Original Study

The Prevalence of *DPYD*9A (c.85T>C)* Genotype and the Genotype-Phenotype Correlation in Patients with Gastrointestinal Malignancies Treated With Fluoropyrimidines: Updated Analysis

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Abstract

In our previous study of a cohort of 28 patients, *DPYD*9A (c.85T>C)* was the most commonly diagnosed variant (46%) and there was a noticeable genotype-phenotype correlation. In this study we genotyped a larger cohort of a mixed racial background to explore the prevalence of *DPYD*9A* variant and to confirm the genotype-phenotype correlation. In this updated analysis, the prevalence of heterozygous and homozygous *DPYD*9A* genotypes were 41% and 10%, respectively; the correlation between *DPYD*9A* genotype and dihydropyrimidine dehydrogenase clinical phenotype was not reproduced. The noticeable correlation that we previously reported is likely because of small sample size and selection bias.

Introduction: The dihydropyrimidine dehydrogenase gene *(DPYD)*9A (c.85T>C)* genotype is relatively common. The correlation between *DPYD*9A* genotype and dihydropyrimidine dehydrogenase (DPD) deficiency phenotype is controversial. In a cohort of 28 patients, *DPYD*9A* was the most commonly diagnosed variant (13 patients [46%]) and there was a noticeable genotype-phenotype correlation. In this study we genotyped a larger cohort of a mixed racial background to explore the prevalence of *DPYD*9A* variant and to confirm the genotype-phenotype correlation. **Patients and Methods:** Between 2011 and 2018, in addition to genotyping for high-risk DPYD variants (*DPYD*2A, DPYD*13* and *DPYD*9B*), genotyping for *DPYD*9A* variant was performed on 113 patients with gastrointestinal malignancies treated with fluoropyrimidines. Fluoropyrimidines-associated toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0). Fisher exact test was used for statistical analysis. **Results:** Heterozygous and homozygous *DPYD*9A* genotypes were identified in 46 (41%) and 11 (10%) patients, respectively. Among patients with *DPYD*9A* genotypes (n = 57), men and women represented 30 (53%) and 27 (47%) patients, respectively. Caucasian, African American, and other ethnicities represented 29 (50.9%), 26 (45.6%), and 2 (3.5%) patients, respectively. Grade 3/4 toxicities were experienced in 26 patients with *DPYD*9A* genotype (3 patients had homozygous status) and in 20 patients with wild type *DPYD*9A* ($P = .4405$). In patients who received full-dose fluoropyrimidines (n = 85), Grade 3/4 toxicities were experienced in 22 patients with *DPYD*9A* genotype (2 patients had homozygous status), and in 17 patients with wild type *DPYD* ($P = .8275$).

These data were presented, in part, at the Gastrointestinal American Society of Clinical Oncology in San Francisco, California, in January 2019.

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Submitted: Mar 4, 2019; Revised: Apr 8, 2019; Accepted: Apr 26, 2019; Epub: May 3, 2019

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1533-0028/$ - see frontmatter
https://doi.org/10.1016/j.clcc.2019.04.005

Conclusion: In our updated analysis, the prevalence of heterozygous and homozygous *DPYD*9A* genotypes were 41% and 10%, respectively. The correlation between *DPYD*9A* genotype and DPD clinical phenotype was not reproduced. The noticeable correlation that we previously reported is likely because of small sample size and selection bias.

Clinical Colorectal Cancer, Vol. 18, No. 3, e280-6
Keywords: Dihydropyrimidine dehydrogenase, *DPYD*9A (c.85T>C)* variant, Fluoropyrimidines, Gastrointestinal malignancy, Germline pharmacogenomics

Introduction

Fluoropyrimidines are a class of chemotherapy drugs that include intravenous 5-fluorouracil (5-FU), oral capecitabine, and oral tegafur.[1,2] Fluoropyrimidines are widely used in the treatment of patients with colorectal cancer and other gastrointestinal (GI) tract malignancies.[3] They are also used in the treatment of breast and head and neck cancers.[4,5] Moreover, as a topical agent, 5-FU is used in the treatment of actinic keratosis.[6]

Dihydropyrimidine dehydrogenase (DPD) is an enzyme (Enzyme Commission 1.3.1.2) encoded by the dihydropyrimidine dehydrogenase gene (*DPYD)* gene. DPD enzyme is the rate-limiting enzyme in fluoropyrimidines catabolism and eliminates >80% of administered or formed fluorouracil.[7] Treatment with fluoropyrimidines can result in severe (Grade 3-4) toxicities in up to 25% to 30% of patients.[8] Among patients with DPD deficiency, the incidence of severe toxicities has been reported to be as high as 88%. In those patients, fluoropyrimidines can be fatal.[9-12]

Dihydropyrimidine dehydrogenase deficiency has an ethnic and gender demographic characteristic. In one study, partial DPD deficiency was present in 12.3%, 4.0%, 3.5%, and 1.9% of African American women and men, and Caucasian women and men, respectively.[13] Molecular analysis of patients with DPD deficiency has identified >128 mutations and polymorphisms in the *DPYD* gene that might result in partial or total loss of DPD activity.[14-16] Only 4 *(DPYD*2A*, *DPYD*13*, *DPYD*9B*, and a collection of single nucleotide polymorphisms, termed *HapB3*) have been consistently associated with enhanced fluoropyrimidines toxicity and marked decrease in DPD activity.[14,16-18]

The *DPYD*9A (c.85T>C)* genotype is relatively common. The estimated prevalence of heterozygous and homozygous *DPYD*9A* genotypes is approximately 31% and 3%, respectively.[14] Previously, *DPYD*9A* genotype was considered pathogenic and several reports showed a genotype-phenotype correlation.[19-23] However, none of the large cohort and case-control studies showed marked decreased DPD activity and increased fluoropyrimidines toxicity.[14,23-31] On the basis of those results, the 2017 updated Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline stated that, similar to several other common DPYD variants, there is strong or moderate evidence that *DPYD*9A* does not affect DPD function in a clinically relevant manner in the context of fluoropyrimidines-associated toxicities.[32]

In our cohort of 28 patients, *DPYD*9A* was the most commonly diagnosed variant (46%) and there was a noticeable genotype-phenotype correlation.[33] In the current study we genotyped a larger cohort of a mixed racial background to explore the prevalence of *DPYD*9A* genotype and to further explore the genotype-phenotype correlation.

Patients and Methods

Patient Population

This was a retrospective study conducted at the University of South Alabama Mitchell Cancer Institute in Mobile, Alabama, United States, in collaboration with ARUP Laboratories (Salt Lake City, UT), The University of Utah, Salt Lake City, Utah, United States. The cohort was identified through searching our cancer center tumor registry for patients genotyped for *DPYD* variants 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 publication of deidentified data.

Dihydropyrimidine Dehydrogenase Gene Genotyping

Germline DNA was obtained from peripheral blood specimens and genotyped for *DPYD* variants in ARUP Laboratories. Between 2011 and 2014, ARUP Laboratories provided results for 5 variants (*IVS14+1G>A [DPYD*2A]*, *DPYD c.1679T>G [DPYD*13A]*, *DPYD c.2846A>T [DPYD*9B]*, *DPYD c.85T>C [DPYD*9A]*, and *DPYD c.-1590T>C*). Between 2015 and 2018, ARUP Laboratories provided results for only 3 variants (*IVS14+1G>A [DPYD*2A]*, *DPYD c.1679T>G [DPYD*13A]*, and *DPYD c.2846A>T [DPYD*9B]*). In July 2018, patients who were screened for high-risk *DPYD* variants but not for *DPYD*9A* variant were retrospectively genotyped for *DPYD*9A* variant. When a mutation was identified, heterozygous or homozygous status was included in the final result report.

Toxicity Grading and Statistical Analysis

Demographic and clinical data were extracted from the patients' charts. Toxicity experienced in the first 4 cycles of treatment was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0).[34] Association between dichotomous fluoropyrimidine-related toxicities and *DPYD* variants status was performed using Fisher exact test. Analyses with P values $< .05$ were considered significant. Tests were performed using GraphPad software QuickCalcs 2016 (GraphPad Software, San Diego, CA).

Results

Patient Characteristics

Between 2011 and 2018, a total of 113 patients with GI malignancies were genotyped for *DPYD* variants. The baseline characteristics of the patients are summarized in Table 1. Median age was 59 years. Men represented 55% of the patients whereas women represented 45%. In our cohort, 66% were Caucasian, 31% were African American, and 3% were other ethnicities (Hispanic, Asian, and

Table 1 Patient Baseline Characteristics (n = 113)

Patient Characteristic	Value
Median Age (Range), Years	59 (21-90)
Sex	
Female	51 (45)
Male	62 (55)
Ethnicity	
African American	35 (31)
Other ethnicity[a]	3 (3)
Caucasian	75 (66)
Diagnosis	
Anal SCC	6 (5)
Appendix adenocarcinoma	3 (3)
Cholangiocarcinoma	3 (3)
Colon adenocarcinoma	43 (38)
Esophageal adenocarcinoma	1 (1)
Gastric adenocarcinoma	7 (6)
Neuroendocrine tumor (SB)	2 (2)
Pancreatic adenocarcinoma	12 (10)
Rectal adenocarcinoma	36 (32)
Chemotherapy Regimen	
Fluorouracil-based	79 (70)
Capecitabine-based	34 (30)

Data are presented as n (%) except where otherwise noted.
Abbreviations: SB = small bowel; SCC = squamous cell carcinoma.
[a]Hispanic, Asian, and American Indian.

American Indian). Colon adenocarcinoma represented the most common malignancy in our cohort (38%). Other patients had anal squamous cell carcinoma, appendix adenocarcinoma, cholangiocarcinoma, esophageal adenocarcinoma, gastric adenocarcinoma, neuroendocrine tumor of the small bowel, pancreatic adenocarcinoma, and rectal adenocarcinoma. A fluorouracil-based chemotherapy regimen was administered in 79 patients (70%) whereas 34 patients (30%) received a capecitabine-based chemotherapy regimen.

Dihydropyrimidine Dehydrogenase Gene Genotyping Analysis

The genotyping analysis of the patients included in our cohort is summarized in Table 2. *DPYD* mutant variants were identified in 61 patients (54%). Among patients with mutant *DPYD* genotype (n = 61), 3 patients (2.7%) had *DPYD IVS14+1G>A (DPYD*2A)* variant, 2 patients (1.8%) had *DPYD c.2846A>T (DPYD*9B)* variant, 46 patients (40.7%) had heterozygous *DPYD*9A* variant, and 11 patients (9.7%) had homozygous *DPYD*9A* variant. One patient was double heterozygous for *DPYD*9A* and *DPYD*9B* variants. *DPYD*13A* and *c.-1590T>C* variants were not identified in any of our patients.

Gender and Ethnic Considerations

Sex and ethnic background of the patients included in our cohort are summarized in Table 2. High risk variants (*DPYD*2A* and *DPYD*9B*) were seen mostly in men (4 patients). Only 1 patient with high-risk variants (*DPYD*2A*) happened to be female. *DPYD*9A* variant was seen in both sexes. In our cohort, high-risk variants (*DPYD*2A* and *DPYD*9B*) were identified only in Caucasian participants. However, heterozygous and homozygous *DPYD*9A* variants were seen in Caucasian, African American, Asian, and Hispanic ethnicities.

Adverse Events

The frequency of Grade 1/2 and Grade 3/4 toxicities in patients with mutant *DPYD* genotypes (*DPYD*9A*, *DPYD*2A*, and *DPYD*9B*) and patients with wild type *DPYD* genotype are summarized in Table 3. None of the patients have died as a consequence of fluoropyrimidines-induced toxicities. The most common experienced Grade 3/4 toxicity in *DPYD*-mutant patients and *DPYD* wild type patients was diarrhea.

Statistical Analysis

In patients with no high-risk *DPYD* variants, the association between *DPYD*9A* variant (heterozygous and homozygous) and Grade 3/4 toxicities in all patients and in patients who received full-dose fluoropyrimidines Are summarized in Table 4. Statistical analysis was performed using Fisher exact test.

Table 2 Dihydropyrimidine dehydrogenase gene (*DPYD*) Genotyping in Patients With GI Malignancies With Mixed Racial and Gender Background (n = 113)

		Sex		Ethnicity		
***DPYD* Genotyping**	**Patients**	**Male**	**Female**	**Caucasian**	**AA**	**Other**
DPYD Wild Type	52 (46)	29 (25.7)	23 (20.4)	42 (37.2.)	9 (8.0)	1 (0.9)[a]
DPYD Mutant (High Risk)	5 (4.4)	4 (3.5)	1 (0.9)	5 (4.4)	0 (0)	0 (0)
*IVS14+1G>A [DPYD*2A]*	3 (2.7)	2 (1.8)	1 (0.9)	3 (2.7)	0 (0)	0 (0)
*c.1679T>G [DPYD*13A]*	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
*c.2846A>T [DPYD*9B]*	2 (1.8)	2 (1.8)	0 (0)	2 (1.8)	0 (0)	0 (0)
c.-1590T>C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
DPYD Mutant *(DPYD*9A)*	57 (50.4)	30 (26.5)	27 (23.9)	29 (25.7)	26 (23)	2 (1.8)
Heterozygous	46 (40.7)	24 (21.2)	22 (19.5)	27 (23.9)	18 (15.9)	1 (0.9)[b]
Homozygous	11 (9.7)	6 (5.3)	5 (4.4)	2 (1.8)	8 (7.1)	1 (0.9)[c]

Data are presented as n (%). One patient had double heterozygous *9A and *9B.
Abbreviations: AA = African American; *DPYD* = dihydropyrimidine dehydrogenase gene; GI = gastrointestinal.
[a]Hispanic.
[b]Asian.
[c]Native American.

Table 3 The Frequency of Grade 1/2 and Grade 3/4 Toxicities in Patients With *DPYD* Wild Type, *DPYD*9A*, *DPYD*2A*, and *DPYD*9B* Variants

	DPYD Wild Type (n = 52)		*DPYD* Mutant (n = 61)					
			*DPYD*9A* (n = 57[a])		*DPYD*2A* (n = 3)		*DPYD*9B* (n = 2[a])	
Adverse Events	**G 1/2**	**G 3/4**	**G 1/2**	**G 3/4**	**G 1/2**	**G 3/4**	**G 1/2**	**G 3/4**
Hematological								
Neutropenia	14 (27)	4 (8)	14 (25)	4 (7)	2 (67)	1 (33)	1 (50)	0 (0)
Anemia	12 (23)	0 (0)	11 (19)	0 (0)	1 (33)	0 (0)	1 (50)	0 (0)
Thrombocytopenia	4 (8)	0 (0)	3 (5)	0 (0)	1 (33)	0 (0)	1 (50)	0 (0)
Neutropenic Fever	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nonhematological								
Mucositis	4 (8)	2 (4)	4 (7)	2 (4)	1 (33)	0 (0)	0 (0)	0 (0)
Nausea	13 (25)	3 (6)	20 (35)	2 (4)	1 (33)	0 (0)	2 (100)	0 (0)
Vomiting	4 (8)	2 (4)	6 (11)	2 (4)	0 (0)	0 (0)	2 (100)	0 (0)
Diarrhea	5 (10)	10 (19)	4 (7)	11 (19)	0 (0)	3 (100)	0 (0)	2 (100)
Neurotoxicity	4 (8)	2 (4)	1 (2)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Skin toxicity	1 (2)	3 (6)	2 (4)	6 (11)	1 (33)	0 (0)	0 (0)	1 (50)

Data are presented as n (%).
Abbreviation: *DPYD* = dihydropyrimidine dehydrogenase gene.
[a]One patient had double heterozygous status for *DPYD*9A* and *DPYD*9B* variants.

Table 4 In Patients With No High-Risk *DPYD* Variants, the Association Between the *DPYD*9A* Variant (Heterozygous and Homozygous) and Grade 3/4 Toxicities Is Shown in All Patients and in Patients Who Received Full-Dose Fluoropyrimidines

Patients With No High-Risk *DPYD* Variants	Grade 3/4 Toxicities	*P*
All Patients (n = 108)		
*DPYD*9A* wild type (n = 52)	20/52 (38)	
*DPYD*9A* mutant (n = 56)	26/56 (46)	.4405
*DPYD*9A* wild type (n = 52)	20/52 (38)	
*DPYD*9A* mutant (heterozygous) (n = 45)	23/45 (51)	.2264
*DPYD*9A* wild type (n = 52)	20/52 (38)	
*DPYD*9A* mutant (homozygous) (n = 11)	3/11 (27)	.7319
Received full dose chemotherapy (n = 85)		
*DPYD*9A* wild type (n = 39)	17/39 (44)	
*DPYD*9A* mutant (n = 46)	22/46 (48)	.8275
*DPYD*9A* wild type (n = 39)	17/39 (44)	
*DPYD*9A* mutant (heterozygous) (n = 39)	20/39 (51)	.6505
*DPYD*9A* wild type (n = 39)	17/39 (44)	
*DPYD*9A* mutant (homozygous) (n = 7)	2/7 (29)	.6823

Data are presented as n (%).
Statistical analysis was performed using Fisher exact test.
Abbreviation: *DPYD* = dihydropyrimidine dehydrogenase.

Grade 3/4 toxicities were experienced in 26 patients (46%) with mutant *DPYD*9A* and in 20 patients (38%) with wild type *DPYD*9A* genotype ($P = .4405$). Among patients with mutant *DPYD*9A* genotype, the experienced Grade 3/4 toxicities were seen in 23 of 45 patients (51%) with heterozygous *DPYD*9A* genotype ($P = .2264$) and 3 of 11 patients (27%) with homozygous *DPYD*9A* genotype ($P = .7319$).

In patients who received full-dose fluoropyrimidines (n = 85), Grade 3/4 toxicities were experienced in 22 patients (48%) with mutant *DPYD*9A* and in 17 patients (44%) with wild type *DPYD*9A* genotype ($P = .8275$). Among patients who received full-dose fluoropyrimidines with mutant *DPYD*9A* genotype, the experienced Grade 3/4 toxicities were seen in 20 of 39 patients (51%) with heterozygous *DPYD*9A* genotype ($P = .6505$) and 2 of 7 patients (29%) with homozygous *DPYD*9A* genotype ($P = .6823$). All patients with *DPYD*2A* (n = 3) and *DPYD*9B* (n = 2) received full-dose chemotherapy and experienced Grade 3/4 toxicities.

Discussion

Dihydropyrimidine dehydrogenase gene (*DPYD)*9A* genotype is considered one of the common DPYD variants. The estimated prevalence of heterozygous and homozygous *DPYD*9A* genotypes is approximately 31% and 3%, respectively.[14] Table 5 shows a summary of several studies that explored the prevalence of *DPYD*9A* genotype.[14,21-28,30,31,33] When the heterozygous or homozygous status was reported, it is indicated in the table. In our cohort of 28 patients, the prevalence of heterozygous and homozygous *DPYD*9A* genotype was 42.9% and 3.6%, respectively.[33] In this updated analysis, the prevalence of heterozygous and homozygous *DPYD*9A* genotype was 40.7% and 9.7%, respectively.

Several studies have shown that women experienced more Grade 3/4 fluoropyrimidines-associated toxicities compared with men. An underlying explanation is yet to be identified. The reported sex differences was present across a range of treatment regimens, patient characteristics, and cancer trial settings.[35,36] The difference in *DPYD*9A* genotype prevalence between men and women has not been well studied. A sex-specific *DPYD*9A* genotype prevalence was not reported in the studies summarized in Table 5. In our updated analysis, the prevalence of heterozygous and homozygous *DPYD*9A* genotypes was 21.2% and 5.3%, respectively, in men and was 19.5% and 4.4%, respectively, in women (Table 2). Among patients with *DPYD*9A* genotypes (n = 57), men and women represented 30 (53%) and 27 (47%) patients, respectively.

Dihydropyrimidine dehydrogenase deficiency has ethnic demographic characteristics. In one study, partial DPD deficiency

Table 5 Summary of Several Studies That Explored the Prevalence of *DPYD*9A (c.85T>C)* Genotype and the Correlation Between *DPYD*9A* Genotype and Fluoropyrimidines-Associated Toxicities

Reference	Population (Cancer)	n	Prevalence Het: n (%), Hom: n (%)	*DPYD*9A* Genotype-Phenotype Correlation (Toxicity)	*P*
Khushman et al[33]	Gastrointestinal	28	Het: 13 (42.9), Hom: 1 (3.6)	Yes (diarrhea)	.0055
Zhao et al[21]	Acute lymphoblastic leukemia	147	Het: 49 (33), Hom: 11 (7.5)	Yes (infection, renal, hepatic)	<.05
Joerger et al[22]	Colorectal	64	Het: 25 (39), Hom: 3 (4.7)	Yes (hand-foot syndrome)	.033
Joerger et al[22]	Gastric	76	Het: 22 (29), Hom: 4 (5.3)	Yes (diarrhea)	.023
Zhang et al[23]	Gastric and colorectal	75	Het: 7 (9.3), Hom: 0 (0)	Yes (nausea and vomiting)	<.05
Amirfallah et al[24]	Colorectal (Turkish)	85	Het: 25 (29.4), Hom: 6 (7.1)	No	NR
Boisdron-Celle et al[26]	Colorectal (French)	252	79 (31)	No	.362
Deenen et al[30]	Colorectal	45	Het: 12 (26.7), Hom: 5 (11)	No	.89
Li et al[31]	Colorectal (Chinese)	117	Het: 14.3, Hom: 0	Nr	NR
Kleibl et al[27]	Colorectal cancer	76	Het: 24 (31.6), Hom: 2 (2.6)	No (protective effect)	.04
Morel et al[14]	Cancer (French)	487	Het: 150 (31), Hom: 15 (3)	No	NR
Amstutz et al[28]	Cancer Patients	111	Het: 37 (33.3), Hom: 6 (5.4)	No	.56
McLeod et al[25]	Colorectal cancer	546	Het: 192 (35), Hom: 35 (6.4)	No	NR

When the heterozygous or homozygous status was reported, it is indicated in the table.
Abbreviations: *DPYD* = dihydropyrimidine dehydrogenase gene; Het = heterozygous; Hom = homozygous.

was present in 12.3%, 4.0%, 3.5%, and 1.9% of African American women and men, and Caucasian women and men, respectively.[13] The difference in *DPYD*9A* genotype prevalence between different ethnic backgrounds has not been well studied. In our updated analysis, the prevalence of heterozygous and homozygous *DPYD*9A* genotype was 23.9% and 1.8%, respectively in Caucasian and 15.9% and 7.1%, respectively, in African American participants. One Asian patient had heterozygous *DPYD*9A* genotype and one Native American patient had homozygous *DPYD*9A* genotype. Among patients with *DPYD*9A* genotypes (n = 57), Caucasian, African American, and other ethnicities represented 29 (50.9%), 26 (45.6%), and 2 (3.5%) patients, respectively. All of the patients with high-risk variants were Caucasian (Table 2).

The correlation between *DPYD*9A* genotype and fluoropyrimidines-associated toxicities has been shown in several studies. However, several other studies showed no correlation. Table 5 summarizes several studies that explored the correlation between *DPYD*9A* genotype and fluoropyrimidines-associated toxicities. On the basis of these, the 2017 updated CPIC guideline stated that there is strong or moderate evidence that *DPYD*9A* genotype does not affect DPD function in a clinically relevant manner in the context of fluoropyrimidines-associated toxicities. In our updated analysis, the correlation between the *DPYD*9A* variant genotype and DPD deficiency clinical phenotype that was noticeable in our earlier analysis was not reproduced.[33] There was no statistically significant association between *DPYD*9A* genotype and Grade 3/4 toxicities in all patients and in patients who received full-dose fluoropyrimidines. A statistically significant association was not shown in either heterozygous or homozygous *DPYD*9A* genotypes.

Individuals with homozygous or double heterozygous high-risk *DPYD* variants might have complete DPD deficiency. They are at increased risk of severe or even fatal fluoropyrimidines-associated toxicities. In those patients, fluoropyrimidines use should be avoided and if needed alternative agents are encouraged to be used instead. In patients with heterozygous high-risk *DPYD* variants, *DPYD* genotype-guided individualized dosing showed improved patient safety.[32,37] In our cohort, 11 patients had homozygous *DPYD*9A* genotype and all of them received treatment before genotyping (7 patients received full dose). Among those patients, only 3 of 11 patients developed Grade 3/4 fluoropyrimidines-associated toxicities. The rest tolerated the treatment very well.

Since ARUP Laboratories stopped reporting the status of *DPYD*9A* in 2015, only 1 patient had the status of homozygous *DPYD*9A* genotype available to the treating oncologist while undergoing treatment. The status of DPYD*9A was not available for the rest of the patients with homozygous *DPYD*9A* genotype until this updated analysis was performed in September 2018. At that time, all patients with homozygous *DPYD*9A* genotype had already received 4 or more cycles of fluoropyrimidines-based chemotherapy regimens. The patient who was diagnosed before 2015 experienced Grade 3 diarrhea on day 3 of cycle 1 adjuvant xeloda [capecitabine] and oxaliplatin (XELOX) given for stage III colon adenocarcinoma. Capecitabine was stopped immediately after the experienced toxicity. Because of toxicity, the planned adjuvant chemotherapy (XELOX) was discontinued and surveillance was started for the patient. Unfortunately, the patient developed disease recurrence 2 years later. Since then, alternate regimens that omit fluoropyrimidines (irinotecan and oxaliplatin and bevacizumab) and trifluridine and tipiracil have been used.

Our study has several limitations. This study was a retrospective study and there are inherent limitations with a retrospective analysis of this sort, particularly regarding selection bias. The decision to genotype patients for *DPYD* was at the discretion of the treating oncologist. The selection bias was more pronounced before 2015. At that time *DPYD* genotyping, including *DPYD*9A* genotype, was only performed for patients experiencing fluoropyrimidines-associated toxicities or when the treating oncologist had concerns about patients' fitness to handle potential toxicities because of their age or the presence of significant comorbidities. After 2015, routine upfront genotyping for *DPYD* was adopted more by the treating oncologists but not all patients treated with fluoropyrimidines were genotyped. After 2015, *DPYD* genotyping did not include *DPYD*9A* genotyping. When *DPYD*9A* genotyping was done retrospectively in September 2018, it was carried out only in patients who had their DNA available at ARUP Laboratories at the time of routine *DPYD* genotyping.

Misclassification bias is a systematic error due to incorrect categorization. The process of attributing an experienced toxicity to 5-FU or capecitabine when they were part of fluoropyrimidines-based chemotherapy regimens was quite challenging sometimes. Every effort was made to make that attribution as accurate as possible. For example, in patients treated with leucovorin, fluorouracil and irinotecan (FOLFIRI), if diarrhea improved after reducing the dose of irinotecan and keeping the same dose of 5-FU, it was attributed to irinotecan and not 5-FU. In patients who tolerated 5-FU as part of first line FOLFIRI and bevacizumab and developed neutropenia or neuropathy with second-line leucovoring, fluorouracil and oxaliplatin, and bevacizumab, the neutropenia or neuropathy were attributed to oxaliplatin and not 5-FU or bevacizumab.

This study represents a single-institution experience. The cohort included patients diagnosed with different and heterogenous primary tumors. The treating oncologist had different genotyping strategies and the selected treatment included several different fluoropyrimidine-based regimens. The oncologists followed the recommended dose management guidelines per package insert when they managed fluoropyrimidines-associated toxicities. However, they still had a degree of variation in their practice. All of these factors need to be kept in mind when interpreting our updated analysis.

Conclusion

In our updated analysis, the prevalence of heterozygous and homozygous *DPYD*9 A*genotypes were 41% and 10%, respectively. Among patients with *DPYD*9A* genotypes (n = 57), men and women represented 30 (53%) and 27 (47%) patients, respectively. Caucasian, African American, and other ethnicities represented 29 (50.9%), 26 (45.6%), and 2 (3.5%) patients, respectively. The correlation between *DPYD*9A* genotype and DPD clinical phenotype was not reproduced. The noticeable correlation that we previously reported is likely because of small sample size and selection bias. Several patients with homozygous *DPYD*9A* genotype received full-dose fluoropyrimidines and tolerated treatment very well.

Clinical Practice Points

- In our previous study of a cohort of 28 patients, *DPYD*9A* was the most commonly diagnosed variant (46%) and there was a noticeable genotype-phenotype correlation.
- In this study we genotyped a larger cohort of a mixed racial background to explore the prevalence of *DPYD*9A* variant and to confirm the genotype-phenotype correlation.
- In this updated analysis, the prevalence of heterozygous and homozygous *DPYD*9A* genotypes were 41% and 10%, respectively; the correlation between *DPYD*9A* genotype and DPD clinical phenotype was not reproduced.
- The noticeable correlation that we previously reported is likely because of small sample size and selection bias.

Acknowledgments

Secondary (non-clinical) testing was supported by an internal grant from ARUP Laboratories.

Disclosure

The authors have stated that they have no conflicts of interest.

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