5. Genetic Polymorphisms and Response Rate

	Response	No Response	Total No. of	p
	N (%)	N (%)	Patients	
CES 2 5'UTR 823 C/G (n=98)				
WT	23 (32)	48 (68)	71	0.015
HT + HM	16 (59)	11 (41)	27	
CES 2 5'UTR 823 C/G and hepatic metastasis (n=48)				
WT	14 (41)	20 (59)	34	0.02
HT + HM	11 (78)	3 (22)	14	

WT: wild type, HT: heterozygous, HM: homozygous

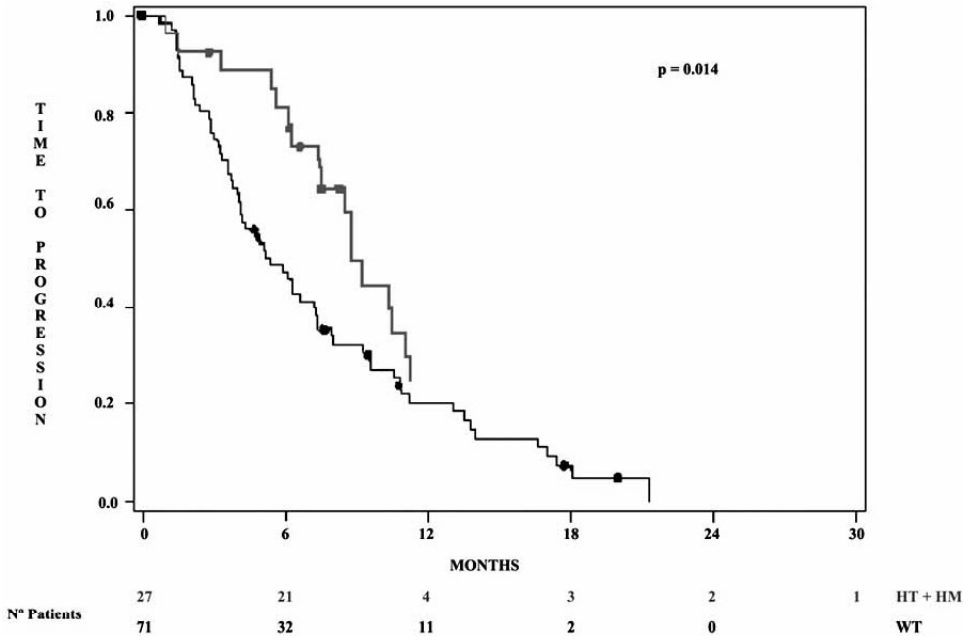

Fig. (1). Time to progression following capecitabine treatment in patients stratified by CES 2 5'UTR 823 C/G genotype.

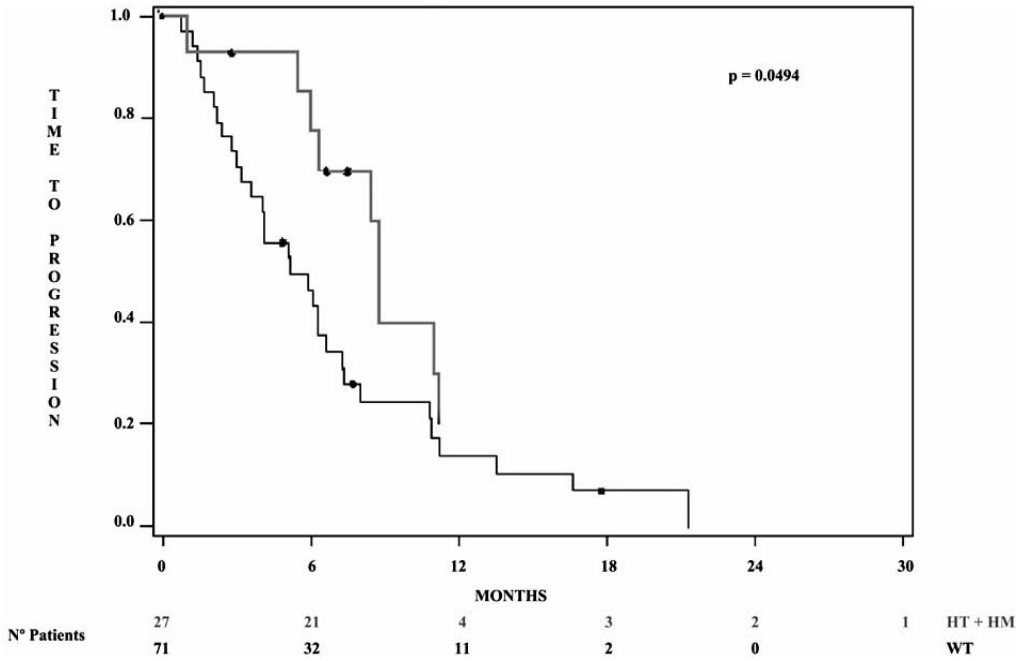

Fig. (2). Time to progression following capecitabine treatment in patients with hepatic metastases stratified by CES 2 5'UTR 823 C/G genotype.

95% CI: 1.3-9.7, p=0.0096) proved to have an independent predictive value on the response to capecitabine treatment.

With regard to time to progression, the only two variables that proved to be independent prognosis factors were ECOG (Hazard ratio 4.4, 95% CI: 2.0-9.6, p=0.0002) and the presence of the CES 2 5'UTR 823 C/G polymorphism (Hazard ratio 0.56, 95% CI: 0.32-0.96, p=0.036).

DISCUSSION

Over the last few years, pharmacogenomics has become one of the basic methods for optimising cancer treatments. The results of investigations carried out in this field not only produce obvious benefits for patients [45], but also have positive repercussions on the cost-effectiveness of these treatments [46].

Capecitabine is an especially attractive drug in which to study the effects of interpersonal variability on its efficacy and toxicity, because each is affected not only by the action of the enzymes involved in the metabolic route of 5FU (i.e. TS, methylenetetrahydrofolate reductase [MTHFR] and DPD), but also by the three enzymes that intervene in the conversion of capecitabine to 5FU (i.e. CES 2, CDD and TP). In contrast to the situation with enzymes responsible for the metabolism of 5FU [8], there is hardly any information about the genetic variability of CES 2 [37, 47] or CDD [38], and no data in the literature which relate their genetic variability with the efficacy and toxicity of capecitabine.

Our aim was to study prospectively any possible correlation between the genetic variants of *CES 2*, *CDD*, *DPD* and *TS* and the toxicity and efficacy of capecitabine treatment. We are now presenting the final results which have been communicated earlier [48].

Park *et al.* [18] conducted a retrospective study to evaluate the relationship between TS genotypes and efficacy of capecitabine in 24 patients with metastatic colorectal cancer. Of these, 75% of TSER2R/2R patients responded to treatment, compared to 8% of TSER2R/3R patients and 25% of the TSER3R/3R group (p=0.036). However, Sharma *et al.* [20] was not able to confirm the predictive value of TS genotypes on response or survival in 56 patients with advanced colorectal cancer treated with capecitabine. These results were similar to those reported by Largillier *et al.* [31] in 57 patients diagnosed with advanced breast cancer. In agreement with this, our data also do not demonstrate an association between TS genotype and response rate. However, TS genotyping in our patients was carried out only in normal tissue, and in heterozygous patients, this genotype could be different from the TS genotype present in the tumour tissue. It is known that there is a high incidence of loss of heterozygosity in the TS locus of tumour tissue [16, 49]. However, patients homozyogus for the TSER2R/2R alleles experienced a longer time to progression than TSER3R/3R patients (7.3 months compared to 4.8 months), although the results were not statistically significant (p=0.13). **Nevertheless, we consider of great importance to point out that a much more sensitive TS 5' polymorphism, i.e. a C/G single nucleotide polymorphism in the second repeat of 3R allele, has been published [50,51]. Unfortunately it was not possible to perform the analysis of this TS 5' polymorphism in our patients, due to the fact that we designed and planned our study before to its publication [50].**

It was not possible to carry out any type of analysis related to the DPD genotype because of the absence of DPYD *2A mutations in our patients. This is probably due to the size of our sample and the extremely low incidence of this mutation [34]. **Moreover, the lack of importance of DPYD *2A mutations in the decrease of DPYD enzyme activity has been published recently. It seems that is DPYD *5A gene mutation which contribute to reduce the enzyme activity [52].**

It is possible that some of the polymorphisms identified in the *CDD* gene could have an effect on the *in vivo* sensitivity of other pyrimidine antagonists such as ara-C [38]. However, there are no data in the literature about the possible influence of the genetic variants of *CDD* on capecitabine activity. In our study, we observed an absolute difference of 13% in the incidence of grade 3 HFS in the group of patients heterozygous and homozygous for the polymorphism CDD 943insC, and its incidence in patients with the wild type sequence. The lack of statistical significance (p=0.07) could be due to the low number of patients in our study and the decision to only consider toxicity grade 3-4 because we considered that only this grade of toxicity was a relevant indicator of toxicity in the clinical setting.

Studies indicate that the existence of genetic variants of *CES 2* could influence the expression of the gene [47] as well as the activity of the enzyme in the conversion of irinotecan into its active metabolite [37], but there is no information available about capecitabine. Our results indicate that at least two polymorphisms present in the *CES 2* gene are related to toxicity and efficacy of capecitabine. With respect to grade 3-4 diarrhoea, despite there being an absolute difference of 46% between patients who were heterozygous or homozygous for the polymorphism CES 2 Exon3 6046 G/A and patients with wild type sequence, this difference did not reached statistical significance. This is probably due, at least in part, to the low frequency of the incidence of this polymorphism (2%).

Most interesting was the association observed between the polymorphism CES 2 5'UTR 823 C/G and the efficacy of capecitabine. The response rate in the carriers of this polymorphism was nearly double that of patients with the wild type sequence of the gene, and this difference led to a longer time to progression of disease (8.7 vs 5.3 months respectively). Curiously, the response rate was higher in the group of patients with hepatic metastases irrespective of whether they were carriers or non-carriers of the polymorphism, than in patients without hepatic metastases. Nevertheless, the response rate was significantly higher in patients with the CES 2 5'UTR 823 C/G polymorphism and hepatic metastasis than in those without the polymorphism. It is possible that the gene with this polymorphism affects the levels of enzyme in tissue, because its location in the 5'UTR region may alter the secondary structures of translational control. On the other hand, it is known that the activity of CES 2 is 10-20 times greater in hepatic tissue, whether normal or cancerous, compared to the tissue of other organs [1]. We postulate that there may be a higher local hepatic concentration of 5FU in patients who are carriers of the CES 2 5'UTR 823 C/G polymorphism, thus simulating an intra-arterial infusion of the drug.

This study is the first to prospectively investigate the correlation of 14 genetic variants in 4 enzymes implicated in the routes of activation of capecitabine. We have demonstrated an association between the existence of a polymorphism in the *CES 2* gene and the activity of capecitabine, both in terms of response rate and time to progression of disease. Moreover, the evidence of this relationship was clearly reinforced by the fact that the presence of the CES 2 5'UTR 823 C/G variant was the only factor with independent value in both multivariate analyses of response and time to progression. Nevertheless, we cannot forget that these results must be viewed as preliminary and need additional confirmation. Studies are now under way on a larger number of patients to confirm these results, and to include the evaluation of the genetic variants of the *MTHFR* gene.

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ABBREVIATIONS

5FU	=	5-Fluorouracil
CES	=	Carboxylesterase
CDD	=	Cytidine deaminase
TP	=	Thymidine phosphorylase
TS	=	Thymidylate synthase
DPD	=	Dihydropyrimidine dehydrogenase
TSER:	=	Thymidylate synthase enhancer region
NCI CTC	=	National Cancer Institute Common Toxicity Criteria
RECIST	=	Response Evaluation Criteria in Solid Tumors
PCR	=	Polymerase chain reactions
HFS	=	Hand-foot syndrome
HT	=	Heterozygote
HM	=	Homozygote
WT	=	Wild type

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