AMP's Recommendations for Clinical *DPYD* Genotyping Allele Selection

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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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On behalf of the AMP Clinical Practice Committee's Pharmacogenomics (PGx) Working Group

Providing global expertise in molecular testing that drives patient care.

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AMP PGx Working Group

- **Victoria M. Pratt** (Co-Chair), Indiana University School of Medicine, Agena Bioscience
- **Karen E. Weck** (Chair), University of North Carolina
- **Larisa H. Cavallari**, University of Florida
- **Edward D. Esplin**, Invitae, ACMG representative
- **Makenzie L. Fulmer**, ARUP Laboratories and University of Utah School of Medicine
- **Andrea Gaedigk**, Children's Mercy Kansas City, PharmVar representative
- **Houda Hachad**, AccessDx Laboratory
- **Yuan Ji**, ARUP Laboratories and University of Utah School of Medicine
- **Lisa V. Kalman**, Division of Laboratory Systems, Centers for Disease Control and Prevention
- **Reynold C. Ly**, Nationwide Children's Hospital
- **Ann M. Moyer**, Mayo Clinic, CAP representative
- **Stuart A. Scott**, Stanford University Medical Center
- **Amy J. Turner**, Medical College of Wisconsin and RPRD Diagnostics, Junior Member
- **Ron H.N. van Schaik**, Erasmus MC University Medical Center, ESPT and DPWG representative
- **Michelle Whirl-Carrillo**, Stanford University, CPIC and PharmGKB representative

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Goals

- To develop recommendations defining a minimum set of variants (a “Must-Test” list) that should be included in clinical genotyping assays
- Used by the clinical PGx testing community as a reference for test development

- **Members**
  - Subject matter expert representatives from the clinical PGx testing community (US and Europe), including organizational representation from ACMG, CAP, CPIC, DPWG, ESPT, PharmGKB, and PharmVar

- **Projects – PGx Allele Selection for Clinical Genotyping**
  - *CYP2C19* - **Pratt VM, et al. *JMD*, 2018;20:269-276**
  - *CYP2C9* - **Pratt VM, et al. *JMD*, 2019;21:746-755**
  - Warfarin-Related Genes - **Pratt VM, et al. *JMD*, 2020;22:847-859**
  - *CYP2D6* - **Pratt VM, et al. *JMD*, 2021;23:1047-1064**
  - *TPMT/NUDT15* - **Pratt VM, et al. *JMD*, 2022;24:1079-1088**
  - *CYP3A4/CYP3A5* - **Pratt VM, et al. *JMD,* 2023;25:619-629**
  - *DPYD* (today’s presentation) - **Pratt VM, et al. *JMD*, 2024; *in press***

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Considerations in *DPYD* Test Interpretation

- For most genes, an individual will have 2 alleles, each with 1 copy of the gene
  - Reported as a diplotype (e.g., *1/*2)
  - Some individuals may have a gene deletion or duplication/multiplication involving one allele
- Diplotype is used to predict the phenotype (metabolizer status)
  - Poor, intermediate, normal, rapid, ultrarapid metabolizer
- **If no variants are detected, *DPYD* is reported as no variant detected and the phenotype as normal metabolizer**

Variability in *DPYD* Genotyping Tests in the United States

Table 1. Variant Coverage and Estimated Diagnostic Performance of Commercially Available *DPYD* Genotyping Tests in the United States

		DPYD Variants Tested								
Laboratory Name	**Test Name (Order Code)**	***2A**	***13**	**c.2846A>T**	**HapB3**	**c.557A>G**	**Other**	**FN Rate**	**Sensitivity**	**NPV**
Color Health	Color Extended and Color Standard panels	X	X					81.6%	18.4%	95.1%
23andMe	*DPYD* Drug Metabolism	X		X				69.4%	30.6%	95.8%
Fulgent Genetics	Picture PGx	X	X	X				65.3%	34.7%	96.0%
ARUP	Dihydropyrimidine dehydrogenase (*DPYD*), 3 Variants (2012166)	X	X	X				65.3%	34.7%	96.0%
Indiana University	Pharmacogenetic *DPYD* Genotyping (53102299)	X	X	X	X	X		0%	100%	100%
Quest	Pharmacogenomics panel (36943)	X			X	X	*2B, *4, *5, *7, *8, *9A, *9B	20.4%	79.6%	98.7%
LabCorp	*DPYD* Genotyping (512275)	X	X	X	X	X		0%	100%	100%
Mayo Clinic Laboratories	*DPYD* Genotype, Varies (DPYDQ)	X	X	X	X	X	*7, *8, *10	0%	100%	100%
OneOme	The RightMed Test	X	X	X	X	X		0%	100%	100%
RPRD	Precision HealthPGx Panel	X	X	X	X	X	*3, *4, *5, *6, *7, *8, *9A, *9B, *10, *11, *12, p.R21Q, p.R21×, p.M166V, p.R592Q, pQu.N635K, p.R886C	0%	100%	100%
Sanford Laboratories	*DPYD* Genotyping (LBOR0149)	X	X	X	X	X	*3, *7, *8, *10, *12	0%	100%	100%
Sinochips Diagnostics	*DPYD* genotyping	X	X	X	X	X	*7, *8, *10, *12	0%	100%	100%
Tempus	*DPYD* test	X	X	X	X	X		0%	100%	100%

Nguyen et al. 2024. J Natl Compr Canc Netw. PMID: 38754463

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Why standardization of PGx testing is needed?

GOAL: To promote standardization of PGx allele testing across clinical laboratories

Inconsistent test design can lead to discordant interpretation and therapeutic recommendations

Test 1:
Detects *2, *5, *9

Test 2:
Detects *2, *3, *4, *5

Patient's Genotype is *1/*4

Test 1:
Reports *1/*1
Normal Metabolizer

Test 2:
Reports *1/*4
Intermediate Metabolizer

- Both tests performed as expected and produced accurate results.
- Different genotypes reported, different phenotypes reported, different recommendations

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Testing Platforms

- Selection of a molecular platform for testing PGx variants depends on many factors, including:
  - Technical feasibility
  - Cost
  - Laboratory workflow
  - Test turnaround time
- Standard molecular techniques may be used (targeted genotyping or sequencing)
  - Many standard techniques do not allow for phasing of variants
  - Haplotypes and diplotypes from raw genotyping or sequencing data are typically inferred

Commercial Testing Platforms*

Variant	rsID	AMP Tier	Agena Bioscience‡ (RUO)	Illumina Global Diversity Array‖ (RUO)	Thermo Fisher PGx Express† (RUO)	Thermo Fisher PGx 120† (RUO)	Thermo Fisher Pharmacoscan † (RUO)	Thermo Fisher PharmacoFocus † (RUO)
c.61C>T	rs72549310						X	X
c.299_302del (c.295_298delTCAT)	rs72549309	2		X			X	X
c.483+18G>A	rs56276561		X					
c.557A>G	rs115232898	1	X	X		X	X	X
c.680+139G>A	rs6668296		X					
c.703C>T	rs1801266	2		X			X	X
c.868A>G	rs146356975	1						
c.959-51T>C	rs115349832		X					
c.1024G>A	rs183385770							
c.1129-5923C>G	rs75017182	1	X	X			X	X
c.1236G>A	rs56038477		X	X		X	X	X
c.1314T>G	rs186169810	2						
c.1475C>T	rs72549304	2						
c.1679T>G	rs55886062	1	X	X		X	X	X
c.1774C>T	rs59086055	2		X				
c.1905+1G>A	rs3918290	1	X	X		X	X	X
c.2279C>T	rs112766203	1						
c.2639G>T	rs55674432	2						
c.2846A>T	rs67376798	1	X	X		X	X	X
c.2194G>A	rs1801160			X			X	X
c.1627A>G	rs1801159			X			X	X
c.1601G>A	rs1801158			X			X	X
c.496A>G	rs2297595			X			X	X
c.85T>C	rs1801265			X			X	X
c.1898delC	rs72549303			X			X	X
c.2983G>T	rs1801268			X			X	X
g.97539400G>A	rs76387818			X			X	X
c.1906-14763G>A	rs12022243			X			X	X
g.97523004G>C	rs12132152			X			X	X
c.151-69G>A	rs115632870			X			X	X
c.1974+75A>G	rs72728438			X			X	X
c.680+2545T>G	rs12119882			X			X	X
c.1896T>C	rs17376848			X			X	X
c.2678A>G	rs188052243			X				
c.2420A>G	rs1335150891			X				
c.2303C>A	rs56005131			X				
c.1543G>A	rs148994843			X				
g.352261G>C	rs748620513			X				
c.1095del	rs1184321568			X				
c.1003G>T	rs72549306			X			X	X
g.330936C>T	rs143879757			X				
c.325T>A	rs1212037891			X				

*Commercially available platforms as of 10/11/2023; the platforms included do not represent a comprehensive list. Nomenclature as provided by the manufacturer that may not conform to standardized Human Genome Variant Society nomenclature. Inclusion herein does not represent an endorsement of any product or service by AMP. †Thermo Fisher Scientific (Waltham, MA); ‡Agena Bioscience (San Diego, CA); ‖Illumina (San Diego, CA). Abbreviations: RUO - research use only; X - variant included in platform. Thermo Fisher PGx 120 does not test for intronic variant for HapB3. Legacy name in parentheses.

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Practice Gaps: lack of clinical PGx testing standardization

Recommendations by AMP PGx Working Group: to promote standardization of PGx allele testing across clinical laboratories

Expert consensus and joint efforts from subject matter experts and professional organizations

Why recommend genotyping for specific alleles?

- Option 1: Test all known variants/alleles
  - Not practical – for example - *CYP2D6*, there are currently >145 alleles/sub-alleles!
  - Ongoing updates to known allele list
- Option 2: Sequencing instead of targeted genotyping
  - Current state: a few key pharmacogenes are technically challenging by short-read NGS chemistry
  - Challenges in reporting genotypes and interpreting rare variants/alleles
  - Phenotype-driven vs. preemptively
  - Cost effectiveness
- **Option 3: Why not adopting a similar approach to ACMG recommendations for *CFTR* testing?**
  - Define a minimum set of variants based on multiethnic allele frequency to optimize diagnostic test rate

AMP PGx Working Group Genotyping Recommendations

These recommendations are intended to:

- Promote standardization of PGx testing across different laboratories
- Inform clinical PGx testing community when designing and validating clinical PGx assays
- Complement other clinical guidelines, such as those issued by CPIC and DPWG, *which primarily focus on the interpretation of genotyping results and therapeutic recommendations for specific drugs*

Future directions:

- Updates on published recommendations
- Collaboration with additional professional (including international PGx) organizations

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AMP PGx Working Group:

Expert Consensus Recommendation/Opinion Development

A Two-Tier Framework

- **Tier 1 -** Minimum "must-test" variants
  - Known effect on protein function and/or gene expression
  - Appreciable minor allele frequency (Tier 1 - ≥0.1% and Tier 2 ≥0.01% cut-offs for *DPYD*) based on gnomAD v4.0.0
  - Available reference materials
  - Technical feasibility to detect variant in a clinical laboratory
- **Tier 2 -** Extended panel
  - Meet at least one but not all the criteria for inclusion in Tier 1
- **Other**
  - Variants with unknown or uncertain function are not recommended for inclusion in clinical test panels

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How to describe *DPYD* Variants?

- Pharmacogene Variation Consortium (PharmVar)
  - Central repository for PGx genes/alleles including *DPYD*
  - PharmVar lists *DPYD* alleles by rsIDs instead of star-alleles
  - Recommends *DPYD* variants should be described using **standard HGVS nomenclature** instead of legacy star-allele names

Allele Name	Legacy Label	PharmVar ID	Variants (rsIDs, Impact) **variant** = variants with dbSNP rsID	GnomAD Global Frequency
rs55886062.1	DPYD*13	PV01000	**1679T>G** (rs55886062, I560S)	0.07%

Variant Positions

Gene	NG_008807.2:g.410273T>G
Transcript	NM_000110.4:c.1679T>G
GRCh37	NC_000001.10:g.97981343A>C
GRCh38	NC_000001.11:g.97515787A>C

https://www.pharmvar.org/gene/DPYD

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AMP PGx Working Group: *DPYD*

- Part of ongoing effort of the AMP PGx Working Group: 7th deliverable
- Challenges:
  - *DPYD* variants are rarer than CYPs, similar to *TPMT* and *NUDT15*
  - Information on phasing of variants not as well documented
  - *DPYD* testing can be different depending on the clinical indication
    - PGx indication vs. diagnostic testing for autosomal recessive DPD deficiency
  - Limited reference materials for *DPYD*
    - Co-project with CDC GeT-RM to characterize additional DNAs from Coriell Life Sciences (Camden, NJ)
    - https://www.jmdjournal.org/article/S1525-1578(24)00155-7/fulltext

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Existing Clinical Guidelines and Recommendations – Fluoropyrimidines and *DPYD*

- Clinical Pharmacogenomics Implementation Consortium (CPIC)
  - https://cpicpgx.org/guidelines/guideline-for-fluoropyrimidines-and-dpyd/
  - Activity score system for genotype-phenotype translation
- Dutch Pharmacogenomics Working Group (DPWG)
  - https://www.nature.com/articles/s41431-019-0540-0
- Experts from the Spanish Pharmacogenetics and Pharmacogenomics (SEFF) and Spanish Society of Medical Oncology
  - https://link.springer.com/article/10.1007/s12094-021-02708-4
- French National Network of Pharmacogenetics (RNPGx)
  - https://www.sciencedirect.com/science/article/pii/S000745511830053 5?via%3Dihub
- FDA: Table of Pharmacogenetic Associations, Table of Pharmacogenomic Biomarkers in Drug Labeling, and FDA label
  - https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations
  - https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling
  - https://www.accessdata.fda.gov/drugsatfda_docs/label/2024/210124s009lbl.pdf

AMP Guidelines for *DPYD* clinical testing – Tier 1

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%

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AMP Guidelines for *DPYD* clinical testing – Tier 2

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%

Most recommended clinically relevant *DPYD* variants are rare in the population

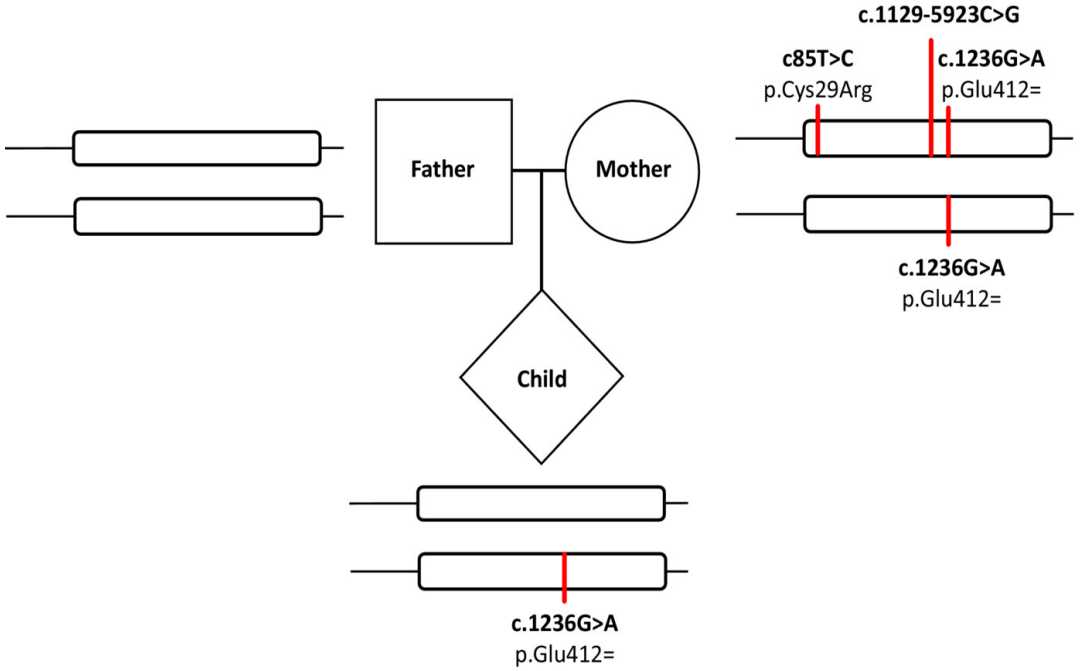
- Due to extreme toxicity associated with DPD deficiency
  - Variants with at least > 0.1% allele frequency in any subpopulation are recommended as Tier 1
  - c.1679T>G, p.Ile560Ser (legacy *DPYD*13)* or did not meet the Tier 1 allele frequency cutoff
    - Included in Tier 1 due to its association with extreme toxicity and the European Medicines Agency (EMA) drug label recommendations
- Overall detection rate of recommended panel to identify individuals with impaired DPD function cannot be reliability determined at this time
  - Overall incidence of partial or complete DPD deficiency is not well defined
  - Large percentage of deleterious variants are rare or novel

DPYD Full Gene Sequencing

- Due to large number of rare variants and potential severe toxicities, clinical labs may choose full gene sequencing rather than genotyping but keep in mind…
  - Current ACMG/AMP guidelines for interpreting sequence variants are not designed for interpreting PGx variants
    - Many rare variants encountered in clinical sequencing may ultimately be classified as variants of unknown significance (VUS).
  - Sequencing may detect both common and rare variants, but Sanger or short-read sequencing will not resolve phasing when more than 1 variant is detected

DPYD HapB3 Haplotype

- HapB3 consists of deep intronic splicing variant, c.1129-5923C>G in cis with the synonymous variant, c.1236G>A.
- c.1129-5933C>G and c.1236G>A have been assumed to be in perfect LD
  - Some labs test for c.1236G>A and not the c.1129-5923C>G to predict an individual's risk of severe 5-FU toxicity.
  - However, recent study shows c.1129-5923C>G is not in perfect LD, as in rare cases the c.1236G>A is without the c.1129-5923C>G.



Turner et al. 2024. Clin Transl Sci. PMID: 38129972

DPYD HapB3 Haplotype Testing

- Using c.1236G>A as a tag variant may not predict an accurate phenotype in rare cases, resulting in false-positive call of decreased function
  - Recommend testing for c.1129-5923C>G and not c.1236G>A, as c.1129-5923C>G is the functional variant responsible for decreased activity
- Laboratories performing exome sequencing may test for c.1236G>A as a proxy variant for c.1129-5923C>G as intronic variants are not detected
  - Laboratories should state limitation in the report acknowledging the incomplete LD and information about c.1129-5923C>G as the underlying causal variant

DPYD Testing in Tumor Diagnostic Testing

- Could be done when performing genomic analysis for other actionable therapeutic markers
  - However, tumor may have additional somatic variants not present in blood and liver where 5-FU metabolism occurs
- We support consideration of *DPYD* testing in tumor diagnostic testing.
  - However, if only the tumor is sequenced, then germline confirmation may be required

Diagnostic Testing for DPD Deficiency

- Germline diagnostic testing for autosomal recessive DPD deficiency in clinical labs may be distinct from *DPYD* PGx testing
  - Sequencing is more commonly used for diagnostic testing
  - Differences include variants analyzed, test design, interpretation, and clinical utilization
- Some labs may offer a single *DPYD* genetic test for both diagnostic and PGx indications

Copy Number Variation: *DPYD* Partial/Whole Gene Deletions

- Exon 4 deletion: Observed at high prevalence in the Finnish population at 2.4% of individuals prescreened for DPD deficiency
  - Another study observed a lower frequency of 0.2% for the deletion in a Canadian population, which included an individual with severe 5-FU toxicity
  - Frequency of deletion likely population specific and vary considerably among different patient populations
- Interstitial deletions of exons 6, 12, and 14-16, and partial and whole gene *DPYD* deletions have been observed with DPD deficiency with variable phenotypes
- Exonic deletions meet the frequency for inclusion in either Tier 1 or 2, but they are not well-defined at this time
  - **Currently no recommendations for routine clinical testing**

DPYD Copy Number Testing

- Multiple technologies can identify recurrent or rare CNVs at the exon level
  - Next-generation sequencing is becoming used as a testing platform for PGx in clinical labs
  - Chromosomal microarray (CMA), Multiplex-ligation dependent probe amplification (MLPA), or Taqman copy number, or exon arrays
- No recommendations for *DPYD* CNV testing at this time
  - Most clinical PGx assays do not currently include CNV testing for *DPYD*
  - Could be considered in cases of 5-FU toxicity or DPD deficiency when a single pathogenic variant cannot explain the phenotype

Proficiency Testing/External Quality Assessment*

- Several proficiency testing (PT) or external quality assessment programs are available for *DPYD* genotyping

Variant (legacy name)	cDNA	Tier	Number (%) CAP	Number (%) RfB	Number (%) SKML	Number (%) EMQN€
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%)

*Information available at time of publication; inclusion does not represent AMP endorsement

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Proficiency Testing/External Quality Assessment*

- Europe's EQA vendors: Reference Institute for Bioanalytics (RfB) and Dutch based-SKML
  - Will need to expand their offerings to meet recommended Tier 1 and 2 variants

Variant (legacy name)	cDNA	Tier	Number (%) CAP	Number (%) RfB	Number (%) SKML	Number (%) EMQN€
rs3918290 (*2A)	c.1905+1G>A	1	63 (98%)	142 (100%)	22 (100%)	73 (100%)
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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%)

Limitations of the AMP PGx Working Group Recommendations

- Only focus on allele recommendations for clinical laboratory PGx genotyping assays that include *DPYD*
  - Not to be interpreted for recommendations for diagnostic testing of autosomal recessive DPD deficiency
- The guidelines do not include:
  - Genotype-phenotype correlation
  - Clinical interpretation of genotypes
  - Recommendations for medication management such as changes of dosing based on genotype
- There are no recommendations or endorsements for any specific molecular methodology, reference materials, proficiency testing services, vendors, or testing platforms

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Conclusions

- This AMP joint consensus recommendation is intended to:
  - Promote test standardization of *DPYD* and genotype concordance between laboratories
  - Inform clinical laboratory professionals of which variants to include when designing and validating clinical PGx assays for *DPYD* testing
  - Complementary to other clinical guidelines, e.g., by CPIC and DPWG
- While designed to be inclusive of admixed population, laboratories should consider genetic variation in their population.
  - Modifications may be considered, and labs should justify variant selection
  - Laboratories should follow best practices for assay validation and adhere to regulatory requirements

Related Paper: *DPYD* Get-RM Study

Characterization of Reference Materials for *DPYD*: A GeT-RM Collaborative Project

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- Limited quality control and reference materials available for clinical *DPYD* testing
- Characterization of 33 DNA samples derived from Coriell cell lines for *DPYD* in four different laboratories
  - 33 distinct *DPYD* variants were identified in the 33 samples characterized
  - These publicly available/well characterized materials can be used to support validation and QA/QC of clinical labs performing clinical PGx testing

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