DPYD IVS14+1G>A and 2846A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis

Aim: In the present study we conducted a systematic review and meta-analysis of published data to quantify the impact of the *DPYD* IVS14+1G>A and 2846A>T variants on the risk of fluoropyrimidine-related toxicities and to determine sensitivity and specificity testing for *DPYD* variants. **Methods:** Relevant studies were identified through PubMed and Web of Knowledge databases, studies included were those published up until to May 2012. Study quality was assessed according to the HuGENET guidelines and Strengthening the Reporting of Genetic Association (STREGA) recommendations. **Results:** Random-effects meta-analysis provided evidence that carriers of *DPYD* IVS14+1G>A are at higher risk of ≥3 degrees of overall grade toxicity, hematological toxicity, mucositis and diarrhea. In addition, a strong association was also found between carriers of the *DPYD* 2846T allele and overall grade ≥3 toxicity or grade ≥3 diarrhea. An inverse linear relationship was found in prospective studies between the odds ratio of *DPYD* IVS14+1G>A and the incidence of overall grade ≥3 toxicity, indicating an higher impact in cohorts in which the incidence of severe toxicity was lower. **Conclusion:** The results of this meta-analysis confirm clinical validity of *DPYD* IVS14+1G>A and 2846A>T as risk factors for the development of severe toxicities following fluoropyrimidine treatment. Furthermore, the sensitivity and specificity estimates obtained could be useful in establishing the cost–effectiveness of testing for *DPYD* variants.

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KEYWORDS: DPYD ■ fluoropyrimidines ■ meta-analysis ■ pharmacogenetics ■ risk factors ■ toxicity

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5-fluorouracil (5-FU) and its prodrugs capecitabine and tegafur are among the most commonly used drugs in oncology (e.g., in gastrointestinal, head and neck, and breast cancers) [1,2]. Approximately 30–40% of treated patients can develop severe toxicities, including myelosuppression, cardiac toxicity, mucositis, hand–foot syndrome and diarrhea [3,4]. Despite the large number of studies attempting to identify pharmacogenetic predictors of severe fluoropyrimidine-related toxicity, there is still no consensus concerning the markers to be used in clinical practice.

DPD plays a key role in the catabolism of 5-FU to its inactive metabolite 5-fluoro-5,6-dihydrouracil and a deficiency of DPD has been recognized as an important risk factor predisposing patients to the development of severe fluoropyrimidine-associated toxicity [5]. The coding gene *DPYD* is highly polymorphic and part of this genetic variation is thought to be responsible for the large variability in DPD activity that is observed in the general population [6]. So far, pharmacogenetic research on *DPYD* in the context of 5-FU toxicity has been mostly focused on the IVS14+1G>A (rs3918290, also known as *DPYD*2A*) mutation, located at the splice donor site of intron 14, which leads to skipping of exon 14 during pre-mRNA splicing and consequently to a truncated protein with absent DPD activity [7,8]. Controversial results have been reported regarding *DPYD* IVS14+1G>A genotyping for the prediction of fluoropyrimidine-related toxicity as some studies report that variant allele carriers are at increased risk of developing severe toxicity [9–11], while other reports fail to confirm such association [12–14]. Furthermore, contrasting results have also been reported on the proportion of toxicities that can be explained by the presence of the *DPYD* IVS14+1G>A variant. In this regard, early studies suggested that this mutation accounts for up to 29% of all grade 3 toxicities in cancer patients receiving 5-FU [15]. However, subsequent studies yielded a lower proportion of cases of fluoropyrimidine-related toxicity that could be explained by the *DPYD* IVS14+1G>A variant [11,16]. Despite the presence of several descriptive reviews on the subject, a quantitative meta-analysis of trials has not been conducted to estimate the impact of *DPYD* IVS14+1G>A on the risk of

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severe fluoropyrimidine-related toxicity and to determine sensitivity of *DPYD* IVS14+1G>A testing.

Among the other deleterious variants of *DPYD*, much attention has been focused on the nonsynonymous 2846A>T (rs67376798, D949V) mutation, located on exon 22 on the 4Fe–4S site, which affects DPD activity through direct interference with cofactor binding and electron transport [17,18]. Although a correlation has been suggested between *DPYD* 2846A>T and the occurrence of severe toxicities after 5-FU administration [9–11], no meta-analytic review to date has been performed to estimate the risk conferred by *DPYD* 2846A>T variant and whether its effect is comparable to the exon 14 skipping mutation.

The aim of this study was to perform a systematic review and meta-analysis on the published data in order to accurately estimate the impact of *DPYD* IVS14+1G>A and 2846A>T on the risk of fluoropyrimidine-related toxicities in cancer patients. In order to assess the clinical utility of screening cancer patients for *DPYD* variants before starting a fluoropyrimidine treatment we also assessed sensitivity and specificity of *DPYD* IVS14+1G>A and 2846A>T genotyping.

Methods

Search strategy & selection criteria

We carried out a computerized literature search of the PubMed and Web of Knowledge databases (we searched for articles published up until 7 May 2012) by using the Boolean combinations of the key terms '*DPYD*' or 'DPD' or 'dihydropyrimidine dehydrogenase' and 'polymorphisms' or 'polymorphism' or 'SNP' or '*DPYD*2A*' or 'IVS14+1G>A' or '1905+1G>A' or '2846A>T' or 'D949V'. We then searched the resulting hits for primary studies of cancer patients treated with fluoropyrimidines including 5-FU, capecitabine and tegafur–uracil that reported on patients with and without grade ≥3 toxicities and genotyping of *DPYD* IVS14+1G>A and/or *DPYD* 2846A>T (D949V) variants. The primary outcome measure of interest was any grade ≥3 toxicity (overall grade ≥3 toxicity). The secondary outcomes included: any grade ≥3 hematologic toxicity; grade ≥3 diarrhea; and grade ≥3 mucositis, as these represent the most important adverse reactions related to fluoropyrimidine treatment [19,20]. Exclusion criteria were the following: studies that did not report data on the entire population treated with fluoropyrimidine (e.g., presented data only for patients with toxicity or only for patients with *DPYD* variants); studies with no patients carrying *DPYD* IVS14+1G>A or 2846A>T variants; studies that were not published in English; and review articles. The retrieved studies were assessed for their appropriateness for inclusion in the meta-analysis. All references cited in the eligible studies were also reviewed to identify additional published works that were not initially retrieved. If two or more studies shared part of the same patient population, the more complete or the one with the larger sample size was included.

Data extraction

The following information was abstracted from included publications: the first author's last name, year of publication, study design (prospective or retrospective), geographic origin, age and gender of patients, cancer type, chemotherapy regimen, toxicity classification criteria, type of adverse effects reported (overall toxicity, hematological toxicity, diarrhea or mucositis) and its respective grade ≥3 incidence, *DPYD* variant analyzed (IVS14+1G>A and/or 2846A>T), number of patients with and without grade ≥3 toxicity for each level of *DPYD* genotype (with and without the variant allele) and method of SNP detection. When publications reported results for multiple types of adverse effects that matched different genotype groups, we included the study in each relevant group. All studies were independently analysed by two reviewers (S Terrazzino and S Cargnin) and any discrepancies in data extraction were resolved through consensus.

Assessment of study quality

The scientific quality of the included studies was evaluated according to the HuGENET guidelines [101] and Strengthening the Reporting of Genetic Association (STREGA) recommendations for reports on genetic association studies [21]. We evaluated the quality of the studies based on the 11n criteria described in Supplementary Table 1 (see www.futuremedicine.com/doi/suppl/10.2217/pgs.13.116). Specifically, the quality criteria were a clear definition of each of the following points: objectives and hypothesis; study design; eligibility criteria for study participants; ethnicity; outcome of interest; Hardy–Weinberg equilibrium; statistical power; descriptive clinical data (e.g., age and sex); statement of genotype frequencies; statement of clinical outcomes; and consideration of study limitation and potential bias. Consistent with current guidelines, we did not weigh studies by quality scores or exclude studies with low-quality scores.

Yet, to assess whether our results were influenced by studies rated as of lower quality, we repeated meta-analyses including only studies with a quality score equal to or above the median value.

Statistical analysis

The effect measure of interest was the odds ratio (OR), which was calculated from the number of patients with and without grade ≥3 toxicity in each genotype group (with and without the variant *DPYD* allele under consideration). When any zero cell occurred in the two-by-two contingency table, we added a Woolf–Haldane continuity correction of 0.5 to generate a finite OR. Measures of pooled OR were expressed as point estimates with 95% CIs. We conducted meta-analyses only for clinical outcomes reported in at least three independent studies. Data were combined using random-effects (DerSimonian and Laird) models, which incorporate the between-study heterogeneity and allow for a different effect in each population [22]. In case of lack of heterogeneity, the random-effects model coincides with the fixed-effect model [23]. We estimated the between-study heterogeneity across all eligible comparisons by using the Cochran's Q χ^2 test (significant for $p < 0.10$) [24]. We also reported the I^2 index, which quantifies heterogeneity irrespective of the number of studies (range: 0–100%; values of 75% imply extreme heterogeneity). Leave-one-out sensitive meta-analyses were performed to assess the contribution of each study to the pooled estimate by excluding individual results one at a time and recalculating the pooled OR estimates for the remaining results. To assess the robustness of our findings and explore possible reasons for heterogeneity, four sensitivity analyses were performed using the following inclusion criteria: prospective studies, higher quality studies, sample size ≥200, and 5-FU-based regimens. Meta-regression analysis was carried out to assess the impact of continuous variables (mean age, sample size, percentage of male subjects and incidence of grade ≥3 toxicity) on the pooled estimate.

By means of true-positive, true-negative, false-positive and false-negative rates, we also computed sensitivity and specificity of *DPYD* IVS14+1G>A and 2846A>T genotyping, respectively. Sensitivity was defined as the proportion of patients experiencing grade ≥3 toxicity who were found to be positive for the *DPYD* variant allele under consideration (IVS14+1G>A or 2846A>T), whereas specificity was defined as the proportion of patients without grade ≥3 toxicity who were negative for the mutant *DPYD* allele. Measures of diagnostic accuracy were calculated for each individual study and reported as point estimates with 95% CIs, and then combined using a random-effects model (DerSimonian–Laird method). Data analysis was performed with Open Meta-Analyst available at [102]. The presence of publication bias was assessed using three tests: the Egger regression asymmetry test for funnel plot [25], the Begg–Mazumdar adjusted rank correlation test [26], and the Harbord's test (similar to Egger's test), which uses a modified linear regression method to reduce the false-positive rate [27]. Calculations of publication bias were performed using StatsDirect statistical software version 2.7.8b (Stats-Direct Ltd, Cheshire, UK) and p-values <0.10 were considered to indicate statistically significant publication bias.

Results

Study characteristics & methodological quality

The search identified 381 records and, of these, 15 studies fulfilled our inclusion criteria [9–14,16,28–35]; for the number of studies evaluated at each stage see the flowchart shown in Figure 1. Details on the demographic and clinical characteristics of eligible studies are summarized in Table 1 and study quality details are provided in Supplementary Table 1. Studies were published between 2004 and 2011, sample sizes ranged from 50 to 750 patients and approximately half of studies (eight out of 15, 53%) included less than 200 participants. Ten of 15 studies (66.6%) were conducted prospectively [9–13,16,28,31,33,34]. Colorectal cancer was the most represented tumor, and other cancers included gastrointestinal, head and neck or breast cancer. In two studies all patients were treated with capecitabine alone or in combination therapy [28,35]; in one study all patients received tegafur–uracil [34]. In the remaining studies, the patient cohort received either 5-FU alone or 5-FU-based regimens [9–12,16,29,31], or were treated with 5-FU or capecitabine [13,14,30,32,33], with the former being the predominant. Most of the studies (ten out of 15) did not clearly report the ethnic origin of patients [11–13,16,28–30,32,34,35], however it was assumed to be predominantly Caucasian because all the studies were conducted in Europe and because the *DPYD* IVS14+1G>A variant has not been found in Asiatic populations [36,37]. Toxicities were evaluated on the basis of National Cancer Institute Common Toxicity Criteria in 13 studies [9,10,12–14,16,28–33,35], while two studies used WHO criteria [11,34]. For the *DPYD* IVS14+1G>A polymorphism, 13 studies,

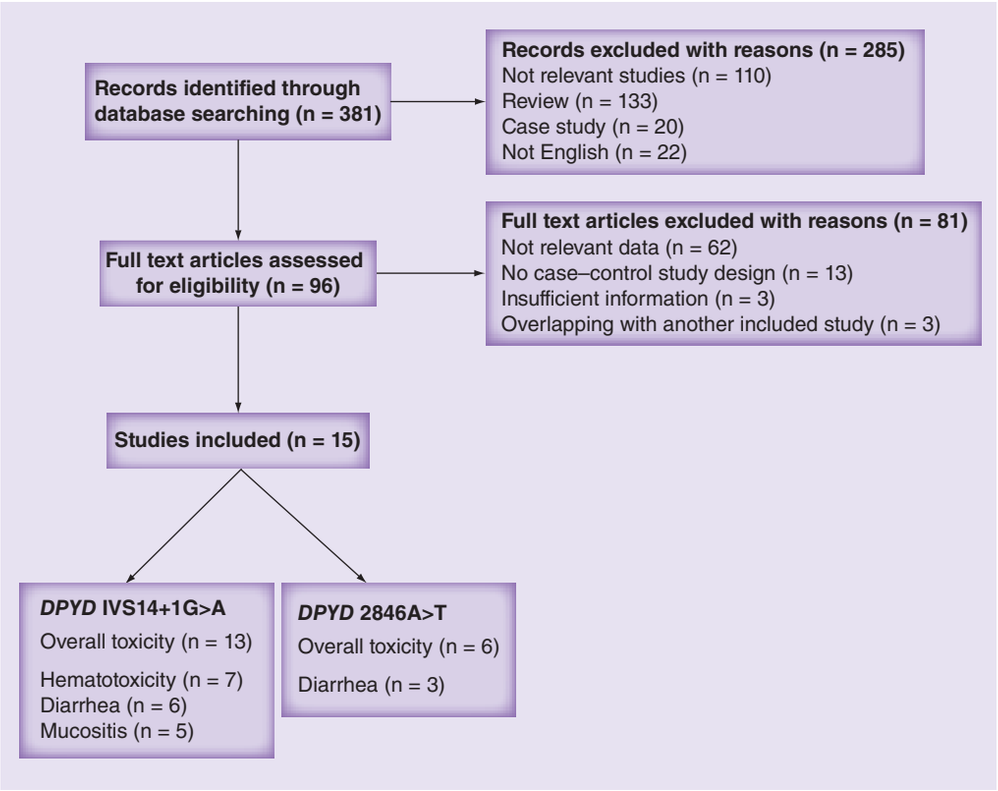


Figure 1. Study selection process.

including 3499 patients, compared the risk of overall grade ≥3 toxicity between *DPYD*2A* carriers and patients with a wild-type genotype [9–14,16,28,30,32–35]. Seven studies (n = 1554 subjects) evaluated the association of *DPYD* IVS14+1G>A with grade ≥3 hematologic toxicity [11,16,29,31–34]; six studies (n = 1526 patients) with grade ≥3 diarrhea [11,16,32–35] and five studies (n = 1015 subjects) with grade ≥3 mucositis [11,16,30,33,34]. For the *DPYD* 2846A>T variant, we identified seven studies (n = 2308 patients) assessing the risk of overall grade ≥3 toxicity between 2846T carriers and those with a wild-type genotype (2846AA) [9–11,14,32,34,36]. Among these, three studies including 721 patients evaluated the association between *DPYD* 2846A>T and grade ≥3 diarrhea [32,34,35].

Association between *DPYD* IVS14+1G>A & severe fluoropyrimidine-related toxicities

Sixty patients out of 4094 (1.46%) carried the *DPYD*2A* allele. The pooled results from random-effects meta-analysis provided strong evidence of association between carriers of the *DPYD* IVS14+1G>A variant allele and overall grade ≥3 toxicity following fluoropyrimidine treatment (OR = 5.42; 95% CI: 2.79–10.52; p < 0.001; Figure 2 & Table 2). It is of note that some of the studies included in the analysis [13,16,28,33,34] had only a single patient that carried the variant allele (Figure 2). The Q-statistic indicated no significant heterogeneity among studies (p < 0.31; I^2: 13%) and no indication of significant publication bias was found (Begg–Mazumdar's test: p = 0.952; Egger's test: p = 0.965; Horbold–Egger: p = 0.221; see Supplementary Figure 1). Exclusion of any single result from the analysis (leave-one-out sensitivity meta-analysis) did not substantially alter the overall result (Supplementary Figure 2). Pooled OR ranged from 4.05 (95% CI: 2.11–7.78), when the study of Morel *et al.* [9] was excluded from the analysis, to 7.32 (95% CI: 3.89–13.79), when the study of Braun *et al.* [12] was omitted. When studies were stratified into lower [9–11,13,16,28,32] and higher [12,14,30,33–35] percentage of cases with overall grade ≥3 toxicity, no significant heterogeneity was observed in either subgroups and a significant association with *DPYD* IVS14+1G>A was found only among studies with a lower percentage (OR: 8.31; 95% CI: 3.63–19.06; Figure 2 & Table 2). Two studies with a nested case–control design included a cohort of additional patients with severe toxicity [14,30]. As recruitment of additional cases might affect the impact of continuous variables (mean age, sample size, percentage of male subjects and incidence of grade ≥3 toxicity) on the pooled

Table 1. Demographic and clinical characteristics of studies included in the meta-analysis.

Study (year)	Location	Study design	Patients, n (male %)	Age, mean (SD)	Cancer type	Chemotherapy regimens	Toxicity classification criteria	Toxicity type and percentage of cases with grade 3–4 toxicity (%)	*DPYD* SNP	Method of *DPYD* SNP analysis	Ref.
Salgueiro *et al.* (2004)	Portugal	Prospective[†]	73 (46.6)	59 (31–85)[‡]	CRC	5-FU based	NCI-CTC	Overall (11.0), hematological (8.2), gastrointestinal (1.4) and mucositis (2.7)	IVS14+1G>A	Sequencing	[16]
Morel *et al.* (2006)	France	Prospective	487 (65.9)	63 (11.5)	CRC, head and neck and breast	5-FU alone (168), FOLFIRI (99), FOLFOX (91), FU/cisplatin (99) and FEC (30)	NCI-CTC	Overall toxicity at the first two cycles (9.0)	IVS14+1G>A and 2846A>T	Pyrosequencing	[9]
Largillier *et al.* (2006)	France	Prospective	105 (0)	61 (33–84)[‡]	Advanced breast cancer	Capecitabine alone	NCI-CTC	Overall toxicity at the first cycle (17.9% of 84 patients)	IVS14+1G>A	PCR-RFLP	[28]
Boisdron-Celle *et al.* (2007)	France	Prospective	252 (55.6)	67 (11.4)	Advanced CRC	LV5FU2 (157) and FUFOL (79)	NCI-CTC	Overall toxicity at the first two cycles (6.4% of 236 patients)	IVS14+1G>A and 2846A>T	Pyrosequencing	[10]
Schwab *et al.* (2008)	Germany	Prospective	683 (56.1)	64 (25–89)[§]	Colon, other gastrointestinal, breast and CUP	5-FU alone	WHO	Overall (16.1), leukopenia (4.7), diarrhea (8.6) and mucositis (7.6)	IVS14+1G>A and 2846A>T	dHPLC and sequencing	[11]
Sulzyc-Bielicka *et al.* (2008)	Poland	Retrospective[¶]	252 (57.9)	62	CRC	5-FU based	NCI-CTC v2.0	Myelotoxicity (1.6)	IVS14+1G>A	PCR-RFLP	[29]

[†][Salgueiro N, Pers. Comm.].
[‡]*Mean (range).*
[§]*Median (range).*
[¶][Sulzyc-Bielicka V, Pers. Comm.].
5-FU: 5-fluorouracil; BMD: Bone marrow depression; CMF: Cyclophosphamide, methotrexate, 5-fluorouracil; CRC: Colorectal cancer; CUP: Cancer of unknown primary origin; dHPLC: Denaturing HPLC; FEC: Bolus 5-fluorouracil, epirubicin, cyclophosphamide; FOLFIRI: Irinotecan, 5-fluorouracil and leucovorin; FOLFOX: Oxaliplatin, 5-fluorouracil, folinic acid; FUFOL: 5-fluorouracil + folinic acid; Mayo protocol: 5-fluorouracil, folinic acid; mCRC: Metastatic colorectal cancer; NCI-CTC: National Cancer Institute Common Terminology Criteria; NCI-CTC AE: National Cancer Institute Common Terminology Criteria for Adverse Events; NS: Not specified; OLF: Oxaliplatin, 5-fluorouracil, folinic acid; PLF: Cisplatin, 5-fluorouracil, folinic acid; SD: Standard deviation.

Table 1. Demographic and clinical characteristics of studies included in the meta-analysis (cont.).

Study (year)	Location	Study design	Patients, n (male %)	Age, mean (SD)	Cancer type	Chemotherapy regimens	Toxicity classification criteria	Toxicity type and percentage of cases with grade 3–4 toxicity (%)	*DPYD* SNP	Method of *DPYD* SNP analysis	Ref.
Gross *et al.* (2008)	Germany	NS	181 (55.2)	60.0 (10.5)	CRC (64), gastro-esophageal (82), breast (29) and not specified (6)	PLF ± paclitaxel (49), OLF/ FOLFOX (32), 5-FU-chemoradiation (30), FEC (18), capecitabine ± oxaliplatin (14), CMF (8), FOLFIRI (7), Mayo protocol (7) and other/not available (16)	NCI-CTC AE v3.0	Overall toxicity within the first three cycles (50.8)	IVS14+1G>A and 2846A>T	dHPLC and sequencing	[14]
Braun *et al.* (2009)	UK	Prospective	1188 (68.0)	64 (27–85)§	Advanced CRC	5-FU alone (688/1035), FOLFOX (172/1035) and FOLFIRI (175/1035)	NCI-CTC AE v2.0	Overall toxicity during the first 12 weeks of treatment (57.5% of 750 patients)	IVS14+1G>A	PCR-RFLP	[12]
Kleibl *et al.* (2009)	Czech Republic	Retrospective	124 (49.2)	60.3 (9.6)	CRC (91), orofacial (1), esophageal (3), gastric (5), biliary (3), pancreatic (2), pharyngeal (1), breast (15) and CUP (3)	5-FU alone (36), 5-FU/capecitabine (52), FOLFIRI (7), FOLFOX (23) and other (6)	NCI-CTC	Overall toxicity during the first two cycles (61.3) and mucositis (19.5)	IVS14+1G>A	Sequencing	[30]
Amstutz *et al.* (2009)	Switzerland	Prospective	111 (64.0)	63 (32–89)§	CRC (66), other GI (29) and non-GI (16)	5-FU alone (26), FOLFOX (38), FOLFIRI (9), 5-FU/cisplatin or carboplatin (13), capecitabine alone (13), and 5-FU/epirubicin and CMF (12)	NCI-CTC AE v3.0	Overall toxicity during the first two cycles (21.6)	IVS14+1G>A	Sequencing	[13]

†[Salgueiro N, Pers. Comm.].
‡*Mean (range).*
§*Median (range).*
¶[Sulzyc-Bielicka V, Pers. Comm.].
5-FU: 5-fluorouracil; BMD: Bone marrow depression; CMF: Cyclophosphamide, methotrexate, 5-fluorouracil; CRC: Colorectal cancer; CUP: Cancer of unknown primary origin; dHPLC: Denaturing HPLC; FEC: Bolus 5-fluorouracil, epirubicin, cyclophosphamide; FOLFIRI: Irinotecan, 5-fluorouracil and leucovorin; FOLFOX: Oxaliplatin, 5-fluorouracil, folinic acid; FUFOL: 5-fluorouracil + folinic acid; Mayo protocol: 5-fluorouracil, folinic acid; mCRC: Metastatic colorectal cancer; NCI-CTC: National Cancer Institute Common Terminology Criteria; NCI-CTC AE: National Cancer Institute Common Terminology Criteria for Adverse Events; NS: Not specified; OLF: Oxaliplatin, 5-fluorouracil, folinic acid; PLF: Cisplatin, 5-fluorouracil, folinic acid; SD: Standard deviation.

Table 1. Demographic and clinical characteristics of studies included in the meta-analysis (cont.).

Study (year)	Location	Study design	Patients, n (male %)	Age, mean (SD)	Cancer type	Chemotherapy regimens	Toxicity classification criteria	Toxicity type and percentage of cases with grade 3–4 toxicity (%)	*DPYD* SNP	Method of *DPYD* SNP analysis	Ref.
Boige *et al.* (2010)	France	Prospective	346 (61.8)	67.5 (7.7)	mCRC	LV5FU2, FOLFOX and FOLFIRI	NCI-CTC v2.0	Hematological (21.6% of 343 patients) and gastrointestinal (12.5% of 343 patients)	IVS14+1G>A	Real-time PCR	[31]
Kristensen *et al.* (2010)	Denmark	Retrospective	68 (55.9)	67 (11.5)	Advanced CRC	5-FU alone (24), FOLFOX (27) and capecitabine alone (17)	NCI-CTC AE v3.0	Overall (13.2), diarrhea (4.4) and BMD (4.4)	IVS14+1G>A and 2846A>T	PCR-RFLP	[32]
Cerić *et al.* (2010)	Bosnia Herzegovina	Prospective	50 (54.0)	60.0 (8.6)	CRC (41), pancreas (1), breast (3), ventricle (3) and gall bladder (2)	5-FU alone (42) and capecitabine alone (8)	NCI-CTC	Overall (50), neutropenia (26.0), diarrhea (24.0) and mucositis (24.0)	IVS14+1G>A	PCR-RFLP	[33]
Cellier *et al.* (2011)	France	Prospective	85 (66.0)	67.1 (25–81)§	Advanced rectal	Tegafur–uracil with leucovorin	WHO	Overall (43.5), hematological (3.5), diarrhea (21.2) and mucositis (1.2)	IVS14+1G>A and 2846A>T	Real-time mini sequencing	[34]
Deenen *et al.* (2011)	The Netherlands	Retrospective	568 (60.7)	63 (31–83)‡	mCRC	Capecitabine-based with cetuximab (287) and capecitabine-based without cetuximab (281)	NCIC-CTC v3.0	Overall (85.4% of 567 patients) and diarrhea (24.5% of 567 patients)	IVS14+1G>A and 2846A>T	Sequencing and real-time PCR	[35]

†[Salgueiro N, Pers. Comm.].
‡*Mean (range).*
§*Median (range).*
¶[Sulzyc-Bielicka V, Pers. Comm.].
5-FU: 5-fluorouracil; BMD: Bone marrow depression; CMF: Cyclophosphamide, methotrexate, 5-fluorouracil; CRC: Colorectal cancer; CUP: Cancer of unknown primary origin; dHPLC: Denaturing HPLC; FEC: Bolus 5-fluorouracil, epirubicin, cyclophosphamide; FOLFIRI: Irinotecan, 5-fluorouracil and leucovorin; FOLFOX: Oxaliplatin, 5-fluorouracil, folinic acid; FUFOL: 5-fluorouracil + folinic acid; Mayo protocol: 5-fluorouracil, folinic acid; mCRC: Metastatic colorectal cancer; NCI-CTC: National Cancer Institute Common Terminology Criteria; NCI-CTC AE: National Cancer Institute Common Terminology Criteria for Adverse Events; NS: Not specified; OLF: Oxaliplatin, 5-fluorouracil, folinic acid; PLF: Cisplatin, 5-fluorouracil, folinic acid; SD: Standard deviation.

estimate, only prospective studies were included in the meta-regression analysis [9–13,16,28,33,34]. An inverse linear relationship was found in prospective studies between OR and incidence of overall grade ≥3 toxicity (p = 0.003; Figure 3), while no linear relationship was found for sample size (p = 0.264), mean age (p = 0.884) or percentage of male subjects (p = 0.365). To further evaluate the robustness of the results, we conducted four sensitivity analyses (inclusion criteria: prospective studies, higher quality studies, studies with sample size ≥200 and studies using 5-FU-based regimens). As summarized in Table 2, no substantial change in the OR estimate was found across the four assumptions tested and pooled OR ranged from 5.57 (95% CI: 2.14–14.48) to 7.24 (95% CI: 2.06–25.40). It is of note that when analysis was limited to patients receiving 5-FU alone, only three prospective studies were identified [10–12], however statistical significance of *DPYD* IVS14+1G>A was still retained (Table 2). Surprisingly, the three studies included two studies with low incidence of adverse effects [10,11] and one study with high incidence [12].

When analysis was performed for each of the three major toxicity types related to fluoropyrimidine treatment (Figure 4 & Table 2), the pooled results provided evidence that patients carrying the *DPYD* IVS14+1G>A allele displayed an increased risk of grade ≥3 hematological toxicity (OR: 15.77; 95% CI: 6.36–39.06; p < 0.001), grade ≥3 diarrhea (OR: 5.54; 95% CI: 2.31–13.29; p < 0.001), and grade ≥3 mucositis (OR: 7.48; 95% CI: 3.03–18.47; p < 0.001). For all the three end points considered, the I^2-statistic indicated the absence of heterogeneity among studies (I^2 = 0%).

Sensitivity & specificity of *DPYD* IVS14+1G>A testing

Pooled summary results of diagnostic performances of *DPYD* IVS14+1G>A genotyping in fluoropyrimidine-treated patients are shown in Table 2 & Supplementary Figure 3. The pooled sensitivity and specificity estimates of *DPYD* IVS14+1G>A genotyping for the prediction of overall grade ≥3 toxicity were 5.2% (95% CI: 3.0–8.9) and 99.2% (95% CI: 98.8–99.4), respectively. Heterogeneity was detected in the estimates of sensitivity (p < 0.001), but not for specificity (p = 0.908). In the four sensitivity analyses conducted, sensitivity estimates were similar to the overall analysis, ranging from 4.0 to 6.6%, and significant heterogeneity among studies was still present across all comparisons. After stratification of studies according to the incidence of overall grade ≥3 toxicity, no heterogeneity (I^2 = 0) was detected among studies with a lower incidence of toxicity and a sensitivity estimate of 9.0% (95% CI: 5.7–13.9) was found. The pooled sensitivity estimates of *DPYD* IVS14+1G>A genotyping for severe hematological toxicity, diarrhea and mucositis were 13.0% (95% CI: 6.6–24.1), 5.6% (95% CI: 3.2–9.7) and 11.5% (95% CI: 6.2–20.5), respectively. No significant heterogeneity was found in the sensitive estimate for the prediction of grade ≥3 hematological toxicity (Q value p = 0.197), and no heterogeneity (I^2 = 0) was found in sensitivity estimates for the prediction of severe degrees of diarrhea or mucositis.

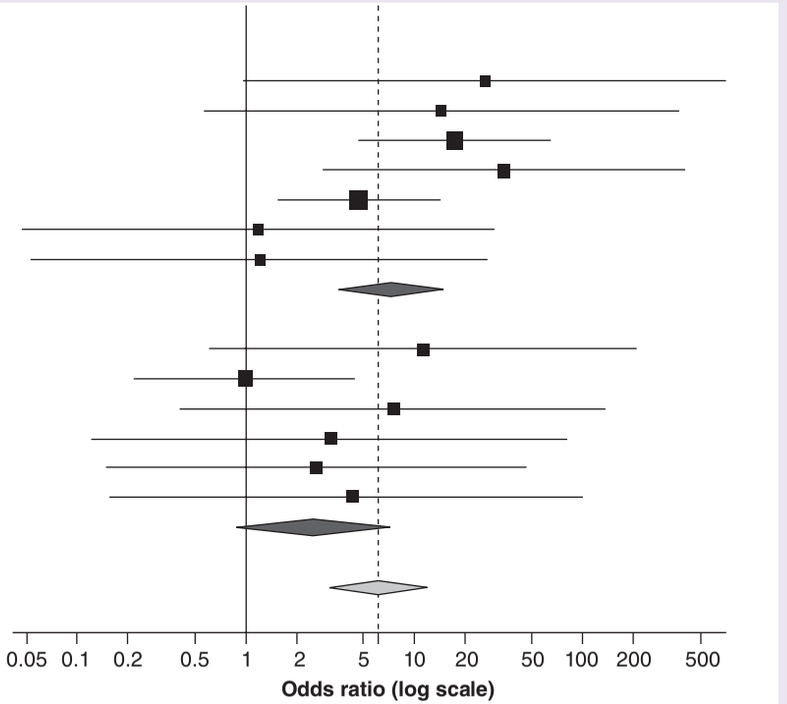
Association between *DPYD* 2846A>T & severe fluoropyrimidine-related toxicities

Thirty-four patients out of 2308 (1.47%) carried the *DPYD* 2846T allele. The pooled results provided evidence of association between carriers of the *DPYD* 2846T allele and overall grade ≥3 toxicity (OR: 8.18; 95% CI: 2.65–25.25; p < 0.001; Figure 2 & Table 2). Exclusion of any single result from the analysis (leave-one-out sensitivity meta-analysis) did not substantively alter the overall estimate (Supplementary Figure 2). However, the significant result of *DPYD* 2846A>T was limited by the presence of moderate heterogeneity, as evident from the I^2 value of 47% or p-value of Cochran's Q-test (<0.076) and possible publication bias (Begg–Mazumdar's test: p = 0.069; Egger's test: p = 0.167; Horbold–Egger: p = 0.946; Supplementary Figure 1). When studies were stratified into lower [9–11,32] and higher [14,34,35] incidence of overall grade ≥3 toxicity, no significant heterogeneity was observed in either subgroups, and a significant association with *DPYD* 2846A>T was found only among studies with a lower incidence of overall grade ≥3 toxicity (OR: 16.59; 95% CI: 5.06–54.43; Figure 2 & Table 2).

None of the studies identified had a nested case–control design or included additional cases with severe toxicity. Results of meta-regression analysis showed an inverse linear relationship between OR and incidence of overall grade ≥3 toxicity (p = 0.006), while no linear relationship was found for the other variables considered (sample size, mean age and percentage of male subjects). However, when the meta-regression analysis was limited to the four prospective studies [9–11,34] the statistical significance of this inverse linear relationship was lost (p = 0.138) (Figure 3).

***DPYD* IVS14+1G>A**

Overall grade 3–4 toxicity	Estimate (95% CI)	Ev/*2A carriers	Ev/*2A WT
Salguerio 2004 [16]	26.200 (0.978–702.240)	1/1	7/72
Largillier 2006 [28]	14.379 (0.557–370.916)	1/1	14/83
Morel 2006 [9]	17.329 (4.686–64.084)	6/10	38/477
Boisdron-Celle 2007 [10]	33.846 (2.878–398.043)	2/3	13/233
Schwab 2008 [11]	4.665 (1.537–14.159)	6/13	104/670
Amstutz 2009 [13]	1.177 (0.046–29.805)	0/1	24/110
Kristensen 2010 [32]	1.211 (0.054–27.221)	0/2	9/66
Subgroup L (I^2 = 14%; p = 0.325)	**8.314 (3.626–19.065)**	**16/31**	**209/1711**
Gross 2008 [14]	11.251 (0.613–206.532)	5/5	87/176
Braun 2009 [12]	0.987 (0.219–4.440)	4/7	427/743
Kleibl 2009 [30]	7.462 (0.403–138.053)	5/5	71/119
Cerić 2010 [33]	3.122 (0.121–80.386)	1/1	24/49
Deenen 2011 [35]	2.623 (0.148–46.359)	7/7	477/560
Cellier 2011 [34]	3.986 (0.158–100.695)	1/1	36/84
Subgroup H (I^2 = 0%; p = 0.680)	**2.417 (0.885–6.606)**	**23/26**	**1122/1731**
Overall (I^2 = 13%; p = 0.313)	**5.417 (2.789–10.521)**	**39/57**	**1331/3442**



***DPYD* 2846A>T**

Overall grade 3–4 toxicity	Estimate (95% CI)	Ev/2846T carriers	Ev/2846AA
Morel 2006 [9]	17.329 (4.686–64.084)	6/10	38/477
Boisdron-Celle 2007 [10]	73.000 (12.896–413.236)	6/8	9/228
Schwab 2008 [11]	8.005 (1.322–48.476)	3/5	107/678
Kristensen 2010 [32]	2.053 (0.078–54.178)	0/1	9/67
Subgroup L (I^2 = 40%; p = 0.174)	**16.591 (5.057–54.426)**	**15/24**	**163/1450**
Gross 2008 [14]	2.934 (0.118–72.988)	1/1	91/180
Cellier 2011 [34]	3.986 (0.158–100.695)	1/1	36/84
Deenen 2011 [35]	1.201 (0.146–9.888)	7/8	478/560
Subgroup H (I^2 = 0%; p = 0.797)	**1.946 (0.414–9.142)**	**9/10**	**605/824**
Overall (I^2 = 47%; p = 0.076)	**8.179 (2.650–25.245)**	**24/34**	**768/2274**

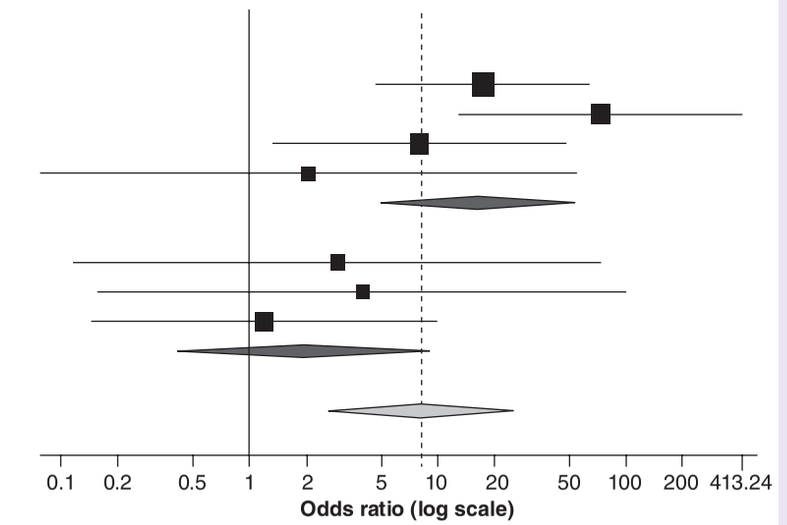


Figure 2. Forest plots for the association of *DPYD* variants with overall grade ≥3 toxicity and following stratified subgroups according to the incidence level of severe toxicity. IVS14+1G>A: data from [9–14,16,28,30,32–35]; 2846A>T: data from [9–11,14,32,34,35]. Pooled estimates (odds ratios) are from the random-effects model.
Ev: Events; Subgroup H: Higher; Subgroup L: Lower.

In the sensitivity analyses conducted, a significant heterogeneity was still found among studies with higher quality (p = 0.046; I^2: 59%) and studies including ≥200 patients (p = 0.190; I^2: 67%), but not among prospective studies (p = 0.249; I^2: 27%) or studies using 5-FU-based regimens (p = 0.205, I^2: 37%; Table 2). The pooled results from random-effects meta-analysis provided evidence of strong association between carriers of the *DPYD* 2846A>T variant allele and overall grade 3–4 toxicity when analysis was restricted to prospective studies (OR: 18.14; 95% CI: 6.26–52.58) or to studies using 5-FU-based regimens (OR: 21.38; 95% CI: 6.71–68.15). The pooled OR estimate for the association between *DPYD* 2846A>T and grade ≥3 diarrhea was 6.04 (95% CI: 1.77–20.66; p = 0.004; Figure 4 & Table 2) and the I^2-statistic indicated the absence of heterogeneity among studies (I^2 = 0%).

Sensitivity & specificity of *DPYD* 2846A>T testing

Pooled summary results of diagnostic performances of *DPYD* 2846A>T genotyping in fluoropyrimidine-treated patients are shown in Table 2 & Supplementary Figure 3. The sensitivity and specificity estimates of *DPYD* 2846A>T genotyping for the prediction of overall grade ≥3 toxicity were 5.4% (95% CI: 1.7–16.1) and 99.1% (95% CI: 98.7–99.4), respectively. Similarly to the *DPYD*2A* variant, sensitivity but not specificity estimate of *DPYD* 2846A>T genotyping was limited by the presence of significant heterogeneity, as evident from p-value of Cochran's Q-test (<0.001).

In the sensitivity analyses conducted, sensitivity estimates ranged from 6.7 to 12.7%, and significant heterogeneity among studies was still present across all comparisons. In the subgroup analysis, a sensitivity estimate of 11.2% (95% CI: 2.8–35.1) was found among studies with a lower incidence of overall grade 3–4 toxicity, however the Q-statistic indicated the presence of high and significant heterogeneity (p < 0.001). The pooled sensitivity and specificity estimates of *DPYD* 2846A>T genotyping for the prediction of grade ≥3 diarrhea were 4.6% (95% CI: 2.2–9.4) and 99.2% (95% CI: 98.4–99.6), respectively. No heterogeneity (I^2: 0) was found in sensitivity and specificity estimates of *DPYD* 2846A>T genotyping for the prediction of severe degrees of diarrhea.

Discussion

Despite recommendations by regulatory agencies, such as the US FDA warning in 2003 stating that 5-FU and capecitabine are contraindicated in patients with a known DPD deficiency, clinical usefulness of routine testing of deleterious *DPYD* genetic variants prior to fluoropyrimidine chemotherapy is still not established. Indeed, there are numerous reports on the impact of deleterious *DPYD* variants on fluoropyrimidine chemotherapy, but to our knowledge the literature lacks pooled estimates on the association between these variants and fluoropyrimidine-induced adverse reactions, and of the sensitivity and specificity of diagnostic pharmacogenetic testing of this gene. The present meta-analysis intends to fill this gap, as we feel that a discussion on whether this pharmacogenetic testing is warranted must start from these data.

This systematic review presents pooled data from primary pharmacogenetic studies that have evaluated the association between *DPYD* IVS14+1G>A or *DPYD* 2846A>T, plausible candidates for predictive pharmacogenetic tests [7,8,17,18], and the risk of grade ≥3 toxicity following fluoropyrimidine treatment. Results of pooled data for *DPYD* IVS14+1G>A (OR: 6.11; 95% CI: 3.11–11.98) and 2846A>T (OR: 8.18; 95% CI: 2.69–25.25) provided consistent evidence for an increased risk of overall severe toxicity. The robustness of these findings was assessed by four sensitivity analyses that substantially confirmed the validity of the overall result. It is noteworthy that an inverse correlation was found in prospective studies between the effect size of *DPYD* IVS14+1G>A and the frequency of overall grade ≥3 toxicity in the patient cohort studied. In other words, the impact of *DPYD* IVS14+1G>A was higher in patient cohorts in which the incidence of severe toxicity was lower. Furthermore, pooled data showed evidence that *DPYD* IVS14+1G>A is a strong risk factor of grade ≥3 hematologic toxicity and to a lesser extent of grade ≥3 mucositis or grade ≥3 diarrhea. Finally, the data suggest that specificity estimates for the prediction of overall grade ≥3 toxicity were above 99%, while sensitivity estimates of *DPYD* IVS14+1G>A and 2846A>T variants were approximately 5% of all overall grade ≥3 toxicities. It should, furthermore, be highlighted that over two-thirds of patients represented in this study that displayed either the *DPYD* IVS14+1G>A or 2846A>T variants developed severe toxicity (39 out of 51 and 24 out of 34, respectively).

We recognize several limitations to the present study. First, despite the large sample size, the number of patients carrying a *DPYD* variant allele is limited and this may account for the large

Table 2. Summary of random-effects meta-analyses for the association between *DPYD* variants and severe fluoropyrimidine-related toxicities.

End point	Studies included (n)	Patients (n)	OR (95% CI)	p-value	Heterogeneity			Sensitivity, % (95% CI)	p-value (Q-test)	Specificity, % (95% CI)	p-value (Q-test)
					Q	*p-value Q test*	*I² %*				
DPYD *IVS14+1G>A and overall grade 3–4 toxicity*											
All studies	13	3499	5.42 (2.79–10.52)	<0.001	13.81	0.31	13	5.2 (3.0–8.9)	<0.001	99.2 (98.8–99.4)	0.908
Prospective studies	9	2559	5.87 (2.42–14.22)	<0.001	12.36	0.14	35	5.9 (2.9–11.9)	0.003	99.0 (98.5–99.4)	0.966
Higher quality studies	7	2932	5.57 (2.14–14.48)	<0.001	10.48	0.11	43	4.5 (2.0–9.8)	<0.001	99.1 (98.8–99.4)	0.846
Sample size ≥200 patients	5	2723	5.62 (1.69–18.73)	0.005	10.40	0.03	62	4.0 (1.4–11.7)	<0.001	99.2 (98.8–99.5)	0.657
5-FU-based chemotherapy	5	2229	7.24 (2.06–25.40)	0.002	10.92	0.03	63	6.6 (2.2–18.2)	<0.001	99.1 (98.5–99.4)	0.844
5-FU alone	3	1324	4.81 (1.01–22.93)	0.049	4.57	0.10	56	4.3 (1.2–14.7)	0.028	99.0 (98.2–99.5)	0.514
Percentage of cases with toxicity											
Higher (≥40%)	6	1757	2.42 (0.88–6.61)	0.085	3.13	0.68	0	3.2 (1.5–6.7)	0.005	99.2 (98.8–99.5)	0.707
Lower (<40%)	7	1742	8.31 (3.63–19.06)	<0.001	6.95	0.32	14	9.0 (5.7–13.9)	0.524	99.0 (98.4–99.4)	0.836
DPYD *IVS14+1G>A and hematological grade 3–4 toxicity*											
All studies	7	1554	15.77 (6.36–39.06)	<0.001	5.04	0.54	0	13.0 (6.6–24.1)	0.197	99.3 (98.6–99.7)	0.150
DPYD *IVS14+1G>A and grade 3–4 diarrhea*											
All studies	6	1526	5.54 (2.31–13.29)	0.001	1.51	0.91	0	5.6 (3.2–9.7)	0.619	99.1 (98.4–99.5)	0.481
DPYD *IVS14+1G>A and grade 3–4 mucositis*											
All studies	5	1015	7.48 (3.03–18.47)	<0.001	0.92	0.93	0	11.5 (6.2–20.5)	0.771	99.4 (96.9–99.2)	0.984
DPYD *2846A>T and overall grade 3–4 toxicity*											
All studies	7	2308	8.18 (2.65–25.25)	<0.001	11.43	0.076	47	5.4 (1.7–16.1)	<0.001	99.1 (98.7–99.4)	0.662
Prospective studies	4	1491	18.14 (6.26–52.58)	<0.001	4.12	0.249	27	10.0 (2.7–31.0)	<0.001	99.3 (98.7–99.6)	0.537
Higher quality studies	5	2059	10.36 (2.76–38.90)	<0.001	9.68	0.046	59	6.7 (1.6–23.5)	<0.001	99.1 (98.6–99.4)	0.516
Sample size ≥200 patients	4	1974	11.58 (2.60–51.61)	0.001	9.16	0.027	67	7.4 (1.4–30.9)	<0.001	99.1 (98.6–99.5)	0.359
5-FU-based chemotherapy	3	1406	21.38 (6.71–68.15)	<0.001	3.17	0.205	37	12.7 (2.7–43.3)	<0.001	99.3 (98.7–99.6)	0.367
Percentage of cases with toxicity											
Higher (≥40%)	3	834	1.95 (0.41–9.14)	0.399	0.45	0.797	0	1.7 (0.1–3.1)	0.529	99.0 (98.1–99.4)	0.690
Lower (<40%)	4	1474	16.59 (5.06–54.43)	<0.001	4.98	0.174	40	11.2 (2.8–35.1)	<0.001	99.3 (98.7–99.6)	0.433
DPYD *2846A>T and grade 3–4 diarrhea*											
All studies	3	721	6.04 (1.77–20.66)	0.004	0.19	0.911	0	4.6 (2.2–9.4)	0.525	99.2 (98.4–99.6)	0.750

5-FU: 5-fluorouracil; OR: Odds ratio.

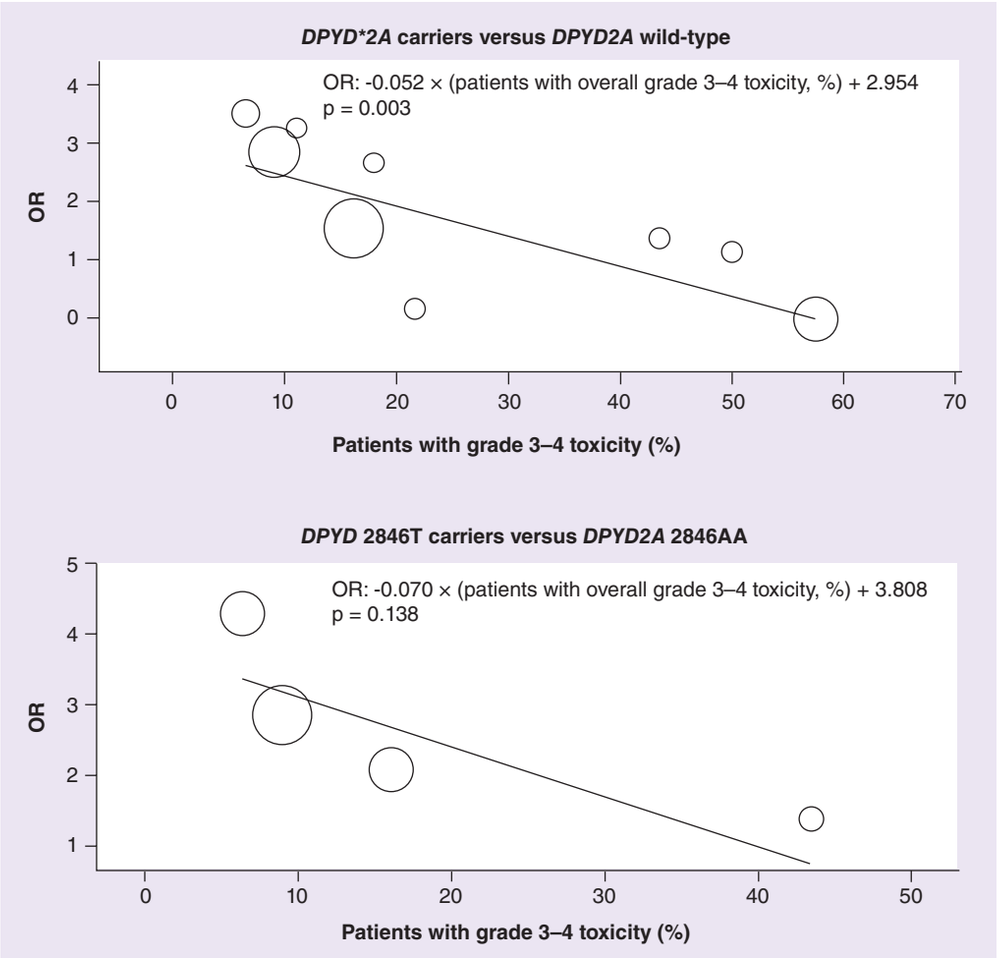


Figure 3. Meta-regression analyses of odds ratios against the incidence of overall grade ≥3 toxicity. Each circle represents a study and the size of each circle is proportional to the study-specific statistical weight. In this analysis only prospective studies were included. IVS14+1G>A: data from [9–13,16,28,33,34]; 2846A>T: data from [9–11,34].
OR: Odds ratio.

confidence intervals and inherent uncertainty in the estimates. Other important limitations include the observational nature of the original data and the lack of uniformity between studies in terms of solid tumor type, treatment protocols and reported period of fluoropyrimidine-related adverse effect. All these factors might have contributed to the heterogeneity observed among studies in sensitivity estimates of *DPYD* testing. In addition, partial reporting of outcome examined by a single study cannot be excluded as this particular form of publication bias may be particularly high for adverse effects [38]. Under such circumstances, the recommended approach for meta-analyses is to avoid focusing on the single overall estimate but to focus on assessing the consistency of effects and evaluating variables that influence outcome measures. Subgroup and sensitivity analyses conducted for *DPYD* IVS14+1G>A did not substantially alter the results or affect our overall conclusions, indicating robustness of our findings to different assumptions regarding study validity and inclusion of data. Finally, the lack of individualized data, which is a general problem of meta-analyses when pooling data from primary studies, also precluded the analysis of combined effects of the two *DPYD* variants on overall grade ≥3 toxicity or following stratification according to each type of adverse effect.

Routine screening of deleterious *DPYD* variants before starting fluoropyrimidine treatment has been proposed for the selection of patients requiring dose reductions [35]. According to the guidelines of The Pharmacogenetics Working Group of the Royal Dutch Association for the Advancement of Pharmacy, alternative drugs to fluoropyrimidines should be recommended for homozygous carriers of a nonfunctional *DPYD* allele, while a 50% reduction of fluoropyrimidine dose should be advised for heterozygous carriers

***DPYD* IVS14+1G>A**

Grade 3–4 hematotoxicity	Estimate (95% CI)	Ev/*2A carriers	Ev/*2A WT
Salguerio 2004 [16]	36.818 (1.336–1014.795)	1/1	5/72
Schwab 2008 [11]	10.190 (2.958–35.111)	4/13	28/670
Sulzyc-Bielicka 2008 [29]	213.000 (7.325–6193.350)	1/1	3/251
Cerić 2010 [33]	9.000 (0.344–235.370)	1/1	12/49
Boige 2010 [31]	18.586 (0.883–391.419)	2/2	72/341
Kristensen 2010 [32]	3.629 (0.145–90.940)	0/2	3/66
Cellier 2011 [34]	99.000 (3.178–3084.370)	1/1	2/84
Overall (I^2 = 0%; p = 0.538)	**15.767 (6.364–39.063)**	**10/21**	**125/1533**

Grade 3–4 diarrhea	Estimate (95% CI)	Ev/*2A carriers	Ev/*2A WT
Salguerio 2004 [16]	15.889 (0.440–573.319)	0/1	1/72
Schwab 2008 [11]	3.289 (0.880–12.299)	3/13	56/670
Cerić 2010 [33]	10.043 (0.383–263.628)	1/1	11/49
Kristensen 2010 [32]	3.626 (0.145–90.940)	0/2	3/66
Cellier 2011 [34]	11.571 (0.452–296.481)	1/1	17/84
Deenen 2011 [35]	7.948 (1.524–41.435)	5/7	134/560
Overall (I^2 = 0%; p = 0.912)	**5.538 (2.307–13.292)**	**10/25**	**222/1501**

Grade 3–4 mucositis	Estimate (95% CI)	Ev/*2A carriers	Ev/*2A WT
Salguerio 2004 [16]	9.400 (0.301–293.197)	0/1	2/72
Schwab 2008 [11]	5.759 (1.711–19.387)	4/13	48/670
Kleibl 2009 [30]	7.000 (1.100–44.527)	3/5	21/119
Cerić 2010 [33]	24.273 (0.877–671.985)	1/1	5/49
Cellier 2011 [34]	18.556 (0.515–668.819)	0/1	1/84
Overall (I^2 = 0%; p = 0.920)	**7.480 (3.029–18.469)**	**8/21**	**77/994**

***DPYD* 2846A>T**

Grade 3–4 diarrhea	Estimate (95% CI)	Ev/2846T carriers	Ev/2846AA
Kristensen 2010 [32]	6.143 (0.210–179.786)	0/1	3/67
Cellier 2011 [34]	11.571 (0.452–296.481)	1/1	17/84
Deenen 2011 [35]	5.299 (1.250–22.463)	5/8	134/560
Overall (I^2 = 0%; p = 0.911)	**6.045 (1.769–20.659)**	**6/10**	**154/711**

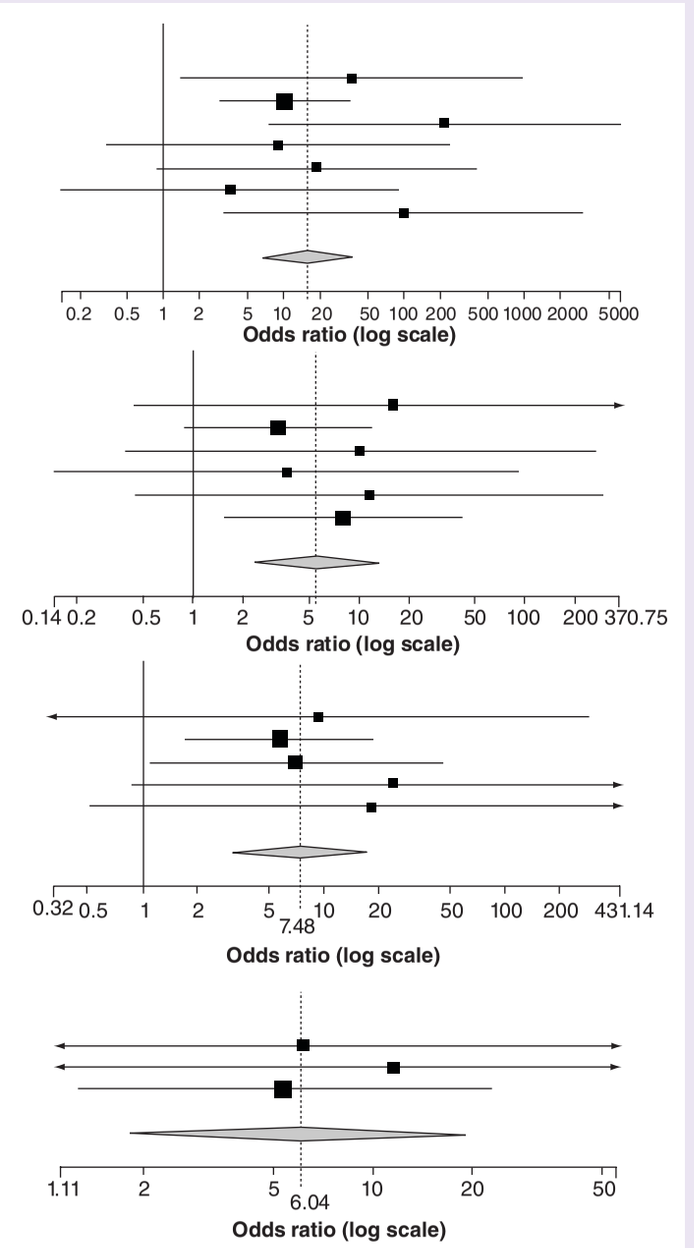


Figure 4. Forest plots for the association of *DPYD* variants with major toxicity types related to fluoropyrimidine treatment. IVS14+1G>A and grade 3–4 hematotoxicity: data from [11,16,29,31–34]; IVS14+1G>A and grade 3–4 diarrhea: data from [11,16,32–35]; IVS14+1G>A and grade 3–4 mucositis: data from [11,16,30,33,34]. 2846A>T and grade 3–4 diarrhea: data from [32,34,35]. Pooled estimates (odds ratios) are from the random-effects model.
Ev: Events.

of a nonfunctional *DPYD* allele [39]. The present meta-analysis supports the assessment of *DPYD* IVS14+1G>A 2846A>T in routine clinical practice and suggests that a dose-reduction recommendation may be appropriate for carriers of *DPYD* variants.

An observation that might appear surprising in this paper was that prospective studies included displayed a very variable incidence of overall grade ≥3 toxicity, ranging from 6 to 57%. This observation has been reported previously by others as well. For example, a meta-analysis investigating toxicity of bolus fluorouracil in advanced colorectal cancer included studies with reported hematological grade 3–4 toxicities ranging from 4 to 49% [40]. In the present study an even wider range of studies were included, with different cancer types and chemotherapy regimens (at times this difference was intra-study). Furthermore, the studies included used different classification criteria of side effects, assessed them at different times and reported them differently. Another issue is that the discrepancy in side effects for less objective adverse effects, for example diarrhea and mucositis, appeared large and it was not always obvious that cancer-related symptoms had been excluded. Notwithstanding these considerations, our study found that *DPYD* IVS14+1G>A has an increased impact in clinical settings in which lower incidence of severe fluoropyrimidine-related toxicities emerge. This was a consistent result, which was present both when including all studies and when including only prospective studies and led to a threefold increase in sensitivity in the population with a lower incidence of grade 3–4 toxicity. This might be correlated with the fact that IVS14+1G>A is present in only 1.5% of the general population. It would therefore appear that deleterious *DPYD* alleles with low frequency increase their relative contribution in clinical settings in which side effects have been reduced by other means. As we are unable to identify the reason for the variability in adverse effects, further studies are necessary as these may pave the way to finding a patient population in which the cost–effectiveness of *DPYD* IVS14+1G>A screening is substantially increased.

While *DPYD* IVS14+1G>A genotyping is routinely available from several laboratories, this test is not currently used in clinical practice owing, in large part, to uncertainties regarding its clinical utility. In addition, the cost–effectiveness of testing for *DPYD* variants still remains to be determined. The data presented here will represent a starting point to establish the cost–effectiveness and clinical usefulness of *DPYD* screening in the different clinical settings. Yet, the crucial element arising from the present analysis is that *DPYD* IVS14+1G>A and *DPYD* A2846T variants each account for 5% of patients with severe toxicities. In this context, it should also be noted that screening for both variants should account for an increased proportion of patients, as the two variants are not in linkage disequilibrium [14]. In addition, the simultaneous presence of *DPYD* IVS14+1G>A and 2846A>T variants was shown to be lethal in some patients shortly after initiation of treatment with fluoropyrimidine [25,41]. Thus, it is likely that simultaneous analysis of deleterious DPD variants and functional SNPs in other relevant genes would improve both the clinical and economic impact of genotyping. Indeed, numerous *DPYD* variants have been described. Alongside those considered in this article, the nonsynonymous 1679T>G (rs55886062) substitution, resulting in the change of isoleucine to serine at codon 560, is among the most studied and appears to be associated with 5-FU toxicity [6]. However, this deleterious variant has been mainly described in case reports [42,43], in studies that included only patients with severe toxicity [44,45] or in patients with reduced DPD activity only [46] and the insufficient number of studies with a case–control design [9,13] precluded the possibility to include the 1679T>G variant in our meta-analysis. On the other hand, epigenetic and regulatory factors affecting DPD activity and contributing to fluoropyrimidine toxicity have eluded detection so far. Although preliminary findings supported the possibility that partial methylation of the *DPYD* promoter may be associated with downregulation of *DPYD* activity [45], *DPYD* promoter hypermethylation was not detected in subsequent larger studies [11,47,48]. Similarly, large *DPYD* intragenic rearrangements do not seem to contribute significantly to the development of 5-FU severe toxicity in gastrointestinal cancer patients [47,49,50]. The rapid development in next-generation sequencing will probably contribute to identify other rare *DPYD* variants with strong effects on fluoropyrimidine toxicity. Alongside *DPYD* genotyping, several phenotypic methods have been proposed for establishing, directly or indirectly, the DPD deficiency status of cancer patients. Among these are evaluation of DPD activity in peripheral blood mononuclear cells, measurement of uracil in plasma and urea, evaluation of the dihydrouracil:uracil and dihydrothymine:thymine ratio in plasma and urea, [2-C13]-uracil breath test, and analysis of fluorouracil and dihydrofluorouracil in plasma

after the administration of a test dose of 5-FU [51,52]. The pros and cons of these phenotypic methods and the issue regarding the circadian rhythm in the expression and activity of DPD have been discussed elsewhere [51]. Although some of these approaches have now been developed with a cost– and time–effectiveness perspective, the sensitivity of determining DPD status on a genotypic, a phenotypic or a mixed basis still remains an unsolved question [53].

Adverse drug reactions to 5-FU-based chemotherapy have been reported to also be influenced by polymorphic variants of genes encoding the drug-related enzymes *TYMS* and *MTHFR*, however conflicting results have been reported so far. In this regard, of note are conclusions of a recent meta-analysis showing that in colorectal cancer patients, homozygous carriers of the 2R allele of the *TYMS* 5′-UTR repeat polymorphism (rs45445694) have an increased risk of developing severe toxicity following fluoropyrimidine treatment, compared to carriers of the *TYMS* 3R allele [54]. By contrast, pooled data showed no significant association between the *MTHFR* 677C>T (rs1801133) variant and the risk of adverse effects following fluoropyrimidine treatment in colorectal cancer patients [54]. Notably, Afzal *et al.* showed that MTHFR activity and a specific combination of the *TYMS* 3′-UTR insertion/deletion (1494del TTAAAG, rs34489327) and *MTHFR* 1298A>C (rs1801131) polymorphisms are possible predictors of 5-FU treatment-related toxicity [55]. As the *DPYD* IVS14+1G>A and *DPYD* A2846T variants each account for a minority of patients with severe toxicities, a pathway-based approach analyzing the combined effect of multiple variant alleles of genes involved in 5-FU metabolism and mechanism of action (e.g., *DPYD*, *TS* and *MTHFR*) may be a more appropriate strategy for the identification of patients at higher risk. This approach should help to decipher the additive, synergistic or compensating effects of these genes, as well as increase the cost–effectiveness and clinical impact of pharmacogenetic testing. However, this will be possible only by recruiting large prospective cohorts of cancer patients treated with homogeneous chemotherapy regimens and this approach would benefit from multicenter and international collaborations.

Conclusion

The results of the present meta-analysis confirm clinical validity of *DPYD* IVS14+1G>A and 2846A>T as risk factors for the development of severe toxicities following fluoropyrimidine treatment. We recommend that further retrospective studies are unnecessary and unlikely to add to the evidence base. Second, the toxicity risk conferred by *DPYD* IVS14+1G>A is more consistent in clinical settings that display lower incidence of severe fluoropyrimidine-related adverse effects. The reason for this inverse relationship should be investigated further in prospective studies. Last, the sensitivity and specificity estimates obtained could be used clinically to determine the cost–effectiveness of *DPYD* variant screening in different settings. In order to convince the policymakers to support such genetic testing, a formal cost–effectiveness analysis is warranted.

Future perspective

Research during the last 10 years has provided a large amount of data on the clinical validity of *DPYD* IVS14+1G>A as a risk factor of severe fluoropyrimidine-related toxicity. However, evidence that *DPYD* testing prior to fluoropyrimidine treatment effectively reduces fluoropyrimidine-induced adverse effects in cancer patients is still lacking. Indeed, despite a potential clinical utility for the identification of patients requiring a fluoropyrimidine dose reduction, for most patients it is unlikely that knowledge of *DPYD* gene status will be sufficient alone to guide treatment decision-making.

It must be acknowledged that other genes (e.g., *TYMS*) have also been shown to be moderate predictors of response or adverse effects, and that future genome-wide association studies with large patient populations will identify polymorphisms in other genes that will be equally or more relevant for individual differences in safety and efficacy of fluoropyrimidine-based regimens. Furthermore, a number of groups are moving forward with a phenotypic approach to reduce side effects. It is therefore likely that in the not so distant future composite pharmacological predictors (genotype and phenotype) together with relevant clinical variables will be used in the clinical setting for the optimization of fluoropyrimidine treatment, yielding higher response rates with a lower incidence of adverse effects.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- Controversial results have been reported so far on the association between *DPYD* IVS14+1G>A and 2846A>T polymorphisms and the risk of developing severe toxicities following fluoropyrimidine treatment.
- No conclusive results have been also reported on the proportion of toxicities that can be explained by the presence of *DPYD* variant alleles.

Methods

- A systematic review and meta-analysis of the available literature was carried out to quantify the impact of the *DPYD* IVS14+1G>A and 2846A>T variants on the risk of fluoropyrimidine-related toxicities and to determine sensitivity and specificity of the test.

Results

- On the basis of currently available data, the increased risk of overall grade ≥3 toxicity for carriers of the *DPYD* IVS14+1G>A and *DPYD* 2846A>T is five- and eight-fold, respectively, compared with wild-type treated with fluoropyrimidines.
- Pooled data showed evidence that *DPYD* IVS14+1G>A is a strong risk factor of grade ≥3 hematologic toxicity and to a lesser extent of grade ≥3 mucositis or grade ≥3 diarrhea. In addition, a strong association was also found between carriers of the *DPYD* 2846T allele and grade ≥3 diarrhea.
- Pooled specificities estimates of *DPYD* IVS14+1G>A and 2846A>T genotyping for the prediction of overall grade ≥3 toxicities were >99% and sensitivity estimate for each variant was about 5%.

Discussion & conclusion

- This meta-analysis confirms clinical validity of *DPYD* IVS14+1G>A and 2846A>T as risk factors for the development of severe toxicities following fluoropyrimidine treatment.
- The results obtained on sensitivity and specificity estimates of testing for *DPYD* variants represent a starting point to establish the cost–effectiveness and clinical usefulness of *DPYD* screening in the different clinical settings.

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