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DPYD polymorphisms *c.496A>G*, *c.2194G>A* and *c.85T>C* and risk of severe adverse drug reactions in patients treated with fluoropyrimidine-based protocols

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Abstract

Aim. Cancer patients with reduced dihydropyrimidine dehydrogenase (DPD) activity are at increased risk of severe fluoropyrimidine (FP)-related adverse events (AE). Guidelines recommend FP dosing adjusted to genotype-predicted DPD activity based on four *DPYD* variants (rs3918290, rs55886062, rs67376798, rs56038477). We evaluated relationship between three further *DPYD* polymorphisms [*c.496A>G* (rs2297595), **6 c.2194G>A* (rs1801160) and **9A c.85T>C* (rs1801265)] and the risk of severe AEs. Methods. Consecutive FP-treated adult patients were genotyped for "standard" and tested *DPYD* variants, and for *UGT1A1*28* if irinotecan was included, and were monitored for the occurrence of grade [?]3 (National Cancer Institute Common Terminology Criteria) vs. grade 0-2 AEs. For each of the tested polymorphisms, variant allele carriers were matched to respective wild type controls (optimal full matching combined with exact matching, in respect to: age, sex, type of cancer, type of FP, *DPYD* activity score, use of irinotecan/*UGT1A1*, adjuvant therapy, radiotherapy, biological therapy and genotype on the remaining three tested polymorphisms). Results. Of the 503 included patients (82.3% colorectal cancer), 283 (56.3%) developed grade [?]3 AEs, mostly diarrhea and neutropenia. Odds of grade [?]3 AEs were higher in *c.496A>G* variant carriers (n=127) than in controls (n=376) [OR=5.20 (95%CI 1.88-14.3), Bayesian OR=5.24 (95% CrI 3.06-9.12)]. Odds tended to be higher in **6 c.2194G>A* variant carries (n=58) than in controls (n=432) [OR=1.88 (0.95-3.73), Bayesian OR=1.90 (1.03-3.56)]. **9A c.85T>G* did not appear associated with grade [?]3 AEs (206 variant carriers vs. 284 controls). Conclusion. *DPYD c.496A>G* variant might need to be considered for inclusion in the *DPYD* genotyping panel.

DPYD polymorphisms *c.496A>G*,*c.2194G>A* and *c.85T>C* and risk of severe adverse drug reactions in patients treated with fluoropyrimidine-based protocols

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

Four *DPYD* variants (rs3918290, rs55886062, rs67376798, rs56038477) are practically relevant biomarkers of fluoropyrimidine-related toxicity but explain only a part of interindividual variability in dihydropyrimidine dehydrogenase (DPD) activity.

Three further *DPYD* polymorphisms *c.496A>G*(rs2297595), **6 c.2194G>A* (rs1801160) and **9A c.85T* >C (rs1801265) have been investigated in this setting, but with conflicting results.

WHAT THIS STUDY ADDS

- *DPYD* c.496A>G variant is associated with 5-fold higher odds of grade [?]3 fluoropyrimidine-related adverse events.
- Association between polymorphisms c.2194G>A and c.85T>G and grade [?]3 toxicity is uncertain, but if existed, it is likely mild.
- *DPYD c.496A>G* variant might need to be considered for inclusion in the *DPYD* genotyping panel.

Abstract

Aim.

Cancer patients with reduced dihydropyrimidine dehydrogenase (DPD) activity are at increased risk of severe fluoropyrimidine (FP)-related adverse events (AE). Guidelines recommend FP dosing adjusted to genotype-predicted DPD activity based on four *DPYD* variants (rs3918290, rs55886062, rs67376798, rs56038477). We evaluated relationship between three further *DPYD* polymorphisms [*c.496A>G* (rs2297595), **6c.2194G>A* (rs1801160) and **9A c.85T* >C (rs1801265)] and the risk of severe AEs.

Methods.

Consecutive FP-treated adult patients were genotyped for "standard" and tested *DPYD* variants, and for *UGT1A1*28* if irinotecan was included, and were monitored for the occurrence of grade [?]3 (National Cancer Institute Common Terminology Criteria) vs. grade 0-2 AEs. For each of the tested polymorphisms, variant allele carriers were matched to respective wild type controls (optimal full matching combined with exact matching, in respect to: age, sex, type of cancer, type of FP, *DPYD* activity score, use of irinotecan/*UGT1A1* , adjuvant therapy, radiotherapy, biological therapy and genotype on the remaining three tested polymorphisms).

Results.

Of the 503 included patients (82.3% colorectal cancer), 283 (56.3%) developed grade [?]3 AEs, mostly diarrhea and neutropenia. Odds of grade [?]3 AEs were higher in *c.496A>G* variant carriers (n=127) than in controls (n=376) [OR=5.20 (95%CI 1.88-14.3), Bayesian OR=5.24 (95% CrI 3.06-9.12)]. Odds tended to be higher in **6c.2194G>A* variant carries (n=58) than in controls (n=432) [OR=1.88 (0.95-3.73), Bayesian OR=1.90

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(1.03-3.56)].*9A c.85T>G* did not appear associated with grade [?]3 AEs (206 variant carriers vs. 284 controls).

Conclusion .

DPYD c.496A>G variant might need to be considered for inclusion in the *DPYD* genotyping panel.

Introduction

Fluoropyrimidines (FPs), 5-Fluorouracil (5-FU) and its oral prodrug capecitabine, are the backbone of several treatment regimens for common solid organ cancer types, including colorectal, breast and head and neck cancer [1]. However, around 20-30% of the treated patients experience severe adverse events classified as grade 3-5 by the Common Terminology Criteria for Adverse Events (CTCAE)[2,3], and FP toxicity can be fatal in up to 1% of the affected patients [4]. The most common toxicities are diarrhea, nausea, vomiting, mucositis, neutropenia, and hand-foot syndrome; the latter especially with capecitabine [2,3]. Fluoropyrimidine toxicity/tolerability is largely driven by the extent of exposure to 5-FU and its cytotoxic metabolites. This is mainly regulated by the activity of the hepatic dihydropyrimidine dehydrogenase (DPD), which metabolizes around 80-90% of the administered 5-FU to inactive 5,6-dihydro-5-fluorouracil and is the rate-controlling enzyme for inactivation of 5-FU [1,5,6]. The activity of the DPD enzyme, coded by the *DPYD* gene, may vary across individuals largely as a consequence of single nucleotide polymorphisms (SNPs). In Caucasians, the prevalence of individuals with a reduced DPD activity is estimated at around 3-7%, while 0.01-0.1% are thought to lack DPD activity completely. Several approaches to prediction of FP toxicity and dose-individualization have been developed, including *DPYD*genotyping to predict DPD activity [7–11]. The first reported and the most well-known practically relevant *DPYD*variant was *DPYD*2A* , a splice-site variant (c.*1905 + 1G>A; IVS14+1G>A* ; rs3918290)[12]. Upfront genotyping for *DPYD*2A* and consequent FP dose-adjustment improves patient safety and is cost-effective [13]. Currently, four genetic *DPYD*variants are considered worth implementing into daily clinical care:*DPYD*2A* (*c.1905 + 1G>A, IVS14 + 1G>A*), *DPYD*13*(*c.1679T>G*), *c.2846A>T* and*c.1236G>A* (in linkage disequilibrium with*c.1129 - 5923C>G*) [14,15]. Of those,*c.1905+1G>A* and *c.1679T>G* result in greatly reduced DPD activity, whereas *c.2846A>T*and *c.1129–5923C>G* result in moderately reduced DPD activity. Considering all four variants combined, ˜7% of Europeans carry at least one decreased function *DPYD* variant[15]. Preemptive screening for these *DPYD*variants and subsequent genotype-guided dose individualization results in a significant reduction of severe adverse reactions and is feasible and cost-effective in daily practice [9,16]. Dosing guidelines – driven by the predicted remaining DPD activity based on the four variants - are provided by the Clinical Pharmacogenetics Implementation Consortium (CPIC) [15], and the Dutch Pharmacogenetics Working Group (DPWG) [14], as well the French National Network of Pharmacogenetics (RNPGx) [17]and the Swiss Group of Pharmacogenomics and Personalised Therapy[18]. They pertain to patients who are heterozygous carriers of a single *DPYD* variant. For homozygous *DPYD*variant allele carriers (two identical variants) and for compound heterozygous *DPYD* variant allele carriers (two or more different variants), dosing guidelines are not yet available (or treatment with an alternative drug is advised), although safe treatment with low-dose FPs was demonstrated in a case series [19]. However, although clinically important, these *DPYD* risk variants are estimated to account for only 20-30% of toxicity cases and cannot fully explain variability in DPD activity [15,20,21].*DPYD* is a very polymorphic gene, with more than 550 missense variants and 40 predicted loss-of-function variants identified in the Genome Aggregation Database (gnomAD) [22]. Although not many have been studied functionally and/or for clinical association with FP toxicity, around 41% variants are thought to reduce DPD enzyme activity and likely contribute to increased risk of FP toxicity[23]. We aimed to assess whether three of these*DPYD* SNPs (in addition to the four variants recommended by CPIC and DPWG) - *c.496A>G* (rs2297595), **9A c.85T>C* (rs1801265) and **6c.2194G>A* (rs1801160) – were associated with the risk of serious adverse drug reactions in cancer patients treated with FP-based protocols.

Patients and Methods

Study outline

This prospective observational study was conducted at a single tertiary centre (January 2016 to December

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2020) and was approved by the Institutional Ethics Committee (approval class: 8.1.-19/232-2; registration number: 02/21 AG). Consecutive adults suffering from solid organ malignancy with an indication for 5-FU/capecitabine-based chemotherapy were genotyped for *DPYD* polymorphisms [**2Ac.1905+1G>A* , (rs3918290);*c.2946A>T* (rs67376798),*c.1236G>A* (rs56038477) and **13 c.1679T>C* (rs55886062)] recommended by the actual guidelines,[14,15,17,18] (residual) enzyme activitiy score was derived (DPYD activity score), and FP doses were adjusted accordingly. Where disease course necessitated, genotyping could be completed only after the treatment commencement and doses were adjusted*post-hoc* . Patients were genotyped also for the investigated*DPYD* polymorphisms [*c.496A>G* (rs2297595),**9A c.85T>C* (rs1801265) and **6c.2194G>A* (rs1801160)] and, if irinotecan was a part of the scheduled treatment, for the *UGT1A1*28* (rs3064744) polymorphism (associated with higher exposure to- and toxicity of irinotecan). Investigated polymorphisms were disclosed only after completion of the study. Patients were observed over the scheduled treatment period, and the association of the polymorphisms of primary interest with the occurrence of severe adverse drug reactions was assessed.

Patients

Included were consecutive adults ([?]18 years of age) meeting the following criteria: a) verified diagnosis of a solid organ malignancy; b) scheduled for FP-based chemotherapy regimen; c) written informed consent to genotyping beyond *DPYD* variants recommended by the guidelines; d) life expectancy of at least 6 months; e) not suffering from inflammatory bowel disease, irritable bowel syndrome, any hematological malignancy or other blood dyscrasias, epilepsy or chronic liver diseases (hepatitis of any etiology, alcoholic or non-alcoholic fatty liver disease).

Monitoring of adverse events and overall follow-up

Patients were followed up from commencement of the scheduled FP-based protocol until 3-4 weeks after the last cycle, death or occurrence of severe toxicity, whichever first. Patient care was delivered following standard in-house protocols in line with the respective professional guidelines. Selected toxicities – anemia, leukopenia, neutropenia, thrombocytopenia, asthenia, diarrhoea, mucositis/stomatitis, vomiting, nausea, hepatic toxicity, skin toxicity and neurotoxicity – were assessed at the start of each cycle and after the last scheduled cycle using the Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0. All adverse events were monitored continuously. In the case of X3 or persistent grade 2 hematologic toxicity, all drug doses were reduced by 25%. In the case of grade X3 non-hematologic toxicity, the dose of the related drugs was reduced by 50%. In the case of grade X3 or persistent grade 2 neurotoxicity, oxaliplatin dose was reduced by 20%. Oxaliplatin was stopped if grade X2 neurosensory symptoms persisted between cycles.

Outcome of interest

The outcome of interest was the incidence of CTCAE grade [?]3 AEs at any time during the observed period, as opposed to no AEs (grade 0) or grade 1-2 AEs. A patient not experiencing AEs or experiencing only grade 1-2 AEs was counted as "grade 0-2 AE". Patients experiencing grade [?]3 AEs at any time (with or without prior grade 1-2 AEs) were counted as "grade [?]3 AE".

Genotyping

Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Genotyping of *DPYD *2A* (c.1905+1G>A, IVS14+1G>A, rs3918290), **13* (c.1679T>G, I560S, rs55886062), *c.2846A>T* (D949V, rs67376798),*c.1236G>A/HapB3* (rs56038477),*c.496A>G* (rs2297595), **6 c.2194G>A* (rs1801160) and **9A c.85T>C*(rs1801265), was performed using TaqMan® SNP Genotyping assays by real-time PCR genotyping on the 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA). *UGT1A1*28* (rs3064744) was genotyped using LightSNiP Genotyping assay (TIB Molbiol GmbH, Berlin, Germany) by real-time PCR genotyping on the LightCycler® instrument (Roche Diagnostics, Mannheim, Germany). Genotyping was performed with methods implemented and validated for routine pharmacogenetic testing in the laboratory that regularly participates in external quality assessment (EQA) schemes (EMQN and RfB).

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Data analysis

Data are summarized for all patients and separately for those with grade [?]3 or grade 0-2 AEs. To evaluate the association between the polymorphisms of primary interest [*c.496A>G*(rs2297595), **9A c.85T>C* (rs1801265) and **6c.2194G>A* (rs1801160)] and the risk of grade [?]3 AEs, the following approach was implemented: a) for*c.496A>G* , variant allele carriage (AG or GG) was considered a "treatment" and wild type (wt) genotype (AA) was considered a "control". Age and sex, type of cancer (colorectal or "other"), FU or capecitabine-based protocol, use of irinotecan (IR) [in combination with the *UGT1A1*28* genotype categorized as "IR not used", "IR used, wt (**1/*1*)" and "IR used, variant allele (*1/*28 or *28/*28)], *DPYD* activity score and*c.2194G>A* and *c.85T>C* genotypes (dichotomized as "variant allele" or "wt") were considered as covariates; *c.496A>G* variant and wt patients were subjected to optimal full matching based on Mahalanobis distance considering all of the covariates, with exact matching regarding DPYD activity score and *c.85T>C* genotype; b) for*c.2194G>A* , optimal full matching of treated (variant) and control patients was undertaken as described, with exact matching regarding DPYD activity score and type of cancer; c) for*c.85T>C* genotype, optimal full matching between treated (variant) and wt subjects was performed as described, with exact matching regarding DPYD activity score and *c.496A>G*genotype. Exact matching was always undertaken in respect to DPYD activity score as a known predictor of the risk of AEs. The choice of other covariates for exact matching was driven by (i) imbalances between variant and wt subjects; (ii) practical relevance of the covariate for the asked questions; and (iii) possibility of retaining all enrolled patients. Matched datasets were then used to fit weighted generalized mixed (hierarchical) models (binary distribution, logit link) with "subclass" (resulting from the matching process) as a random effect: frequentist (maximum likelihood estimation with Gaus-Hermite quadrature approximation and empirical [robust] sandwich estimation) and Bayesian models (4 chains, 4000 iterations, 8000 samples of the posterior, highest posterior density [HPD] confidence intervals, vaguely informative normal priors for ln[odds] and the intercept [0, 4; scaled], and priors on the terms of a decomposition of the covariance matrices [Gamma shape=1, scale=1; LKJ for correlation matrix, regularization=1; Dirichlet for the simplex vectors, concentration=1]), with the evaluated genotype (variant allele) as the effect of interest and with further adjustment for covariates for which adequate balance was not achieved by the matching procedure (standardized difference between matched treated and control subjects [?]0.1). For the frequentist analysis, effects, variances and covariance were retained and used to adjust model-derived confidence intervals and P-values for multiplicity by the simulation method. For optimal full matching [24], we used package *MatchIt*[25] in R. We used SAS 9.4 for Windows (SAS Inc., Cary, NC) (proc glimmix, proc plm) to fit frequentist models, and package*nstanarm* (https://mc-stan.org/rstanarm/) in R to fit Bayesian models. We used CubeX [26] to evaluate linkage disequilibrium between investigates polymorphisms, and package*Evalue* in R to evaluate the sensitivity of the generated estimates to unmeasured confounding, i.e., to determine *E-value*– a minimum strength of association (on a relative risk scale) that an unmeasured confounder needs to have with the treatment and the outcome to fully explain away a specific treatment-outcome association[27].

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20[28].

Results

Patients

A total of 503 patients (age 27-86 years), all of European descent, were enrolled, comparably men and women (Table 1) mostly suffering from colorectal cancer (82.3%) and sporadically from a variety of other cancers (stomach & esophagus was the most common "other" location, 8.3%) (Table 1). No toxicity-related deaths (grade 5 AE) were observed. Grade 3-4 AEs were observed in 283 (56.3%) patients who were comparable in age and prevalence of colorectal cancer to those developing grade 0-2 AEs (Table 1). 5-FU-based protocols were predominantly used (68.4%), while irinotecan was included in 180 (35.8%) patients, comparably in

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those with grade 3-4 and those with 0-2 AEs (Table 1). Treatment was adjuvant in 212 (42.2%) patients, was combined with radiotherapy in 50 (9.9%) patients, and included also biological therapy in 146 (29.0%) patients, comparably in those who developed grade 3-4 and those with grade 0-2 AEs (Table 1). Forty-nine (9.6%) patients experienced no AEs, but most of the others experienced multiple AEs. Grade 1-2 AEs were reported in 361 patients, including 190/283 (67.1%) of those who eventually developed grade 3-4 AEs (Table 1). Fatigue and diarrhea were the most common grade 1-2 AEs, while neutropenia and diarrhea were the most common grade 3-4 AEs (Table 1).

Considering the four *DPYD* SNPs recommended by the guidelines for the derivation of the activity score, genotyping of **13, c.2846A>T* and *c.1236G>A* failed for technical reasons in 2 patients (one developed grade 3-4 AEs, the other one grade 0-2 AEs) (Table 2). Overall, variant alleles were rare across the 4 SNPs (variant allele carriage 0.3% to 4.4%) (Table 2). There was a clear imbalance between grade 3-4 AE patients and grade 0-2 AE patients regarding the prevalence of **2A* variant allele carriers (6.4% vs. 0), while the two patient subsets were comparable regarding the other 3 SNPs (Table 2). Most of the patients (90.7%) had an activity score of 2.0 (Table 2), but activity scores of 0, 0.5 or 1.0 were observed only in patients with grade 3-4 AEs (Table 2).

Considering the polymorphisms of primary interest, genotyping for*c.2194G>A* failed for technical reasons in 10 patients, and in additional 3 (for a total of 13) patients it also failed for **9A c.85T>C* (Table 2). There was a clearly higher prevalence of *c.496A>G* variant allele carriers and a tendency of higher prevalence of*c.2194G>A* variant allele carriers among grade 3-4 AE patients compared to grade 0-2 AE patients (Table 2), while prevalence of **9A c.85T>C* variant allele carriers was comparable across the two subsets (Table 2).

Of the patients receiving irinotecan, 105 (58.3%) were variant allele carriers with a clearly higher prevalence in grade 3-4 AE patients (72.0%) than in grade 0-2 AE patients (41.2%).

DPYD polymorphism c.496A>G is associated with an increased risk of grade 3-4 AEs

Polymorphism *c.496A>G* was in a linkage disequilibrium with **9A c.85T>C* (D'=0.627, r^2=0.1877, Chi^2=91.97), but not in respect to polymorphisms **2A* , **13* ,*c.2846A>T* , *c.1236G>A* or **6 c.2194G>A* (not shown). Before matching,*c.496A>G* variant allele and wt subjects differed in a number of characteristics, particularly (standardized difference d>0.1) in respect to prevalence of those with colorectal cancer, **6 c.2194G>A* and **9A c.85T>A* genotypes (Table 3). Incidence of grade 3-4 AEs was considerably higher in variant (78.7%) than in wt subjects (48.7%), d=0.658 (Table 3). After matching (Table 3), the subsets were virtually identical in respect to all matching covariates (all d<0.1) except for prevalence of men (59.2% vs. 49.5%, d=0.195) (Table 3) and incidence of grade 3-4 AEs remained higher in variant allele carriers (77.4% vs. 47.6%, d=0.646). With further adjustment for sex, *c.496A>G* variant vs. wt remained associated with around 5-fold higher odds of grade 3-4 AEs, in both frequentist and Bayesian models (Table 3). Considering the Bayesian estimate (OR=5.24, 95%CI 3.06-9.12), the association appeared rather robust – a strong unmeasured confounder effect (on a relative risk scale) would be needed to move the point estimate (E-value/RR=4.01) or the lower limit of the confidence interval (E-value/RR=2.89) to 1.0.

*DPYD polymorphism *6 c.2194G>A does not appear associated with the risk of grade 3-4 AEs*

Polymorphism **6 c.2194G>A* did not appear in LD with any of the other *DPYD* polymorphisms (not shown). The 10 patients missing **6 c.2194G>A* and **9A c.85T>C* genotypes (plus 3 additional missing the*c.85T>C* genotype) were excluded from the analysis. Before matching, **6 c.2194G>A* variant and wt subjects differed (d>0.1) in a range of characteristics, including type of cancer, FU-based/capecitabine-based treatments and prevalence of **9A c.85T>C* and*c.496A>G* variant allele carriers (Table 5). Incidence of grade 3-4 AEs was higher in variant than in wt subjects (67.2% vs. 54.6%, d=0.261). After matching, variant and wt subjects were fairly balanced (all d<0.1) for most of the covariates, except for age (d=0.20) and **9A* (d=0.157) and *c.496*genotypes (d=0.225). Incidence of grade 3-4 AEs was still higher in variant than in wt subjects (65.9% vs. 54.8%, d=0.229). After adjustment for the imbalanced genotypes and age, **6 c.2194G>A* variant allele carriage appeared weakly associated with a higher risk of grade 3-4 AEs:

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frequentist OR=1.88 (95%CI 0.95-3.42; P=0.071; and P=0.191 with multiplicity adjustment) (Table 4). The Bayesian estimate (OR=1.90, 95% CrI 1.03-3.56) also suggested uncertainty about the effect: a) probability of OR >1.0 was 98.0% (Table 4); b) a mild unmeasured confounder effect would shift the lower limit of the 95%CrI to <1.0 (E-value/RR=1.25, to shift it to 0.95). With the limitation of missing data on 13 subjects, the present observations do not support a relevant association between this polymorphism and the risk of severe AE.

*DPYD polymorphism *9A c.85T>C does not appear associated with the risk of grade 3-4 AEs*

Besides the c.*496A>G* polymorphism,*c.85T>C* also appeared in LD with the*c.1236G>A* polymorphism (D'=0.923, r^2=0.061, Chi^2=29.89). Subjects missing genotype data on this SNP were excluded from the analysis. Before matching, *c.85T>C* variant and wt subjects differed (d>0.10) in a range of characteristics, including the prevalence of FU-/capecitabine-based protocols, use of radiotherapy, DPYD activity scores and **6* c.*2194G>A* and c.*496A>G* genotypes (Table 6). Incidence of grade 3-4 AEs appeared comparable in variant and in wt (58.7% vs. 54.2%, d=0.091). After matching, variant and wt subjects were closely similar to all matching covariates (Table 6). Incidence of grade 3-4 AEs appeared mildly lower in variant allele carriers (50.6% vs. 57.6%, d=-0.141) (Table 6), yielding OR=0.67 with 95%CI/CrI embracing unity (frequentist P=0.179, Bayesian probability of OR <1.0 96.2%) (Table 4).

Discussion

Present data strongly suggest an association between the *DPYD*polymorphism (variant allele) *c.496A>G* (rs2297595) and the risk of severe (CTCAE grade [?]3) adverse events in adults of European descent receiving FP-based chemotherapy protocols for solid organ malignancies: (i) raw incidence of grade [?]3 AEs was 78.7% in 127 variant allele carriers vs. 48.7% in 376 wild type (wt) homozygotes; (ii) the relationship was similar (77.4% vs. 47.6%) when variant and wt patients were matched on a range of demographic, morbidity, therapy and genetic DPYD characteristic and with further control of bias by exclusion criteria (comorbidity with symptoms that might mimic FP adverse effects); (iii) with further adjustment for sex (which could not be adequately balanced by matching), the strength of association was marked although odds ratio (around 5.2) might not be too intuitive for interpretation: (fully) adjusted probabilities of grade [?]3 AE were 80.9% in variant allele carriers and 44.9% in wt subjects (absolute difference of 36%, 95%CI 16.9-49.2; risk ratio 1.803, 95%CI 1.341-2.293); (iv) this SNP was not in a linkage disequilibrium (LD) with any of the CPIC-DPWG recommended SNPs or with*c.2194G>A* . It was in LD with **9A c.85T>C* polymorphisms, where variant allele carriage numerically tended towards a lower risk of severe AEs. Inevitably, there is unmeasured (residual) confounding due to the fact that a number of missense *DPYD* SNPs with potentially reduced DPD activity were not accounted for. However, E-values indicate that strong unmeasured confounding would be needed to explain away the observed association.

The observed LD between *c.496A>G* (rs2297595) and*c.85T>C* (rs1801265) was reported by others[29] as well. Both SNPs are exonic and result in amino acid changes in the DPD protein (p.C29R and p.M166V, respectively).*In vitro* studies have yielded inconclusive data regarding their effects on DPD function [30–32]. However, a recent study based on plasma dihydrouracil/uracil ratios indicated a significant impact of both SNPs on DPD activity *in vivo*[33], while another phenotyping study showed their association with decreased metabolism of 5-FU [34]. Data from the Genotype-Tissue Expression (GTEx) project [35]indicate that *c.85T>C* is associated with higher*DPYD* gene expression in certain tissues, but no link has been made between *c.496A>G* and *DPYD* expression[35]. Data on association with FP toxicity have been contradictory for both SNPs. Regarding *c.496A>G* , several studies have associated it with a higher risk of FP toxicity[36–39], while others failed to demonstrate an association [40–43], or even suggested a protective effect [44]. The *c.85T>C* variant was described as function-reducing upon discovery because it was initially observed in DPD deficient patients [45]. However, later studies failed to corroborate such a conclusion [46], and some showed association with increased DPD activity[30,47]. In respect to FP toxicity, two clinical studies suggested a protective effect for the variant allele (higher DPD activity?) [40,41], several others failed to do so[36,38,48], and some even suggested association with an increased risk of FP toxicity, although observations could not be replicated [49,50]. Currently, CPIC Guidelines grade both variants as normal function alleles due to the lack of clear

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evidence linking these variants with 5-FU toxicity [15]. In respect to *c.496A>G* (rs2297595), the present estimate seems robust, likely accurate and practically relevant. Regarding *c.85T>C* (rs1801265), present data are inconclusive. There is a numerical tendency suggesting a possible protective effect of this SNP, but uncertainty is high, and even if such an effect existed, it is likely modest and of questionable relevance.

*c.2194G>A, *6* (p.V732I, rs1801160) is another extensively evaluated *DPYD* SNP, but with a plethora of conflicting results. One *in vitro* study suggested the SNP did not affect DPD enzyme activity [23], while an *in vivo*study indicated a mild reduction in DPD activity which, however, might have been due to LD with c.557A>G (p.Y186C)[30]. Several, generally smaller, studies failed to detect a relationship between this SNP and FP-related toxicity[29,43,51–53], while several others indicated an association between the variant allele and occurrence of leukopenia, neutropenia or diarrhea [39,44]. However, larger studies[54]and in particular, analyses of the Pan-European Trials in Alimentary Tract Cancer (PETACC-8) [38,55], demonstrated an association between the *c.2194G>A*variant and clinically relevant adverse drug reactions in colorectal cancer patients treated with FOLFOX4 or XELOX adjuvant chemotherapy. Interestingly, the OR for variant allele and grade [?]3 adverse events in PETACC-8 [55] – OR=1.7 (95%CI 1.3-2.4, based on grade [?]3 AEs in 119/199 variant and in 644/1346 wt patients) - was closely similar to the presently reported OR=1.88 (1.90 in Bayesian analysis). However, the present sample was considerably smaller, hence CIs were wider, resulting in uncertainty about the effect. The Bayesian estimate (OR=1.90, 1.03-3.56) was more *convincing* - but in terms of relative risks, the present and the published [55]effects are around 1.25. The present effect was judged highly susceptible to unmeasured confounding. Hence, we opted to declare this variant "apparently not associated" with the risk of grade [?]3 AEs: the uncertainty about the effect was high. In reality, the present observations are in line with the results from PETACC-8 – however, the effect appears to be quite modest.

The present study is limited mainly by a moderate sample size resulting in: a) limited power. This affected the conclusions about the effects of*c.85T>G* and *c.2194G>A* ; b) matching based on DPYD activity score instead of the four SNPs recommended by the guidelines (very low number of variant allele carriers). However, it is unlikely that this fact had biased the estimates: the entire concept is based on the idea of SNPs reflecting on DPD activity and, consequently, on AEs; c) matching on "FU-based/capecitabine-based" protocols instead of exact matching on specific treatment schemes. This fact is also unlikely to have relevantly affected the estimates: the possible capecitabine/5-FU distinction is accounted for; we matched for adjuvant/non-adjuvant setting, radiotherapy and biological therapy; and use of irinotecan (accounting also for the relevant SNP), where irinotecan "non-use" refers to combinations with oxaliplatin or a small number of cases on mono treatment with 5-FU or capecitabine.

In conclusion, present data strongly suggest a marked association between *DPYD c.496A>G* (rs2297595) and an increased risk of severe FP-related toxicity and support a view that this SNP might need to be reconsidered for inclusion into the*DPYD* genotyping panel.

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COMPETING INTERESTS

The authors have no conflicts of interest to declare.

CONTRIBUTORS

N.B., V.T., and L.G. were involved in the conception and design of the study and drafted the article. I.B., S.P., L.G., L.Š. and L.L. organized and carried out clinical data collection and genetic analyses. V.T. provided expertise in statistical modelling and performed the statistical analysis. N.B., V.T., L.G. and S.P. were involved in the analysis and interpretation of data. All authors revised the manuscript and approved the final version for submission.

DATA AVAILABILITY STATEMENT

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

LITERATURE

1. Longley DB, Harkin DP, Johnston PG. 5-Fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* . 2003;3(5):330-338.

2. Rosmarin D, Palles C, Church D, 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(10):1031-1039.

3. Twelves C, Wong A, Nowacki MP, et al. Capecitabine as adjuvant treatment for stage III colon cancer. *N Engl J Med* . 2005;352(26):2696-2704.

4. Hoff PM, Ansari R, Batist G, et al. Comparison of oral capecitabine versus intravenous fluorouracil plus leucovorin as first-line treatment in 605 patients with metastatic colorectal cancer: results of a randomized phase III study. *J Clin Oncol* . 2001;19(8):2282-2292.

5. van Kuilenburg ABP. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. *Eur J Cancer* . 2004;40(7):939-950.

6. Diasio RB, Harris BE. Clinical pharmacology of 5-fluorouracil.*Clin Pharmacokinet* . 1989;16(4):215-237.

7. Amstutz U, Froehlich TK, Largiadèr CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. *Pharmacogenomics* . 2011;12(9):1321-1336.

8. Ciccolini J, Gross E, Dahan L, Lacarelle B, Mercier C. Routine dihydropyrimidine dehydrogenase testing for anticipating 5-fluorouracil-related severe toxicities: hype or hope? *Clin Colorectal Cancer* . 2010;9(4):224-228.

9. Henricks LM, Lunenburg CATC, Man FM De, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer : a prospective safety analysis. 2018;19:1459-1467.

10. Henricks LM, Lunenburg CATC, Meulendijks D, et al. Translating DPYD genotype into DPD phenotype: Using the DPYD gene activity score.*Pharmacogenomics* . 2015;16(11):1277-1286.

11. Meulendijks D, Henricks LM, Sonke GS, 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(16):1639-1650.

12. Wei X, McLeod HL, McMurrough J, Gonzalez FJ, Fernandez-Salguero P. Molecular basis of the human dihydropyrimidine dehydrogenase deficiency and 5-fluorouracil toxicity. *J Clin Invest* . 1996;98(3):610-615.

13. Deenen MJ, Meulendijks D, Cats A, et al. Upfront genotyping of DPYD*2A to individualize fluoropyrimidine therapy: A safety and cost analysis. *J Clin Oncol* . 2016;34(3):227-234.

14. Lunenburg CATC, van der Wouden CH, Nijenhuis M, et al. Dutch Pharmacogenetics Working Group (DPWG) guideline for the gene–drug interaction of DPYD and fluoropyrimidines. *Eur J Hum Genet* . 2020;28(4):508-517.

15. Amstutz U, Henricks LM, Offer SM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing: 2017 Update.*Clin Pharmacol Ther* . 2018;103(2):210-216.

16. Henricks LM, Lunenburg CATC, de Man FM, et al. A cost analysis of upfront DPYD genotype–guided dose individualisation in fluoropyrimidine-based anticancer therapy. *Eur J Cancer* . 2019;107(2019):60-67.

17. Quaranta S, Thomas F. Pharmacogenetics of anti-cancer drugs: State of the art and implementation – recommendations of the French National Network of Pharmacogenetics. *Therapie* . 2017;72(2):205-215.

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18. Hamzic S, Aebi S, Joerger M, et al. Fluoropyrimidine chemotherapy: recommendations for DPYD genotyping and therapeutic drug monitoring of the Swiss Group of Pharmacogenomics and Personalised Therapy.*Swiss Med Wkly* . 2020;150:w20375.

19. Henricks LM, Kienhuis E, de Man FM, et al. Treatment Algorithm for Homozygous or Compound Heterozygous DPYD Variant Allele Carriers With Low-Dose Capecitabine. *JCO Precis Oncol* . 2017;(1):1-10.

20. Ciccolini J, Milano G, Guchelaar HJ. Detecting DPD deficiency: when perfect is the enemy of good. *Cancer Chemother Pharmacol* . 2021;(0123456789):2-4.

21. Froehlich TK, Amstutz U, Aebi S, Joerger M, Largiader CR. Clinical importance of risk variants in the dihydropyrimidine dehydrogenase gene for the prediction of early-onset fluoropyrimidine toxicity. *Int J Cancer* . 2015;136(3):730-739.

22. Genome Aggregation Database (gnomAD). DPYD dihydropyrimidine dehydrogenase. https://gnomad.broadinstitute.org/gene/ENSG00000188641?dataset=gnomad_r2_1. Published 2021. Accessed August 30, 2021.

23. Shrestha S, Zhang C, Jerde CR, et al. Gene-Specific Variant Classifier (DPYD-Varifier) to Identify Deleterious Alleles of Dihydropyrimidine Dehydrogenase. *Clin Pharmacol Ther* . 2018;104(4):709-718.

24. Hansen BB. Full matching in an observational study of coaching for the SAT. *J Am Stat Assoc* . 2004;99(467):609-618.

25. Ho, DE; Imai, K; King, G; Stuart E. MatchIt : Nonparametric Preprocessing for Parametric Causal Inference. *J Stat Softw* . 2011;42(8):1-28.

26. Gaunt TR, Rodriguez S, Day IN. Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool 'CubeX'. *BMC Bioinformatics* . 2007;8:428.

27. VanderWeele TJ, Ding P. Sensitivity Analysis in Observational Research: Introducing the E-Value. *Ann Intern Med* . 2017;167(4):268-274.

28. Alexander SPH, Fabbro D, Kelly E, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2019/20: Enzymes. *Br J Pharmacol* . 2019;176 Suppl:S297-S396.

29. Amstutz U, Farese S, Aebi S, Largiader CR. Dihydropyrimidine dehydrogenase gene variation and severe 5-fluorouracil toxicity: a haplotype assessment. *Pharmacogenomics* . 2009;10(6):931-944.

30. Offer SM, Wegner NJ, Fossum C, Wang K, Diasio RB. Phenotypic Profiling of DPYD Variations Relevant to 5-Fluorouracil Sensitivity Using Real-time Cellular Analysis and In Vitro Measurement of Enzyme Activity. 2013;73(6):1958-1969.

31. 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(9):2545-2554.

32. van Kuilenburg ABP, Meijer J, Tanck MWT, et al. Phenotypic and clinical implications of variants in the dihydropyrimidine dehydrogenase gene. *Biochim Biophys Acta - Mol Basis Dis* . 2016;1862(4):754-762.

33. Hamzic S, Scharer D, Offer SM, et al. Haplotype structure defines effects of common DPYD variants c.85T > C (rs1801265) and c.496A > G (rs2297595) on dihydropyrimidine dehydrogenase activity: Implication for 5-fluorouracil toxicity. *Br J Clin Pharmacol* . 2021;(August 2020):3234-3243.

34. Gentile G, Botticelli A, Lionetto L, et al. Genotype-phenotype correlations in 5-fluorouracil metabolism: A candidate DPYD haplotype to improve toxicity prediction. *Pharmacogenomics J* . 2016;16(4):320-325.

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35. Lonsdale, J; Thomas, J; Salvatore M et al. The Genotype-Tissue Expression (GTEx) project. *Nat Genet* . 2013;45(6):580-585.

36. Gross E, Busse B, Riemenschneider M, et al. Strong association of a common dihydropyrimidine dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. *PLoS One* . 2008;3(12):e4003.

37. Falvella FS, Cheli S, Martinetti A, et al. DPD and UGT1A1 deficiency in colorectal cancer patients receiving triplet chemotherapy with fluoropyrimidines, oxaliplatin and irinotecan. *Br J Clin Pharmacol* . 2015;80(3):581-588.

38. Ruzzo A, Graziano F, Galli F, 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(9):1269-1277.

39. Deenen MJ, Tol J, Burylo AM, 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(10):3455-3468.

40. Etienne-Grimaldi M-C, Boyer J-C, Beroud C, et al. New advances in DPYD genotype and risk of severe toxicity under capecitabine. *PLoS One* . 2017;12(5):e0175998.

41. Madi A, Fisher D, Maughan TS, et al. Pharmacogenetic analyses of 2183 patients with advanced colorectal cancer; potential role for common dihydropyrimidine dehydrogenase variants in toxicity to chemotherapy.*Eur J Cancer* . 2018;102:31-39.

42. Loganayagam A, Arenas Hernandez M, Corrigan A, et al. Pharmacogenetic variants in the DPYD, TYMS, CDA and MTHFR genes are clinically significant predictors of fluoropyrimidine toxicity. *Br J Cancer* . 2013;108(12):2505-2515.

43. Toffoli G, Giodini L, Buonadonna A, et al. Clinical validity of a DPYD-based pharmacogenetic test to predict severe toxicity to fluoropyrimidines. *Int J Cancer* . 2015;137(12):2971-2980.

44. Kleibl Z, Fidlerova J, Kleiblova P, 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(4):303-316.

45. Vreken P, Van Kuilenburg AB, Meinsma R, van Gennip AH. Identification of novel point mutations in the dihydropyrimidine dehydrogenase gene. *J Inherit Metab Dis* . 1997;20(3):335-338.

46. van Kuilenburg ABP, De Abreu RA, van Gennip AH. Pharmacogenetic and clinical aspects of dihydropyrimidine dehydrogenase deficiency.*Ann Clin Biochem* . 2003;40:41-45.

47. Sistonen J, Buchel B, Froehlich TK, et al. Predicting 5-fluorouracil toxicity: DPD genotype and 5,6-dihydrouracil:uracil ratio.*Pharmacogenomics* . 2014;15(13):1653-1666.

48. Milano G. Highlight on DPYD gene polymorphisms and treatment by capecitabine. *Scand J Clin Lab Invest Suppl* . 2016;245:S30-3.

49. Maharjan AS, McMillin GA, Patel GK, et al. The Prevalence of DPYD*9A(c.85T>C) Genotype and the Genotype-Phenotype Correlation in Patients with Gastrointestinal Malignancies Treated With Fluoropyrimidines: Updated Analysis. *Clin Colorectal Cancer* . 2019;18(3):e280-e286.

50. Khushman M, Patel GK, Hosein PJ, et al. Germline pharmacogenomics of DPYD*9A (c.85T>C) variant in patients with gastrointestinal malignancies treated with fluoropyrimidines. *J Gastrointest Oncol* . 2018;9(3):416-424.

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51. Schwab M, Zanger UM, Marx C, 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(13):2131-2138.

52. Wei X, Elizondo G, Sapone A, et al. Characterization of the human dihydropyrimidine dehydrogenase gene. *Genomics* . 1998;51(3):391-400.

53. Ridge SA, Sludden J, Wei X, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in patients with colorectal cancer. *Br J Cancer* . 1998;77(3):497-500.

54. Del Re M, Cinieri S, Michelucci A, et al. DPYD*6 plays an important role in fluoropyrimidine toxicity in addition to DPYD*2A and c.2846A>T: a comprehensive analysis in 1254 patients. *Pharmacogenomics J* . 2019;19(6):556-563.

55. Boige V, Vincent M, Alexandre P, et al. DPYD genotyping to predict adverse events following treatment with fluorouracil-based adjuvant chemotherapy in patients with stage III colon cancer. *JAMA Oncol* . 2016;2(5):655-662.

Table 1 . Patient characteristics and recorded adverse events (AE). Differences between grade 3-4 AE patients and grade 0-2 AE patients are mean differences or prevalence ratios.

	All patients	Grade 3-4 AE	Grade 0-2 AE	3-4 vs. 0-2
N	503	283	220	—
Age (years)	63.0±10.6 (27-86)	63.3±10.8	62.6±10.3	0.7 (-1.2, 2.6)
Men	252 (50.1)	128 (45.2)	124 (56.4)	0.80 (0.68-0.95)
Colorectal cancer	414 (82.3)	228 (80.6)	186 (84.6)	0.95 (0.88-1.03)
Other cancer locations	89 (17.7)	55 (19.4)	34 (15.4)	1.26 (0.86-1.86)
Stomach & esophagus	42 (8.3)	32 (11.3)	10 (4.5)	—
Pancreas & biliary	16 (3.2)	11 (3.9)	6 (2.7)	—
Breast	13 (2.6)	6 (2.1)	7 (3.2)	—
Other	18 (3.6)	6 (2.1)	11 (5.0)	—
5-FU-based protocol	344 (68.4)	200 (70.7)	144 (64.4)	1.08 (0.96-1.22)
FOLFIRI	175 (34.8)	95 (33.6)	80 (36.4)	—
MAYO	102 (20.3)	64 (22.6)	38 (17.3)	—
FOLFOX	67 (13.3)	41 (14.5)	26 (11.8)	—
Capecitabine-based	159 (31.6)	83 (29.3)	76 (34.6)	0.85 (0.66-1.10)

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	All patients	Grade 3-4 AE	Grade 0-2 AE	3-4 vs. 0-2
Mono capecitabine	99 (19.7)	48 (17.0)	51 (23.2)	—
CAPOX	30 (6.0)	17 (6.0)	13 (5.9)	—
XELOX	25 (5.0)	13 (4.6)	12 (5.5)	—
XELIRI	5 (1.0)	5 (1.8)	0	—
Includes irinotecan	180 (35.8)	100 (35.3)	80 (36.4)	0.97 (0.77-1.23)
Adjuvant treatment	212 (42.2)	121 (42.8)	91 (41.4)	1.03 (0.84-1.28)
Radiotherapy	50 (9.9)	28 (9.9)	22 (10.0)	0.99 (0.59-1.68)
Biological therapy	146 (29.0)	83 (29.3)	63 (28.6)	1.02 (0.78-1.35)
Bevacizumab	112 (22.3)	63 (22.3)	49 (22.3)	—
Cetuxi-/panitumumab	34 (6.8)	20 (7.1)	14 (6.4)	—
Non-Ca co-medication	249 (49.6)	134 (47.5)	115 (52.3)	0.91 (0.76-1.08)
No AE (grade=0)	49 (9.6)	0	49 (22.3)	—
Grade 1-2 AE[a]	361 (71.8)	190 (67.1)	171 (77.7)	—
Fatigue	147 (29.3)	81 (28.7)	66 (30.0)	—
Diarrhea	131 (26.1)	58 (20.5)	73 (33.2)	—
Vomiting	124 (28.7)	89 (31.5)	55 (25.0)	—
Neutropenia	94 (18.7)	29 (10.2)	65 (29.5)	—
Abdominal pain	67 (13.4)	50 (17.8)	17 (7.7)	—
Oral mucositis	61 (12.2)	36 (12.8)	25 (11.4)	—
Hand & foot	48 (9.5)	22 (7.8)	26 (11.8)	—
Infection	47 (9.4)	24 (8.5)	24 (10.9)	—
Grade [?]3 AE[a]	283 (56.3)	283 (100)	0	—
Neutropenia	178 (35.5)	178 (63.1)	—	—

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	All patients	Grade 3-4 AE	Grade 0-2 AE	3-4 vs. 0-2
Diarrhea	102 (20.3)	102 (36.2)	—	—
Infection	90 (17.9)	90 (31.6)	—	—
Oral mucositis	46 (9.2)	46 (16.4)	—	—
Fatigue	38 (7.6)	38 (13.4)	—	—
Vomiting	28 (5.5)	28 (9.9)	—	—
Hand & foot	15 (3.0)	15 (5.4)	—	—
Abdominal pain	13 (2.6)	13 (4.6)	—	—

Abbreviatioins: CAPOX – capecitabin + oxaliplatin; 5-FU – 5-fluorouracil; FOLFIRI – 5-fluorouracil + leucovorin + irinotecan; FOLFOX – 5-fluorouracil + leucovorin + oxaliplatin; MAYO – 5-fluorouracil + low-dose leucovorin; XELIRI – capecitabin + irinotecan; XELOX – capecitabin + oxaliplatin

[a]Most patients suffered multiple adverse sevent

Table 2 . Genotyping data for all patients and across subsets by severity of adverse events (AE).

	Genotype /score	All N=503	Grade 3-4 n=283	Grade 0-2 n=220	Prevalence ratio 3-4/0-2	P
DPYD guide-line SNPs						
**2A* (rs3918290)	GG	485 (96.4)	265 (93.6)	220 (100)	0.94 (0.90-0.96)	<
	GA	17 (3.4)	17 (6.0)	0	19.4 (1.10-349)	0
	AA	1 (0.2)	1 (0.4)	0	—	–
	Variant	18 (3.6)	18 (6.4)	0	56.8 (3.28-995)	<
**13* (rs55886062)	TT	500 (99.4)	281 (99.3)	219 (99.6)	0.99 (0.98-1.02)	0
	TG	1 (0.2)	1 (0.35)	0	—	–
	GG	0	0	0	—	–
	Variant	1 (0.2)	1 (0.35)	0	—	–
	Missing	2 (0.4)	1 (0.35)	1 (0.4)	—	–

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	Genotype /score	All N=503	Grade 3-4 n=283	Grade 0-2 n=220	Prevalence ratio 3-4/0-2	P
c.2846A>T (rs67376798)	AA	497 (98.8)	278 (98.2)	219 (99.6)	0.99 (0.96-1.01)	0
	AT	4 (0.8)	4 (1.4)	0	13.2 (0.73-241)	0
	TT	0	0	0	—	-
	Variant	4 (0.8)	4 (1.4)	0	—	-
	Missing	2 (0.4)	1 (0.35)	1 (0.4)	—	-
c.1236G>A (rs56038477)	GG	479 (95.2)	272 (96.1)	207 (94.1)	1.02 (0.98-1.07)	0
	GA	22 (4.4)	10 (3.5)	12 (5.5)	0.65 (0.30-1.44)	0
	AA	0	0	0	—	-
	Variant	22 (4.4)	10 (3.5)	12 (5.5)	—	-
	Missing	2 (0.4)	1 (0.35)	1 (0.4)	—	-
DPYD activity score	2.0	456 (90.7)	249 (88.0)	207 (94.1)	0.94 (0.88-0.99)	0
	1.5	26 (5.2)	14 (4.9)	12 (5.5)	0.91 (0.44-1.88)	0
	1.0	18 (3.6)	18 (6.4)	0	56.8 (3.28-995)	<
	0.5	0	0	0	—	-
	0	1 (0.2)	1 (0.35)	0	—	-
	Missing	2 (0.4)	1 (0.35)	1 (0.4)	—	-
Investigated DPYD SNPs						
c.496A>G (rs2297595)	AA	376 (74.7)	183 (64.7)	193 (87.7)	0.74 (0.66-0.81)	<
	AG	120 (23.9)	94 (33.2)	26 (11.8)	2.79 (1.90-4.16)	<

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	Genotype /score	All N=503	Grade 3-4 n=283	Grade 0-2 n=220	Prevalence ratio 3-4/0-2	P
	GG	7 (1.4)	6 (2.1)	1 (0.4)	3.89 (0.71-21.4)	0
	Variant	127 (25.3)	100 (35.3)	27 (12.2)	2.86 (1.96-4.22)	<
*6 c.2194G>A (rs1801160)	GG	435 (86.5)	237 (83.7)	198 (90.0)	0.93 (0.87-0.99)	0
	GA	54 (10.7)	37 (13.1)	17 (7.7)	1.68 (0.99-2.88)	0
	AA	4 (0.8)	2 (0.7)	2 (0.9)	—	–
	Variant	58 (11.5)	39 (13.8)	19 (8.6)	1.59 (0.96-2.65)	0
	Missing	10 (2.0)	7 (2.5)	3 (1.4)	—	–
*9A c.85T>C (rs1801265)	TT	284 (56.5)	154 (54.4)	130 (59.1)	0.92 (0.79-1.08)	0
	TC	174 (34.6)	102 (36.0)	72 (32.7)	1.01 (0.86-1.41)	0
	CC	32 (6.4)	19 (6.7)	13 (5.9)	1.13 (0.58-2.20)	0
	Variant	206 (40.0)	121 (42.7)	85 (38.6)	1.11 (0.90-1.37)	0
	Missing	13 (2.5)	8 (2.8)	5 (2.3)	—	–
Irinotecan-treated		N=180	n=100	n=80		
*UGT1A1*28* (rs3064744)	*1/*1	75 (41.7)	28 (28.0)	47 (58.8)	0.48 (0.33-0.68)	<
	*1/*28	70 (38.9)	45 (45.0)	25 (31.2)	1.44 (0.98-2.14)	0
	*28/*28	35 (19.4)	27 (27.0)	8 (10.0)	2.65 (1.32-5.44)	0
	Variant	105 (58.3)	72 (72.0)	33 (41.2)	1.74 (1.32-2.36)	<

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Abbreviations: DPYD – dihydropyrimidine dehydrogenase; SNP – single nucleotide polymorphism; UGT – uridine 5'-diphospho-glucoronosyltransferase

Table 3 . Characteristics of *c.496A>G*variant allele carriers and wild type patients before and after matching.[a] Covariates used in matching procedure are shaded. Differences between variant allele carriers and wild type patients are depicted as standardized differences (d). Values <0.1 indicate irrelevant differences.

	Before matching	Before matching	Before matching	Before matching	Before matc
	Variant		Wild type		d
N	127		376		—
Age (years)	62.6±11.2		63.2±10.4		-0.049
Men	67 (52.8)		185 (49.2)		0.071
Colorectal cancer	99 (78.0)		315 (83.8)		-0.149
5-FU-based treatment	85 (66.9)		259 (68.9)		-0.042
Capecitabine-based treatment	42 (33.1)		117 (31.1)		0.042
Irinotecan (IR) not included	81 (63.8)		242 (64.4)		-0.012
IR included, UGT1A1 variant	26 (20.5)		79 (21.0)		-0.013
IR Included, UGT1A1 wild type	20 (15.8)		55 (14.6)		0.031
Adjuvant therapy	57 (44.9)		155 (41.2)		0.074
Radiotherapy	12 (9.5)		38 (10.1)		-0.022
Biological therapy	34 (26.8)		127 (26.8)		-0.067
DPYD activity score 0-1	4 (3.2)		15 (4.0)		-0.045
DPYD activity score 1.5-2	122 (96.1)		360 (95.7)		0.016
DPYD activity score missing	1 (0.8)		1 (0.3)		0.072
DPYD **6 c.2194G>A* variant	11 (8.7)		47 (12.5)		-0.125
DPYD **6 c.2194G>A* wild type	112 (88.2)		323 (85.9)		0.068
DPYD **6 c.2194G>A* missing	4 (3.2)		6 (1.6)		0.102
DPYD **9A c.85T>C* variant	94 (74.2)		112 (29.8)		0.982
DPYD **9A c.85T>C* wild type	29 (22.8)		255 (67.8)		-1.023
DPYD **9A c.85T>C* missing	4 (3.2)		9 (2.4)		0.046
Adverse events grade [?]3	100 (78.7)		183 (48.7)		0.658

Abbreviations: DPYD – dihydropyrimidine dehydrogenase; 5-FU – 5-fluorouracil; UGT – uridine 5'-diphospho-glucoronosyltransferase

[a]Variant allele carriers and wild type patients were matched using optimal full matching based on Mahalanobis distance with exact matching regarding DPYD activity score and **9A c.85T>C* genotype (see Patients and Methods for details).

Table 4 . Summary of models (frequentist and Bayesian) fitted to probability of grade 3-4 adverse events (as opposed to grade 0-2) in matched sets. A separate model was fitted for each of the three investigated *DPYD* polymorphisms in the respective matched set with adjustment for covariates that were not adequately balanced (standardized difference [?]0.1) by the matching procedure.

	Frequentist	Frequentist	Frequentist	Frequentist	Frequentist	Fre
	Model-generated OR (95%CI)	Model-generated	Model-generated P		Multiplicity adjusted[a] 95%CI	Mu
Model for c.496[b]						
c.496A>G variant	5.20 (1.88-14.3)		0.001		1.61-16.7	
*Model for *6 c.2194*[c]						

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	Frequentist	Frequentist	Frequentist	Frequentist	Frequentist	Fre
c.2194G>A variant	1.88 (0.95-3.73)		0.071		0.78-4.54	
*Model for *9A c.85*[d]						
c.85T>G variant	0.67 (0.37-1.21)		0.179		—	

Abbreviations: DPYD – dihydropyrimidine dehydrogenase

[a]In models with additional adjustments for non-balanced covariates, effects and covariances from the fitted models were retained and used to further adjust confidence intervals and P-values for multiplicity.

[b]Adjustment for sex

[c]Adjustment for age and *c.496A>G*(rs2297595) and **9A c.85T>C* (rs1801265) polymorphisms

[d]All covariates were well balanced – no additional adjustment

Table 5 . Characteristics of **6c.2194G>A* variant allele carriers and wild type patients before and after matching.[a] Covariates used in matching procedure are shaded. Differences between variant allele carriers and wild type patients are depicted as standardized differences (d). Values <0.1 indicate irrelevant differences. Analysis pertains to 490 patients: 10 patients missing data on **6 c.2194G>A* genotype and additional three missing data on **9A c.85T>C* genotype were excluded.

	Before matching	Before matching	Before matching	Before matching	Before matcl
	Variant		Wild type		d
N	58		432		—
Age (years)	65.8±8.9		62.9±10.6		0.303
Men	30 (51.7)		219 (50.7)		0.021
Colorectal cancer	50 (86.2)		353 (81.7)		0.123
FU-based treatment	35 (60.3)		298 (69.0)		-0.181
Capecitabine-based treatment	23 (39.7)		134 (31.0)		0.181
Irinotecan (IR) not included	38 (65.5)		276 (63.9)		0.034
IR included, UGT1A1 variant	14 (24.1)		90 (20.8)		0.079
IR Included, UGT1A1 wild type	6 (10.3)		66 (15.3)		-0.148
Adjuvant therapy	25 (39.7)		180 (41.7)		0.029
Radiotherapy	8 (13.8)		40 (9.3)		0.142
Biological therapy	16 (27.6)		125 (28.9)		-0.030
DPYD activity score 0-1	2 (3.4)		17 (3.9)		-0.026
DPYD activity score 1.5-2	56 (96.6)		415 (96.1)		0.026
DPYD **9A c.85T>C* variant	19 (32.8)		187 (43.3)		-0.218
DPYD **9A c.85T>C* wild type	39 (67.2)		245 (56.7)		0.218
DPYD *c.496A>G* variant	11 (19.0)		112 (25.9)		-0.167
DPYD *c.496A>G* wild type	47 (81.0)		320 (74.1)		0.167
Adverse events grade [?]3	39 (67.2)		236 (54.6)		0.261

Abbreviations: DPYD – dihydropyrimidine dehydrogenase; 5-FU – 5-fluorouracil; UGT – uridine 5'-diphospho-glucoronosyltransferase

[a]Variant allele carriers and wild type patients were matched using optimal full matching based on Mahalanobis distance with exact matching regarding DPYD activity score and type of cancer (colorectal vs. "other") (see Patients and Methods for details).

Table 6 . Characteristics of **9A c.85T>C*variant allele carriers and wild type patients before and after

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matching.[a] Covariates used in matching procedure are shaded. Differences between variant allele carriers and wild type patients are depicted as standardized differences (d). Values <0.1 indicate irrelevant differences. Analysis pertains to 490 patients: 13 patients missing data on **9A c.85T>C* genotype were excluded.

		Before matching	Before matching	Before matching	Before matching
	Variant	Variant		Wild type	
N	206	206		284	
Age (years)	62.4±10.9	62.4±10.9		63.8±10.1	
Men	99 (48.1)	99 (48.1)		150 (52.8)	
Colorectal cancer	166 (80.6)	166 (80.6)		237 (83.4)	
FU-based treatment	146 (70.9)	146 (70.9)		187 (65.8)	
Capecitabine-based treatment	60 (29.1)	60 (29.1)		97 (34.2)	
Irinotecan (IR) not included	131 (63.6)	131 (63.6)		183 (64.4)	
IR included, UGT1A1 variant	48 (23.3)	48 (23.3)		56 (19.7)	
IR Included, UGT1A1 wild type	27 (13.1)	27 (13.1)		45 (15.9)	
Adjuvant therapy	84 (40.8)	84 (40.8)		121 (42.6)	
Radiotherapy	26 (12.6)	26 (12.6)		22 (7.7)	
Biological therapy	61 (29.6)	61 (29.6)		80 (28.2)	
DPYD activity score 0-1	5 (2.4)	5 (2.4)		14 (4.9)	
DPYD activity score 1.5-2	201 (97.6)	201 (97.6)		270 (95.1)	
DPYD **6 c.2194G>A* variant	19 (9.2)	19 (9.2)		39 (13.7)	
DPYD **6 c.2194G>A* wild type	187 (90.8)	187 (90.8)		245 (86.3)	
DPYD *c.496A>G* variant	94 (45.6)	94 (45.6)		29 (10.2)	
DPYD *c.496A>G* wild type	112 (54.4)	112 (54.4)		255 (89.8)	
Adverse events grade [?]3	121 (58.7)	121 (58.7)		154 (54.2)	

Abbreviations: DPYD – dihydropyrimidine dehydrogenase; 5-FU – 5-fluorouracil; UGT – uridine 5'-diphospho-glucoronosyltransferase

[a]Variant allele carriers and wild type patients were matched using optimal full matching based on Mahalanobis distance with exact matching regarding DPYD activity score and *c.496A>G* genotype (see Patients and Methods for details).