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SPECIAL ARTICLE

Recommendations for Clinical *CYP2D6* Genotyping Allele Selection

A Joint Consensus Recommendation of the Association for Molecular Pathology, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, and the European Society for Pharmacogenomics and Personalized Therapy

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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 to determine a minimal set of variants that should be included in clinical PGx genotyping assays. This document series provides recommendations on a minimal panel of variant alleles (Tier 1) and an extended panel of variant alleles (Tier 2) that will aid clinical laboratories in designing assays for PGx testing. When developing these recommendations, 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, as well as other technical considerations with regard to PGx testing. The ultimate goal of this Working Group is to promote standardization of PGx gene/allele testing across clinical laboratories. This document is focused on

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

The Pharmacogenomics (PGx) Working Group of the Clinical Practice Committee, Association for Molecular Pathology (AMP) with organizational representation from the College of American Pathologists (A.M.M.), Clinical Pharmacogenetics Implementation Consortium (M.W.-C.), Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association (R.H.N.V.S.), and European Society for

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clinical *CYP2D6* PGx testing that may be applied to all cytochrome P450 2D6–metabolized medications. These recommendations are not meant to be interpreted as prescriptive but to provide a reference guide for clinical laboratories that may be either implementing PGx testing or reviewing and updating their existing platform. *(J Mol Diagn 2021, 23: 1047–1064; https://doi.org/10.1016/j.jmoldx.2021.05.013)*

To address variability in clinical pharmacogenomics (PGx) testing, and to facilitate standardization across laboratories, the Association for Molecular Pathology (AMP) PGx Working Group has developed a series of documents that recommend a minimal set of variant alleles for inclusion in clinical PGx assays. Previous documents have covered *CYP2C19*,[1] *CYP2C9*,[2] and genes important for warfarin PGx testing.[3] The current document is focused on *CYP2D6* and is intended to provide guidance for clinical laboratorians and assay manufacturers who develop, validate, and/or offer clinical *CYP2D6* genotyping assays, and therefore to promote the standardization of PGx testing across clinical laboratories. This document should be implemented together with other relevant clinical guidelines, including those published by the Clinical Pharmacogenetics Implementation Consortium (CPIC),[4–9] Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association (DPWG) Canadian PGx Network for Drug Safety,[10] and the American College of Medical Genetics and Genomics,[11] which have mostly been focused on the interpretation of PGx test results and therapeutic recommendations on specific drug-gene pairs.

The AMP PGx Working Group uses a two-tier strategy and selection criteria for recommending PGx variants for clinical testing.[1–3] Tier 1 PGx variant alleles are a minimal set recommended for clinical testing, while Tier 2 variant alleles do not meet all criteria for inclusion in Tier 1 but may be considered for clinical testing. As defined in previous guidelines,[1–3] Tier 1–recommended variant alleles 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/ethnicity group, and iii) have publicly available reference materials (RMs). Tier 2–recommended variant alleles meet at least one but not all of the Tier 1 criteria, and may be moved to Tier 1 if RMs or additional information becomes available.

CYP2D6

The cytochrome P450 (CYP) 2D6 enzyme, encoded by *CYP2D6*, is a member of the 2D subfamily of CYP enzymes and is involved in the metabolism of many commonly prescribed medications, including some antidepressants, atypical and typical antipsychotics, β-blockers, opioids, antiemetics, atomoxetine, and tamoxifen.[12,13] About 21% of currently approved medications are metabolized by CYP2D6.[14] Interindividual variation in CYP2D6 activity varies substantially and can be attributed, at least in part, to numerous genetic variants in *CYP2D6* in the general population. These variants cause increased-function, decreased-function, or nonfunctional CYP2D6 enzyme.

Clinical *CYP2D6* testing involves the determination of sequence and structural variants by targeted genotyping or sequencing, followed by empirical assignment of detected variants into star (*) alleles or haplotypes. A *haplotype* is a combination of sequence variants from multiple loci on a single chromosome, and a *diplotype* is composed of two haplotypes from the maternal and paternal chromosomes. Although the terms *genotype* and *diplotype* are often used interchangeably in PGx literature, in this document, *genotype* refers to sequence variants identified by molecular platforms that will then be assembled to haplotypes (alleles) and diplotypes. Both genotype and copy number results are combined to assemble haplotypes and diplotypes. *CYP2D6* diplotypes are typically stratified into four groups that represent a patient's predicted CYP2D6 metabolizer

Pharmacogenomics and Personalized Therapy (R.H.N.V.S.). The AMP 2019 and 2020 Clinical Practice Committee consisted of Daniel Jones (2019 to 2020 Chair), Josh Deignan, Jianling Ji, Pinar Bayrak-Toydemir, Fatimah Nahhas, Noah A. Brown, Marian Harris, Rashmi Goswami, Pranil Chandra, Jonathan Earle, Susan Hsiao, Kenneth L Muldrew, Daniel Cohen, Joseph Yao, Justin Zook, Annette Meredith, Joshua Coleman, Megan Wachsmann, Celeste Eno, and Andres Madrigal.

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phenotype: ultrarapid metabolizer, normal metabolizer (NM), intermediate metabolizer, and poor metabolizer.[15] Importantly, CYP2D6 metabolizer phenotype has significant consequences on drug safety and effectiveness, prompting the *CYP2D6* gene to be broadly included in the US Food and Drug Administration's (FDA) Table of Pharmacogenetic Associations (*https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations*, last accessed September 27, 2020), and Table of Pharmacogenomic Biomarkers in Drug Labeling (*https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling*, last accessed September 27, 2020).

CYP2D6 has nine exons and is located on chromosome 22q13.2 next to two highly homologous pseudogenes, *CYP2D7* and *CYP2D8*.[16] It is highly polymorphic, with over 130 star allele haplotypes defined by the Pharmacogene Variation Consortium (PharmVar, *www.pharmvar.org*, last accessed September 23, 2020).[17] Most of the *CYP2D6* star alleles are defined by single-nucleotide variants or small insertion/deletions. *CYP2D6* can also harbor copy-number variants (CNVs; deletions and duplications) and gene conversions with the nearby *CYP2D7* pseudogene, leading to *CYP2D6-2D7* and *CYP2D7-2D6* hybrid alleles.[16,18–20]

Clinical laboratories offering *CYP2D6* PGx testing mostly use a targeted genotyping approach. However, the large numbers of *CYP2D6* alleles and structural variants make analysis of this gene challenging.[16,19,20] A Genetic Testing Reference Material Program study[21] found little consistency in alleles included in *CYP2D6* clinical tests across multiple laboratories. In addition, many assays were not designed for the detection of CNVs or other structural variants. A study in over 100,000 patient samples across all regions of the United States showed that structural variants, including CNVs, accounted for 7% of all *CYP2D6* variants and could affect CYP2D6 metabolizer status.[22] Therefore, the variability in assay design, with or without the detection of CNVs and structural variants, can result in discrepancies in star allele calls and diplotype assignment, which directly affect phenotype translation, interpretation, and ultimately a patient's care.[23,24]

Clinical Testing

According to the National Institutes of Health Genetic Testing Registry (*https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=1565%5bgeneid%5d*, last accessed October 29, 2020), *CYP2D6* variants tested in US clinical laboratories range from a few targeted haplotype-defining variants to the analysis of selected exons or the entire coding region of the gene. Testing methods include targeted genotyping employing various laboratory-developed procedures or commercial platforms, single-nucleotide polymorphism–based microarrays, and full-gene Sanger or next-generation sequencing approaches, with or without deletion/duplication and structure variant analysis. For gene duplications, some laboratories offer additional testing, for example using long-range PCR[12,25–27] to determine which allele is duplicated in order to provide a more accurate activity score assignment and phenotype prediction.[15,28] Regardless of whether a targeted genotyping or a full-gene sequencing approach is used, most laboratories specifically test for haplotype-defining variants and empirically assign diplotypes. Next-generation, short-read sequencing presents many challenges, including the accurate distinction of *CYP2D6* sequence variants versus interfering pseudogenes, the characterization of structural variants, phasing of variants for the assignment of haplotype and diplotype, and the interpretation of novel or rare haplotypes. Recent studies have addressed some of these technical challenges by using full-gene, single-molecule, real-time sequencing with the Pacific Biosciences platform[21,29] and allele-specific amplification combined with long-range PCR sequencing for the comprehensive characterization of full-length *CYP2D6* haplotypes.[21,29] In addition, bioinformatics tools have been developed to computationally infer *CYP2D6* star allele diplotypes from next-generation sequencing–derived variant call format and binary alignment map files.[30–36]

Existing Guidelines

Clinical PGx guidelines are available from professional societies, including the CPIC, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association (DPWG), Canadian PGx Network for Drug Safety, and American College of Medical Genetics and Genomics. At the time of the publication of the present article, CPIC has published six *CYP2D6*-related guidelines with dosing recommendations on 14 medications.[4–9] Although some clinical PGx guidelines have included summaries of known *CYP2D6* alleles, frequencies in various populations, and their functional and/or clinical relevance, they do not explicitly recommend specific variant alleles for inclusion in *CYP2D6* genotyping in clinical laboratories. Specific considerations for diagnostic laboratories, such as allele selection, testing platforms, and the availability of RMs, have not been the focus of the other clinical practice guidelines. However, consistency of genotyping tests among clinical laboratories will facilitate the use of these important clinical PGx practice guidelines and the clinical implementation of PGx testing in general.

Materials and Methods

The AMP PGx Working Group comprises PGx experts from the CPIC, College of American Pathologists (CAP), DPWG and the PGx clinical testing and research communities. *CYP2D6* alleles, including the *5 gene deletion, alleles with gene duplications, and alleles having one or more gene copies consisting of a combination of portions of *CYP2D6* and *CYP2D7* (commonly referred to as *hybrid genes* or

Table 1 Reference Materials

Allele	Coriell cell line no.	Diplotype	Coriell cell line no.	Diplotype
*2	HG00373	*2/*2	NA19226	*2/*2×2
*3	HG00111	*3/*3	NA17176	*3/*45
*4	NA15245	*4×2/*4	NA19174	*4/*40
*5	NA19317	*5/*5	HG03246	*5/*43
*6	NA24027	*2×2/*6	NA20289	*6/*11
*7	HG03643	*2/*7		
*9	NA06989	*9/*9		
*10	NA19143	*2(*45)/*10	NA19207	*2×2/*10
*11	NA20289	*6/*11		
*13	NA19790	*1/*13+*2	NA19785	*1/*13+*2
*14	NA18552	*1/*14	HG02373	*14/*36+*10
*15	NA19239	*15/*17	NA23877	*15/*41
*17	NA23297	*10×2/*17	NA17169	*17/*56
*21	NA18973	*1/*21	HG00589	*1/*21
*22	HG00337	*2×2/*22	NA20803	*2/*22
*28	HG01680	*28/*59	NA17448	*1/*28
*29	NA17137	*29/*45	NA19109	*2×2/*29
*31	HG01086	*1/*31	HG01094	*1/*31
*33	NA12154	(*68)+*4/*33		
*35	NA07029	*1/*35	NA12003	*4/*35
*36	NA18563	*1/*36+*10	NA18565	*10/*36×2
*39	NA23878	(*4N)+*4/*39		
*40	NA19917	*1/*40	NA19174	*4/*40
*41	NA18544	*10/*41	NA18540	(*36+)10/*41
*43	HG03246	*5/*43	NA17128	*1/*43
*45	NA17137	*29/*45	NA17176	*3/*45
*46	NA19908	*1/*46		
*52	NA18632	*36×2+*10/*52		
*56	NA17169	*17/*56	HG03225	*5/*56
*58	NA17185	*4/*58	NA19180	*1/*58
*59	HG01680	*28/*59		
*68	HG01190	*68+*4/*5	NA21781	*2×2/*68+*4
*71	HG00436	*2×2/*71		
*82	NA19777	*1/*82		
*83	NA17287	*1/*83		
*90	NA18642	*36+*10/*1+*90		
*99	HG03703	*1/*99	HG03781	*2/*99
*106	HG03259	*5/*106	HG01108	*2/*106
*108	NA17222	*2/*108		
*111	NA20875	*1/*111	NA21105	*3/*111
*112	HG03780	*1/*112	HG03882	*1/*112
*113	HG04206	*2/*113	HG03619	*2/*113
Other structural variants (gene deletion, duplication, hybrids, and tandem arrangements)	*1×2, *2×2, *4×2, *4N+*4, *5, *10×2, *13+*2, *17×2, *36×2, *36+*1, *36+*10, *36×2+*10, *36×2+*10×2, *41×3, and *68+*4			

Additional reference materials for many of these alleles are also available[22,37,38] (Centers for Disease Control and Prevention, *https://www.cdc.gov/labquality/get-rm/inherited-genetic-diseases-pharmacogenetics/pharmacogenetics.html*, last accessed July 27, 2021). Genotypes in parentheses were determined in only one laboratory and not confirmed.

hybrid alleles), were reviewed and stratified into tiers on the basis of four criteria: i) functional characterization status of the allele, that is, whether it is known to affect the function of the gene or encoded protein; ii) presence at an appreciable minor allele frequency (MAF) in a population/ethnicity [in this particular document on *CYP2D6*, the Working Group used a MAF of ≥1% for Tier 1 alleles, and ≥0.1% for Tier 2 alleles, based on currently available information from applicable resources (PharmGKB, *https://www.pharmgkb.org/gene/pa128*, last accessed October 29, 2020)]; iii) the availability of RMs (Table 1)[22,37,38]; and iv) the technical feasibility of clinical laboratories to detect the variant.

These criteria received equal weight in the deliberations of the AMP PGx Working Group. Of note, the fourth

criterion regarding technical feasibility was added to this document due to the complexity of *CYP2D6* testing of some of the variants (*Results*). This additional criterion was not included in previous recommendations published by this Working Group because the variants in the genes described in those recommendations were detectable using standard clinical laboratory methods.[1–3] Additionally, commercially available genotyping platforms (Supplemental Table S1) were reviewed for an understanding of the capacity of laboratories to implement the Working Group's recommendations; however, these data were not used as part of the determination of Tier assignment.

The AMP PGx Working Group utilized information on MAF and function from CPIC, PharmGKB, and the scientific literature. Because part of the process that the CPIC uses for developing clinical dosing guidelines includes curating allele frequencies and function assignments,[39,40] the PGx Working Group used the CPIC's curated information. In the case of *CYP2D6*, in which some star alleles are a combination of multiple variants and certain variants may be present in multiple alleles in different combinations with other variants, sequence variant frequencies from databases such as the Genome Aggregation Database (gnomAD, *https://gnomad.broadinstitute.org*, last accessed September 17, 2020) and the International Genome Sample Resource (commonly referred to as *1000 Genomes*, *https://www.internationalgenome.org*, last accessed September 17, 2020) may not accurately represent *CYP2D6* star allele frequencies. CPIC relied on studies that specifically examined star allele haplotype frequencies. Dependence on the literature results in several caveats with regard to the calculated allele frequencies, including that: i) most studies test for a limited number of alleles, and some alleles are rarely assayed or reported in the literature; ii) some studies do not test for CNVs and structural variants, which can result in inaccurate allele assignment; and iii) some studies test for a limited number of sequence variants and default allele assignment (eg, assigning all alleles with the c.100C>T variant as *CYP2D6*10*). As a result, allele frequencies can be under- or overestimated or simply not detected. The assignment of the clinical function of alleles by CPIC is based on findings from literature reviews of allele function. Of note, CPIC-defined clinical function may not be the same as the biochemical function of the allele. Although PharmVar (*https://www.pharmvar.org/gene/cyp2d6*, last accessed October 6, 2020) listed over 130 *CYP2D6* alleles, the CPIC had evaluated alleles up to *114 as of mid-2020. Therefore, the present recommendation document from the AMP PGx Working Group considers CYP2D6 alleles through only *114.

The percentage of laboratories that included specific variants in clinical *CYP2D6* testing was calculated based on data obtained from CAP Proficiency Testing data. Laboratories self-report whether their test is designed to detect each of the indicated alleles. Survey data were obtained from the 2019-A and -B mailings. While many subscribers to the CAP PGx proficiency testing program are US-based, there are also international participants. Additionally, data were obtained from the German-based Reference Institute for Bioanalytics Molecular Genetics Group 2, which has a panel that includes *CYP2D6*.

Results

Tier 1 *CYP2D6* Variant Alleles

CYP2D6 variant star alleles recommended for inclusion in Tier 1 include *CYP2D6* *2 to *6, *9, *10, *17, *29, and *41, and the presence or absence of a gene duplication or multiplication (Table 2).

*CYP2D6*2*

*CYP2D6*2* is a commonly reported allele with normal function and is characterized by two common missense variants, one in exon 6 [NM_000106.6:c.886C>T (dbSNP, *https://www.ncbi.nlm.nih.gov/snp*, last accessed May 10, 2021; p.Arg296Cys, rs16947, legacy 2850C>T) and one in exon 9 (NM_000106.6:c.1457G>C, p.Ser486Thr, rs1135840, legacy 4180G>C). The *CYP2D6*2* allele frequency varies among different populations with approximately 28% in Europeans, 16% to 20% in those of African ancestry, and 12% to 29% in Asian populations (Central/South and East Asian; PharmGKB, *https://www.pharmgkb.org/gene/pa128*). This allele would not typically meet the Working Group criteria for Tier 1 variants, as it does not result in altered *CYP2D6* function unless it is duplicated or multiplicated, which does meet criteria; however, the Working Group decided to include *CYP2D6*2* in Tier 1 because c.886C>T and/or c.1457G>C is often tested for in laboratories to disambiguate *CYP2D6*2* and other *CYP2D6* alleles that include these variants (Tables 2 and 3).

*CYP2D6*3*

The no-function *CYP2D6*3* allele is characterized by a single-nucleotide deletion (NM_000106.6:c.775del, p.Arg259fs, rs35742686, legacy 2549delA) in exon 5 that causes a frameshift and premature truncation of the CYP2D6 protein, and subsequently a loss of enzyme function.[41–43] *CYP2D6*3* occurs at a frequency of approximately 1.6% in populations of European ancestry, but <1% in other ancestral populations (PharmGKB, *https://www.pharmgkb.org/gene/pa128*, last accessed October 29, 2020).

*CYP2D6*4*

The *CYP2D6*4* allele is a no-function allele characterized by a splicing defect variant (NM_000106.6:c.506-1G>A, rs3892097, legacy 1846G>A) located in the splice junction between intron 3 and exon 4 that results in a nonfunctional protein.[44,45] *CYP2D6*4* is the allele that contributes most commonly to the CYP2D6 poor metabolizer phenotype.

Table 2 *CYP2D6* Tier 1 Variant Alleles

Allele	Allele functional status assigned by CPIC[†]	Core variant(s)[‡]	Legacy nomenclature (M33388) ATG start[§,¶]	RefSeqGene LRG_303 (NG_008376.4) ATG start[§]
*2	Normal function	rs16947, rs1135840	2850C>T, 4180G>C	2851C>T, 4181G>C
*3	No function	rs35742686	2549delA	2550delA
*4	No function	rs3892097	1846G>A	1847G>A
*5	No function	*CYP2D6* full gene deletion		
*6	No function	rs5030655	1707delT	1708delT
*9	Decreased function	rs5030656	2615delAAG	2616delAAG
*10	Decreased function	rs1065852, rs1135840	100C>T, 4180G>C	100C>T, 4181G>C
*17	Decreased function	rs28371706, rs16947, rs1135840	1023C>T, 2850C>T, 4180G>C	1022C>T, 2851C>T, 4181G>C
*29	Decreased function	rs59421388, rs61736512+ rs1058164, rs16947, rs1135840	3183G>A, 1659G>A, 1661G>C, 2850C>T, 4180G>C	3184G>A, 1660G>A, 1662G>C, 2851C>T, 4181G>C
*41	Decreased function	rs28371725, rs16947, rs1135840	2988G>A, 2850C>T, 4180G>C	2989G>A, 2851C>T, 4181G>C
×N	Variable, depending the duplicated alleles	Duplications		

(*table continues*)

[†]Citations for assignment of function can be found on the Pharmacogene Variation Consortium website (*https://www.pharmvar.org*, last accessed October 28, 2020); HGVS nomenclature can be found on the National Center for Biotechnology Information website (*https://www.ncbi.nlm.nih.gov/snp* and *http://www.ncbi.nlm.nih.gov/clinvar*, both last accessed October 28, 2020).

[‡]Core variant(s) can be found on the Pharmacogene Variation Consortium website (*https://www.pharmvar.org*, last accessed October 28, 2020); the characteristic variant associated with altered function and corresponding HGVS nomenclature for each star allele are underlined.

[§]Count from ATG start site.

[¶]The reference sequence initially used for defining variants, M33388, differs from the current RefSeq NG_008376.4 at four positions, of which three are insertion/deletions and thus causes variant coordinates to shift among these reference sequences (*https://www.pharmvar.org/gene/cyp2d6*, last accessed October 6, 2020). Positions on M33388 are referred to as "legacy."

CPIC, Clinical Pharmacogenetics Implementation Consortium; HGVS, Human Genome Variation Society.

This allele is found in European populations at a frequency of approximately 18.5%, whereas its frequency ranges between 3% and 5% in those of African ancestry and from 0.5% to 9.1% in Asian (Central/South and East Asian) populations (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). The c.100C>T variant (p.Pro34Ser, rs1065852, legacy 100C>T) is also present in all but one *CYP2D6*4* haplotype (ie, the *4.012 suballele). Additionally, c.100C>T, which causes decreased function, is present in the *CYP2D6* *10 and *36 alleles and in several other *CYP2D6* alleles/haplotypes.

*CYP2D6*5*

The no-function *CYP2D6*5* allele is characterized by a full gene deletion, resulting in the absence of CYP2D6 protein and loss of enzyme function.[46] The allele frequencies of *CYP2D6*5* are approximately 3% to 5% in African, Asian (Central/South and East Asian), and European populations (PharmGKB, *https://www.pharmgkb.org/gene/pa128*).

*CYP2D6*6*

The no-function *CYP2D6*6* allele is characterized by a single-nucleotide deletion in exon 3 (NM_000106.6:c.454del, p.Trp152fs, rs5030655, legacy 1707delT), causing a frameshift and premature truncation of the CYP2D6 protein, resulting, in turn, in the loss of enzyme function.[47] The frequencies of *CYP2D6*6* are approximately 1% in European populations and 0.5% or lower in other populations (PharmGKB, *https://www.pharmgkb.org/gene/pa128*).

*CYP2D6*9*

The *CYP2D6*9* allele is a decreased-function allele characterized by the deletion of three nucleotides that results in the loss of a lysine at amino acid position 281 in exon 5 (NM_000106.6: c.841_843del, p.Lys281del, rs5030656, legacy 2615delAAG) and decreased enzyme function.[48,49] The *CYP2D6*9* frequencies are approximately 2.8% and 1.6% in European and Latino populations, respectively, and <0.5% in African ancestry and Asian (Central/South and East Asian)

Table 2 (*continued*)

RefSeqGene LRG_303 (NG_008376.4)	HGVS genomic nomenclature: GRCh38 (NC_000022.11)	HGVS cDNA nomenclature: LRG_303 (NM_000106.6[8])	HGVS protein nomenclature: LRG_303 (NP_000097.3)	Reference material available	Multiethnic allele frequency
g.7870C>T, g.9200G>C	g.42127941G>A, g.42126611C>G	c.886C>T, c.1457G>C	p.Arg296Cys, p.Ser486Thr	Yes	3.9%–29.5%
g.7569del	g.42128242del	c.775del	p.Arg259fs	Yes	<0.1%–1.6%
g.6866G>A	g.42128945C>T	c.506-1G>A	(splicing defect)	Yes	0.5%–18.5%
				Yes	1.6%–5.4%
g.6727del	g.42129084del	c.454del	p.Trp152fs	Yes	0%–1.1%
g.7635_7637del	g.42128176_42128178del	c.841_843del	p.Lys281del	Yes	0%–2.8%
g.5119C>T, g.9200G>C	g.42130692G>A, g.42126611C>G	c.100C>T, c.1457G>C	p.Pro34Ser, p.Ser486Thr	Yes	1.4%–43.6%
g.6041C>T, g.7870C>T, g.9200G>C	g.42129770G>A, g.42127941G>A, g.42126611C>G	c.320C>T, c.886C>T, c.1457G>C	p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	<0.1%–19.3%
g.8203G>A, g.6679G>A g.6681G>C, g.7870C>T, g.9200G>C	g.42127608C>T, g.42525132_42525134delinsGAT, g.42127941G>A, g.42126611C>G	c.1012G>A, c.406_408delinsATC, c.886C>T, c.1457G>C	p.Val338Met, p.Val136Ile, p.Arg296Cys, p.Ser486Thr	Yes	0%–12.1%
g.8008G>A, g.7870C>T, g.9200G>C	g.42127803C>T, g.42127941G>A, g.42126611C>G	c.985+39G>A, c.886C>T, c.1457G>C	N/A (splicing defect), p.Arg296Cys, p.Ser486Thr	Yes	0.8%–15.4%
				Yes	Variable

populations (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). The c.841_843del variant is also found in combination with other sequence variants on two other rare and not well-characterized alleles, *109 and *115.

*CYP2D6*10*

The decreased-function *CYP2D6*10* allele is characterized by a missense variant in exon 1 (NM_000106.6: c.100C>T, p.Pro34Ser, rs1065852, legacy 100C>T).[50–52] This allele also contains the common c.1457G>C variant. *CYP2D6*10* is most common in the Asian population (South/Central and East Asian), with an allele frequency ranging from 9% to 44%, whereas the frequencies are 4% to 6% in those of African ancestry and <2% in the European population (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). Accurately assigning *CYP2D6*10* is challenging given that c.100C>T is also present in many other *CYP2D6* allele haplotypes, including *4, *36, *37, *47, *49, *52, *54, *56, *57, *64, *65, *69, *72, *87, *94, *95, *99, *100, *101, *114, and *132 (PharmVar, *https://www.pharmvar.org/gene/cyp2d6*).

*CYP2D6*17*

The decreased-function *CYP2D6*17* allele is characterized by a missense variant in exon 2 (NM_000106.6:c.320C>T, p.Thr107Ile, rs28371706, legacy 1023C>T), and includes the two common variants c.886C>T and c.1457G>C. *CYP2D6*17* is most common in those of African ancestry, with an allele frequency of 17% to 19%, but is <0.5% in European and Asian (Central/South and East Asian) populations. Additionally, this allele occurs in approximately 2.3% in the Latino population (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). The variant c.320C>T is also present in the *40 allele (in Tier 2), as well as in *58 and *64.

*CYP2D6*29*

The decreased-function *CYP2D6*29* allele is characterized by two missense variants, NM_000106.6:c.1012G>A

Table 3 *CYP2D6* Tier 2 Variant Alleles

Allele	Allele functional status assigned by CPIC[†]	Core variant(s)[‡]	Legacy nomenclature (M33388) ATG start[§,¶]	RefSeqGene LRG_303 (NG_008376.4) ATG start[§]	RefSeqGene LRG_303 (NG_008376.4)
*7	No function	rs5030867	2935A>C	2936A>C	g.7955A>C
*8	No function	rs5030865, rs16947, rs1135840	1758G>T, 2850C>T, 4180G>C	1759G>T, 2851C>T, 4181G>C	g.6778G>T, g.7870C>T, g.9200G>C
*12	No function	rs5030862, rs16947, rs1135840	124G>A, 2850C>T, 4180G>C	124G>A, 2851C>T, 4181G>C	g.5143G>A, g.7870C>T, g.9200G>C
*14	Decreased function	rs5030865, rs16947, rs1135840	1758G>A, 2850C>T, 4180G>C	1759G>A, 2851C>T, 4181G>C	g.6778G>A, g.7870C>T, g.9200G>C
*15	No function	rs774671100	137_138insT	137_138insT	g.5156dup
*21	No function	rs72549352, rs16947, rs1135840	2579_2580insC, 2850C>T, 4180G>C	2580_2581insC, 2851C>T, 4181G>C	g.7599dup, g.7870C>T, g.9200G>C
*31	No function	rs267608319, rs16947, rs1135840	4042G>A, 2850C>T, 4180G>C	4043G>A, 2851C>T, 4181G>C	g.9062G>A, g.7870C>T, g.9200G>C
*40	No function	rs72549356, rs28371706, rs16947, rs1135840	1863_1864insTTTCGCCCCT TTCGCCCC, 1023C>T, 2850C>T, 4180G>C	1864_1865insTTTCGCCC CTTTCGCCCC, 1022C>T, 2851C>T, 4181G>C	g.6875_6883TTTCGCCCC [3], g.6041C>T, g.7870C>T, g.9200G>C
*42	No function	rs72549346, rs16947, rs1135840	3260_3261insGT, 2850C>T, 4180G>C	3261_3262insGT, 2851C>T, 4181G>C	g.8279_8280dup, g.7870C>T, g.9200G>C
*49	Decreased function	rs1135822, rs1065852, rs1135840	1611T>A, 100C>T, 4180G>C	1612T>A, 100C>T, 4181G>C	g.6631T>A, g.5119C>T, g.9200G>C
*56	No function	rs72549347, rs1135840	3201C>T, 4180G>C	3202C>T, 4181G>C	g.8221C>T, g.9200G>C
*59	Decreased function	rs79292917, rs16947, rs1135840	2939G>A, 2850C>T, 4180G>C	2940G>A, 2851C>T, 4181G>C	g.7959G>A, g.7870C>T, g.9200G>C
Hybrid genes	No function	Variable			

(*table continues*)

[†]Citations for assignment of function can be found on the Pharmacogene Variation Consortium website (*https://www.pharmvar.org*, last accessed October 28, 2020); HGVS nomenclature can be found on the National Center for Biotechnology Information website (*https://www.ncbi.nlm.nih.gov/snp* and *http://www.ncbi.nlm.nih.gov/clinvar*, last accessed October 28, 2020).

[‡]Core variant(s) can be found on the Pharmacogene Variation Consortium website (*https://www.pharmvar.org*, last accessed October 28, 2020); the characteristic variant associated with altered function and corresponding HGVS nomenclature for each star allele are underlined.

[§]Count from ATG start site.

[¶]The reference sequence initially used for defining variants, M33388, differs from the current RefSeq NG_008376.4 at four positions, of which three are insertion/deletions, and thus causes variant coordinates to shift among these reference sequences (PharmVar, *https://www.pharmvar.org/gene/cyp2d6*, last accessed October 6, 2020). Positions on M33388 are referred to as legacy.

CPIC, Clinical Pharmacogenetics Implementation Consortium; HGVS, Human Genome Variation Society.

(p.Val338Met, rs59421388, legacy 3183G>A) and c.406_408delinsATC (p.Val136Ile, rs61736512 + rs1058164, legacy 1659G>A and 1661G>C). This allele also includes the two common variants, c.886C>T and c.1457G>C. Functionally, c.1012G>A (p.Val338Met) and c.406_408delinsATC (p.Val136Ile) together alter CYP2D6 enzyme function.[53] *CYP2D6*29* has allele frequencies of 9% to 12% in those of African ancestry, 1.5% in Latinos, and <0.5% in European and Asian (Central/South and East Asian) populations (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). Note that many platforms interrogate only c.1012G>A due to the difficulty in testing for c.406_408delinsATC. In addition, c.1012G>A is also present in the *CYP2D6* *70 and *109 haplotypes in combination with

Table 3 (*continued*)

HGVS genomic nomenclature: GRCh38 (NC_000022.11)	HGVS cDNA nomenclature: LRG_303 (NM_000106.6[§])	HGVS protein nomenclature: LRG_303 (NP_000097.3)	Reference material available	Multiethnic allele frequency
g.42127856T>G	c.971A>C	p.His324Pro	Yes	0%–0.6%
g.42129033C>A, g.42127941G>A, g.42126611C>G	c.505G>T, c.886C>T, c.1457G>C	p.Gly169Ter, p.Arg296Cys, p.Ser486Thr	No	0%–0.1%
g.42130668C>T, g.42127941G>A, g.42126611C>G	c.124G>A, c.886C>T, c.1457G>C	p.Gly42Arg, p.Arg296Cys, p.Ser486Thr	No	0%–1.7%
g.42129033C>T, g.42127941G>A, g.42126611C>G	c.505G>A, c.886C>T, c.1457G>C	p.Gly169Arg, p.Arg296Cys, p.Ser486Thr	Yes	0%–0.3%
g.42130655dup	c.137dup	p.Leu47fs	Yes	0%–0.6%
g.42128218dup, g.42127941G>A, g.42126611C>G	c.805dup, c.886C>T, c.1457G>C	p.Arg269fs, p.Arg296Cys, p.Ser486Thr	Yes	0%–0.4%
g.42126749C>T, g.42127941G>A, g.42126611C>G	c.1319G>A, c.886C>T, c.1457G>C	p.Arg440His, p.Arg296Cys, p.Ser486Thr	Yes	0%–0.8%
g.42128934_42128942AAAGGGGCG[3], g.42129770G>A, g.42127941G>A, g.42126611C>G	c.514_522TTTCGCCCC[3], c.320C>T, c.886C>T, c.1457G>C	p.172_174FRP[3], p.Thr107Ile, p.Arg296Cys, p.Ser486Thr	Yes	0%–1.3%
g.42127532_42127533dup, g.42127941G>A, g.42126611C>G	c.1088_1089dup, c.886C>T, c.1457G>C	p.Gln364fs, p.Arg296Cys, p.Ser486Thr	No	0%–0.5%
g.42129180A>T, g.42130692G>A, g.42126611C>G	c.358T>A, c.100C>T, c.1457G>C	p.Phe120Ile, p.Pro34Ser, p.Ser486Thr	No	0%–1.1%
g.42127590G>A, g.42126611C>G	c.1030C>T, c.1457G>C	p.Arg344Ter, p.Ser486Thr	Yes	0%–0.2%
g.42127852C>T, g.42127941G>A, g.42126611C>G	c.975G>A, c.886C>T, c.1457G>C	p.Pro325= (splicing defect), p.Arg296Cys, p.Ser486Thr	Yes	0%–0.7%

other sequence variants, but these alleles are rare, and their function has not been well defined.

*CYP2D6*41*

The decreased-function *CYP2D6*41* allele is characterized by a variant in intron 6 that causes a splice defect (NM_000106.6: c.985+39G>A, rs28371725, legacy 2988G>A), resulting in decreased canonical CYP2D6 mRNA expression levels and enzyme function.[54,55] This haplotype also includes the common c.886C>T and c.1457G>C variants. *CYP2D6*41* has allele frequencies of 4% to 11.5% among individuals of African ancestry, 2% to 12% in Asian populations (East and Central/South Asian), and approximately 9% in Europeans (PharmGKB, *https://*

Table 4 Copy Number Detection for Selected Hybrid Alleles Composed of Portions of *CYP2D6* and *CYP2D7*

Allele	Allele functional status[†]	Reference material available	Multiethnic allele frequency	Hybrid type	5′ UTR[‡]	Exon 1[‡]	Intron 2[‡]	Intron 5[‡]	Intron 6[‡]	Exon 9[‡]
*4.013 (*4N)	No function			*2D6-2D7*	Yes	Yes	Yes	Yes	Yes	No
*13	No function	Yes	0%–0.4%	*2D7-2D6*	No	No	Yes/no	Yes/no	Yes/no	Yes[§]
*36	No function	Yes	0%–1.2%	*2D6-2D7*	Yes	Yes	Yes	Yes	Yes	No
*68	No function	Yes	Not available	*2D6-2D7*	Yes	Yes	No[¶]	No[¶]	No[¶]	No
*83	Uncertain function	Yes	Not available	*2D6-2D7*	Yes	Yes	Yes	Yes	Yes	No

[†]Allele functional status corresponds to CPIC clinical allele function assignments as listed in the *CYP2D6* Allele Functionality Table available through PharmGKB (*https://www.pharmgkb.org/page/cyp2d6refmaterials*, last accessed June 2, 2021); these function assignments are also displayed by the Pharmacogene Variation Consortium.

[‡]Signal present on copy number analysis for this allele.

[§]A hybrid with a switch to *CYP2D6* past exon 9 has been described in tandem arrangements; these are technically also *CYP2D7-2D6* hybrids and produce no signal across any of the listed regions.

[¶]It cannot be ruled out that rare/undefined hybrids switch in different regions affecting the copy number call in that region.

CPIC, Clinical Pharmacogenetics Implementation Consortium; HGVS, Human Genome Variation Society.

www.pharmgkb.org/gene/pa128). c.985+39G>A is also present in the *CYP2D6* *32, *69, *91, *119, *123, and *138 alleles.

CYP2D6xN (Gene Duplications/Multiplications)

The *CYP2D6* locus is prone to structural variation, which can result in two or more gene copies on a single haplotype as a result of meiotic nonallelic homologous recombination (*https://www.pharmvar.org/gene/cyp2d6*).[16] Allelic variants that are duplicated/multiplicated are annotated by PharmVar as "×N", if the number of duplicated/multiplicated gene copies is unknown. For duplicated allelic variants with known copy number, it is commonly annotated as ×2 (two copies), ×3 (three copies), and so on. However, this nomenclature is not standardized, and as a result, not every laboratory adheres to this annotation for *CYP2D6* duplication/multiplication alleles. Thus, gene copy number may be reported by some clinical laboratories as *CYP2D6*1/*2/×N, (*1/*2)dup*, or *(*1/*2)×N*, for example. It is important to note that the functional effect of a *CYP2D6* duplication depends on which allele is duplicated. The most commonly observed are **1×N*, **2×N*, **4×N* and **41×N*[20] (*https://www.pharmvar.org/gene/cyp2d6*). Some laboratories can determine which allele is duplicated and may use nomenclature that specifies this information (eg, **1×2/*4 vs *1/*4×2).* The frequencies of duplicated alleles in different populations are variable, and can be found at *https://www.pharmvar.org/gene/cyp2d6*. Interrogation of *CYP2D6* gene duplications/multiplications is included in Tier 1 due to the frequency of *CYP2D6* gene-duplication events in the general population, their effect on enzyme function, and the feasibility of detection using common copy number assays (eg, TaqMan, quantitative PCR, multiplex ligation-dependent probe amplification).

Tier 2 *CYP2D6* Variant Alleles

The *CYP2D6* variant alleles recommended for inclusion in Tier 2 include *CYP2D6* *7, *8, *12, *14, *15, *21, *31, *40, *42, *49, *56, and *59, and hybrid genes containing portions of *CYP2D6* and *CYP2D7* (Table 3).

*CYP2D6*7*

The *CYP2D6*7* allele is a no-function allele characterized by a missense variant in exon 6 (NM_000106.6: c.971A>C, p.His324Pro, rs5030867, legacy 2935A>C) that results in a nonfunctional protein.[56] This allele occurs at frequencies of <0.05% in European and African ancestry populations and between 0.01% and 0.6% in Asian populations (Central/South and East Asian) (PharmGKB, *https://www.pharmgkb.org/gene/pa128*).

*CYP2D6*8*

The *CYP2D6*8* allele is a no-function allele characterized by a nonsense variant in exon 3 (NM_000106.6: c.505G>T, p.Gly169Ter, rs5030865, legacy 1758G>T) that results in a nonfunctional protein.[57] This allele also contains the common c.886C>T and c.1457G>C variants. It has been identified in the European and American populations with frequencies of 0.02% and 0.1%, respectively (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). Note that the *CYP2D6*8* c.505G>T variant is triallelic with *CYP2D6*14* (below).

*CYP2D6*12*

The no-function *CYP2D6*12* allele is characterized by a missense variant in exon 1 (NM_000106.6: c.124G>A, p.Gly42Arg, rs5030862, legacy 124G>A), which results in a nonfunctional protein.[58,59] This allele also contains the common c.886C>T and c.1457G>C variants. It has been identified in the indigenous American populations with a frequency of 1.7%, but less in Europeans, with a 0.02% frequency, and in African ancestry populations with frequencies of 0.08% to 0.3% (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). Because RMs are not available for *CYP2D6*12*, it is currently classified as a Tier 2 allele. This categorization is subject to change should RMs become available.

Table 5 Number/Percentage of Laboratories that Report Testing for Specific *CYP2D6* Alleles from Proficiency Testing Data

Allele	Tier	CAP PGx, *n* (%)	MG2, *n* (%)
*2	1	135 (96.4)	37 (74.0)
*2A		29 (20.7)	N.D.
*3	1	131 (93.6)	50 (100)
*4	1	138 (98.6)	50 (100)
*5 (Deletion)	1	122 (87.1)	49 (98.0)
*6	1	134 (95.7)	48 (96.0)
*7	2	124 (88.6)	26 (52.0)
*8	2	122 (87.1)	26 (52.0)
*9	1	132 (94.3)	34 (68.0)
*10	1	134 (95.7)	38 (76.0)
*11	No tier	67 (47.9)	N.D.
*12	2	99 (70.7)	N.D.
*14	2	88 (62.9)	N.D.
*15	2	60 (42.9)	N.D.
*17	1	130 (92.9)	28 (56.0)
*21	2	N.D.	N.D.
*29	1	118 (84.3)	N.D.
*31	2	N.D.	N.D.
*35	No tier	71 (50.7)	16 (32.0)
*40	2	N.D.	N.D.
*41	1	133 (95.0)	40 (80.0)
*42	2	N.D.	N.D.
*49	2	N.D.	N.D.
*56	2	N.D.	N.D.
*59	2	N.D.	N.D.
Duplication	1	123 (87.9)	47 (94.0)
Hybrid alleles *36 (alone or as part of *36+*10)	2	24 (17.1)	N.D.

Specific *CYP2D6* alleles that are queried in the College of American Pathologists (CAP) PGx and/or the German Reference Institute for Bioanalytics Molecular Genetics Group (MG)-2 proficiency testing surveys are listed, along with the tier assigned in the present article. The numbers and percentages of laboratories that reported that they test for the allele are provided. All alleles included in the CAP and/or MG2 surveys were listed, although *11 and *35 were not assigned to tier 1 or 2. Additionally, several alleles assigned to tier 2 are included in this table despite not being part of the CAP or MG2 survey (eg, *21, *31, *40, *42, *49, *56, *59).

N.D., no data on that allele were available from the survey.

*CYP2D6*14*

The decreased-function *CYP2D6*14* allele is characterized by a missense variant in exon 3[60] (NM_000106.6:c.505G>A, p.Gly169Arg, rs5030865, legacy 1758G>A), causing decreased enzyme function.[51] A G>T (c.505G>T) change in the same triallelic position (rs5030865) is observed in the *CYP2D6*8* allele. The common c.886C>T and c.1457G>C variants are both present in the *CYP2D6*14* haplotype. This allele has been detected primarily in the East Asian population, with a reported frequency of 0.3% (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). The variant c.505G>A is also present on the *CYP2D6*114* no-function allele in combination with the c.100C>T and the common c.886C>T and c.1457G>C variants. *CYP2D6*14A* was changed to *CYP2D6*114.001* because the function of this allele (no function) is different from the more common decreased-function *14B (*14.001) allele. *CYP2D6*114* has been detected primarily in the East Asian population, with a reported frequency of 0.08% (PharmGKB, *https://www.pharmgkb.org/gene/pa128*).

*CYP2D6*15*

*CYP2D6*15* is a no-function allele characterized by a T insertion in exon 1 causing a frameshift (NM_000106.6:c.137dup, p.Leu47fs, rs774671100, legacy 137_138insT) and resulting in a premature stop codon, truncation of the CYP2D6 protein, and loss of enzyme function.[61] This allele has frequencies of approximately 0.6% in African ancestry populations and <0.05% in European and Asian populations (Central/South and East Asian) (PharmGKB, *https://www.pharmgkb.org/gene/pa128*).

*CYP2D6*21*

The no-function *CYP2D6*21* allele is characterized by an insertion of a C in exon 5, causing a frameshift (NM_000106.6:c.805dup, p.Arg269fs, rs72549352, legacy 2579_2580insC) that results in a premature stop codon, truncation of the CYP2D6 protein, and nonfunctional enzyme.[62,63] *CYP2D6*21* also contains c.886C>T and c.1457G>C. This allele has been identified in the East Asian

population with an allele frequency of approximately 0.4% (PharmGKB, https://www.pharmgkb.org/gene/pa128).

*CYP2D6*31*

The no-function *CYP2D6*31* allele is characterized by a missense variant in exon 9 (NM_000106.6:c.1319G>A, p.Arg440His, rs267608319, legacy 4042G>A), resulting in nonfunctional protein.[64,65] This haplotype also contains c.886C>T and c.1457G>C. The allele frequency of *CYP2D6*31* among most populations is currently unclear; however, it has been found at a frequency of <1% in European and Latino populations (PharmGKB, https://www.pharmgkb.org/gene/pa128). GnomAD reports this variant, c.1319G>A, at a frequency of <0.1% in African, European (Finnish and non-Finnish), Latino, and Asian populations (gnomAD browser, https://gnomad.broadinstitute.org/variant/22-42522751-c-t?dataset=gnomad_r2_1, last accessed October 29, 2020).

*CYP2D6*40*

The no-function *CYP2D6*40* allele is characterized by an in-frame insertion of 18 bp consisting of a duplication of a 9-bp sequence (TTTCGCCCC × 2) in exon 4 (NM_000106.6:c.514_522TTTCGCCCC[3], p.172_174FRP[3], rs72549356, legacy 1863_1864insTTTCGCCCCTTTCGCCCC), rendering the protein nonfunctional.[66] This allele also contains the variant in *CYP2D6*17*, c.320C>T, and the common c.886C>T and c.1457G>C variants. The frequency of *CYP2D6*40* ranges from 0.5% to 1.3% in those of African ancestry but is <0.1% in the European population (PharmGKB, https://www.pharmgkb.org/gene/pa128). Importantly, if the in-frame 18-bp insertion is not specifically tested, the c.320C>T allele receives a *CYP2D6*17* default assignment, which is notable given that *CYP2D6*40* is a no-function allele and *CYP2D6*17* is a decreased-function allele. In addition, a similar 9-bp (TTTCGCCCC) insertion variant is present in the *CYP2D6* *30 and *58 alleles. As such, the *CYP2D6*40* allele was reassigned to Tier 2 despite having RMs, a >1% minor allele frequency, and well-defined function due to the difficulties of detecting and discriminating the 9- and 18-bp insertions.

*CYP2D6*42*

The no-function *CYP2D6*42* allele is characterized by an insertion of two nucleotides in exon 7 (NM_000106.6:c.1088_1089dup, p.Gln364fs, rs72549346, legacy 3260_3261insGT) that cause a frameshift and premature stop codon, resulting in a truncated and nonfunctional CYP2D6 protein.[67] *CYP2D6*42* also contains the c.886C>T and the c.1457G>C variants. This allele has been identified in those of African ancestry and in the Central/South Asian population with an allele frequency of <0.5% (PharmGKB, https://www.pharmgkb.org/gene/pa128).

*CYP2D6*49*

The decreased-function *CYP2D6*49* allele is characterized by a missense variant in exon 3[68] (NM_000106.6:c.358T>A, p.Phe120Ile, rs1135822, legacy 1611T>A). The haplotype also includes the c.100C>T and the c.1457G>C variants. The c.358T>A and c.100C>T together contribute to decreased enzyme function.[51,52] The *CYP2D6*49* allele has been found in the East Asian population with a frequency of about 1% (PharmGKB, https://www.pharmgkb.org/gene/pa128). The defining variant of *CYP2D6*49*, c.358T>A, is also present in the *CYP2D6*53* allele. The variant c.358T>A is also found in combination with another variant on the *CYP2D6*53* allele. Because RMs are not available for *CYP2D6*49*, it is currently classified as a Tier 2 allele. This categorization is subject to change should RMs become available.

*CYP2D6*56*

The no-function *CYP2D6*56* allele is characterized by a nonsense variant in exon 7 (NM_000106.6:c.1030C>T, p.Arg344Ter, rs72549347, legacy 3201C>T) that generates a stop codon resulting in nonfunctional truncated protein.[69,70] This haplotype also contains the common c.1457G>C variant. The *CYP2D6*56* allele has been identified in those of African ancestry or European populations with a frequency of <0.2% (PharmGKB, https://www.pharmgkb.org/gene/pa128). Some suballeles of *CYP2D6*56* contain the c.100C>T variant, such as *56.002 and *56.003. If the *CYP2D6*56* defining variant (c.1030C>T) is not tested, these suballeles are defaulted to *CYP2D6*10*.

*CYP2D6*59*

The decreased-function *CYP2D6*59* allele is characterized by a splice variant at the end of exon 6 (NM_000106.6:c.975G>A, p.Pro325=, rs79292917, legacy 2939G>A) that results in decreased canonical mRNA expression levels, protein, and enzyme activity.[71] This haplotype also contains the common c.886C>T and c.1457G>C variants. This allele has been found in the European population with a frequency of 0.7% (PharmGKB, https://www.pharmgkb.org/gene/pa128).

Hybrid Alleles

Due to the high-sequence homology between *CYP2D6* and *CYP2D7*, hybrid alleles with gene conversions are also present in the general population. The hybrid alleles can contain the 5′ region of *CYP2D6* followed by conversion to *CYP2D7* sequence, or the 5′ region of *CYP2D7* followed by conversion to *CYP2D6* sequence, which have been consolidated under the *CYP2D6*13* designation by PharmVar.[72] These hybrid alleles can be independent or in tandem with one or more full copies of the *CYP2D6* gene[17,73] (PharmVar, https://www.pharmvar.org/gene/cyp2d6). Hybrid alleles have a frequency of approximately 1% in East Asians and are less common in other populations (PharmGKB, https://www.pharmgkb.org/gene/pa128).

Because hybrid alleles are technically more challenging to characterize, the Working Group included these alleles in Tier 2. While most *CYP2D6* hybrid alleles are generally considered to be nonfunctional, they can complicate *CYP2D6* copy number analysis, particularly if the test queries *CYP2D6* gene copy number at only one position.[27,73,74] For example, if a laboratory's copy number assay is designed to query exon 1 of *CYP2D6* and a *CYP2D6-CYP2D7* hybrid gene is present, the laboratory may mistake the hybrid gene for a full copy of the *CYP2D6* gene (Table 4). Using only one location within *CYP2D6* for copy number can satisfy the Tier 1 recommendation to identify the *CYP2D6*5* deletion or gene duplication; however, it is recommended at least two, if not multiple locations, be used for detecting hybrid alleles. Ideally, targeting the 5′ untranslated region or exons 1 and 9, thus covering both ends of the gene, is advised to differentiate full copies of *CYP2D6* from hybrid alleles. While the Working Group believes that it is important to detect hybrid alleles for Tier 2 recommendations, complete characterization of the hybrid alleles is less important as most are nonfunctional.

Quality Assessments

Proficiency testing programs are commercially available for some of the *CYP2D6* alleles. Recent data from CAP proficiency testing and the German Reference Institute for Bioanalytics Molecular Genetics Group 2 were assessed to determine which *CYP2D6* alleles are currently tested by participating laboratories, and to assess the potential challenges in the implementation of these recommendations.

The numbers and percentages of laboratories that reported testing for each *CYP2D6* allele in the CAP 2019 PGx-B and Molecular Genetics Group 2 (MG21/20; April 2020) surveys are presented in Table 5. While 247 laboratories participated in the CAP 2019 PGX-B survey, data are based on the 140 (56.7%) that indicated which alleles are included in their clinical *CYP2D6* test. The European Molecular Genetics Quality Network (*https://www.emqn.org*, last accessed October 6, 2020) has a pilot panel for PGx (Pharmaco-20), covering 45 variants in various pharmacogenes, including *CYP2D6*. No information is provided regarding which *CYP2D6* alleles were characterized, nor is an outcome report publicly available.

Several supplemental questions were asked to facilitate a better understanding of the processes that laboratories use for *CYP2D6* testing. Of the 140 laboratories that provided information on the *CYP2D6* alleles included in their testing, 138 responded to the supplemental question regarding whether they perform gene-duplication/CNV testing. Of those 138 laboratories, 125 (90.6%) indicated that they perform CNV testing, while 13 laboratories responded that they do not. Twenty-nine did not respond to the question about how many positions within the *CYP2D6* gene are tested in their CNV assay. Of the 111 laboratories that responded, 63 (56.8%) indicated that they query a single position within the gene, 41 (36.9%) evaluate multiple positions, and 7 indicated "other." When asked whether the laboratory was able to differentiate between a whole gene duplication/multiplication versus the presence of a hybrid allele, 124 laboratories responded. Of the 124, 72 laboratories (58.1%) indicated that they are not able to, while 37 (29.8%) indicated that they can, and 15 (12.1%) responded that they were unsure.

Discussion

In the present article, the AMP PGx Working Group describes recommendations on alleles to be included in clinical *CYP2D6* genotyping assays. As with other published AMP PGx guidelines, alleles were selected and stratified as Tier 1 or 2 recommendations, based on priority-based criteria for testing as defined by the Working Group. The Tier 1 variant alleles are recommended to be included as a minimum panel in all clinical *CYP2D6* genotyping tests and were selected for inclusion based on their effects on the *CYP2D6* gene, CYP2D6 protein expression or enzyme function, frequency in a subset of the population, and availability of RMs. In addition, the Working Group considered the feasibility of detecting Tier 1 alleles using current molecular methods that are widely available to clinical laboratories, acknowledging that it is not practical to expect laboratories to detect complex, rare variants that are not readily detectable by routine methods (eg, gene conversions, structural variants, phasing of variants, or determination of the type and number of gene-duplication events).

The goals of these recommendations are to promote standardization among clinical laboratories and to ensure that clinical PGx tests include, at a minimum, those variant alleles that are present in approximately 1% or more of a human subpopulation and have a well-documented effect on enzyme function, unless not technically feasible. Failure to test for any of the Tier 1 alleles could result in inaccurate prediction of phenotype function in a significant proportion of the tested population. The Tier 2 alleles are included for clinical laboratories that desire to provide more comprehensive testing for rarer alleles with a defined effect on function and/or those without RMs readily available for assay validation. Of note, the Tier 1 recommendations detect the majority of known non−wild-type CYP2D6 variation (ie, non-*1 alleles) in major ethnic groups (≥78% of African Americans, approximately 84% of European Caucasians, and approximately 85% of East Asians), based on published data on allele frequency (PharmGKB, *https://www.pharmgkb.org/gene/pa128*). When both Tier 1 and Tier 2 recommended alleles are tested, the capacity to detect known non−wild-type CYP2D6 variation is higher (≥80% African Americans, approximately 85% European Caucasians, approximately 87% East Asians). The Working Group acknowledges that functionally important, rare alleles may occur in isolated populations, and that

clinical laboratories may also choose to include additional alleles that are representative of their tested population. *CYP2D6* variants or alleles without sufficient evidence of functional effect are not recommended for routine clinical testing. All clinical laboratories should follow best practices for assay validation and adhere to the applicable regulatory requirements of their location.

Genetic testing of pharmacogenes, such as CYP genes, relies on inferring star alleles or haplotypes, based on the results of genotyped variants. For a highly polymorphic gene such as *CYP2D6*, interrogation of a single sequence variant is often not sufficient to call an allele/haplotype. As with all PGx genotyping, it is recognized that a lack of detection of rare sequence variants that are not included in the assay will result in a default wild-type call or normal allele (eg, *CYP2D6*1*) assignment. Similarly, rare variants that occur on a specific haplotype and that are not tested will result in a default call of the more common haplotype that includes a subset of the variants, which may, in turn, result in an incorrect phenotype prediction. For example, the Tier 1 alleles *CYP2D6* *17, *29, and *41 should be identified not only by the variant that characterizes the allele, but also by the presence of the c.886C>T variant. On the other hand, the two common variants, c.886C>T and c.1457G>C, are present in many Tier 1 (*2, *17, *29, and *41) and Tier 2 (*8, *12, *14, *21, *31, *40, and *59) alleles, as well as many other rare alleles. Thus, lack of detection of the core variants for these alleles will result in a default *CYP2D6*2* allele assignment (normal function). This is a caveat of all genetic testing based on haplotype calling, and emphasizes the need for inclusion of the most common variant alleles in clinical genotyping assays. It is recommended that laboratories include a caveat statement in their reports that additional or rare variants may be present that could affect a patient's predicted phenotype.

CYP2D6 genotyping is technically challenging due to the presence of CNVs and more complex gene-conversion events associated with some variant alleles in the general population. Through homologous recombination, gene deletion and duplication events have occurred through evolution, with reports of some individuals having as many as 13 tandem copies of a functional *CYP2D6* gene.[75] Therefore, the identification of gene deletion (*CYP2D6*5*) and gene duplications/multiplications (*CYP2D6×N*) is considered an essential aspect of clinical *CYP2D6* testing and is achievable using commercially available copy number assays. However, many methods/platforms cannot determine the exact number of gene copies on an allele or which of the two chromosomal alleles harbors the gene-duplication event. Therefore, in some cases it may not be possible to determine whether a functional or decreased-/no-function allele is duplicated (eg, to discriminate between *CYP2D6* *2×2/*4 and *2/*4×2, or *2×3/*4 and *2×2/*4×2). This is a caveat of many clinically available tests that is recognized by the Working Group, and the determination of the number of gene copies and function is not considered as Tier 1 at this time. Similarly, the detection of hybrid alleles is not considered Tier 1, as their accurate detection is technically challenging. The Working Group includes information on the detection of hybrid alleles in Tier 2 for clinical laboratories that desire to incorporate a more comprehensive genotyping analysis of these complex alleles. Such analyses can be useful for determining the assessment of *CYP2D6* gene function in individual patients and thereby improve the accuracy of phenotype predictions.

Clinical Applications

There are six published CPIC guidelines that involve CYP2D6-metabolized medications: tamoxifen,[4] ondansetron and tropisetron,[5] tricyclic antidepressants,[6] selective serotonin reuptake inhibitors,[7] select opioids including codeine,[8] and atomoxetine.[9] In addition, FDA-approved labels of over 20 medications include dosing recommendations based on CYP2D6 phenotype, and the labels of over 70 additional medications include informative statements on the impact of CYP2D6 phenotype on drug exposure (CPIC, *https://cpicpgx.org/genes-drugs*, last accessed November 13, 2020). The importance of CYP2D6 phenotype on drug therapy in patients with psychiatric disease was underlined in a recent review of PGx testing in psychiatry,[76] as well as in a recommendation from the International Society of Psychiatric Genetics: genetics information on *CYP2D6* along with *CYP2C19* would likely be most beneficial in patients who have experienced an inadequate response or an adverse reaction to a previous trial of an antidepressant or antipsychotic (International Society of Psychiatric Genetics, *https://ispg.net/genetic-testing-statement*, last accessed October 29, 2020).

CYP2D6 Alleles Not Included in Tier 1 or 2 Recommendations

According to the data on frequency from CPIC, the *CYP2D6*11* allele (NM_000106.6:c.181-1G>C, rs201377835, legacy 883G>C) has a MAF of up to 0.1% in the European population. However, according to gnomAD, the variant that characterizes this allele is most common in the non-Finnish European population, with an MAF of 0.025%, which falls below the defined cutoff of 0.1% MAF for inclusion in Tier 2. Although the gnomAD data report the MAF of a single variant, it is recognized that no haplotype can be present in the population more frequently than any individual variant contained within the haplotype. Therefore, despite the data on frequency from CPIC, this allele is not recommended in either tier at this time due to its overall low frequency based on data on population from gnomAD.

*CYP2D6*69* is a no-function allele that is difficult to discriminate from other alleles and is therefore not included in the Tier 1 or 2 recommendations. The variants that are present in *CYP2D6*69* (c.100C>T, c.886C>T, c.985+39G>A, and c.1457G>C) are also present in many other commonly detected *CYP2D6* alleles, including *2, *10, and *41. When

these four variants are heterozygous, it is difficult to discriminate the rare *CYP2D6* *1/*69 diplotype from the more commonly observed *10/*41 diplotype unless additional testing is undertaken, such as testing for c.352+7A>G, rs267608289, which appears to be unique to *69, or establishing that c.100C>T, c.886C>T, c.985+39G>A, and c.1457G>C are on the same haplotype (eg, by using methods for establishing linkage, eg, allele-specific amplification combined with real-time PCR amplification, or parent testing).[77] Thus, it is common practice to default to *CYP2D6*10/*41*, based on observed allele frequencies that may underestimate the frequency of *69 (PharmGKB, https://www.pharmgkb.org/gene/pa128). Given that both *CYP2D6* *1/*69 and*10/*41 are associated with intermediate metabolism, this distinction does not affect phenotype assignment. A *CYP2D6*69* allele may be identified by atypical or unexpected variants present in a patient, that cannot otherwise be reconciled. A case in point is a patient in whom this allele was first discovered and ultimately genotyped as *CYP2D6*4/*69*.[77] Sequence analysis would also facilitate the identification of *CYP2D6*69*.

A *CYP2D6* enhancer variant (NM_152613.3:c.63-2604G>A, rs5758550), located 116 Kbp downstream of the *CYP2D6* gene, was suggested to affect CYP2D6 activity by modulating expression levels, and through this mechanism account for unexplained variability in CYP2D6 activity *in vivo*.[78,79] The investigators proposed that this enhancer variant, in combination with c.886C>T, which is present on *CYP2D6*2* and many other alleles, should be included in PGx test panels as it may be predictive of functional activity. The findings from one subsequent study, however, contradicted these findings.[80] Specifically, in the latter publication, the investigators did not find an effect of rs5758550 on the formation of endoxifen in breast cancer patients taking tamoxifen. Additional studies are needed for corroborating the initial hypothesis that the enhancer variant has a clinically relevant impact on CYP2D6 activity, which would warrant its inclusion in clinical PGx genotyping panels. Furthermore, rs5758550 not only is present in *CYP2D6*2*, where it was originally discovered, but also was recently shown to occur in many other haplotypes including decreased- and no-function alleles.[81] Therefore, due to limited evidence, the Working Group does not recommend testing for the rs5758550 variant.

Limitations

This document is focused only on recommendations on alleles to include in clinical laboratory genotyping assays for *CYP2D6*. This document does not include, for example, the mapping of genotypes to phenotype, clinical interpretation of genotypes, or recommendations on changes to medication therapy based on genotype, as these were considered to be outside the scope of this document and/or available from other sources, such as CPIC and PharmGKB.[82,83] Although technical challenges related to interrogating *CYP2D6* were discussed in this document, the Working Group does not recommend or endorse any particular molecular testing platforms for *CYP2D6* genotyping.

Conclusion

This document provides recommendations on PGx alleles to include in clinical tests for *CYP2D6* genotyping. These recommendations are intended to facilitate the design and implementation of genetic testing by clinical laboratories. In addition, these recommendations are intended to promote test standardization and genotype concordance between laboratories.

Disclaimer

The 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 Guideline or Report should not be considered inclusive of all proper approaches or methods, or exclusive of others. The Guideline or Report cannot guarantee any specific outcome, nor does it establish a standard of care. The Guideline or Report is not intended to dictate the treatment of a particular patient. Treatment decisions must be made based on the independent judgment of health care providers and each patient's individual circumstances. The AMP makes no warranty, express or implied, regarding the Guideline or Report 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.

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Supplemental Data

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