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Association between *DPYD* c.1129-5923 C>G/hapB3 and severe toxicity to 5-fluorouracil-based chemotherapy in stage III colon cancer patients: NCCTG N0147 (Alliance)

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Abstract

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Lee, A.M., Shi, Q., Alberts, S.R., Sargent D.J., Sinicrope, F.A., Berenberg, J., Goldberg, R.M., Diasio, R.B. HapB3 and the deep intronic variant c.1129-5923 C>G of the dihydropyrimidine dehydrogenase gene (DPYD) as predictors of toxicity in stage III colon cancer (CC) patients (pts) (NCCTG Alliance N0147). Abstract 508. ASCO: GI Symposium. January 17 2015. San Francisco, CA

Severe (grade≥3) adverse events (AEs) to 5-fluorouracil (5-FU)-based chemotherapy regimens can result in treatment delays or cessation, and, in extreme cases, life-threatening complications. Current genetic biomarkers for 5-FU toxicity prediction, however, account for only a small proportion of toxic cases. In the current study, we assessed *DPYD* variants suggested to correlate with 5-FU toxicity, a deep intronic variant (c.1129-5923 C>G) and four variants within a haplotype (hapB3), in 1953 stage III colon cancer patients who received adjuvant FOLFOX +/- cetuximab. Logistic regression was used to assess multivariable associations between *DPYD* variant status and AEs common to 5-FU (5FU-AEs). In our study cohort, 1228 patients (62.9%) reported any grade≥3 AE (overall AE), with 638 patients (32.7%) reporting any grade≥3 5FU-AE. Only 32 of 78 (41.0%) patients carrying *DPYD* c.1129-5923 C>G and the completely linked hapB3 variants c.1236 C>G and c.959-51 T>C displayed at least one grade≥3 5FU-AE, resulting in no statistically significant association ($OR_{adj.}$=1.47, 95%CI=0.90-2.43, p=0.1267). No significant associations were identified between c.1129-5923 C>G/hapB3 and overall grade≥3 AE rate. Our results suggest that c.1129-5923 C>G/hapB3 have limited predictive value for severe toxicity to 5-FU-based combination chemotherapy.

Keywords

dihydropyrimidine dehydrogenase; polymorphism; pharmacogenetics; 5-fluorouracil; colon cancer; toxicity

Severe (grade≥3) adverse events (AEs) to 5-fluorouracil (5-FU)-based chemotherapy regimens can result in treatment delays or cessation, and, in extreme cases, life-threatening complications. 5-FU-related pharmacogenetic studies have traditionally focused on the rate-limiting enzyme of 5-FU catabolism, dihydropyrimidine dehydrogenase (DPD), which is encoded by the *DPYD* gene. To date, only three functionally-deleterious *DPYD* gene variants have been consistently shown to affect DPD activity and correlate with severe 5-FU toxicity: DPYD*2A (c.1905+1 G>A; rs3918290), D949V (c.2846A>T; rs67376798), and I560S (c.1679 T>G, DPYD*13, rs55886062) [1-7]. Though well substantiated, these three variants alone account for a relatively small percentage of severe toxicity in 5-FU-treated patients [8-10], indicating a need to identify other genetic markers that can increase the predictive value of pharmacogenetic testing.

Recent studies have focused on the intronic region of *DPYD* and have identified a haplotype (hapB3) that is detected in a proportion of patients who developed 5-FU toxicity. HapB3 is comprised of three intronic variants (c.483+18 G>A - rs56276561, c.680+139 G>A - rs6668296, and c.959-51 T>C - rs115349832) and one synonymous variant (c.1236 G>A, E412E; rs56038477) [11]. A subsequent study identified a deep intronic variant (c.1129-5923 C>G; rs75017182) in tight linkage with HapB3 that was shown to affect pre-mRNA splicing [12]. Though evidence suggests a correlation between c.1129-5923 C>G / hapB3 and severe 5-FU toxicity [13,14], other studies have shown no significant associations [6, 15-17].

Discrepant results across multiple studies may be partially due to inadequately sized cohorts that include a diversity of cancer types, stages and treatments. Due to previous discrepancies and the need for validation in larger homogenous patient populations uniformly treated with

current standard combination therapies, we genotyped the *DPYD* variants of hapB3 and the deep intronic variant c.1129-5923 C>G in a large cohort of stage III colon cancer patients treated in a randomized adjuvant trial of FOLFOX, alone or combined with cetuximab, to test their individual associations with grade≥3 toxicity.

We utilized whole blood-derived DNA obtained from prospectively collected stage III colon cancer patients in a randomized phase III trial by the North Central Cancer Treatment Group (NCCTG N0147, NCT00079274) [18]. NCCTG is now part of the Alliance for Clinical Trials in Oncology. AEs were assessed biweekly according to NCI, Common Toxicity Criteria (v.3) and classified as common to 5-FU treatment (5FU-AEs) by the study chair who was blinded to SNP data and included: fatigue, anorexia, dehydration, diarrhea, stomatitis/mucositis, nausea/vomiting, leukopenia, neutropenia, febrile neutropenia, thrombocytopenia, and pain [10]. Genotyping was performed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry (Sequenom MassARRAY, San Diego CA) as previously described [10]. Due to the enrichment of *DPYD* c.1129-5923 C>G/hapB3 in the European population, only Caucasian patients treated with FOLFOX, with or without the addition of cetuximab, and with available DNA, were included in this study (n=2017). Additionally, any patient identified as a carrier or with an undetermined status for any of the three deleterious *DPYD* variants (DPYD*2A, D949V, and I560S) were excluded, resulting in a total of 1953 patients available for analysis. Each participant signed an IRB-approved, protocol-specific informed consent in accordance with federal and institutional guidelines.

The primary objective was to associate *DPYD* c.1129-5923 C>G/hapB3 with the rate of AEs commonly attributable to 5-FU treatment (5FU-AE, primary outcome), defined as the proportion of patients with at least one grade 3 or greater (grade≥3) 5FU-AE during the entire course of the treatment. The secondary objective was to associate *DPYD* c.1129-5923 C>G/hapB3 with any grade≥3 AE (overall AE). The frequency for each variant was compared to published frequencies from the 1000 Genomes Project [19], tested for departure from Hardy-Weinberg equilibrium, and assessed for linkage disequilibrium (Haploview) [20]. Chi-squared or Fisher's exact test, unequal variance two-sample t-test, and Wilcoxon rank sum test were used to compare categorical variables, continuous variables, and counts between patients' *DPYD* variant status [21,22]. Logistic regression was used to assess the association between SNP status and AE rates, adjusting for clinicopathological factors [22]. All multivariable models were adjusted for age, sex, performance score, stratification factors (T/N stage and grade), primary tumor site, *KRAS*, *BRAF*, mismatch repair status (MMR), treatment, total number of treatment cycles, and dose modifications. Analyses were based on data frozen at December 3, 2014, and were performed in SAS v9. A two-sided p-value <0.05 was considered statistically significant. Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center.

Patient characteristics in relation to grade≥3 5FU-AEs are provided in Table 1. A total of 1228 patients (62.9%) reported any grade≥3 AE (overall AE), with 638 patients (32.7%) reporting any grade≥3 5FU-AE. Most frequent 5FU-AEs included: diarrhea (12.5%), neutropenia (10.3%), pain (5.4%), fatigue (5.2%), nausea/vomiting (4.7%), and mucositis (4.1%). Older patients were more likely to experience 5FU-AEs than younger patients

(p<0.0001). Women reported higher 5FU-AEs than men (38.1% vs. 28.2%, p<0.0001). Other factors significantly associated with higher rates of 5FU-AEs were N1 (vs. N2) tumors, proximal (vs. distal) tumors, *BRAF* MUT (vs. WT) tumors, and patients receiving cetuximab (vs. not). Patients who discontinued treatment before completing 12 cycles were more likely to have experienced 5FU-AEs, compared to those who completed all 12 cycles (36.5% vs. 31.4%, p=0.034). Patients with grade≥3 5FU-AEs were also more likely to have received a dose modification (no modification 12.2% vs. planned modification 40.3% vs. unplanned modification 29.6%, p<0.0001).

DPYD c.1129-5923 C>G displayed complete linkage with hapB3 variants c.1236 G>A and c.959-51 T>C which was consistent with previously reported results. *DPYD* variant frequencies were comparable to those observed in the 1000 Genomes European super-population, and all tested variants were in Hardy-Weinberg equilibrium (supplemental table S1). Three patients heterozygous for c.1129-5923 C>G and hapB3 variants c.1236 G>A and c.959-51 T>C were wild-type for the other two hapB3 variants c.680+139 G>A and c. 483+18 G>A. One patient heterozygous for hapB3 variants c.680+139 G>A and c.483+18 G>A were wild-type for the deep intronic variant c.1129-5923 C>G and the other two hapB3 variants c.1236 G>A and c.959-51 T>C. A total of 75 patients carried the complete haplotype hapB3 with only one homozygous patient.

A total of 32 of 78 (41.0%) patients carrying *DPYD* c.1129-5923 C>G and the completely linked hapB3 variants c.1236 C>A and c.959-51 T>C displayed at least one grade≥3 5FU-AE; however, the association was not statistically significant in the multivariable model ($OR_{adj.}$=1.47, 95%CI=0.90-2.43, p=0.1267; Figure 1). Neither the remaining hapB3 variants, c.680+139 G>A and c.483+18 G>A, nor the aggregated hapB3 classification (i.e., minor alleles present for all four individual variants) showed significant associations with grade≥3 5FU-AE. No significant associations were detected between grade≥3 overall-AE and either individual *DPYD* variants or the aggregated hapB3 classification. All individual hapB3 variants, *DPYD* c.1129-5923 C>G, and the aggregated hapB3 classification were significantly associated with the individual grade≥3 5FU-AE neutropenia (p≤0.01, supplemental table S2).

To our knowledge, our study is the largest conducted to date that assesses the association between c.1129-5923 C>G/hapB3 and severe grade≥3 AEs in a Caucasian-only cohort of stage III colon cancer patients treated in the adjuvant setting with a standardized regimen of FOLFOX with or without the addition of cetuximab collected under a single clinical trial. All patients utilized in the current analysis were previously determined to be negative for the three deleterious *DPYD* variants in order to assess the individual relationship between hapB3/c.1129-5923 C>G and severe toxicity without the potential influence from DPYD*2A, D949V, and I560S.

Though a trend towards increased grade≥3 5FU-AE was observed for c.1129-5923 C>G/ hapB3 carriers, our results displayed no statistical significance, contradicting a recently published meta-analysis [23]. These discordant results may be due, in part, to heterogeneity in toxicity assessment. Though our study utilized well-defined and standardized toxicity endpoint collection protocols, only grade≥3 AEs were recorded cummulatively over the

entire treatment whereas other studies demonstrating a significant association between *DPYD* hapB3 and toxicity were restricted to severe AEs that occurred during early therapy cycles. Regardless, calculated effect sizes in both the current study and recently published meta-analysis are similar, however smaller in comparison to reported effect sizes for DPYD*2A and D949V. Based on these observations, c.1129-5923 C>G/hapB3 may not significantly alter DPD function in the same severity as other known deleterious *DPYD* variants. One patient in our study cohort was homozygous for c.1129-5923 C>G/hapB3, but presented with only grade≥3 nausea/vomiting. This observation is in contrast to those observed by Froehlich et al., who identified one patient homozygous for c.1129-5923 C>G with lethal toxicity [14]. Previous functional assessment showed that c.1129-5923 C>G does not result in the exclusive generation of a mutant transcript as the wild-type mRNA remained present in a homozygous patient [12], indicating that other molecular mechanisms may be involved.

DPYD contains over 16,000 reported variants with almost 98% located within the non-coding intronic sequence [24]. HapB3 variants and c.1129-5923 C>G encompass a large genomic region spanning over 22kb between intron 5 through exon 11 of the *DPYD* gene; therefore the possibility that other variants within this region could better define a toxicity-associated haplotype cannot be excluded. Additionally, recent evidence has shown that micro RNA mir-27a can regulate DPD expression and that a common polymorphism (rs895819) in the *MIR27A* gene associates with an increased risk of fluoropyrimidine toxicity in patients harboring *DPYD* risk variants [25,26]. Though our results suggest a limited role for c.1129-5923 C>G/hapB3 in 5-FU toxicity prediction, future studies utilizing *MIR27A* rs895819 in addition to a deep sequencing approach covering the expansive genomic region of *DPYD* hapB3 may aid in identifying a refined, 5-FU toxicity-associated haplotype.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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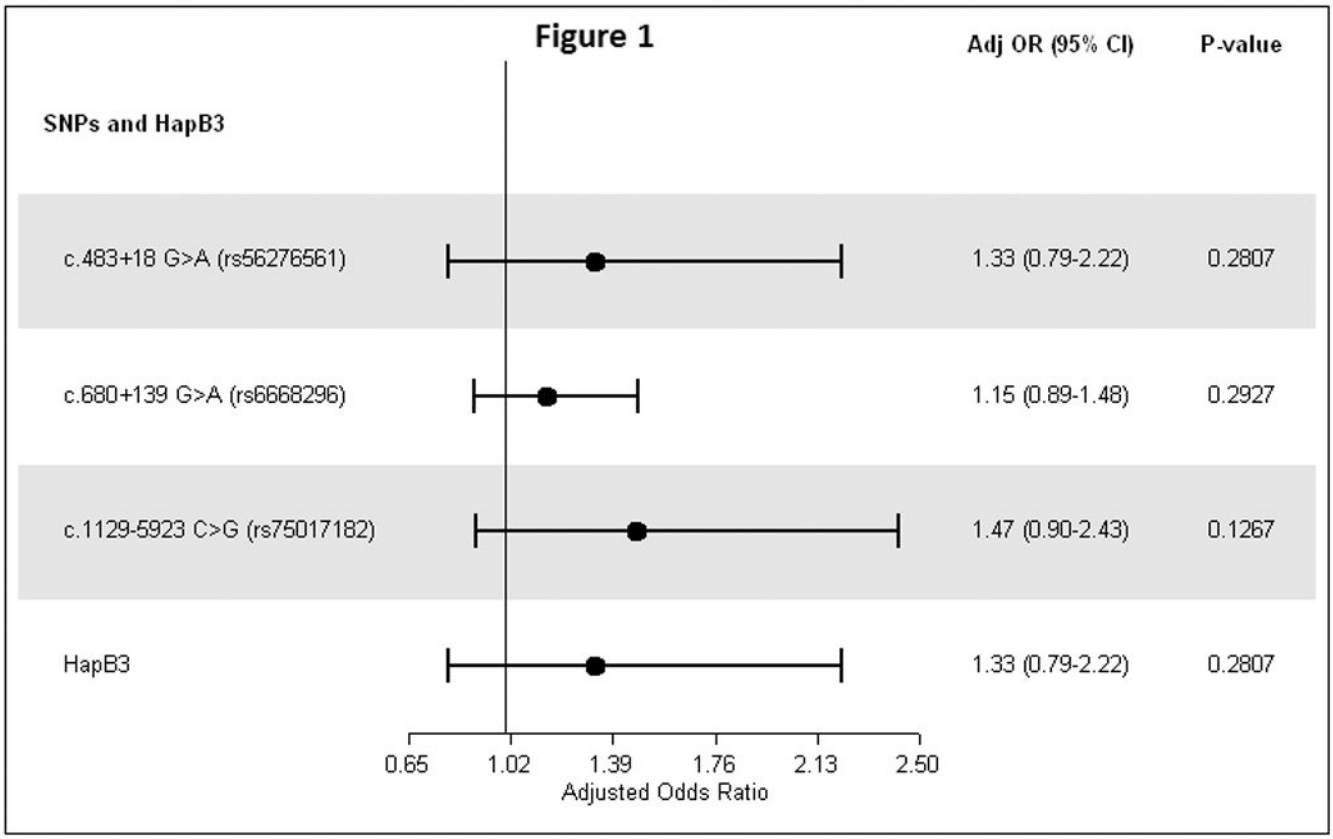

Figure 1. Multivariable logistic regression analysis between *DPYD* variants and grade≥3 5FU-AE in the N0147 study population

Multivariable models were adjusted for age, sex, stratification factors (positive lymph nodes and number of lymph nodes examined), primary tumor site, *KRAS*, *BRAF*, MMR, treatment, total number of treatment cycles, and dose modifications. Two-sided p-values were calculated using a logistic regression model. Genotype counts are as followed: c.483+18 G>A: 29 of 76 carriers (38.2%) and 609 of 1877 (32.5%) wild-type patients experienced grade≥3 5FU-AEs; c.680+139 G>A: 143 of 412 carriers (34.7%) and 495 of 1541 (32.1%) wild-type patients experienced grade≥3 5FU-AEs; c.1129-5923 C>G: 32 of 78 carriers (41.0%) and 606 of 1875 (32.3%) wild-type patients experienced grade≥3 5FU-AEs; HapB3: 29 of 75 carriers (38.7%) and 609 of 1878 (32.4%) wild-type patients experienced grade≥3 5FU-AEs. CI = confidence interval; 5FU-AEs = grade≥3 adverse events common to 5-FU; OR = odds ratio. Complete linkage was observed between c.1129-5923 C>G, c.1236 G>A, and c.959-51 T>C therefore only c.1129-5923 C>G is displayed.

Table 1
Patient characteristics with grade≥3 adverse events common to 5-FU

Characteristic	Grade≥3 5FU-AE No (N=1315)	Yes (N=638)	Total (N=1953)	p value
Age, in years				<0.0001[1]
Mean (SD)	56.5 (10.8)	60.2 (11.1)	57.7 (11.1)	
Median	57.0	62.0	59.0	
Range	19.0 - 85.0	29.0 - 84.0	19.0 - 85.0	
Sex, n (%)				<0.0001[2]
Female	547 (61.9%)	337 (38.1%)	884 (45.3%)	
Male	768 (71.8%)	301 (28.2%)	1069 (54.7%)	
T stage, n (%)				0.6829[2]
Missing	1	0	1	
T1 or T2	205 (68.3%)	95 (31.7%)	300 (15.4%)	
T3 or T4	1109 (67.1%)	543 (32.9%)	1652 (84.6%)	
Positive LNs				0.0204[2]
1-3	729 (65.2%)	389 (34.8%)	1118 (57.2%)	
≥4	586 (70.2%)	249 (29.8%)	835 (42.8%)	
LNs Examined				<0.0029[1]
Missing	2	1	3	
Mean (SD)	20.48 (11.50)	18.88 (10.53)	19.96 (11.22)	
Median	18.0	17.0	18.0	
Range	1.0 - 96.0	2.0 - 92.0	1.0 - 96.0	
Histology Grade, n (%)				0.0557[2]
High	322 (63.9%)	182 (36.1%)	504 (25.8%)	
Low	993 (68.5%)	456 (31.5%)	1449 (74.2%)	
ECOG PS, n (%)				0.1081[2]
Missing	2	0	2	
0	1032 (68.5%)	475 (31.5%)	1507 (77.2%)	
1	273 (63.5%)	157 (36.5%)	430 (22.0%)	
2	8 (57.1%)	6 (42.9%)	14 (0.7%)	
Primary Tumor Side, n (%)				0.0109[2]
Missing	18	8	26	
Right	626 (64.6%)	343 (35.4%)	969 (50.3%)	
Left	671 (70.0%)	287 (30.0%)	958 (49.7%)	
KRAS, n (%)				0.8535[2]
Missing	50	20	70	
Mutant	349 (67.5%)	168 (32.5%)	517 (27.5%)	
Wildtype	916 (67.1%)	450 (32.9%)	1366 (72.5%)	
BRAF, n (%)				0.0167[2]

Characteristic	Grade≥3 5FU-AE No (N=1315)	Yes (N=638)	Total (N=1953)	p value
Missing	84	34	118	
Mutant	166 (60.8%)	107 (39.2%)	273 (14.9%)	
Wild-Type	1065 (68.2%)	497 (31.8%)	1562 (85.1%)	
MSI, n (%)				0.0734[2]
Missing	51	22	73	
pMMR	1120 (68.0%)	528 (32.0%)	1648 (87.7%)	
dMMR	144 (62.1%)	88 (37.9%)	232 (12.3%)	
Received Cetuximab, n (%)				<0.0001[2]
No	730 (72.9%)	271 (27.1%)	1001 (51.3%)	
Yes	585 (61.4%)	367 (38.6%)	952 (48.7%)	
Treatment cycles completed, n (%)				0.0341[2]
Missing	2	0	2	
< 12 cycles	316 (63.5%)	182 (36.5%)	498 (25.5%)	
12 cycles	997 (68.6%)	456 (31.4%)	1453 (74.5%)	
Dose Modification, n (%)				<0.0001[2]
Missing	9	12	21	
No	412 (87.8%)	57 (12.2%)	469 (24.3%)	
Yes_planned	763 (59.7%)	514 (40.3%)	1277 (66.1%)	
Yes_unplanned	131 (70.4%)	55 (29.6%)	186 (9.6%)	

[1] two-sided Wilcoxon Rank Sum test;

[2] two-sided chi-squared test (or Fisher Exact test)

Abbreviations: 5-FU = 5-fluorouracil; AE = adverse events; T stage = tumor stage; LN = lymph node; ECOG PS = Eastern Cooperative Oncology Group performance status; MSI = microsatellite instability; pMMR = proficient mismatch repair; dMMR = deficient mismatch repair.