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TYMS 2R3R polymorphism and *DPYD* [IVS]14+1G>A gene mutation in Mexican colorectal cancer patients

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Objective: To examine the association between *TYMS 2R3R* polymorphism and *DPYD* [IVS]14+1G>A mutation by comparing healthy subjects with colorectal cancer (CRC) patients in the Mexican population. Method: Genotyping of the *2R/3R* was performed by polymerase chain reaction (PCR) and [IVS]14+1G>A mutation by real-time PCR analysis. Results: The observed frequencies of the *TYMS 2R3R* polymorphism and the -[IVS]14+1G>A mutation in *DPYD* did not indicate an increased risk for CRC (*p*>0.05). However we observed an association of the *2R/2R* (OR 3.08, 95% CI 1.66-6.08, *p*=0.0017) and heterozygous (OR 1.98, 95% CI 1.32-2.97, *p*=0.0012) genotypes as risk factors when comparing controls and CRC patients that were also tobacco consumers. An association between the genotype and the disease was evident. The distribution of the *2R/2R* genotype and hematological toxicity (adjusted OR 2.26, 95% CI 1.54-4.45, *p*=0.0259), heterozygous (*2R/3R*) with tumor stage III-IV (OR 1.81, 95% CI 1.11-2.94, *p*=0.020) and 2R/2R-2R/3R in non-chemotherapy response CRC patients with hematological (OR 2.3, 95% CI 1.21-4.4, *p*=0.014) and gastric toxicities (OR 3.11, 95% CI 1.18-8.2, *p*=0.035) confirmed that this factor may significantly contribute to the CRC susceptibility. Conclusion: *TYMS 2R3R* polymorphism and the -[IVS]14+1G>A mutation in *DPYD* was not associated with susceptibility to CRC. However, the *2R/2R* and *2R/3R* genotypes of *TYMS* polymorphism could significantly contribute to hematological and gastric toxicity in CRC patients in this sample population.

Key words: TYMS 2R/3R, colorectal cancer, polymorphism, DPYD [IVS]14+1G>A, Mexican population

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Abbreviations: CRC, colorectal cancer; DPD, dihydropyrimidine dehydrogenase; *MTHFR*, methylenetetrahydrofolate reductase; TS, thymidylate synthase; *TYMS*, TS gene; VNTR, variable tandem repeat sequence

INTRODUCTION

Colorectal cancer (CRC) is one of the most common diseases in the world and presents a significant public health problem due to its social implications, high healthcare costs, and increasing rates of diagnosis (Pourhoseingholi, 2014; Alberts *et al*., 2016; Gutiérrez *et al*., 2016). Approximately 60% of all CRC cases are diagnosed in the developed countries, and its incidence varies between different ethnic groups (Pourhoseingholi, 2014; Siegel *et al*., 2014; Gutiérrez *et al*., 2016; Ferlay *et al*., 2015). In Mexico, CRC is responsible for 4% of all cancer-related mortalities and is considered as one of the major causes of mortality (García *et al*., 2015). CRC is also considered to be a multifactor disease that may result from interactions among genes, proteins and environmental factors. It develops through a gradual accumulation of genetic and epigenetic changes that transform normal colonic mucosa into invasive cancer tissue, while also modifying the chemotherapy response (Alberts *et al*., 2016; Gutiérrez *et al*., 2016; Song *et al*., 2016). Chemotherapy resistance has been associated with factors that can influence the therapeutic potential of a given drug (Tecza *et al*., 2016). The thymidylate synthase enzyme (TS) has been reported to participate in the synthesis of pyrimidines, which is essential for DNA synthesis (Balboa *et al*., 2015). Mechanisms of DNA hypomethylation and hypermetilation are important for the colon carcinogenesis process.

This donation is made by S-adenosylmethionine (SAM), which provides the link among the metabolisms of carbon, folic acid and the DNA methylation process (Grem, 2012). In this sense, methylenetetrahydrofolate reductase (MTHFR), TS and dihydropyrimidine dehydrogenase (DPD) have been reported to compete for folic acid and affect homocysteine levels. It is thought that alteration in the genes that encode the afore mentioned enzymes can cause hyperhomocysteinemia due to intracellular folate deficiency and decreased SAM, which in turn can alter DNA methylation patterns, activate proto-oncogenes and induce cellular transformation. In addition, they may erroneously incorporate nitrogenous bases into DNA and cause chromosomal instability. Moreover, it has been observed that DNA hypomethylation in both lymphocytes and colon tissue is associated with low folate consumption in both, animal and human models (Grem, 2012). The TS gene (*TYMS*), which is located on chromosome 18p, contains seven exons, and encodes a TS enzyme (EC 2.1.1.45) that uses 5,10-methylenetetrahydrofolate (methylene-THF) as a cofactor to maintain the dTMP (thymidine-5-prime monophosphate) pool, which is critical for DNA replication and repair. This enzyme has been of interest as a target for cancer chemotherapeutic agents. It is considered to be the primary site of action for 5-fluorouracil, 5-fluoro-2-prime-deoxyuridine, and some folate analogs (OMIM:Entry 188350).

The *2R/3R* polymorphism is a variable tandem repeat sequence (VNTR; rs45445694) associated with the regulation of *TYMS* expression. It is localized at 5'-untranslated region (UTR) and has 2 to 9 repeats, of which 2 and 3 repeats (*2R* and *3R*) are the most frequent alleles, with an increment of expression from 2- to 4-fold of the *3R/3R* genotype when compared with the *2R/2R* genotype (Trinh *et al*., 2002; Zhu *et al*., 2012; Wang *et al*., 2016,).

It has been hypothesized that the *TYMS* polymorphisms can influence the enzyme activity, which would affect plasma folate levels and, indirectly, the plasma homocysteine levels (Trinh *et al*., 2002; Zhu *et al*., 2012; Wang *et al*., 2016). The *2R* allele (rs45445694) showed a frequency of 18% among Hispanic infants and 26.3% in non-Hispanic white infants (Zhu *et al*., 2012).

Another genetic variant is [IVS]14+1G>A mutation in the *DPYD* gene, which is found in DPD deficient patients who are prone to develop life-threatening toxicities from capecitabine and 5-fluorouracil (5-FU) therapy. Different studies have shown that 13% of the patients with these mutations suffered severe toxicities (mucositis, granulocytopenia, neuropathy, and diarrhea), including lethal events (Wang *et al*., 2016). A cause of this toxicity seems to be a decreased drug clearance, which can result in extended 5-FU exposure. DPD-deficient patients exhibit a normal phenotype until administered with 5-FU or capecitabine. It has been estimated that nearly 3-5% of the general population has the DPD activity below normal (Cerić *et al*., 2010).

The *TYMS 2R/3R* and *DPYD* [IVS]14+1G>A polymorphisms have displayed a significant association between the response to and/or toxicity of capecitabine and 5-fluorouracil (5-FU) in some studies (Balboa *et al*., 2015; Wang *et al*., 2016; Romiti *et al*., 2016). However, in the Mexican population, these associations remain unknown. Thus, the aim of this investigation was to determine the frequency and association of *TYMS 2R3R* polymorphism and DPYD [IVS]14+1G>A gene mutation in Mexican colorectal cancer patients.

MATERIALS AND METHODS

DNA was extracted from peripheral blood lymphocytes using standard protocols (Miller *et al*., 1988). Blood samples were collected from 456 healthy blood donor volunteers with an average age of 37.71 years. These volunteers were not age-matched with the patient group. Blood samples were also collected from 347 patients with clinically and histologically confirmed CRC. All patients were residents of the metropolitan area of Guadalajara and were recruited from June 2013 to November 2016. All samples were obtained after the patients provided written informed consent, as approved by the ethical committee (1305). All procedures performed in studies involving human participants were in accordance with the 1964 Helsinki declaration. No familial samples were included. Clinical and demographical data were obtained using written questionnaires. All patients were also interviewed to determine their occupational exposure and current drug regimens. The CRC patient database and patient DNA samples were examined for other polymorphisms (Gutiérrez *et al*., 2016). The response to treatment with 5-FU and capecitabine was based on changes in the penetration of the intestinal layers of the tumor from the pathology report, clinical evaluation and physical examination. The pathological response was evaluated according to the Ryan's pathological classification described as follows: 0. Complete response (no viable cancer cells are observed); 1. Moderate response (single cells or small groups of cancerous cells); 2. Minimum response (residual cancer surrounded by fibrosis), and 3. Poor response (minimal or no tumor destruction, extensive residual cancer) (Ryan *et al*., 2005). Amplification of the *TYMS* promoter region for rs45445694 was performed *via* PCR using the following primers: 5'CGTGGCTCCTGCGTTTCC-3' and 5'-GAGCCGGCCACAGGCAT-3', as described previously by Hishida and coworkers (Hishida *et al*., 2003). The PCR amplifications were performed in a total volume of 15 µL containing 0.2 mM dNTPs (Invitrogen, Carlsbad, CA USA), 5 pmol of primers, 2.0 mM $MgCl_2$, 2.5 U of Taq polymerase (Invitrogen, Carlsbad, CA USA), and 50 ng of genomic DNA. The following PCR conditions were used: 94°C (4 min), followed by 35 cycles at 94°C (1 min), 58°C (1 min) and 72°C (1 min), with a final extension at 72°C (7 min). Using this procedure, fragments of 210 bp, 238 bp, and 266 bp were obtained (Fig. 1). To facilitate allelic discrimination, the amplified products were separated on 6% polyacrylamide gels (29:1), followed by silver staining (Sanguinetti *et al.,* 1994). We determined that the 210 bp fragment alone represented the polymorphic-type genotype (*2R/2R*), the 238 bp fragment represented the wild type genotype (*3R/3R*), the 210 and 238 bp fragments indicated the heterozygous genotype (*2R/3R*), and the 266 bp fragments represented the *4R* allele.

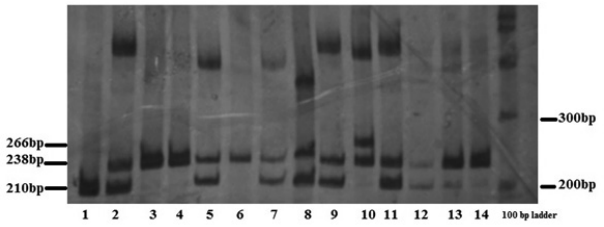

Figure 1. Detection of *TYMS 2R/3R* genotypes.
Electrophoretic separation on 6% polyacrylamide gels (29:1). Line 1: *2R/2R* genotype (210 bp). Lines 2, 5, 7-9, and 11-12: heterozygous genotype (*2R/3R*; 210 bp and 238 bp). Lines 3-4, 6, 13-14: genotype *3R*/3R (238 bp). Line 10: genotype *3R/4R* (238 bp and 266 bp).

The DPYD [IVS]14+1G>A mutation was identified by real-time PCR using the following probes: VIC-5'-TGT TTT AGA TGT TAA ATC ACA CTT A [T/C]-3' and FAM-5'-GTT GTC TGG AAA GTC AGC CTT T-3'(C_30633851_20), as validated and designed by Applied Biosystem (Thermo Fisher Scientific, Waltham, MA, USA). The reactions included a volume of 5 µL (~ 10 µg genomic DNA), 6.25 µL of TaqMan universal buffer, 0.32 µL of VIC and FAM TaqMan labeled probe and 3.43 µL of water per sample. They were always placed in 96-well plates in a light-covered system and were read in the ABI 7300 Real-Time PCR System (Applied Biosystem/Thermo Fisher Scientific, Waltham, MA, USA). The amplification conditions were as follows – 40 cycles: 95°C for 10 minutes, 92°C for 10 seconds, and 60°C for 1 minute. As an internal control, 10% of the reactions were analyzed in duplicate to observe concordance in all of the samples analyzed.

Allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit Chi-square test to compare the observed genotype frequencies to the expected frequencies among control subjects. Odds ratios and 95% confidence intervals (CI) were also calculated. A two-tailed $p<0.05$ was considered to be statistically significant. All statistical

Table 1. Demographic data for the study group

CRC patients(n=347)			Controls(n=456)	OR (CI 95%)*	*p*-value	
Age (years)						
Mean (S.D.)**	59.49	(12.04)	31.71	(10.54)		<0.0001
	(n)	%	(n)	%		
< 50 years	(69)	20.0	(415)	91.0		
≥ 50 years	(278)	80.0	(41)	19.0	40.7 (26.9–61.7)	<0.0001
Gender						
Male	(171)	49.0	(239)	52.5		0.379
Female	(176)	51.0	(217)	47.5		
Tobacco Consumption						
Yes	(162)	47	(123)	30,0	2.3 (1.7–3.1)	<0.0001
No	(185)	53	(333)	70,0		
Alcohol Consumption						
Yes	(159)	46	(70)	15	4.6 (3.3–6.4)	<0.0001
No	(188)	54	(386)	85		
Tobacco and Alcohol Consumption***						
Yes	(117)	45	(20)	7	11.5 (6.8–19.3)	<0.0001
No	(144)	55	(284)	93		

S.D. (standard deviation), NS (no significant difference), *OR (odds ratio) from the adjusted regression analysis, **Student's *t*-test ***only groups that either indicated both, alcohol and tobacco consumption, or no alcohol and no tobacco consumption, were considered.

analyses were performed using the PASW Statistic Base 18 software, 2009 (Chicago, IL, USA).

RESULTS

Table 1 shows the comparative epidemiological data from the CRC patients and the control individuals. In the patient group, the observed average age was 59.49 years, ranging from 23 to 92 years of age. Fifty-one percent (176/347) of these patients were female. Tobacco consumption (adjusted OR 2.3, 95% CI 1.7–3.1, $p<0.0001$), alcohol consumption (OR 4.6, 95% CI 3.3–6.4, $p<0.0001$) and both, the tobacco-alcohol consumption were observed (OR 11.5, 95% CI 6.8–19.3, $p<0.0001$) as risk factors for CRC.

Table 2 shows the general clinical characteristics of the patient group. We observed that 70% of the patients had rectum cancer, approximately 12% had diverticulitis, 72% had stage III–IV tumors, 45.5% had metastasis, 91% had prognoses of 1–4 years, 54% were not responsive to chemotherapy, 64% had gastric toxicity and 60% had hematologic toxicity. Some patients had higher than normal CEA (58%), glutamate oxaloacetate transaminase (SGOT) (27%), alkaline phosphatase (ALP) (42%), or glucose (20%) levels.

The genotypes and allele frequencies of the *TYMS 2R/3R* polymorphism were not significantly different between patients and controls, and the *DPYD [IVS]14+1G>A* mutation was not present in either CRC patients or controls (Table 3). The genotype distribution of *TYMS 2R/3R* polymorphism was in Hardy-Weinberg equilibrium in the control group.

Comparison of the *TYMS 2R/3R* polymorphism and frequency of *DPYD [IVS]14+1G>A* mutation in Mexican controls with some control populations is shown in Table 4.

Significant differences were evident when comparing the CRC group and the control tobacco consumers according to the *2R/2R* (OR 3.08, 95% CI 1.66–6.08, $p=0.0017$) and heterozygous genotypes (OR 1.98, 95% CI 1.32–2.97, $p=0.0012$) as risk factors (data not shown).

In addition, significant differences were found with regards to clinical characteristics of the CRC group and genotype, with respect to the 2R/*2R* genotype *TYMS* polymorphism and hematological toxicity (adjusted OR 2.26, 95% CI 1.54–4.45, $p=0.0259$), whereas heterozygosity (*2R/3R*) with tumor stage III–IV (OR 1.81, 95% CI 1.11–2.94, $p=0.020$) was a risk factor for CRC (Table 5). Additionally, a difference was observed in the non-chemotherapy response of CRC patients with *TYMS 2R/2R-2R/3R* genotype and those with gastric (OR 3.11, 95% CI 1.18–8.2, $p=0.035$) and hematological (OR 2.3, 95% CI 1.21–4.4, $p=0.014$) toxicities.

DISCUSSION

CRC is a multifactor disease with a complex etiology. In Mexico, as in other parts of the world, the incidence of CRC has increased over the last 20 years and is currently one of the leading causes of death for both, women and men (Gallegos *et al.*, 2009; Pourhoseingholi, 2014; Sigel *et al.*, 2014; Alberts *et al.*, 2016, Gutiérrez *et al.*, 2016). These facts are consistent with observations made in the study we present here, where 80% of the CRC patients were ≥ 50 years old (average patient age was 59.49 ± 12.04 years). Many studies have observed a high incidence of CRC in patients who were approximately 50 years old (Pourhoseingholi, 2014; Sigel *et al.*, 2014; Ferlay *et al.*, 2015; Alberts *et al.*, 2016, Gutiérrez *et al.*, 2016). An increased frequency of this disease may be due to lifestyle changes in the Mexican population in terms of diet (including food additives), exposure to

Table 2. Clinical data for CRC patients

	(n)	%		(n)	%
Type			Chemotherapy response		
Colon	(103)	30	Yes	(159)	46
Rectum	(244)	70	No	(188)	54
Personal medical history			Chemo-toxicity***		
No	(206)	59	Gastric	(221)	64
DM-AH	(98)	29	Dermatologic	(106)	31
Diverticulitis	(43)	12	Hematologic,	(207)	60
			Cardiologic	(11)	3
Body mass Index (BMI)**			Neurologic	(45)	13
18.5–19.9 (underweight)	(49)	14	Laboratory test		
20–24.9 (normal weight)	(157)	45	CEA (mg/L)		
25–29.9 (overweight)	(100)	29	Normal	(147)	42
30–34.9 (obesity I)	(34)	10	Elevated	(200)	58
≥35 (obesity II- IV)	(7)	2	Hemoglobin (g/dL)		
Tumor stage			Normal	(179)	52
I–II	(97)	28	Low	(168)	48
III–IV	(250)	72	SGOT (ml/L)		
Adenocarcinoma Histology			Normal	(153)	73
Differentiated	(90)	26	High	(94)	27
Little differentiated	(220)	63	SGPT (µl/L)		
Non differentiated	(37)	11	Normal	(303)	87
Lymph node status			High	(44)	13
Yes	(135)	39	LDH ((µl/L)		
No	(212)	61	Normal	(211)	61
Metastasis			High	(136)	39
Yes	(158)	45.5	ALP (µl/L)		
No	(189)	54.5	Normal	(202)	58
Diagnostic time			High	(145)	42
1–4 years	(317)	91	Glucose (g/dL)		
5–9 years	(24)	7	Normal	(277)	80
≥10 years	(6)	2	High	(70)	20

Carcinoembryonic antigen (CEA), glutamate oxalate transaminase (SGOT), glutamic pyruvic transaminase (SGPT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), **according to OMS classifications. (Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Geneva (Switzerland): World Health Organization, 2004). ***Gastric (mucositis, diarrhea, abdominal pain, dysphagia, proctitis, nausea, vomiting), Dermatologic (hair loss, oncolysis, pigmentation, dermatitis), Hematologic (Anaemia, Thrombocytopenia), Cardiologic (chest pain, myocardial ischemia, spasm myocardial), Neurologic (ataxia, dementia, somnolence, motor debility)

toxic substances and changes in longevity (Gutiérrez *et al*., 2016).

In this study and that of Gutiérrez *et al*., 2016, the consumption of tobacco and alcohol, respectively, were found to be risk factors in approximately 46% of the CRC patients. CRC is not considered to be a strictly tobacco-related malignancy but an association between smoking habit and this disease has been observed (Johnson *et al*., 2013; Gutiérrez *et al*., 2016). The relationship between high alcohol intake and CRC risk remains controversial, but some recent studies have suggested that it might reduce the folate levels, thereby contributing to abnormal DNA methylation and induction of cytochrome P450 enzymes to activate carcinogens (Gallegos *et al*., 2009; Chan *et al*., 2010; Gutiérrez *et al*., 2016).

New techniques and approaches that allow a better discrimination of CRC are now being used in developing countries with a high incidence of this disease (Gallegos *et al*., 2009; Gutiérrez, *et al*., 2016). In the present study, rectal cancer was found to be present in 70% of the patients. This frequency is consistent with the overall rates reported in the literature concerning cancer in individuals with or without a family history of CRC (Gallegos *et al*., 2009; Henrikson *et al*., 2015; Gutiérrez *et al*., 2016). The body mass index (BMI) of most patients in this study was categorized as normal. A possible explanation for this phenomenon may be that many of these individuals presented advanced-stage CRC, as reflected in the percentage of patients diagnosed with stage III or IV tumors (72%). In addition, gastric and hematologic

Table 3. Genotype and allelic distribution of the *TYMS 2R/3R* and DPYD [IVS]14+1G>A polymorphisms in CRC patients and controls

Polymorphism	CRC		Controls		OR	CI (95%)	*p*-value
TYMS 2R/3R							
Genotype	(n=347)	%	(n=456)	%			
2R/2R	(77)	22	(85)	18.81	1.2	(0.88–1.7)	0.21
3R/3R	(108)	31	(137)	30	1.0*		
2R/3R	(155)	45	(230)	50	0.7	(0.59–1.0)	0.10
2R/4R	(2)	0.5	(1)	0.2			
3R/4R	(4)	1	(3)	1			
4R/4R	(1)	0.5	(0)	0			
Alleles (2n)							
2R	(311)	0.448	(401)	0.439	1.03	(0.84–1.2)	0.73
3R	(375)	0.540	(507)	0.555	0.93	(0.77–1.1)	0.53
4R	(8)	0.012	(4)	0.006	2.6	(0.79–8.8)	0.14
DPYD [IVS]14+1G>A	(n=286)	%	(n=286)	%			
Wild type	(286)	100	(286)	100	1.0		
Mutation type	(0)	0	(0)	0			

*Control genotype. Hardy-Weinberg equilibrium in controls (chi-square test=0.0036; p=0.7881 for *TYMS 2R3R* polymorphism).

complications secondary to chemotherapy (64% and 60%, respectively) may be responsible for the BMI data in this study.

Advances in molecular and genetic epidemiology have increased our knowledge on the mechanisms behind colorectal carcinogenesis, the relationship between disease susceptibility and exposure to carcinogens, and individual genetic variations (Gutiérrez *et al*., 2016). Several relevant studies have associated different polymorphisms of enzymes that metabolize drugs, such as TS and DPD as the drug target enzymes, with clinical outcomes treated with commonly prescribed drugs in chemotherapy, such as 5-FU and capecitabine (Leung & Chan, 2015; Wang *et al.,* 2016; Romiti *et al*., 2016; Saif *et al*., 2016; Yang *et al*., 2016; Lee *et al*., 2016). Studies have demonstrated that the underexpression of TYMS predicts that patients carrying the *2R* allele who are treated with capecitabine to be at an increased risk for severe fluoropyrimidine-associated toxicity (Meulendijks *et al*., 2016) and with an increased severity and susceptibility to various diseases (Bezerra *et al*., 2014; Dunna *et al*., 2014). However, the relationship between these polymorphisms and cancer remains controversial and depends on the population studied (Wang *et al*., 2012). Moreover, little is known regarding this association in the Mexican CRC patients.

In our study group, the frequency of *2R/2R* variant was 18.81% in controls, and 22% in CRC patients, which indicates that this polymorphism is not a risk factor for CRC. These data are consistent with a recent meta-analysis that examined different types of cancer (Tang *et al*., 2012; Kim *et al*., 2015). However, when comparing the *TYMS 2R/2R* polymorphism of our Mexican control group with the control groups of other populations, differences were observed with the Asians, Slovenes, and Indo-Chinese with respect to the distribution of the 3 genotypes of this polymorphism. Similarities in the 2R/2R genotype frequency were also observed between American Hispanics and Caucasians, Brazilians, Danish, Germans, Iranians, and Hungarians with respect to the Mexican population, which points to the genetic heterogeneity of this polymorphism in other populations (Adleff *et al*., 2004; Zhu *et al*., 2012; Qiao *et al*., 2017).

DPYD [IVS]14+1G>A mutation was not found in the present study, and these data are similar to those described in other populations, such as Caucasians, African-Americans, Egyptians, Turks, and Taiwanese (Sulzyc *et al*., 2008). Many studies have also reported presence of the *DPYD* [IVS]14+1G>A mutation in CRC patients suffering from severe toxicity after the 5-FU administration. However, some studies suggest that *DPYD* [IVS]14+1G>A mutation is present only in Caucasians (Sulzyc *et al*., 2008).

The association of the *2R/2R* and *2R/3R* genotypes of the *TYMS* polymorphism as risk factors with tobacco consumption was also demonstrated. As is well known, smoked tobacco contains many toxic substances that can damage DNA by formation of adducts, and the individuals with *2R/2R* genotype or heterozygotes could be unable to metabolize these adducts formed by tobacco carcinogens, as our data suggest. Shi et al., 2005, demonstrated an association of the TS3'UTR polymorphism with tobacco use in patients with lung cancer.

Nevertheless, *TYMS 2R/2R* genotype was assessed to be a risk factor for hematological toxicity, and the heterozygous *2R3R TYMS* genotype was found in CRC patients with stage III-IV tumors. Previous studies have observed that chemotherapy influences drug response, toxicities and clinical outcomes in patients (Wang *et al*., 2012; Rosmarin *et al.,* 2015; Zhao *et al*., 2015; Balboa *et al*., 2015; Wang *et al*., 2016). Capecitabine (Xeloda, Roche, oral drug) and 5-FU (pro-drug) are chemotherapeutic drugs commonly given to patients with colorectal cancer (CRC). These drugs commonly causes cytotoxicity by inhibiting thymidine synthesis and by being converted to metabolites that are incorporated into nucleic acids. The most common toxicities include hand-foot syndrome, diarrhea, neutropenia, thrombocytopenia, nausea, vomiting, mucositis and stomatitis (Rosmarin *et al*., 2015).

In addition to these mechanisms, several factors may influence the development of toxicities or the clinical

Table 4. Frequency comparison of the *TYMS 2R/3R* genotype polymorphisms in controls and other populations.

Polymorphism	Genotypes			*p*-value		
TYMS 2R/3R						
	2R/2R	*3R/3R*	*2R/3R*	*2R/2R*	*3R/3R*	*2R/3R*
Mexicans (present study)	85	137	234			
			Vs.			
American Hispanics (Zhu *et al*., 2012)	70	141	165	0.9355	0.03882	0.02813
Brazilians (Qiao *et al*., 2017)	53	78	169	0.8081	0.2612	0.2011
Brazilians (Qiao *et al*., 2017)	66	130	194	0.5753	0.3410	0.6987
American Caucasians (Zhu *et al*., 2012)	34	41	54	0.0721	0.7866	0.0723
Dutches (Qiao *et al*., 2017)	116	123	252	0.0721	0.06648	0.9500
Germans (Qiao *et al*., 2017)	111	141	289	0.5073	0.1849	0.5491
Englishes (Qiao *et al*., 2017)	181	205	368	0.0346	0.3158	0.4314
Iranians (Qiao *et al*., 2017)	27	52	30	0.1906	0.0006	0.0000
Slovenians (Qiao *et al*., 2017)	76	52	124	0.0006	0.0087	0.3414
Hungarians (Adleff *et al*., 2004)	18	25	59	0.9262	0.3209	0.2784
Asian (Qiao *et al*., 2017)	0	153	24	0.0000	0.0000	0.0000
Indonesians (Qiao *et al*., 2017)	0	40	4	0.0083	0.0000	0.0000
Asian Singapureans (Qiao *et al*., 2017)	15	483	154	0.0000	0.0000	0.0000
Indians (Qiao *et al*., 2017)	14	83	47	0.0170	0.0000	0.0000
DPYD Mutation						
[IVS]14+1G>A	Heterozygous		Sample	Frequency (%)		
Mexicans (present study)	0		286	0		
			Vs.			
Dutches (Sulzyc *et al*., 2008)	24		1357	1.76		
Finns (Sulzyc *et al*., 2008)	2		90	2.22		
Afro-Americans (Sulzyc *et al*., 2008)	0		105	0		
American Caucasians (Sulzyc *et al*., 2008)	0		157	0		
Egyptians (Sulzyc *et al*., 2008)	0		247	0		
Turks (Sulzyc *et al*., 2008)	0		250	0		
Taiwaneses (Sulzyc *et al*., 2008)	0		300	0		

outcome of CRC, including other gene polymorphisms and alternative cytotoxic paths that are capable of increasing activity and inducing changes in cell physiology to result in neoplastic progression (Gutiérrez *et al*., 2016). Different studies have observed an association of *TYMS 2R/3R* with different clinical outcome manifestations (Wang *et al*., 2012; Rosmarin *et al*., 2015; Zhao *et al*., 2015; Balboa *et al*., 2015; Wang *et al*., 2016). In this study, we also observed an association of gastric and hematological toxicities in the non-chemotherapy responses of patients carrying *2R/2R-2R/3R* genotypes. With respect to the administration of therapeutic drugs, similar dosages generated a wide range of clinical outcomes and toxicities. Different clinical variables have been associated with drug response (age, gender, diet, ethnicity, organ function, tumor biology, genetic factors). Drug-drug interaction and environmental factors contribute significantly to response variability. In contrast, genetic factors (pharmacological targets, drug metabolizing enzymes and/or drug transporters) also have a great impact on the drug response Genetic differences between individuals affect the kinetics of the

Table 5. Association between *TYMS 2R3R* polymorphism and more than one clinical variable in colorectal cancer patients.

Genotype		OR	CI	*p*-value
2R/2R	Hematological toxicity	2.26	(1.54–4.45)	0.025
2R/3R	Stage III–IV	1.81	(1.11–2.94)	0.020
2R/2R-2R/3R				
Poor chemotherapy response				
	Gastric toxicity	3.11	(1.18–8.2)	0.035
	Hematological toxicity	2.3	(1.21–4.4)	0.014

drug and the speed of movement through the organism (López *et al*., 2008). This heterogeneity is reflected both, in its toxicity and in its therapeutic efficacy. It has been also observed that the response to drugs not only is related to the monogenic inheritance of a protein but also depends on genes encoding proteins involved in multiple metabolic pathways, posttranslational modifications, gene interactions, and epigenetics (Pinedo & Peters, 1988).

In conclusion, our results do not support an association of the *TYMS 2R/3R* polymorphism between the CRC patients and controls, and the *DPYD [IVS]14+1G>A* mutation was not observed in either group. The *TYMS 2R/3R* polymorphism was associated in patients with: a) *2R/2R* genotypes and hematological toxicity, b) heterozygous (*2R/3R*) and stage III-IV of cancer, and c) *2R/2R-2R/3R* non-chemotherapy-responsive CRC patients with hematological and gastric toxicities. These findings confirmed that *TYMS 2R/3R* polymorphism may significantly contribute to CRC susceptibility, depending on the clinical outcomes in this population. Further studies are required to confirm or reject these observations.

Conflicts of interest

The authors declare no conflict of interest.

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