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Association of C677T and A1298C *MTHFR* Polymorphisms and Fluoropyrimidine-induced Toxicity in Mestizo Patients With Metastatic Colorectal Cancer

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Abstract. *Background/Aim: Enzymatic variants involved in fluoropyrimidine metabolism have been associated with adverse events (AEs). We assessed the association between C677T (rs1801133) and A1298 (rs1801131) methylenetetrahydrofolate reductase (MTHFR) polymorphisms and AEs in patients with first-line fluoropyrimidine-based chemotherapy. Patients and Methods: Fifty patients with metastatic colorectal cancer were prospectively followed-up during the first 4 cycles of fluoropyrimidine-based treatment to assess AEs. Germline DNA was analyzed to determine the C677T and A1298C MTHFR polymorphisms. The associations between MTHFR polymorphisms and toxicity were examined. Results: Individuals carrying at least one mutant allele of the MTHFR C677T polymorphism had increased risk to experience anemia (OR=1.69, 95% CI=1.13-2.53, p=0.005), neutropenia (OR=2.27, 95% CI=1.47-3.42, p<0.001) thrombocytopenia (OR=1.91, 95% CI=1.30-2.70, p<0.001), neuropathy (OR=1.77, 95% CI=1.16-2.70, p=0.02), diarrhea (OR=1.69, 95% CI=1.13-2.53, p=0.005), and hand-foot syndrome (OR=1.56, 95% CI=1.08-2.27, p=0.013), compared to patients carrying the wild type alleles. The presence of the mutant allele C of the MTHFR A1298C polymorphism was associated with increased risk of anemia (OR=2.75, 95% CI=1.01-7.48, p=0.02) and thrombocytopenia (OR=3.14, 95% CI=1.01-9.78, p=0.03); however, the prevalence of this allele in the sample was quite low (20%). Conclusion: MTHFR C677T and A1298C polymorphisms predicted toxicity in a subset of Mestizo patients with colorectal adenocarcinoma.*

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Key Words: Metastatic colorectal cancer, methylenetetrahydrofolate reductase, SNPs, fluoropyrimidine-based chemotherapy, side effects, Mestizo population.

Colorectal cancer (CRC) is the second most prevalent neoplastic disorder worldwide, and the second leading cause of cancer-related death in both sexes (1). Although during the last decades new therapies have become available for the treatment of this disease, fluoropyrimidine-based chemotherapy still remains the backbone of treatment for these patients (2). However, adverse drug reactions to fluoropyrimidine-based chemotherapy (*i.e.*, diarrhea, mucositis, vomiting, myelosuppression, neuropathy, and hand-foot syndrome) represent a clinical challenge due to the relatively high frequency of dose reductions or treatment discontinuations among affected patients (3).

Fluoropyrimidines, mainly capecitabine and its active metabolite 5-fluorouracil (5-FU), exert their cytotoxic effects in two different ways (Figure 1). First, 5-FU metabolites are extensively incorporated into RNA and DNA molecules, disrupting their normal functions. In addition, 5-FU inhibits the thymidylate synthase, in a reaction where the 5,10-methylenetetrahydrofolate (MTHF) acts as a methyl donor by forming a stable ternary complex with both the active 5-FU metabolite (5-fluoro-2-deoxyuridine-5-monophosphate), and the target enzyme (4). This inhibition precludes the *de novo* synthesis of thymidylate, which is required for DNA replication and repair. Intracellular concentrations of 5,10-MTHF are highly regulated by the MTHF reductase (MTHFR). This enzyme catalyzes the irreversible conversion of 5,10-MTHF to 5-methyltetrahydrofolate, and reduces the concentration of 5,10-MTHF available for binding to the ternary complex (5).

It has been previously shown that two common single-nucleotide polymorphisms (SNPs), C677T (rs1801133) and A1298C (rs1801131) reduce enzyme activity and lead to an altered distribution of intracellular folates (6). Individuals homozygous for the *MTFHR* C677T variant (TT) produce a

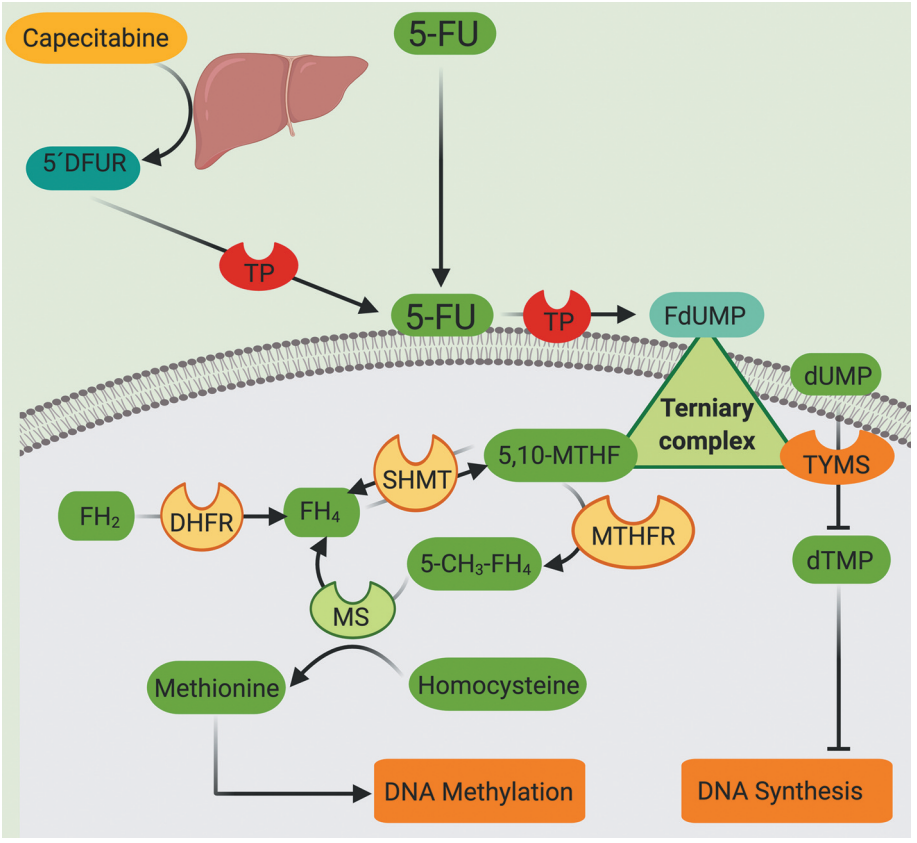

Figure 1. *Simplified overview of Capecitabine and 5-Fluorouracil metabolic pathways and targets. Enzymes: DHFR: dihydrofolate reductasa; MS: methionine synthase; MTHFR: 5,10-methylenetetrahydrofolate reductase; SHMT: serine-hydroxymethyltransferase; TP: thymidine phosphorylase; TYMS: thymidylate synthase. Metabolites: 5-CH3-FH4: 5'-methyl-tetrahydrofolate; 5-FU: 5-fluourouracil; 5,10-MTHF: 5,10-methylenetetrahydrofolate; 5'DFUR: 5'- deoxyfluorouracil;; dTMP: deoxythymidine 5'-monophosphate; dUMP: deoxyuridine 5'-monophosphate; FdUMP: 5-fluoro-2-deoxyuridine-5'- monophosphate; FH2: dihydrofolate; FH4: tetrahydrofolate.*

thermolabile form of the protein with reduced catalytic activity as a result of an Ala-to-Val substitution at codon 222 (6, 7). The C677T variant is associated with a reduction in *MTHFR* activity by 30% in heterozygotes (CT) and 70% in homozygotes (TT) (7). Similarly, the *MTHFR* A1298C polymorphism induces a Glu-to-Ala substitution (Glu429Ala) in a regulatory domain resulting in a mild reduction (30%-40%) in the catalytic activity of the enzyme (8). Heterozygote patients for both SNPs (677CT/1298AC) also share a synergic decrease in the MTHFR activity (9).

Decreased enzyme activity results in an increased intracellular concentration of 5,10-MTHF that could enhance the stable formation of the ternary complex, resulting in increased toxicity of fluoropyrimidines (5). Indeed, clinical data support this hypothesis (10-12), although with conflicting results (13-16). Previous studies have reported that frequencies of mutant alleles of the aforementioned SNPs vary according to ethnic origin (17), with described frequencies for the *MTFHR* C677T mutant allele ranging from 10% (in Asians) to 57% (in Mexicans Mestizos), and for the 1298 CC allele in a range from 14% (in Asian) to 37% (in Caucasians) (18-20). This high variability is considered an important cause of inconsistencies among available data regarding the toxicity of fluoropyrimidine-based chemotherapy and its relationship with *MTHFR* polymorphisms (21). Therefore, in this prospective study we aimed to estimate the effect of the germline *MTHFR* polymorphisms C677T and A1298C on the toxicity induced by fluoropyrimidine-based chemotherapy in patients with metastatic colorectal adenocarcinoma from a Mestizo cohort in Costa Rica.

Patients and Methods

Patients and clinical data. A prospective study was carried out at Hospital San Juan de Dios, San José, Costa Rica (Caja Costarricense de Seguro Social), from January to July 2019. We included patients with histologically confirmed diagnosis of stage IV adenocarcinoma of the colon or rectum, who were treatment

naïve in the metastatic setting. Eligible patients were required to have adequate organ function according to their attending oncologist, life expectancy more than 3 months, and good performance status (ECOG performance status 0-2). Patients received one of the following chemotherapy schemes: i) FOLFOX (5-FU 400 mg/m^2 as a bolus, plus leucovorin 400 mg/m^2 and oxaliplatin 85 mg/m^2 on day one; followed by 5-FU 2400 mg/m^2 as a 46-h continuous infusion every 15 days); ii) CAPEOX (Capecitabine 1000 mg/m^2 twice daily for 14 days and oxaliplatin 130 mg/m^2 every 21 days); iii) FOLFIRI (5-FU 400 mg/m^2 as a bolus, plus leucovorin 400 mg/m^2 and irinotecan 180 mg/m^2 on day 1, followed by 5-FU 2400 mg/m^2 as a 46-h continuous infusion every 15 days).

The assessment of treatment-induced toxicity was monthly performed by the oncologist in charged by determining patient symptoms, physical examination, and haemogram and biochemical tests during the first four cycles of treatment (84 days). Patients were followed up from the start of their treatment until the end of their fourth cycle of cytotoxic chemotherapy. Toxic effects were graded according to the National Cancer Institute – Common Terminology Criteria for Adverse Events version 4 criteria (CTCAE v.4) (19). Toxicity grades were dichotomized as either mild to moderate (grade 0-2), or severe (grade 3 or 4). Hand-foot syndrome was categorized into mild to moderate (grade 0 or 1), or severe (grade 2 or 3).

Ethical approval was obtained from the Institution's Ethics Committee (Comité Ético Científico Central, Caja Costarricense de Seguro Social, protocol No. R017-SABI-00126). All patients signed informed consents before inclusion. (NCT registration number: 03852290).

MTHFR polymorphisms. Venous blood samples were collected into tubes containing 1.7 mM EDTA and stored at -20^{o} C until testing. Germline DNA was extracted automatically using the QIAamp DNA Blood Mini Kit with QIAcube (Qiagen, Valencia, CA, USA). DNA quality was evaluated by Nanodrop 260/280 and 260/230 ratios (Thermo Fisher Scientific®) and concentration was quantified with a Qubit Fluorometer (Life Technologies®) according to the manufacturer's recommendations.

The *MTHFR* C677T and A1298C SNPs were determined by polymerase chain reaction (PCR) – restriction fragment length polymorphism (RFLP) analysis. For the *MTHFR* C677T polymorphism the sequences of primers were 5'-TGAAGGAGAAGGTGTCTGCG GGA-3' and 5'-AGGACGGTGCGGTGAGAGTG-3'. The PCR products were amplicons of 198 bp and were digested with 1U of *Hinf* I for 8 h. For the *MTHFR* A1298C polymorphism, the primer sequences were 5' CTTTGGGGAGCTGAAGGACTACTAC 3' and 5' CACTTTGTGACCATTCCGGTTTG 3'. The PCR products were amplicons of 163 bp and were digested by 1U *Mbo* II. The PCR was performed in thermal cycler (Verity, Applied Biosystem) using 50-100 ng/sample and PCR conditions were an initial preheating step of 95˚C for 3 min, followed by 30 cycles of 95˚C for 1 min, an annealing step at 55˚C for 1 min and an extension step at 72˚C for 1 min. A last step of extension was performed at 72˚C for 7 min. Regarding the C677T SNP, since mutation creates a *Hinf* I restriction site, the amplicon of the wild-type allele (198 bp) is not cut, while the mutant allele is cut into 2 fragments of 175 and 23 bp, respectively. The A1298C mutation abolishes an *Mbo* II restriction site, therefore, the amplicon of the wild-type allele showed fragments of 56, 30, 28 and 19 bp, while the mutant allele only showed three segments of 84, 30 and 19 bp. The digested PCR products were separated on ethidium bromide-stained 3% gels and visualized under ultra-violet light. A positive control was included in all the electrophoresis samples for comparative purposes.

Statistical analysis. Genotype distributions were tested for agreement with those expected under the Hardy-Weinberg equilibrium using chi-squared test. The association between genotypes under a dominant model (TT+CT *vs*. CC for the *MTHFR* C677T and AC+CC *vs*. AA for the *MTHFR* A1298C) and the presence of severe (grade 3 or 4) side effects were performed using the chi-squared or Fisher's exact test when applicable. *t*-Test analysis was used to determine whether age was significantly different between individuals who experienced at least one serious adverse event and those who did not. Univariate logistic regression analysis was performed to calculate the odds ratio (OR) for toxicity and its corresponding 95% confidence interval (95% CI). A *p*-value less than 0.05 was considered statistically significant. Analyses were performed with the SPSS software version 21.0 for Mac (Chicago, IL, USA).

Results

Clinical characteristics. A total of 50 patients were included in this study. Their demographic and clinical characteristics are resumed in Table I. The majority of recruited individuals were female (n=27, 54%) with good performance status at treatment initiation. Most patients had left-sided or rectal primary tumors (n=38, 76%) that were usually removed before therapy (n=32, 64%). Synchronous metastatic disease with liver and/or lung involvement was the most frequent clinical presentation (n=38, 76%). The majority of patients were treated with a chemotherapy combination of 5-FU or capecitabine and oxaliplatin (n=43, 86%).

Genotyping. C677T *MTHFR* genotypic frequencies were distributed as follows: CC (30%), CT (50%), and TT (20%). The C allele frequency was 55%, following the Hardy-Weinberg equilibrium (HWE) (p=0.91). A1298C *MTHFR* genotypic frequencies were: AA (70%), AC (26%), and CC (4%). The A allele frequency was 80%, also following the HWE (p=0.58). No patients were found to be homozygous for both loci. Figure 2 shows representative agarose gel electrophoresis profiles of PCR-RFLP for the *MTHFR* A1298C and C677T polymorphisms, respectively.

Toxicity. Overall, the frequency of severe toxicity was 52% (n=26). Mild or moderate adverse events were recorded in 80% of the patients (n=40). Mean age did not differ between patients who experienced at least one severe adverse event and those who did not (60.8±10.3 *vs*. 57.3±13.2 years, p=0.32). Neuropathy, blood marrow suppression, and diarrhea were the most incident side effects (Table II).

The correlations between *MTHFR* polymorphisms and grades of toxicity are presented in Table II. Significant correlations were found between the presence of at least one mutant allele of the *MTHFR* C677T polymorphism and the occurrence of severe neutropenia (p<0.001), anemia

Table I. *Demographic and clinical characteristics of the studied population (n=50).*

Variable	Frequency (%)
Age* (years)	58.9±12.6
Gender	
Female	27 (54)
Male	23 (46)
Performance status (ECOG)	
0	35 (70)
1	15 (30)
Primary tumour location	
Right colon	12 (24)
Left colon	21 (42)
Rectum	17 (34)
Pathological tumour size stage (T)	
II	1 (2)
III	26 (52)
IV	14 (28)
Unknown	9 (18)
Pathological nodal status (N)	
0	9 (18)
I	26 (52)
II	6 (12)
Unknown	9 (18)
Metastasis	
Metachronous	12 (24)
Synchronous	38 (76)
Site of metastasis	
Liver	30 (60)
Lung	16 (32)
Lymph nodes	13 (26)
Peritoneum	11 (22)
Central nervous system	1 (2)
Primary tumour resected	32 (64)
Chemotherapy scheme	
FOLFOX	30 (60)
CAPEOX	13 (26)
FOLFIRI	7 (14)
Patients experiencing at least one side effect	
Mild to moderate	40 (80)
Severe	26 (52)

CAPEOX: Capecitabine, oxaliplatin; ECOG: Eastern Collaborative Oncology Group; FOLFIRI: 5-fluourouracil, irinotecan, and leucovorin; FOLFOX: 5-fluourouracil, oxaliplatin, and leucovorin. *Data presented as mean±SD.

(p=0.003), thrombocytopenia (p<0.001), neuropathy (p=0.007), fatigue (p=0.007), diarrhea (p=0.009), hand-foot syndrome (p=0.012), nausea (p=0.016), and vomiting (p=0.011). Similarly, we found a statistical significant difference in the occurrence of severe anemia (p=0.031) and thrombocytopenia (p=0.025) among patients carrying at least one C mutant allele of the *MTHFR* A1298C polymorphism in comparison with wild-type individuals.

Table III shows the univariate regression analysis for toxicity in a dominant model for each SNP (TT+CT *vs*. CC and CC+AC *vs*. AA, for the 677 and 1298 *MTHFR* genotypes, respectively). Patients with at least one mutant allele for the *MTHFR* C677T genotype were more likely to experience hematological toxicity [neutropenia (OR=2.27, 95% CI=1.47-3.42, p<0.001), anemia (p=0.005), and thrombocytopenia (OR=1.91, 95% CI=1.30-2.70, p<0.001)], neuropathy (OR=1.77, 95% CI=1.16-2.70, p=0.02), diarrhea (OR=1.69, 95% CI=1.13-2.53, p=0.005), hand-foot syndrome (OR=1.56, 95% CI=1.08-2.27, p=0.013), nausea (OR=1.55, 95% CI=1.15-2.10, p=0.021), and vomiting (OR=1.48, 95% CI=1.11-1.99, p=0.041). Similarly, the presence of the mutant allele C of the *MTHFR* A1298C polymorphism was significantly associated with anemia (OR=2.75, 95% CI=1.01-7.48, p=0.02) and thrombocytopenia (OR=3.14, 95% CI=1.01-9.78, p=0.03). However, the prevalence of the mutant allele C of the *MTHFR* A1298C polymorphism was quite low in the studied population (20%).

Discussion

Our findings support the association between *MTHFR* C677T (rs1801133) and A1298C (rs1801131) SNPs and fluoropyrimidine-related toxicity in a cohort of Costa Rican Mestizo patients with metastatic colorectal cancer. Specifically, patients with at least one mutated allele were more likely to experience severe toxicity than those with wild-type alleles. Although these findings have been described previously with contradictory results, few reports have evaluated this association in Latin-American populations. Indeed, our findings are novel to show the prevalence and influence of these SNPs on 5-FU-related toxicity in a Mestizo population.

The association between toxicity and *MTHFR* polymorphisms is controversial. In line with our results, Chua and colleagues reported higher gastrointestinal toxicity of 5-FU based chemotherapy in TT homozygous patients with metastatic colorectal cancer in comparison with heterozygous and wild-type subjects of the *MTHFR* C677T polymorphism (23). Similarly, Derwinger *et al*., reported that colorectal cancer patients carrying one mutated allele of the aforementioned SNP had more dose reductions or treatment modifications than those carrying the *MTHFR* 677 CC genotype, as a consequence of side effects and poor tolerability to 5-FU based chemotherapy (24).

Although other authors have also described analogous results to our findings (11, 25), some researchers have published no association between *MTHFR* SNPs and toxicity (13-16). It is important to highlight that the majority of studies that did not report any association between toxicity and SNPs had a lower percentage of patients with grade 3 to 4 toxicity than those studies in which this correlation was found (15 *vs*. 50%). From a pharmacological point of view, patients carrying the mutated forms of the *MTHFR* C677T and A1298C SNPs have a decreased enzyme activity resulting in higher inhibition of the thymidylate synthase by fluoropyrimidines, which could explain the increase of side effects (4-6).

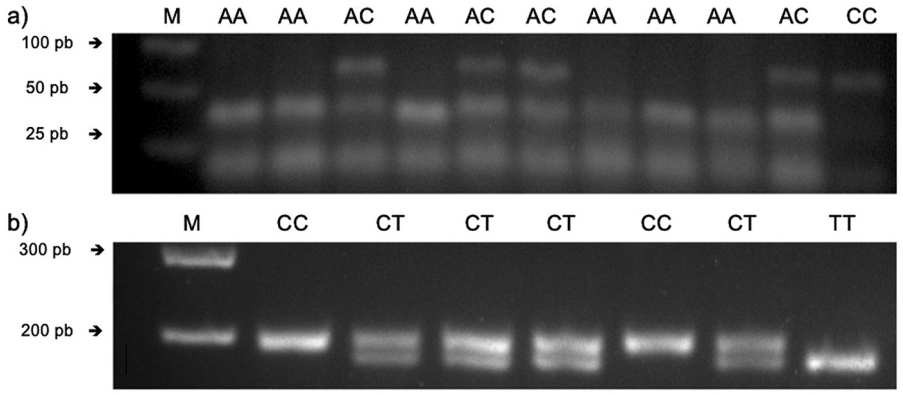

Figure 2. *Representative electrophoretic profiles of PCR-restriction fragment length polymorphism (RFLP) for 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms. Each column represents an individual patient; the last three columns represent controls (wild type homozygous, heterozygous, and mutant homozygous, respectively). (a) MTHFR A1298C and (b) MTHFR C677T. M: DNA ladder marker.*

Table II. *Frequency of mild to moderate (n=40) and severe (n=26) adverse events according to 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms.*

Toxicity	Total (n)	*MTHFR* C677T (%)			*p*-Value*	*MTHFR* A1298C (%)			*p*-Value*
		CC	CT	TT		AA	AC	CC	
Neutropenia									
Mild to moderate	27	55.6	3.7	40.7	0.430	33.3	7.4	59.3	0.35
Severe	23	0	39.1	60.9	**<0.001**	17.4	0	82.6	0.14
Anemia									
Mild to moderate	25	48.0	4.0	48.0	0.760	40.0	4.0	56.0	0.470
Severe	25	12.0	36.0	52.0	**0.003**	12.0	4.0	81.0	**0.031**
Thrombocytopenia									
Mild to moderate	28	50.0	3.6	46.4	0.770	35.7	7.1	57.1	0.390
Severe	22	4.5	40.9	54.5	**<0.001**	13.6	0	86.4	**0.025**
Neuropathy									
Mild to moderate	24	50.0	8.3	41.7	0.630	66.7	8.3	25.0	0.41
Severe	26	11.5	30.8	57.7	**0.007**	73.1	0	26.9	0.32
Fatigue									
Mild to moderate	35	37.1	8.6	54.3	0.390	65.7	5.7	28.6	0.30
Severe	15	13.3	46.7	40.0	**0.007**	80.0	0	20.0	0.48
Diarrhea									
Mild to moderate	25	48.0	8.0	44.0	0.670	60.0	8.0	32.0	0.24
Severe	25	12.0	32.0	56.0	**0.009**	80.0	0	20.0	0.18
Mucositis									
Mild to moderate	29	37.9	13.8	48.3	0.31	65.5	3.4	31.0	0.36
Severe	21	19.0	28.6	52.4	0.24	76.2	4.8	19.0	0.63
Hand-foot syndrome									
Mild to moderate	27	44.4	7.4	48.1	0.670	63.0	7.4	29.6	0.29
Severe	23	13.0	34.8	52.2	**0.012**	78.3	0	21.7	0.30
Nausea									
Mild to moderate	35	40.0	11.4	48.6	0.610	62.9	5.7	31.4	0.42
Severe	15	6.7	40.0	53.2	**0.016**	86.7	0	13.3	0.22
Vomiting									
Mild to moderate	37	37.8	10.8	51.4	0.550	62.2	5.4	32.4	0.39
Severe	13	7.7	46.2	46.2	**0.011**	92.3	0	7.7	0.08
Weight loss									
Mild to moderate	40	32.5	20.0	47.5	0.48	65.0	5.0	30.0	0.37
Severe	10	20.0	20.0	60.0	0.72	90.0	0	10.0	0.29

**p*-Values were calculated for the comparisons between patients carrying the wild-type alleles (*MTHFR* 677 CC or *MTHFR* 1298 AA) and those carrying at least one mutant allele. Bold values indicate statistical significance.

Table III. *Univariate analysis of mild/moderate or severe adverse events according to the presence of 5,10-methylenetetrahydrofolate reductase (MTHFR) polymorphisms.*

Toxicity	*MTHFR* C677T CT/TT *vs.* CC	*p*-Value	*MTHFR* A1298C AC/CC *vs.* AA	*p*-Value
	Odds ratio (95% CI)		Odds ratio (95% CI)	
Neutropenia	2.27 (1.47-3.42)	**<0.001**	2.34 (0.86-6.87)	0.12
Anemia	1.69 (1.13-2.53)	**0.005**	2.75 (1.01-7.48)	**0.02**
Thrombocytopenia	1.91 (1.30-2.70)	**<0.001**	3.14 (1.01-9.78)	**0.03**
Neuropathy	1.77 (1.16-2.70)	**0.02**	1.24 (0.53-2.89)	0.63
Fatigue	1.38 (0.98-1.92)	0.09	1.72 (0.56-5.20)	0.50
Diarrhea	1.69 (1.13-2.53)	**0.005**	2.00 (0.80-5.01)	0.12
Mucositis	1.30 (0.91-1.85)	0.14	1.45 (0.48-3.62)	0.61
Hand-foot syndrome	1.56 (1.08-2.27)	**0.013**	1.70 (0.68-4.27)	0.24
Nausea	1.55 (1.15-2.10)	**0.021**	2.78 (0.71-10.86)	0.17
Vomiting	1.48 (1.11-1.99)	**0.041**	4.92 (0.73-33.81)	0.08
Weight loss	1.18 (0.81-1.72)	0.70	3.50 (0.52-23.56)	0.24

Bold values indicate statistical significance.

The inconsistency among our data and other studies can also rely in several variables such as age, sample size, the chemotherapy scheme, individual folate status, and ethnic origin (21). Among these factors, ethnic background could be considered of paramount importance, since the prevalence of mutant alleles has been shown to vary greatly between different ethnic populations. Specifically, previous studies have reported that the frequency of the *MTFHR* C677T mutant allele ranged from 10% in Asian populations to 57% in Mexicans Mestizos, and that of the 1298 CC allele ranged from 14% (in Asian) to 37% (in Caucasians) (17-20).

Indeed, previous studies have revealed an association between the *MTHFR* SNPs and clinical outcomes. Martin and colleagues have demonstrated that these variant alleles have different effects on breast cancer prognosis depending on the studied population (26). In addition, data from Schwab and colleagues, which included patients with distinct types and stages of cancer, as well as different dosing regimens, showed that *MTHFR* polymorphisms played a limited role for 5-FU related toxicity (13). Similarly, Ruzzo and colleagues reported a lack of association between the presence of mutant alleles of the aforementioned SNPs and chemotherapy-induced toxicity with either FOLFOX or FOLFIRI, in advanced colorectal cancer patients (14, 15). However, it is worth noting that the incidence of severe toxicity was considerably lower in these studies in comparison with our results (15-23% *vs.* 50%). This could be due to the different populations that were analyzed (ethnicity, sample size). In line with our results, those studies showing a relationship between the *MTHFR* SNPs and fluoropyrimidine-related toxicity share a similar percentage of blood marrow suppression, diarrhea, and neuropathy (11, 23-25).

Combined analysis of both SNPs in our cohort did not show any patient with both mutant variants, suggesting linkage disequilibrium between C677T and A1298C variants, as previously described by other authors (27). However, the small sample size of our study could disregard the association between both SNPs.

Although some authors have acknowledged that in colorectal cancer the influence of *MTHFR* polymorphisms on toxicity can be blurred by the effect of oxaliplatin or irinotecan (28), our findings, as well as previous data, consistently described the association between the *MTHFR* 677 CT/TT genotype and increased toxicity in patients treated with either FOLFOX or FOLFIRI (29).

Regarding the association between the A1298C SNP and toxicity, the results of previous studies are also controversial. Although some authors have shown that individuals with advanced colorectal cancer and presence of the *MTHFR* 1298 CC genotype have significantly higher fluoropyrimidine-related toxicity compared to those with *MTHFR* 1298 AA genotype (14, 30), other authors have not shown any significant relationship between the occurrence of adverse events and this particular SNP (10, 13, 15-16). In our study, only a significant association between this SNP and anemia and thrombocytopenia was detected; however, we cannot claim this result as confirmatory due to the low prevalence of the mutant allele C in our cohort.

The mutant allelic frequency of the *MTHFR* C677T polymorphism in this study was slightly higher than that reported for Caucasian populations, and very similar to that described for Mestizos from Mexico (18). Similarly, the mutant allele frequency of the *MTHFR* A1298C polymorphism was very similar to that reported for Latin American populations, and lower than that described for patients of Caucasian origin (17, 18). These differences support the hypothesis of different toxicities and outcomes in patients treated with fluoropyrimidines, as a consequence of genetic variants of each studied population.

Our study has some caveats such as the relatively small sample size and unicenter design. Nevertheless, our population was in Hardy-Weinberg equilibrium, and we were able de detect a high number of individuals with the variant allele of the *MTHFR* C677T polymorphism. Although we did not test for other SNPs in fluoropyrimidines-metabolizing enzymes such as dihydropyrimidine dehydrogenase, and thymidylate synthase, we consider that our results provide a first approach to identify potential genetic markers of fluoropyrimidine toxicity in our population.

In summary, our results suggest that *MTHFR* C677T SNP may predict toxicity in Costa Rican patients with metastatic colorectal cancer and treated with fluoropyrimidine-based chemotherapy. These findings could help clinicians to foresee severe adverse events related to the use of this cytotoxic therapy among patients with metastatic colorectal cancer. Besides, our results provide a valuable tool in clinical practice to identify patients at risk, who require a closed follow-up in order to anticipate potential life-threatening toxicities. Further larger studies are warranted to validate these clinical findings.

Conflicts of Interest

None to declare.

Authors' Contributions

Allan Ramos-Esquivel: Conceptualization, methodology, data acquisition, statistical analyses, interpretation of results, writing, and editing. Ricardo Chinchilla: data acquisition, reviewing, and editing. Marta Valle: supervision, conceptualization, methodology, reviewing, and editing.

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Ethical Disclosure

The Authors have obtained appropriate institutional review board approval for all human experimental investigations (IRB Approval Number: R017-SABI-00126). An informed consent was obtained from the participants involved.

References

1 GLOBOCAN 2018. Lyon, France, 2018. World Health Organization. International Agency for Research on Cancer. Available at: https://gco.iarc.fr/today/data/factsheets/cancers/10_8_9-Colorectum-fact-sheet.pdf [Last accessed January 16 2020]

2 Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, Aranda E, Bardelli A, Benson A, Bodoky G, Ciardello F, D'Hoore A, Díaz-Rubio E, Douillard JY, Ducreux M, Falcone A, Grothey A, Gruenberger T, Haustermans K, Heinemann V, Hoff P, Köhne CH, Labianca R, Laurent-Puig P, Ma B; Maughan T, Muro K, Normanno N, Österlund P, Oyen WJG, Papamichael D, Pentheroudakis G, Pfeiffer P, Price TJ, Punt C, Ricke J, Roth A, Salazar R, Scheithauer W, Schmoll HJ, Tabernero J, Taïeb J, Tejpar S, Wasan H, Yoshino T, Zaanan A and Arnold D: ESMO Consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol *27(8)*: 1386-1422, 2016. PMID: 27380959. DOI: 10.1093/annonc/mdw235

3 Meta-Analysis Group In Cancer; Lévy E, Piedbois P, Buyse M, Pignon JP, Rougier P, Ryan L, Hansen R, Zee B, Weinerman B, Pater J, Leichman C, Macdonald J, Benedetti J, Lokich J, Fryer J, Brufman G, Isacson R, Laplanche A, Quinaux E and Thirion P: Toxicity of fluorouracil in patients with advanced colorectal cancer: effect of administration schedule and prognostic factors. J Clin Oncol *16(11)*: 3537-3541, 1998. PMID: 9817272. DOI: 10.1200/JCO.1998.16.11.3537

4 Vodenkova S, Buchler T, Cervena K, Veskrnova V, Vodicka P and Vymetalkova V: 5-Fluorouracil and other fluoropyrimidines in colorectal cancer: Past, present and future. Pharmacol Ther *206*: 107447, 2020. PMID: 31756363. DOI: 10.1016/j.pharmthera.2019.107447

5 Longley DB, Harkin P and Johnston PG: 5-Fluorouracil: mechanism of action and clinical strategies. Nat Rev Cancer *3(5)*: 330-338, 2003. PMID: 12724731. DOI: 10.1038/nrc1074

6 Rosenblatt DS: Methylenetetrahydrofolate reductase. Clin Invest Med *24(1)*: 56-59, 2001. PMID: 11266034.

7 Bagley PJ and Selhub J: A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci *95(22)*: 13217-13220, 1998. PMID: 9789068. DOI: 10.1073/pnas.95.22.13217

8 Weisberg I, Tran P, Christensen B, Sibani S and Rozen R: A second genetic polymorphism in methylenetetrahydrofolate reductase (*MTHFR*) associated with decreased enzyme activity. Mol Genet Metab *64(3)*: 169-172, 1998. PMID: 9719624. DOI: 10.1006/mgme.1998.2714

9 Martin YN, Salavaggione OE, Eckloff BW, Wieben ED, Schaid DJ and Weinshilboum RM: Human methylenetetrahydrofolate reductase pharmacogenomics: gene resequencing and functional genomics. Pharmacogenet Genomics *16(4)*: 265-277, 2006. PMID: 16538173. DOI: 10.1097/01.fpc.0000194423.20393.08

10 Afzal S, Jensen SA, Vainer B, Vogel U, Matsen JP, Sørensen JB, Andersen PK and Poulsen HE: *MTHFR* polymorphisms and 5-FU-based adjuvant chemotherapy in colorectal cancer. Ann Oncol *20(10)*: 1660-1666, 2009. PMID: 19465420. DOI: 10.1093/annonc/mdp046

11 Sharma R, Hoskins JM, Rivory LP, Zucknick M, London R, Liddle C and Clarke S: Thymidylate synthase and methylenetetrahydrofolate reductase gene polymorphisms and toxicity to capecitabine in advanced colorectal cancer patients. Clin Cancer Res *14(3)*: 817-825, 2008. PMID: 18245544. DOI: 10.1158/1078-0432.CCR-07-0425

12 Rosmarin D, Palles C, Church D, Domingo E, Jones A, Johnstone E, Wang H, Love S, Julier P, Scudder C, Nicholson G, Gonzalez-Neira A, Martin M, Sargent D, Green E, McLeod H, Zanger UM, Schwab M, Braun M, Seymour M, Thompson L, Lacas B, Boige V, Ribelles N, Afzal S, Enghusen H, Jensen SA, Etienne-Grimaldi M, Milano G, Wadelius M, Glimelius B,

Garmo H, Gusella M, Lecomte T, Laurent-Puig T, Martinez-Balibrea E, Sharma R, Garcia-Foncillas J, Kleibl Z, Morel A, Pignon J, Midgley R, Kerr D and Tomlinson I: Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol *32(10)*: 1031-1039, 2014. PMID: 24590654. DOI: 10.1200/JCO.2013.51.1857

13 Schwab M, Zanger UM, Marx C, Schaeffeler E, Klein K, Dippon J, Kerb R, Blievernicht J, Fischer J, Hofmann U, Bokemeyer C, Eichelbaum M and German 5-FU Toxicity Study Group: Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. J Clin Oncol *26(13)*: 2131-2138, 2008. PMID: 18299612. DOI: 10.1200/JCO.2006.10.4182

14 Capitain O, Boisdron-Celle M, Poirier AL, Abadie-Lacourtoisie S, Morel A and Gamelin E: The influence of fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer. Pharmacogenomics J *8(4)*: 256-267, 2008. PMID: 17700593. DOI: 10.1038/sj.tpj.6500476

15 Ruzzo A, Graziano F, Loupakis F, Rulli E, Canestrari E, Santini D Catalano V, Ficarelli R, Maltese P, Bisonni R, Masi G, Schiavon G, Giordani P, Giustini L, Falcone A, Tonini G, Silva R, Mattioli R, Floriani I and Magnani M: Pharmacogenomic profiling in patients with advanced colorectal cancer treated with first-line FOLFOX-4 chemotherapy. J Clin Oncol *25(10)*: 1247-1254, 2007. PMID: 17401013. DOI: 10.1200/JCO.2006.08.1844

16 Ruzzo A, Graziano F, Loupakis F, Santini D, Catalano V, Bisonni R, Ficarelli R, Fontana A, Andreoni F, Falcone A, Canestrari E, Tonini G, Mari D, Lippe P, Pizzagalli F, Schiavon G, Alessandroni P, Giustini L, Maltese P, Testa E, Menichetti ET and Magnani M: Pharmacogenetic profiling in patients with advanced colorectal cancer treated with first-line FOLFIRI chemotherapy. Pharmacogenomics J *8(4)*: 278-288, 2008. PMID: 17549067. DOI: 10.1038/sj.tpj.6500463

17 Wilcken B, Bamforth F, Li Z, Zhu H, Ritvanen A, Redlund M, Stoll C, Alembik Y, Dott B, Czeizel AE, Gelman-Kohan Z, Scarano G, Bianca S, Ettore G, Tenconi R, Bellato S, Scala I, Mutchinick OM, López MA, de Walle H, Hofstra R, Joutchenko L, Kavteladze L, Bermejo E, Martínez-Frías ML, Gallagher M, Erickson JD, Vollset S, Mastroiacovo P, Andria G and Botto LD: Geographical and ethnic variation of the 677C>T allele of 5,10 methylenetetrahydrofolate reductase (*MTHFR*): findings from over 7000 newborns from 16 areas world wide. J Med Genet *40(8)*: 619-625, 2003. PMID: 12920077. DOI: 10.1136/jmg.40.8.619

18 López-Cortés A, Paz-y-Niño C, Guerrero S, Jaramillo-Koupermann G, León A, Intriago-Baldeón DP, García-Cárdenas JM, Guevara-Ramírez P, Armendáriz-Castillo I, Leone PE, Quiñones LA, Cayún JP and Soria NW: Pharmacogenomics, biomarker network, and allele frequencies in colorectal cancer. Pharmacogenomics J *20(1)*: 136-158, 2020. PMID: 31616044. DOI: 10.1038/s41397-019-0102-4

19 Lin KM, Yang MD, Tsai CW, Chang WS, Hsiao CL, Jeng LB, Yueh TC, Lee MC and Bau DT: The role of MTHFR genotype in colorectal cancer susceptibility in Taiwan. Anticancer Res *38(4)*: 2001-2006, 2018. PMID: 29599316. DOI: 10.21873/anticanres.12438

20 Gallegos-Arreola MP, Garcí-Ortiz JE, Figuera LE, Puebla-Pérez AM, Morgan-Villela G and Zúñiga-González GM: Association of the 677C —> polymorphism in the MTHFR gene with colorectal cancer in Mexican patients. Cancer Genomic Proteomics *6(3)*: 183-188, 2009. PMID: 19487547. DOI:

21 De Mattia E and Toffoli G: C677T and A1298C *MTHFR* polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy personalisation. Eur J Cancer *45(8)*: 1333-1351, 2009. PMID: 19144510. DOI: 10.1016/j.ejca.2008.12.004

22 Common Terminology Criteria for Adverse Events version 4.0 (CTCAE), 2019. National Cancer Institute. Available at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcaev4.pdf [Last accessed on 2nd January 2019]

23 Chua W, Goldstein D, Lee C, Dhillon H, Michael M, Mitchell P, Clarke SJ and Iacopetta B: Molecular markers of response and toxicity to FOLFOX chemotherapy in metastatic colorectal cancer. Br J Cancer *101(6)*: 998-1004, 2009. PMID: 19672255. DOI: 10.1038/sj.bjc.6605239

24 Derwinger K, Wettergren Y, Odin E, Carlsson G and Gustavsson B: A study of the *MTHFR* gene polymorphism C677T in colorectal cancer. Clin Colorectal Canc *8(1)*: 43-48, 2009. PMID: 19203896. DOI: 10.3816/CCC.2009.n.007

25 Taflin H, Wettergreen Y, Odin E, Carlsson G and Derwinger K: Gene polymorphisms MTHFRC677T and MTRA2756G as predictive factors in adjuvant chemotherapy for stage III colorectal cancer. Anticancer Res *31(9)*: 3057-3062, 2011. PMID: 21868559

26 Martin DN, Boersma BJ, Howe TM, Goodman JE, Mechanic LE, Chanock SJ and Ambs S: Association of *MTHFR* gene polymorphisms with breast cancer survival. BMC Cancer *6*: 257, 2006. PMID: 17069650. DOI: 10.1186/1471-2407-6-257

27 Van der Put NM, Gabreels F, Stevens EM, Smeitink JA, Trijbels FJ, Eskes TK, van den Heuvel LP and Blom HJ: A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet *62(5)*: 1044-1051, 1998. PMID: 9545395. DOI: 10.1086/301825

28 Etienne-Grimaldi MC, Francoual M, Formento JL and Milano G: Methylenetetrahydrofolate reductase (*MTHFR*) variants and fluorouracil-based treatments in colorectal cancer. Pharmacogenomics *8(11)*: 1561-1566, 2007. PMID: 18034621. DOI: 10.2217/14622416.8.11.1561

29 Nahid NA, Apu MNH, Islam MR, Shabnaz S, Chowdhury SM, Ahmed MU, Nahar Z, Islam MS, Islam MS and Hasnat A: *DPYD**2A and *MTHFR* C677T predict toxicity and efficacy, respectively, in patients on chemotherapy with 5-fluorouracil for colorectal cancer. Cancer Chemother Pharmacol *81(1)*: 119-129, 2018. PMID: 29134491. DOI: 10.1007/s00280-017-3478-3

30 van Huis-Tanja LH, Gelderblom H, Punt CJA and Guchelaar HJ: *MTHFR* polymorphisms and capecitabine-induced toxicity in patients with metastatic colorectal cancer. Pharmacogenet Genomics *23(4)*: 208-218, 2013. PMID: 23407049. DOI: 10.1097/FPC.0b013e32835ee8e1

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