The Journal of Molecular Diagnostics, Vol. ■, No. ■, ■ 2024

ELSEVIER

the Journal of Molecular Diagnostics

jmdjournal.org

SPECIAL ARTICLE

DPYD Genotyping Recommendations

A Joint Consensus Recommendation of the Association for Molecular Pathology, American College of Medical Genetics and Genomics, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, Pharmacogenomics Knowledgebase, and Pharmacogene Variation Consortium

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Supported exclusively by the Association for Molecular Pathology.

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Standard of practice is not defined by this article, and there may be alternatives. See *Disclaimers* for further details.

https://doi.org/10.1016/j.jmoldx.2024.05.015

Accepted for publication
May 21, 2024.

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The goals of the Association for Molecular Pathology Clinical Practice Committee's Pharmacogenomics (PGx) Working Group are to define the key attributes of pharmacogenetic alleles recommended for clinical testing and a minimum set of variants that should be included in clinical PGx genotyping assays. This document series provides recommendations for a minimum set of variant alleles (tier 1) and an extended list of variant alleles (tier 2) that will aid clinical laboratories when designing assays for PGx testing. The Association for Molecular Pathology PGx Working Group considered the functional impact of the variant alleles, allele frequencies in multiethnic populations, the availability of reference materials, and other technical considerations for PGx testing when developing these recommendations. The goal of this Working Group is to promote standardization of PGx testing across clinical laboratories. This document will focus on clinical *DPYD* PGx testing that may be applied to all dihydropyrimidine dehydrogenase–related medications. These recommendations are not to be interpreted as prescriptive but to provide a reference guide. *(J Mol Diagn 2024, ■: 1–13; https://doi.org/10.1016/j.jmoldx.2024.05.015)*

Clinical pharmacogenomics (PGx) tests can aid clinicians with medication management based on a patient's individual genetic profile, which is an increasingly adopted strategy for implementing personalized medicine.[1] However, previous studies have shown that available clinical PGx tests have wide variability with the alleles they include,[2,3] which can range from interrogating a limited number of variants/alleles in a pharmacogene to sequencing selected exons or the entire coding region of a gene. This can lead to an individual's genotype being reported as normal or negative for the interrogated variants, although clinically relevant variants may still be present if not interrogated and may have the effect of reducing clinical sensitivity and negative predictive value. Discrepancies in interpretation and implementation of PGx testing may result in inconsistent or erroneous clinical management. Until recently, there has been little effort to standardize the content or specific variants/alleles that should be included in clinical PGx tests.

To address this issue, the Association for Molecular Pathology (AMP) PGx Working Group has developed a series of documents that recommend a minimum set of variants to include in clinical PGx assays to facilitate standardization across laboratories and ensure that clinically relevant variant alleles are included in clinical PGx assays. The previous AMP PGx Working Group documents covered *CYP2C19*,[4] *CYP2C9*,[5] genes important for warfarin PGx testing,[6] *CYP2D6*,[7] *TPMT/NUDT15*,[8] and *CYP3A4/CYP3A5*.[9]

This current document focuses on *DPYD* and is intended to provide guidance to clinical laboratories and assay manufacturers who develop, validate, and/or offer clinical *DPYD* pharmacogenomic testing. Although the goal of this document is specifically to aid in allele selection for targeted genotyping assays, laboratories may also consider sequencing the *DPYD* gene to cover the large number of reportable variants associated with severe fluoropyrimidine-related toxicity in patients with dihydropyrimidine dehydrogenase (DPD) deficiency. However, these recommendations are not intended for diagnostic *DPYD* genetic testing of the autosomal recessive DPD deficiency disorder. This document should be implemented together with other relevant clinical guidelines, including those published by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG), both of which focus primarily on the interpretation of PGx test results and therapeutic recommendations for specific drug-gene pairs (*https://www.pharmgkb.org/guidelineAnnotations*, last accessed January 5, 2024).

The AMP PGx Working Group uses a two-tier strategy for selection criteria in recommending PGx variants for clinical testing.[4–9] Briefly, tier 1 recommended variants are those that meet the following criteria: i) have a well-characterized effect on the function of the protein and/or gene expression, ii) have an appreciable minor allele frequency in a population/ancestral group, iii) have publicly available reference materials (RMs), and iv) are technically feasible for clinical laboratories to interrogate using standard molecular testing methods. Tier 2 recommended variants meet at least one but not all the tier 1 criteria. Tier 2 variants may be reclassified to tier 1 if additional information or RM(s) become available. Variants with unknown effect on protein function or gene expression are not included in these recommendations for clinical genotyping assays.

DPD and the *DPYD* Gene

DPD [NADP(+); other names include DPYD; EC:1.3.1.2] is involved in the reversible reduction of uracil and thymine as well as the chemotherapeutic drug 5-fluorouracil (5-FU). The DPD protein is encoded by the *DPYD* gene, which is located on chromosome 1p21.3, spans 843 kilobases in length, and contains 23 exons.[10–13] The DPD protein is expressed in many tissues, with liver and peripheral blood having the highest expression levels and enzymatic activity.[14] Biallelic loss-of-function variants in *DPYD* cause severe DPD deficiency, which is a rare autosomal recessive disorder characterized by thymine-uraciluria and other variable features, including failure to thrive, microcephaly, seizures, and both motor impairment and intellectual disability (Online Mendelian Inheritance in Man number 274270, *https://omim.org/clinicalSynopsis/274270*, last accessed January 5, 2024).

Fluoropyrimidines, antimetabolite drugs including 5-FU, capecitabine, and tegafur, are widely used to treat a variety

of cancers.[15] Both capecitabine and tegafur are prodrugs of 5-FU, which are metabolized to the active form through several enzymatic reactions. The rate-limiting step of conversion from 5-FU to inactive metabolites is catalyzed by DPD. Individuals with decreased DPD activity are less able to break down fluoropyrimidines to inactive metabolites, thereby increasing exposure to active drug moieties. Compromised DPD activity increases an individual's risk of experiencing potentially life-threatening fluorouracil toxicity, including bone marrow suppression, gastrointestinal toxicity, and neurotoxicity (*https://www.ncbi.nlm.nih.gov/books/NBK385155* and *https://omim.org/clinicalSynopsis/274270*, last accessed January 5, 2024).[15,16]

Over 1598 sequence variants in the *DPYD* gene have been identified (Genome Aggregation Database version 4.0.0),[17] of which a selection is listed by the Pharmacogene Variation Consortium (PharmVar; *https://www.pharmvar.org/gene/DPYD*, last accessed January 5, 2024). Although some variants have been characterized as having normal, decreased, or no DPD enzyme activity, the functional and clinical impacts remain uncertain for most of these variants, especially those that are rare. *DPYD* variants known to have pharmacogenomic significance were historically described using star alleles or alternative names [eg, **2A*, haplotype B3 (HapB3)]; however, this has recently transitioned toward using the standard Human Genome Variation Society nomenclature (*https://hgvs-nomenclature.org/stable*, last accessed May 6, 2024) as the actionable variants are relevant whether found as part of a larger haplotype or independently as rare variants. 5-FU toxicity may also be observed in patients with partial DPD deficiency, which has a frequency of approximately 3% to 8% that varies among populations. However, severe DPD deficiency is a rare autosomal recessive disorder with large phenotypic variability, including intellectual disability, motor impairment, and seizures (*https://medlineplus.gov/genetics/condition/dihydropyrimidine-dehydrogenase-deficiency/#frequency*, last accessed January 5, 2024).

Testing for variants in the *DPYD* gene can help identify patients at risk of developing fluoropyrimidine toxicity who should receive reduced doses or avoid treatment with capecitabine and fluorouracil, as recommended by CPIC and DPWG guidelines. The association of *DPYD* with 5-FU and capecitabine toxicity are also included in the US Food and Drug Administration Table of Pharmacogenomics Biomarkers in Drug Labeling with indications for warnings and precautions (*https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling*, last accessed January 5, 2024). Germline diagnostic testing for autosomal recessive DPD deficiency at clinical laboratories may be distinct from pharmacogenomic *DPYD* testing, as sequencing is more commonly implemented for diagnostic testing. As such, the variants analyzed, test design, interpretation, and clinical use are different; however, some laboratories may offer a single *DPYD* genetic test for both diagnostic and pharmacogenomic indications.

DPYD Variants and Haplotypes

Some *DPYD* variants were assigned star (*) allele numbers when first published (*DPYD*1* to *DPYD*13*), as proposed by McLeod et al in 1998,[18] whereas others were reported and referred to by a descriptive name (eg, Hap3B) or a Single Nucleotide Polymorphism Database reference SNP cluster ID (dbSNP rsID) (*https://www.ncbi.nlm.nih.gov/snp*, last accessed January 5, 2024). However, the use of star nomenclature based on haplotypes capturing all variants within a defined gene region was deemed impractical by the PharmVar *DPYD* Gene Expert panel because of the size of the gene (843 kilobases) and the presence of recombination between exons, which makes haplotype phasing across all exonic regions extremely difficult. Furthermore, the PharmVar *DPYD* experts also argued that many of the functionally relevant variants are rare, and if detected, clinicians may act on their presence regardless of whether the haplotype has other variants. Contrary to the star allele nomenclature used for other pharmacogenes, such as *CYP2C19* or *CYP2D6*, which captures all variants on an allele across a defined region, the star designation for *DPYD* was originally used to describe individual variants rather than haplotypes. To address these gene-specific challenges, PharmVar lists rsIDs for *DPYD* (*https://www.pharmvar.org/gene/DPYD*, last accessed January 5, 2024) with star designations being shown as legacy names. *DPYD* variants should be described using standard Human Genome Variation Society nomenclature instead of the legacy star allele names. Because more than two variants can be found in an individual, the CPIC guideline uses a DPD activity score to facilitate standardized reporting for predicted overall DPD activity. Briefly, normal function variants have an activity value of 1, nonfunctional variants have a value of 0, and decreased activity variants are assigned a value of 0.5. The two lowest scoring variants are used to calculate the activity score, which is mapped to the prescribing recommendation (*https://www.pharmgkb.org/page/dpydRefMaterials*, last accessed January 5, 2024).

Existing Clinical Guidelines and Recommendations

Drug-gene pair-based clinical guidelines have been developed for 5-FU and capecitabine with *DPYD* by CPIC (2017 update[19]; for additional updates, see *https://www.pharmgkb.org/chemical/PA448771/guidelineAnnotation/PA166109594*, last accessed January 5, 2024), DPWG (*https://www.pharmgkb.org/chemical/PA448771/guidelineAnnotation/PA166104963*, last accessed January 5, 2024),[20] and experts from the Spanish Pharmacogenetics and Pharmacogenomics Society, the Spanish Society of

Medical Oncology, and the French National Network of Pharmacogenetics.[21,22] All recommend that an alternative drug be used for patients who are predicted to be DPD poor metabolizers with an activity score of 0 or 0.5, and a genotype-guided dosing adjustment is recommended for individuals who are predicted to be DPD intermediate metabolizers (activity score of 1 or 1.5). The DPWG considers *DPYD* genotyping as essential before fluoropyrimidine therapy. The French National Network of Pharmacogenetics publication considers the serum dihydrouracil/uracil ratio or lymphocyte DPD activity-based phenotyping essential, and targeted *DPYD* genotyping should be performed if biochemical phenotyping is not available.

Testing Platforms

Selection of a molecular platform or assays to use for testing PGx variants can be based on many factors that include, but are not limited to, the spectrum of sequence variants, technical feasibility of analysis of the genomic region of interest, cost, laboratory workflow, and test turnaround time required. As the *DPYD* gene resides on a genomic region that is amenable to interrogation using standard molecular techniques, clinical molecular laboratories may use targeted genotyping or sequencing (Sanger sequencing or next-generation sequencing) approaches, determined at the discretion of the testing laboratory. Almost all commonly used molecular platforms, except for long-read sequencing technologies,[23–25] are unable to provide phasing information of the detected variants. In addition, as described previously, pathogenic/nonfunctional/reduced function variants in *DPYD* have both a pharmacogenomic indication and clinical implications for diagnosing autosomal recessive DPD deficiency. Therefore, the testing platform chosen, approach for variant classification, result interpretation, post-test recommendations, and clinical implementation can be different for *DPYD* testing for these different clinical indications. This may impact clinical test selection as some laboratories may only perform testing and interpretation for one of these indications, whereas other laboratories will provide an interpretation for both diagnostic and PGx indications.

There are 122 clinical tests for *DPYD* from 47 laboratories worldwide listed in the Genetic Testing Registry (*https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=1806[geneid]*, last accessed January 5, 2024). Except for phenotyping tests (analyte or enzyme activity assays), all other clinical tests are molecular method–based analysis of *DPYD* using targeted genotyping or sequencing. Gene copy number variation (CNV) analysis (ie, testing for the presence of deletions and/or duplications) is included in 63 of 122 *DPYD* tests. In terms of test purpose, diagnosis (93 tests), screening (23 tests), and drug response (19 tests) are the three primary applications of *DPYD* testing.

Materials and Methods

The AMP PGx Working Group is composed of subject matter experts from the American College of Medical Genetics and Genomics, CDC, CPIC, College of American Pathologists, DPWG, European Society for Pharmacogenomics and Personalized Therapy, Pharmacogenomics Knowledgebase, PharmVar, and the PGx clinical testing and research communities. *DPYD* variants were reviewed and classified into two tiers based on four criteria:

(1) Functional characterization of the variant (ie, whether it is known to affect expression of the gene or function of the encoded protein).
(2) Presence at an appreciable minor allele frequency in a population/ancestral group. In this *DPYD* recommendation document, the Working Group used a minor allele frequency in at least one subpopulation of $\geq$0.1% for tier 1 variants, and $\geq$0.01% for tier 2 variants (*https://gnomad.broadinstitute.org* version 4.0.0, last accessed February 11, 2024).
(3) Availability of RMs (Table 1).[26]
(4) Technical feasibility for clinical laboratories to interrogate using standard molecular testing methods. This criterion was determined to not be relevant for these *DPYD* recommendations, as none of the reviewed variants were considered difficult to interrogate using standard methods.

These criteria received equal weight during the AMP PGx Working Group deliberations. Additionally, commercially available genotyping platforms (Supplemental Table S1) were reviewed for assessing the ability of laboratories to implement the Working Group recommendations; however, these data were not used as a determinant of tier assignment. The European Medicines Agency recommendations were also reviewed. Variants that were listed in the European Medicines Agency capecitabine drug label that normally would not meet the frequency cutoff for tier 1 were included, specifically c.1679T>G (rs55886062, legacy name *DPYD*13*) (*https://www.pharmgkb.org/labelAnnotation/PA166104905*, last accessed May 7, 2024). The AMP PGx Working Group used functional information from CPIC. CPIC assigned clinical function (*https://cpicpgx.org/resources*, last accessed January 5, 2024) may not be the same as the biochemical function of the variant or the American College of Medical Genetics and Genomics/AMP recommendations for interpretation of sequence variants widely adopted by clinical molecular genetics laboratories for inherited disorders.[27]

College of American Pathologists proficiency testing (PT) program data were obtained from the Pharmacogenetics, PGX-A 2023 mailing.[28] PT program participants include both US-based and international laboratories. Laboratories self-reported whether they clinically tested for *DPYD* as well as which variants their test is designed to

Table 1 Reference Materials

Coriell ID	Consensus genotype NM_000110.4[†]	Variant rsID (legacy name)
HG01631	c.299_302del het	rs72549309 (*7)
NA19207	c.557A>G het	rs115232898
NA20362	c.557A>G het	rs115232898
HG02645	c.868A>G het	rs146356975
HG02772	c.868A>G het	rs146356975
NA20362	c.1129-5923C>G het	rs75017182 (Hap3B)
HG00118	c.1129-5923C>T het	rs75017182 (HapB3)
HG00129	c.1129-5923C>T het	rs75017182 (HapB3)
NA20362	c.1236G>A het	rs56038477 (HapB3)
HG00118	c.1236G>T het	rs56038477 (HapB3)
HG00129	c.1236G>T het	rs56038477 (HapB3)
HG00613	c.1314T>G het	rs186169810
HG01631	c.1627A>G het	rs1801159 (*5)
NA12248	c.1679T>G het	rs55886062 (*13)
HG00332	c.1679T>G het	rs55886062 (*13)
NA18956	c.1774C>T het	rs59086055
NA20901	c.1905+1G>A het	rs3918290 (*2A)
HG00185	c.1905+1G>A het	rs3918290 (*2A)
HG03645	c.2279C>T het	rs112766203
HG03716	c.2279C>T het	rs112766203
NA06991	c.2846A>T het	rs67376798
HG00118	c.2846A>T het	rs67376798

Information about additional reference materials and variants is also available from Gaedigk et al.[26]

SensID (*https://www.sens-id.com/shop/gdna-en/sid-000110*, last accessed January 10, 2024; Rostock, Germany) has commercial *DPYD* controls. Inclusion herein does not represent an endorsement of any product or service by the Association for Molecular Pathology.

[†]According to Gaedigk et al.[26]

Hap3B, haplotype B3; Het, heterozygous; ID, identifier.

detect. Additionally, data were obtained from the Germany-based Reference Institute for Bioanalytics, the UK-based European Molecular Genetics Quality Network, and the Dutch-based Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek.

Results

Tier 1 *DPYD* Variant Alleles

DPYD variants recommended for inclusion in tier 1 include NM_000110.4:c.1905+1G>A, c.1679T>G, c.1129-5923C>G, c.557A>G, c.868A>G, c.2279C>T, and c.2846A>T (Table 2). Human Genome Variation Society nomenclature was used throughout (*https://www.ncbi.nlm.nih.gov/snp* and *http://www.ncbi.nlm.nih.gov/clinvar*, last accessed January 5, 2024); variant frequency by population information is from *https://gnomad.broadinstitute.org* version 4.0.0 (last accessed February 11, 2024) unless otherwise specified.

DPYD c.1905+1G>A

The no function c.1905+1G>A variant is located at the exon 14/intron 14 splice junction causing aberrant splicing, which has been associated with absence of activity (NM_000110.4: c.1905+1G>A, rs3918290, legacy name *DPYD*2A*).[11,29–32] This variant is found in the Middle Eastern, South Asian, and European (non-Finnish) populations at allele frequencies between 0.3% and 0.5%. It is less prevalent in those of African ancestry at an allele frequency of 0.05% and is not typically found in East Asian populations.

DPYD c.1679T>G

The no function c.1679T>G variant in exon 13 is a missense variant (NM_000110.4:c.1679T>G, p.Ile560Ser, rs55886062, legacy name *DPYD*13*).[33,34] This rare variant has been primarily observed in the European (non-Finnish) population at a frequency of 0.08%, followed by those of African ancestry at 0.02% frequency. It has not been found in Middle Eastern, South Asian, and East Asian populations. Although the population frequency of this variant is below the tier 1 frequency threshold of 0.1%, it was elevated to tier 1 based on the European Medicines Agency drug label and European guidelines for *DPYD* testing.

DPYD c.1129-5923C>G

The haplotype previously designated as HapB3 is a decreased function allele. It is currently defined by two variants in *cis*, c.1129-5923C>G (NM_000110.4:c.1129-5923C>G, rs75017182) and c.1236G>A (NM_000110.4:c.1236G>A, rs56038477, p.Glu412=). The c.1129-5923C>G variant is in intron 10 and introduces a cryptic splice site, which has been associated with decreased DPD activity in individuals with the HapB3 haplotype.[35–37] Previous studies have reported that the synonymous variant c.1236G>A is in linkage disequilibrium (LD) with c.1129-5923C>G in Europeans, and thus, c.1236G>A has been used as a tag to detect the presence of c.1129-5923C>G.[19,38] However, recent data have found that the two variants are not in complete LD in all populations, and that using the c.1236G>A variant alone to infer the haplotype may lead to rare false-positive at-risk phenotype assignments as c.1236G>A can occur without the underlying functional variant c.1129-5923C>G.[39] The c.1129-5923C>G is the most common decreased function variant in Middle Eastern, European (non-Finnish), and South Asian populations at frequencies of 2.4%, 2.1%, and 1.6%, respectively. It is rare in individuals of East Asian ancestry at 0.06% and slightly more prevalent in those of African/African American ancestry, at approximately 0.3%.

DPYD c.557A>G

The NM_000110.4:c.557A>G (rs115232898, p.Tyr186Cys) variant is a missense variant in exon 3 that encodes a tyrosine-to-cysteine amino acid change reported to impact function.[40] This decreased function variant is most prevalent among those of African genetic ancestry,[41] with a multiethnic allele frequency range of 0% to 2.1%.

Table 2 Tier 1 *DPYD* Variants

Variant (NM_000110.4)	Legacy name	CPIC defined function	Activity value	rsID	*DPYD* RefSeqGene (LRG_722)	GRCh38.p13 chr 1	HGVS protein nomenclature	Reference material available[†]	Multiethnic allele frequency, %
c.1905+1G>A	*2A	No function	0	rs3918290	NG_008807.2: g.476002G>A	NC_000001.11: g.97450058C>T	N/A	Yes	0–0.5
c.1679T>G	*13	No function	0	rs55886062	NG_008807.2: g.410273T>G	NC_000001.11: g.97515787A>C	NP_000101.2: p.Ile560Ser	Yes	0–0.08
c.1129-5923C>G, c.1236G>A	HapB3	Decreased function	0.5	rs75017182, rs56038477	NG_008807.2: g.346167C>G, NG_008807.2: g.352197G>A	NC_000001.10: g.97579893G>C, NC_000001.10: g.97573863C>T	N/A, NP_000101.2: p.Glu412=	Yes	0.06–2.4
c.557A>G	N/A	Decreased function	0.5	rs115232898	NG_008807.2: g.226586A>G	NC_000001.11: g.97699474T>C	NP_000101.2: p.Tyr186Cys	Yes	0–2.1
c.868A>G	N/A	Decreased function	0.5	rs146356975	NG_008807.2: g.330911A>G	NC_000001.11: g.97595149T>C	NP_000101.2: p.Lys290Glu	Yes	0–0.2
c.2279C>T	N/A	Decreased function	0.5	rs112766203	NG_008807.2: g.620781C>T	NC_000001.11: g.97305279G>A	NP_000101.2: p.Thr760Ile	Yes	0–0.5
c.2846A>T	N/A	Decreased function	0.5	rs67376798	NG_008807.2: g.843669A>T	NC_000001.11: g.97082391T>A	NP_000101.2: p.Asp949Val	Yes	0–0.6

Citations for *DPYD* variant function assignments can be found at https://www.pharmgkb.org/page/dpydRefMaterials; and for HGVS nomenclature, at https://www.ncbi.nlm.nih.gov/snp and http://www.ncbi.nlm.nih.gov/clinvar (last accessed January 5, 2024).

[†]Table 1 and Gaedigk et al.[26] The characteristic variant and corresponding HGVS nomenclature associated with altered function of the HapB3 allele is underlined.

Chr, chromosome; CPIC, Clinical Pharmacogenetics Implementation Consortium; GRCh38, genome reference consortium human build 38; Hap3B, haplotype B3; HGVS, Human Genome Variation Society; ID, identifier; LRG, locus reference genomic; N/A, not applicable; RefSeqGene, Reference Sequence.

DPYD c.868A>G

The decreased function c.868A>G variant is a missense variant in exon 9 (NM_000110.4:c.868A>G, p.Lys290Glu, rs146356975).[40,42] This variant is observed in the African ancestry population at an overall frequency of 0.2% and is rare in the Middle Eastern population at a frequency of <0.02%. This variant has not been found in those of European, East Asian, or South Asian populations.

DPYD c.2279C>T

The decreased function c.2279C>T variant in exon 18 is a missense variant that changes a threonine to isoleucine (NM_000110.4: c.2279C>T, p.Thr760Ile, rs112766203).[40,43] This variant has been observed mostly in the South Asian population at a frequency of 0.5% and is rare in the East Asian population at a frequency of <0.01%. This variant has not been found in those of African ancestry, or in Middle Eastern and European populations. Of note, rs112766203 is tri-allelic and can also occur as NM_000110.4: c.2279C>G (p.Thr760Ser), with a frequency of 0.0003% (*https://gnomad.broadinstitute.org/variant/1-97305279-G-C?dataset=gnomad_r4*, last accessed January 5, 2024); however, this alternate nucleotide is not included as a tier 1 or tier 2 allele.

DPYD c.2846A>T

The decreased function NM_000110.4:c.2846A>T (rs67376798, p.Asp949Val) variant in exon 22 is a missense variant, leading to an aspartic acid–to–valine amino acid change in exon 22 that is associated with decreased function[44] and increased toxicity.[45,46] The c.2846A>T variant occurs in the European (non-Finnish) population at 0.6% and in those of African ancestry at approximately 0.1%. It is extremely rare in South Asian or Middle Eastern populations at allele frequencies of 0.05% and 0.03%, respectively.

Tier 2 *DPYD* Variant Alleles

DPYD variants recommended for inclusion in tier 2 include NM_000110.4:c.299_302del, c.703C>T, c.1314T>G, c.1475C>T, c.1774C>T, and c.2639G>T (Table 3).

DPYD c.299_302del

The no function c.299_302del variant is a frameshift variant in exon 4, resulting in a nonfunctional protein product (NM_000110.4:c.299_302del, p.Phe100Serfs*15, rs72549309, legacy name *DPYD*7*).[47,48] This variant is observed in the non-Finnish European population at an overall frequency of 0.015%.

DPYD c.703C>T

The no function c.703C>T variant is a missense variant in exon 7 (NM_000110.4: c.703C>T, p.Arg235Trp, rs1801266, legacy name *DPYD*8*).[40,49,50] This variant has been observed in the South Asian, East Asian, and European (non-Finnish) populations at frequencies of 0.03%, 0.004%, and 0.004%, respectively, but has not been found in individuals of Middle Eastern or African ancestry.

DPYD c.1314T>G

The decreased function c.1314T>G variant is a missense variant in exon 11 (NM_000110.4:c.1314T>G,

Table 3 Tier 2 *DPYD* Variants

Variant (NM_000110.4)	Legacy name	CPIC defined function	Activity value	rsID	*DPYD* RefSeqGene (LRG_722)	GRCh38.p13 chr 1	HGVS protein nomenclature	Reference material available[†]	Multiethnic allele frequency, %
c.299_302del	*7	No function	0	rs72549309	NG_008807.2: g.185642TCAT[1]	NC_000001.11: g.97740411ATGA[1]	NP_000101.2: p.Phe100fs	Yes	0–0.01
c.703C>T	*8	No function	0	rs1801266	NG_008807.2: g.234284C>T	NC_000001.11: g.97691776G>A	NP_000101.2: p.Arg235Trp	No	0–0.03
c.1314T>G	N/A	Decreased function	0.5	rs186169810	NG_008807.2: g.352275T>G	NC_000001.11: g.97573785A>C	NP_000101.2: p.Phe438Leu	Yes	0–0.05
c.1475C>T	N/A	No function	0	rs72549304	NG_008807.2: g.376451C>T	NC_000001.11: g.97549609G>A	NP_000101.2: p.Ser492Leu	No	0–0.02
c.1774C>T	N/A	No function	0	rs59086055	NG_008807.2: g.475870C>T	NC_000001.11: g.97450190G>A	NP_000101.2: p.Arg592Trp	Yes	0–0.08
c.2639G>T	N/A	No function	0	rs55674432	NG_008807.2: g.827444G>T	NC_000001.11: g.97098616C>A	NP_000101.2: p.Gly880Val	No	0–0.08

Citations for *DPYD* variant function assignments can be found at https://www.pharmgkb.org/page/dpydRefMaterials; and for HGVS nomenclature, at https://www.ncbi.nlm.nih.gov/snp and http://www.ncbi.nlm.nih.gov/clinvar (last accessed January 5, 2024).

[†]Table 1 and Gaedigk et al.[26]

Chr, chromosome; CPIC, Clinical Pharmacogenetics Implementation Consortium; GRCh38, genome reference consortium human build 38; HGVS, Human Genome Variation Society; ID, identifier; LRG, locus reference genomic; N/A, not applicable; RefSeqGene, Reference Sequence.

p.Phe438Leu, rs186169810).[40] This variant is observed in the East Asian population at an overall frequency of 0.05%.

DPYD c.1475C>T

The no function c.1475C>T variant is a missense variant in exon 12 (NM_000110.4:c.1475C>T, p.Ser492Leu, rs72549304).[40,45,51] This variant is observed in the South Asian population and those of African ancestry at allele frequencies of 0.02% and 0.01%, respectively. Note that rs72549304 is quad-allelic: c.1475C>G (p.Ser492Trp, 0% to 0.0009%) and c.1475C>A (p.Ser492Ter, 0% to 0.005%); however, these alternate nucleotides are not included as tier 1 or tier 2 alleles based on frequency.

DPYD c.1774C>T

The no function c.1774C>T variant in exon 14 is a missense variant that changes an arginine to tryptophan (NM_000110.4: c.1774C>T, p.Arg592Trp, rs59086055).[40,52,53] This variant has been observed in the East Asian population at a frequency of 0.08% but <0.01% in South Asian and European (non-Finnish) populations, as well as those of African ancestry. It has not been found in Middle Eastern populations.

DPYD c.2639G>T

The no function c.2639G>T variant is a missense variant in exon 21 (NM_000110.4:c.2639G>T, p.Gly880Val, rs55674432).[40] This variant is observed in the South Asian population at an overall frequency of 0.08%.

Discussion

In this document, the AMP PGx Working Group recommends inclusion of specific *DPYD* variants in clinical PGx genotyping assays as either tier 1 or tier 2 variants. The goal of this recommendation and other related Working Group recommendations is to promote standardization and to ensure that laboratories conducting PGx testing include the most clinically relevant variants. Although these recommendations are designed to be inclusive of admixed populations, laboratories should consider the genetic variation present in their population. Modification of these recommendations may be considered, and laboratories should justify their variant selection. Clinical laboratories should follow best practices for assay validation and adhere to the applicable regional regulatory requirements, as well as considering the technical recommendations from the American College of Medical Genetics and Genomics.[54]

DPYD is a polymorphic gene with approximately 1600 variants described in Genome Aggregation Database version 4.0.0 to date; however, most of these variants are rare. After excluding noncoding and synonymous variants, the average and median frequencies of the remaining 800 variants are 0.145% and 0.00078%, respectively. Although *DPYD* has many rare variants, collectively they may impact a significant number of individuals. For example, in a recent study of >10,000 individuals, if a panel of the three most commonly tested *DPYD* variants [NM_000110.4:c.1905+1G>A (*2A*); c.1679T>G (*13*); and c.2846A>T (rs67376798)] was used instead of sequencing, 112 potentially significant variants present in 630 individuals (6.3% of the cohort) would have gone undetected.[55] However, many of these rare variants currently have unknown function.

Most of the recommended clinically relevant *DPYD* variants are rare in the general population. Because of the extreme toxicity associated with DPD deficiency, *DPYD* variants with at least 0.1% allele frequency in any human subpopulation are recommended as tier 1 to include in pharmacogenetic testing. In addition,

NM_000110.4:c.1679T>G, p.Ile560Ser, rs55886062 (legacy name *DPYD*13*) does not meet the PGx Working Group allele frequency cutoff for tier 1; however, it is recommended for inclusion in tier 1 because of its association with extreme toxicity and the European Medicines Agency drug label recommendations for this variant. All variants recommended for tier 2 had a frequency between 0.1% and 0.01%. Additionally, three variants (NM_000110.4:c.703C>T, NM_000110.4:c.1775G>A, and NM_000110.4:c.2639G>T) in tier 2 do not have an identified RM. The overall detection rate of the recommended tier 1 and tier 2 variants to identify individuals with impaired DPD function could not be reliably determined at this time, as the overall incidence of partial or complete DPD deficiency is not well defined, and a large percentage of deleterious variants are rare or novel.

Although the Working Group focused on variants previously identified in the literature and included in the list of variants curated by CPIC as associated with 5-FU toxicity, additional variants are present in the Genome Aggregation Database that may also be associated with DPD deficiency and/or 5-FU toxicity, such as the c.2043_2058del (p.Leu682IlefsTer24, rs773499329; minor allele frequency, 0.006%) that was identified during the Genetic Testing Reference Materials Coordination Program (GeT-RM) study.[26] Although the overall minor allele frequency is 0.006%, it is observed predominantly in the South Asian population at 0.1%. Laboratories may choose to include these additional variants as they are identified.

Because of the large number of rare variants and potentially severe toxicities, clinical laboratories may choose to conduct full gene sequencing rather than genotyping to identify variants in the *DPYD* gene. However, laboratories performing sequencing should be aware that the current American College of Medical Genetics and Genomics/AMP guidelines for interpretation of sequence variants are not designed for interpreting pharmacogenomic variants.[27] As such, many rare variants encountered during clinical sequencing may ultimately be classified as variants of uncertain significance. Although sequencing may allow for detection of both common and rare variants, use of Sanger sequencing or short-read next-generation sequencing will not resolve the phase of variants when more than one variant is detected.

The haplotype known as HapB3 (legacy name) consists of a deep intronic variant, NM_000110.4:c.1129-5923C>G (rs75017182), that causes alternative splicing and results in decreased enzyme activity, and a synonymous variant in *cis*, NM_000110.4:c.1236G>A (rs56038477, NP_000101.2: p.Glu412=). The original definition of the HapB3 haplotype included three additional intronic variants, NM_000110.4:c.483+18C>T (rs56276561), c.680+139 C>T (rs6668296), and c.959-51C>T (rs115632870).[56] However, the latter are not in complete LD, are not known to alter function, and thus are not suitable proxies for detection of c.1129-5923C>G. In contrast, c.1129-5923C>G and c.1236G>A have been assumed to be in perfect LD. On the basis of the assumption of perfect LD, some laboratories test the synonymous variant c.1236G>A, and not the intronic splice variant c.1129-5923C>G, to predict an individual's risk of severe fluoropyrimidine-related toxicity. However, recent findings demonstrate that c.1129-5923C>G and c.1236G>A are not in perfect LD, as some rare cases harbor the c.1236G>A variant without c.1129-5923C>G.[39] Using c.1236G>A as a tag variant may not predict an accurate phenotype in rare cases. Although these cases are rare, it emphasizes the importance of assaying for the functional variant that causes decreased activity (ie, c.1129-5923C>G). Some laboratories performing exome sequencing may test c.1236G>A as a proxy for the presence of the deep intronic functional variant c.1129-5923C>G, as intronic variants such as this are not detected in the setting of exome sequencing. However, for clarity, it is recommended that laboratories using this strategy include a limitation in their report acknowledging the incomplete LD as well as information pertaining to c.1129-5923C>G (rs75017182) as the underlying causal variant.

Patient advocacy groups, such as Advocates for Universal DPD/DPYD Testing (*https://test4dpd.org*, last accessed January 5, 2024), have emerged and are working to raise awareness about fluoropyrimidine toxicity and the availability of *DPYD* testing, as well as advocating for universal testing. Some testing proponents have suggested the possibility of including *DPYD* when performing genomic analysis of tumors for other actionable therapeutic markers to detect patients at risk of toxicity to chemotherapeutic agents because of DPD deficiency. Although tumor tissue may have additional somatic variants not present in blood, and thus are also absent from the liver, where the bulk of the fluoropyrimidine metabolism occurs, a small study suggested concordance between blood and tumor testing.[57] As tumor sequencing is becoming more routine in cancer care, the PGx Working Group supports consideration of *DPYD* testing in the setting of tumor diagnostic testing; however, if tumor tissue is sequenced, germline confirmation may be required. The PGx Working Group recognizes that either targeted genotyping or sequencing approaches may be used by laboratories and does not recommend a particular method for testing, nor does it explicitly recommend for or against testing.

In vitro functional assays and *in silico* predictors of protein function can be useful in gauging the effect of *DPYD* variants.[58] Functional assays for DPD activity have the potential to identify all individuals with DPD deficiency, regardless of the variants present, and could be an alternative to *DPYD* genotyping. However, there is currently no standard for DPD functional testing, and current assays have shown conflicting results.[59]

Copy Number Variation and Partial Gene Deletions

CNVs, including deletions and duplications of *DPYD*, have been observed in individuals with DPD deficiency or 5-FU

toxicity. Most recently, a study identified a high prevalence of an exon 4 deletion in the Finnish population at a frequency of 2.4% in individuals prescreened for DPD deficiency.[60] This was followed by another study that observed a lower frequency of 0.2% for the exon 4 deletion in a Canadian population. Notably, the latter study found the exon 4 deletion in an individual with severe 5-FU toxicity.[61] These studies suggested that the exon 4 deletion may be relatively common and found in up to 7% of individuals with DPD deficiency; frequencies of exon 4 deletions are likely population specific and may vary considerably among different patient populations.[62,63] Interstitial deletions of exons 6, 12, and 14 to 16, in addition to partial and whole gene *DPYD* deletions, have been observed in individuals with DPD deficiency with variable phenotypes, including speech delay, autism-like symptoms, intellectual delay, seizures, and/or obesity.[61] Notably, exon 4 and 11 deletions have been found among the *DPYD* RMs, whereas no materials with exon 6, 12, or 14 to 16 deletions were found.[26] Although the exonic deletions meet the frequency for inclusion in either tier 1 or tier 2, as they are not clearly well defined at this time, the PGx Working Group does not currently have recommendations for routine clinical testing.

With increasing use of next-generation sequencing as the testing platform for PGx in clinical laboratories, it may be possible to identify recurrent or rare CNVs at the exon level in *DPYD*. Other technologies, including chromosome microarray, multiplex ligation-dependent probe amplification, TaqMan copy number assays, and exon arrays, can also be used to detect CNVs as well.[61,64] The AMP PGx Working Group has no recommendations for *DPYD* CNV testing at this time, and most clinical PGx assays do not currently include CNV analysis; however, CNV testing could be considered in cases of 5-FU toxicity or DPD deficiency in which a single pathogenic sequence variant could not explain the phenotype.

Proficiency Testing and External Quality Assessment

Several PT or external quality assessment programs are available for *DPYD* genotyping. College of American Pathologists PT data were evaluated to gain a better understanding of the testing practices of laboratories, including which *DPYD* alleles are currently included in clinical testing.[28] Of the 245 participants in the College of American Pathologists PGX-A 2023 mailing, 69 (28.2%) responded to questions related to *DPYD* testing. Among those 69 laboratories, 64 (92.8%) indicated that they offer a clinical *DPYD* test. The number and percentage of the 64 laboratories that reported testing 10 listed *DPYD* alleles are presented in Table 4. The single laboratory not testing for the c.1905+1G>A allele tests for only c.1679T>G (rs55886062, *DPYD*13*). The most common combinations of alleles included in testing were as follow: i) four tier 1 variants: c.1905+1G>A (rs3918290), c.1679T>G (rs55886062), c.1129-5923C>G (rs75017182), and c.2846A>T (rs67376798), tested by 14 (20.3%) of laboratories; ii) three tier 1 variants: c.1905+1G>A (rs3918290), c.1679T>G (rs55886062), and c.2846A>T (rs67376798), tested by 11 (15.9%) of laboratories; and iii) all 10 listed variants, tested by 11 (15.9%) of laboratories.

In Europe, there are three major external quality assessment vendors: the Germany-based Reference Institute for Bioanalytics, the UK-based European Molecular Genetics Quality Network, and the Dutch-based Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek (Table 4). The PGx Working Group acknowledges that most clinical laboratories and PT/external quality assessment (EQA) providers will need to expand their offerings to meet the recommended tier 1 and 2 variants.

Limitations

This document focuses only on recommendations of variants to include in clinical pharmacogenomic genotyping assays for *DPYD*; as such, these recommendations should not be interpreted as recommendations for clinical diagnostic testing for autosomal recessive DPD deficiency. In addition, this document does not include mapping of genotypes to phenotypes, clinical interpretation of genotypes, or recommendations for changes to medication therapy based on genotype, as these were determined to be out of scope for this document and/or available from other resources, such as CPIC and Pharmacogenomics Knowledgebase. Although technical challenges related to interrogating *DPYD* were discussed in this document, the Working Group does not recommend or endorse any molecular testing platforms for *DPYD* genotyping.

Conclusions

This document provides recommendations for variants to include in clinical pharmacogenomic *DPYD* genotyping assays. These recommendations are intended to facilitate the design and implementation of pharmacogenomic testing by clinical laboratories. In addition, these recommendations are intended to promote test standardization and genotype concordance between laboratories.

Disclaimers

The Association for Molecular Pathology (AMP) Clinical Practice Guidelines and Reports are developed to be of assistance to laboratory and other health care professionals by providing guidance and recommendations for particular areas of practice. The Guidelines or Reports should not be considered inclusive of all proper approaches or methods, or exclusive of others. The Guidelines or Reports cannot

Table 4 Variants Included in Proficiency Testing

Variant (legacy name)	cDNA	Tier	CAP, *N* (%)	RfB, *N* (%)	SKML, *N* (%)	EMQN, *N* (%)[†]
rs3918290 (**2A*)	c.1905+1G>A	1	63 (98)	142 (100)	22 (100)	73 (100)
rs72549303 (**3*)	c.1898del	None	13 (20)			
rs1801158 (**4*)	c.1601G>A	None				1 (1)
rs1801159 (**5*)	c.1627A>G	None				1 (1)
rs1801160 (**6*)	c.2194G>A	None				47 (64)
rs72549309 (**7*)	c.299_302del	2	15 (23)		2 (9)[‡]	5 (7)
rs1801266 (**8*)	c.703C>T	2	18 (28)			3 (4)
rs1801265 (**9A*)	c.85T>C	None				2 (3)
rs1801267 (**9B*)	c.2657G>A	None	13 (20)			2 (3)
rs1801268 (**10*)	c.2983G>T	None				3 (4)
rs72549306 (**11*)	c.1003G>T	None				1 (2)
rs115232898	c.557A>G	1	27 (42)			3 (4)
rs78060119 (**12*)	c.1156G>T	None	14 (22)			2 (3)
rs55886062 (**13*)	c.1679T>G	1	59 (92)	138 (97)	22 (100)	71 (97)
rs72549310	c.61C>T	None				1 (1)
rs67376798	c.2846A>T	1	54 (84)	138 (97)	22 (100)	70 (96)
rs75017182 (HapB3)	c.1129-5923C>G	1	36 (56)	50 (35)	22 (100)	44 (60)
rs56038477 (HapB3)	c.1236G>A	None				28 (38)

The number (percentage) of laboratories that reported testing for specific *DPYD* variants was provided on the basis of the CAP proficiency testing Pharmacogenetics, PGX-A 2023 mailing,[28] the German RfB MG21/23 proficiency testing, the Dutch SKML Farmacogenetica 2023 testing, and the UK EMQN 2022 external quality assessment. Empty cells indicate these variants were not available in the proficiency testing.

[†]Unpublished data from 2022 assessment.

[‡]Information from the 2021 Dutch Pharmacogenetics Network survey because this variant is not part of the SKML testing scheme.

CAP, College of American Pathologists; cDNA, coding DNA; EMQN, European Molecular Genetics Quality Network; HapB3, haplotype B3; RfB, Reference Institute for Bioanalytics; SKML, Stichting Kwaliteitsbewaking Medische Laboratoriumdiagnostiek.

guarantee any specific outcome, nor do they establish a standard of care. The Guidelines or Reports are not intended to dictate the treatment of a particular patient. Treatment decisions must be made on the basis of the independent judgment of health care providers and each patient's individual circumstances. The AMP makes no warranty, express or implied, regarding the Guidelines or Reports and specifically excludes any warranties of merchantability and fitness for a particular use or purpose. The AMP shall not be liable for direct, indirect, special, incidental, or consequential damages related to the use of the information contained herein.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the CDC/Agency for Toxic Substances and Disease Registry. Use of trade names and commercial sources is for identification only and does not imply endorsement by the CDC, the Public Health Service, or the US Department of Health and Human Services.

Acknowledgments

We thank Ed Esplin for contributions to the Pharmacogenomics (PGx) Working Group; Jacob Ruden and Robyn Temple-Smolkin for support of the PGx Working Group; and the European Molecular Genetics Quality Network for providing unpublished data for the EQA assessment for 2022.

Disclosure Statement

The University of North Carolina Medical Genetics Laboratory, RPRD Diagnostics, AccessDx Laboratory, and the Stanford Medicine Clinical Genomics Laboratory are fee-for-service clinical laboratories that offer clinical pharmacogenomic testing. V.M.P. is the director of Scientific Affairs for Agena Bioscience, is a member of the Pharmacogene Variation Consortium (PharmVar) Steering Committee and PharmVar *CYP2C* and *CYP3A* Gene Expert Panels, and is the Association for Molecular Pathology liaison to the National Academy of Medicine Roundtable on Genomics and Precision Health. L.H.C. is supported by NIH/National Human Genome Research Institute (NHGRI) grant U01 HG007269 and NIH/National Center for Advancing Translational Sciences grant UL1 TR001427 and serves on the Clinical Pharmacogenetics Implementation Consortium (CPIC) steering committee. A.G. is the director of PharmVar, a member of CPIC, and a member of the CPIC and Pharmacogenomics Clinical Annotation Tool Scientific Advisory Boards. H.H. is an employee of AccessDx Holdings and serves on the CPIC Scientific Advisory Board and on the PharmVar *CYP2D6* Gene Expert Panel. Y.J. serves as the Vice Chair of the American College of Medical Genetics and Genomics (ACMG) Membership Committee. R.C.L. is a member of the PharmVar *CYP2D6* Gene Expert Panel. A.M.M. is a member of the College of American Pathologists (CAP)/ACMG Biochemical and Molecular Genetics Committee

and Pharmacogenetics Workgroup, the PharmVar *CYP2D6* Gene Expert Panel, the ClinGen Pharmacogenomics (PGx) Working Group, and the ClinPGx Scientific Advisory Board. S.A.S. serves on the steering committees of CPIC and PharmVar and is a member of the PharmVar *CYP2C* Gene Expert Panel. A.J.T.'s efforts are supported in part by RPRD Diagnostics, an independent clinical laboratory offering pharmacogenetic testing services; she also serves on the PharmVar *CYP1A2*, *CYP2D6*, *DPYD*, and *NUDT15* Gene Expert Panels. R.H.N.v.S. is a member of the Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, is a board member and past president of the European Society for Pharmacogenomics and Personalized Therapy, serves on the PharmVar *CYP3A* Gene Expert Panel, and is a member of the CPIC Scientific Advisory Board. M.W.-C. is supported by NIH/NHGRI/National Institute of Child Health and Human Development/National Institute on Drug Abuse grant U24 HG010615 and NIH/NHGRI grant U24 HG013077, is a co-investigator of CPIC, is co−principal investigator and director of the Pharmacogenomics Knowledgebase, and serves on the steering committee and multiple Gene Expert Panels for PharmVar. K.E.W. serves as the CAP liaison to the National Academy of Medicine Roundtable on Genomics and Precision Health. The remaining authors have declared no related conflicts of interest.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2024.05.015*.

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