Frecuencia e impacto clínico de las variantes del gen de la DPYD

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RESUMEN

Introducción y objetivo: El objetivo de este estudio fue determinar la prevalencia de variantes de pérdida de función en el gen DPYD en pacientes con tumores sólidos a los que se les ha realizado un genotipado y establecer los resultados clínicos de esta implementación en la práctica clínica en cuatro hospitales de las Islas Baleares.

Método: El estudio fue descriptivo, observacional, retrospectivo y multicéntrico, realizado en pacientes cuyo genotipado había sido determinado por el GENIB entre septiembre de 2020 y abril de 2022. Las variantes genotipadas fueron rs3918290 (c.1905+1G>A, DPYD*2A), rs55886062 (c.1679T>G, DPYD*13), rs67376798 (c.2846A>T) y rs56038477 (c.1236G>A/HapB3).

Resultados: Se incluyeron 349 pacientes, 22 (6,3%) eran portadores de al menos un polimorfismo. Del total de pacientes que recibieron tratamiento antineoplásico a base de fluoropirimidinas (294), 19 (6,46%) eran portadores de al menos una de las variantes analizadas. En el grupo de pacientes no portadores, 28 (10,2%) sufrieron toxicidad de grado 3 o superior, frente a 5 pacientes de los 19 (26,3%) portadores. Los pacientes portadores que comenzaron con dosis reducidas tuvieron menos toxicidad.

Conclusión: Este estudio muestra una prevalencia de portadores de variantes del gen DPYD en nuestra población similar a la estimada en otras poblaciones. Además, se confirma la relevancia clínica de la presencia de estas variantes genéticas con aparición de toxicidad grave y potencialmente letal.

Palabras clave: **polimorfismo, farmacogenética, dihidropirimida-dehidrogenasa, fluorouracilo, capecitabina**

Frequency and clinical impact of DPYD gene variants

SUMMARY

Abstract: Introduction and objective: The aim of this study was to determine the prevalence of loss-of-function variants in the DPYD gene in patients with solid tumours who have undergone genotyping and establish the clinical results of this implementation in clinical practice in four hospitals in the Balearic Islands.

Methods: The study was descriptive, observational, retrospective and multicentre carried out in patients which genotype of DPYD gene had been determined by the GENIB between September 2020 and April 2022. Genotyped variants were rs3918290 (c.1905+1G>A, DPYD*2A), rs55886062 (c.1679T>G, DPYD*13), rs67376798 (c.2846A>T), and rs56038477 (c.1236G>A/HapB3).

Results: 349 patients were included, 22 (6.3%) were carriers of at least one polymorphism. Of all the patients who received antineoplastic treatment based on fluoropyrimidines (294), 19 (6.46%) were carriers of at least one of the variants analysed. In the group of non-carrier patients, 28 (10.2%) suffered grade 3 or higher toxicity, compared to 5 patients of the 19 (26.3%) carriers. Carrier patients who started with re-duced doses had less toxicity.

Conclusion: This study shows a prevalence of carriers of DPYD gene variants in our population similar to that estimated in other populations. Further, the clinical relevance of the presence of these genetic variants with the appearance of serious and potentially lethal toxicity is confirmed.

Keywords: **polymorphism, pharmacogenetics, dihydropyrimidine-dehydrogenase fluorouracil, capecitabine**

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INTRODUCTION

Fluoropyrimidines are anticancer drugs that include 5-fluorouracil (FU), its oral prodrug capecitabine, and the oral prodrug tegafur. They are commonly prescribed for the adju-vant and palliative treatment of various types of solid malignancies, including gastroin-testinal, breast, and head and neck cancers[1-3] .

These drugs are usually well-tolerated (approximately 30% of patients present some de-gree of toxicity), although there is a low percentage of patients (around 1-3% according to the summary of product characteristics) who present severe toxicity (grades 3-4) to these agents[4,5] thereby affecting their quality of life[6,7].

Dihydropyrimidine dehydrogenase (DPD) is the fundamental enzyme in the metabolism of FU. It is estimated to metabolise 80% of FU and its activity is subject to interindividual variability and genetic polymorphisms. Complete deficiency of DPD activity is very rare, estimated at 0.01% to 0.5% of Caucasian individuals, while partial deficiency has been estimated at 3% to 8% of the Caucasian population[8].

Several studies have shown that patients with DPD enzyme deficiency are at increased risk of developing serious adverse reactions such as diarrhoea, mucositis, or neutropenia when treated with fluoropyrimidines[9-11].

Treatment of severe toxicity is often associated with interruption or even discontinuation of potentially effective treatment and often requires hospitalisation. This has a major impact on a patient's prognosis and quality of life, and also generates significant healthcare costs.

The European Medicines Agency (EMA) published a recommendation in 2020 to test for lack of DPD activity before starting treatment with these agents using genotype and/or phenotype tests for DPD deficiency[12]. Specifically, they recommend genotyping the most studied loss- of-function variants in the dihydropyrimidine dehydrogenase (DPYD) gene, which are rs3918290 (c.1905+-1G>A, DPYD*2A), rs55886062 (c.1679T>G, DPYD*13), rs67376798 (c.2846A>T), and rs56038477 (c.1236G>A/HapB3). A specific reduced dose has not been established for these cases, although 25-50% reductions in the initial dose of fluoropyrimidines have been recommended[13-15].

In clinical practice, since September 2020, genotyping of these four variants of the DPYD gene has been carried out centrally at the healthcare level at the Genetics and Genomics Unit of the Balearic Islands (GENIB) for patients who are candidates for treatment with fluoropyrimidines.

Primary Objective:

To determine the prevalence of loss-of-function variants in the DPYD gene in patients with solid tumors who had undergone genotyping by the GENIB.

Secondary Objectives:

To establish the clinical results of this implementation in clinical practice in hospitals in the Balearic Islands, evaluated by severity of toxicity. To compare our results with published studies.

MATERIALS AND METHODS INCLUSION CRITERIA

Patients ≥18 years, from three hospitals in Mallorca and one in Ibiza, diagnosed with any solid tumour who underwent genotyping of the DPYD gene between September 2020 and April 2022.

Exclusion criteria

Information not available in the medical records.

Design

Descriptive, observational, retrospective, multicentre study carried out in four hospitals in the Balearic Islands (Son Espases University Hospital, Son Llàtzer University Hospital, Manacor Hospital, and Can Misses Hospital), in patients diagnosed with any solid tu-mour in whom the genotype of the DPYD gene had been determined by the GENIB.

Data collection and statistical analysis

Through the application used for the management of laboratory samples (Gestlab®, v2.20.2.1482), patients who were requested for the determination and its result were identified. Patients' clinical data were collected from the computerised clinical history program of each hospital and the data related to cancer treatment from the Farmis-Oncofarm® software. Data processing was carried out using an Excel sheet (Mi-crosoft Office, Redmond, WA, USA, 2010).

The following data were collected: sex, age, type of cancer, type of chemotherapy treat-ment, whether or not they had started treatment before genotyping, dose reduction in the case of being a carrier and/or as a consequence of toxicity, admission for toxicity, use of granulocyte colony stimulation factors (G-CSF), treatment interruption due to toxicity, toxicity developed during the first three cycles, and death.

The prevalence of loss-of-function variants in the DPYD gene was defined as the pro-portion of patients who presented at least one variant of the DPYD gene out of the total number of patients who had undergone genotyping. Regarding toxicity, all the data available in the clinical history were collected subjectively by the physician, but only grade 3 or higher toxicity of any type was taken into account.

A descriptive analysis of all the variables was performed. Categorical variables were es-timated using global percentages and frequencies. Quantitative variables were expressed as median and interquartile range. The study was authorised by the regional Research Ethics Committee (IB 4905/2 2 PI) in July 2022. Variables were recorded in a coded database and data were collected up to April 2022.

Genotyping

Genomic DNA from all included patients was extracted from a 6 ml blood sample in an EDTA tube using the InviGenius® PLUS kit (Invitek Molecular). Variants rs3918290 (c.1905+1G>A, DPYD*2A), rs55886062 (c.1679T>G, DPYD*13), rs67376798 (c.2846A>T), and rs56038477 (c.1236G>A/HapB3) were genotyped. The sequence flanking the genetic polymorphisms of the DPYD gene was amplified by polymerase chain reaction (PCR). The PCR product was studied by bidirectional Sanger sequencing to reveal the pres-ence/absence of the variants.

RESULTS

A total of 349 patients, who had been diagnosed with any solid tumour and genotyped for the DPYD gene from September 2020 to April 2022, were included. Twenty-two (6.3%) were carriers of at least one polymorphism, all of them heterozygous. Half of the carrier patients were fe-

males; with a median age of 67 years (range 58 to 91). The baseline characteristics of the patients included are detailed in Table 1.

The most frequent variant was rs56038477 (c. 1236G>A/HapB3) which was identified in 13 patients (13/349 3.7%). The second most predominant was rs67376798 (c.2846A>T), present in four patients (4/349 1.1%), followed by variants rs3918290 (c.1905+1G>A, DPYD*2A) and rs55886062 (c .1679T>G), each identified in two patients (2/349 0.6%). Lastly, one patient was a heterozygous carrier of two variants (rs67376798 and rs56038477).

Of all the patients who received antineoplastic treatment based on fluoropyrimidines (294), 19 (6.46%) were carriers of at least one of the variants analysed. In the group of non-carrier patients, 28 (10.2%) suffered grade 3 or higher toxicity, none causing death, compared to five patients of the 19 (26.3%) carriers. The carrier with compound hetero-zygosity started capecitabine at 100% dose, but was admitted seven days later due to in-testinal perforation and died shortly due to complications. Table 2 shows in detail the DPYD variant, dose received, and grade ≥ 3 toxicity among carrier patients. Dose reduction was based on medical criteria.

Regarding non-carriers, 125 patients started treatment before knowing the genotyping, 53 of whom (19.3%) were admitted or whose dose had to be reduced, while 62 (22.5%) of the 150 patients who started after knowing the result required admission or dose reduction.

Table 1. Baseline patient characteristics, treatments, and tumours (n = 349)

	Median (IQR)	n (%)
Patients		
Sex (Females)		153 (43.8%)
Age (years)	67 (58 to 91)	
Treatment		
Capecitabine based		206 (59%)
5-FU based		88 (25.2%)
No treatment		55 (15.8%)
Primary tumour		
Colon		175 (50.1%)
Rectum		68 (19.5%)
Pancreas		35 (10%)
Stomach		31 (8.9%)
Breast		19 (5.4%)
Oesophagus		8 (2.3%)
Anal		6 (1.8%)
Head and Neck		5 (1.4%)
Others		2 (0.6%)

5-FU: 5-Fluorouracil; IQR: interquartile range

DISCUSSION

In our sample, 22 patients carrying a variant of the DPYD loss-of-function gene were identified. The frequency found, 6.3%, is similar to that estimated in the Caucasian population (3-8%)[14] although recent studies found a different prevalence: 0.95% in a prospective study from Quebec[15] 10% in a retrospective study in Barcelona[16] and 4.9% in a recent study in the population of Spain[17].

Of these variants, the most frequent we found was rs56038477 at 3.7% – within the range described for the Caucasian population (2.6%-6.3%)[18] followed by rs67376798 at 1.1%, for which allele frequencies of 0.4-1.4% have been described. In the other two variants, rs3918290 and rs55886062, we found 0.5% in each one, in which frequencies of 0.8-2.2%[18] and 0.06- 1%[18] have been described, respectively. The rs3918290 variant was found to be slightly lower than in published studies (1.7%)[18]. It should be noted that one pa-tient was found to be a heterozygous carrier of two variants: rs67376798 and rs56038477.

According to the dosage of fluoropyrimidines for patients carrying variables of the DPYD gene, there is no consensus recommendation. Initially, it was recommended that patients with the rs3918290 and rs55886062 variants begin treatment with fluoropyrimidines at 50% of the standard dose, while carriers of the rs67376798 and rs75017182 variants were to start treatment at 75% of the standard dose. However, more recent publications recommend reducing the total dose by 50% regardless of the gene variable found[19,20] as in a prospective study it was shown that a 25% reduction in the rs67376798 and rs75017182 variants was insufficient and a 50% reduction was suggested as well[18]. Similar results have also been found in subsequent studies[16,21,22].

In our results, seven carrier patients started with standard doses and three of them had grade 3 or higher toxicity (42.9%), leading to treatment interruption and even death in one of them. The other 12 patients started at a reduced dose, producing grade 3 or higher toxicity in only two (16.6%). This fact consolidates the clinical relevance of reducing the dose of fluoropyrimidines in patients carrying said gene.

If we compare these data with previous studies, we find that the rate of toxicity in carrier patients in whom the dose has previously been reduced is similar, 13%19 and 22.7%[22]. However, it is much higher for those who started with standard doses (42.8%) than in studies similar to ours (24% and 23.5%)[21,22] maybe due to a limited number of cases. Interestingly, two patients initially received a 100% dose despite knowing they were car-riers before starting treatment, and they did not develop toxicity.

Regarding non-carrier patients, 28 had grade 3 or higher toxicity (10.2%). These results are slightly lower than those found in the aforementioned studies (21.1% and 13.6%)[21,22].

This study has several limitations. First of all, it is a retrospective study; so many data may have been lost. Secondly, the toxicities, as previously mentioned, were mainly col-lected based on the information available in medical records. It is also necessary to note that genotyping is much more established in digestive tumors than, for example, in tu-mors such as breast cancer, so the sample size of this study may be less than that of our real population.

Table 2. DPYD variant, dose received, and grade 3 toxicity among carrier patients

DPYD variant	Patients	5-FU/Cape based treatment	% Initial dose	Start before knowing DPYD variant	Toxicity grade ≥ 3 (Hem/ No hem)	Hospital admission due to toxicity	Stop treatment due to toxicity	Dead¥
rs56038477	11	Cape	50%	No	Yes (No hem)	No	No	No
		Cape	50%	No	Yes (No hem)	Yes	No	No
		5-FU	100%	Yes	Yes (Hem)	Yes	Yes	Yes
		5-FU	50%	No	No	No	No	No
		5-FU	100%	Yes	No	No	No*	No
		Cape	60%	No	No	No	No	No
		Cape	75%	No	No	Yes	No	No
		Cape	75%	No	No	No	No	No
		Cape	75%	No	No	No	No	No
		Cape	75%	Yes	Yes (Hem)	No	No	No
		Cape	50%	No	No	No	No	No
rs67376798	4	Cape	75%	Yes	No	No	No	No
		Cape	50%	No	No	No	No	No
		5-FU	100%	Yes	Yes	No	No*	No
		5-FU	100%	No	No	No	No	No
rs3918290	2	Cape	100%	Yes	Yes (Hem)	Yes	Yes	No
		5-FU	50%	No	No	No	No	No
rs55886062	1	Cape	100%	No	No	No	No	No
rs67376798 + rs56038477	1	Cape	100%	Yes	No	No	Yes	No

5-FU: 5-fluorouracil; Cape: capecitabine; Hem: haematology toxicity; *Dose had to be reduced due to toxicity; ¥ Death related to treatment

An important strength of our work is that it is multicentre study, with the participation of several hospitals and a significant sample size.

All things considered, our results support the clinical relevance of performing DPYD genotyping in all patients who are going to be treated with fluoropyrimidines, mainly due to their association with the appearance of severe toxicity, a fact that potentially affects patients' quality of life.

CONCLUSIONS

In conclusion, this study shows a prevalence of carriers of DPYD gene variants in our population similar to that estimated in the Caucasian population. In addition, the clinical relevance of the presence of these genetic variants with the appearance of serious and potentially lethal toxicity is confirmed. We believe that the implementation of genotyping shows the benefit of using pharmacogenetics in multidisciplinary teams to improve pa-tient care through precision medicine.

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Institutional Review Board Statement: The study was authorised by the regional Research Ethics Committee (IB 4905/2 2 PI) in July 2022.

Informed Consent Statement: Patient consent was waived due to the variables were recorded in a coded database and data were collected up to April 2022.

Data Availability Statement: the results are not public but the main author can be contacted.

Conflicts of Interest: The authors declare no conflict of interest.

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