Article

Novel Genetic Variants Explaining Severe Adverse Drug Events after Clinical Implementation of DPYD Genotype-Guided Therapy with Fluoropyrimidines

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Novel genetic variants explaining severe adverse drug events after Clinical implementation of *DPYD* genotype-guided therapy with fluoropyrimidines

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Abstract: Fluoropyrimidines (FPs) are commonly prescribed in many cancer streams. The *DPYD**2A (rs3918290), *13 (rs55886062), *HapB3 (rs56038477), alleles, and *DPYD* rs67376798, had shown the highest level of evidence about their association with FPs response. The EMA and FDA-approved drug labels for FPs, recommend genotyping these variants before treatment starts. We implemented the *DPYD* genotyping in our daily clinical routine, but we still find patients showing severe adverse drug events (ADEs) to FPs. We studied among these patients the association with the response to FPs of *DPYD* rs1801265, rs17376848, rs1801159, rs1801160, rs1801158, and rs2297595; as explanatory candidates of the interindividual differences for the FP-related toxicities. We also studied the impact of *DPYD**2A, *13, *HapB3, and rs67376798 genotype for FP dose tailoring in our clinical practice and characterized the *DPYD* gene in our population. We found that FP dose lowering based on *DPYD* genotype does not affect the treatment efficacy. Also, the *DPYD**4 (defined by rs1801158) allele was associated with a higher risk of ADEs (severity grade ≥ 3) in both the univariate (O.R.= 5.66; 95% C.I.= 1.35 – 23.67; p= 0.014) and multivariate analyses (O.R.= 5.73; 95% C.I.= 1.41 – 28.77; p= 0.019). This makes it a candidate variant for implementation in clinical practice.

Keywords: Fluoropyrimidines; DPYD; Personalized medicine; Pharmacogenetics; clinical implementation

1. Introduction

Fluorouracil and its oral prodrug capecitabine, both fluoropyrimidines (FPs), are anticancer drugs used in the treatment of many solid tumors such as breast and gastric, but especially in colorectal cancer. Despite the wide use of these drugs, among FP-treated patients with standard doses, up to 30% have severe (grade ≥ 3) treatment-related toxicity [1,2], which can lead to treatment-related death in up to 1% of patients [2–4]. The most common severe treatment-related toxicity includes diarrhea, oropharyngeal mucositis, hand-foot syndrome, and myelosuppression [2,5].

Fluorouracil is administered intravenously while capecitabine is an oral prodrug metabolized by carboxylesterase, cytidine deaminase, and thymidine phosphorylase resulting respectively in 5'-deoxy-5-fluorocytidin (5'dFCR), 5'-deoxy-5-fluorouridine (5'dFUR), and finally 5-Fluorouracil (5-FU) [6] (Figure 1). After this, around 80% of fluorouracil is inactivated, and the remaining 20% is

converted into cytotoxic metabolites or excreted in the urine [5]. FPs are catabolized by different enzymes leading to their inactivation and the dihydropyrimidine dehydrogenase (DPD) is the main enzyme responsible for this [7].
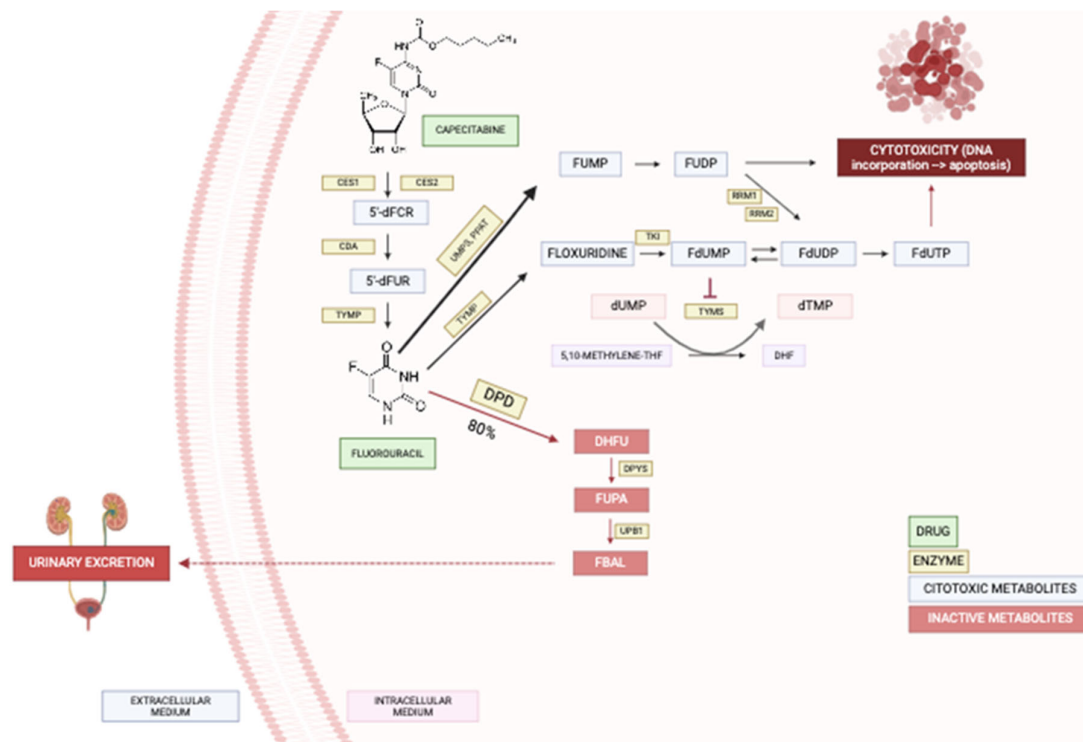
Figure 1. Capecitabine/Fluorouracil Pathway.

Patients with reduced DPD activity catabolize less fluorouracil, leading to more than 20% of cytotoxic/therapeutic metabolites, thus increasing the risk of supratherapeutic drug availability toxicity. In European and North American patients, reduced and complete lack of DPD activity are present in 3-5% and 0.01-0.1%, respectively [2].

1.1. The dihydropyrimidine dehydrogenase gene (DPYD)

Many genetic variants in the gene encoding the DPD enzyme (*DPYD* gene) partially explained the interindividual differences in the toxicity of FPs. The most relevant genetic variants in this regard are the *DPYD* rs67376798 (c.2846A>T, D949V), the rs3918290 (c.1905+1G>A, IVS14+1G>A) defining the *DPYD**2A allele, the rs55886062 (c.1679T>G, I560S) defining the *DPYD**13, and the rs75017182 (c.1129-5923C>G) and rs56038477 (c.1236G>A, E412E) characterizing the *DPYD**haplotype (Hap) B3. All of them have been associated with a reduction in DPD activity, resulting in higher concentrations of fluorouracil cytotoxic metabolites and an increased risk of severe or fatal toxicities.

The *DPYD**2A (rs3918290) was the first allele known to have a functional impact on DPD activity [8,9]. It was stated that homozygous carriers of this variant have a complete loss of function of DPD while heterozygous carriers showed a significant reduction of mean DPD activity [9,10]. Several studies reported a significant association of this allele with the toxicity of FPs, even, the FPs dose tailoring based on *DPYD**2A genotype was demonstrated to be an effective strategy for the prevention of severe or fatal toxicity events [11]. In this study, Deene *et al.* showed in a cohort of n=1631 patients that a 50% dose reduction in *DPYD**2A (rs3918290) carriers may decrease the FP-related toxicity from 73% to 28% (p<0.001). Thereafter, when studied *DPYD**2A (defined by rs3918290) combined with *DPYD**13 (defined by rs55886062), *DPYD**Hap B3 (defined by rs56038477), and rs67376798, it was found a 25.6-fold increased risk of death in FP treated patients [12], and a systematic review and meta-analysis including n=7365 patients concluded that *DPYD* genotyping for

these four *DPYD* variants will identify a significant ratio of patients who are DPD deficient [13]. Finally, Henricks L.M. *et al.* [2] found the prospective *DPYD* genotyping feasible in routine clinical practice, and dose reductions based on *DPYD* genotype improved patient safety of FP-treated patients. In this prospective multicenter study, they tailored the FP treatment dose based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477) and rs67376798 in n=1103 patients. *DPYD**2A and *13 carriers received a 50% dose reduction while rs67376798 and *DPYD**HapB3 carriers received a reduced dose of 25%. Results indicated that FP-related severe toxicity (grade ≥ 3) was more prevalent among *DPYD* variant carriers compared to wild-type patients (p=0.0013), and a significantly lower relative risk for severe FP-related toxicity for *DPYD* genotype-guided dosing compared with historical cohorts for each genetic variant carrier.

On the other hand, *DPYD* rs3918290, rs55886062, and rs56038477, characterizing the *DPYD**2A, *13, and *HapB3 alleles, respectively, and rs67376798, are not the only *DPYD* variants associated with the toxicity of FPs. There are many others that showed the highest level of evidence according to the PharmGKB [14] on this association (Table 1).

Table 1. *DPYD* variants associated with any fluoropyrimidines phenotype with level of evidence 1A (Obtained from PharmGKB [14]).

*allele	DPYD Variant	Major Nucleotide Variation	MAF			Molecules	Toxicity
			Ibs	Europe	Global		
-	rs115232898	c.557A>G	100/0	100/0	99/1	fluorouracil	x
-	rs148994843	c.1543G>A	No data	100/0	100/0	fluorouracil	
-	rs17376848	c.1896T>C	98/2	96/4	94/5	capecitabine / fluorouracil	x
*4	rs1801158	c.1601G>A	94/6	97/3	99/1	capecitabine / fluorouracil	x
*5	rs1801159	c.1627A>G	79/21	81/19	82/18	capecitabine / fluorouracil	x
*6	rs1801160	c.2194G>A	95/5	95/5	96/4	capecitabine / fluorouracil	x
*9A	rs1801265	c.85T>C	79/21	79/21	74/26	capecitabine / fluorouracil	x
*8	rs1801266	c.703C>T	No data	100/0	100/0	fluorouracil	
*10	rs1801268	c.2983G>T	No data	100/0	100/0	fluorouracil	
-	rs2297595	c.496A>G	88/12	88/12	94/6	capecitabine / fluorouracil	x
*2A	rs3918290	c.1905+1G>A	100/0	99/1	99/1	capecitabine / fluorouracil / tegafur	x
*13	rs55886062	c.1679T>G	100/0	100/0	100/0	capecitabine / fluorouracil / tegafur	x
-	rs56005131	c.2303C>A	100/0	100/0	100/0	fluorouracil	
*HapB3	rs56038477	c.1236G>A	98/2	98/2	99/1	capecitabine / fluorouracil	x
-	rs59086055	c.1774C>T	100/0	100/0	100/0	fluorouracil	x

		c.2846A>T rs67376798	100/0	99/1	99/1	capecitabine / fluorouracil / tegafur	x
*3	rs72549303	c.1898del	No data	100/0	100/0	fluorouracil	
*11	rs72549306	c.1003G>T	No data	100/0	100/0	fluorouracil	
*7	rs72549309	c.299_302del	No data	100/0	100/0	fluorouracil	
*HapB3	rs75017182	c.1129-5923C>G	98/2	98/2	99/1	capecitabine / fluorouracil	x
*12	rs78060119	c.1156G>T	No data	100/0	100/0	fluorouracil	x
MAF: Minor Allele Frequency; Ibs: Iberian peninsula; *MAFs obtained from the 1000 Genomes Project [15].							

1.2. Pharmacogenetics of fluoropyrimidines

All the evidence regarding the association of *DPYD* variants with FP-related toxicity, the reduction of toxicity with dose tailoring based on *DPYD* genotype, and the clinical impact of this practice, has led to updates in the drug labels approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA), incorporating this information, as well as the development of pharmacogenetic (PGx) dosing guidelines for these drugs.

The European Public Assessment Report (EPAR) [16] for capecitabine by the EMA states that capecitabine is contraindicated in patients with no DPD activity and recommends a reduction in starting dose in reduced DPD activity patients to avoid serious toxicity. This EPAR also reports that the reduced DPD activity may be stated considering the *DPYD* genotype and highlights the *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 (also known as c.2846A>T), as main responsible of DPD reduced/complete absence activity but considering that there may be other *DPYD* variants associated with an increased risk of life-threatening toxicity. The FDA table of PGx [17] includes information about the capecitabine and fluorouracil/*DPYD* drug-gene interactions reporting that genotype-translated DPD intermediate or poor metabolizer (IM or PM) phenotypes result in higher severe or life-threatening risk toxicities. It considers that there is no dosage safe in PMs and recommends withholding or discontinuing in the presence of early-onset or unusually severe toxicity.

Carrying these *DPYD* variants alone or combined results in different translated phenotypes for DPD activity, thus different dosing recommendations. As commented above, there are available PGx dosing guidelines including this information. Both, the Clinical Pharmacogenetics Implementation Consortium (CPIC) [18], and Dutch Pharmacogenomics Working Group (DPWG) guidelines [19] categorize patients' *DPYD* gene activity score (GAS) depending on *DPYD* genotype as shown in Table 2. Furthermore, CPIC guidelines refer to the DPD metabolizing status and categorize patients in normal metabolizers (NM), not carrying any of these variants (GAS=2), IM if *DPYD* GAS= 1 or 1.5, and PM when the GAS is lower than 1.

Table 2. Phenotype translation from considered allele combinations for fluoropyrimidines dose tailoring.

DPYD Allele	wildtype	*DPYD**2A	*DPYD**13	*DPYD**HapB3	c.2846A>T
wildtype	GAS = 2	GAS = 1	GAS = 1	GAS = 1.5	GAS = 1.5
DPYD*2A		GAS = 0	GAS = 0	GAS = 0.5	GAS = 0.5
DPYD*13			GAS = 0	GAS = 0.5	GAS = 0.5
DPYD*HapB3				GAS = 1	GAS = 1
c.2846A>T					GAS = 1

> *DPYD**2A defined by rs3918290; c.2846A>T defined by rs67376798; *DPYD**13 defined by rs55886062; *DPYD**HapB3 defined by rs56038477; GAS: Gene (*DPYD*) Activity Score

Also, both the DPWG and CPIC guidelines (Table 3), recommend an alternative drug, if possible, in patients with *DPYD* GAS lower than 1 (DPD PMs, in CPIC guidelines), and start with 50% of the standard dose in patients with GAS of 1 or 1.5 (DPD IMs). Also, the CPIC guideline reports that IM patients carrying the c.[2846A>T]/[2846A>T] genotype (GAS=1) may require a dose reduction higher than 50%. The main difference between CPIC and DPWG guidelines is the *DPYD* alleles considered for analysis. While the DPWG considers only the *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 (also known as c.2846A>T), the CPIC guideline for FPs and *DPYD* provides a list with n=83 variants resulting in diplotypes translated into IM or PM (GAS<2) phenotypes, thus in DPD reduced activity and FPs dose tailoring.

Table 3. CPIC and DPWG PGx guidelines recommendations for *DPYD*/Fluoropyrimidines drug-gene interactions.

PGx guideline	*DPYD* Variant	Phenotype	Recommendation
CPIC	*2A c.2846A>T *13 *HapB3 Others^	GAS = 2 (NM)	Use the standard dose
		GAS = 1.5 (IM)	50% dose reduction followed by dose titration, based on clinical judgment and ideally therapeutic drug monitoring
		GAS = 1 (IM)	50% dose reduction followed by dose titration, based on clinical judgment and ideally therapeutic drug monitoring. In homozygous for c.2846A>T a dose reduction of more than 50% may be required
		GAS = 0.5 (PM)	Alternative drug. If no other therapeutic option, strongly reduce the dose with early therapeutic drug monitoring
		GAS = 0 (PM)	Alternative drug
DPWG	*2A c.2846A>T *13 *HapB3	GAS = 2	Use the standard dose
		GAS = 1.5	Start with 50% of the standard dose or avoid fluorouracil and capecitabine. Adjust the subsequent doses guided by toxicity and effectiveness
		GAS = 1	Start with 50% of the standard dose or avoid fluorouracil and capecitabine. Adjust the subsequent doses guided by toxicity and effectiveness
		GAS = 0	Avoid fluorouracil and capecitabine. If not possible, Determine DPD activity and adjust the dose

PGx: Pharmacogenetic; CPIC: Clinical Pharmacogenetics Implementation Consortium; DPWG: Dutch Pharmacogenomics Working Group; GAS: Gene Activity Score; NM: Normal metabolizer; IM: Intermediate metabolizer; PM: Poor metabolizer. ^See genetic variants in Supplementary Table S1 of the CPIC Guideline for Fluoropyrimidines and DPYD (18).

In Spain, the Spanish Agency for Medicine and Health Products (*Agencia Española del Medicamento y Productos Sanitarios*, AEMPS), Spanish Society of Pharmacogenetics and Pharmacogenomics (*Sociedad Española de Farmacogenética y Farmacogenómica,* SEFF), and Spanish Society of Medical Oncology (*Sociedad Española de Oncología Médica*, SEOM), recommend genotyping the *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 (also known as c.2846A>T), prior to FP treatment, avoid its use in DPD PM patients (GAS<1), and a 50% dose reduction in DPD IM patients (1<GAS<2).

Since 2021 we have implemented in our hospital the PGx test of *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 (also known as c.2846A>T), before capecitabine and fluorouracil treatment initiation and the dose tailoring based on guidelines by the DPWG. Despite this implementation in clinical practice and its acceptance by sanitary authorities and physicians in daily routine, we still have patients experiencing severe or life-threatening FP-related toxicities.

1.3. Hypothesis and Objectives

The EMA, AEMPS, SEFF, SEOM, and the drug label for capecitabine and 5-FU, recommend *DPYD* genotyping to state the DPD metabolizing status before the treatment starts with these drugs. Besides, there are available dosing guidelines based on PGx information as those from the DPWG [19] and CPIC [18].

We have implemented in our daily clinical practice the *DPYD* genotyping before the treatment starts with FPs. Despite this, many patients still show severe adverse drug events.

With this study, we aim to state the impact in our clinical routine of capecitabine and 5-FU dose tailoring based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 (also known as c.2846A>T) studying its association with the treatment efficacy and toxicity.

Also, we aim to explain the toxicity events in the cohort of patients receiving the FP treatment tailored by *DPYD* genotype. In this regard, we studied the association with the FP toxicity and efficacy of other relevant *DPYD* variants, previously associated with FP response, but not recommended by the scientific societies and sanitary authorities.

Finally, we aimed to characterize in our population the *DPYD* gene, studying its genotypes and phenotypes distribution, the minor allele frequency (MAF), Hardy-Weinberg (H-W) equilibrium of included variants, and possible linkage disequilibrium (LD).

2. Materials and Methods

2.1. Study design

Observational retrospective study including patients treated with capecitabine or fluorouracil and tested for *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 (also known as c.2846A>T) before treatment start in our hospital between Mar/01/2021 and Dec/31/2021.

The inclusion criteria were, first, patients diagnosed with cancer and prescribed capecitabine or fluorouracil; tested for *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798; capecitabine or fluorouracil dose tailored based on DPWG guidelines; with available 6-month follow-up period based on medical records, and non-previously treated with FPs. Patients not signing the informed consent or asking for the withdrawal of the study, would be excluded.

The Research Ethics Committee of Granada approved the study (Code: 1605-N_22; Date of approval: Sep/14/2022) and the principles of the Declaration of Helsinki were followed.

The main endpoints were the toxicity and efficacy of capecitabine or 5-FU. This data was obtained from electronic medical records and confirmed by physicians in case of discrepancies.

The toxicity endpoint was adverse drug events (ADEs) to capecitabine or 5-FU. The causality and severity of ADEs were stated using the Liverpool Causality Assessment Tool (LCAT) [20], and

Common Terminology Criteria for Adverse Events (CTCAE) [21] respectively. ADEs categorized as probable or definite showing a severity grade 3 or higher were considered for the study only.

The efficacy endpoint was achieved if patients received a positive clinical assessment by the oncologist regarding the progression of the illness recorded in the medical records, and the non-discontinuation of FP treatment during follow-up if it was not because of an ADE. For the positive clinical assessment by the oncologists in metastatic patients it was used The Response Evaluation Criteria in Solid Tumors (RECIST 1.1) within 6 months of initiating therapy [22]. Radiological and imaging studies, such as Computed Tomography (CT), were employed to monitor the evolution of both target and non-target lesions in treated patients. These assessments were conducted every 3 to 4 months (1-2 times during follow-up), unless patients exhibited clinical signs of progression earlier (e.g., pain, dyspnea, sweating, elevated tumor markers in blood tests).

2.2. Procedures for the inclusion of DPYD variants in the study

To include candidate *DPYD* variants as explanatory factors of remaining toxicity events in our study population, receiving dose-tailored FP treatment based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798, we searched in PharmGKB [14] for genetic variants in *DPYD* reported as clinical annotations with the highest level of evidence (1A) about their association with any phenotype to capecitabine, 5-FU or tegafur (Table 1). We included those genetic variants related to the toxicity of these drugs, with MAF higher than 1% in the Iberian Peninsula population according to the 1000 Genomes project [15].

2.3. Management of patients

As part of the clinical practice at our hospital, whenever a doctor considers prescribing capecitabine or 5-FU in patients diagnosed with whatever cancer, they may request the *DPYD* PGx test from the Hospital Pharmacy.

Once the request for the test is received, a nurse takes a saliva sample with sterile cotton swabs, the DNA is extracted, and *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 tested in less than 48h from the saliva sample collection. The pharmacists upload in the electronic medical records a PGx report including the therapeutic recommendation translated of the PGx result within 72h of sample collection.

The genotype-phenotype-therapeutic recommendation translation process (dose tailoring) is performed following the instructions by the DPWG guidelines and shown in Tables 2 and 3. This means patients not carrying any of the tested variants are assigned a *DPYD* GAS=2 and treated with the standard dose. Those carrying a single mutated allele are assigned GAS=1-1.5 and start the treatment with a 50% reduction of the standard dose. Patients with two mutated alleles are assigned GAS=0 and the treatment is switched.

The remaining DNA and saliva samples are stored as a private biosamples collection registered with the Carlos III Health Institute (C.0007322). Once the follow-up of patients finished, we retrospectively tested, using this remaining DNA, the *DPYD* variants meeting the criteria explained in "2.2 Procedures for the inclusion of genetic variants in the study".

Those patients not treated with capecitabine or fluorouracil after the PGx test were excluded from the analysis. Included patients were followed up for six months.

2.4. Data Management, Statistical Analysis, and Genotyping

First, a descriptive analysis of the population included in the study was performed. The impact and usefulness of the *DPYD* PGx test for capecitabine and 5-FU dose tailoring in our clinical practice was assessed by studying the association with the toxicity and efficacy endpoints of the dose tailoring based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798.

After that, to explain ADEs that occurred during follow-up in patients receiving an FP-*DPYD*-dose tailored treatment, we performed a genotype association study with the toxicity and efficacy endpoint.

Moreover, we carried out a multivariate analysis to discard possible confounding factors on the association of genetic variants with the toxicity and efficacy endpoints.

Finally, we characterized the *DPYD* variants in our population, including both those used in our daily clinical practice and those studied for their association with the toxicity endpoint after PGx FPs dose tailoring. In this regard, the distribution (number, n; and percentage, %) of genotypes, phenotypes, and MAFs were calculated, a LD analysis was carried out and the H-W equilibrium tested.

The descriptive analysis, MAFs, genotype/phenotype distribution, and multivariate analysis were conducted using R commander. For the association studies of genetic variants with the endpoints, LD analysis, and H–W equilibrium analyses we used the SNPstats online tool [23].

For the multivariate analysis, we considered for inclusion all the study variables recorded, including clinical parameters, genetic variants, and concomitant treatments. We build different multivariate models using the backward, forward, and stepwise methods for the association with the efficacy and toxicity endpoints. These models were upgraded and compared using the Akaike Information Criterion (AIC). The final model showing the lower AIC was chosen.
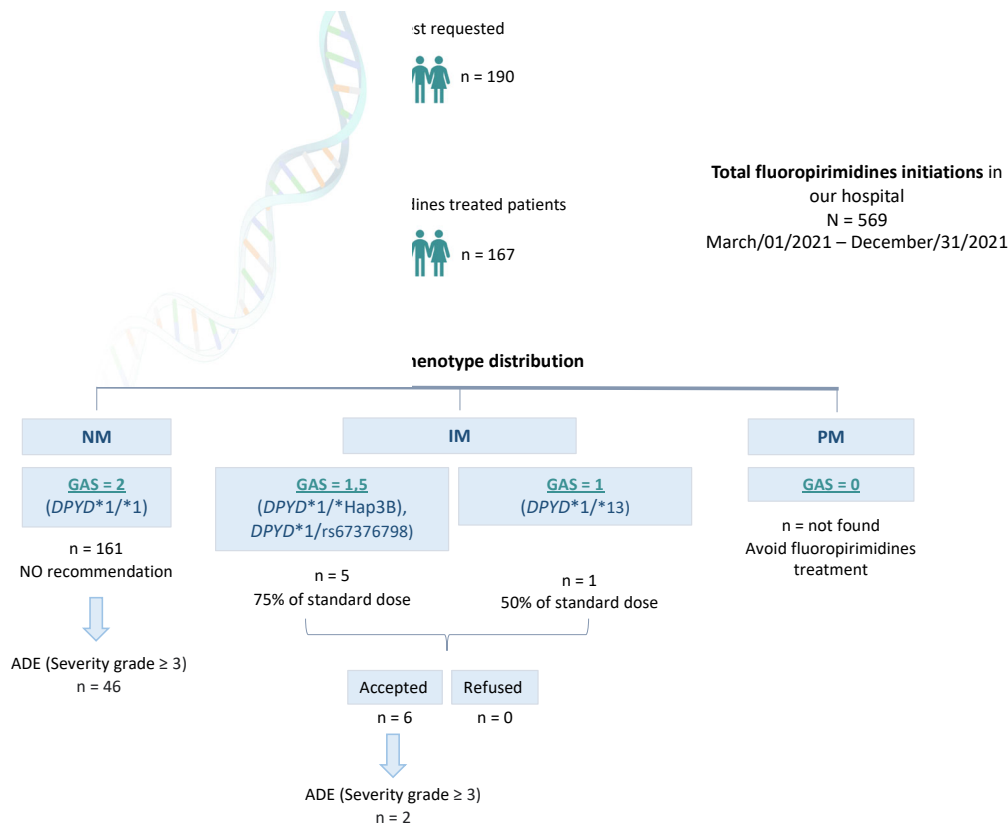
The Chi-square test or Fisher exact test was used, and odds ratios (OR) and p-values calculated considering $p<0.05$ statistically significant.

For genotyping, DNA was isolated from saliva samples using standard procedures. DNA extraction was carried out following the method by Freeman et al. [24], a non-organic (proteinase K and salting out) protocol including modifications from the method described by Gomez-Martín A. et al. [25]. The included genetic variants were genotyped using Taqman assay technology (Thermo Fisher Scientific, Waltham, MA, USA) and analyzed with QuantStudio 12K Flex de Applied Biosystems (California, USA).

3. Results

Between Mar/01/2021 and Dec/31/2021, N=569 patients were prescribed Capecitabine or 5-FU in *Hospital Universitario Clínico San Cecilio* (Granada, Spain). In total, n=190 *DPYD* PGx tests were requested, and n=167 patients were finally treated with FPs. This means n=402 patients were prescribed capecitabine or 5-FU in our Hospital but not *DPYD* tested, and n=23 were *DPYD* tested but not treated with FPs.

Among n=167 *DPYD*-tested patients finally treated with capecitabine or 5-FU we found n=161 *DPYD**1/*1 (wildtype) patients, translated into GAS=2 phenotype receiving normal doses of FPs. We also found n=1 *DPYD**1/*13, n=3 *1/*HapB3, and n=2 *1/c.2846A>T genotypes, translated into n=5 GAS=1.5, and n=1 GAS=1, who were recommended to be treated with the 50% of the standard dose. All these patients were dose-adjusted based on our recommendation (Figure 2).

st requested

n = 190

lines treated patients

n = 167

Total fluoropirimidines initiations in
our hospital
N = 569
March/01/2021 – December/31/2021

enotype distribution

NM	IM		PM
GAS = 2 (*DPYD**1/*1)	**GAS = 1,5** (*DPYD**1/*Hap3B), *DPYD**1/rs67376798)	**GAS = 1** (*DPYD**1/*13)	**GAS = 0**

n = 161
NO recommendation

n = 5
75% of standard dose

n = 1
50% of standard dose

n = not found
Avoid fluoropirimidines
treatment

ADE (Severity grade ≥ 3)
n = 46

Accepted	Refused
n = 6	n = 0

ADE (Severity grade ≥ 3)
n = 2

Figure 2. Recruitment and management of patients.

Among those n=23 non-treated patients we found n=21 *DPYD**1/*1 (wildtype), and n=2 *1/*HapB3.

All the n=167 FP treated patients after dose tailoring based on *DPYD* genotyping were prescribed because of digestive tumors including colon (n=81), stomach (n=13), pancreas (n=10), duodenum (n=1), esophageal (n=1), and rectal cancer (n=45); breast cancer (n=12), and other kind of tumors (n=4). All the patients received capecitabine or 5-FU as a first-line treatment except n=12 breast cancer patients who were prescribed after cytotoxic chemotherapy failure and locally advanced tumor or metastasis. The mean age was 64.26 ± 10.89 years old, and 37.13 % women (Table 4).

Table 4. Baseline characteristics of fluoropyrimidine-treated patients.

Parameter	N = 167 n (%) or mean ± sd
Women	62 (37.13)
Age	64.26 ± 10.89
BMI	26.69 ± 4.92
BS	1.80 ± 0.19
Ethnicity (European)	167 (100)
Tumor Location	
Colorectal	126 (75.45)
Gastric	13 (7.78)
Pancreas	10 (5.99)
Breast	12 (7.19)
Others	6 (3.59)
Tumor stage	

I	3 (1.80)
II	29 (17.36)
III	64 (38.32)
IV	71 (42.51)
Chemotherapy treatment	
Capecitabine (monotherapy)	72 (43.11)
XELOX	39 (23.35)
FOLFOX	40 (23.95)
FLOT	6 (3.59)
FOLFIRINOX	10 (5.99)
Initial doses (1000mg/m2)	
100	143 (85.63)
90	2 (1.20)
85	2 (1.20)
80	11 (6.59)
75	3 (1.80)
50	6 (3.59)
Associated antibody	16 (9.58)
Toxicity endpoint	48 (28.74)
Efficacy endpoint	127 (71.86)
DPYD **genotype**	
DPYD*1/*1	161 (96.40)
DPYD*1/*HapB3	3 (1.80)
DPYD*1/rs67376798	2 (1.20)
DPYD*1/*13	1 (0.60)
DPD phenotype	
NM (GAS: 2)	161 (96.40)
IM (GAS: 1.5 or GAS:1)	6 (3.60)
PM (GAS: 0)	0 (0.00)
BMI: Body Mass Index; BS: Body Surface; XELOX: Capecitabine and oxaliplatin; FOLFOX: 5-fluorouracil, leucovorin and oxaliplatin; FLOT: 5-fluorouracil, leucovorin, oxaliplatin and docetaxel; FOLFIRINOX: 5-fluorouracil, leucovorin, irinotecan and oxaliplatin; NM: Normal Metabolizer; IM: Intermediate Metabolizer; PM: Poor Metabolizer; GAS: Gen Activity Score.	

3.1. Association study of DPYD variants with the response

3.1.1. Association with response of dose tailoring based on DPYD.

We found no association between *DPYD* variants used for FP dose tailoring and the toxicity endpoint. We neither found an association with the efficacy endpoint. This means that carrying a genotype translated into GAS lower than 2 (DPD IM or PM) and receiving adjusted doses is not related to variable response to FPs.

3.1.2. Association with the response of new DPYD variants

Among treated patients based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798, we still found n=47 patients meeting the toxicity endpoint.

As commented above we retrospectively studied n=6 *DPYD* variants that had been related to the toxicity of FPs, and with MAF higher than 1% in the Iberian Peninsula population according to the 1000 Genomes project.

We found that carrying the *DPYD* rs1801158 is associated with a higher risk of ADEs with severity grade ≥ 3 (OR=5.66; 95% C.I.=1.35–23.67; p=0.014).   No other of those *DPYD* variants was associated with the toxicity endpoint. We neither found an association of any of the included SNPs with the efficacy endpoint.

3.1.3. Multivariate analysis

The following variables were included as possible explanatory parameters of the efficacy and toxicity endpoints in the multivariate analysis: clinical variables including age, sex, and body surface area; concomitant drugs in the therapeutic scheme including monoclonal antibodies, oxaliplatin, irinotecan, and radiotherapy; and all the *DPYD* genetic variants included in the study, those used and non-used for FPs dose tailoring.

For the multivariate analysis to explain the toxicity endpoint the model using the stepwise (backward/forward) method showed the lower AIC, and finally included. After adjustment, the model showed that the *DPYD* rs1801158 is associated with ADEs (severity grade ≥ 3) to FPs (OR=5.73; 95% CI=1.41–28.77; p=0.019) in *DPYD* dose tailored patients based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798.

In the multivariate analysis to explain the efficacy endpoint we found that the concomitant treatment with irinotecan is associated with lower rates of efficacy (p= <0.001).

3.2. DPYD characterization.

In total, n=190 patients were requested to be tested and dose-tailored based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798.   Among them, n=189 were tested for *DPYD* variants considered candidates to explain differences in the response to FPs. This means that n=1 patient could not be tested for these variants because we did not have enough stored DNA.

Among all the tested variants in our population, we found no differences with the MAF for the Iberian Peninsula population reported by the 1000 Genomes Project [15], and all the SNPs were in the Hardy-Weinberg equilibrium.

We found many significant differences in the LD analysis. The *DPYD* rs1801265 was linked to the rs56038477 (r=0.214; p<0.001) and the rs2297595 (r=0.604; p<0.001) in our population. More in detail, we found that all the patients carrying the rs56038477 also carried the rs1801265, and n=31 (83.8%) of patients carrying the rs2297595 also carried the rs1801265. We found other p-values lower than 0.05 in the LD analyses but showing r values close to r=1.

4. Discussion

FPs, including 5-FU and the oral prodrug capecitabine, are commonly prescribed antimetabolite chemotherapies utilized across many cancer streams.

Among FP-treated patients with standard doses, up to 30% show severe (grade ≥ 3) treatment-related toxicity. Many genetic variants in the *DPYD* gene encoding the DPD enzyme partially explained these toxicities. The *DPYD**2A (defined by rs3918290), *13 (defined by rs55886062), *HapB3 (defined by rs56038477), alleles, and *DPYD* rs67376798, had shown the highest level of evidence about their association with FPs response. The EMA and FDA-approved drug labels for FPs, and the SEFF and SEOM in Spain, recommend genotyping these variants before treatment starts.

Depending on *DPYD* genotype patients may be categorized as DPD NM, IM, or PM and receive a PGx dose-tailored treatment (50% of standard doses in DPD IM patients, and alternative therapies in DPD PMs).

Moreover, different studies concluded that dose adjustments based on *DPYD* genotype don´t influence the treatment efficacy. Deenen M.J. *et al.* did not find a relationship between *DPYD* variants and progression-free survival or overall survival despite a 50% dose reduction in *DPYD**2A carriers [26], and Lam S.W. *et al.* [27] observed no differences in response in seven more clinical studies

examining *DPYD* polymorphisms with a dose reduction, time to progression, progression-free survival, and/or overall survival, concluding that there is no evidence that a priori dose adjustments for *DPYD* carriers decreases FP efficacy, and low-activity variant carriers treated with standard of care appear to have similar efficacy once an acceptable dose is found.

We implemented the *DPYD* genotyping in our daily clinical routine, but we still find patients showing severe toxicities to FPs. Thus, we hypothesized that there might be other variants influencing the FP-related toxicities.

We found six *DPYD* variants (rs1801265, rs17376848, rs1801159, rs1801160, rs1801158, and rs2297595) as explanatory candidates of the interindividual differences for the FP-related toxicities since these had been related to the toxicity of FPs with the highest level of evidence, and they have a MAF higher than 1% in the Iberian Peninsula population.

In this study, we assessed the association with response to FPs of these novel candidate variants to explain suboptimal patient response for the first time in a cohort that received FP treatment based on the *DPYD**2A (defined by rs3918290), *13 (defined by rs55886062), *HapB3 (defined by rs56038477), and rs67376798.

This way, we could determine whether these new *DPYD* variants explain the remaining toxicities despite a PGx dose-tailored treatment and would be potentially useful in daily clinical practice.

This study may also be used as a guide for the clinical implementation of the FP-*DPYD* drug-gene interaction.

4.1 Limitations

This is not a comparative clinical trial; rather, it is an observational cohort study. We did not consider all the variants in the *DPYD* gene. However, for the recruited cohort, it was not useful to study further. In fact, we examined all variants with a MAG higher than 1% in our population. Rare variants do not make sense in a cohort of n=167 patients.

Nevertheless, it is necessary to investigate rare variants in larger cohorts to study the different interactions between them in detail. In particular, conducting an association study between *DPYD* haplotypes and response to FPs would be especially interesting. Also, studying the epigenetics of the *DPYD* gene as a key gene in the PGx of FPs would be also interesting. This might elucidate discrepancies between association studies of *DPYD* variants with FP response.

We did not study all the clinical variables that influence the response to FPs, especially the baseline condition of recruited patients, as commented above. Besides that, this study is based on real-world data obtained from daily clinical practice, and we are limited by the reliable information collected in the patients' medical records.

In the same regard, we recruited a wide range of different patients, including metastatic/adjuvant-treated patients, different stage of tumor, chemotherapy scheme, etc. Anyway, the aim was to perform the study considering real world data based on our daily clinical practice, and we found significant differences about the influence of the *DPYD**4 alle on FP toxicities.

Also, considering the inclusion criteria of candidate *DPYD* variants as explanatory factors of FP-related toxicities, we should study the *DPYD* rs75017182 (Table 1). It was not genotyped since this is, within the *DPYD* rs56038477, the variants characterizing the *DPYD**HapB3, and it has a lower MAF.

4.2 Association of genetic variants with response to fluoropyrimidines.

In the study of the association of FP dose tailoring according to the *DPYD* genotype with the toxicity and efficacy endpoints, we found no significant differences (Table 5). These results make sense since significant differences would have meant an underestimation of dose modifications resulting from the presence of the *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798 variants.

Table 5. Association study of *DPYD* variants used for fluoropyrimidines dose tailoring with the response.

		ADE severity ≥ 3		OR (95% CI)	p-value
		Yes n (%)	NO n (%)		
DPYD GAS <2	YES n (%)	2 (4.3)	4 (3.3)	1.29 (0.11 – 9.34)	0.674
	NO n (%)	45 (95.7)	116 (96.7)		
		Efficacy		OR (95% CI)	p-value
		Yes n (%)	NO n (%)		
DPYD GAS <2	YES n (%)	3 (2.3)	3 (7.9)	0.28 (0.04 – 2.19)	0.132
	NO n (%)	126 (97.7)	35 (92.1)		
ADE: Adverse drug event; OR= Odds ratio; CI: Confidence interval; GAS= Gene activity score					

Table 6. Association study with the efficacy/toxicity endpoints of *DPYD* variants not used for FPs dose tailoring.

		ADE severity ≥ 3		OR (95% CI)	p-value
		Yes n (%)	NO n (%)		
DPYD rs1801265	A/G - G/G	15 (31.9)	38 (31.9)	1.00 (0.48 – 2.06)	1
	A/A	32 (68.1)	81 (68.1)		
DPYD rs17376848	A/G	0 (0)	6 (5)	0.00 (0 - NA)	0.043
	A/A	47 (100)	113 (95)		
DPYD rs1801159	C/T - T/T	18 (38.3)	53 (44.5)	0.77 (0.39 – 1.54)	0.46
	C/C	29 (61.7)	66 (55.5)		
DPYD rs1801160	C/T	6 (12.8)	15 (12.6)	1.01 (0.37 – 2.80)	0.98
	C/C	41 (87.2)	104 (87.4)		
DPYD rs1801158	C/T	6 (12.8)	3 (2.5)	5.66 (1.35 – 23.67)	0.014
	C/C	41 (87.2)	116 (97.5)		
DPYD rs2297595	C/T	11 (23.4)	24 (20.2)	1.21 (0.54 – 2.72)	0.65
	C/C	36 (76.6)	95 (79.8)		
		Efficacy		OR (95% CI)	p-value
		Yes n (%)	NO n (%)		
DPYD rs1801265	A/G - G/G	41 (31.8)	12 (32.4)	0.97 (0.44 – 2.12)	0.94
	A/A	88 (68.2)	25 (67.6)		
DPYD rs17376848	A/G	6 (4.7)	0 (0)	NA (0.00-NA)	0.079
	A/A	123 (95.3)	37 (100)		
DPYD rs1801159	C/T - T/T	56 (43.4)	15 (40.5)	1.13 (0.54 – 2.37)	0.76
	C/C	73 (56.6)	22 (59.5)		
DPYD rs1801160	C/T	16 (12.4)	5 (13.5)	0.91 (0.31 – 2.66)	0.86
	C/C	113 (87.6)	32 (86.5)		
DPYD rs1801158	C/T	6 (4.7)	3 (8.1)	0.55 (0.13 – 2.33)	0.43
	C/C	123 (95.3)	34 (91.9)		

DPYD rs2297595	C/T	28 (21.7)	7 (18.9)	1.19 (0.47 – 2.99)	0.71
	C/C	101 (78.3)	30 (81.1)		
ADE: Adverse drug event; OR= Odds ratio; CI: Confidence interval					

In the association study with the toxicity and efficacy endpoints of the new variants, which are not currently being used to guide treatment with FPs in our population, we have observed the following:

The *DPYD**4 (defined by rs1801158) allele is associated with a higher risk of severe ADEs (severity grade ≥ 3) in both the univariate (OR=5.66; 95% CI=1.35–23.67; p=0.014) and multivariate analyses (OR= 5.73; 95% CI= 1.41–28.77; p= 0.019) after adjusting the model.

This variant had been previously associated with FP response. The *DPYD**4 CT genotype was associated with decreased catalytic activity of DPD [28], and increased risk of drug toxicity when treated with capecitabine or fluorouracil in colorectal cancer patients [29], as compared to CC genotype.   On the other hand, many other studies showed contradictory results [30] in this regard. An interesting study by André B. P. van Kuilenburg *et al.* [31] found that *DPYD**4 allele T is associated with decreased activity of DPD when expressed in mammalian cells (HEK293 Flp-In) as compared to allele C but highlighting the conflicting data about this association as they found no association when DPD activity was assessed within a healthy cohort of n=100 individuals.

These, and the results described below, reveal the need for further studies, especially considering the expression of genes, and not just categorizing patients on carriers/non-carriers of single or combined *DPYD* variants.

We found an association of chemotherapy schemes including irinotecan with a lower efficacy in the multivariate analysis (Table 7). We observed that the n=10 patients treated with FOLFIRINOX (including irinotecan) are the same n=10 patients with pancreatic cancer, with the worse baseline condition and prognosis among recruited patients.

Table 7. Association study with the toxicity and efficacy endpoint of study variables included in the multivariate model after adjustment.

Toxicity endpoint		
Variable	**OR (95% CI)**	**p-value**
DPYD rs1801158 (CT or TT)	5.73 (1.41 – 28.77)	0.019
Irinotecan	2.32 (0.92 – 5.81)	0.071
Age	NA	0.090
DPYD rs56038477 (CT or TT)	6.99 (0.64 – 155.45)	0.120
Monoclonal antibody	0.00 (NA - Inf)	0.990
DPYD rs17376848 (AG or GG)	0.00 (NA - Inf)	0.992
Efficacy endpoint		
Variable	**OR (95% CI)**	**p-value**
Irinotecan	0.15 (0.06 – 0.35)	<0.001
DPYD rs56038477 (CT or TT)	0.096 (0.01 – 1.05)	0.062
Radiotherapy	2.31 (0.79 – 8.48)	0.16
ADE: Adverse drug event; OR= Odds ratio; CI: Confidence interval; NA: Not applicable		

Table 8. *DPYD* characterization in our population.

*allele	*DPYD* Variant	Major Nucleotide Variation	Genotype N=190 ^			H-W	MAF	MAF Ibs	Comparison with 1000 Genomes
			Wt	Het	Hom				
-	rs17376848	c.1896T>C	181 (95.77)	8 (4.23)	0 (0)	1	0.021	98/2	1
*4	rs1801158	c.1601G>A	177 (93.65)	12 (6.35)	0 (0)	1	0.032	94/6	0.149
*5	rs1801159	c.1627A>G	109 (57.67)	69 (36.51)	11 (5.82)	1	0.241	79/21	0.475
*6	rs1801160	c.2194G>A	168 (88.89)	21 (11.11)	0 (0)	1	0.056	95/5	0.643
*9A	rs1801265	c.85T>C	128 (67.73)	58 (30.69)	3 (1.59)	0.3	0.169	79/21	0.170
-	rs2297595	c.496A>G	152 (80.42)	37 (10.58)	0 (0)	0.2	0.098	88/12	0.470
*2A	rs3918290	c.1905+1G>A	190 (100)	0 (0)	0 (0)	-	0	100/0	1
*13	rs55886062	c.1679T>G	189 (99.47)	1 (0.53)	0 (0)	1	0.003	100/0	1
*HapB3	rs56038477	c.1236G>A	185 (97.37)	5 (2.63)	0 (0)	1	0.013	98/2	0.729
-	rs67376798	c.2846A>T	188 (98.95)	2 (1.05)	0 (0)	1	0.011	100/0	0.538

Wt: Wildtype; Het: Heterozygous; Hom: recessive homozygous; H-W: Hardy Weinberg equilibrium analysis; MAF: Minor Allele Frequency; Ibs: Iberian Peninsula; *MAFs obtained from the 1000 Genomes Project [15]. ^N=189 for *DPYD* variants not used for fluoropyrimidines dose tailoring.

Table 8. Linkage disequilibrium analysis.

Linkage disequilibrium									
n=190^									
	rs1801265 (*9A)	rs17376848	rs1801159 (*5)	rs1801160 (*6)	rs1801158 (*4)	rs2297595	rs55886062 (*13)	rs67376798	rs56038477 (*HaPB3)
rs1801265 (*9A)	-	0.9463 2e-04 0.012 0.0037	0.6052 -0.0045 0.1142 -0.0284	0.0369 0.0103 0.1943 0.1145	0.1844 -0.0044 0.9901 -0.0728	0 0.0684 0.7748 0.6035	0.0272 0.0024 0.9751 0.1212	0.5667 -9e-04 0.9191 -0.0314	1e-04 0.0074 0.9903 0.2139

rs173768 48	-	-	0.8753 5e-04 0.0358 0.0086	0.5391 -0.0011 0.9562 -0.0337	0.7111 -4e-04 0.8977 -0.0203	0.6804 -9e-04 0.4854 -0.0226	0.9834 0 0.0028 0.0011	0.9584 0 0.2721 -0.0029	0.897 -1e-04 0.5501 -0.0071
rs180115 9 (*5)	-	-	-	0.9568 -3e-04 0.0203 -0.003	0.0887 -0.0065 0.9933 -0.0934	0.0141 -0.0177 0.6966 -0.1347	0.0848 0.0022 0.9725 0.0946	0.9315 2e-04 0.0342 0.0047	0.2636 0.0025 0.3631 0.0613
rs180116 0 (*6)	-	-	-	-	0.4411 0.0017 0.9746 -0.0423	0.6994 0.0016 0.2376 -0.0212	0.8619 -1e-04 0.6704 -0.0095	0.0191 0.0024 0.4308 0.1287	0.1098 0.0025 0.2396 0.0878
rs180115 8 (*4)	-	-	-	-	-	0.3038 -0.0028 0.9848 -0.0564	0.9693 0 0.231 -0.0021	0.9033 -1e-04 0.5147 -0.0067	0.8394 -2e-04 0.7001 -0.0111
rs229759 5	-	-	-	-	-	-	0.7833 -3e-04 0.8023 -0.0151	0.6709 -6e-04 0.8752 -0.0233	0.4257 0.0013 0.1577 0.0437
rs558860 62 (*13)	-	-	-	-	-	-	-	0.8471 0 0.0149 0.0106	0.8998 0 0.0122 0.0069
rs673767 98	-	-	-	-	-	-	-	-	0.950

								0.0042 0.0034
rs56038477 (*HapB3)	-	-	-	-	-	-	-	-	p-value D D′ r

^For rs1801265 (*9A), rs17376848, rs1801159 (*5), rs1801160 (*6), rs1801158 (*4), rs2297595 n=189 patients were genotyped because we had not enough DNA of n=1 patient. Green data: Linkages with p-value < 0.05

Also, regarding our results, no other variants were significantly associated with either toxicity or efficacy endpoints. The *DPYD* rs17376848 showed a confounding association with the response. According to significance criteria based on p-values, carriers of this variant (Genotype AG or GG vs. AA) were associated with a lower risk of the toxicity endpoint (p=0.043) and a certain trend toward the efficacy of FPs (p=0.079). However, upon closer examination of these results, we find that only n=6 patients carry the *DPYD* rs17376848 variant, and none of them experienced an ADE (severity grade ≥ 3) during follow-up, preventing us from confirming this association. Furthermore, this statistical significance is lost in the multivariate analysis (p=0.992; Table 7) when considering the influence of concomitant treatments, other clinical variables, and interactions with other *DPYD* variants.

As happens with *DPYD**4 (defined by rs1801158), those other five variants suggested as candidate variants to explain the remaining toxicity events in our population showed inconclusive results in previous studies [30,32]. In any case, further studies with larger cohorts and treatment guidance based on these variants are necessary to confirm their utility or lack of influence in clinical practice.

Anyway, despite of the baseline characteristics of recruited patients, stage of the tumor, different fluoropyrimidines schemes, interactions between clinical and PGx variables, and receiving a PGx dose tailored treatment, the *DPYD**4 (defined by rs1801158) showed an association with a higher risk of severe ADEs to FPs in our population. This makes it a candidate variant for potential implementation in clinical practice.

4.3 Insights on Clinical Practice.

We have observed that in the clinical practice of our hospital, there is an important association between the profile of the health professional with the degree of acceptance and requests for PGx tests. As also happened to us in previous studies [33], there is a bias between the hospital departments prescribing the drugs and those requesting the PGx tests. In this case, we have observed that 96.3% (n=183) of the n=190 requests for the *DPYD* test were made by the medical oncology department, and n = 7 (3.7%) by other units of our hospital. Furthermore, we observed that, of the n=170 patients who were prescribed capecitabine or 5-FU in the medical oncology unit during the time of recruitment, n=167 (98.2%) received the treatment guided by the PGx test. On the other hand, n=402 patients in total in our hospital received treatment with FPs without having been genotyped for the variants recommended in the drug label, by the EMA, FDA, SEFF, and SEOM. Among these, only n=3 were treated by doctors assigned to the medical oncology service, and n=399 by other units.

As we can see, despite the level of evidence regarding the *DPYD*-FPs interaction and the recommendations in the drug label, by health authorities, and scientific societies, the degree of implementation of these tests is strongly linked to the profile of the healthcare professional and internal procedures or protocols of their hospital department.

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On the other hand, it is true that until a few months ago, PGx tests, including the *DPYD*-FPs interaction described in this study, had not been included in the portfolio of common services of the national health system in Spain, which is the set of health procedures that must be available to any citizen. The recent update of the service portfolio institutionally supports the performance of PGx tests and invites health professionals to perform them.

Another aspect that we observed in our results is that the level of acceptance of the therapeutic recommendations that emerge from the PGx results is total and dose reductions do not translate into a decrease in the treatment efficacy. Furthermore, based on our results and as we mentioned above, *DPYD* rs1801158 could be implemented in clinical practice. This variant has a relatively high frequency (MAF=0.032; 6% carriers) and was the only genetic or clinical variant that was associated with an increased risk of toxicity secondary to FPs in the univariate and multivariate analyses.

The *DPYD*-FP interaction, regardless of many contradictory results, has been demonstrated to be useful in clinical practice in different studies. Furthermore, recent studies demonstrated the cost-effectiveness of PGx tests [34,35]. The potential benefit, actual related costs, and support by sanitary authorities should be enough reasons for the large implementation of PGx tests, especially the *DPYD*-FP interaction.

5. Conclusions

Based on our results we concluded that FP dose lowering based on *DPYD* genotype does not affect the treatment efficacy. The *DPYD**4 (defined by the rs1801158) is associated with FP toxicity in patients receiving a PGx dose tailored treatment based on *DPYD**2A (defined by rs3918290), *DPYD**13 (defined by rs55886062), *DPYD**HapB3 (defined by rs56038477), and rs67376798. Based on this, the *DPYD**4 (defined by the rs1801158) is an explanatory factor of remaining ADEs among FP-treated and PGx dose-tailored patients, and its genotyping should be implemented in daily clinical practice.

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