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DPYD Genotyping in Greek Cancer Patients: Clinical Validation and Implementation of EMA-Recommended Variants for Fluoropyrimidine Dose Individualisation

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ABSTRACT

Dihydropyrimidine dehydrogenase (DPD), encoded by the DPYD gene, is the key enzyme regulating the breakdown of fluoropyrimidines (FPs). Variants in DPYD substantially alter DPD function and are firmly established predictors of FP-related adverse reactions. Four specific DPYD polymorphisms—rs3918290, rs55886062, rs67376798, and rs75017182—are incorporated into genotype-guided FP dosing recommendations and are advised by the European Medicines Agency (EMA) for testing prior to therapy. In Greece, however, information on DPYD screening remains unavailable. This work aimed to determine the occurrence of rs3918290, rs55886062, rs67376798, rs75017182, and additionally rs1801160, and to examine whether these variants correlate with FP-associated toxicities in Greek oncology patients. The cohort included 313 individuals receiving FP-based regimens. Genotyping of DPYD was performed with the QuantStudio™ 12K Flex Real-Time PCR instrument (ThermoFisher Scientific) using TaqMan® assays C__30633851_20 (rs3918290), C__11985548_10 (rs55886062), C__27530948_10 (rs67376798), C_104846637_10 (rs75017182), and C__11372171_10 (rs1801160).

Toxic effects of any grade (1–4) were observed in 208 participants (66.5%), and 25 of them (12%) showed grade 3–4 complications. EMA-listed DPYD variants appeared in 9 subjects (2.9%), all of whom developed toxicity ($p = 0.031$, specificity 100%). Their frequency was higher in those with grade 3–4 events (12%, $p = 0.004$, specificity 97.9%). Reduced DPYD function increased the likelihood of severe reactions (OR: 6.493, $p = 0.014$) and of grade 1–4 gastrointestinal (OR: 13.990, $p = 0.014$), neurologic (OR: 4.134, $p = 0.040$), and metabolic/nutritional (OR: 4.821, $p = 0.035$) toxicities. Dose intensity of FP treatment was notably lower in DPD-impaired patients ($\beta = -0.060$, $p < 0.001$). The rs1801160 variant showed no meaningful association with toxicity or dosing. A combined DPYD–TYMS–MTHFR effect was linked to high-grade toxicity (OR: 3.725, $p = 0.007$). These results reinforce that DPYD reduced-activity alleles serve as significant indicators of FP-related toxicities in the Greek population. Incorporating DPYD genotyping prior to FP therapy is advisable for safer dose selection. Interactions between DPYD and other relevant genes warrant additional study to evaluate whether they enhance predictive power for toxicity in FP-treated patients.

Keywords: DPYD, Pharmacogenomics, Fluoropyrimidines, 5-fluorouracil, Capecitabine, Clinical implementation

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Introduction

Fluoropyrimidines (FPs), including 5-fluorouracil (5-FU) and its orally administered precursor capecitabine (CAP), are routinely applied in managing solid tumors. Although they are effective, toxicity remains a major limitation of FP therapy [1]. Reported toxicity rates span 10%–40%. Mortality associated with FP reactions is also notable, with an estimated 150 deaths per year in France and about 1,300 annually in the United States [2, 3].

Pharmacogenomic testing enhances FP treatment due to the established link between DPYD variants, severe FP toxicity, and personalized dose adjustments. DPYD codes for DPD, the rate-controlling enzyme for 5-FU

metabolism. It is among the most comprehensively studied pharmacogenes, with more than 80 known variants and a detailed characterization of their impact on DPD function [4]. Among these, DPYD *2A (rs3918290) and *13 (rs55886062) produce total loss of DPD activity (value 0), while c.2846T>A (rs67376798) and c.1129–5923C>G (rs75017182, HapB3) result in partial activity (value 0.5). Guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) recommend FP dose reductions between 25% and 50% for patients with impaired DPD function (activity score 0.5–1.5) or complete avoidance of FP when activity is absent (score 0) [4, 5].

The capacity of DPYD screening to lower the incidence of fluoropyrimidine (FP)-related adverse reactions has been confirmed across numerous investigations [see overview in [1], and its economic value has also been demonstrated [6, 7]. Collectively, published work indicates that the variants rs3918290, rs55886062, rs67376798, and rs75017182 increase susceptibility to treatment toxicity, whereas applying dose adjustments based on genotype substantially limits this risk without diminishing therapeutic benefit [1]. Since April 2020, the EMA has advised clinicians to test patients for DPD deficiency before initiating therapy with fluorouracil or its analogues, capecitabine and tegafur, using either genetic or phenotypic measurements [8]. Routine pre-therapeutic genotyping of these four DPYD loci has been implemented in several European healthcare systems—among them Germany, Austria, the Netherlands, Switzerland, the UK, Spain, and Italy [9–16].

In Italy, an extra marker—rs1801160 (c.2194G>A, p.Val732Ile, DPYD*6)—is incorporated into ethnicity-tailored dosing recommendations [16]. Although CPIC classifies DPYD6 as a normal-function allele, a recent meta-analysis reported that carriers face a 1.73-fold higher likelihood of overall FP toxicity [17]. Consequently, beyond the four EMA-endorsed variants, the **DPYD6** allele may also merit routine testing to further reduce FP toxicity risk [18].

Although DPYD variation is recognized as a major factor in fluoropyrimidine dose optimization, data remain insufficient in various countries, including Greece, regarding both allele frequencies and population-specific toxicity patterns. To address this information gap, the present study aimed to quantify the frequency of **DPYD *2A, 13, c.2846T>A, HapB3, and 6 among Greek cancer patients receiving 5-FU or CAP, and to evaluate their relation to (severe) FP toxicity and dose intensity. Additionally, building on our earlier proposal of a polygenic dosing framework [1], previously noted genotype–toxicity associations in this cohort [19, 20] were reassessed in the context of DPYD variation.

Materials and Methods

Study population

A full description of the cohort has been published previously [19, 20]. Briefly, the study included 313 unrelated patients with cancer (160 men, 153 women; mean age 64.2 ± 10.6, range 34–88) treated with 5-FU or CAP, either alone or combined with other antineoplastic medications. All participants were hospitalized in the Oncology Department of the Academic General Hospital of Alexandroupolis (Greece) and were retrospectively enrolled between February 2018 and December 2019.

Information on demographics, treatment regimen, drug dosage, duration of therapy, method of administration, toxicity events, and treatment impact was collected for each participant. Toxicity grading followed the CTCAE v5.0 criteria [21]. A single oncology team monitored all patients and made decisions regarding drug type, dosing schedules, administration intervals, and cessation of therapy.

All individuals provided written informed consent. The protocol received approval from both the Scientific Council and the Ethics Committee of the Academic General Hospital of Alexandroupolis and adhered to the Declaration of Helsinki.

Genotyping

Blood samples of approximately 3 mL were drawn into EDTA tubes. Genomic DNA was isolated from whole blood using either the Gentra Puregene Blood Kit (QiagenR/Qiagen, MD, United States) or the MagCore Automated Nucleic Acid Extractor (RBC Bioscience, New Taipei City, Taiwan). Samples were stored at −20°C until further processing. DNA yield and purity were assessed using a Q5000 UV-Vis spectrophotometer (Quawell) and a Qubit 4 fluorometer (ThermoFisher Scientific).

Genotyping of DPYD was performed on 96-well plates using the QuantStudio™ 12K Flex Real-Time PCR System (ThermoFisher Scientific) with the following TaqMan® assays:

C__30633851_20 (rs3918290), C__11985548_10 (rs55886062), C__27530948_10 (rs67376798), C_104846637_10 (rs75017182), C__11372171_10 (rs1801160).
Each PCR reaction (total 10 μL) consisted of 5 μL TaqMan Genotyping Master Mix, 0.5 μL of 20× assay, and 4.5 μL DNA (~20 ng).
Thermal cycling for rs3918290, rs55886062, rs67376798, and rs1801160 involved:
– 60°C, 30 s (pre-read);
– 95°C, 10 min;
– 50 cycles of 95°C, 15 s, and 60°C, 1:30 min;
– final 60°C, 30 s (post-read).

For rs75017182, 40 cycles of 95°C (15 s) and 60°C (1 min) were used. Each plate included triplicate non-template controls. Ten percent of samples were re-genotyped independently, yielding 100% agreement.
Genotype assignment was performed with QuantStudio 12K Flex Software v1.5. When a variant allele was present, allelic discrimination plots were generated automatically; for samples without detected variants, calls were based on multicomponent signal profiles.

Statistical analysis
Normality of continuous measurements was evaluated using the Shapiro–Wilk test. Variables following a normal distribution are reported as mean ± standard deviation (SD), whereas non-normally distributed data are summarized as median (25th, 75th percentiles). Depending on distributional properties, comparisons between groups were performed using parametric tests (independent t-test or one-way ANOVA) or non-parametric tests (Mann–Whitney or Kruskal–Wallis). For categorical variables, either the χ^2 test or Fisher's exact test was applied, determined by sample size constraints. Allelic proportions were derived through gene counting, and deviations from Hardy–Weinberg equilibrium were examined using an exact two-tailed probability calculation according to Weir (1996) [22]. Sensitivity, specificity, and predictive values (PPV, NPV) for DPYD genotyping in relation to FP-induced toxicity (any grade and grade 3–4) were calculated from true/false positive and negative rates.
A stepwise forward logistic regression adjusted for sex, age, and weight was used to estimate odds ratios (ORs), with overall or severe toxicity as the dependent variable and either individual DPYD variants or DPYD–other-gene interactions as independent predictors. To evaluate the probability of altered FP dose intensity linked with DPYD genotypes, β-coefficients with 95% confidence intervals were obtained via multivariable linear regression (stepwise forward), using FP dose intensity as the outcome and sex, age, weight, and DPYD deficiency as covariates. Statistical significance was set at $p < 0.05$. All analyses were run in IBM SPSS Statistics for Windows, Version 27.0 (IBM Corp., NY, United States).

Results and Discussion

Population characteristics
A summary of demographic and clinical features is shown in **Table 1**, and an expanded description of clinicopathological variables—including sex-specific profiles—has been published earlier [19, 20]. The occurrence of any FP-related toxicity (grades 1–4) was 66.5%, corresponding to 208 individuals, with gastrointestinal events representing the most frequent AEs (37.1%). A total of 26 grade 3–4 toxicities occurred among 25 patients (12% of those with grades 1–4 effects). In 79 patients, toxicity necessitated treatment modification: 44 required dose reductions and/or 59 experienced delayed administration or therapy discontinuation. Adjustments were distributed across chemotherapy cycles: n = 21 (cycle 1), n = 25 (cycle 2), n = 14 (cycle 3), n = 12 (cycle 4), n = 9 (cycle 5), and n = 22 (cycle 6+).

Table 1. Main characteristics of the patient cohort.

Characteristic	FP-treated patients (N = 313)
Demographic data	
Male sex, n (%)	160 (51.1%)
Age, years (mean ± SD)	64.2 ± 10.6
Body surface area, m² (median [25th–75th percentile])	1.79 [1.60–1.90]

Current or former smokers, n (%)	72 (23.0%)
Treatment regimen	
Capecitabine-containing regimen, n (%)	205 (65.5%)
5-Fluorouracil or capecitabine monotherapy, n (%)	10 (3.2%)
Sequential or concurrent 5-FU + capecitabine, n (%)	55 (17.6%)
XELOX (capecitabine + oxaliplatin), n (%)	144 (46.0%)
CAP + oxaliplatin + monoclonal antibody, n (%)	41 (13.1%)
CAP + oxaliplatin + taxanes, n (%)	34 (10.9%)
CEF (cyclophosphamide + epirubicin + 5-FU), n (%)	41 (13.1%)
CMF (cyclophosphamide + methotrexate + 5-FU), n (%)	17 (5.4%)
FOLFOX or FOLFIRI, n (%)	16 (5.1%)
5-FU + monoclonal antibody, n (%)	3 (1.0%)
5-FU + taxanes, n (%)	4 (1.3%)
Toxicity and dose modifications	
Any grade 1–4 toxicity, n (%)	208 (66.4%)
Severe (grade 3–4) toxicity, n (%)	26 (12.5%)
Dose reduction required, n (%)	44 (14.0%)
Treatment delay or permanent discontinuation, n (%)	59 (18.8%)
Relative dose intensity, % (median [25th–75th percentile])	100 [96–100]

FP: fluoropyrimidine; SD: standard deviation; CAP: capecitabine; 5-FU: 5-fluorouracil; XELOX: capecitabine + oxaliplatin; MA: monoclonal antibody; CEF: 5-FU + epirubicin + cyclophosphamide; CMF: 5-FU + methotrexate + cyclophosphamide; FOLFOX: 5-FU + leucovorin + oxaliplatin; FOLFIRI: 5-FU + leucovorin + irinotecan + MA.

Frequency of DPYD polymorphisms

Among the four EMA-endorsed DPYD loci, 9 patients were identified as heterozygous carriers (2.9% total): 1 individual carried the deleterious *2A allele (0.3%), and 8 harbored the reduced-function rs75017182 variant (2.6%). Neither DPYD *13 nor rs67376798 was detected in the cohort (95% CI: 0.0001%–0.6%). The rs1801160 variant appeared far more frequently, with 42 heterozygotes (13.4%) and 1 homozygote (0.3%) **(Table 2)**.

Table 2. Prevalence of DPYD variants in the overall cohort and across toxicity categories.

DPYD variant / haplotype	Total cohort (N = 313)	Grade 1–4 toxicity (n = 208)	p-value (vs. no toxicity)	Grade 3–4 toxicity (n = 25)	p-value (vs. grade 0–2)
rs3918290 (*2A)	1 (0.3%)	1 (0.5%)	0.031	1 (4.0%)	0.004
rs75017182 (HapB3, c.1129-5923C>G)	8 (2.5%)	8 (3.8%)	0.031	2 (8.0%)	0.004
rs55886062 (*13, c.1679T>G)	0	0	—	0	—
rs67376798 (c.2846A>T)	0	0	—	0	—
rs1801160 (*6, c.2194G>A)	43 (13.7%)	29 (13.9%)	0.775	2 (8.0%)	0.675
Any EMA-recommended reduced-function variant (*2A, *13, HapB3, c.2846A>T)	9 (2.9%)	9 (4.3%)	0.031	3 (12.0%)	0.004
Combined *2A / HapB3 / *6 carriage	52 (16.6%)	38 (18.3%)	0.365	5 (20.0%)	0.567

Association of DPYD variants with FP-induced toxicity and dose intensity

All 9 carriers of DPYD *2A or rs75017182 experienced toxicity (p = 0.031). For grades 1–4 toxicity, the PPV was 100%, the NPV was 34.5%, the specificity was 100%, and sensitivity 4.3%. DPYD deficiency significantly elevated the odds of gastrointestinal toxicity (OR: 13.99, 95% CI 1.71–114.22, p = 0.014), neurological toxicity

(OR: 4.13, 95% CI 1.07–16.04, p = 0.040), and nutrition/metabolism issues (OR: 4.82, 95% CI 1.12–20.73, p = 0.035).

Among cases with grade 3–4 toxicity, 3 of 25 patients (12.0%) carried *2A or rs75017182 (p = 0.028), yielding PPV 33.3%, NPV 92.8%, specificity 97.9%, and sensitivity 12%. DPYD deficiency raised the odds of severe toxicity across all systems (OR: 6.49, 95% CI 1.45–29.06, p = 0.014). For severe gastrointestinal events, the impact was especially high (OR: 45.94, 95% CI 4.69–449.95, p = 0.001). No severe (grade 3–4) toxicities in other organ systems occurred among variant carriers.

Adjusted linear regression showed that DPYD-deficient patients had significantly lower FP dose intensity (β = −0.060, 95% CI −0.085 to −0.035, p < 0.001).

The rs1801160 variant showed no independent association with toxicity (p = 0.78 for any grade; p = 0.68 for grade 3–4), nor after accounting for *2A and rs75017182 (p = 0.77, p = 0.51, respectively). rs1801160 also had no measurable effect on dose intensity (p = 0.37).

*Characteristics of patients carrying DPYD *2A and rs75017182 variants*

Eight of the patients carrying DPYD *2A or rs75017182 received CAP-based combination therapy: 5 on XELOX, 3 on CAP + MA, while one individual alternated between CAP and 5-FU combined with taxanes. Most carriers (n = 7, 77.8%) had colorectal cancer. Gastrointestinal toxicity occurred in 8 of 9 carriers. Treatment adjustments were made in 4 of these individuals (5% of all dose/therapy modifications). A dose reduction occurred only for the *2A carrier during cycle 2 due to grade 3–4 toxicity, described further in Section 3.5. Two carriers with grade 1–2 toxicity experienced delayed chemotherapy at cycle 4, whereas the remaining carrier was switched to a different regimen after the first cycle.

*Case report of the DPYD*2A carrier patient*

Only one individual in the cohort carried the pathogenic DPYD2A allele, which corresponds to markedly diminished DPD function (activity score 1). This patient was a 63-year-old man diagnosed with colorectal cancer and pulmonary metastatic disease. His initial CAP regimen was 3,500 mg per day. During the second treatment cycle, grade 1–2 diarrhea appeared, prompting a dosage reduction to 3,000 mg. When the third cycle began, diarrhea escalated to grade 4, leading clinicians to lower the CAP dose again to 2,000 mg. At this level—43% below the original prescription—the patient no longer showed any adverse effects. CPIC recommendations specify that heterozygous DPYD2A carriers should initiate therapy at roughly 50% of the standard FP dose. Had his genotype been known beforehand and the CAP dose been adjusted accordingly, the severe diarrhea would likely have been avoided.

*Interaction of DPYD*2A and rs75017182 with TYMS and MTHFR polymorphisms*

Earlier publications from our group on the same cohort reported two findings: the TYMS-TSER 2R/2R genotype was linked to FP dose reduction among female patients [19], and the MTHFR 665C>T variant increased both the likelihood of FP dose reduction (OR 5.05) and the magnitude of that reduction (β = 3.318) in women [20]. In this updated analysis, we investigated whether these loci interact with DPYD variants to enhance the prediction of FP-associated toxicity **(Table 3)**. Individually, neither TYMS nor MTHFR reached significance in relation to grade 3–4 toxicity across the entire sample. However, combined genetic profiles revealed important patterns: DPYDTYMS TSER 2R/2R and DPYDMTHFR 665T+ combinations increased the odds of severe toxicity (OR 2.89, 95% C.I. 1.23–6.84, p = 0.015; and OR 6.18, 95% C.I. 1.97–19.42, p = 0.002, respectively). A three-gene interaction model (DPYDTYMSMTHFR) also showed a significant association with grade 3–4 toxicity (OR 3.73, 95% C.I. 1.43–9.71, p = 0.007). No significant interactions emerged for dose intensity (data not shown).

Table 3. Logistic regression—adjusted for age, sex, and body weight—estimating odds of grade 3–4 toxicity across genetic models.

Model	Predictor	Odds Ratio (OR)	95% Confidence Interval	p-value
Predictive models for grade 3–4 fluoropyrimidine toxicity				

Model A	DPYD reduced-function variant (*2A or HapB3)	6.49	1.45–29.06	0.014
	Sex (male vs. female)	1.42	0.60–3.38	0.43
	Age (per 10-year increase)	0.61	0.26–1.46	0.27
	Weight (per kg)	0.99	0.96–1.02	0.37
Model B	DPYD*TYMS TSER 2R/2R interaction	2.89	1.23–6.84	0.015
	Sex	1.49	0.63–3.56	0.37
	Age	0.62	0.26–1.47	0.28
	Weight	0.99	0.96–1.02	0.35
Model C	DPYD*MTHFR 665C>T (T allele carrier) interaction	6.18	1.97–19.42	0.002
	Sex	1.56	0.64–3.76	0.33
	Age	0.61	0.25–1.47	0.27
	Weight	0.99	0.96–1.02	0.34
Model D	DPYD*TYMS TSER 2R/2R*MTHFR 665T+ triple interaction	3.73	1.43–9.71	0.007
	Sex	1.52	0.63–3.65	0.35
	Age	0.63	0.27–1.51	0.30
	Weight	0.99	0.96–1.02	0.34
Non-significant models				
Model A	DPYD*rs1801160 (*6) interaction	1.43	0.50–4.04	0.51
	Sex	1.48	0.63–3.47	0.37
	Age	0.67	0.29–1.56	0.35
	Weight	0.98	0.96–1.01	0.28
Model B	TYMS TSER 2R/2R alone	1.15	0.48–2.78	0.76
	Sex	1.47	0.63–3.44	0.38
	Age	0.66	0.28–1.54	0.33
	Weight	0.99	0.96–1.01	0.30
Model C	MTHFR 665C>T (T carrier) alone	1.12	0.49–2.56	0.80
	Sex	1.47	0.62–3.44	0.38
	Age	0.66	0.28–1.54	0.34
	Weight	0.99	0.96–1.01	0.30

We genotyped 313 Greek patients treated with 5-FU or CAP for five DPYD variants: *2A, rs75017182, *13, rs67376798, and rs1801160. Carriers of the four variants listed by EMA represented 2.9% of the sample, and these genotypes showed clear relationships with FP-related toxicity (any grade and severe) and with dose intensity. Inclusion of rs1801160 did not improve predictive performance, despite a higher carrier frequency (13.7%). When we integrated DPYD with TYMS and MTHFR as a combined model, multiple significant gene–gene interactions emerged for grade 3–4 toxicity.

The variants *2A, rs75017182, *13, and rs67376798 form the basis of current FP pharmacogenomic decision-making, and many European healthcare systems have implemented genotype-guided FP dosing. For Greece, however, information on the prevalence and clinical relevance of these alleles has been limited. In our cohort, nine individuals were heterozygous for *2A or rs75017182, corresponding to a DPYD-deficient rate of 2.9%, which aligns with reported European estimates (<5%) [23]. The absence of *13 and rs67376798 mirrors their very low frequency in European populations (0–0.5%). The rs1801160 allele was more common (7% frequency; 13.7% carriers), consistent with approximately 5% reported elsewhere [17].

Both *2A and rs75017182 were strongly linked to the presence of toxicity; every carrier experienced FP-related side effects. For severe events (grade 3–4), three of the nine carriers were affected. Predictive specificity for toxicity was high (100% for any grade, 97.9% for severe), while sensitivity remained low (4.3% and 12%), a pattern similar to earlier work showing low combined sensitivity (5.3%) but >99% specificity for major DPYD variants [24]. Overall, the findings confirm that DPYD genotyping of EMA-listed alleles provides clinically useful guidance for predicting FP-induced toxicity in Greek patients and supports dosing optimization.

In addition to the EMA-endorsed DPYD variants, we also evaluated the rs1801160 polymorphism. Although this allele appeared relatively common in our cohort, no link emerged between rs1801160 and the various FP-related toxicity outcomes. The rs1801160 change is generally considered a normal-activity variant, yet several reports suggest that, after adjusting for the four EMA-listed loci, it may still elevate the probability of FP toxicity [17, 18]. Its prevalence in the Greek population mirrors that of other European groups, but incorporating rs1801160 into our predictive framework led to a drop in PPV (from 100% to 72% for toxicity of any grade) and in specificity (from 100% to 86.7%). Based on our sample size, an effect of the magnitude reported by Kim *et al*. (2022) would have been detectable with statistical power exceeding 75% [17], had it existed. While rs1801160 has not consistently demonstrated a direct association with FP intolerance [25, 26], some studies indicate a possible connection with neutropenia risk [27]. In Kim *et al*.'s meta-analysis, this polymorphism was linked to increased odds of neutropenia (OR 1.87) and displayed a slightly lower, though still elevated, risk for overall toxicity (OR 1.72). The absence of neutrophil count data in our cohort may partly obscure this relationship. It is also noteworthy that non-DPYD factors, including microRNA regulation [28], influence FP toxicity; miR-27A variants, for instance, have shown relevance in DPYD carriers [29]. Thus, any modest effect of rs1801160 might be overshadowed by other genetic contributors. Validating such findings across diverse ethnic backgrounds is crucial to establishing robust models for FP-toxicity prediction. Without population-specific evidence, inclusion of multiple minor-effect variants risks exaggerating toxicity estimates and leading to inappropriate dose reductions. Ethnic variability must therefore be considered, as demonstrated by various pharmacogenomic-based dosing schemes such as CYP2C9/VKORC1-guided warfarin therapy [30]. For FPs specifically, DPYD plays a limited role in toxicity among Asian populations [31].

Although DPYD status remains central to individualized FP dosing, a major challenge is the low prevalence of these deleterious alleles, resulting in limited sensitivity. This underscores the need to identify additional damaging DPYD variants and to integrate multi-gene information into dosing protocols. DPYD is well studied, and numerous rare variants that substantially heighten FP-toxicity risk have been documented. Broader screening could help explain more cases of DPD deficiency and address the persistent gap in heritability [32–34]. Still, even with added rare alleles, sensitivity is expected to remain modest. We have detailed previously that several genes beyond DPYD influence FP response and that practice is slowly shifting from a single-gene to a multigene dosing strategy [1]. In line with this, we reassessed our cohort to evaluate the combined effects of TYMS and MTHFR variants with DPYD polymorphisms. Earlier analyses revealed sex-dependent impacts of TYMS and MTHFR on FP response [19, 20]. While neither gene independently predicted FP toxicity in the full cohort, interaction modeling showed that, once adjusted for DPYD, variants in TYMS and MTHFR contribute additional risk. Notably, the joint DPYDTYMSMTHFR pattern was linked to nearly a fourfold increase in grade 3–4 toxicity, independent of sex, and improved both confidence interval boundaries and p-values relative to DPYD alone. Given that TYMS and MTHFR influence FP action rather than drug metabolism—thymidylate synthase being the FP target and MTHFR modulating folate pathways that interact with TS inhibition [1]—their combined effect with DPYD suggests additive pharmacokinetic and pharmacodynamic contributions. Larger patient samples are needed to explore this further. Ultimately, the field is moving toward integrated pharmaco-omics approaches in oncology [35], and computational methods such as artificial intelligence and machine learning will be essential for variant classification and constructing clinically actionable multigene models [1, 36].

The study benefits from several strengths: toxicity assessments were consistent due to monitoring by a small oncology team; full TYMS and MTHFR genotyping enabled interaction testing; and all DPYD alleles were analyzed with validated TaqMan assays, achieving complete concordance across operators. Nonetheless, limitations remain. The retrospective nature of the work prevents evaluation of the impact of upfront DPYD genotyping on avoiding FP toxicities. Rare DPYD variants were not sequenced, and consequently, potentially informative alleles were missed. Other FP-related loci, such as ENOSF1, were outside the scope of the interaction analyses. Although all patients received FP-based regimens, contributions from additional agents cannot be excluded, and carriers of DPYD variants were not treated with FP monotherapy.

Conclusion

In summary, we report that roughly 3% of Greek cancer patients exhibit reduced DPD activity. Our data reaffirm the clinical relevance of DPYD for predicting FP-related adverse events. Dose adjustments based on 2A, rs75017182, 13, and rs67376798 can be applied in Greek clinical practice before FP initiation. The combined DPYDTYMSMTHFR model shows promise but requires broader validation across multiple populations.

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