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Research article

Clinical impact of DPYD genotyping and dose adjustment in candidates for fluoropyrimidine treatment

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1. Introduction

Fluoropyrimidines (5-fluorouracil, capecitabine, and tegafur) are widely used in the treatment of different malignant tumors, including cancer of the stomach, bowel, pancreas, esophagus, and breast; and squamous cell carcinoma of the head and neck [1–6]. More than two million people per year are diagnosed with cancers that are treated with fluoropyrimidines, usually in combination with other antineoplastic agents [1–4,7].

Up to 40 % of people who receive these drugs experience severe toxicities (neutropenia, nausea, vomiting, diarrhea, stomatitis, mucositis, and hand-foot syndrome), and fluoropyrimidine toxicities are life-threatening in around 1 % of cases [1–5,8–10]. These toxicities can occur from the first treatment cycle and lead to treatment delays, interruptions, or hospital admissions, increasing health costs and reducing patients' quality of life and treatment effectiveness [2–4,8].

Although fluoropyrimidine toxicity has many causes, experts agree that the main cause is reduced activity of dihydropyrimidine dehydrogenase (DPD). DPD is the main enzyme responsible for metabolizing fluoropyrimidines (at least 80 % of the dose in the case of 5-fluorouracil). Reduced DPD activity leads to reduced drug clearance, accumulation of the active metabolite, and increased half-life of 5-fluorouracil, which can cause severe or even fatal toxicities when the drug is administered at standard doses [1–4,8,11–19].

Partial DPD deficiency is present in 3 %–8 % of the population [2,4,6,8]. Complete deficiency of the enzyme is much rarer, affecting an estimated 0.01 %–0.5 % of people [2,4,8]. Different studies have shown that between 39 % and 61 % of people with severe fluoropyrimidine-associated toxicities have reduced DPD activity [8].

The high inter- and intra-individual variation in DPD activity is mainly due to polymorphisms in *DPYD*, the gene that encodes the enzyme [1,4,18–21]. *DPYD* has multiple polymorphisms, but recent studies have shown that four main variants are associated with a clinically relevant decrease in DPD activity [1–4,8,22–24].

1. *c.1905+1G > A* (*rs3918290*, *DPYD*2A*, *IVS14+1G > A*)
2. *c.1679T > G* (*rs55886062*, *DPYD*13*, *p.I560S*)

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3 *c.2846A > T* (*rs67376798*, *p.D949V*)
4. *c.1236G > A/HapB3* (*rs56038477*, *E412E*, *in haplotype B3*)

The recommendations in current guidelines and from health regulatory agencies focus on these four variants [1,3,4,8,25,26], as research suggests they are responsible for around half of all DPD deficiencies [8]. A safety notice published in 2020 by the Spanish Agency of Medicines and Medical Devices (AEMPS) recommended performing *DPYD* genotyping in candidates for fluoropyrimidine treatment [27].

However, there is some variation between the *DPYD* genotype and the DPD phenotype, possibly due to other less well studied polymorphisms such as *c.2194G > A* (**6*, rs1801160, *p.V732I*) [23,28–31], or the *MIR27A* variant *rs895819A > G* [23,30]. It is of considerable scientific interest to study new variants and evaluate their association with toxicity [11]. However, based on current evidence, performing prospective genotyping of the four main variants and personalizing fluoropyrimidine dose before the start of treatment is a promising strategy to prevent severe and life-threatening fluoropyrimidine-related toxicity without reducing efficacy [2,8,32].

Henricks and colleagues showed that reducing fluoropyrimidine dose based on *DPYD* genotyping before the start of treatment leads to a decrease in severe toxicity [2]. The aim of our study was to evaluate the clinical impact, on toxicity, of individualizing fluoropyrimidine dose according to the results of active genotyping for the four main *DPYD* variants [1,3,4].

2. Methods

We conducted a single-center, ambispective, quasi-experimental study in a tertiary hospital in Spain. For the intervention group, we included adults (aged ≥18 years) with any type of cancer who were scheduled to begin treatment with fluoropyrimidines, alone or in combination with other agents, radiotherapy, or both. For ethical reasons, and in view of current recommendations in clinical guidelines and from health regulatory agencies, we used a retrospective control group, meaning participants had already started therapy at study inclusion. The control group had not undergone *DPYD* genotyping because they started treatment in a period prior to the implementation of this strategy in routine clinical practice. People who had undergone previous chemotherapy with agents other than fluoropyrimidines were eligible for inclusion. We applied no restrictions related to functional status, life expectancy, or comorbidities.

This was a feasibility study with a secondary, under-powered analysis of the overall toxicity pre-vs. post-implementation.

In the intervention group, we tested for the four main variants (*c.1905+1G > A*, *c.16T79>G*, *c.2846A > T*, and *c.1236G > A*) and adjusted the initial fluoropyrimidine dose in variant carriers according to clinical guidelines. We also tested for the polymorphisms *c.2194G > A* and *MIR27A* rs895819 as part of an observational study not reported in this publication.

We compared toxicity and other consequences in the intervention arm (genotyping) and the control arm (no genotyping). An optimal statistical analysis would have evaluated the effect of adjusting fluoropyrimidine dose versus not adjusting fluoropyrimidine dose in variant carriers only. However, because genotyping was not part of usual practice in our hospital before we began this project, we were unable to select a retrospective control group of variant carriers. Selecting a control group to undergo prospective genotyping without the corresponding dose adjustments in variant carriers would have been unethical. For these reasons, we were unable to conduct our primary comparison of interest.

We defined the intervention as genotyping and preparation of the pharmacokinetic reports with the corresponding dose adjustments.

We collected the same variables from both groups (except *DPYD* variants, which we could only collect in the intervention group) to minimize potential bias and maximize the quality and reliability of our results.

An oncologist evaluated and classified toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 [33]. Follow-up was from the first to the sixth treatment cycle. We also recorded consequences of toxicity, such as hospital admissions related to toxicity, dose reductions, delays, and treatment discontinuation. As per usual clinical practice, blood tests were performed before the beginning of treatment and before each new cycle to assess treatment safety. We used the initial blood sample for *DPYD* genotyping, meaning participants did not have to undergo an additional blood draw.

The reference dose was used according to standard clinical practice, following the drug label and clinical guidelines, always at the discretion of the oncologist.

For the analysis of genetic variants in DPYD, we used real-time polymerase chain reaction (PCR) genotyping with Thermo Fisher TaqMan probes. The first step of genotyping was extraction and purification of genomic DNA from whole blood samples obtained by venipuncture and collected in EDTA-coated tubes. We used the Canvax commercial kit (Higher Purity TM Blood DNA extraction kit) with silica microarrays and spectrophotometric determination of DNA concentration within 24 h of collection. The sample was stored at −20 °C after purification. Subsequently, the six variants were genotyped by real-time PCR using the TaqMan Drug Metabolism Enzyme assay, providing a robust and reproducible signal. Templates were amplified in Rotor Gene 3000 (Hold 95 °C/10′ 40 cycles 95 °C/15″ 60 °C/90″) using TaqMan drug metabolism genotyping assays, in a final volume of 10 μL, with negative water blanks and positive gBlock (IDT). Each sample was amplified in duplicate, and 10 % of the samples analyzed were randomly replicated. All assays produced concordant results. As positive controls, we designed and used gBlocks Gene Fragments (IDT), which are sequence-verified genomic blocks with high sequence fidelity (reference 76686492 of 609 bp), including the mutated forms of the single nucleotide polymorphisms (SNPs) rs3918290-*2A, rs5588606262-*13, and rs67376798-D949V.

The frequency equilibrium develops according to principles and conditions elucidated by Hardy and Weinberg. Taking in account that this equilibrium is valid only under certain conditions and that in small populations (our case) the proportion of genotypes may be

shifted due to casual fluctuations.

We performed a descriptive analysis of the collected data. For categorical variables, we reported absolute and relative frequencies; and for continuous variables, we reported minimum and maximum values and means with standard deviations. We used contingency tables to analyze the homogeneity of the intervention and control groups, applying Fisher's exact test. We analyzed the categorical variables associated with toxicity and its consequences (hospital admissions related to toxicity, dose reduction, treatment delay or suspension) with contingency tables, using Fisher's exact test. We planned to fit a multivariable logistic model to measure the associations between toxicity and each group after adjusting for the other explanatory variables. We would have estimated odds ratios (ORs) with their 95 % confidence intervals (CIs). Within the intervention group, we evaluated the grade of toxicity and health outcome for each polymorphism. We used SPSS v.26 for the statistical analysis.

The hospital ethics committee approved the study protocol, patient information sheet, and informed consent form (code 20/006).

3. Results

We included a total of 308 adults, divided into two groups. The intervention group had 163 participants, who we recruited consecutively from May 2022 until reaching the predefined sample size. For the control group (n = 145), we consecutively included all people who met our eligibility criteria from January 1, 2019 until reaching the predefined sample size.

The intervention group consisted of adult patients (≥18 years) with cancer who were intended to begin fluoropyrimidine based anticancer therapy, either as a single agent or in combination with other chemotherapeutic agents, radiotherapy, or both. Patients with all tumour types for which fluoropyrimidine based therapy was considered in their best interest were eligible. Previous chemotherapy was allowed, except for previous use of fluoropyrimidines. Whereas those in the control group consisted adult patients (≥18 years) with cancer who had received at least one cycle of fluoropyrimidine-based chemotherapy prior to the implementation of the pharmacogenetic protocol. In both groups there were no restrictions on comorbidities, performance status or life expectancy. Patients were excluded from the study if the necessary variables for data collection were not available or those with limited comprehension capacity to provide informed consent were also excluded.

In the intervention group, 22 participants (13.5 %) did not start treatment with fluoropyrimidines for reasons unrelated to our study, so most of the study variables were missing. We considered this data missing at random. We had full data for the remaining 286 people (141 in the intervention group and 145 in the control group).

Table 1 shows the baseline characteristics of the participants, comparing the intervention group with the control group. Using the data collected from the initial blood test, we recorded whether hemoglobin, neutrophil, and platelet levels were within the normal range.

The two study groups had similar clinical and sociodemographic characteristics. We only found significant differences in the variables sex and tumor type.

Table 2 presents variables related to treatment (previous chemotherapy, treatment line, regimen, fluoropyrimidine, and other concomitant antineoplastic drugs).

Forty-two participants (29.8 %) in the intervention group and 77 (53.1 %) in the control group completed more than six cycles of treatment. A smaller proportion completed exactly six cycles (the follow-up of this study): 25 participants (17.7 %) in the intervention group and 18 (12.4 %) in the control group.

Sixteen participants (11.4 %) in the intervention group had one of the four main *DPYD* variants. Table 3 shows the distribution of variants in the intervention group.

Therefore, 11.4 % of participants required a dose adjustment before starting treatment. The medical oncology team accepted the

Table 1
Baseline characteristics of the study population.

Baseline characteristics		Control n (%)[a]	Intervention n (%)[a]	P value
Mean age (SD), years		67.4 (10.3)	66.0 (12.2)	0.30
Sex	Male	87 (60.0 %)	68 (48.2 %)	0.046
	Female	58 (40.0 %)	73 (51.8 %)	
Tumor type	Colorectal	96 (66.2 %)	75 (53.2 %)	0.035
	Gastric	36 (24.8 %)	38 (27.0 %)	
	Breast	10 (6.9 %)	18 (12.8 %)	
	Other	3 (2.1 %)	10 (7.1 %)	
Stage	I	6 (4.3 %)	9 (6.8 %)	0.199
	II	21 (15.1 %)	16 (12.0 %)	
	III	35 (25.2 %)	49 (36.8 %)	
	IV	77 (55.4 %)	59 (44.4 %)	
Performance status	0	39 (26.9 %)	50 (35.5 %)	0.286
	1	91 (62.8 %)	77 (54.6 %)	
	2	15 (10.3 %)	14 (9.9 %)	
	4	0	0	
Normal Hb/neutrophils/platelets	No	104 (72.7 %)	94 (66.7 %)	0.266
	Yes	39 (27.3 %)	47 (33.3 %)	

Abbreviations: Hb hemoglobin; SD: standard deviation.
[a] Unless otherwise specified in the Baseline characteristics column.

Table 2
Treatment variables in the study population.

Treatment variables		Intervention n (%)	Control n (%)	P value
CTx-naïve	Yes	95 (67.4 %)	101 (69.7 %)	0.67
	No	46 (32.6 %)	44 (30.3 %)	
Type of treatment	Neoadjuvant	28 (19.9 %)	20 (13.8 %)	0.284
	Adjuvant	52 (36.9 %)	64 (44.1 %)	
	Palliative	61 (43.3 %)	61 (42.1 %)	
Fluoropyrimidine	Capecitabine	93 (66.0 %)	95 (65.5 %)	0.937
	Fluorouracil	48 (34.0 %)	50 (34.5 %)	
Treatment regimen	Combination	97 (68.8 %)	104 (71.7 %)	0.588
	Monotherapy	44 (31.2 %)	41 (28.3 %)	
CTx regimen	5FU + other anticancer drugs	26 (18.4 %)	20 (13.8 %)	–
	5FU + oxaliplatin and folinic acid (with or without bevacizumab)	19 (13.5 %)	29 (20.0 %)	
	5FU + radiotherapy (with or without mitomycin)	2 (1.4 %)	1 (0.7 %)	
	Capecitabine monotherapy (with or without bevacizumab)	34 (24.1 %)	36 (24.8 %)	
	Capecitabine + other anticancer drugs	12 (8.5 %)	6 (4.1 %)	
	Capecitabine + oxaliplatin (with or without bevacizumab)	34 (24.1 %)	45 (31.0 %)	
	Capecitabine + radiotherapy (with or without mitomycin)	14 (9.9 %)	8 (5.5 %)	

Abbreviations: 5FU, 5-fluorouracil; CTx: chemotherapy.

Table 3
Distribution of main *DPYD* variants in the intervention group.

DPYD status	Wild-type	c.1905+1G > A	c.1679T > G	c.2846A > T	DPYD c.1236G > A/HapB3
Total	125 (88.6 %)	0	0	10 (7.1 %)	6 (4.3 %)
Heterozygous	0	0	0	7 (5.0 %)	2 (1.4 %)
Homozygous	0	0	0	2 (1.4 %)	3 (2.1 %)
Compound heterozygous	0	0	0	1 (0.7 %)	1 (0.7 %)

pharmacogenetic recommendation for 15 of the 16 patients. For the remaining patient, the starting dose was reduced by 70 % due to an error in the interpretation of the report. This error was detected after the first cycle, and the dose was corrected for the subsequent cycles.

Table 4 presents the genetic variables and activity scores in the intervention group.

Table 4 shows that 11 patients had an activity score of 1.5. Nine of these patients had an initial 50 % dose reduction, in accordance with CPIC guideline updates; one patient started with a 70 % dose reduction due to a medical error that was corrected in the second cycle; and one patient with activity score of 1.5, was the only one who started with a 30 % dose reduction. The other five mutated patients had an activity score of 1 and started with a 50 % dose reduction, as per the pharmacogenetic recommendation.

Table 5 compares the grades of toxicity and consequences of toxicity between the intervention and control group. Our aim was to show the impact of pharmacogenetic studies on the reduction of toxicity, comparing the incidence and severity of adverse events with those observed in a similar population that received the same treatment but was not subjected to pharmacogenetic studies. However, we found no differences in toxicity between the groups; for this reason, we did not fit a multivariate logistic model.

Table 6 shows the associations of the different polymorphisms with toxicity and its consequences in the intervention group.

Table 7 compares the toxicity between patients DPYD-mutated patients in whom the dose was adjusted before the start of treatment, and the control group (no genotyping).

Eleven carriers of *DPYD* decreased/no function variants experienced no or clinically tolerable toxicity during the first two cycles of treatment (with a reduced starting dose of fluoropyrimidine). For these patients, we increased the dose in subsequent cycles, as recommended in the clinical guidelines. A standard dose titration was used at the discretion of the oncologist, following clinical practice. With the increased doses, three participants (27.3 %) experienced toxicities of grade 3 or higher, two (18.2 %) were admitted

Table 4
Genetic variables and activity score in the intervention group.

Genotype	n	AS	Phenotype
c.1905+1G > A	0	–	–
c.1679T > G	0	–	–
c.2846A > T heterozygous	7	1.5	Intermediate metabolizer
c.2846A > T homozygous	2	1	Intermediate metabolizer
c.2846A > T/c.2194G > A compound heterozygous	1	1.5[a]	Intermediate metabolizer
c.1236G > A/HapB3 heterozygous	2	1.5	Intermediate metabolizer
DPYD c.1236G > A/HapB3 homozygous	3	1	Intermediate metabolizer
DPYD HapB3/c.2194G > A compound heterozygous	1	1.5[a]	Intermediate metabolizer

Abbreviations: AS: activity score.
[a] No dose adjustment recommendation for *c.2194G>A* as it is a variable under investigation. It was considered as wild-type *1.

Table 5
Comparison of toxicity and consequences of toxicity between the intervention and control groups.

Toxicity and consequences		Intervention n (%)	Control n (%)	P value
Toxicity	Yes	139 (98.6 %)	139 (96.5 %)	0.447[a]
Toxicity grade	1–2	75 (54.0 %)	84 (60.4 %)	0.275
	≥3	64 (46.0 %)	55 (39.6 %)	
Hospital admission	No	106 (75.2 %)	119 (82.1 %)	0.155
	Yes	35 (24.8 %)	26 (17.9 %)	
Dose reduction	No	100 (70.9 %)	100 (69.0 %)	0.718
	Yes	41 (29.1 %)	45 (31.0 %)	
Delay	No	83 (59.3 %)	88 (60.7 %)	0.809
	Yes	57 (40.7 %)	57 (39.3 %)	
Discontinuation	No	84 (60.0 %)	95 (65.5 %)	0.335
	Yes	57 (40.4 %)	50 (34.5 %)	
Reason for discontinuation	Unacceptable toxicity	31 (22.0 %)	22 (15.2 %)	–
	Progression	18 (12.8 %)	19 (13.1 %)	
	Change of regimen	5 (3.5 %)	0	
	Patient request	3 (2.1 %)	3 (2.1 %)	
	Not stated	0	2 (1.4 %)	

[a] Fisher's exact test.

Table 6
Association of polymorphisms with toxicity and its consequences in the intervention group.

Polymorphism	n	Toxicity grade		Consequences of drug toxicity			
		1/2	≥3	Hosp. adm.	Dose red.	Delay	Discontinuation
Wild-type	125 (100.0 %)	67 (53.6 %)	58 (46.4 %)	32 (25.6 %)	36 (28.8 %)	51 (40.8 %)	51 (40.8 %)
c.1905+1G > A	0	–	–	–	–	–	–
c.1679T > G	0	–	–	–	–	–	–
c.2846A > T heterozygous	7 (100.0 %)	5 (71.4 %)	2 (28.6 %)	2 (28.6 %)	2 (28.6 %)	2 (28.6 %)	3 (42.9 %) (2 unacceptable toxicity, 1 progression)
c.2846A > T homozygous	2 (100.0 %)	2 (100.0 %)	0	0	0	0	1 (50.0 %) (progression)
c.2846A > T compound heterozygous	1 (100.0 %)	0	1 (100.0 %)	0	1 (100.0 %)	1 (100.0 %)	1 (100.0 %) (unacceptable toxicity)
DPYD c.1236G > A/HapB3 heterozygous	2 (100.0 %)	2 (100.0 %)	0	0	0	1 (50.0 %)	0
DPYD c.1236G > A/HapB3 homozygous	3 (100.0 %)	2 (66.7 %)	1 (33.3 %)	0	1 (33.3 %)	1 (33.3 %)	1 (33.3 %) (progression)
DPYD c.1236G > A/HapB3 compound heterozygous	1 (100.0 %)	0	1 (100.0 %)	1 (100.0 %)	1 (100.0 %)	1 (100.0 %)	0

Abbreviations: Dose red., dose reduction; Hosp. adm., hospital admission.

to hospital, none died, four (36.4 %) did not tolerate treatment and had their dose reduced again, four (36.4 %) had dose delays, and five (45.4 %) discontinued treatment (due to unacceptable toxicity in three cases and cancer progression in two cases).

Six of the seven heterozygous *c.2846A > T* carriers (activity score 1.5) started treatment with a 50 % dose reduction (the other patient started with a 70 % reduction due to a medical error). For five of these patients, the dose was increased in subsequent cycles, up to a maximum of 75 % of the standard dose (in two cases). Of the seven patients in this group, only the two with 75 % doses experienced toxicities of grade 3 or higher (28.6 %, a lower frequency compared with the whole intervention group or the control group), and both had consequences of toxicity such as dose reductions, delays, and eventually discontinuation of treatment due to unacceptable toxicity. In addition, one of the two patients was admitted to hospital.

The two homozygous *c.2846A > T* carriers (activity score 1) started treatment with a 50 % dose reduction. One of the two had their dose increased from the fifth cycle to 75 % of the standard dose. Neither of them experienced toxicities of grade 3 or higher or any of the consequences of toxicity collected in our study, so the reduction was sufficient in these cases.

The two heterozygous *c.1236G > A/HapB3* carriers started treatment with a 50 % dose reduction. One patient had their dose increased in the third cycle and the other in the fourth cycle, up to a maximum of 75 % of the standard dose (still within the initial recommendation). Neither patient presented grade 3 toxicity or relevant events.

The three homozygous *c.1236G > A/HapB3* carriers started treatment with a 50 % dose reduction. Two of these patients had their dose increased to 75 %, and neither experienced toxicity of grade 3 or higher. In contrast, the third patient did not have their dose increased but did experience severe toxicity, leading to treatment delay.

One patient was a compound heterozygous carrier of *c.2846A > T/c.2194G > A*. They started treatment with a 50 % dose reduction, which was increased to 75 % of the standard dose in the third cycle (still within initial recommendation). The reduction was

insufficient in this case, because the patient experienced toxicities of grade 3 or higher, had their dose reduced, then had their treatment delayed, and finally had the drug discontinued owing to unacceptable toxicity. One patient was a compound heterozygous carrier of *c.1236G > A/HapB3/c.2194G > A*. Their initial dose was reduced by 30 % and was not increased in subsequent cycles. This person also experienced toxicity of grade 3 or higher, was admitted to hospital, had their dose reduced, and had treatment delayed; however, in this case, the drug was not discontinued. In these two compound heterozygotes, we followed the dosing recommendation for single heterozygotes because one of the variants was *c.2194G > A* (collected for the observational study), which is not one of the four main variants covered in the clinical guidelines.

In the five carriers of *DPYD* decreased/no function variants in which the reduced starting dose was not increased in subsequent cycles, toxicity of grade 3 or higher was 40.0 %, hospital admissions related to toxicity 20.0 %, dose reduction 20.0 %, dose delays 40.0 %, and 20.0 % discontinued treatment (1 cases due to cancer progression).

4. Discussion

To the best of our knowledge, this is the first ambispective, quasi-experimental study conducted in Spain that evaluates the clinical impact on toxicity of a *DPYD* genotyping protocol.

When the project began, *DPYD* testing was not implemented in daily clinical practice. Therefore, a fundamental part of the project involved implementing pharmacogenetics in the daily practice of the center through an effective and coordinated pathway between the departments involved. We standardized the pharmacogenetic reports with the corresponding dose adjustments and incorporated them into the computer program [11]. Our priority was to implement this process for all patients without delaying the start of treatment.

Despite the retrospective inclusion of our control participants, the two study groups had similar clinical and sociodemographic characteristics, with no significant differences in most of the variables collected, excepted sex and tumor type. The most common type of cancer in both groups was colorectal cancer. Regarding treatment, most people in both groups were chemotherapy-naïve (P = 0.678). The most commonly used fluoropyrimidine in both groups was capecitabine (P = 0.937). In general, fluoropyrimidines were used in combination with other drugs, with no significant differences between the groups. Among the participants receiving fluorouracil, most also received other antineoplastic drugs, and nobody was on monotherapy. In contrast, the two largest groups of people receiving capecitabine were either on monotherapy or also receiving oxaliplatin (with or without bevacizumab).However, the overall distribution of regimens is different between the two groups, which could influence the frequency and type of toxicities observed.

Regarding the results of genotyping, we detected *DPYD* variants in 16 patients (11.4 %). This proportion is much higher than in most previous studies (3 %–8 %) [1–4,8,34], but similar to the proportion observed in another Spanish study by Riera and colleagues (9.6 %) [35]. In a study conducted in France by Loriot and colleagues, up to 15 % of participants showed partial DPD deficiency [31]. No participants in our study had variants that caused complete DPD deficiency, in line with the current literature (0.01 %–0.5 %) [1–4,8,34].

In studies conducted in Europe [1,4], the most common variant is *DPYD c.1236G > A/HapB3*, with a frequency of 4.1 %–4.8 %, followed by *c.1905+1G > A* (1.0 %–1.2 %), *c.2846A > T* (0.8 %–1.4 %), and *c.1679T > G* (0.1 %). The frequency of *DPYD c.1236G > A/HapB3* was similar in our study (4.3 %), and no participants had *c.1905+1G > A* or *c.1679T > G*. However, the most common variant in our population was *c.2846A > T*, (7.1 %). In the Spanish study by Riera and colleagues [35], *c.1236G > A/HapB3* and *c.2846A > T* were also the most common variants.

For dosing, we followed the recommendations presented in clinical guidelines [1,3,4]. Five people (3.5 %) had an activity score of 1, so the oncology team reduced the starting dose by 50 %. Eleven people (7.8 %) had an activity score of 1.5. For these cases, the recommendation in the 2017 Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline was to reduce the starting dose by 25 %–50 % [1], whereas the 2018 update states: "*DPYD* Intermediate Metabolizers should receive a 50 % dose reduction from the full standard starting dose, whether the activity score is 1 or 1.5 followed by dose titration, based on clinical judgement and ideally therapeutic drug monitoring." This discrepancy affected only one patient (activity score 1.5), who started with a 30 % reduction in accordance with the 2017 guidelines. The team accepted our recommendation for 10 participants: nine began therapy with a 50 % reduction and one with a 30 % reduction. Due to a medical error, the remaining patient had their starting dose reduced by 70 % (this was corrected in subsequent cycles).

Our initial hypothesis was that implementing *DPYD* genotyping in routine clinical practice would reduce toxicity in people treated with fluoropyrimidines. However, we found no significant differences in the occurrence of toxicities and their consequences between the intervention and control group.

As shown in Table 7, lower incidence of severe toxicity is observed is observed in the DPYD-mutated patients in whom the dose was adjusted before the start of treatment, compared to the control group. The magnitude of the reduction is 6.6 %, which, although a small effect, may be clinically relevant because of the consequences for the patients, but no statistically significant differences are detected (p-value: 0.599).

Table 7
Comparison of toxicity between patients with DPD-deficiency and control group.

	DPD-deficiency	Control n (%)	P value
Total	16	145	0.599
Toxicity grade ≥ 3	5 (31.3 %)	55 (37.9 %)	

The CPIC guideline recommends that carriers of variants with reduced or no enzyme activity who experience no or clinically tolerable toxicity should have their dose increased by a dose titration, based on clinical judgement and ideally therapeutic drug monitoring to maintain efficacy [1,34]. In most *DPYD* variant carriers (n = 11, 68.8 %), the reduced starting dose was increased in subsequent cycles. Three patients had their dose increased in the second cycle: up to 75 % of the standard dose in two cases, and up to 50 % in one case (the person who had had their starting dose reduced by 70 % because of a medical error). The remaining eight participants had their dose increased from the third cycle, according to the recommendations.

Among the 11 patients who had their dose increased after the first two cycles, there was a lower frequency of grade 3 or higher toxicities (27.3 %) compared with the whole intervention group (46.0 %), as well as fewer hospital admissions related to toxicity and fewer treatment delays. However, they required more dose reductions compared with the intervention group (36.4 % versus 29.1 %) and were more likely to have the drug discontinued (45.5 %), due to unacceptable toxicity in more than half of the cases (60.0 %). When we compared carriers of decreased/no function *DPYD* variants in whom the reduced initial dose was increased in subsequent cycles versus carriers of *DPYD* decreased/no function variants without subsequent dose increases, we found a lower frequency of grade 3 or higher toxicities in those with dose increases (27.3 % versus 40.0 %). However, among participants without dose increases, there were fewer dose reductions (20.0 % versus 36.4 %) and fewer drug discontinuations (20.0 % versus 45.5 %), just one case and that was due to cancer progression. To establish concise recommendations regarding dose increases in *DPYD* variant carriers, there is a need for more studies with a larger sample size.

The main limitation of our study is the use of a retrospective cohort without genotyping as the control group. While we know how many people in the intervention group were variant carriers, we do not have this information for the control participants. Our analysis compared toxicity and other consequences in the intervention (testing) and control (no testing) arm. This is not the appropriate primary comparison. From a statistical point of view, the primary analysis should compare toxicity and other clinical consequences in variant carriers with (intervention group) versus without (control group) dose adjustment. However, this was not possible because patients treated in our hospital before we started this project (and before the recommendations of the Spanish health authorities) did not receive genotyping, so we were unable to select a retrospective control group of variant carriers. It would have been unethical to evaluate toxicity in a prospective control group (genotyping) without the corresponding dose adjustments, as this would have gone against the recommendations of the Spanish health authorities and current clinical guidelines. The risk of severe or even fatal drug toxicity in people with DPD deficiency precluded a fully prospective study, which would not have been approved by the hospital ethics committee. The ambispective study design may have led to differences between the two groups. We found no significant differences in most of the variables measured, but the overall distribution of regimens was different, which could influence the frequency and type of toxicities observed. In addition, retrospective studies carry the risk of missing data.

Another limitation is that our study was conducted in a single center with relatively few participants. Because of the low prevalence of polymorphisms leading to dose adjustment in the intervention group, our analysis had low statistical power. Multicenter studies with larger sample sizes could draw more solid conclusions.

Our study focused on toxicity and its consequences. We did not assess survival or efficacy outcomes owing to the wide variety of tumor types, treatment regimens, and stages, as well as the small sample size.

In addition to the four main *DPYD* variants, there are other less common variants and other factors that could influence fluoropyrimidine toxicity. However, a lack of evidence on the clinical effects and cost-effectiveness of typing these variants limits the implementation of this intervention in clinical practice [4].

Our study demonstrates the feasibility of implementing prospective genotyping in routine clinical practice in a tertiary hospital even if it has no pharmacogenetics unit, as it is possible to outsource pharmacogenetic testing without delaying treatment. We also showed it is possible to implement this intervention for all patients before beginning treatment with the corresponding dose adjustment based on genotyping. These results, together with the high degree of acceptance by the oncology department, demonstrate the strong coordination and collaboration between the departments involved, with an efficient pathway and short response times. In our study, the hospital pharmacist played a key role in coordinating the implementation of pharmacogenetic testing in routine clinical practice.

The prevalence of *DPYD* variants with reduced DPD activity detected in our study was higher than in most of the published literature. Although we were unable to demonstrate a reduction in severe toxicity in the intervention group after *DPYD* genotyping compared to the control group, a reduction in toxicity was observed in the subgroup of patients with *DPYD* mutations whose doses were adjusted prior to treatment initiation, compared to the control group, although this difference was not statistically significant. The lack of statistically significant results may be due to several factors, including the small sample size and retrospective control group.

In conclusion, our study demonstrates successful implementation of prospective genotyping in routine practice. In our case, to support routine testing and preemptive dosing strategies, a study with a larger sample size and a control group undergoing *DPYD* genotyping is necessary.

CRediT authorship contribution statement

Ana Hernández-Guío: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Investigation, Data curation, Conceptualization. **Miguel Ángel Calleja-Hernández:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition. **Andrés Corno-Caparrós:** Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Marta Zayas-Soriano:** Visualization, Formal analysis, Data curation, Conceptualization. **Mª Ángeles Bernabéu-Martínez:** Writing – review & editing, Formal analysis, Data curation. **Fernando Gutiérrez-Nicolás:**

Writing – review & editing, Validation, Supervision, Project administration, Formal analysis, Conceptualization.

Data availability statement

The data associated with the study have not been deposited in a publicly available repository because they are confidential. However, these data are available upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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