Clinical Pharmacogenetics Implementation Consortium Guidelines for Dihydropyrimidine Dehydrogenase Genotype and Fluoropyrimidine Dosing

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The fluoropyrimidines are the mainstay chemotherapeutic agents for the treatment of many types of cancers. Detoxifying metabolism of fluoropyrimidines requires dihydropyrimidine dehydrogenase (DPD, encoded by the DPYD gene), and reduced or absent activity of this enzyme can result in severe, and sometimes fatal, toxicity. We summarize evidence from the published literature supporting this association and provide dosing recommendations for fluoropyrimidines based on DPYD genotype (updates at http://www.pharmgkb.org).

The purpose of this guideline is to provide information to allow the interpretation of clinical dihydropyrimidine dehydrogenase (*DPYD*) genotype tests so that the results can be used to guide dosing of fluoropyrimidines (5-fluorouracil, capecitabine, and tegafur). Detailed guidelines for use of fluoropyrimidines, their clinical pharmacology (see ref. 1 for review), and analyses of the cost-effectiveness are beyond the scope of this article. The Clinical Pharmacogenetics Implementation Consortium guidelines consider the situation of patients for whom genotype data are already available.[2]

FOCUSED LITERATURE REVIEW

A systematic literature review focused on the *DPYD* genotype and the use of 5-fluorouracil, capecitabine, and tegafur (details in **Supplementary Material** online) was conducted, with reviews used as summaries of earlier literature.

GENE: *DPYD*

Background

Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme for fluoropyrimidine catabolism and eliminates >80% of administered 5-fluorouracil.[3] DPD levels show high inter- and intraindividual variation, and this variability is likely to influence response of patients to 5-fluorouracil with respect to toxicity, resistance, and efficacy.[4] In patients who are deficient in DPD, 5-fluorouracil can cause profound toxicity, such as myelosuppression, mucositis, neurotoxicity, hand–foot syndrome, and diarrhea. Familial studies have demonstrated that this is an autosomal codominantly inherited trait.[5]

DPYD, the gene encoding DPD, is a large gene with 4,399 nucleotides in 23 coding exons spanning 950 kb on chromosome 1p22.[6] The most well-studied variant is *DPYD*2A* (also known as *DPYD*:IVS14 + 1G>A, c.1905+1G>A, or rs3918290).[7] It is a single-nucleotide polymorphism at the intron boundary of exon 14 that results in a splicing defect, skipping of the entire exon, and a nonfunctional protein.[8] Recently, Offer *et al.* measured the relative sensitivity to 5-fluorouracil of cells expressing DPD variations and confirmed that *DPYD*2A* is catalytically inactive.[9] This allele is considered relatively rare, although it is more common than most other known inactivating variants in *DPYD*. Estimates of the frequency of the **2A* allele range from <0.005 (in the HapMap CEU, YRI, JPT, and HCB populations and several other studies) to 3.5% in a Swedish population.[10]

The most frequently observed variants are **5* (rs1801159 T>C), **6* (rs1801160 C>T), and **9A* (rs1801265 A>G) at frequencies of 11.5–30, 0.7–9, and 2.9–13.7%, respectively, and data regarding their effects on DPD activity are contradictory.[6,11–14] However, the Dutch Pharmacogenetics Working Group has designated these alleles as "functional" on the basis of the lack of an association with toxicity reported in studies and/or decreased clearance or activity.[15] *DPYD*3* (rs72549303 C>del), **13* (rs55886062 A>C), and rs67376798T>A are also relatively

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rare but result in low DPD activity and/or 5-fluorouracil toxicity (see **Supplementary Tables S1 and S2** online).[5,16,17] Moreover, most variants of phenotypic consequence in *DPYD* are of very low frequency, and several studies did not observe any individuals with these variants.[11,13,14,18,19] Recently, a novel *DPYD* variant (*Y186C*) was identified only in the African-American population (found in 26% of African-American patients with reduced DPD activity). Individuals carrying this allele had a 46% reduction in DPD activity as compared with noncarriers.[20]

Patients with <70% of the mean observed leukocyte DPD protein activity in the normal population are considered at risk for the development of severe toxicity after administration of 5-fluorouracil (or its prodrugs).[21] The relationship between *DPYD* genotype and phenotype is complicated; although several variants have been associated with low DPD activity (**2A*, **13*, and rs67376798) and fluoropyrimidine toxicity (**2A*, **13*, and rs67376798) or have been observed in other cases of toxicity (**2A*, **4*, **6*, **9A*, **13*, and rs67376798), the presence of these variants does not always result in toxicity, and associations have not been consistently replicated (discussed in refs. 9,11,19,22 and other publications).

The inconsistency in study results may be explained by the substantial variation in treatment regimens across studies. In a study by Schwab *et al.*[11] including 683 cancer patients, *DPYD*2A* was found to play a limited role in 5-fluorouracil-related toxicity; of note, only patients receiving 5-fluorouracil monotherapy were included. By contrast, Morel *et al.* found strong associations in a cohort of 487 patients between both *DPYD*2A* and rs67376798 and severe 5-fluorouracil toxicity in patients receiving combination therapy. Studies linking *DPYD*2A* to toxicity generally include patients on combination therapies, suggesting that concomitant drugs may enhance the effect of *DPYD* risk alleles. Furthermore, Schwab *et al.* also observed a higher rate of severe toxicities in patients receiving bolus-based 5-fluorouracil than in patients receiving continuous infusion, suggesting a dose-dependent effect of 5-fluorouracil. Moreover, several studies have shown that only ~50% of heterozygote carriers of a low-activity allele develop severe 5-fluorouracil toxicity.[11,18,19] This may indicate allelic regulation of *DPYD* or compensation by another *DPYD* variant on the second allele, resulting in greater DPD activity.[22] Recently, *DPYD* haplotypes (e.g., haplotype B3) have been considered to be more predictive in identifying patients at risk for severe 5-fluorouracil-related[19] and capecitabine-related toxicities (grade ≥3).[23] However, data on functional consequences of these haplotypes are so far incomplete. Promoter methylation altered expression in cell lines;[24] however, methylation was not associated with toxicity in patients.[11] MicroRNAs have also been implicated in the regulation of *DPYD*, but their relevance for the modulation of DPD phenotypes with respect to drug response has not been tested.[25] Nevertheless, >20% (23.3–38%) of 5-fluorouracil toxicities can be explained by combining multiple *DPYD* variants, suggesting a significant importance of *DPYD* variation for the risk of 5-fluorouracil-related toxicities.[18,19,26,27]

Genetic test interpretation

Each named * allele is defined by the genotype at one or more specific single-nucleotide polymorphisms (**Supplementary Table S1** online). DPD function associated with the most common allelic variants is summarized in **Supplementary Table S2** online. **Table 1** summarizes the assignment of the probable DPD phenotype on the basis of the * allele diplotypes, and these assignments are used to link genotypes with fluoropyrimidine dosing. Briefly, homozygotes of **2A*, **13*, and rs67376798 are considered deficient in DPD; heterozygotes for any combination of **2A*, **13*, and rs67376798 have intermediate or partial DPD activity; and those with none of these alleles are likely to have normal, high activity. *DPYD* alleles have been extensively studied in multiple geographically, racially, and ethnically diverse groups and are summarized in **Supplementary Tables S3 and S4** online. Because of conflicting data or weak evidence for alleles other than **2A*, **13*, and rs67376798, this guideline does not currently report dosing recommendations for other variants of *DPYD*. Reports of other variants and phenotypes are discussed in the **Supplementary Material** online.

Available genetic test options

There are several testing options for *DPYD* genotype, although, at present most test only for the *DPYD*2A* variant. A list of testing services is provided in an online linked format at PharmGKB (http://www.pharmgkb.org/gene/PA145) and the National Institutes of Health Genetic Testing Registry (http://www.ncbi.nlm.nih.gov/gtr/conditions/C2720286/ or http://www.ncbi.nlm.nih.gov/gtr/conditions/CN077983/).

Incidental findings

Individuals who harbor one copy of variant *DPYD* can be considered to have carrier status for an inborn error of metabolism,

Table 1 Assignment of likely DPD phenotype based on genotype

Likely phenotype	Genotypes	Examples of diplotypes
Homozygous for wild-type allele or normal, high DPD activity	An individual carrying two or more functional (**1*) alleles	**1/*1*
Heterozygote or intermediate activity (~3–5% of patients); may have partial DPD deficiency; at risk for toxicity with drug exposure	An individual carrying one functional allele (**1*) plus one nonfunctional allele (**2A*, **13*, or rs67376798)	**1/*2A*; **1/*13*; or *1/rs67376798
Homozygous variant or mutant; DPD deficiency (~0.2% of patients); at risk for toxicity with drug exposure	An individual carrying two nonfunctional alleles (**2A*, **13*, or rs67376798)	*2A/*2A; 13/*13; or rs67376798/rs67376798

DPD, dihydropyrimidine dehydrogenase.

and consideration should be given to its potential effects on offspring. Patients homozygous for inactivating variants of the *DPYD* gene have DPD deficiency, a disease that shows large phenotypic variability, ranging from no symptoms to severe convulsive disorders with motor and mental retardation.[28,29]

Other considerations

Several other genes may influence responses to 5-fluorouracil[3,11] (see **Supplementary Material** online). The well-studied genes among these are *ABCB1*, *MTHFR*, and *TYMS*, although results have been inconsistent to date, and predictive dosing strategies have yet to be successfully applied. Some of the testing options for 5-fluorouracil toxicity and *DPYD* also include testing for other gene variants in *TYMS* and *MTHFR*. For a summary of pharmacogenomic studies of 5-fluorouracil, see the PGx Research tab at http://www.pharmgkb.org/drug/PA128406956.

There are alternatives to genotyping of *DPYD* that assess DPD activity directly, including dihydrouracil/uracil ratio determination in plasma, the uracil breath test method, measurement of DPD activity in peripheral mononuclear cells, and pharmacokinetically guided strategies such as the 5-fluorouracil test dose method (see ref. 30 for further information). Studies using dose reduction of 5-fluorouracil in patients with DPD deficiency, as evidenced by the use of one of these functional tests, have shown a reduction in drug-related toxicities while maintaining efficacy in these patients.[31,32]

DRUGS: FLUOROPYRIMIDINES

Background

Fluoropyrimidines such as 5-fluorouracil, capecitabine, and tegafur are widely used in the treatment of solid tumors, including colorectal and breast cancer and cancers of the aerodigestive tract. More than 2 million patients receive these types of drugs annually.[19] Approximately 10–40% of 5-fluorouracil patients develop severe, and sometimes life-threatening, toxicity (neutropenia, nausea, vomiting, severe diarrhea, stomatitis, mucositis, hand–foot syndrome, and neuropathy).[19]

Only 1–3% of the administered 5-fluorouracil dose has been found to be metabolized to cytotoxic metabolites, with ~80% of the administered dose being degraded or excreted in the urine. DPD is the first and rate-limiting step in the catabolic pathway converting 5-fluorouracil to dihydrofluorouracil.[3] Dihydrofluorouracil is then converted to fluoro-β-ureidopropionate and fluoro-β-alanine, which are then excreted in the urine (http://www.pharmgkb.org/pathway/PA150653776).[3] Capecitabine and tegafur are prodrugs of 5-fluorouracil that are converted to 5-fluorouracil and then metabolized by DPD as described above.

Fluoropyrimidines are mostly used in combination with various other antineoplastic drugs. Disease and treatment regimens (which are also related to disease background—for example, breast cancer patients tend to receive bolus 5-fluorouracil, whereas colorectal cancer patients tend to receive infusion 5-fluorouracil) may also influence the importance of DPD activity to risk for toxicity.

Linking genetic variability to variability in drug-related phenotypes

There is substantial evidence linking *DPYD* genotype with phenotypic variability in DPD enzyme activity, 5-fluorouracil clearance, and subsequently 5-fluorouracil toxicity (**Supplementary Table S5** online). Evidence providing the basis for the dosing recommendations (**Table 2**) is from two large prospective studies,[11,18] small studies with retrospective genotyping of patients with severe toxicity, and case studies (see **Supplementary Table S5** online).

5-Fluorouracil has a relatively narrow therapeutic window, resulting in a small difference between an efficacious dose and the maximum tolerable dose. Reduced activity of DPD, resulting in reduced clearance and increased half-life of 5-fluorouracil, results in increased risk of dose-dependent severe toxicities.[33–35] Morel *et al.*[18] compared the 5-fluorouracil clearances of patients with *DPYD*2A* ($n = 8$ heterozygotes; $n = 1$ homozygote), **13* ($n = 1$ heterozygote), and rs67376798 ($n = 10$ heterozygotes) with those of patients without a

Table 2 Recommended dosing of fluoropyrimidines by DPD phenotype

Phenotype (genotype)	Implications for phenotypic measures	Dosing recommendations	Classification of recommendations[a]
Homozygous for wild-type allele, or normal, high DPD activity	Normal DPD activity and "normal" risk for fluoropyrimidine toxicity	Use label-recommended dosage and administration	Moderate
Heterozygous, or intermediate activity	Decreased DPD activity (leukocyte DPD activity at 30–70% that of the normal population) and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Start with at least a 50% reduction in starting dose, followed by titration of dose based on toxicity[b] or pharmacokinetic test (if available)	Moderate
Homozygous, or deficient activity	Complete DPD deficiency and increased risk for severe or even fatal drug toxicity when treated with fluoropyrimidine drugs	Select alternative drug	Strong

Fluoropyrimidines: 5-fluorouracil, capecitabine, and tegafur.

DPD, dihydropyrimidine dehydrogenase.

[a]Rating scheme is described in **Supplementary Material** online. [b]Increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy; decrease the dose in patients who do not tolerate the starting dose to minimize toxicities.

known variant or with *DPYD*9A* or c.1590T>C. Mean clearances were 74.9 ± 38.3 l/h·m^2(range: 21.2–183.5 l/h·m^2) and 132.3 ± 46.6 l/h·m^2 (range: 36.8–369.7 l/h·m^2), respectively. They were statistically different ($P < 0.001$). Boisdron-Celle *et al.*[35] compared the 5-fluorouracil clearances in patients with *DPYD*2A* ($n = 2$ heterozygotes) and rs67376798 ($n = 7$ heterozygotes) with the clearances in those with no relevant variant ($n = 163$). 5-Fluorouracil clearances in patients heterozygous for either *DPYD*2A* or rs67376798 were 80 and 40–58% (depending on treatment regimen), respectively, less than the clearances in the group with no variant. One patient had three heterozygote variants: *DPYD*2A*, rs67376798, and 85 T > C, resulting in a 5-fluorouracil plasma clearance close to zero. Both studies found a significant difference in grade 3–4 toxicities in patients with these variants as compared with patients lacking variants. These data indicate that patients heterozygous for these variants have significantly reduced 5-fluorouracil clearances, ranging from 40 to 80% less than the clearances in patients without these variants.

Dosage recommendations

Table 2 summarizes the genetics-based dosing recommendations for *DPYD* genotype and fluoropyrimidines. The strength of the dosing recommendations is based on the facts that some variants (*DPYD*2A*, **13*, and rs67376798) clearly affect DPD activity, DPD activity is clearly related to 5-fluorouracil clearance, and 5-fluorouracil exposure is associated with its toxic effects. Therefore, reduction of fluoropyrimidine dosage in patients with these variants may prevent severe and possibly life-threatening toxicities. However, available evidence does not clearly indicate a degree of dose reduction needed to prevent fluoropyrimidine-related toxicities. **Supplementary Table S6** online summarizes the effects of these variants on 5-fluorouracil clearance and DPD activity. Although the data suggest that patients with the *DPYD*2A* variant may need a greater dose reduction than a patient with the rs67376798 variant, it is unclear to what extent the dose should be reduced. Furthermore, patients who are heterozygous for the nonfunctional *DPYD* variants mostly demonstrate partial DPD deficiency (leukocyte DPD activity at 30–70% that of the normal population).[16,21,26,34,35] Thus, our recommendation is to start with at least a 50% reduction of the starting dose; followed by an increase in dose in patients experiencing no or clinically tolerable toxicity, to maintain efficacy; and a decrease in dose in patients who do not tolerate the starting dose, to minimize toxicities. An alternative is pharmacokinetic-guided dose adjustment (if available). Patients who are homozygous for *DPYD*2A, *13,* or rs67376798 may demonstrate complete DPD deficiency,[17,21,23] and the use of 5-fluorouracil or capecitabine is not recommended in these patients. Because capecitabine and tegafur are converted to 5-fluorouracil and then metabolized by DPD, the clearance of and exposure to 5-fluorouracil, in addition to its toxic effects, are similar in patients with these variants.[23,36]

The US Food and Drug Administration (FDA) has added statements to the drug labels for 5-fluorouracil (topical only) and capecitabine that contraindicate use in patients with DPD enzyme deficiency. The FDA drug label also warns to use precaution with intravenous 5-fluorouracil in these patients. The Dutch Pharmacogenetics Working Group has evaluated therapeutic dose recommendations for 5-fluorouracil, capecitabine, and tegafur (5-fluorouracil prodrug combined with uracil; not available in United States).[15] The Working Group recommends the use of an alternative drug for homozygous carriers of a decreased-activity allele and a reduced dose or alternative drug to capecitabine or 5-fluorouracil for heterozygous carriers of a decreased-activity allele.[15]

At the time of this writing, there are no data available on the possible role of *DPYD*2A, *13,* or rs67376798 in 5-fluorouracil toxicities in pediatric patient populations; however, there is no reason to suspect that variant *DPYD* alleles would affect 5-fluorouracil metabolism differently in children as compared with adults.

Recommendations for incidental findings

DPD deficiency is a clinically heterogeneous autosomal recessive disorder of pyrimidine metabolism resulting in wide variability of clinical presentations.[28] These symptoms generally present in childhood, with the majority of patients showing symptoms within the first year of life. Currently, there is no correlation between symptom severity and DPD function and/or genetics. However, early diagnosis is crucial because of the potential of life-threatening defects. Therefore, early phenotypic (e.g., urine screening of uracil and its degradation products) and/or genetic testing (pre- or postnatal) of offspring of *DPYD*-variant carriers could aid in early diagnosis and prevent unnecessary and costly diagnostic testing.[29,37]

Other considerations

Some studies have suggested that the patient's gender may influence the likelihood of 5-fluorouracil toxicity, although the results have been contradictory and the mechanism is unknown. Several studies showed increased numbers of women, as compared with men, among patients with 5-fluorouracil toxicity,[11,26] although in one study, this was not significant when excluding patients with breast cancer, suggesting in that instance that the effect may have been an artifact of different treatment regimens.[26] Early studies showed lower DPD activity and lower clearance of 5-fluorouracil in women as compared with men.[38,39] In one of the largest studies of *DPYD* variants and 5-fluorouracil toxicity, the association of *DPYD*2A* with increased risk for toxicity was more significant in men than in women, even though there was no difference between DPD activity and protein content in histologically normal liver tissues of male and female donors.[11] The use of folinic acid and the mode of 5-fluorouracil infusion have also been associated with 5-fluorouracil toxicity and should be considered when estimating a patient's individual risk of toxicity with 5-fluorouracil (see ref. 11 for further information).

There is some evidence from the work of Gamelin *et al.*[40] that individual pharmacokinetically guided dosing of 5-fluorouracil therapy in patients with metastatic colorectal cancer improves treatment outcome with a reduced number of

5-fluorouracil-related adverse drug reactions. Of note, in a recent paper by Deenen *et al*., data are provided indicating that the cumulative dose of capecitabine per course was significantly decreased in patients heterozygous for *DPYD*2A* (~50%) or rs67376798 (~25%) as compared with that in patients with the wild-type allele, and therapy could be continued safely in variant cases. Unfortunately, no data on capecitabine pharmacokinetics are included. Indirect determination of the endogenous uracil/dihydrouracil plasma ratio as a surrogate for DPD activity could be used to titrate 5-fluorouracil-based chemotherapy with reduction of severe 5-fluorouracil-related toxicities.[31]

POTENTIAL BENEFITS AND RISKS FOR THE PATIENT

The main benefit to the patient would be the potential to avoid toxicity by using either alternative therapy or lower fluoropyrimidine doses. The aim is to prevent the most severe and fatal instances of toxicity, but some patients who would not have experienced this degree of toxicity and who would have benefited from fluoropyrimidine therapy may be advised against it. Moreover, heterozygous patients who receive a lower dose of a fluoropyrimidine and who would not have experienced this degree of toxicity may not experience the full benefit of fluoropyrimidine therapy; therefore, it is important to increase the dose in patients experiencing no or clinically tolerable toxicity to maintain efficacy. Patients who proceed with 5-fluorouracil therapy may still experience lower-grade toxicity that may be acceptable and even necessary in order to achieve efficacy. Some patients without a variant *DPYD* allele may still experience severe toxicity due to other genetic, environmental, or other factors.

A possible risk is the misreporting or misinterpretation of genotype test results. This mistake could be recorded in the patient record and could also influence further treatments.

CAVEATS: APPROPRIATE USE AND/OR POTENTIAL MISUSE OF GENETIC TESTS

The positive predictive value and negative predictive value of *DPYD*2A* genotyping to predict development of severe toxicity (grade 3) are ~50 and ~95%, respectively;[11] however, taking into account other variant alleles, such as rs67376798 and *DPYD*13,* increases the positive predictive value to 62% (the negative predictive value remains unchanged).[18] Furthermore, the sensitivity calculated in this study for this genotype test was only 31%; therefore, the absence of these variants does not rule out DPD defects. Although many additional variants of *DPYD* are known (see **Supplementary Tables S1, S3, and S4** online), the frequencies are often very low, and evidence for their functionality is limited.

DISCLAIMER

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines reflect expert consensus based on clinical evidence and peer-reviewed literature available at the time they are written and are intended only to assist clinicians in decision making and to identify questions for further research. New evidence may have emerged since the time a guideline was submitted for publication. Guidelines are limited in scope and are not applicable to interventions or diseases that are not specifically identified. Guidelines do not account for individual variations among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It remains the responsibility of the health-care provider to determine the best course of treatment for a patient. Adherence to any guideline is voluntary, with the ultimate determination regarding its application to be made solely by the clinician and the patient. CPIC assumes no responsibility for any injury or damage to persons or property arising out of or related to any use of CPIC's guidelines or for any errors or omissions.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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