Original Article

Germline pharmacogenomics of DPYD*9A (c.85T>C) variant in patients with gastrointestinal malignancies treated with fluoropyrimidines

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Background: The correlation between DPYD*9A (c.85T>C) genotype and dihydropyrimidine dehydrogenase (DPD) deficiency clinical phenotype is controversial. Reference laboratories either did not perform DPYD*9A genotyping or have stopped DPYD*9A genotyping and limited genotyping to high-risk variants (DPYD*2A, DPYD*13 and DPYD*9B) only. This study explored DPYD*9A genotype and clinical phenotype correlation in patients with gastrointestinal (GI) malignancies treated with fluoropyrimidines.

Methods: Between 2011 and 2017, 67 patients with GI malignancies were genotyped for DPYD variants. Fluoropyrimidines-associated toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0). Fisher's exact test was used for statistical analysis.

Results: DPYD variants were identified in 17 out of 67 (25%) patients. One patient was homozygous for DPYD*9A variant and one patient was double heterozygous for DPYD*9A and DPYD*9B variants. In patients with identified DPYD variants, 13/17 (76%) patients had DPYD*9A variant, 3/17 (18%) patients had DPYD*2A variant and 2/17 (12%) patient had DPYD*9B variant. Only patients genotyped prior to 2015 were genotyped for DPYD*9A variant (N=28). Of those, 13/28 patients (46%) had DPYD*9A variant. Grade 3–4 diarrhea was associated with DPYD*9A variant in patients treated with full dose fluoropyrimidines (P=0.0055).

Conclusions: In our cohort, DPYD*9A variant was the most common diagnosed variant. The correlation between DPYD*9A genotype and DPD deficiency in clinical phenotype was noticeable in patients who received full dose fluoropyrimidines as they all experienced grade 3–4 toxicities (diarrhea).

Keywords: Germline pharmacogenomics; dihydropyrimidine dehydrogenase (DPD); DPYD*9A (c.85T>C) variant; fluorouracil (FU); gastrointestinal malignancy

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Introduction

Fluoropyrimidines includes intravenous fluorouracil (FU), its oral pre-prodrug capecitabine and the oral prodrug tegafur [component of tegafur-uracil and Teysuno (S1)] (1,2). Fluoropyrimidines are considered the cornerstone of most chemotherapeutic regimens approved for the treatment of gastrointestinal (GI) tract tumors. They are also commonly used in the treatment of other types of solid malignancies including breast and head and neck cancers (3,4).

Dihydropyrimidine dehydrogenase (DPD) is an enzyme (EC 1.3.1.2) encoded by DPYD gene. DPD is the rate-limiting enzyme for catabolism of uracil, thymine and their analogue fluoropyrimidine and eliminates >80% of administered or formed FU (5). Generally, treatment of cancer patients with fluoropyrimidines is relatively well tolerated. However, around 5–10% of the treated patients develop severe, potentially life threatening toxicity such as GI toxicity, skin toxicity, myelosuppression and neurotoxicity (6-8). Among patients with DPD deficiency, the incidence of grade 3 or greater toxicities has been reported to be as high as 88%. In those patients, fluoropyrimidines can be fatal (9-12). Moreover, toxicity develops significantly earlier in patients with low DPD activity than in patients with normal DPD activity (13).

The prevalence of DPD deficiency in Caucasian is approximately 3–5% (14). Profound deficiency of DPD is less frequent occurring in approximately 0.2% of individuals (15-20). Molecular analysis of patients with DPD deficiency has identified over 128 mutations and polymorphisms in the DPYD gene that may result in partial or total loss of DPD activity (17,21,22). Three DPYD variants (DPYD*2A, DPYD*13 and DPYD*9B) have consistently been reported to be associated with fluoropyrimidines-associated toxicity and impaired DPD enzyme activity (12,21,23-26)

The correlation between DPYD*9A (c.85T>C) genotype and DPD deficiency clinical phenotype is controversial (27,28). Reference laboratories either did not perform DPYD*9A genotyping or have stopped DPYD*9A genotyping and limited genotyping to high-risk variants (DPYD*2A, DPYD*13 and DPYD*9B) only. DPYD*9A (c.85T>C) variant was the most common variant diagnosed in our cohort and a genotype-clinical phenotype correlation was noticeable. Thus, here we explored DPYD*9A genotype and clinical phenotype correlation in patients with GI malignancies treated with fluoropyrimidines.

Methods

Patient population

This is a retrospective study conducted at the University of South Alabama Mitchell Cancer Institute in Mobile, Alabama, USA. Cohort was identified through searching our cancer center tumor registry for patients genotyped for DPYD variants between 2011 and 2017. 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 de-identified data (IRB #836682-3).

DPYD Genotyping

Germline DNA was obtained from peripheral blood specimen and genotyped for DPYD variants in ARUP laboratories (Salt lake city, UT, USA) or LabCorp laboratories (Burlington, NC, USA) depending on patients' specific health insurance. 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 2017, 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)]. LabCorp Laboratories provided results for IVS14+1G>A (DPYD*2A) only. 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 was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 3.0) (29). Association between dichotomous fluoropyrimidine-related toxicities and DPYD variants status 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, California, USA).

Results

Patients characteristics

Between 2011 and 2017, a total of 67 patients with GI

Table 1 Patients baseline characteristics (N=67)

Characteristics	Number subject, N [%]
Age (years)	
Median [range]	60 [30–87]
Sex	
Female	33 [49]
Male	34 [51]
Ethnicity	
African American	15 [22]
Hispanic	1 [2]
White	51 [76]
Diagnosis	
Anal SCC	5 [7]
Cholangiocarcinoma	1 [2]
Colon adenocarcinoma	33 [49]
Esophageal adenocarcinoma	1 [2]
Gastric adenocarcinoma	4 [6]
Neuroendocrine tumor (SB)	1 [2]
Pancreatic adenocarcinoma	3 [4]
Rectal adenocarcinoma	19 [28]
Chemotherapy regimen	
Fluorouracil-based	34 [51]
Capecitabine-based	33 [49]

SCC, squamous cell carcinoma; SB, small bowel.

malignancies were genotyped for DPYD variants. The baseline characteristics of the patients are summarized in *Table 1*. Median age is 60 years. Males represented 51% of the patients while females represented 49%. In our cohort, 76% were Caucasian, 22% were African Americans and 2% were Hispanics. Colon adenocarcinoma represented the most common malignancy in our cohort. Other patients had anal squamous cell carcinoma (SCC), cholangiocarcinoma, esophageal adenocarcinoma, gastric adenocarcinoma, neuroendocrine tumor of the small bowel (jejunum), pancreatic adenocarcinoma and rectal adenocarcinoma. A Fluorouracil-based chemotherapy regimen was administered in 34 (51%) patients while 33 (49%) patients received a capecitabine-based chemotherapy regimen.

DPYD Genotyping

In 24 patients (36%), the treating oncologist considered DPYD genotyping prior to the initiation of treatment with fluoropyrimidines. Such decision to perform upfront genotyping was mainly done due to concerns about patients' fitness to handle potential toxicities due to their age or the presence of significant comorbidities. The other, 43 patients (64%), were genotyped for DPYD after they have experienced grade 3 or greater toxicities.

All patients (N=67) were genotyped for IVS14+1G>A (DPYD*2A) variant. Genotyping for DPYD c.1679T>G (DPYD*13A) and DPYD c.2846A>T (DPYD*9B) variants was performed in 55 patients (82%) only. Genotyping for DPYD c.85T>C (DPYD*9A) and DPYD c.1590T>C variants was performed in 28 patients (42%) only. The strategy of genotyping by the treating oncologist and the DPYD variants genotyped are summarized in *Table 2*.

DPYD genotyping analysis

The genotyping analysis of the patients included in our cohort is summarized in *Table 2*. DPYD variants were identified in 17 out of 67 patients (25%). One patient was homozygous for DPYD*9A (c.85T>C) variant and one patient was double heterozygous for DPYD*9A (c.85T>C) and DPYD*9B variants. The remaining of the patients were heterozygous. Among the patients with identified DPYD variants (N=17), 13 (76%) patients had DPYD c.85T>C (DPYD*9A) variant, 3 (18%) patients had DPYD IVS14+1G>A (DPYD*2A) variant and 2 (12%) patient had DPYD c.2846A>T (DPYD*9B) variant. Neither DPYD*13A nor DPYD c.1590T>C variants were identified.

Only 28 patients were genotyped for DPYD*9A (c.85T>C) variant since LabCorp laboratories did not perform DPYD*9A genotyping and since ARUP laboratories have stopped DPYD*9A genotyping and limited genotyping to high-risk variants (DPYD*2A, DPYD*13 and DPYD*9B) only. Among patients screened for DPYD*9A (c.85T>C) variant (N=28), 13 patients (46%) had DPYD*9A (c.85T>C) variant and 1 (4%) patient had DPYD IVS14+1G>A (DPYD*2A) variant. None of the other variants (DPYD*9B, DPYD*13A and DPYD c.1590T>C) were identified.

Adverse events

The frequency of grade 1–2 and grade 3–4 toxicities in

Table 2 The strategy of genotyping by the treating oncologist and the DPYD variants genotyped

Characteristics	Number subject, N [%]
Testing Strategy	
Upfront	24 [36]
After experiencing toxicities	43 [64]
Tested variants	
IVS14+1G>A [DPYD*2A]	67 [100]
c.1679T>G [DPYD*13A]	55 [82]
c.2846A>T [DPYD*9B]	55 [82]
c.85T>C [DPYD*9A]	28 [42]
c.1590T>C	28 [42]
Mutation status	
Wild type	50 [75]
Mutant DPYD	17 [25]
Mutant variant	
IVS14+1G>A [DPYD*2A]	3/17 [18]
c.1679T>G [DPYD*13A]	0/17 [0]
c.2846A>T [DPYD*9B]	2[#]/17 [12]
c.85T>C [DPYD*9A]	13[#]/17 [76]
c.1590T>C	0 [0]
Alleles involved	
Homozygous	1/17 [6]
Heterozygous	15/17 [88]
Double heterozygous	1/17 [6]

[#], one patient had double heterozygous status for DPYD*9A and DPYD*9B variants

DPYD-mutant patients and DPYD-wild type patients is 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 both DPYD-mutant patients and DPYD-wild type patients was diarrhea.

The frequency of grade 1–2 and grade 3–4 toxicities in patients with DPYD*9A, DPYD*2A and DPYD*9B variants is summarized in *Table 4*. In our cohort, 8 patients out of 13 patients (62%) with DPYD*9A (c.85T>C) variant developed grade 3–4 diarrhea. The five patients who did not develop grade 3–4 diarrhea received dose reduced chemotherapy as they were genotyped by the treating oncologist prior to initiation of treatment. All patients with DPYD*2A (N=3) and DPYD*9B (N=2) received full dose chemotherapy and experienced grade 3–4 diarrhea. Of note, 3 patients with DPYD*9A variant developed grade 3–4 skin toxicity. The skin toxicity in 2 of those 3 patients was manifested as balanitis.

Statistical analysis

In all patients, grade 3–4 diarrhea was associated with DPYD mutant status (any variant) (P=0.0045). In patients genotyped for DPYD*9A (c.85T>C) variant (N=28), patients with DPYD*9A (c.85T>C) variant tend to experience more grade 3–4 diarrhea (62%) compared to patients with DPYD*9A (c.85T>C) wild-type (36%). However, the association between DPYD*9A (c.85T>C) status and grade 3–4 diarrhea did not reach statistical significance (P=0.256) likely due to small sample size. In patients genotyped for DPYD*9A and received full dose fluoropyrimidines (N=23), grade 3–4 diarrhea was associated with DPYD*9A (c.85T>C) variant (P=0.0055). The association between grade 3–4 diarrhea and patients genotyped for any DPYD variant (*2A, *13A, *9B, *9A and /or DPYD c.1590T>C) and patients genotyped for DPYD*9A (c.85T>C) variant is summarized in *Table 5*.

Discussion

The correlation between DPYD*9A (c.85T>C) genotype and impaired DPD activity has been demonstrated in laboratory and clinical studies. In *Escherichia coli*, DPYD*9A (c.85T>C) mutation lead to a mutant DPD protein (C29R) leading to significant decrease in enzymatic activity (30). In patients with DPYD*9A (c.85T>C) variant, DPD activity has been reported to be decreased (27,31,32). In one study of patients with GI malignancies, DPYD*9A (c.85T>C) variant was associated with fluoropyrimidines-associated toxicity. Patients experienced diarrhea (P<0.05) and hand foot syndrome (HFS) (P<0.05) (27).

Understandably so, the correlation between DPYD*9A (c.85T>C) genotype and impaired DPD activity continued to be controversial as other clinical studies reported no correlation between DPYD*9A (c.85T>C) genotype and DPD deficiency clinical phenotype (33-35). Moreover, additional studies suggested that DPYD*9A (c.85T>C) may serve as a protective allele against fluoropyrimidines-associated toxicity (28). Based on the current limited knowledge, the 2017 updated Clinical Pharmacogenetics

Table 3 The frequency of grade 1–2 and grade 3–4 toxicities in DPYD-mutant patients and DPYD-wild type patients

Adverse events	DPYD mutant (N=17)		DPYD wild type (N=50)	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Hematological				
Neutropenia	9 [53]	1 [6]	14 [28]	2 [6]
Anemia	7 [41]	0 [0]	15 [30]	0 [0]
Thrombocytopenia	3 [18]	0 [0]	5 [10]	0 [0]
Neutropenic fever	–	1 [6]	0 [0]	0 [0]
Non-hematological				
Mucositis	3 [18]	1 [6]	7 [14]	2 [4]
Nausea	9 [53]	0 [0]	11 [22]	3 [6]
Vomiting	6 [35]	0 [0]	4 [8]	3 [6]
Diarrhea	1 [6]	12 [71]	8 [16]	15 [30]
Neurotoxicity	0 [0]	0 [0]	3 [6]	1 [2]
Skin toxicity	2 [12]	3 [18]	4 [8]	5 [10]

Table 4 The frequency of grade 1–2 and grade 3–4 toxicities in patients with DPYD*9A, DPYD*2A and DPYD*9B variants

Adverse events	DPYD Mutant (N=17), N [%]					
	DPYD*9A (N=13[#])		DPYD*2A (N=3)		DPYD*9B (N=2[#])	
	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4	Grade 1–2	Grade 3–4
Hematological						
Neutropenia	6 [46]	0 [0]	2 [67]	1 [33]	1 [50]	0 [0]
Anemia	6 [46]	0 [0]	1 [33]	0 [0]	1 [50]	0 [0]
Thrombocytopenia	1 [8]	0 [0]	1 [33]	0 [0]	1 [50]	0 [0]
Neutropenic fever	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
Non-hematological						
Mucositis	2 [15]	1 [8]	1 [33]	0 [0]	0 [0]	0 [0]
Nausea	7 [54]	1 [8]	1 [33]	0 [0]	2 [100]	0 [0]
Vomiting	5 [38]	1 [8]	0 [0]	0 [0]	2 [100]	0 [0]
Diarrhea	1 [8]	8 [62]	0 [0]	3 [100]	0 [0]	2 [100]
Neurotoxicity	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]	0 [0]
Skin toxicity	1 [8]	3 [23]	1 [33]	0 [0]	0 [0]	1 [50]

[#], one patient had double heterozygous status for DPYD*9A and DPYD*9B variants.

Implementation Consortium (CPIC) guideline for DPD genotype and fluoropyrimidine dosing, it was stated that the DPYD*9A (c.85T>C), among other variants, doesn't affect DPD activity in a clinically relevant manner (36).

In our cohort, a correlation between the DPYD*9A (c.85T>C) variant genotype and DPD deficiency clinical phenotype was noticeable. All patients (N=8) who received full dose fluoropyrimidines experienced grade 3–4 diarrhea.

Table 5 The association between grade 3–4 diarrhea and patients genotyped for any DPYD variant (*2A, *13A, *9B, *9A and/or DPYD c.1590T>C) and patients genotyped for DPYD*9A variant. Statistical analysis was performed using Fisher's exact test

Patients	Grade 3–4 diarrhea, N [%]	P
Patients genotyped for any variant (*2A, *13A, *9B, *9A and /or DPYD c.1590T>C) (N=67)		0.0045
DPYD Mutant (any variants) (N=17)	12/17 [71]	
DPYD wild-type (N=50)	15/50 [30]	
Patients genotyped for DPYD*9A (N=28), all patients (N=28)		0.256
Patients with DPYD*9A variant (N=13)	8/13 [62]	
Patients with DPYD*9A wild type (N=14[#])	5/14 [36]	
Received full dose chemotherapy (N=23)		0.0055
Patients with DPYD*9A variant (N=8)	8/8 [100]	
Patients with DPYD*9A wild type (N=14[#])	5/14 [36]	

[#], one patient had DPYD*2A variant.

Among those patients, three patients, in addition to diarrhea, developed skin toxicity manifested as balanitis in two patients and HFS in one patient. None of the patients with DPYD*9A (c.85T>C) variant developed grade 3–4 hematological toxicities.

In our cohort of patients with DPYD*9A (c.85T>C) variant (N=13), only one patient was found to be homozygous for DPYD*9A. This patient experienced grade 3 diarrhea on day 3 of cycle #1 adjuvant XELOX given for stage III colon adenocarcinoma. Capecitabine was stopped immediately after the experienced toxicity. Due to toxicity, the planned adjuvant chemotherapy (XELOX) was discontinued and the patient was started on surveillance. Unfortunately, the patient developed disease recurrence 2 years later. Since then alternate regimens that omit fluoropyrimidines (IROX and bevacizumab) and trifluridine and tipiracil have been used.

One patient was found to be double heterozygous for DPYD (DPYD*9A (c.85T>C) and DPYD*9B). This patient experienced grade 3 balanitis toward the end of neoadjuvant concurrent chemoradiation with capecitabine given for stage II rectal adenocarcinoma. DPYD genotyping was not considered at that time. After surgical resection, the patient was started on adjuvant chemotherapy with XELOX. Shortly after cycle #2, he experienced grade 3 diarrhea. Genotyping for DPYD was considered then and revealed double heterozygous status. The treating oncologist continued the planned adjuvant chemotherapy but he reduced the dose of capecitabine by 50%. The patient completed the rest of the planned adjuvant chemotherapy and it was well tolerated. Patient continues to do well without evidence of disease recurrence.

The remaining eleven patients were found to be heterozygous for DPYD variants. Five patients were diagnosed to have heterozygous DPYD*9A (c.85T>C) variant prior to initiation of therapy. In those patients fluoropyrimidines-based regimens were administered at a reduced dose followed by dose adjustment at the discretion of the treating oncologist discretion. Treatment was well tolerated. Six patients were diagnosed to have heterozygous DPYD*9A (c.85T>C) variant after initiation of full dose fluoropyrimidines-based regimens and after they experienced grade 3–4 toxicity. Subsequently, fluoropyrimidines-based regimens were administered at a reduced dose followed by dose adjustment at the discretion of the treating oncologist discretion. Treatment was well tolerated.

In addition to the noticeable correlation between DPYD*9A (c.85T>C) variant genotype and clinical phenotype of DPD deficiency, our study represents one other study that shows the association between diarrhea and DPYD*9A (c.85T>C) variant. Joerger *et al.*, reported that grade 1–4 diarrhea was associated with DPYD*9A (c.85T>C) in patients with gastroesophageal cancer (p=0.0023). In the same study grade 1–4 HFS was associated with DPYD*9A (c.85T>C) variant in patients with colorectal cancer (P=0.0033) (27).

The current genotyping strategy adopted by ARUP laboratories (that limits genotyping to high risk variants only) and LabCorp (that limits genotyping to DPYD*2A variant only) has several limitations. High-risk variants (DPYD *2A, *13 and *9B) were identified in only 30% of patients who developed grade 3–4 toxicities

after exposure to fluoropyrimidines (21). The Clinical Pharmacogenetics Implementation Consortium concluded that 23–38% of severe fluoropyrimidines-associated toxicity could be attributed to DPYD variants (clinical sensitivity approximately 31%) (37). In a prospective study, DPYD*2A variant was identified in only 5% of patients experiencing grade 3–4 fluoropyrimidines-associated toxicity. Moreover, less than 50% of patients with DPYD*2A variant developed grade 3–4 toxicity (the positive predictive value was 46%) (26). In another study, only 6% of patients experiencing grade 3–4 toxicity had a high-risk mutant DPYD variant but the positive predictive value was >99% (38). Moreover, it is important to recognize that DPD deficiency has been identified in patients with wild-type DPYD alleles probably due to epigenetic mechanisms (39,40).

The limitations observed by the current adopted genotyping strategy, that limit screening to DPYD*2A variant only or high-risk variants (DPYD *2A, *13 and *9B), are important to recognize. The patients treated with fluoropyrimidines and their treating oncologist would certainly benefit from adopting a better screening approach for DPD deficiency. The controversy about the correlation between DPYD*9A (c.85T>C) genotype and DPD deficiency clinical phenotype is understandable. However, with the available data that show DPYD*9A (c.85T>C) genotype-clinical phenotype correlation, the decision of not including DPYD*9A (c.85T>C) variant in the screening panel maybe premature at this point. Our study showed that DPYD*9A (c.85T>C) variant genotyping has helped medical oncologists in the clinic to identify patients with an underlying genetic alteration that could predispose them to experience grade 3–4 fluoropyrimidines-associated toxicity. If our cohort was only genotyped for DPYD*2A or high-risk variants only, many patients would have been undiagnosed and their outcomes could have been different. By including DPYD*9A (c.85T>C) variant to the screening panel by reference laboratories, the oncology community would have more data about its clinical significance.

Our study has several limitations. This study is a retrospective study and there are inherent limitations and selection bias associated with a retrospective analysis of this sort, particularly regarding the oncologist discretion to conduct upfront screening in particular subjects. In 24 patients (36%), the treating oncologist considered DPYD genotyping prior to the initiation of treatment with fluoropyrimidines mainly due to concerns about patients' fitness to handle potential toxicities due to their age or the presence of significant comorbidities. Five patients with DPYD*9A variants were genotyped prior to initiation of treatment.

This study represents a single institution experience and the sample size is relatively small. Moreover, there is significant heterogeneity in the patient population, including inclusion of multiple different tumor types and a variety of fluoropyrimidine-based regimens (many of which were, presumably, chemotherapy combinations that may have differentially contributed to the observed toxicity). Despite the correlative findings that we were able to generate, studies of larger cohorts and ideally conducted prospectively will likely provide more solid data about such genotype and clinical phenotype correlation. Overall, our study should be considered hypothesis-generating rather than definitive due to its limitations.

Conclusions

DPD enzyme deficiency is a pharmacogenetic syndrome associated with dose-limiting toxicity to fluoropyrimidines. In our cohort, DPYD*9A (c.85T>C) variant was the most common diagnosed variant. The correlation between DPYD*9A genotype and DPD deficiency clinical phenotype was noticeable in patients who received full dose fluoropyrimidines as they all experienced grade 3–4 toxicities (diarrhea). Oncologists should consider DPYD genotyping for patients experiencing grade 3–4 diarrhea after exposure to fluoropyrimidines. The prevalence of partial DPD deficiency in the general population is approximately 3–5%. Genotyping for high-risk DPYD variants (*2A, 13A and 9B) only has several limitations and is suboptimal as it leaves most patients with DPD deficiency undiagnosed. A more comprehensive approach would include testing for additional DPYD variants [including but not limited to DPYD*9A (c.85T>C) variant] or ideally screening the entire coding region and potentially additional regions responsible for regulating DPYD gene expression and translation. Despite the correlative findings that we were able to generate, studies of larger cohorts and ideally conducted prospectively will likely provide more data about DPYD*9A genotype and clinical phenotype correlation.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The University of South Alabama Institutional Review Board (IRB) (No. 836682-3) approved this study and the IRB-approved database provided a waiver of the requirement for informed consent and allowed for publication of de-identified data.

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