



The prevalence and clinical relevance of *2R/2R TYMS* genotype in patients with gastrointestinal malignancies treated with fluoropyrimidine-based chemotherapy regimens

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Abstract

Introduction The prevalence of *2R/2R TYMS* genotype is variable but estimated to be around 20–30% in Caucasians. The clinical relevance of *TYMS 2R/2R* genotype in predicting severe fluoropyrimidine-related adverse events (FrAE) is controversial. Here, we explored the prevalence and clinical relevance of *2R/2R TYMS* genotype.

Methods Between 2011 and 2018, 126 patients were genotyped for *TYMS*. FrAEs were graded according to CTCAE version 5.0. Fisher's exact test was used for statistical analysis.

Results The prevalence of *TYMS 2R/2R* genotype was 24.6%. Among patients with *TYMS* genotypes ($N = 71$) that predict decreased TS expression, *2R/2R TYMS* genotype was the most common *TYMS* genotype seen in female (57%) and African American (60%) patients. Among patients with genotypes that predict increased TS expression ($N = 55$), 12 patients had grade 3–4 FrAEs (22%), while among patients with genotypes that predict decreased TS expression ($N = 71$), 30 patients had grade 3–4 FrAEs (42%) ($p = 0.0219$). Compared to patients with genotypes predicting increased TS expression, 17 out of 31 patients (55%) with *TYMS 2R/2R* genotype had grade 3–4 FrAEs ($p = 0.0039$) and 15 out of 40 patients (38%) with *TYMS 2R/3RC* and *TYMS 3RC/3RC* genotype had grade 3–4 FrAEs ($p = 0.1108$).

Conclusion The prevalence of *TYMS 2R/2R* genotype was 24.6%, and it had a unique sex and ethnic distribution. Polymorphism in the promoter region of *TYMS* gene that predicts decreased TS expression due to *2R/2R* variant was associated with grade 3–4 FrAEs. These data suggest that genotyping patients who are not DPD deficient for *TYMS* might identify patients at risk of severe FrAEs.

Introduction

Fluoropyrimidines are antimetabolite chemotherapy drugs that are widely used in the treatment of cancer. There are three fluoropyrimidine drugs in clinical use: intravenous 5-fluorouracil (5-FU), oral capecitabine, and oral tegafur. Capecitabine and tegafur are precursors of 5-FU [1, 2]. Fluoropyrimidines are considered the backbone of most chemotherapeutic regimens approved for the treatment of gastrointestinal (GI) malignancies [3]. They also represent treatment options in other malignancies such as breast and head and neck cancer [4, 5].

Among patients treated with 5-FU or capecitabine, approximately 20–25% of patients experience severe (grade 3–4) fluoropyrimidine-related adverse events (FrAEs) [6]. Severe FrAEs lead to patients' hospitalization and treatment interruption or discontinuation. The inter-individual variation

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in the occurrence and severity of FrAEs is partly due to genetic factors [7, 8].

Dihydropyrimidine dehydrogenase (DPD) enzyme, encoded by *DPYD* gene, is the rate-limiting enzyme for 5-FU catabolism, eliminating approximately 80% of administered or formed 5-FU [9]. Any variation in DPD activity can result in a cytotoxic accumulation of free 5-FU. The prevalence of DPD deficiency in Caucasians is approximately 3–5% [10, 11]. African Americans, especially women, seem to have a higher prevalence of approximately 4–12% [12]. Genomic analysis of patients with DPD deficiency has identified over 128 mutations and polymorphisms in the *DPYD* gene, but only four high-risk variants (*DPYD*2A*, *DPYD*13*, *DPYD*9B*, and *HapB3*) have been consistently associated with DPD deficiency and FrAEs. Genotyping for *DPYD* helps in identifying patients with DPD deficiency and guide the dosing of fluoropyrimidines. However, genotyping is limited to high-risk variants, and most patients who experience FrAEs are not DPD deficient [13–15].

In addition to *DPYD*, polymorphism in the *TYMS* gene that encodes thymidylate synthase (TS) may be associated with increased risk of FrAEs. TS is potently inhibited by 5-FU. Cells convert 5-FU to the metabolite fluorodeoxyuridine monophosphate, which binds to TS and inhibits the production of deoxythymidine monophosphate (dTMP). dTMP is essential for DNA replication and repair, so the lack of it leads to cell death [16, 17]. Fig. 1 shows the cascade of metabolic reactions where fluoropyrimidines inhibit TS and eventually lead to DNA damage.

TYMS gene expression is regulated by transcription factors that bind to the promoter region. The 5' untranslated region contains a 28-base-pair variable number of tandem repeats (VNTRs), which act to enhance the promoter and transcriptional activity (Fig. 2). Most patients have either 2 (2R) or 3 (3R) repeats. Homozygous *TYMS 3R/3R* genotype has a higher level of TS, while homozygous *TYMS 2R/2R* genotype has low TS level and may be at greater risk of FrAEs [18, 19]. A single-nucleotide polymorphism of the second repeat of the 3R allele (3RC) abolishes a binding site in the 3R second repeat allele and reduces TS activity compared to wild-type 3R allele (3RG) [20].

The prevalence of 2R/2R *TYMS* genotype in different ethnic background is variable but estimated to be around 20–30% in Caucasians [21]. The clinical relevance of *TYMS 2R/2R* genotype in predicting severe FrAEs is controversial [7, 19, 22–26]. Here, in a cohort of patients with GI malignancies treated with fluoropyrimidine-based chemotherapy regimens, we explored the prevalence and clinical relevance of 2R/2R *TYMS* genotype. Moreover, given the different racial and sex background in our cohort, ethnic and sex differences were explored.

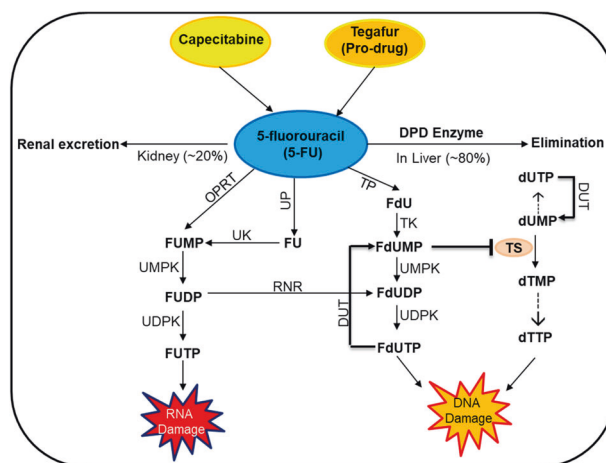


Fig. 1 Schematic representation of fluoropyrimidine-based drug metabolic pathway. The capecitabine and tegafur are the oral pro-drug and pro-drug, respectively, which in turn converted into 5-FU, while 5-FU is directly administered as IV. In normal condition of DPD and TS activity, maximum drug is eliminated from body while minimal amount is functionally active and inhibits the DNA and RNA synthesis leading to cell death during cancer treatment. Patients possessing the DPD deficiency show grade 3–4 toxicity as maximum drug is accumulated in the body that inhibit the *TYMS*. The *TYMS 2R/2R* genotype has low TS level and correlated with severe fluoropyrimidines-related adverse events. DPD dihydropyrimidine dehydrogenase, UP uridine phosphorylase, UK uridine kinase, TK thymidine kinase, TP thymidine phosphorylase, TS thymidylate synthetase, OPRT orotate phosphoribosyltransferase, RNR ribonucleotide reductase, NME1-NME2 nucleoside diphosphate kinase.

Materials and methods

Patient population

This is a retrospective study conducted at the University of South Alabama Mitchell Cancer Institute in Mobile, Alabama, USA in collaboration with ARUP Laboratories, The University of Utah, Salt Lake City, Utah, USA. Cohort was identified through searching our cancer center tumor registry for patients with GI malignancies genotyped for *TYMS* gene between 2011 and 2018. The University of South Alabama Institutional Review Board (IRB) approved this study and the IRB-approved database provided a waiver of the requirement for informed consent and allowed for the publication of de-identified data.

Fluoropyrimidine-based chemotherapy

The fluoropyrimidine-based chemotherapy regimens that the patients in this cohort received include FOLFIRINOX, FOLFOX with or without bevacizumab, cetuximab or panitumumab, XELOX, FOLFIRI with or without bevacizumab, cetuximab or panitumumab, XELIRI with or without bevacizumab, cetuximab or panitumumab, FLOT, 5-FU and mitomycin, 5-FU and liposomal irinotecan,

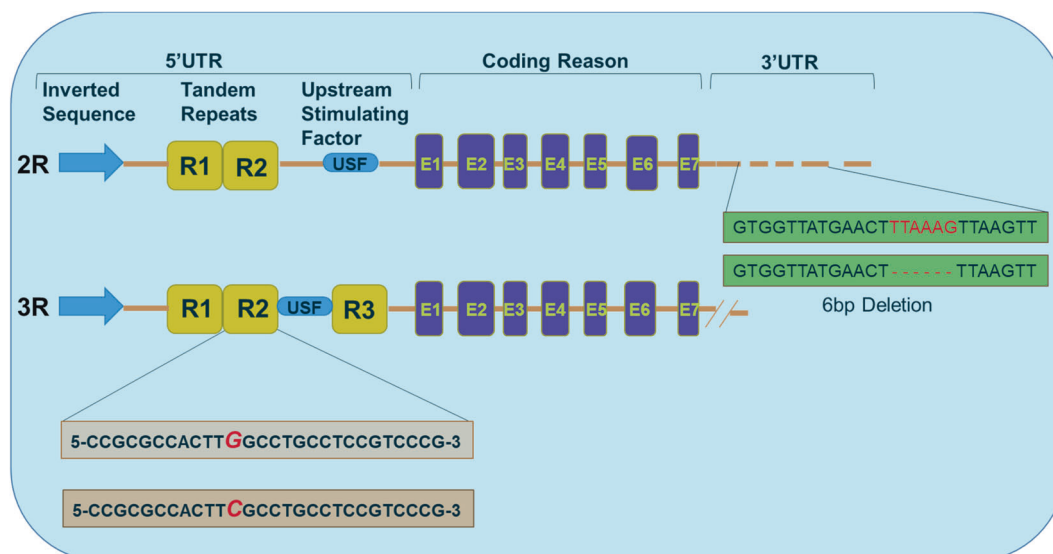


Fig. 2 Regulation of TYMS gene expression by 5' and 3' untranslated regions (UTRs). Upstream or 5'UTR of the thymidylate synthase gene (TYMS) contains either two tandem repeats (2R) or three tandem repeats (3R) of 28-bp sequences. These tandem repeats regulate the transcription and translation of TYMS gene with the impaired enzyme activity. Moreover, other functional variants of the

capecitabine and gemcitabine, single agent 5-FU with or without concurrent radiotherapy, and capecitabine with or without concurrent radiotherapy. FOLFIRINOX consists of 5-FU bolus of 400 mg/m², 5-FU continuous infusion 2400 mg/m² for 46 h, leucovorin 400 mg/m², oxaliplatin 85 mg/m², and irinotecan 180 mg/m² every 2 weeks. FOLFOX consists of 5-FU bolus of 400 mg/m², 5-FU continuous infusion 2400 mg/m² for 46 h, leucovorin 400 mg/m², and oxaliplatin 85 mg/m² every 2 weeks. XELOX consists of capecitabine 1000 mg/m² twice daily for 2 weeks and oxaliplatin 130 mg/m² every 3 weeks. FOLFIRI consists of 5-FU bolus of 400 mg/m², 5-FU continuous infusion 2400 mg/m² for 46 h, leucovorin 400 mg/m², and irinotecan 180 mg/m². XELIRI consists of capecitabine 1000 mg/m² twice daily for 2 weeks and irinotecan 250 mg/m² every 3 weeks. FLOT consists of 5-FU continuous infusion 2600 mg/m² for 24 h, leucovorin 400 mg/m², oxaliplatin 85 mg/m², and docetaxel 50 mg/m². 5-FU and mitomycin consist of 5-FU 4000 mg/m² and mitomycin 10 mg/m². 5-FU and nanoliposomal irinotecan consists of 5-FU continuous infusion 2400 mg/m² for 46 h, leucovorin 400 mg/m², and nanoliposomal irinotecan 70 mg/m². Capecitabine and gemcitabine consist of capecitabine 1660 mg/m² for 21 days and gemcitabine 1000 mg/m² day 1, 8, and 15 every 4 weeks.

Genotyping strategy

Genotyping strategies were quite variable. The most common genotyping strategy was to genotype patients prior to initiating treatment with fluoropyrimidines-based

TYMS gene have been also identified such as single-nucleotide polymorphism (SNP) G>C within the second repeat of the 3R allele. Thymidylate synthase promoter 3RC/3RC genotype causes lower transcriptional activity of TYMS, comparable with the TS 2R/2R genotype. The six nucleotide insertion or deletion also identified to affect the RNA stability of TYMS gene.

chemotherapy. Between 2016 and 2018, genotyping was conducted almost universally on all patients with GI malignancies treated with fluoropyrimidines-based chemotherapy. Prior to 2016, only 24 patients were genotyped and genotyping was conducted at the discretion of the treating medical oncologist. Those patients were genotyped either because they experienced toxicities or because they had significant comorbidities and their medical oncologist decided to do upfront genotyping.

TYMS genotyping

Germline DNA was obtained from peripheral blood specimens and genotyped TYMS gene in ARUP laboratories (Salt Lake City, UT, USA). The VNTRs 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 (rs2863542, 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. A 6-bp deletion variant at the 3'UTR region of TYMS (rs151264360) was genotyped as well.

DPYD genotyping

Germline DNA was obtained from peripheral blood specimens and genotyped for high-risk DPYD variants (IVS14+IG>A

[DPYD*2A], DPYD c.1679T>G [DPYD*13A] and DPYD c.2846A>T [DPYD*9B] in ARUP laboratories (Salt Lake City, UT, USA). Only patients with no mutant high-risk DPYD variants were included in this cohort. Patients with mutant DPYD*9A (c.85T>C) were included in our cohort since the 2017 updated Clinical Pharmacogenetics Implementation Consortium guidelines for DPD genotype and fluoropyrimidine dosing and other studies stated that the DPYD*9A (c.85T>C) does not affect DPD activity in a clinically relevant manner [14, 15].

Toxicity grading and statistical analysis

Demographic and clinical data were extracted from the patients' charts. FrAEs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0). Association between dichotomous fluoropyrimidine-related toxicities and TYMS genotype was performed using Fisher's exact test. Analyses with *p* values ≤ 0.05 were considered significant. Tests were performed using GraphPad software QuickCalcs (GraphPad software 2016, San Diego, CA).

Results

Patient characteristics

Between 2011 and 2018, a total of 126 patients with GI malignancies were genotyped for TYMS and had no identifiable high-risk DPYD variants. The baseline characteristics of the patients are summarized in Table 1. Median age is 59 years. Males represented 55% of the patients, while females represented 45%. In our cohort, 63% were Caucasian, 35% were African Americans, and 2% were other ethnicities (Hispanics, Asians, and Indian Americans). Colon adenocarcinoma represented the most common malignancy in our cohort. Other patients had anal squamous cell carcinoma, appendix adenocarcinoma, cholangiocarcinoma, esophageal adenocarcinoma, gastric adenocarcinoma, neuroendocrine tumor, pancreatic adenocarcinoma, and rectal adenocarcinoma. A fluorouracil-based chemotherapy regimen was administered in 74% of the patients, while 26% of the patients received a capecitabine-based chemotherapy regimen.

TYMS genotyping

TYMS genotypes that predict increased TS expression (3RG/3RG, 3RG/3RC, 2R/3RG, 2R/4R, 3R/4R, 4R/3RG) were identified in 55 patients (44%). TYMS genotypes that predict decreased TS expression (2R/2R, 2R/3RC, 3RC/3RC) were seen in 71 patients (56%). In our cohort, patients with

Table 1 Patient baseline characteristics.

Patient characteristics	Number subject, <i>N</i> [%]
Age (years)	
Median (range)	59 (21–90)
Sex	
Female	57 [45%]
Male	69 [55%]
Ethnicity	
African American	44 [35%]
Other ethnicities ^a	3 [2%]
Caucasians	79 [63%]
Diagnosis	
Anal SCC	6 [5%]
Appendix	3 [2%]
Cholangiocarcinoma	4 [3%]
Colon adenocarcinoma	50 [40%]
Esophageal adenocarcinoma	2 [2%]
Gastric adenocarcinoma	8 [6%]
Neuroendocrine tumor (SB)	3 [3%]
Pancreatic adenocarcinoma	13 [10%]
Rectal adenocarcinoma	37 [29%]
Chemotherapy regimen	
Fluorouracil-based	93 [74%]
Capecitabine-based	33 [26%]

SCC squamous cell carcinoma, SB small bowel.

^aHispanics, Asians, and Indian Americans

2R/2R TYMS genotype represented 24.6% of the total cohort and 44% of the patients with genotypes that predict decreased expression of TS. TYMS genotyping in patients with genotypes that predict increased and decreased TS expression is summarized in Table 2.

Sex differences

In our cohort, the distribution of 2R/3RG TYMS genotype was similar between males and females. The distribution of all other genotypes apart from 2R/2R and 3RG/3RC TYMS genotypes was more frequent in males than females. The 2R/2R TYMS genotype had a very unique sex distribution where 20 out of 31 patients (65%) were females. This is the only TYMS genotype where females were twice as common as males. In fact, among all the female patients in our cohort (*N* = 57), the 2R/2R TYMS genotype was present in 20 female patients (35%). Moreover, among patients with TYMS genotypes that predict decreased TS expression, 2R/2R TYMS genotype was the most common TYMS genotype seen in female patients (57%). Of note, the three patients with 4R containing TYMS genotypes (2R/4R, 3R/4R, and 4R/3RG) were all males. TYMS genotyping in patients with

Table 2 *TYMS* genotyping in patients with GI malignancies with different racial and sex backgrounds.

TYMS genotyping (<i>N</i> = 126)	Number of subjects <i>N</i> [%]	Sex <i>N</i> [%]		Ethnicity <i>N</i> [%]		
		Male	Female	Caucasians	AA	Others
Genotypes predictive of increased TS expression	55 [44%]	33 [26.2%]	22 [17.5%]	30 [23.8%]	23 [18.3%]	2 [1.6%]
3RG/3RG	12 [9.5%]	11 [8.7%]	1 [0.8%]	6 [4.8%]	5 [4.0%]	1 ^a [0.8%]
3RG/3RC	22 [17.5%]	10 [7.9%]	12 [9.5%]	19 [15.1%]	3 [2.4%]	0 [0.0%]
2R/3RG	18 [14.3%]	9 [7.1%]	9 [7.1%]	5 [4.0%]	12 [9.5%]	1 ^b [0.8%]
2R/4R	1 [0.8%]	1 [0.8%]	0 [0.0%]	0 [0.0%]	1 [0.8%]	0 [0.0%]
3R/4R	1 [0.8%]	1 [0.8%]	0 [0.0%]	0 [0.0%]	1 [0.8%]	0 [0.0%]
4R/3RG	1 [0.8%]	1 [0.8%]	0 [0.0%]	0 [0.0%]	1 [0.8%]	0 [0.0%]
Genotypes predictive of decreased TS expression	71 [56%]	36 [28.6%]	35 [27.8%]	50 [39.7%]	20 [15.9%]	1 [0.8%]
2R/2R	31 [24.6%]	11 [8.7%]	20 [15.9%]	19 [15.1%]	12 [9.5%]	0 [0.0%]
2R/3RC	31 [24.6%]	19 [15.1%]	12 [9.5%]	24 [19.0%]	7 [5.6%]	0 [0.0%]
3RC/3RC	9 [7.1%]	6 [4.8%]	3 [2.4%]	7 [5.6%]	1 [0.8%]	1 ^c [0.8%]

AA African American.

^aNative American.^bAsian.^cHispanic.**Table 3** The frequency of grade 1–2 and grade 3–4 fluoropyrimidines-related adverse events in patients with *TYMS* genotypes that predict increased and decreased TS expression.

Adverse events	TYMS genotypes that predict increased TS expression (<i>N</i> = 55)		TYMS genotypes that predict decreased TS expression (<i>N</i> = 71)	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Hematological	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)
Neutropenia	16 (29)	3 (5)	21 (30)	4 (6)
Anemia	10 (18)	0 (0)	16 (23)	0 (0)
Thrombocytopenia	4 (7)	0 (0)	6 (8)	0 (0)
Neutropenic fever		0 (0)	0 (0)	0 (0)
Non-hematological	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)
Mucositis	4 (7)	3 (5)	4 (6)	3 (4)
Nausea	17 (31)	2 (4)	23 (32)	2 (3)
Vomiting	3 (5)	2 (4)	5 (7)	1 (1)
Diarrhea	4 (7)	9 (16)	7 (13)	11 (15)
Neurotoxicity	3 (5)	0 (0)	3 (4)	4 (6)
Skin toxicity	3 (5)	3 (5)	1 (1)	8 (11)
Fatigue	30 (55)	2 (4)	27 (38)	5 (7)
Vasospasm	0 (0)	1 (2)	0 (0)	4 (6)

GI malignancies with different sex background is summarized in Table 2.

Ethnic differences

Caucasians were the majority in both genotypes that predict decreased TS expression and in genotypes (3RG/3RG and 3RG/3RC) that predict increased TS expression. Among patients with *TYMS* genotypes that predict

decreased TS expression, 2R/2R *TYMS* genotype was the most common *TYMS* genotype seen in African American patients (60%). Of note, the three patients with 4R containing *TYMS* genotypes (2R/4R, 3R/4R, and 4R/3RG) were all African Americans. There was minimal representation of other ethnic backgrounds in our cohort. *TYMS* genotyping in patients with GI malignancies with mixed racial background is summarized in Table 2.

Table 4 The frequency of grade 1–2 and grade 3–4 fluoropyrimidines-related adverse events in patients with TYMS genotypes that predict decreased TS expression (2R/2R, 2R/3RC, and 3RC/3RC genotypes).

Adverse events	TYMS genotypes that predict decreased TS expression (<i>N</i> = 71)					
	2R/2R (<i>N</i> = 31)		2R/3RC (<i>N</i> = 31)		3RC/3RC (<i>N</i> = 9)	
	G 1–2	G 3–4	G 1–2	G 3–4	G 1–2	G 3–4
Hematological	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)
Neutropenia	11 (35)	1 (3)	9 (29)	1 (3)	1 (11)	2 (22)
Anemia	8 (26)	0 (0)	6 (19)	0 (0)	2 (22)	0 (0)
Thrombocytopenia	3 (10)	0 (0)	3 (10)	0 (0)	0 (0)	0 (0)
Neutropenic fever	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Non-hematological	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)
Mucositis	2 (6)	3 (10)	1 (3)	0 (0)	1 (11)	0 (0)
Nausea	13 (42)	2 (6)	9 (29)	0 (0)	1 (11)	0 (0)
Vomiting	2 (6)	1 (3)	2 (6)	0 (0)	1 (11)	0 (0)
Diarrhea	4 (13)	6 (19)	2 (6)	3 (10)	1 (11)	2 (22)
Neurotoxicity	0 (0)	2 (6)	3 (10)	2 (6)	0 (0)	0 (0)
Skin toxicity	1 (3)	3 (10)	0 (0)	4 (13)	0 (0)	1 (11)
Fatigue	17 (54)	2 (6)	8 (26)	3 (10)	2 (22)	0 (0)
Vasospasm	0 (0)	1 (3)	0 (0)	1 (3)	0 (0)	2 (22)

Table 5 The association between grade 3–4 fluoropyrimidines-related adverse events (FrAEs) and TYMS genotypes.

Patients	Grade 3–4 FrAEs, <i>N</i> [%]	<i>p</i>
Patients with genotypes that predict increased TS expression (<i>N</i> = 55)	12 [22%]	<i>p</i> = 0.0219
Patients with genotypes that predict decreased TS expression (<i>N</i> = 71)	30 [42%]	
Patients with genotypes that predict increased TS expression (<i>N</i> = 55)	12 [22%]	<i>p</i> = 0.0039
Patients with 2R/2R genotype that predict decreased TS expression (<i>N</i> = 31)	17 [55%]	
Patients with genotypes that predict increased TS expression (<i>N</i> = 55)	12 [22%]	<i>p</i> = 0.1108
Patients with 2R/3RC and 3RC/3RC TYMS genotypes that predict decreased TS expression (<i>N</i> = 40)	15 [38%]	

Statistical analysis was performed using Fisher's exact test.

Adverse events

The frequency of grade 1–2 and grade 3–4 FrAEs in patients with TYMS genotypes that predict increased TS expression (3RG/3RG, 3RG/3RC, 2R/3RG, 2R/4R, 3R/4R, 4R/3RG) and TYMS genotypes that predict decreased TS expression (2R/2R, 2R/3RC, 3RC/3RC) is summarized in Table 3. None of the patients have died as a consequence of fluoropyrimidine-induced toxicities. The most commonly experienced adverse event in both group of patients with grade 3–4 FrAEs was diarrhea. Among patients with TYMS genotypes that predict decreased TS expression (2R/2R, 2R/3RC, 3RC/3RC), the frequency of grade 1–2 and grade 3–4 FrAEs was further explored. Diarrhea was the most experienced grade 3–4 FrAE in patients with 2R/2R TYMS genotype, while skin toxicity was the most experienced grade 3–4 FrAE in patients with TYMS 2R/3RC. Of note, grade 3–4 neutropenia and vasospasm were more experienced in patients with TYMS 3RC/3RC compared to 2R/2R and 2R/3RC TYMS genotypes. The frequency of grade 1–2 and grade 3–4 FrAEs in patients with TYMS genotypes that

predict decreased TS expression (2R/2R, 2R/3RC, 3RC/3RC) is summarized in Table 4.

Statistical analysis

Among patients with TYMS genotypes that predict increased TS expression (*N* = 55), 12 patients (22%) had grade 3–4 FrAEs, while among patients with TYMS genotypes that predict decreased TS expression, 30 patients (42%) had grade 3–4 FrAEs (*p* = 0.0219). Given the observed statistically significant difference, we explored the impact of the different TYMS genotypes that predict decreased TS expression (2R/2R, 2R/3RC, 3RC/3RC) on the observed results. Compared to patients with genotypes predicting increased TS expression, 17 out of 31 patients (55%) with 2R/2R TYMS genotype had grade 3–4 FrAEs (*p* = 0.0039), while only 15 out 40 patients (38%) with 2R/3RC or 3RC/3RC TYMS genotypes had grade 3–4 FrAEs (*p* = 0.1108). The association between grade 3–4 FrAEs and TYMS genotypes is shown in Table 5. Statistical analysis was performed using Fisher's exact test.

Table 6 Summary of several studies that explored the prevalence of 2R/2R *TYMS* genotype.

Author	Population	<i>N</i>	Prevalence <i>N</i> (%)
Zhu	Case and control infants (American Caucasians)	78 and 132	16 (21) and 34 (26)
Zhu	Case and control infants (American Hispanics)	144 and 396	25 (17) and 70 (18)
De Jonge	Children with ALL and matched control (Netherlands)	244 and 491	51 (21) and 116 (24)
Gast	Children with ALL and matched control (Germany)	457 and 541	95 (21) and 111 (21)
Lightfoot	Children with ALL and matched control (UK)	759 and 754	193 (25) and 181(24)
Petra	Children with ALL and matched control (Slovenia)	68 and 252	17 (25) and 76 (30)
Adleff	Colorectal (Hungary)	102	18 (17.6)
Kristensen	Colorectal (Denmark)	122	34 (28)
Vazquez	Healthy volunteers (Argentina)	199	43 (26.1)
Canalle	Children with ALL and matched control (Brazil)	126 and 300	33 (26) and 53 (18)
Silva	Children with ALL and matched control (Brazil)	140 and 390	25 (18) and 66 (17)
Gallegos-Arreola	Colorectal and healthy subjects (Mexico)	347 and 456	77 (22) and 85 (19)
Chan	Children with ALL and matched control (Indonesia)	184 and 177	30 (16) and 0 (0)
Giovanetti	Children with ALL and matched control (Indonesia)	71 and 44	1 (1) and 0 (0)
Yeoh	Children with ALL and matched control (Singapore)	518 and 652	12 (2) and 15 (2)
Nazki	Children with ALL and matched control (India)	43 and 144	8 (19) and 14 (10)

Discussion

The prevalence of 2R/2R *TYMS* genotype in different ethnic background is quite variable. In Caucasian Americans, the prevalence of 2R/2R *TYMS* genotype in infants with conotruncal heart defects and control group was 21% and 26%, respectively. In the same study, the prevalence of 2R/2R *TYMS* genotype in American Hispanics was 17% and 18%, respectively [27]. In children with acute lymphoblastic leukemia (ALL) and matched control, the prevalence of 2R/2R *TYMS* genotype, respectively, was 21% and 24% in the Netherlands, 21% and 21% in Germany, 25% and 24% in United kingdom, and 25% and 30% in Slovenia [28–31]. In patients with colorectal cancer, the prevalence of 2R/2R *TYMS* genotype was 18% in Hungary and 28% in Denmark [17, 32].

Many studies have explored the prevalence of 2R/2R *TYMS* genotype in Hispanic population as well. In healthy volunteers from Argentina, the reported prevalence is 26% [33]. In Brazil, the prevalence of 2R/2R *TYMS* genotype in children with ALL and matched control was 26% and 18%, respectively, in one study and 21% and 24%, respectively, in another study [34, 35]. In Mexico, the prevalence of 2R/2R *TYMS* genotype in patients with colorectal cancer and healthy subjects is 22% and 19%, respectively.

The prevalence of 2R/2R *TYMS* genotype in other ethnicities (Asians and Indians) showed lower reported prevalence compared to Caucasians and Hispanics. In children with ALL and matched control, the prevalence of 2R/2R

TYMS genotype, respectively, was 16% and 0% in one study and 1% and 0% in another study from Indonesia. The prevalence was 2% and 2% in Singapore and 19% and 10% in India [36–39]. Table 6 summarizes several studies that explored the prevalence of 2R/2R *TYMS* genotype.

In our cohort of 126 patients, the prevalence of 2R/2R *TYMS* genotype was 24.6% of the total cohort and 44% of the patients with genotypes that predict decreased expression of TS. Among Caucasians ($N = 80$), 19 patients (24%) had 2R/2R *TYMS* genotype, which is not very different from the reported prevalence of 2R/2R *TYMS* genotype in Caucasians in America or Europe. Among African Americans ($N = 43$), 12 patients (28%) had 2R/2R *TYMS* genotype, which is slightly higher than the prevalence of 2R/2R *TYMS* genotype in Caucasians. The prevalence of 2R/2R *TYMS* genotype in African Americans is not well established. In one study that explored pharmacogenomics in patients with colorectal cancer, 36 patients were African Americans, and among this group, 25 patients (69%) had either 2R/2R or 2R/3R *TYMS* genotypes [40].

Among male patients ($N = 69$), 11 patients (16%) had 2R/2R *TYMS* genotype. Among female patients ($N = 57$), 20 patients (35%) had 2R/2R *TYMS* genotype. It is important to emphasize that 60% (12 patients) of the female patients in our cohort with 2R/2R *TYMS* genotype are African Americans. Several studies showed that women, especially African Americans, experienced more grade 3–4 fluoropyrimidine-associated toxicities compared to men. An

underlying explanation is yet to be identified. The reported higher prevalence of 2R/2R TYMS genotype in female African American patients in our study might represent one possible explanation. Certainly, this should be considered hypothesis-generating observation.

The role of 2R/2R TYMS genotype in predicting severe FrAEs is controversial. The association between 2R/2R TYMS genotype and FrAEs has been demonstrated in many but not all studies. In the positive studies, the sensitivity and positive predictive value were of limited clinical benefit. Among unselected 200 patients treated with 5-FU, grade 3–4 FrAEs were experienced in 44 patients (22%). In this group of patients ($N = 44$), 13 patients had 2R/2R TYMS genotype (sensitivity 30%). Among all the patients with 2R/2R TYMS genotype ($N = 25$), 13 patients experienced grade 3–4 FrAEs (positive predictive value 52%) [7, 19, 22, 23].

On the other hand, several other studies failed to show a positive association between 2R/2R TYMS genotype and FrAEs [23–26]. In one prospective study where TYMS genotyping was used to select the chemotherapy of choice in patients with rectal cancer, the rate of grade 3–4 FrAEs was less in patients with 2R/2R, 2R/3R, or 2R/4R TYMS genotypes compared to patients with 3R/3R or 3R/4R TYMS genotypes (30% vs 54%). Moreover, the hospitalization rate was lower at 16% vs 34% [26].

Our patients with 2R/2R TYMS genotype experienced different grade 3–4 hematological and non-hematological FrAEs. Diarrhea was the most common experienced grade 3–4 FrAEs. Other adverse events include neutropenia, mucositis, nausea, vomiting, neurotoxicity, skin toxicity, fatigue, and vasospasm. In our cohort, the association between 2R/2R TYMS genotype and FrAEs was noticeable. Compared to patients with genotypes predicting increased TS expression, 2R/2R TYMS genotype was the only genotype among genotypes predicting decreased TS expression that had statistically significant association with grade 3–4 FrAEs ($p = 0.0039$). The association between the other genotypes (2R/3RC and 3RC/3RC TYMS genotypes) and grade 3–4 FrAEs did not reach statistical significance ($p = 0.1108$).

Our study has several limitations. This study represents a single-institution experience with limited cohort of ethnic diversity. Our cohort was made of Caucasians and African Americans for the most part, and only three patients were from other ethnic backgrounds (Asian, Hispanic, and Indian American). Our cohort is also quite heterogenous regarding the primary site of the tumor and stage. It is also important to recognize that this study is a retrospective study and there are inherent limitations with a retrospective analysis, particularly regarding selection bias. TYMS genotyping strategies were quite variable as TYMS genotyping was at the discretion of the treating medical oncologist, and the selected treatment

included several different fluoropyrimidine-based regimens. The medical oncologists followed the recommended dose management guidelines per package insert when they managed FrAEs. However, they still had a degree of variation in their practice. The process of attributing an experienced toxicity to 5-FU or capecitabine when they were part of fluoropyrimidine-based chemotherapy regimens was quite challenging sometimes. Every effort was made to make that attribution as accurate as possible. The aforementioned limitations should be kept in mind prior to drawing any conclusions.

Conclusion

The prevalence of TYMS 2R/2R genotype in our cohort was 24.6%. Among Caucasians and African Americans, it was 24% and 28%, respectively. Polymorphism in the promoter region of TYMS gene that predict decreased TS expression due to 2R/2R variant was associated with grade 3–4 FrAEs. These data suggest that genotyping patients who are not DPD deficient for TYMS might identify patients at risk of severe FrAEs.

The 2R/2R TYMS genotype had a very unique sex and ethnic distribution. Among patients with TYMS genotypes that predict decreased TS expression (2R/2R, 2R/3RC, 3RC/3RC), 2R/2R TYMS genotype was the most common TYMS genotype seen in female patients (57%) and in African American patients (60%). The reported higher prevalence of 2R/2R TYMS genotype in female African American patients in our study might represent one possible explanation for why women, especially African Americans, experience more grade 3–4 FrAEs.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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