ARTICLE

*DPYD**6 plays an important role in fluoropyrimidine toxicity in addition to *DPYD**2A and c.2846A>T: a comprehensive analysis in 1254 patients

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Abstract

Dihydropyrimidine dehydrogenase (*DPYD*) is a highly polymorphic gene and classic deficient variants (i.e., c.1236G>A/ HapB3, c.1679T>G, c.1905+1G>A and c.2846A>T) are characterized by impaired enzyme activity and risk of severe adverse drug reactions (ADRs) in patients treated with fluoropyrimidines. The identification of poor metabolizers by pre-emptive *DPYD* screening may reduce the rate of ADRs but many patients with wild-type genotype for classic variants may still display ADRs. Therefore, the search for additional *DPYD* polymorphisms associated with ADRs may improve the safety of treatment with fluoropyrimidines. This study included 1254 patients treated with fluoropyrimidine-containing regimens and divided into *cohort 1*, which included 982 subjects suffering from gastrointestinal G≥2 and/or hematological G≥3 ADRs, and *cohort 2* (control group), which comprised 272 subjects not requiring dose reduction, delay or discontinuation of treatment. Both groups were screened for *DPYD* variants c.496A>G, c.1236G>A/HapB3, c.1601G>A (*DPYD**4), c.1627A>G (*DPYD**5), c.1679T>G (*DPYD**13), c.1896T>C, c.1905 + 1G>A (*DPYD**2A), c.2194G>A (*DPYD**6), and c.2846A>T to assess their association with toxicity. Genetic analysis in the two cohorts were done by Real-Time PCR of DNA extracted from 3 ml of whole blood. *DPYD* c.496A>G, c.1601G>A, c.1627A>G, c.1896T>C, and c.2194G>A variants were found in both *cohort 1* and *2,* while c.1905+1G>A and c.2846A>T were present only in *cohort 1*. *DPYD* c.1679T>G and c.1236G>A/HapB3 were not found. Univariate analysis allowed the selection of c.1905+1G>A, c.2194G>A and c.2846A>T alleles as significantly associated with gastrointestinal and hematological ADRs ($p < 0.05$), while the c.496A>G variant showed a positive trend of association with neutropenia ($p = 0.06$). In conclusion, c.2194G>A is associated with clinically-relevant ADRs in addition to the already known c.1905+1G>A and c.2846A>T variants and should be evaluated pre-emptively to reduce the risk of fluoropyrimidine-associated ADRs.

Introduction

Fluoropyrimidines are the most widely used chemotherapeutic agents for the treatment of many solid tumors, including gastrointestinal, head and neck, pancreas, and breast cancers [1]. Indeed, 5-fluorouracil (5-FU) and its prodrug capecitabine are the backbone of many combination chemotherapy regimens. Despite their clinical benefit, fluoropyrimidines are associated with adverse drug reactions (ADRs), including gastrointestinal and hematological toxicities and hand-foot syndrome (HFS), which may also be life-threatening [2]. ADRs may limit treatment effectiveness, because they impose modification of treatment schedules and/or their discontinuation. Therefore, there is a critical need for the identification of biomarkers predictive of drug-related toxicities, particularly in patients given adjuvant therapy [3]. Fluoropyrimidine metabolism involves numerous enzymes with many intermediate metabolites, but the

rate-limiting step is dependent on dihydropyrimidine dehydrogenase (DPD), which metabolizes at least 80% of the administered dose of 5-FU or capecitabine into 5-fluoro-5,6-dihydrouracil (5-FDHU) [4]. If DPD is inactive or has reduced activity, the amount of 5-FU for anabolic activation increases, leading to 5-FU-related ADRs [4]. The major cause of DPD deficiency is the presence of mutations within the encoding gene DPYD, affecting splicing process, gene transcription and enzyme activity [5]. Many DPYD variants have been discovered [6–9], but most of them do not impair enzyme activity or their functional effect is unclear, with the exception of the splice site mutation in intron 14 (c.1905+1G>A, DPYD*2A) and the non-synonymous variant c.2846A>T (p.D949V), strongly associated with partial or complete loss of enzymatic activity and severe ADRs [10, 11]. Numerous efforts have been made to investigate the best approach to assess DPD deficiency and reduce the risk of toxicity [12] but, despite a strong laboratory rationale and cost-effectiveness of genotyping [13], the issue is still debated as contrasting recommendations on the implementation of DPYD analysis in clinical practice have been issued [14–16], fueling a debate on the usefulness of this test in the management of patients who are candidates to fluoropyrimidine treatment [17–19]. For these reasons, this study was designed to provide further evidence on the role of DPYD assessment by evaluating a large cohort of patients to discover which mutations should be tested to reduce the risk of ADRs and avoid unjustified costs of screening extremely rare DPYD genotypes.

Materials and methods

Study design and patients

Recruitment of patients started in October 2011 and ended in September 2017 and included a total of 1254 subjects. The study evaluated the possible association of the following DPYD variants selected on the basis of their occurrence in the general population and/or known to be involved in treatment-related ADRs: c.496A>G, c.1236G>A/HapB3, c.1601G>A (DPYD*4), c.1627A>G (DPYD*5), c.1679T>G (DPYD*13), c.1896T>C, c.1905+1G>A (DPYD*2A), c.2194G>A (DPYD*6), and c.2846A>T with ADRs requiring dose modifications, treatment delay or discontinuation. The population of 1254 subjects comprised a group of 982 patients (cohort 1) given fluoropyrimidine-based regimens to treat gastrointestinal, pancreatic, head and neck and breast cancers and suffering from G≥3 hematological or G≥2 gastrointestinal ADRs (CTCAE v.4). Overall, gastrointestinal toxicity is much less manageable than hematological ADRs, which are short lasting with fluoropyrimidines and do not usually require treatment with myeloid growth factors. On the contrary, starting from G2, gastrointestinal toxicity substantially impacts on the quality of life of patients and frequently requires dose modifications [20]. Patients received their first cycle of treatment at standard dosing and regimens as per current best practice guidelines; if irinotecan was also indicated, UGT1A1 analysis was performed and only subjects carrying the UGT1A1*1 or *1/*28 genotypes were included in cohort 1. Patients carrying the UGT1A1*28/*28 were excluded because of the high risk of developing gastrointestinal/hematological toxicities [21]. The same DPYD variants examined in cohort 1 were also examined in a control population of 272 subjects (cohort 2) displaying optimal tolerability to treatment (no toxicity, dose reduction, treatment delay or discontinuation) to better define which DPYD variants are associated with clinically-relevant ADRs. Pharmacogenetic analysis was performed by real-time PCR by using the TaqMan® SNP Genotyping Assay (Life Technologies, Carlsbad, CA).

The study was approved by the Ethics Committee of Pisa University Hospital and conducted in accordance with the principles of the Declaration of Helsinki; all patients gave their signed informed consent before blood collection and DNA analysis.

Statistical analysis

Categorical data were described by absolute and relative frequencies, whereas quantitative data were reported as mean and standard deviation. The association between DYPD variants and ADRs was evaluated by χ^{-2} test and odds ratio was also calculated. To compare the relative frequencies, z-test for two proportions was applied. Finally, all risk factors significantly influencing ADRs in the univariate analysis were assessed together in a binary logistic regression model as multivariate analysis. The results of the regression model were calculated by Wald test and expressed using odds ratio. A p-value < 0.05 was considered significant. All analyses, descriptive and inferential, were performed by the IBM SPSS statistics version 24.

Results

A total of 1254 patients were enrolled in the study; 539 (43.0%) patients were male and 715 (57.0%) female, median age was 62 years (cohort 1 interquartile range [IQR]: 14; cohort 2 IQR: 10). A detailed description of patients is reported in Table 1. Since age and performance status have a significant impact on the occurrence of ADRs at the univariate analysis, the genotypic analysis was adjusted for these variables.

Cohort 1 (Table 1) consisted of 982 patients (590 females [60.1%] and 392 males [39.9%]); gastrointestinal (G≥2) and

Table 1 Characteristics of patients of *cohorts 1 and 2*

Characteristics	Statistics	
	Cohort 1	Cohort 2
Patients	982	272
Gender (M/F)	392/590 (39.9/60.1)	147/125 (54/46)
Age (years)	63.9 ± 9.8	58.7 ± 7.4
Race	Caucasian	Caucasian
Disease		
Colorectal cancer	740 (75.4)	130 (47.8)
Gastric cancer	193 (19.6)	12 (4.4)
Breast cancer	49 (5.0)	130 (47.8)
Treatment[a]		
FU-LV (De Gramont regimen)	170 (17.3)	0 (0)
Capecitabine	210 (21.4)	92 (33.8)
FOLFIRI	182 (18.5)	0 (0)
FOLFOX-4	190 (19.3)	130 (47.8)
FOLFOXIRI	54 (5.5)	0 (0)
CAPOX	160 (16.3)	0 (0)
TPF	0 (0)	0 (0)
XELIRI	8 (0.7)	0 (0)
EOX[b]	8 (0.8)	50 (18.4)
ADRs		
Gastrointestinal	Grade≥2	
Nausea/Vomiting	16%	0 (0)
Diarrhea	39.7%	0 (0)
Stomatitis	14%	0 (0)
Dermatological	Grade≥2	
Hand-foot syndrome	9.3%	0 (0)
Hematological	Grade≥3	
Fever	2.2%	0 (0)
Leucopenia	12.3%	0 (0)
Neutropenia	17.4%	0 (0)
Febrile neutropenia	4.7%	0 (0)
Anemia	4.2%	0 (0)
Thrombocytopenia	5.8%	0 (0)

[a]Abbreviations listed as per NCI Thesaurus v. 16.08e (release 2016-08-29)

[b]Epirubicin, oxaliplatin, capecitabine

hematological toxicities (G≥3) were present in 69.7% and 46.6% of patients, respectively. A control group of 272 patients (147 males [54%] and 125 females [46%], *cohort 2*) receiving standard doses of fluoropyrimidine-based therapies, without dose reduction, delay or discontinuation, were also enrolled (Table 1). The frequencies of c.496A>G, c.1601G>A, c.1627A>G, c.1896T>C, c.1905+1G>A, c.2194G>A and c.2846A>T alleles are reported in Table 2. The c.1679T>G and c.1236G>A/HapB3 variants were not found neither in *cohort 1* nor in *cohort 2*. The comparison

Table 2 Type and frequencies of *DPYD* genotypes in *cohorts 1 and 2*

SNPs	Heterozygous + homozygous mutants (%)		
	Cohort 1	*Cohort 2*	*p*-value
c.496A>G	23.8	18	0.052
c.1601G>A	9.3	6.2	0.136
c.1627A>G	32.6	39.7	0.035[a]
c.1679T>G	Not found	Not found	Not found
c.1896T>C	3.5	4.8	0.415
IVS14+1G>A	6.2	0	**<0.0001**
c.2194G>A	19.7	11.8	**0.004**
c.2846A>T	2.4	0	**0.020**
c.1236G>A/HapB3	Not found	Not found	Not found

[a]Higher frequency in *cohort 2*

between the two cohorts demonstrated that IVS14+1GA/AA, c.2194GA/AA, c.2846AT/TT were significantly higher in *cohort 1* than in *cohort 2*: 6.2% vs. 0% ($p < 0.0001$), 19.7% vs. 11.8% ($p = 0.004$) and 2.4% vs. 0% ($p = 0.020$), respectively (Table 2). The statistical analysis showed that IVS14+1GA and AA genotypes were significantly associated with diarrhea ($p = 0.001$), alopecia ($p = 0.007$), febrile neutropenia ($p < 0.0001$), and thrombocytopenia ($p = 0.012$). c.2194GA/GG were associated with stomatitis ($p = 0.053$), leucopenia ($p = 0.003$), and thrombocytopenia ($p = 0.049$). Finally, c.2846AT/TT were associated with diarrhea ($p = 0.02$, Table 3). The strong association of c.1905+1G>A and c.2846A>T with ADRs was also demonstrated by the absence of c.1905+1A and c.2846T variant alleles in *cohort 2* (Table 2). Borderline associations of c.496AG/GG ($p = 0.068$) and c.1905+1GA/AA ($p = 0.061$) with neutropenia and of 2194GA/AA ($p = 0.062$) with febrile neutropenia were found. On the contrary, c.1601G>A, c.1627A>G and c.1896T>C played no role in fluoropyrimidine toxicities (Table 3). At univariate analysis, the incidence of ADRs was lower in patients treated with fluoropyrimidines alone or in association with oxaliplatin *vs.* all other treatments (Table 1S). However, at multivariate analyses, *DPYD* variants were confirmed as independent factors of ADRs irrespective of treatments received (Table 4).

Discussion

An extensive search of genetic variants of *DPYD* associated with enzyme deficiency and poor-metabolizer status has been performed and several genotypes were identified [5, 8, 22]. In agreement with three meta-analyses [10, 23, 24], our study confirmed the well-known role of c.1905+1G>A and c.2846A>T in fluoropyrimidine-associated ADRs. An additional meta-analysis also found an association between severe ADRs and the non-synonymous variant c.1679T>G

Table 3 DPYD variants and associations with ADRs

ADRs		c.496A>G	c.1601G>A	c.1627A>G	c.1679T>G	c.1896T>C	c.1905+1G>A	c.2194G>A	c.2846A>T	c.1236G>A/HapB3
Nausea/vomiting	p-value	0.773	0.287	0.598	Not found	0.836	0.418	0.830	0.074	Not found
Diarrhea	p-value	0.613	0.074	0.347	Not found	0.111	0.001 (OR 2.317)	0.725	0.020 (OR 2.602)	Not found
Stomatitis	p-value	0.205	0.079	0.236	Not found	0.396	0.076	0.053 (OR 1.514)	0.674	Not found
Dermatitis	p-value	0.215	0.618	0.482	Not found	0.969	0.152	0.152	0.749	Not found
Alopecia	p-value	0.406	0.848	0.689	Not found	0.655	0.007 (OR 4.239)	0.886	0.486	Not found
Leucopenia	p-value	0.100	0.464	0.979	Not found	0.294	0.265	0.003 (OR 1.895)	0.467	Not found
Neutropenia	p-value	0.068 (OR 1.408)	0.638	0.396	Not found	0.339	0.061 (OR 1.757)	0.127	0.658	Not found
Febrile neutropenia	p-value	0.470	0.366	0.745	Not found	0.245	<0.0001 (OR 4.135)	0.062 (OR 1.838)	0.272	Not found
Anemia	p-value	0.620	0.729	0.653	Not found	0.561	0.081	0.595	0.960	Not found
Thrombocytopenia	p-value	0.612	0.080	0.647	Not found	0.984	0.012 (OR 2.686)	0.049 (OR 1.796)	0.592	Not found
HFS	p-value	0.102	0.269	0.911	Not found	0.659	0.081	0.371	0.940	Not found
Fever	p-value	0.513	0.909	0.816	Not found	0.392	0.478	0.084	0.475	Not found

(DPYD*13) as well as with the synonymous variant c.1236G>A in complete linkage with HapB3 [25], a haplotype containing three intronic polymorphisms (IVS5 +18G>A, IVS6+139G>A and IVS9-51T>G) [26, 27]. Several other variants have been associated with fluoropyrimidine toxicities, including c.257C>T, c.1850C>T [28], c.2509-2510insC, c.1801G>C, c.680G>A [29], c.85T>C (p.R29C) [30], and c.496A>G (p.M166V) [31]; however, due to the lack of confirmatory studies, their association with toxicity remains unproven.

The present study found a significant association between the non-synonymous variant c.2194G>A (p.V732I, DPYD*6) with ADRs by fluoropyrimidines. The results of the present work provide additional information on the debate on this pharmacogenetic marker. Despite the c.2194A allele seems to be relatively common, conflicting results have been reported concerning its influence on DPD activity and association with clinically-relevant ADRs. Some studies did not assign a role to c.2194G>A in the occurrence of fluoropyrimidine toxicity [32, 33] and in silico analysis demonstrated a normal enzyme activity [8]. A study found that c.2194G>A showed weak evidence for association with reduced DPD activity in African-American patients; c.2194GA patients displayed a 29% reduction in DPD activity compared to the wild-type, although the linkage with c.557A>G (p.Y186C) may have played a prominent role [34]. In the study by Schwab et al. [35] the role of c.2194G>A was not considered significant, but this result may have been affected by the small number of carriers of this variant. Another study on the role of selected DPYD variants on treatment tolerability showed no association between G≥3 toxicity and c.2194G>A, but, also in this case, this result may have been affected by the small group of patients [36].

On the contrary, the secondary analysis of the Pan-European Trials in Alimentary Tract Cancer (PETACC-8) study provided evidence of the association of c.2194G>A variant with clinically-relevant ADRs in FOLFOX4-treated patients [37] and the same result was observed in the TOSCA randomized trial that enrolled colon cancer patients given 3 or 6 months of either FOLFOX-4 or XELOX adjuvant chemotherapy [38]. In particular, the work by Boige et al. examined a cohort of 1545 patients and found a significant association of ADRs with c.2194G>A variant [37]. The statistical analysis revealed a correlation between G≥3 ADRs by 5-FU and c.2194G>A (OR = 1.7; p < 0.001); in more detail, G≥3 hematologic adverse events (OR = 1.9) and G≥3 neutropenia (OR = 1.8) were associated with c.2194G>A [37]. Data generated within a clinical study have the clear advantage of being obtained in a selected, homogeneous population with strict follow-up. Our study has the limitation of having enrolled a heterogenous population but

Table 4 Multivariate analysis of toxicity risk factors. p and OR values are indicated. OR > 1 if associated with a p-value < 0.05 indicates a toxicity risk factor

ADRs		c.496A>G	IVS14+1G>A	c.2194G>A	c.2846A>T	Treatment combinations other than fluoropyrimidines ± oxaliplatin[a]
Diarrhea	p-value		**0.001** (OR 2.408)		**0.017** (OR 2.777)	**0.008** (OR 0.656)
Stomatitis	p-value		0.067 (OR 1.836)	**0.049** (OR 1.536)		**<0.0001** (OR 0.442)
Leukopenia	p-value			**0.003** (OR 1.958)		**<0.0001** (OR 0.339)
Alopecia	p-value		**0.012** (OR 4.370)			**0.024** (OR 0.344)
Neutropenia	p-value	0.054 (OR 1.452)	**0.042** (OR 1.894)			**<0.0001** (OR 0.301)
Febrile neutropenia	p-value		**<0.0001** (OR 4.241)	0.060 (OR 1.879)		0.079 (OR 0.555)
Thrombocytopenia	p-value		**0.011** (OR 2.863)	**0.040** (OR 1.875)		**<0.0001** (OR 0.319)

[a]FOLFIRI, FOLFOXIRI, TPF, XELIRI, EOX

what can be viewed as a limitation turns to be an important confirmation of PETACC-8 results in different clinical settings, thus demonstrating the usefulness of c.2194G>A screening. It should be noted that the data of the present study are in accordance not only with the results provided by the PETACC-8 trial but also with the previously published biomarker analysis of TOSCA trial, which found a significant association between c.2194G>A and time to neutropenia [38]. A smaller study found a relationship of c.2194G>A with leukopenia (OR = 8.17) and neutropenia (OR = 2.78) [39] and an additional work also positively associated this variant with diarrhea [27]. Finally, a meta-analysis conducted on seven cohort studies, with a total of 946 colorectal cancer patients receiving 5-FU chemotherapy, found a significant association between the c.2194G>A polymorphism, bone marrow suppression ($p < 0.001$) and gastrointestinal ADRs ($p < 0.05$) [23]. Although other mutations of DPYD may represent a risk for patients [14, 37, 40] their extremely low frequency does not suggest their inclusion in routine preemptive screening.

Recommendations are available on which variants to test and which dose adjustement of fluoropyrimidines should be adopted and include c.1236G>A/HapB3, c.1679T>G, c.1905+1G>A, and c.2846A>T [41, 42]. The updated guideline on DPYD genotyping [42] is an extremely valuable instrument to apply targeted genotyping in current clinical laboratory practice. It is not surprising that it includes only mutations (i.e., IVS14+1G>A, c.2846A>T, HapB3, and c.1679T>G) supported by a substantial amount of clinical data and established association with toxicities, while it does not recommend novel variants like c.2194G>A which still lack of clear clinical information and/or controversial data are provided due to small groups of patients examined in some studies. Despite the large number of published works, the present study is one of the few addressing the issue of DPYD variants and treatment safety in a large population. We purposely included patients given various protocols containing fluoropyrimidines to assess the impact of DPYD polymorphisms in different settings and validate the role of gene variants independently of the drugs combined with fluoropyrimidines. A direct comparison between the same regimens would have been statistically more correct and this is a weakness of the present study. However, due to the multitude of drug combinations containing fluoropyrimidines and disease settings, a much larger patient population should have been enrolled. Nonetheless, the role of major variants (IVS14+1G>A, c.2846A>T) has been confirmed in this study, despite the heterogeneity of clinical settings, and the importance of c.2194G>A is further documented by the present work.

It is still a matter of debate when to screen subjects candidate to fluoropyrimidine treatment and if the therapeutic drug monitoring (TDM) has a role in optimizing drug doses. In selected patients in whom dose adaptation is difficult, measurement of 5-FU and of its major metabolite 5-FDHU can be performed. TDM is an extremely useful approach but turnaround time is longer than SNP genotyping and pre-analytical issues may limit its widespread use. In some centers, patients are prospectively screened and dose reductions are made, if necessary. Unfortunately, DPYD screening is still not universally accepted, although it has been demonstrated to be cost-effective [43], and several subjects are examined only after an ADR has occurred, thus abolishing the advantage of a preemptive genotyping to reduce the deleterious consequences of administering a fluoropyrimidine in a poor metabolizer.

In conclusion, the present article provides evidence that c.2194G>A should be examined in addition to well-known deleterious variants; a dose reduction of 20% in homozygous variant patients and a close monitoring of heterozygous subjects for ADRs are thus advisable.

AP, FDB, GA, CZ, RB, AB, EM, CP, AF. Data acquisition: MDR, RD, AM. Quality control of data: MDR, AM, PS. Data analysis and interpretation: MDR, RD. Statistical analysis: SS, RM, SC, VM. Manuscript preparation: MDR, RD. Manuscript editing: all. Manuscript review: all

Compliance with ethical standards

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

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References

1. Meyerhardt JA, Mayer RJ. Systemic therapy for colorectal cancer. N Engl J Med. 2005;352:476–87.
2. Takimoto CH. The clinical pharmacology of the oral fluoropyrimidines. Curr Probl Cancer. 2001;25:134–213.
3. Innocenti F. DPYD variants to predict 5-FU toxicity: the ultimate proof. J Natl Cancer Inst. 2014;106:12.
4. Liu XQ, Zhuang M, Wang Z, Huber RM. Correlation between dihydropyrimidine dehydrogenase and efficacy and toxicity of fluoropyrimidine drugs. Eur Rev Med Pharmacol Sci. 2014;18:2772–6.
5. Amstutz U, Froehlich TK, Largiader CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. Pharmacogenomics. 2011;12:1321–36.
6. Del Re M, Di Paolo A, van Schaik RH, Bocci G, Simi P, Falcone A, et al. Dihydropyrimidine dehydrogenase polymorphisms and fluoropyrimidine toxicity: ready for routine clinical application within personalized medicine? EPMA J. 2010;1:495–502.
7. Offer SM, Fossum CC, Wegner NJ, Stuflesser AJ, Butterfield GL, Diasio RB. Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. Cancer Res. 2014;74:2545–54.
8. Shrestha S, Zhang C, Jerde CR, Nie Q, Li H, Offer SM, et al. Gene-specific variant classifier (DPYD-Varifier) to identify deleterious alleles of dihydropyrimidine dehydrogenase. Clin Pharmacol Ther. 2018;104:709–18.
9. Hishinuma E, Narita Y, Saito S, Maekawa M, Akai F, Nakanishi Y, et al. Functional characterization of 21 allelic variants of dihydropyrimidine dehydrogenase identified in 1070 Japanese individuals. Drug Metab Dispos. 2018;46:1083–90.
10. Terrazzino S, Cargnin S, Del Re M, Danesi R, Canonico PL, Genazzani AA. DPYD IVS14+1G>A and 2846A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. Pharmacogenomics. 2013;14:1255–72.
11. Henricks LM, Lunenburg CA, Meulendijks D, Gelderblom H, Cats A, Swen JJ, et al. Translating DPYD genotype into DPD phenotype: using the DPYD gene activity score. Pharmacogenomics. 2015;16:1277–86.
12. van Staveren MC, Guchelaar HJ, van Kuilenburg AB, Gelderblom H, Maring JG. Evaluation of predictive tests for screening for dihydropyrimidine dehydrogenase deficiency. Pharm J. 2013;13:389–95.
13. Deenen MJ, Meulendijks D, Cats A, Sechterberger MK, Severens JL, Boot H, et al. Upfront genotyping of DPYD*2A to individualize fluoropyrimidine therapy: a safety and cost analysis. J Clin Oncol. 2016;34:227–34.
14. Caudle KE, Thorn CF, Klein TE, Swen JJ, McLeod HL, Diasio RB, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing. Clin Pharmacol Ther. 2013;94:640–5.
15. Caudle KE, Klein TE, Hoffman JM, Muller DJ, Whirl-Carrillo M, Gong L, et al. Incorporation of pharmacogenomics into routine clinical practice: the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline development process. Curr Drug Metab. 2014;15:209–17.
16. Van Cutsem E, Cervantes A, Adam R, Sobrero A, Van Krieken JH, Aderka D, et al. ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2016;27:1386–422.
17. Danesi R, Del Re M, Ciccolini J, Schellens JHM, Schwab M, van Schaik RHN, et al. Prevention of fluoropyrimidine toxicity: do we still have to try our patient's luck? Ann Oncol. 2017;28:183.
18. Deenen MJ, Meulendijks D. Recommendation on testing for dihydropyrimidine dehydrogenase deficiency in the ESMO consensus guidelines for the management of patients with metastatic colorectal cancer. Ann Oncol. 2017;28:184.
19. Ciccolini J, Del Re M, Danesi R, Milano G, Schellens JHM, Raymond E. Predicting fluoropyrimidine-related toxicity: turning wish to will, the PAMM-EORTC position. Ann Oncol. 2018;29:1893–4.
20. Kalsi T, Babic-Illman G, Fields P, Hughes S, Maisey N, Ross P, et al. The impact of low-grade toxicity in older people with cancer undergoing chemotherapy. Br J Cancer. 2014;111:2224–8.
21. Cremolini C, Del Re M, Antoniotti C, Lonardi S, Bergamo F, Loupakis F, et al. DPYD and UGT1A1 genotyping to predict adverse events during first-line FOLFIRI or FOLFOXIRI plus bevacizumab in metastatic colorectal cancer. Oncotarget. 2018;9:7859–66.
22. Matsusaka S, Lenz HJ. Pharmacogenomics of fluorouracil-based chemotherapy toxicity. Expert Opin Drug Metab Toxicol. 2015;11:811–21.
23. Li Q, Liu Y, Zhang HM, Huang YP, Wang TY, Li DS, et al. Influence of DPYD genetic polymorphisms on 5-fluorouracil toxicities in patients with colorectal cancer: a meta-analysis. Gastroenterol Res Pract. 2014;2014:827989.
24. Rosmarin D, Palles C, Church D, Domingo E, Jones A, Johnstone E, et al. Genetic markers of toxicity from capecitabine and other fluorouracil-based regimens: investigation in the QUASAR2 study, systematic review, and meta-analysis. J Clin Oncol. 2014;32:1031–9.
25. Meulendijks D, Henricks LM, Sonke GS, Deenen MJ, Froehlich TK, Amstutz U, et al. Clinical relevance of DPYD variants c.1679T>G, c.1236G>A/HapB3, and c.1601G>A as predictors of severe fluoropyrimidine-associated toxicity: a systematic review and meta-analysis of individual patient data. Lancet Oncol. 2015;16:1639–50.
26. Amstutz U, Farese S, Aebi S, Largiader CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. Pharmacogenomics. 2009;10:931–44.
27. Deenen MJ, Tol J, Burylo AM, Doodeman VD, de Boer A, Vincent A, et al. Relationship between single nucleotide polymorphisms and haplotypes in DPYD and toxicity and efficacy of capecitabine in advanced colorectal cancer. Clin Cancer Res. 2011;17:3455–68.

28. Del Re M, Quaquarini E, Sottotetti F, Michelucci A, Palumbo R, Simi P, et al. Uncommon dihydropyrimidine dehydrogenase mutations and toxicity by fluoropyrimidines: a lethal case with a new variant. Pharmacogenomics. 2016;17:5–9.

29. Del Re M, Michelucci A, Di Leo A, Cantore M, Bordonaro R, Simi P, et al. Discovery of novel mutations in the dihydropyrimidine dehydrogenase gene associated with toxicity of fluoropyrimidines and viewpoint on preemptive pharmacogenetic screening in patients. EPMA J. 2015;6:17.

30. Gentile G, Botticelli A, Lionetto L, Mazzuca F, Simmaco M, Marchetti P, et al. Genotype-phenotype correlations in 5-fluorouracil metabolism: a candidate DPYD haplotype to improve toxicity prediction. Pharm J. 2016;16:320–5.

31. Gross E, Busse B, Riemenschneider M, Neubauer S, Seck K, Klein HG, et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS ONE. 2008;3:e4003.

32. Ridge SA, Sludden J, Wei X, Sapone A, Brown O, Hardy S, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in patients with colorectal cancer. Br J Cancer. 1998;77:497–500.

33. Wei X, Elizondo G, Sapone A, McLeod HL, Raunio H, Fernandez-Salguero P, et al. Characterization of the human dihydropyrimidine dehydrogenase gene. Genomics. 1998;51:391–400.

34. Offer SM, Lee AM, Mattison LK, Fossum C, Wegner NJ, Diasio RB. A DPYD variant (Y186C) in individuals of african ancestry is associated with reduced DPD enzyme activity. Clin Pharmacol Ther. 2013;94:158–66.

35. Schwab M, Zanger UM, Marx C, Schaeffeler E, Klein K, Dippon J, et al. Role of genetic and nongenetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. J Clin Oncol. 2008;26:2131–8.

36. Toffoli G, Giodini L, Buonadonna A, Berretta M, De Paoli A, Scalone S, et al. Clinical validity of a DPYD-based pharmacogenetic test to predict severe toxicity to fluoropyrimidines. Int J Cancer. 2015;137:2971–80.

37. Boige V, Vincent M, Alexandre P, Tejpar S, Landolfi S, Le Malicot K, et al. DPYD genotyping to predict adverse events following treatment with flourouracil-based adjuvant chemotherapy in patients with stage III colon cancer: a secondary analysis of the PETACC-8 randomized clinical trial. JAMA Oncol. 2016;2:655–62.

38. Ruzzo A, Graziano F, Galli F, Galli F, Rulli E, Lonardi S, et al. Dihydropyrimidine dehydrogenase pharmacogenetics for predicting fluoropyrimidine-related toxicity in the randomised, phase III adjuvant TOSCA trial in high-risk colon cancer patients. Br J Cancer. 2017;117:1269–77.

39. Kleibl Z, Fidlerova J, Kleiblova P, Kormunda S, Bilek M, Bouskova K, et al. Influence of dihydropyrimidine dehydrogenase gene (DPYD) coding sequence variants on the development of fluoropyrimidine-related toxicity in patients with high-grade toxicity and patients with excellent tolerance of fluoropyrimidine-based chemotherapy. Neoplasma. 2009;56: 303–16.

40. Collie-Duguid ES, Etienne MC, Milano G, McLeod HL. Known variant DPYD alleles do not explain DPD deficiency in cancer patients. Pharmacogenetics. 2000;10:217–23.

41. Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. Lancet Oncol. 2018;19:1459–67.

42. Amstutz U, Henricks LM, Offer SM, Barbarino J, Schellens JHM, Swen JJ, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update. Clin Pharmacol Ther. 2018;103:210–6.

43. Henricks LM, Lunenburg CATC, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. A cost analysis of upfront DPYD genotype-guided dose individualisation in fluoropyrimidine-based anticancer therapy. Eur J Cancer. 2019;107:60–7.

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