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Brief Communication

Allelic Frequency of *DPYD* Genetic Variants in Patients With Cancer in Spain: The PhotoDPYD Study

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Abstract

Introduction: Identifying polymorphisms in the dihydropyrimidine dehydrogenase (*DPYD*) gene is gaining importance to be able to predict fluoropyrimidine-associated toxicity. The aim of this project was to describe the frequency of the *DPYD* variants *DPYD*2A* (rs3918290); c.1679T>G (rs55886062); c.2846A>T (rs67376798) and c.1129-5923C>G (rs75017182; HapB3) in the Spanish oncological patients.

Material and Methods: Cross-sectional and multicentric study (PhotoDPYD study) conducted in hospitals located in Spain designed to register the frequency of the most relevant *DPYD* genetic variants in oncological patients. All oncological patients with *DPYD* genotype were recruited in the participant hospitals. The measures determined where the presence or not of the 4 *DPYD* previously described variants.

Results: Blood samples from 8054 patients with cancer from 40 different hospitals were used to determine the prevalence of the 4 variants located in the *DPYD* gene. The frequency of carriers of one defective *DPYD* variant was 4.9%. The most frequently identified variant was c.1129-5923C>G (rs75017182) (HapB3), in 2.9%, followed by c.2846A>T (rs67376798) in 1.4%, c.1905 + 1G>A (rs3918290, *DPYD*2A*) in 0.7% and c.1679T>G (rs55886062) in 0.2% of the patients. Only 7 patients (0.08%) were carrying the c.1129-5923C>G (rs75017182) (HapB3) variant, 3 (0.04%) the c.1905 + 1G>A (rs3918290, *DPYD*2A*) and one (0.01%) the *DPYD* c.2846A>T (rs67376798, p.D949V) variant in homozygosis. Moreover, 0.07% were compound heterozygous patients, 3 carrying the *DPYD* variants *DPYD*2A* + c.2846A>T, 2 the *DPYD* c.1129-5923C>G + c.2846A>T and one the *DPYD*2A* + c.1129-5923C>G variants.

Conclusions: Our results demonstrate the relatively high frequency of *DPYD* genetic variants in the Spanish patient with cancer population, which highlights the relevance of their determination before initiating a fluoropirimidine-containing regimen.

Introduction

Fluoropyrimidines, such as capecitabine, tegafur, and 5-fluorouracil (5-FU), are a group of antimetabolites that are widely used in the treatment of several oncological diseases, including colorectal, breast, and gastric cancer, among others.[1] The cytotoxic effect of fluoropyrimidines is due to several active metabolites that are generated after entering the cell, including 5-fluoro-2-dUMP, a metabolite that forms a stable ternary complex with the enzyme thymidylate synthase (TS) and inhibits it. TS inhibition suppresses thymidine synthesis, and this suppression interferes with DNA synthesis and repair, leading to cell apoptosis.[2]

The metabolism of these drugs is marked by the activity of several enzymes, being the dihydropyrimidine dehydrogenase (DPD), which transforms 5-FU into dihydrofluorouracil (DHFU) and removes more than 80% of 5-FU, the most crucial one.

When the activity of this enzyme is reduced, relevant toxicities can occur. Many genetic variants have been described in its encoding gene (*DPYD*), most of them without clinical relevance, but approximately 3-5% of patients are carriers of one genetic variant that reduces or cancels the activity of the enzyme.[3] DPD deficiency leads to an accumulation of the metabolite 5-fluorodeoxyuridine monophosphate (5FdUMP), causing increased gastrointestinal, neurological and hematopoietic toxicity, and may even cause fatal toxicity.

The American regulatory agency, the U.S. Food and Drug Administration (FDA), as well as organizations such as the Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC), have recognized the clinical importance of genotyping the most relevant *DPYD* genetic variants.[4,5] Likewise, both the National Comprehensive Cancer Network (NCCN) and the European Society for Medical Oncology

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(ESMO) highlight the need to determine the patients' genotype prior to starting treatment with fluoropyrimidines. The ESMO includes a series of recommendations on the individualization of treatment with fluoropyrimidines based on the *DPYD* genotype, suggesting the determination of 4 variants before treatment initiation: *DPYD*2A* (rs3918290); c.1679T>G (rs55886062); c.2846A>T (rs67376798); c.1129-5923C>G (rs75017182; HapB3 or c.1236G>A; rs56038477). They also suggest dose adjustment based on the genotype and even propose changing treatment in severe cases. In May 2020, the Spanish Agency for Medicines and Health Products (AEMPS) published an informative note in this regard, recommending carrying out genotyping tests in patients who are candidates for treatment with fluoropyrimidines, which is also suggested in the data sheet of these drugs.[6]

Currently, more centers are performing genotyping tests in oncological patients receiving treatment with fluoropyrimidines; however, there is scarcity of information of the frequency of patients with an impaired DPD activity in most population.

The aim of this project was to describe the frequency of the *DPYD* variants *DPYD*2A* (rs3918290); c.1679T>G (rs55886062); c.2846A>T (rs67376798); and c.1129-5923C>G (rs75017182) (HapB3) in the Spanish patient with cancer population, by recruiting patients in several centers in Spain, with the goal of creating a national registry.

Material and Methods

This is a cross-sectional and multicentric study (PhotoDPYD study) designed to register the frequency of the most relevant *DPYD* genetic variants (*DPYD*2A* (rs3918290); c.1679T>G (rs55886062); c.2846A>T (rs67376798); c.1129-5923C>G (rs75017182) (HapB3) in oncological patients from hospitals located in Spain.

This study was coordinated by the Spanish Society of Hospital Pharmacy (SEFH) through the RedDPYD research group, which facilitated the coordination of all the hospitals involved in the project by means of the RedCap platform. A map depicting the participating institutions in the study is represented in Fig. 1.

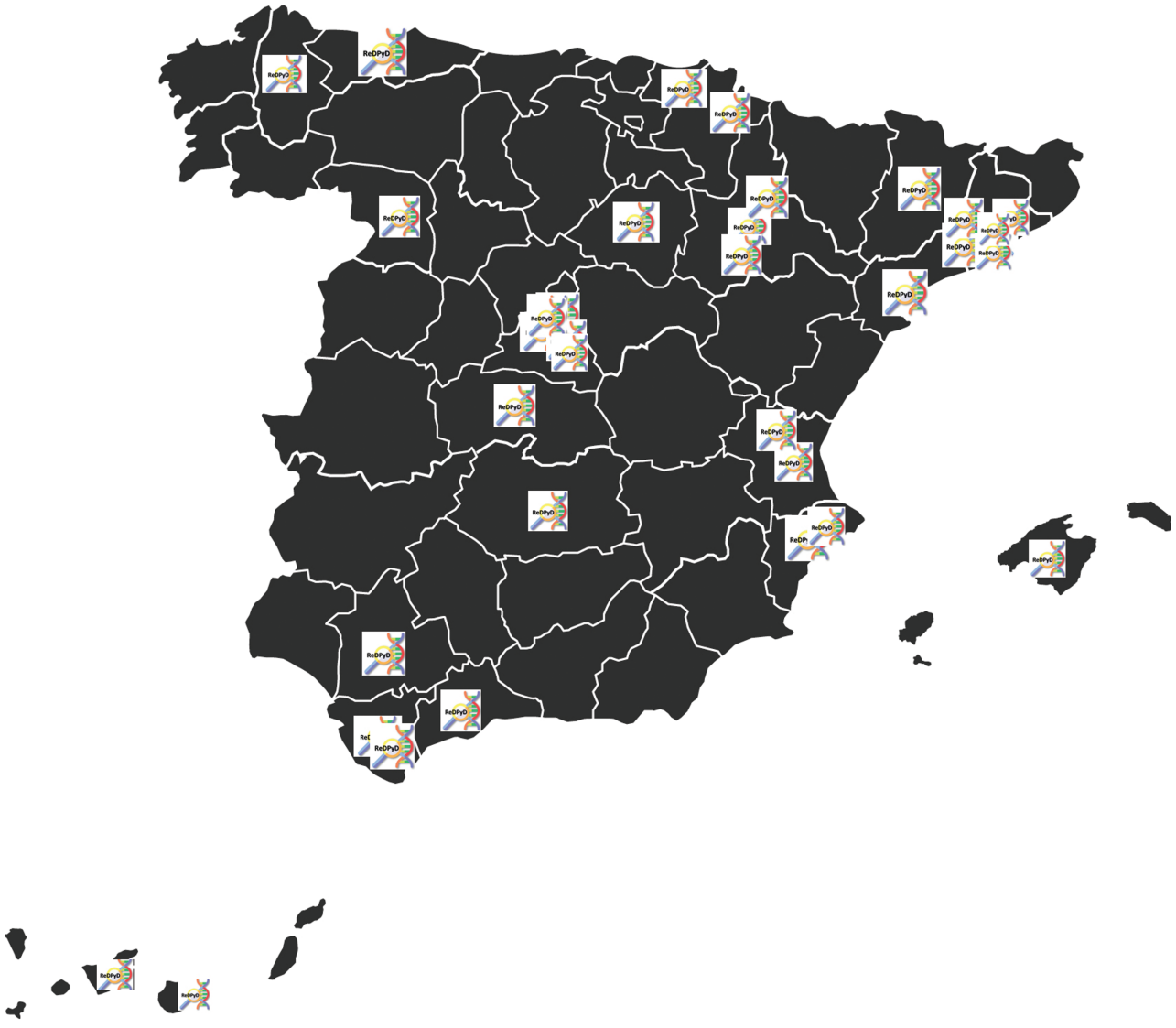

Figure 1. Map despicting the participating institutions in the PhotoDPyD study. This cover has been designed using an image from Freepik. https://www.freepik.es/vector-gratis/mapa-espana_2454242.htm#query=mapaespa%C3%B1a&position=0&from_view=search&track=ais?log-in=google.

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All oncological patients in treatment with fluoropirimidines for colorectal, gastric, gastrointestinal (including esophagus and pancreas), breast and head and neck cancers with *DPYD* genotype were recruited in the participant hospitals after providing written informed consent. The inclusion criteria were the following: cancer diagnosis, age over 18 years old and with a genotyping study of the following *DPYD* variants: *DPYD*2A* (rs3918290); c.1679T>G (rs55886062); c.2846A>T (rs67376798); and c.1129-5923C>G (rs75017182) (HapB3). The study was approved by Complejo Hospitalario Universitario de Canarias regulatory committee and its promoter was the Spanish Foundation of Hospital Pharmacy (FEFH).

Results

Patients Included in the Study

Blood samples from 8054 colorectal, gastric, gastrointestinal (including esophagus and pancreas), patients with breast and head and neck cancer from 40 different hospitals were used to determine the prevalence of the selected genetic variants. Baseline characteristics of the patients (in the 89.1% of the patients was possible to obtain this information) are depicted in Table 1.

Hospital characteristics of the included patients and the number of patients screened for *DPYD* mutations are detailed in Table 2.

Frequency of the *DPYD* Genetic Variants

The 4 previously described variants located in the *DPYD* gene were screened in all patients. Of the overall patients, 95.1% (7663) were wild type, 4.6% (374) were heterozygous for one non-functional allele and 0.2% (17) carried 2 non-functional alleles (12 in homozygosis and 5 were double heterozygosis patients). As described in Table 3, the most frequently identified variant was c.1129-5923C>G (rs75017182) (HapB3), in 209 patients (2.6%), followed by c.2846A>T (rs67376798) in 105 patients (1.3%), c.1905 + 1G>A (rs3918290, DPYD*2A) in 55 patients (0.7%) and c.1679T>G (rs55886062) in 15 patients (0.2%). In 0.1% (12) of the patients, the variants were found in homozygosis; specifically, the c.1129-5923C>G (rs75017182) (HapB3) variant was found in homozygosis in 7 patients (0.09%), the *DPYD* c.2846A>T (rs67376798, p.D949V) in 3 (0.04%) and the c.1905 + 1G>A (rs3918290, *DPYD*2A*) in 2 (0.02%) patients.

Table 1. Baseline characteristics of the patients included (*N* = 7.107, 89.1% of the total; *N* = number).

Age (mean ± SD)	67.5 ± 12.5
Sex (*N*, %)	
Female	3082 (43.4)
Type of tumor (*N*, %)	
Colorectal	5852 (82.3%)
Gastric	215 (3.0%)
Gastrointestinal	390 (5.5%)
Breast	475 (6.7%)
Head and neck	129 (1.8%)
Others	46 (0.6%)

Table 2. Hospital characteristics of the included patients and the number of patients screened for DPYD mutations.

	Percentage of hospitals (*N* of hospitals/total of hospitals)	Mean of patients screened for *DPYD* mutations (*N* of patients screened/*N* of hospitals)
Hospitals of less than 250 beds	22.5% (9/40)	35.0 (314/9)
Hospitals of more than 250 beds but less than 500 beds	27.5% (11/40)	90.2 (992/11)
Hospitals of more than 500 beds but less than 1000 beds	35.0% (14/40)	315.3 (3313/14)
Hospitals of more than 1000 beds	15.0% (6/40)	572.5 (3435/6)

Table 3. Frequency of carriers of functionally relevant DPYD variants in heterozygosis (including compound heterozygous).

dbSNP	Nucleotide change	*DPYD* allele	*N* (%)
rs3918290	c.1905 + 1G>A	*DPYD*2A*	55 (0.7%)
rs55886062	C.1679T>G	*DPYD*13*	15 (0.2%)
rs67376798	c.2846A>T	Non-described	105 (1.3%)
rs75017182	c.1129-5923C>G	HapB3	209 (2.6%)

Abbreviations: SNP: single-nucleotide polymorphism; dbSNP: database SNP. Source: CPIC.[5]

Allelic Frequency of *DPYD* Genetic Variants

Considering the results abovementioned, the allelic frequency of the 4 *DPYD* variants determined in the present study are 0.014 for c.1129-5923C>G, 0.007 for c.2846A>T, 0.004 for c.1905 + 1G>A and 0.0009 for c.1679T>G.

Multiple *DPYD* Variant Carriers

We detected 5 double heterozygous patients (0.06%), 2 carrying the *DPYD* variants c.1129-5923C>G + c.2846A>T, 2 the *DPYD*2A* + c.2846A>T and another one *DPYD*2A* + c.1129-5923C>G (Table 4). It was not possible to detect the phasing (ie, allelic location of variants, *cis* or *trans* orientation) in any of the 6 patients.[7]

Discussion

To the best of our knowledge, this is the largest cohort of unselected patients with cancer in which the most relevant *DPYD* genetic variants (*DPYD*2A* (rs3918290), c.1679T>G (rs55886062), c.2846A>T (rs67376798), and c.1129-5923C>G (rs75017182) (HapB3) have been identified through genotyping. In line with the results previously described in a pilot study,[8] our results confirm that approximately a 5% of the Spanish cancer patient population carries at least a defective *DPYD* variant, highlighting the relevance of performing *DPYD* genotyping before starting a fluoropyrimidine-based regimen.

The mutations that showed the least frequency were those that imply a nullity in the DPD activity (c.1905 + 1G>A (*DPYD*2A)* and c.1679T>G), found in 0.7% and

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Table 4. Compound heterozygous *DPYD* variant allele carriers.

Patient/s	*DPYD* variants	Theoretical DPD activity[1]	Frequency
1 and 2	c.1129-5923C>G + c.2846A>T	1	0.02%
3 and 4	*DPYD*2A* + c.2846A>T	0.5	0.02%
5	*DPYD*2A* + c.1129-5923C>G	0.5	0.01%

Abbreviation: DPD: dihidropyridine deshydrogenase.

0.2% of the patients, respectively, which coincides with those previously described in other studies, where it is indicated that 0.01%-0.5% of the population has a total activity deficit,[9] and between 3% and 8% have a partial deficit.[10] As it was already reported in Caucasians, we found that the variants c.2846A>T and c.1129-5923C>G (rs75017182) (HapB3) were the most common ones, with a frequency of 1.3% and 2.6% of heterozygous carriers, respectively.[11]

Our results also reveal that 12 patients (0.1%) presented one of the variants in homozgyosis, being again the c.1129-5923C>G (rs75017182) (HapB3) the variant most found, and that 0.04% of the population showed a homozygous genotype for the c.1905 + 1G>A (*DPYD**2A) mutation. We detected a frequency of 0.06% of patients carrying multiple *DPYD* variants, which is lower in comparison of what it is described in the literature from data provided from publicly available databases (approximately 0.2%), but not from well-performed studies.[7,12] Therefore, to our knowledge, this is the first study to analyze the frequency of patients carrying multiple *DPYD* variants. Patients carrying multiple *DPYD* variants could be at higher risk of developing severe toxicity; however, standard dose reductions from DPWG and CPIC guidelines cannot be accurately applied, as the phasing of them is generally unknown. If the 2 variants are located in opposite alleles, the risk of toxicity is supposed to be higher. Despite the low population frequency observed, the absolute number of identified compound heterozygous patients will increase as the number of genotyped patients increases and the panel of tested variants expanses. As for homozygous carriers, their higher risk of fluoropyrimidine-induced severe toxicity is well established and reported in several clinical guidelines.[4-6]

This study has some limitations. First, it was not possible to identify the ethnic groups of the patients that composed our study population. However, it is expected that the main ethnic group is Caucasian. Second, as the information regarding patients carrying multiple *DPYD* variants is reported mostly in case series, the comparison of their frequency is challenging, which highlights the importance of designing well-performed epidemiological studies providing valuable information regarding compound heterozygous patients.

Conclusion

This study showed that approximately 5% of the Spanish cancer patient population have a reduced DPD activity, with the clinical implications associated to this fact in terms of fluoropyrimidine treatment-induced toxicity. These results are robust, as our cohort of more than 8000 patients is the largest thus far reported.

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Conflict of Interest

The authors indicated no financial relationships.

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Author Contributions

Conception/design: M.M., F.G.N. Collection and/or assembly of data: All authors. Data analysis and interpretation: All authors. Manuscript writing and final approval of manuscript: All authors.

Data Availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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