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Thymidylate synthase gene variants as predictors of clinical response and toxicity to fluoropyrimidine-based chemotherapy for colorectal cancer

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Abstract

Background: Fluoropyrimidines form the chemotherapy backbone of advanced and metastatic colorectal cancer (CRC). These drugs are frequently associated with toxicity events that result in dose adjustments and even suspension of the treatment. The thymidylate synthase (*TYMS*) gene is a potential marker of response and toxicity to fluoropyirimidines as this enzyme is the molecular target of these drugs. Our aim was to assess the association between variants of *TYMS* with response and toxicity to fluoropyrimidines in patients with CRC in independent retrospective and prospective studies.

Methods: Variants namely rs45445694, rs183205964, rs2853542 and rs151264360 of *TYMS* were genotyped in 105 CRC patients and were evaluated to define their association with clinical response and toxicity to fluoropyrimidines. Additionally, the relationship between genotypes and tumor gene expression was analyzed by quantitative polymerase chain reaction.

Results: The 2R/2R (rs45445694) was associated with clinical response ($p = 0.05$, odds ratio (OR) = 3.45) and severe toxicity ($p = 0.0014$, OR = 5.21, from pooled data). Expression analysis in tumor tissues suggested a correlation between the 2R/2R genotype and low *TYMS* expression.

Conclusions: The allele 2R (rs45445694) predicts severe toxicity and objective response in advanced CRC patients. In addition, the alleles G(rs2853542) and 6bp-(rs151264360) are independent predictors of response failure to chemotherapy. This is the first study made on a Latin American population that points out *TYMS* gene variants have predictive values for response and toxicity in patients with CRC treated with fluoropyrimidine-based chemotherapy.

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Keywords: colorectal cancer; fluoropyrimidines; objective response rate; toxicity; *TYMS*; variants.

Introduction

Tumors of the large bowel are a leading cause of morbidity and mortality due to cancer in Mexico [1]. 5-Fluorouracil (5-FU) and capecitabine are fluoropyrimidines constituting the corner stone for chemotherapy against gastrointestinal tumors. Its application in the treatment of metastatic colorectal cancer has represented a significant development in terms of overall survival, compared to the support therapy for patients [2]. Despite this, there is not one predictive biomarker of response for the current first-line scheme based on fluoropyrimidines that can be applied to patients with advanced colorectal cancer (CRC) and the search for useful predictive biomarkers of response to fluoropyrimidines to guide effective therapeutic decisions is still in progress [3, 4]. In the context of personalized medicine, these types of biomarkers are fundamental to ensure the maximum possible benefit for a specific treatment and to extend the spectrum of potential diseases that could benefit from a specific therapy.

Another key consideration of biomarkers for cancer chemotherapy is their prediction proficiency for adverse events. In the case of fluoropyrimidines, serious toxicity is observed in up to 14% of the cases with oral treatment and up to 42% with systemic therapy [5]. Concerning the predictive markers of toxicity to fluoropyrimidines, variants in the gene dihydropyrimidine dehydrogenase responsible for the limiting step in the metabolism of 5-FU were associated with severe toxic events, resulting in even fatal outcomes. However, the low allelic frequency of these variants in the general population, coupled with their low sensitivity to predict toxic events in the clinic, points out to the presence of other possible biomarkers associated with fluoropyrimidines toxicity [6, 7].

The *TYMS* gene codifies for the thymidylate synthase enzyme involved in the *de novo* synthesis of deoxythymidine monophosphate. As this enzyme is the pharmacological target for the fluoropyrimidines, *TYMS* gene variations are attractive candidates for pharmacogenetic studies. However, the coding region of the *TYMS* gene is highly stable, so only few variants with some functional effect were reported, even in tumor tissues; so attention has turned to the variants present in the noncoding regions of the gene [8]. The potential use of variants in the 5′UTR (untranslated region) and 3′UTR flanks has been investigated in several studies of the *TYMS* gene as genetic biomarkers to predict clinical response in gastrointestinal tumors. However, the results are conflicting and there is no consensus on the clinical value of these variants [9–11]. For instance, while some studies report association between a favorable clinical response and variants at the 5′UTR and 3′UTR regulatory regions of the *TYMS* gene, other reports have failed to replicate such results [12–16]. None of these studies was performed on the Latin-American populations and studies in this group may contribute to the understanding of the biological significance of *TYMS* variants and their pharmacological relevance for CRC patients undergoing chemotherapy.

The present study deals with the identification of germ-line variants in noncoding regions of the *TYMS* gene in Mexican patients with CRC from two independent analyses: retrospective and prospective studies, aimed at evaluating the predictive power of these variants in the development of serious toxicity events and lack of response to first-line chemotherapeutic scheme based on fluoropyrimidines for advanced colorectal tumors. Additionally, we analyzed the expression of the *TYMS* gene in fresh samples of tumor and nontumor tissues of colorectal origin and their association with these variants.

Materials and methods

Study design and patients

We reviewed clinical records and tested samples of 99 patients with advanced CRC (stages III–IV according to the TNM classification) receiving 5-FU or capecitabine in the High Specialties Unit N°25 of the Mexican Institute for Social Security (IMSS) during 2010–2012 for retrospective analyses. These study subjects participated in the genome wide association study entitled “Genetic study of Common Hereditary Bowel Cancers in Hispania and the Americas” (FP7 Program). In addition, 68 patients with similar clinic characteristics and chemotherapeutic regime were recruited for a prospective study to analyze treatment toxicity and clinical response during 2013–2015 in the same hospital. Clinical protocols were approved by the respective Ethics Committees of the following participating institutions: University Hospital of Universidad Autonoma de Nuevo Leon (approval code BI13-002), High Specialties Unit N°25, IMSS and West National Medical Center, IMSS (approval code R-2015-785-024). All patients signed an informed consent for their connection with the described studies. Categorization of objective clinical response was performed following the Guide to Response Evaluation Criteria in Solid Tumors (RECIST v1.1, 2009) and adverse events were evaluated by the Guide to Common Terminology Criteria for Adverse Events (CTCAE v4.03, 2010). Patients for whom they could not get complete clinical information or whose biological samples did not have the necessary

quality for subsequent analysis were excluded. After examining the clinical data and genomic DNA quality, 105 patients were selected for these pharmacogenetic studies (42 and 63 from the two recruiting campaigns).

Chemotherapy

Patients received the FOLFOX scheme consisting of IV infusion of 400 mg/m^2 leucovorin and 100 mg/m^2 oxaliplatin in 2 h and a subsequent bolus of 400 mg/m^2 5-FU, followed by continuous infusion of 2400–3000 mg/m^2 5-FU in 48 h every 2 weeks. As an alternative, some patients received the XELOX scheme consisting of IV infusion of 130 mg/m^2 oxaliplatin every 3 weeks and 1000 mg capecitabine twice daily after meals.

The general physical condition of each patient was determined prior to the therapy according to the Eastern Cooperative Oncology Group (ECOG) scale and laboratory tests were performed to assess the baseline liver and kidney functions [17].

Genotyping

Genomic DNA was prepared from blood samples anticoagulated with etheylenediaminetetraacetic acid and stored at –20 °C until further processing. The DNA was isolated with the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) following manufacturer instructions. The variable number of tandem repeats (VNTR) variants of the 5′UTR flank of *TYMS* (rs45445694), and their additional single nucleotide variant (SNV) G>C in the first repeat of the 2R allele (rs183205964, named 2RG or 2RC), the SNV G>C in the second repeat of the 3R allele (rs2853542, named 3RG or 3RC), and the 6 bp insertion in the second repeat of the 3R allele (rs538469385) (all located into the rs45445694 variant) were performed by polymerase chain reaction-restriction fragment length polymorphism using the primers 5012F19:5′-CGGGAAAAGGCGCGCGGAA-3′ and 5147R18: 5′-GCAGCTCCGAGCCGGCCA-3 and HaeIII (New England Biolabs, Ipswich, MA, USA). A 6-bp deletion variant at the 3′UTR region of *TYMS* (rs151264360) was genotyped as previously reported [18]. Genotyping analyses were verified in duplicate for each specimen.

Quantitative *TYMS* expression analysis

All samples were blind evaluated by a pathologist to separate cancerous and normal fractions. Tumor and adjacent tissue samples were recovered from surgical specimens preserved in Tissue-Tek® Optimal Cutting Temperature (Sakura Finetek, Torrance, CA, USA) at –80 °C until processing. Some 10 μm slices cut at –20 °C were immediately processed for gDNA as previously reported [19] and for total RNA using the TRizol® reagent (Invitrogen, Carlsbad, CA, USA). The quality of the RNA was assessed by electrophoresis and spectrophotometry. The cDNA was synthesized with the SuperScript® III First-Strand Synthesis kit (Invitrogen). The quantitative polymerase chain reaction study was performed using the SYBR® Green Master Mix (Bio-Rad. Hercules, CA, USA) in the CFX96™ Real Time System (Bio-Rad) using the primers T2211F: 5′-TACCTGGGGCAGATCCAACACATC-3′ and T2211R: 5′-ACAGCAACTCCTCCAAAACACCC-3. The *B2M* gene expression was selected for normalization. Expression studies were performed in triplicate for both tested genes. Quantification of the relative expression of the *TYMS* gene was performed by the 2[-Delta Delta C(T)] method previously reported [20].

Statistical analysis

Deviations from the Hardy-Weinberg law were evaluated by the Pearson's χ^2-test. Univariate analyses were performed with one-tailed Fisher's exact test and the Cochran-Armitage trend test. The likelihood ratio test was employed to compare variant effects in different genetic models using the SNPstats program [21]. The selection of the best model was based on the joint analysis of the Akaike information criterion and the Bayesian information criterion. Multivariate logistic regression-based analysis was used for the adjustment of the genetic model selected with clinical or demographic covariates. Comparison of *TYMS* gene expression between tumor and adjacent-tissue samples was performed using the Mann-Whitney *U* test on linearized relative expression data. Two-tailed $p < 0.05$ values were considered significant in the different performed tests. All statistical tests were performed using the IBM SPSS Statistics software v22 (IBM Corp. Armonk, NY, USA).

Results

Association analysis for TYMS variants and toxicity in the retrospective study

A total of 42 patients from the retrospective study were included in these analysis. The variants analyzed in the present study are described in Table 1. Regarding the rs45445694 VNTR variant, only alleles with 2(2R) and 3(3R) repeats were identified in the study sample. In addition, the G>C SNV inside the second repeat of the 3R variant (rs2853542) and the 6-bp deletion variant located in the 3′UTR region (rs151264360) were also identified. Variants ascertained in this study are shown in Figure 1. All other reported variants were not detected in the study subjects.

The patients were classified for toxicity according to CTCAE v4.03 and categorized into mild to moderate toxicity (grades 0–2) and serious toxicity (grades 3–4) groups. Some 26% (11/42) of the patients experienced serious toxicity (Table 2). The main causes of toxicity were gastrointestinal toxicity (nausea, vomiting, diarrhoea), followed by neurological toxicity (dysesthesia and sensory neuropathy). Some 7.1% (3/42) of the patients required dose

Table 1: List of analyzed variants.

dbSNP ID[a]	Chromosome coordinates[b]	Nomenclature[c]	Description
rs45445694	18:657646	LRG_783t1:c.-97_70CCGCGCCACTTGGCCTGCCTCC GTCCCG [1–4, 7–9]	VNTR of 28 nt in the 5′UTR region of the *TYMS* gene
rs183205964	18:657657	LRG_783t1:c.-86G>C	G>C transversion in the 12th nt of the first repeat of the 2R allele of the VNTR
rs2853542	18:657685	LRG_783t1:c.58G>C	G>C transversion in the 12th nt of the second repeat of the 3R allele of the VNTR
rs538469385	18:657697	LRG_783t1:c.-46_45insCCCCCG	Insertion of 6 nt in the second repeat of the 3R allele of the VNTR
rs151264360	18:673444	LRG_783t1:c.447_452delTTAAAG	Deletion of 6 nt in the 3′UTR region of the *TYMS* gene. Also named 1494del6

According with Human Genome Variation Society nomenclature recommendations. [a]dbSNP build 147. [b] RefSeq entry: NC_000018.10 GRCh38.p7. [c]RefSeq entry: NM_001071.

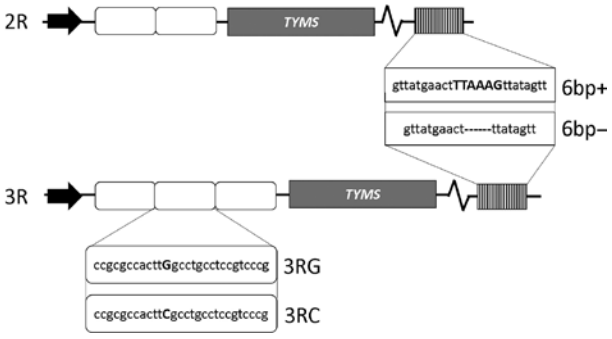

Figure 1: Topological description of the 5′UTR and 3′UTR variants in the *TYMS* gene.
Bold arrows indicate the direction of the coding sequence. Open squares show the variable number repeat variant (rs45445694) with either 2 or 3 repeat alleles. The G>C variant (rs2853542) is located inside the second repeat of the 3R allele. The dashed square shows the position of the del6bp variant (rs151264360) in the 3′UTR region of the gene.

adjustment and treatment was suspended in 11.9% (5/42) of the patients because of severe toxicity. The most important serious toxicities were diarrhoea and sensory neuropathy (14% and 7%, respectively). Death events related to the therapy were not reported.

The univariate analysis did not show associations among serious toxicity and age, sex, tumor stage or type of administered fluoropyrimidine (Supplemental Table 1). The likelihood ratio test determined that the 2R allele of the rs45445694 variant was significantly associated with an increased risk of serious global toxicity in a recessive genetic model (p = 0.023, odds ratio (OR) = 8.29, 95%CI 1.25–54.71) (Table 3). The G>C inside the 3R variant (rs 2853542) was not associated with serious toxicity to the fluoropyrimidines (p = 0.208, 6% vs. 25%).

Prospective analysis of association between variants and objective response

A total of 63 patients met the necessary conditions for their clinical and genetic evaluations in the prospective study. Patients were categorized into responder (partial or complete response) or nonresponder (stable disease, disease progression, or death due to disease) groups, according to RECIST v1.1. The total objective response rate (ORR) was 29%. Univariate analysis for therapeutic response against age (<57 vs. ≥57 years old), gender (male vs. female), and therapy (chemotherapy vs. chemotherapy + radiotherapy) did not show associations (Supplemental Table 2).

The allele discrimination analyses for this cohort identified the 2R (rs45445694), the G>C SNV inside the 3R allele (rs2853542, called 3RC allele) and the 6 bp deletion in the 3′UTR region of *TYMS* (rs151264360) in frequencies of 0.48, 0.46 and 0.33, respectively. Again, the G>C SNV inside the first repetition of the 2R allele (rs183205964) and the 6 bp insertion in the *TYMS* promoter (rs538469385) were not identified. All genotypes for the described *TYMS* variants were in Hardy-Weinberg equilibrium (Supplemental Table 3).

The association analysis for the VNTR (rs45445694) variant and tumor response rate showed that the 2R allele was associated with a positive patient response to chemotherapy in a recessive genetic model (p = 0.05; OR = 3.45, 95% CI 1.00–11.99) (Table 4).

According to the VNTR and the SNV G>C (rs2853542) of the *TYMS* gene patients were categorized into genotypes of low (2R/2R, 2R/3RC, 3RC/3RC) and high (2R/3RG, 3RG/3RC, 3RG/3RG) enzyme expression, based on functional analyses previously reported [17]. This categorization showed a significant association between low gene expression variants and positive tumor response (p = 0.005, OR = 6.84,

Table 2: Distribution of toxicity events in the retrospective and prospective analysis of CRC patients.

Toxicity	n (%)[a]					
	Retrospective (n = 42)			Prospective (n = 63)		
	Total	Grades 1–2	Grades 3–4	Total	Grades 1–2	Grades 3–4
Gastrointestinal	20 (47.6)	13 (30.9)	7 (16.7)	29 (46.0)	20 (31.7)	9 (14.5)
Neurological	15 (35.7)	12 (28.6)	3 (7.1)	24 (38.1)	21 (33.3)	3 (4.8)
Hematological	4 (9.5)	3 (7.1)	1 (2.4)	15 (23.8)	12 (19.0)	3 (4.8)
Asthenia	10 (23.8)	9 (21.4)	1 (2.4)	16 (25.4)	14 (22.2)	2 (3.2)
Hand-foot syndrome	15 (35.7)	14 (33.3)	1 (2.4)	7 (11.1)	7 (11.1)	0 (0)
Other	9 (21.4)	5 (11.9)	4 (9.5)	3 (4.8)	2 (3.2)	1 (1.6)
All toxicities	42 (100)	31 (74.0)	11 (26.0)	63 (100)	45 (71.0)	18 (29.0)

Toxicities grading according with CTCAE v4.03. [a]Percentage with respect to the total population of patients in each study.

Table 3: Association of the VNTR with severe toxicity to fluoropyirimidine-based chemotherapy.

Genetic model	Genotype	n (%)		OR (95% CI)	p-Value[a]
		Toxicity (0–2)	Toxicity (3–4)		
Retrospective study of CRC patients (n = 42):					
Recessive	3R/3R–2R/3R	29 (93.5)	7 (63.6)	1.00	**0.023**
	2R/2R	2 (6.5)	4 (36.4)	8.29 (1.25–54.71)	
Prospective study of CRC patients (n = 63):					
Recessive	3R/3R–2R/3R	37 (86)	12 (60)	1.00	**0.024**
	2R/2R	6 (14)	8 (40)	4.11 (1.19–14.25)	
Pooled data (n = 105):					
Recessive	3R/3R–2R/3R	66 (89.2)	19 (61.3)	1.00	**0.0014**
	2R/2R	8 (10.8)	12 (38.7)	5.21 (1.86–14.59)	

CI, confidence interval. Statistically significant associations are highlighted in bold. [a]Likelihood ratio test.

95% CI 1.73–27.02). In contrast, the number of 3RG alleles in the genotype significantly correlated with therapeutic failure (p = 0.0033) (Figure 2). The 6 bp-(rs151264360) variant at the 3′UTR was significantly associated in an additive genetic model with a positive response to chemotherapy (p = 0.02, OR = 4.81 for each deleted allele, 95% CI 1.56–14.77) (Table 4).

Associations among toxicity and clinical, genetic and demographic factors in the prospective analysis

Some 18 out of 63 of patients who reported toxicity developed serious adverse events due to chemotherapy, according to the CTCAv4.01 Guide. Ten of these patients required dose adjustment and two required definitive therapy suspension (Table 2). Gastrointestinal type toxicity was the most frequent, followed by neurological and hematological toxicities (46%, 38% and 24%, respectively). No death events were reported in this prospective study.

None of the analyzed clinical and demographic factors (gender, age, type of treatment, drug, and ECOG performance status) showed association with serious toxicity to fluoropyrimidines in the univariate analysis (Supplemental Table 4).

Regarding the rs45445694, rs2853542, and rs151264360 *TYMS* variants, the 2R allele of rs45445694 was associated with severe toxicity to the fluoropyrimidine-based chemotherapy in a recessive genetic model in the multivariate analysis (p = 0.0024, OR = 4.11, 95% CI 1.19–14.25) (Table 3). The 3RG and 3RC alleles of rs2853542 were not associated with serious toxicity (27% vs. 22%, p = 0.566) suggesting that this variant lacks informational value for toxicity to fluoropyrimidines.

Combined multivariate analysis

The multivariate logistic regression analysis for combined toxicity data pooled from the retrospective study and the prospective study of CRC patients ($n = 105$, $\alpha = 0.05$,

Table 4: Association of the VNTR and del6bp variants with the objective response of CRC patients to fluoropyrimidine-based chemotherapy in the prospective study.

Genetic model	Genotype	n (%)		OR (95% CI)	p-Value[a]	AIC[b]	BIC[c]
		Nonresponders	Responders				
VNTR (rs45445694)							
Co-dominant	3R/3R	12 (26.7%)	4 (22.2%)	1.00	0.14	77.5	83.9
	2R/3R	26 (57.8%)	7 (38.9%)	0.81 (0.20–3.30)			
	2R/2R	7 (15.6%)	7 (38.9%)	3.00 (0.64–14.02)			
Dominant	3R/3R	12 (26.7%)	4 (22.2%)	1.00	0.71	79.2	83.5
	2R/3R-2R/2R	33 (73.3%)	14 (77.8%)	1.27 (0.35–4.64)			
Recessive	3R/3R-2R/3R	38 (84.4%)	11 (61.1%)	1.00	**0.05**	75.6	79.9
	2R/2R	7 (15.6%)	7 (38.9%)	3.45 (1.00–11.99)			
Over-dominant	3R/3R-2R/2R	19 (42.2%)	11 (61.1%)	1.00	0.17	77.5	81.8
	2R/3R	26 (57.8%)	7 (38.9%)	0.47 (0.15–1.42)			
Log-additive	–	–	–	1.82 (0.80–4.15)	0.15	77.3	81.6
del6bp (rs151264360)							
Co-dominant	6bp–/6bp–	6 (13.3%)	0 (0%)	1.00	0.007	71.3	77.8
	6bp+/6bp–	24 (53.3%)	5 (27.8%)	NA (0.00–NA)			
	6bp+/6bp+	15 (33.3%)	13 (72.2%)	NA (0.00–NA)			
Dominant	6bp–/6bp–	6 (13.3%)	0 (0%)	1.00	0.038	75.1	79.4
	6bp+/6bp– 6bp+/6bp+	39 (86.7%)	18 (100%)	NA (0.00–NA)			
Recessive	6bp–/6bp– 6bp+/6bp–	30 (66.7%)	5 (27.8%)	1.00	0.005	71.4	75.7
	6bp+/6bp+	15 (33.3%)	13 (72.2%)	5.20 (1.56–17.32)			
Over-dominant	6bp–/6bp 6bp+/6bp+	21 (46.7%)	13 (72.2%)	1.00	0.062	75.9	80.2
	6bp+/6bp–	24 (53.3%)	5 (27.8%)	0.34 (0.10–1.10)			
Log-additive	–	–	–	4.81 (1.56–14.77)	**0.002**	69.8	74.1

NA, not applicable; CI, confidence interval. Statistically significant associations are highlighted in bold. [a]Likelihood ratio test. [b]Akaike's information criterion. [c]Bayesian information criterion.

statistical power = 80%), showed a significant association between the 2R/2R genotype adjusted by age and the risk of severe global toxicity to fluoropyrimidines (p = 0.0014, OR = 5.21, 95% CI 1.86–14.59) (Table 3). The *post-hoc* analysis for prediction of chemotherapy toxicity establishes a recessive model for the association of the 2R allele with toxicity. This study ensures significant detection of the toxicity effect in the studied sample.

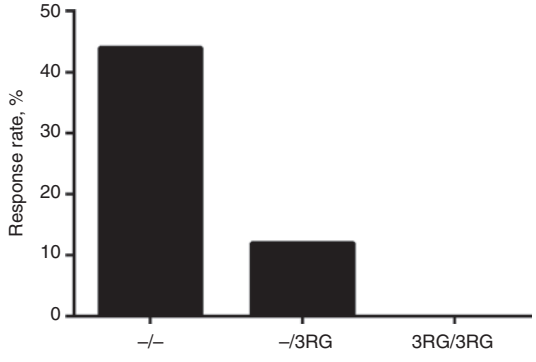

Figure 2: Lack of response to chemotherapy with the number of 3RG alleles in the cohort of colorectal cancer patients.
p = 0.0033, Cochran-Armitage trend test.

TYMS gene expression in colorectal tissues

Tumor and adjacent-tissue samples were available from 16 patients for *TYMS* expression analysis. A trend to lower *TYMS* expression was observed in tumor tissues, although the comparison did not reach significance (Figure 3A). As expected, no associations between *TYMS* expression and rate of response to chemotherapy were found. The 2R/2R genotype of the VNTR was significantly associated with a low relative expression of *TYMS* in tumor tissues (p < 0.05) (Figure 3B). Genotypic analyses in paired tumor and adjacent-tissue samples for the selected *TYMS* variants showed 100% correspondence, suggesting that none of the tumors presented loss of heterozygosity. No other association was found.

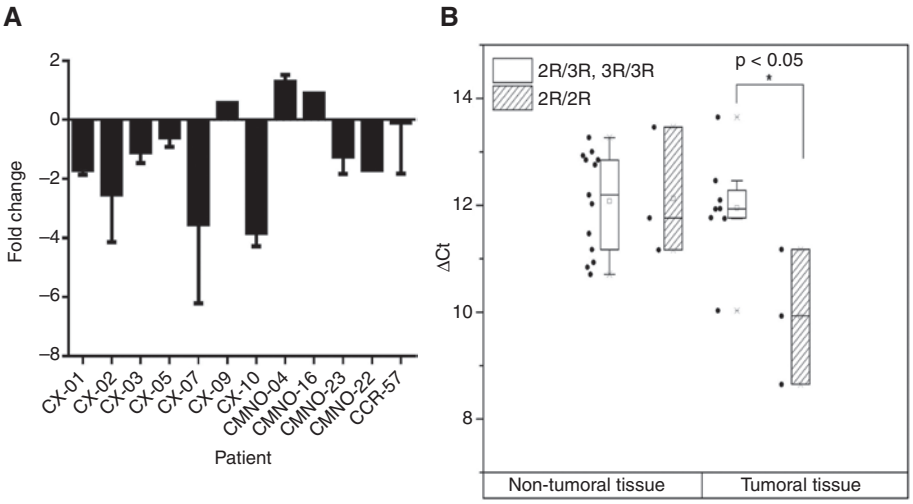

Figure 3: Comparison between *TYMS* gene relative expression in tumor and nontumor colorectal tissues.
(A) Fold change in *TYMS* relative expression between tumor and nontumor adjacent colorectal tissues. p = 0.34, Wilcoxon signed-rank test. Results from three biological replicates. (B) *TYMS* relative expression by genotype of the VNTR variant in tumor (n = 11) and nontumor (n = 16) colorectal tissues. p < 0.05, Mann-Whitney U-test.

Discussion

The TYMS enzyme is the pharmacological target of the fluoropyrimidines and is mainly responsible for the cytotoxic effect. It is reasonable that variants in the regulatory regions of *TYMS* may affect the gene expression and are potential candidates for predicting clinical response and toxicity to fluoropyrimidines [22–24]. Our retrospective analysis of CRC patients shows a significant association between the number of 2R alleles of the VNTR variant in the region 5′UTR of the gene and serious global toxicity to fluoropyrimidines, a result confirmed by the prospective analysis of patients with colorectal tumors. Multivariate analysis of pooled data (prospective and retrospective) shows a recessive model for 2R allele and phenotype of severe toxicity to the fluoropyrimidines with a notorious OR value (5.21). Accordingly, the 2R/2R genotype has been linked to toxicity to fluoropyrimidines [25–28].

Some studies have related the 2R allele with lower gene expression; however, the relationship between the functional effect of two or more repetitions of this variant and the 5-FU effect is unclear [29–31].

We also found a significant association between 2R/2R genotype and ORR of colorectal cancer patients to chemotherapy. This result was reported previously in patients with advanced gastrointestinal cancer treated with fluoropyrimidines [32, 33]. The dual relationship between greater toxicity and better response with the genotype 2R/2R is demonstrated in the clinical context [34–36]. The 2R/2R genotype may potentiate the drug cytotoxic effect by increasing the chemosensitivity of both, tumor and nontumor cells [37].

The variant G>C (rs2853542) showed a tendency towards poorer chemotherapeutic response with each copy of the G allele. Although the clinical effect of this variant has been less studied, our results confirm the report of Meulendijks et al. [33] regarding the negative effect of the G allele on the antineoplastic response. *In vitro* studies suggest that this allele modifies a putative sequence for the binding of the USF-1 transcription factor, decreasing the gene transcriptional activity. This could be associated with increased tumor sensitivity to the fluoropyrimidines and better clinical response [38, 39]. The absence of this allele in the genotypes classified in the low expression category (i.e. 2R/2R, 2R/3RC, 3RC/3RC) may explain the significant association between better clinical response and low expression genotypes in this study [40]. This result was previously reported for esophageal cancer patients but not for CRC patients [41].

The 6bp+/6bp+ genotype of rs151264360 was associated with good response to fluoropyrimidines, as described by Arrazubi et al. [42] but contrasts with the report by Dotor et al. [43]. There are methodological differences in the treatment administration schemes and sample origins that may explain the discrepancies. Our results also differ with two studies performed on Asian populations. An important difference is that 6bp−/6bp− genotype frequencies described in these studies are higher than those found in our cohort of patients [14, 44]. A comparison of VNTR genotype frequencies in similar studies from different populations shows similarities between Mexican and Caucasian populations from Italy, Hungary and Spain (2R/2R: 16%–24%, 2R/3R: 47%–49%, 3R/3R: 29%–35%), but noticeable differences with East

Asian populations (2R/2R: 4%–5%, 2R/3R: 21%–29%, 3R/3R: 67%–73%) [14, 15, 25, 45, 46]. The low frequencies of the 2R allele in the last geographical group may explain the absence of significant association between the VNTR and clinical response in these populations [47]. Similarity between frequencies found in this study with those from Caucasian populations may be explained by the Spaniard contributions to the admixed Mestizo genome in current north-eastern and western Mexicans (38% and 30%, respectively), as was previously established [48, 49].

The *TYMS* expression studies in our colorectal samples showed decreased gene expression in the 2R/2R tumor tissue samples. This observation agrees with reports indicating better ORR to fluoropyrimidines in patients whose colorectal tumors have lower *TYMS* expression [50]. Our studies showed 100% genotypic correspondence between tumor and adjacent-tissue samples for the studied *TYMS* variants, suggesting that none of the tumors presented loss of heterozygosity as previously reported, particularly for the rs45445694 VNTR variant [51, 52]. Functional studies have reported *in vitro* and *in vivo* effects to the G>C (rs2853542) and VNTR variant in *TYMS* expression [38, 53, 54].

Regarding the 6 bp allele in the 3′UTR region of the gene, we did not find association between this allele and TYMS expression. This allele is related with decreased mRNA stability *in vitro* studies which results in lower enzyme expression but this finding requires *in vivo* confirmation [55].

Evidence presented here supports that rs45445694 and rs151264360 are predictive biomarkers of response. However, the role of additional variants in noncoding regions of *TYMS* to chemotherapeutic response and toxicity to fluoropyrimidines cannot be ruled out [56, 57].

Conclusions

The 2R/2R genotype of the VNTR (rs45445694) in the promoter region of the *TYMS* gene is a predictive marker of clinical response and severe toxicity to the fluoropyrimidines in CRC patients. It is possible that this genotype decreases the expression of the gene in the colorectal tumor tissue. The G variant in rs2853542 and the 6bp deletion in rs151264360 (3′UTR region) are independent biomarkers of therapeutic failure.

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