Case Report

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Pharmacogenomics

Case report: severe toxicity in an African–American patient receiving FOLFOX carrying uncommon allelic variants in *DPYD*

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Cancers of the colon are commonly treated with fluoropyrimidines, which often cause severe toxicities in patients with certain variants in *DPYD*. Y186C (rs115232898) and a variant in the 3′ untranslated region (rs12132152) are uncommon alleles previously observed in African–Americans. An African–American female underwent 5-fluorouracil-based therapy (400 mg/m^2 bolus, 1200 mg/m^2/day over 46 h). The patient experienced severe pancytopenia after the first cycle. After 5-fluorouracil (5-FU) dose reduction (600 mg/m^2/day), the steady-state 5-FU plasma concentration became 474 ng/ml (range 301–619 ng/ml) and increased following a subsequence dose increase (800 mg/m^2/day; 1248 ng/ml). After a 1000 mg/m^2/day dose resulted in myelosuppression, 5-FU was again de-escalated for the remaining cycles (600 mg/m^2). The observed complications are likely a function of uncommon genetic variants that affect DPYD metabolism.

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Fluoropyrimidines are major constituents of most combinations of chemotherapy used to treat colorectal cancer, and these medications are generally well tolerated, with few patients developing severe toxicities [1]. Of those who develop severe toxicity, approximately 30–50% are not found to harbor DPYD deficiency [2], although this estimate is likely too high given that most have not studied all *DPYD* variants with functional consequences. Conversely, 50–88% of those carrying a known variant in *DPYD* experience grade III–IV fluoropyrimidine-related toxicity [2], suggesting discovery of new DPYD variants is urgently needed. However, 50–88% of individuals harboring function-reducing genetic variants in *DPYD* are at risk of developing severe hematological toxicities, mucositis and diarrhea associated with fluoropyrimidine treatment [2]. Moderate to strong evidence indicates that ten genetic variants in *DPYD* are associated with a reduction in the rate of fluoropyrimidine metabolism, and at least 17 other *DPYD* genotypes may reduce DPYD function [3]. Several of these variants are observed in specific racial populations, and understanding of genetic variability in specific racial populations will be crucial for future reductions in fluoropyrimidine toxicity. We present a patient who underwent FOLFOX therapy for a colorectal malignancy who developed profound pancytopenia following the first dose of therapy at the NIH Clinical Center in Bethesda, Maryland.

Case presentation

A 63-year old African–American female with newly diagnosed metastatic colon cancer to bilateral lungs, mesenteric and pelvic lymph nodes status post bowel resection and diverting colostomy. Pathology revealed pT4apN1c, mismatch repair proficient, KRAS G12V mutant, BRAF wildtype adenocarcinoma. Past medical history otherwise unremarkable with the exception of a recent pulmonary embolism. Following consent and enrollment onto a clinical trial (ClinicalTrials.gov: NCT03050814), she initiated treatment on the standard of care arm consisting of the following mFOLFOX6 regimen every 2 weeks for 12 planned cycles: oxaliplatin 85 mg/m^2; leucovorin 400 mg/m^2; 5-FU bolus 400 mg/m^2; and 5-FU continuous infusion 2400 mg/m2 (1200 mg/m^2/day) over 46 h.

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Cycle 1 chemotherapy infusions were administered without incident and growth factor support was not given prophylactically. Two weeks later, the patient presented for cycle 2 with complaints of fatigue, mucositis, dysphagia, indigestion and mild nausea controlled with ondansetron. On physician exam she was found to have oral mucositis with a low-grade temperature of 37.8°C. Laboratory results were significant for an ANC of 0 K/μl, WBC 1.09 K/μl and platelets 18 K/μl. Hepatic and renal function were within normal limits. The patient was admitted for intravenous antibiotics, empiric candidiasis treatment and supportive care.

When the patient's WBC count recovered sufficiently to allow DNA extraction, we undertook to genotype genes involved in the metabolism and elimination of 5-FU using the DMET Plus® Array (Thermo Scientific, MA, USA) that tests 1936 variants in 235 different ADME genes [4]. We particularly focused on genetic variants in *DPYD* (18 allelic variants; Table 1), although we also evaluated genes previously associated with 5-FU or oxaliplatin clinical outcome: *ABCB1, CES2*, *GSTP1* and *TYMS* [5,6]. No variants that clearly affect the metabolism or transport rate of any of these genes were detected (Table S1) [3,5–7]. The patient was heterozygous for the I105V variant in *GSTP1* (rs1695) that has been associated with increased incidence of thrombocytopenia in patients treated with capecitabine and oxaliplatin [8]; however, insufficient evidence exists linking this SNP to oxaliplatin-related toxicities [9].

After the patient fully recovered, she was rechallenged with the following changes to the regimen for cycle 2: no 5-FU bolus; 50% 5-FU infusion dose reduction (1200 mg/m^2); approximately 40% oxaliplatin dose reduction (50 mg/m^2) and pegfilgrastim (Table 2). To ascertain whether the patient had low clearance of 5-FU, plasma samples were collected 2, 4 and 24 h after the start of the infusion, and 5-FU plasma concentration was monitored using a previously developed Liquid chromatography–mass spectrometry–mass spectrometry (LC/MS/MS) assay [11]. The steady-state 5-FU plasma concentration during the cycle 2 infusion was 474 ng/ml (range, 301–619 ng/ml; Table 3), which approximates the average 5-FU plasma concentration of those administered over twice that dose [12]. Nevertheless, neutropenia was not observed at this dose.

The patient presented for cycle 3 with no significant complaints of adverse events and there were no notable laboratory abnormalities. Given cycle 2 was tolerated exceedingly well but the underlying cause of the profound toxicities associated cycle 1 was still enigmatic, the oxaliplatin and 5-FU continuous infusion doses were slowly escalated (Table 2). After cycle 3 began (24 h), we observed a high 5-FU plasma concentration (1248 ng/ml; Table 3). Ultimately, the 5-FU was once again de-escalated due to severe myelosuppression following the 2000 mg/m^2 dose. Starting with cycle 5, the patient received oxaliplatin 65 mg/m^2 and 5-FU infusion 1200 mg/m^2. She tolerated this dose well and completed the remainder of the planned 12 cycles with stable disease. At that time, the patient was transitioned to capecitabine maintenance treatment with a preemptive 50% dose reduction. On day 8 of capecitabine, the ANC fell to 0.570 K/μl from 1.96 K/μl 7 days prior. After a discussion of the risks and benefits, the patient opted to continue the 5-FU infusion instead of capecitabine at the last tolerated dose and continues to have stable disease on maintenance treatment.

The Pharmacoscan array (Thermo Scientific) includes several important genetic variants that are not present on the DMET Plus array but are still associated with reduced DPYD function: 1156G >T (*DPYD*12*, rs78060119; a very rare allele), 1129-5923C >G (*DPYD HapB3*, rs75013182, rs56038477 and rs56276561), *DPYD* 2846A >T (rs67376798) and *DPYD* 557A >G (Y186C; rs115232898) [3]. Pharmacoscan also contains additional variants in other genes we tested and three additional genes involved in fluorouracil and oxaliplatin pharmacology: *CES1*, *ERCC2* and *MTHFR* [5,6]. Since several variants remained untested via DMET, we genotyped the patient again using the Pharmacoscan array instead.

Pharmacoscan detected the *DPYD* Y186C variant (rs115232898), an uncommon allele (minor allele frequency (MAF) = 0.032 in African–Americans, MAF = 0.045 in African populations, MAF = 0.006 in the ad mixed American population, and not observed in East or South Asians or European; the latter five population estimates are derived from the 1000 Genomes Project) [13,14] that causes a 46% decrease in the DPYD-mediated fluoropyrimidine metabolic rate [14]. Contrary to the DMET Plus results, the Pharmacoscan array phenotype-interpretation software indicated the patient was actually an intermediate 5-FU metabolizer. She was also heterozygous for a SNP in the 3′ untranslated region of *DPYD* (rs12132152), an uncommon variant (MAF = 0.0061 in African–Americans) [13] that has also been associated with fluorouracil toxicity [6,10]. Thus, although there is only a single report on the latter allele, this patient may be predisposed to fluorouracil toxicity from more than one rare variant in *DPYD*. We did not observe any variants in other genes via the Pharmacoscan array that would unequivocally affect gene function and therefore outcome of FOLFOX therapy, including the commonly tested and less-frequently observed *DPYD*2* allele (Table S1) [5,6].

Table 1. *DPYD* genotypes from DMET Plus and Pharmacoscan.

RSID	Variant	DMET Plus genotype	Pharmacoscan genotype
rs4970722	*DPYD*_c.40-3123T>A		T/A
rs72549310	*DPYD*_c.61C>T (R21X)	C/C	C/C
rs80081766	*DPYD*_c.62G>A (R21Q)		G/G
rs1801265	*DPYD*9*_c.85T>C (C29R)	C/C	C/C
rs115632870	*DPYD*_c.151-69G>A		G/G
rs17378539	*DPYD*_c.234-3075A>G		A/A
rs72549309‡	*DPYD*7*_c.295_298delTCAT (F100Frameshift)	TCAT/TCAT	TCAT/TCAT
rs2297595	*DPYD*_c.496A>G (M166V)	A/A	A/A
rs6670886	*DPYD*_c.525G>A (S175=)	G/G	G/G
rs115232898†	*DPYD*_c.557A>G (Y186C)		A/G
rs12119882	*DPYD*_c.680 + 2545T>C		T/T
rs1801266‡	*DPYD*8*_c.703C>T (R235W)	C/C	C/C
rs6675198	*DPYD*_c.756T>C (G252=)	T/T	T/T
rs45589337	*DPYD*_c.775A>G (K259E)		A/A
rs2786507	*DPYD*_c.850 + 26331C>T		C/C
rs10518636	*DPYD*_c.851-38157C>T		C/C
rs2811196	*DPYD*_c.851-18271A>G		A/G
rs72549306	*DPYD*11*_c.1003G>T (V335L)	G/G	G/G
rs1042478	*DPYD*_c.1035T>C (F345=)	T/T	T/T
rs1042479	*DPYD*_c.1074T>A (R358=)	T/T	T/T
rs75017182†	*DPYD*_c.1129-5923C>G (SpliceVariant)		C/C
rs78060119‡	*DPYD*12*_c.1156G>T (E386X)		G/G
rs56038477†	*DPYD*_c.1236G>A (E412=)		G/G
rs2811219	*DPYD*_c.1340-11501T>C		T/C
rs1801158	*DPYD*4*_c.1601G>A (S534N)	G/G	G/G
rs1801159	*DPYD*5*_c.1627A>G (I543V)		A/A
rs55886062‡	*DPYD*13*_c.1679T>G (I560S)	T/T	T/T
rs17116806	*DPYD*_c.1740 + 8030G>T		G/G
rs12136186	*DPYD*_c.1741-16477A>G		A/A
rs3897854	*DPYD*_c.1741-2527G>A		G/G
rs17376848	*DPYD*_c.1896T>C (F632=)	T/T	T/T
rs72549303‡	*DPYD*3*_c.1898delC (P633Frameshift)	C/C	C/C
rs3918289‡	*DPYD*_c.1905C>G/T (N635K/N)	C/C	C/C
rs3918290‡	*DPYD*2*_c.1905 + 1G>A (SpliceVariant)	G/G	G/G
rs4492658	*DPYD*_c.1906-28506C>G		C/G
rs7548189	*DPYD*_c.1906-19696G>T		G/G
rs2152878	*DPYD*_c.1906-5426A>G		A/A
rs72728438	*DPYD*_c.1974 + 75A>G		A/A
rs1801160	*DPYD*6*_c.2194G>A (V732I)		G/G
rs12140120	*DPYD*_c.2300-23459G>A		G/G
rs2027056	*DPYD*_c.2300-23051C>A		A/A
rs6656660	*DPYD*_c.2622 + 9416C>A		C/C
rs7552825	*DPYD*_c.2623-42576G>A		G/G
rs1760217	*DPYD*_c.2623-38806T>C		T/T
rs147545709	*DPYD*_c.2656C>T (R886C)		C/C

†Moderate to strong evidence that bolded genotype is responsible for reduction in DPYD function in the DPYD_allele_functionality_table contained in the Clinical Pharmacogenetics Implementation Consortium (CPIC) DPYD versus 5-fluorouracil guidelines [3].
‡Moderate to strong evidence that bolded genotype is responsible for loss of DPYD function in the DPYD_allele_functionality_table contained in the CPIC DPYD versus 5-fluorouracil guidelines [3].
§Uncommon allele previously associated with fluorouracil toxicity [10].
CPIC: Clinical Pharmacogenetics Implementation Consortium.

Table 1. *DPYD* genotypes from DMET Plus and Pharmacoscan (cont.).

RSID	Variant	DMET Plus genotype	Pharmacoscan genotype
rs1801267	*DPYD*9B*_c.2657G>A (R886H)	G/G	G/G
rs17471125	*DPYD*_c.2767-5102A>G		A/A
rs290852	*DPYD*_c.2767-2165A>G		G/G
rs67376798†	*DPYD*_c.2846A>T (D949V)		A/A
rs1801268‡	*DPYD*10*_c.2983G>T (V995F)	G/G	G/G
rs76387818	*DPYD*_c.*5132C>T (3′UTR)		C/T
rs12132152§	*DPYD*_c.*21528C>T (3′UTR)		C/T
rs479424			G/G

†Moderate to strong evidence that bolded genotype is responsible for reduction in DPYD function in the DPYD_allele_functionality_table contained in the Clinical Pharmacogenetics Implementation Consortium (CPIC) DPYD versus 5-fluorouracil guidelines [3].
‡Moderate to strong evidence that bolded genotype is responsible for loss of DPYD function in the DPYD_allele_functionality_table contained in the CPIC DPYD versus 5-fluorouracil guidelines [3].
§Uncommon allele previously associated with fluorouracil toxicity [10].
CPIC: Clinical Pharmacogenetics Implementation Consortium.

Table 2. Dose and administration of FOLFOX.

Cycle	Chemotherapy regimen	Notable adverse effects reported on D1 of the subsequent cycle
C1	• Oxaliplatin 85 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • 5-FU bolus 400 mg/m^2 iv. bolus • 5-FU 2400 mg/m^2 (1200 mg/m^2/day) iv. continuous infusion over 46 h	Fatigue, mucositis, dysphagia, indigestion, mild nausea, oral candidiasis, temperature 37.8°C, ANC 0 K/μl, WBC 1.09 K/μl and platelets 18 K/μl
C2 (delayed)	• Oxaliplatin 50 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 1200 mg/m^2 (600 mg/m^2/day) iv. continuous infusion over 46 h • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Tolerated well
C3	• Oxaliplatin 65 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 1600 mg/m^2 (800 mg/m^2/day) iv. continuous infusion over 46 h • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Tolerated well
C4	• Oxaliplatin 65 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 2000 mg/m^2 (1000 mg/m^2/day) iv. continuous infusion over 46 hours • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Lip ulcerations ANC 0 K/μl, WBC 1.84 K/μl and platelets 14 K/μl
C5 (delayed)	• Oxaliplatin 65 mg/m^2 iv. infusion over 2 h • Leucovorin 400 mg/m^2 iv. infusion over 2 h • No 5-FU bolus • 5-FU 1200 mg/m^2 (600 mg/m^2/day) iv. continuous infusion over 46 h • Pegfilgrastim 6 mg subq 24 h after completion of chemotherapy	Tolerated well overall This dose continued through C12.

5-FU: 5-Fluorouracil; ANC: Absolute neutrophil count; iv: Intravenous; WBC: White blood cell count.

Table 3. Plasma concentration at timepoints after 5-fluorouracil infusions.

Hours after start of 5-fluorouracil continuous infusion	Plasma concentration (ng/ml)
Cycle 2	
– 2	301
– 4	619
– 24	501
– Average	474
Cycle 3	
– 24	1248

Discussion

The severe pancytopenia and high fluorouracil plasma concentration this patient experienced is likely related to the inheritance of an uncommon variant in *DPYD*, Y186C (rs115232898), that is observed in African–Americans and significantly reduces the metabolic rate of fluorouracil [14,15]. Although little clinical evidence is available for another uncommon SNP in the 3′-UTR (rs12132152) of *DPYD*, this polymorphism may have also contributed to such toxicity [5,8,10]. Similar to a previous case report, the present patient was heterozygous for the C29R polymorphism (*DPYD*9* [15]); however, several other reports indicate that this variant is associated with normal DPYD function [3,14]. The present data adds to emerging evidence that the Y186C variant is important in patients with African origins who are receiving 5-FU, and future studies should evaluate the consequences of rs12132152 on interindividual variation of DPYD function and its clinical effects on fluoropyrimidine therapy [10,14,15].

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