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SPECIAL ARTICLE

Recommendations for Clinical *CYP2C9* Genotyping Allele Selection

A Joint Recommendation of the Association for Molecular Pathology and College of American Pathologists

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The goals of the Association for Molecular Pathology Pharmacogenomics (PGx) Working Group of the Association for Molecular Pathology Clinical Practice Committee are to define the key attributes of PGx alleles recommended for clinical testing and a minimum set of variants that should be included in clinical PGx genotyping assays. This document provides recommendations for a minimum panel of variant alleles (Tier 1) and an extended panel of variant alleles (Tier 2) that will aid clinical laboratories when designing assays for *CYP2C9* testing. The Working Group considered the functional impact of the variants, allele frequencies in different populations and ethnicities, the availability of reference materials, and other technical considerations for PGx testing when developing these recommendations. Our goal is to promote standardization of testing PGx genes and alleles across clinical laboratories. These recommendations are not to be interpreted as restrictive but to provide a reference guide. The current document will focus on *CYP2C9* testing that can be applied to all *CYP2C9*-related medications. A separate recommendation on warfarin PGx testing is being developed to include recommendations on *CYP2C9* alleles and additional warfarin sensitivity—associated genes and alleles. *(J Mol Diagn 2019, 21: 746—755; https://doi.org/10.1016/j.jmoldx.2019.04.003)*

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The Pharmacogenomics (PGx) Working Group of the Clinical Practice Committee, Association for Molecular Pathology (AMP) with organizational representation from the College of American Pathologists (represented by A.M.M.) and Clinical Pharmacogenetics Implementation Consortium (represented by M.W-C.). The AMP 2017 and 2018 Clinical Practice Committee consisted of Antonia R. Sepulveda (Chair), Monica J. Basehore, Mark Boguski, Noah A. Brown, Susan Butler-Wu, Pranil Chandra, Josh Deignan, Alex Greninger, Meera R. Hameed, Kenneth L Muldrew, Keyur Patel, Jess Friedrich Peterson, Benjamin Pinsky, Mark J. Routbort, Kandelaria Rumilla, Ryan Schmidt, David S. Viswanatha, Megan B. Wachsmann, and Justin Zook.

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The Association for Molecular Pathology (AMP) Pharmacogenomics (PGx) Working Group describes a minimum list of alleles to include in clinical cytochrome P450 2C9 (*CYP2C9*) genotyping panels. These recommendations are developed to guide clinical laboratory professionals who validate and offer clinical PGx assays, with the goal of promoting standardization of PGx testing across different laboratories. This series of AMP PGx Working Group documents should be implemented with other clinical guidelines such as those issued by the Clinical Pharmacogenetics Implementation Consortium (CPIC), which focus primarily on the interpretation of genotyping results and therapeutic recommendations for a specific drug(s).[1] The results of this study suggest variants for inclusion in clinical *CYP2C9* genotyping panels and defines the key attributes of those alleles that were chosen for recommendation in clinical PGx testing.

Clinical PGx testing assays across different laboratories differ with regard to both the star (*) allele haplotypes tested in each pharmacogene and the variants used to define those haplotypes.[2,3] A Genetic Testing Reference Material Program (GeT-RM) study[4] evaluated a number of PGx test panels across 28 genes in 137 genomic DNA samples and found discrepant results mostly attributable to assay design. Without exception, no 2 tests that examined any of the 28 PGx genes included in the study were designed to detect the same set of variants and/or haplotypes (alleles). This genotyping variability can result in discrepancies in haplotype and diplotype assignment, which may affect test interpretation and ultimately patient care. For example, if a patient's genome harbors a heterozygous *CYP2C9* NM_000771.3:c.449G>A, p.Arg150His; rs7900194 variant, which defines the *8 (reduced function) allele common among African Americans and Africans,[5,6] but the patient undergoes a test that does not include this variant, he or she may be assigned a genotype of *1/*1 (normal function) rather than *1/*8, affecting the predicted phenotype and clinical treatment strategy and potentially creating a health care disparity. Variability in the PGx alleles tested by different clinical laboratories has also led to discrepant results on proficiency testing surveys.[3]

The AMP PGx Working Group was formed to derive a minimum set of alleles and variants that should be included in clinical PGx genotyping test panels and to define the key attributes of the selection of these alleles. This group has previously published recommendations for variants that should be included in any clinical *CYP2C19* genotyping assay.[7] Through that effort, the committee developed a two-tier strategy and selection criteria for recommended PGx clinical testing. Tier 1 recommended PGx variant alleles are those that i) have been well characterized and found to significantly affect the function of the protein and/or gene leading to an alteration in a drug response phenotype, ii) have an appreciable minor allele frequency in a population/ethnicity group, and iii) have publicly available reference materials (RMs). Alleles and variants that currently meet at least one but not all three of the tier 1 criteria are included as tier 2 variant alleles, which may be moved to tier 1 if RMs or additional information becomes available. A description on the rationale for these clinical PGx testing recommendations and development of this two-tier classification strategy have been previously described in the *CYP2C19* recommendation document from this group.[7]

CYP2C9

The cytochrome P450 2C9 is a member of the CYP2C subfamily of the cytochrome P450 enzymes and is one of the most abundant and important drug metabolizing enzymes. It has been estimated that approximately 15% of all CYP-related biotransformation is catalyzed by CYP2C9, including several widely prescribed medications with a narrow therapeutic index, such as the anticoagulant warfarin and the anticonvulsant phenytoin.[8] Like other CYP enzymes, CYP2C9 catalyzes a variety of exogenous and endogenous compounds, many of which are also substrates for other phase I and/or phase II enzymes. Medications for which CYP2C9 is responsible for >25% of metabolic clearance have been summarized elsewhere.[9]

CYP2C9 is currently included in the U.S. Food & Drug Administration (FDA) Table of Pharmacogenetic Biomarkers in Drug Labeling for several FDA-approved drugs (U.S. Food & Drug Administration, *https://www.fda.gov/Drugs/ScienceResearch/ucm572698.htm*, last accessed August 15, 2018). The *CYP2C9* gene has nine exons and is located on chromosome 10q23.33, where several CYP2C subfamily members (*CYP2C18*, *CYP2C19*, *CYP2C9*, and *CYP2C8*) are clustered. Like other *CYP450* genes, *CYP2C9* is highly polymorphic, and variant *CYP2C9* star (*) alleles are frequently included in clinical PGx testing assays. The two most well-characterized variant alleles are *CYP2C9*2* (NM_000771.3:c.430C>T, p.Arg144Cys, rs1799853) and *CYP2C9*3* (NM_000771.3:c.1075A>C, p.Ile359Leu, rs1057910), both of which are associated with decreased enzyme activity and impaired drug metabolism phenotypes.[10] Among the 60 variant *CYP2C9* star (*) alleles listed on The Pharmacogene Variation Consortium website (*https://www.pharmvar.org*; formerly the Human Cytochrome P450 (CYP) Allele Nomenclature website),[11] at least 20 are reported to have *in vivo* and/or *in vitro* functional evidence of altered activity (The Pharmacogene Variation Consortium, *https://www.pharmvar.org/gene/CYP2C9*, last accessed August 15, 2018).

According to the Genetic Testing Registry (The National Center for Biotechnology Information, *https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=CYP2C9*, last accessed August 15, 2018) and AMP Test Directory (Association for Molecular Pathology, *https://www.amp.org/resources/test-directory*, last accessed August 15,2018), the alleles included in *CYP2C9* genotyping tests offered by clinical

Table 1 Commercially Available *CYP2C9* Testing Platforms

CYP2C9 allele	Affymetrix PharmacoScan (RUO)*	iPLEX ADME (RUO)†	INFINITI (CE-marked)‡	eSensor (FDA-cleared)§	Defense analyte-specific reagents)¶	OpenArray (V, RUO)*	TrimGen (FDA-cleared)‖
1B		x					
1C		x					
1D		x					
2	x	x	x	x	x		x
2C		x					
3	x	x	x	x	x		x
3A		x					
3B		x					
4	x	x	x				
5	x	x	x				
6	x	x	x				
7		x					
8	x	x					
9	x	x					
10	x	x					
11	x		x				
11A		x					
11B		x					
12	x	x					
13	x	x					
14		x					
15	x	x					
16	x	x					
17	x	x					
18	x	x					
19	x	x					
20	x	x					
21	x	x					
22		x					
23	x	x					
24	x	x					
25	x	x					
26	x	x					
27		x					
28		x					
29	x	x					
30	x	x					
31	x	x					
32	x	x					
33		x					
34	x	x					
36	x						
37	x						
38	x						
39	x						
40	x						
42	x						
43	x						
44	x						
45	x						
46	x						
47	x						
48	x						
49	x						

(*table continues*)

Table 1 (*continued*)

CYP2C9 allele	Affymetrix PharmacoScan (RUO)*	iPLEX ADME (RUO)†	INFINITI (CE-marked)‡	eSensor (FDA-cleared)§	Defense analyte-specific reagents)¶	OpenArray (V, RUO)*	TrimGen (FDA-cleared)‖
50	x						
51	x						
52	x						
53	x						
54	x						
55	x						
56	x						
57	x						
58	x						

Commercially available platforms as of April 2, 2019 and does not represent a comprehensive list. Inclusion herein does not represent an endorsement of any product or service by AMP.

*Thermo Fisher Scientific, (Waltham, MA).

†Agena Biocience (San Diego, CA).

‡AutoGenomics (Carlsbad, CA).

§GenMark Diagnostics (Carlsbad, CA).

¶BioFire Defense, LLC (Murray, UT).

‖TrimGen Genetic Diagnostics (Sparks, MD).

CE, Conformité Européenne; FDA, Food and Drug Administration; RUO, research use only; V, variable.

laboratories in the United States range from a few targeted alleles to interrogation of the entire coding region, and the techniques include targeted genotyping, bidirectional Sanger sequencing, next-generation sequencing, whole genome sequencing, or whole exome sequencing, with or without deletion or duplication analysis. Inconsistent clinical results across laboratories[12] may be attributed to the selection of tested *CYP2C9* alleles, targeted testing of populations with varying ethnic backgrounds, and technical performance of the testing platforms. Differences can also occur postanalytically during PGx interpretation and/or reporting; however, addressing these issues is considered outside the scope of this series of AMP PGx recommendation documents.

Existing Guidelines

Clinical PGx guidelines are available from groups including CPIC (Clinical Pharmacogenetics Implementation Consortium, *https://cpicpgx.org*, accessed August 15, 2018), the Dutch Pharmacogenetics Working Group funded by the Royal Dutch Pharmacists Association,[13] and the Canadian Pharmacogenomics Network for Drug Safety (*http://cpnds.ubc.ca*, last accessed August 15, 2018). The goals ofCPIC (and others) are to address barriers to clinical implementation of pharmacogenetic tests by creating, curating, and posting freely available, peer-reviewed, evidence-based, updatable, and detailed gene and drug clinical practice guidelines (*https://cpicpgx.org*). These guidelines, which were developed using extensive literature review and discussion among experts, are gene-drug pair oriented with an emphasis on interpretation of genotype and phenotype, and genotype-guided therapeutic recommendations. These documents have played a critical role in shaping the clinical implementation of PGx tests and have facilitated development of clinical decision support tools for clinicians to better understand and more efficiently use PGx testing results. There are currently 10 guidelines available on CYP2C9-metabolized medications from the CPIC, Dutch Pharmacogenetics Working Group, and Canadian Pharmacogenomics Network for Drug Safety (PharmGKB,[14] *https://www.pharmgkb.org/gene/PA126/guideline*, last accessed August 15, 2018). Although some clinical PGx guidelines include summaries of known *CYP2C9* alleles, frequencies in various populations, and their functional and/or clinical relevance, they do not explicitly recommend specific variant alleles for clinical laboratories to include in *CYP2C9* genotyping panels. Moreover, althoughthe FDA recognizes the role of *CYP2C9* genetic variability for a number of medications, limited information is usually available in FDA product labels regarding the testing methods used by drug manufacturers while conducting PGx studies. Despite the fact that many commercial platforms are available, clinical laboratories often develop their own laboratory tests for *CYP2C9* genotyping. Specific considerations from a diagnostic laboratory perspective, such as allele selection, testing platforms, and availability of RMs, have not been the focus of the consortia guidelines. However, consistency in clinical genotyping panels among clinical laboratories could further promote the use of these important clinical PGx practice guidelines.

The AMP PGx Working Group reviewed the variant *CYP2C9* star (*) alleles currently cataloged by PharmVar,[11] including allele function, multiethnic allele frequencies, the availability of RMs, and commercially available genotyping platforms (Table 1). Tier 1 recommended *CYP2C9* variant

alleles were defined as those that have i) well-characterized alteration of CYP2C9 activity that has an effect on drug response[15] and for which the functional variant is known, ii) appreciable minor allele frequency in a population (PharmGKB, *https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx*, last accessed August 15, 2018), and iii) publicly available RMs (Table 2). Tier 2 *CYP2C9* variant alleles are defined as alleles that meet at least one but not all of the criteria for inclusion in Tier 1 and are considered optional for inclusion in expanded clinical genotyping panels. Some of the Tier 2 alleles may be recommended as Tier 1 in the future if RMs or additional information becomes available. Variants with unknown or uncertain function are not recommended for inclusion in targeted clinical *CYP2C9* genotyping test panels, although it may be useful to include these in research panels to clarify functional and/or clinical outcomes.

Tier 1 *CYP2C9* Variant Alleles

CYP2C9 variant alleles recommended as Tier 1 by the AMP PGx Working Group include *CYP2C9* **2*, **3*, **5*, **6*, **8*, and **11*. This recommendation was based on their well-established functional effects on CYP2C9 activity and drug response,[15] availability of RMs, and their appreciable allele frequencies in major ethnic groups (PharmGKB, *https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx*, last accessed August 15, 2018). The *CYP2C9*2* and **3* alleles are the most common *CYP2C9* alleles interrogated by commercially available platforms (Table 1). Importantly, the inclusion of these two variants accounts for 98% to 100% of the currently defined variation in *CYP2C9*, leading to decreased function in European, Middle Eastern, and Asian populations but only approximately 25% of the currently defined *CYP2C9* variation in populations with African ancestry (Table 3). Thus, genotyping for only the *CYP2C9*2* and **3* variants will not detect most *CYP2C9* genomic variation leading to decreased enzymatic activity in populations with African ancestry. The *CYP2C9*5*, **6*, **8*, and **11* alleles have a combined frequency of approximately 10% in populations with African ancestry and collectively are more common than the **2* and **3* alleles in these populations, accounting for approximately 75% of the currently defined *CYP2C9* variation in African and African Americans. In contrast, these alleles have frequencies <0.4% in European and Asian populations (PharmGKB, *https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx*, last accessed August 15, 2018), accounting for <3% of currently defined *CYP2C9* variation in those populations.

Table 2 Current Publicly Available Reference Materials for *CYP2C9*

Allele	Coriell number (diplotype)
**2*	NA10854 (*2/*2)
	NA10831 (*1/*2)
**3*	NA10855 [*2/*3 (*18)]
	NA17290 [*1/*3 (*18)]
**4*	None
**5*	NA23275 (*5/*5)
	NA19908 (*1/*5)
	NA19178 (*5/*9)
**6*	NA19213 (*1/*6)
	NA19143 (*1/*6)
**7*	None
**8*	NA19226 (*1/*8)
	NA12815 (*1/*8)
**9*	NA07439 (*1/*9)
	NA19178 (*5/*9)
**10*	NA15245 (*10/*12)
**11*	NA19122 (*1/*11)
**12*	NA15245 (*10/*12)
**13*	None
**15*	None
**18*	NA19917 [*1/*1 (*18)]
	NA23405 [*1/*3 (*18)]

This is not a comprehensive list. Inclusion herein does not represent an endorsement of any product or service by AMP. For a complete list, see CDC website (*https://wwwn.cdc.gov/clia/Resources/GETRM/default.aspx*, last accessed August 15, 2018).

CYP2C9*2

The decreased function *CYP2C9*2* allele is characterized by the presence of a missense variant in exon 3 (NM_000771.3:c.430C>T, p.Arg144Cys, rs1799853) that causes a decrease in CYP2C9 enzymatic activity toward most of its substrates.[16–18] *CYP2C9*2* has an allele frequency that ranges from 11% to 13% in European, Middle Eastern, and South/Central Asian populations but only approximately 2% in populations with African ancestry and <1% in the East Asian population (PharmGKB, *https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx*, last accessed August 13, 2018). This variant is also present in the *CYP2C9*35* allele that is defined by the additional presence of an arginine to leucine change at amino acid 125 (NM_000771.3:c.374G>T, p.Arg125Leu, rs72558189)[19] (The Pharmacogene Variation Consortium, *https://www.pharmvar.org/gene/CYP2C9*, accessed August 15, 2018). The functional effect of the *CYP2C9*35* defining variant is yet unknown.

CYP2C9*3

The decreased function *CYP2C9*3* allele has a missense variant in exon 7 (NM_000771.3:c.1075A>C, p.Ile359Leu, rs1057910) that causes significant reduction in enzymatic activity.[16,20] The magnitude of enzyme impairment caused by *CYP2C9*3* is more pronounced than that of *CYP2C9*2* for most important drug substrates.[21] Its frequency ranges

Table 3 *CYP2C9* Tier 1 Variant Alleles

Allele	Allele functional status[†]	Defining functional variant	HGVS nomenclature: NM_000771.3	HGVS nomenclature: NG_008385.1[‡]	Reference material available[§]	Multiethnic allele frequency
*2[¶]	Decreased function	rs1799853	c.430C>T, p.Arg144Cys	g.8633C>T	Yes	0%–12%
*3[‖]	Decreased function	rs1057910	c.1075A>C, p.Ile359Leu	g.47639A>C	Yes	1%–11%
*5	Decreased function	rs28371686	c.1080C>G, p.Asp360Glu	g.47644C>G	Yes	0%–1%
*6	No function	rs9332131	c.818del, p.Lys273Argfs*34	g.15625delA	Yes	0%–1%
*8	Decreased function	rs7900194	c.449G>A, p.Arg150His	g.8652G>A	Yes	0%–5%
*11	Decreased function	rs28371685	c.1003C>T, p.Arg335Trp	g.47567C>T	Yes	0%–2%

[†]Citations for assignment of function can be found at *https://www.pharmvar.org/gene/CYP2C9* (last accessed August 15, 2018).
[‡]CYP2C9 RefSeqGene.
[§]Table 2.
[¶]Note that the defining variant of the *35 allele (c.374G>T, p.Arg125Leu) is likely in linkage disequilibrium with the defining *2 variant (c.430C>T, p.Arg144Cys)).
[‖]Note that the defining *18 variant of the allele (c.1190A>C, p.Asp397Ala, rs72558193) is likely in linkage disequilibrium with the defining variant of *3 variant (c.1075A>C, p.Ile359Leu, rs1057910).
HGVS, Human Genome Variation Society.

from 7% to 10% in populations of European, Middle Eastern, and South/Central Asian ancestry but is much lower in populations of African (approximately 1%) and East Asian (approximately 3%) ancestry (PharmGKB, *https://api.pharmgkb.org/v1/download/file/attachment/CYP2C9_frequency_table.xlsx*, last accessed August 13, 2018). The *CYP2C9*3* defining variant (NM_000771.3:c.1075A>C, p.Ile359Leu, rs1057910) is also present in *CYP2C9*18*, which additionally harbors a missense variant (NM_000771.3:c.1190A>C, p.Asp397Ala, rs72558193). Very limited *in vitro* data are available on the functional effects of *CYP2C9*18*, which was initially identified in Indians.[22,23]

CYP2C9*5

The *CYP2C9*5* allele, which has been found almost exclusively in individuals of African descent, is characterized by a missense variant in exon 7 (NM_000771.3:c.1080C>G, p.Asp360Glu, rs28371686) and is associated with reduced enzymatic activity.[23]

CYP2C9*6

The *CYP2C9*6* allele is defined by a single nucleotide deletion in exon 5 that causes a frameshift (NM_000771.3:c.818delA, p.Lys273Argfs, rs9332131).[24] Although this allele has a lower frequency than the other decreased activity alleles among African Americans and Africans (Table 3), its null activity and association with central nervous system phenytoin toxicity[24] and reduced warfarin dose requirements[25] make it an important allele to interrogate when clinically genotyping *CYP2C9*.

CYP2C9*8

The *CYP2C9*8* allele is defined by a missense variant in exon 3 (NM_000771.3:c.449G>A, p.Arg150His, rs7900194) and is the most frequent decreased function allele among African Americans and Africans (Table 3). The c.449G>A variant is very rare in most other populations (gnomAD browser, *https://gnomad.broadinstitute.org/variant/10-96702066-G-C*, last accessed July 10, 2019). Although this allele results in decreased enzymatic function toward warfarin and phenytoin, it has been reported to exhibit substrate specificity. For example, an *in vitro* study reported that it may confer increased enzymatic activity toward tolbutamide.[26] However, two promoter variants, NM_000771.3:c.-1766T>C (rs9332094) and NM_000771.3:c.-1188T>C (rs4918758), which are in strong linkage disequilibrium with the defining *CYP2C9*8*

Table 4 *CYP2C9* Tier 2 Variant Alleles

Allele	Allele functional status[†]	Defining functional variant	HGVS nomenclature: NM_000771.3	HGVS nomenclature: NG_008385.1[‡]	Reference material available[§]	Multiethnic allele frequency
*12	Decreased function	rs9332239	c.1465C>T, p.Pro489Ser	g.55363C>T	Yes	0%–0.3%
*13	Decreased function	rs72558187	c.269T>C, p.Leu90Pro	g.8301T>C	No	0%–0.2%
*15	No function	rs72558190	c.485C>A, p.Ser162*	g.14125C>A	No	0%–0.01%

[†]Available from PharmVar (*https://www.pharmvar.org/gene/CYP2C9*, last accessed August 15, 2018).
[‡]CYP2C9 RefSeqGene; forward relative to chromosome.
[§]Table 2.
HGVS, Human Genome Variation Society.

variant (rs7900194) and have been associated with decreased *CYP2C9* expression, may also contribute to the effects of the **8* allele.[27] Moreover, given the high homology in DNA sequence at the NM_000771.3:c.449G>A locus among CYP2C genes, genotyping for the NM_000771.3:c.-1766T>C polymorphism has been proposed as an alternative means of identifying *CYP2C9*8*.[28] Substrate specificity may limit the classification of this allele as a decreased function allele when assigning a likely phenotype if used to inform medications other than phenytoin or warfarin (eg, sulfonylureas).[6,29,30] Both *in vivo* and *in vitro* studies have found that decreased warfarin clearance is associated with *CYP2C9*8*.[29] Although its functional impact is less well characterized than the **2* and **3* alleles, the **8* allele was chosen as a tier 1 allele because of its prevalence in populations of African descent, among whom warfarin has been widely used. Because ofconcerns of substrate specificity for the *CYP2C9*8* allele, clinicians should be cautious when interpreting genotypic results and taking clinical action for any substrate other than those that have been extensively characterized. Also of note, NM_000771.3:c. 449G>A/C/T is a tetra-allelic variant also reported as NM_000771.3:c.449G>T, p.Arg150Leu, rs7900194 (*CYP2C9*27*), which should be taken into consideration when designing new assays or interpreting analytic results.

CYP2C9*11

The *CYP2C9*11* allele is defined by a missense variant (NM_000771.3:c.1003C>T, p.Arg335Trp, rs28371685) in exon 7. This allele has been reported across all ethnicities and is also prevalent among African Americans and Africans (Table 3). Consistent with other tier 1 recommended *CYP2C9* alleles, *CYP2C9*11* has been associated with impaired warfarin metabolism and lower dose requirements.[31]

Tier 2 *CYP2C9* Variant Alleles

The following *CYP2C9* alleles are recommended for inclusion in tier 2: *CYP2C9*12*, **13*, and **15* (Table 4). The *CYP2C9*12* allele is defined by a missense variant in exon 9 (NM_000771.3:c.1465C>T, p.Pro489Ser, rs9332239); *CYP2C9*13* is defined by a missense variant in exon 2 (NM_000771.3:c.269T>C, p.Leu90Pro, rs72558187); and *CYP2C9*15* is defined by a nonsense variant in exon 4 (NM_000771.3:c.485C>A, p.Ser162*, rs72558190). These alleles have been shown to have either decreased function or no function (The Pharmacogene Variation Consortium, *https://www.pharmvar.org/gene/CYP2C9*, last accessed August 15, 2018) toward major CYP2C9 substrates and therefore may be included in more comprehensive clinical genotyping panels. However, they were not included in the tier 1 recommendations because ofeither very low multiethnic minor allele frequencies (<0.5%) and/or a lack of currently available RMs (**13* and **15*). As further information is available for these variants and RMs become available, they may be promoted to tier 1 *CYP2C9* recommended alleles.

Discussion

Professional organizations such as AMP devote resources and efforts to establishing recommendations for professional practice because it is important for molecular diagnostic laboratories to have resources for developing and validating clinical diagnostic testing. AMP members are among the early adopters and users of PGx testing in clinical settings and have accumulated substantial knowledge and expertise that is useful for laboratories beginning to implement these tests. This document offers a two-tier categorization of recommended *CYP2C9* alleles for inclusion in clinical *CYP2C9* genotyping assays.

The AMP PGx Working Group has proposed a recommended minimum set of alleles and their defining variants (tier 1) that should be included in clinical *CYP2C9* genotyping tests based on allele function, population frequency, and the availability of RMs. In addition, the Working Group has defined selected *CYP2C9* alleles that do not currently meet one or more of the criteria for inclusion in tier 1 and are thus considered optional for clinical testing (tier 2). These recommendations are intended to facilitate standardization of testing by laboratories and to improve genotyping concordance across laboratories.

The tier 1 alleles recommended for clinical testing were selected based on their reported clinical relevance for CYP2C9-associated medications, their frequency, and the availability of RMs. Tier 1 alleles include *CYP2C9*2*, **3*, **5*, **6*, **8* and **11*. *CYP2C9*2* and **3* are the most common alleles in whites and Asians (Table 3) and have been extensively investigated among CYP2C9-metabolized medications with narrow therapeutic ranges, such as warfarin and phenytoin. Similar data exist for the **5*, **6*, **8*, and **11 alleles, which occur predominately in populations of African descent.

There are currently two published CPIC practice guidelines that involve *CYP2C9*, and both also incorporate additional pharmacogene(s): warfarin (*CYP2C9*, *VKORC1*, *CYP4F2*, and rs12777823)[15] and phenytoin (*CYP2C9* and *HLA-B*).[32] A CPIC practice guideline for *CYP2C9* and celecoxib is in progress (K. Caudle, personal communication). A second PGx expert group led by the Royal Dutch Association for the Advancement of Pharmacy, the Dutch Pharmacogenetics Working Group, offers additional guidance for use of *CYP2C9* genotyping for prescribing acenocoumarol (not FDA approved), glibenclamide, gliclazide, glimepiride, phenoprocoumon (not FDA approved), and tolbutamide.[13] Moreover, FDA-approved labeling information also includes dosing recommendations based on *CYP2C9* genotype for the following medications: celecoxib (U.S. Food & Drug Administration, *https://www.*

accessdata.fda.gov/drugsatfda_docs/label/2016/020998s048lbl.pdf, last accessed August 15, 2018), flibanserin (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/022526lbl.pdf, last accessed August 15, 2018), flurbiprofen (https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/018766s020lbl.pdf, last accessed August 15, 2018), lesinurad (https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/207988lbl.pdf, last accessed August 15, 2018), phenytoin (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/084349s081s082s084lbl.pdf, last accessed August 15, 2018), siponimod (https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/209884s000lbl.pdf, last accessed April 2, 2019), and warfarin (https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/009218s118lbl.pdf, last accessed August 15, 2018). As such, *CYP2C9* testing has the potential to guide clinicians when considering the use of at least 12 different medications, some of which are among the top 200 most prescribed medications in the US (http://clincalc.com/DrugStats, last accessed August 15, 2018).

Although this document provides allele recommendations for all clinical *CYP2C9* genotyping indications, *CYP2C9* testing is closely associated with warfarin dosing. The CYP2C9 enzyme metabolizes the more potent *S*-warfarin enantiomer, and the *CYP2C9 *2*, *3*, *5*, *6*, *8*, and *11* alleles are associated with reduced *S*-warfarin clearance.[23,29,31,33–35] Data consistently demonstrate reduced warfarin dose requirements in individuals who carry these variant alleles, and a recent clinical trial found that warfarin dosing guided by *CYP2C9*, *VKORC1*, and *CYP4F2* genotypes reduced the composite end point of venous thromboembolism, major bleeding, supratherapeutic anticoagulation, and death compared with a nongenotype guided approach.[36] The FDA-approved warfarin labeling includes dosing recommendations based on the *CYP2C9* and *VKORC1* genotypes.[15] However, the prescribing information does not include the alleles that are important for warfarin response in patients of African descent. This is especially relevant for ethnically diverse and admixed populations. African alleles may be present in individuals whomay not consider themselves of African descent; thus, determination of African ethnicity may not be required to test for these alleles. When these alleles are present in any patient regardless of known African descent, they would be associated with reduced metabolism and could be used for therapeutic decisions. Failing to account for the *CYP2C9*5*, *6*, *8*, and *11* alleles in warfarin PGx dosing algorithms may lead to increased risk for overdosing in African Americans.[15,37,38] In addition, current CPIC guidelines for warfarin dosing underscore the importance of accounting for African alleles and provide separate recommendations for patients of African versus non-African ancestry.[15] As such, we recommend that laboratories include all tier 1 alleles, including the major African alleles, in clinical *CYP2C9* testing.

The CYP2C9 enzyme is also involved in the metabolism of phenytoin. Patients with a reduced or no function allele (eg, *2, *3, *5, *6, *8, *11) are more likely to have impaired metabolism of phenytoin and require lower doses of the drug to prevent neurologic toxic effects.[30,32,39] TheCPIC guidelines recommend consideration of lower phenytoin doses in patients who carry reduced function *CYP2C9* alleles.[32]

The tier 2 recommended *CYP2C9* alleles are additional variant alleles that laboratories may choose to include in expanded clinical genotyping assays. The three tier 2 *CYP2C9* alleles have reduced or no function. However, *CYP2C9*13* and *15* lack available RMs, and all three alleles have very low minor allele frequencies (<0.3%) in major ethnic groups as described above. In particular, the *CYP2C9*15* no function allele has been found in East Asian (Exome Aggregation Consortium, http://exac.broadinstitute.org, last accessed August 9, 2018) and South Asian populations[22,40] at very low frequencies (<0.01%); however, testing of this allele may more accurately assign phenotype for these ethnic groups.

The AMP PGx Working Group considered additional *CYP2C9* star (*) alleles for possible inclusion in tier 2, including *CYP2C9*4*, *7*, *9*, *10*, *14*, *18*, *25*, *31*, and *35*. These alleles were not included in tier 2 at this time for the following reasons: *CYP2C9*4*, *10*, and *31* are extremely rare and have no appreciable allele frequency in multiethnic populations. *CYP2C9*7*, *10*, and *14* are of uncertain function currently and therefore are not recommended for inclusion in clinical testing panels, although it may be useful to include these alleles in research studies designed to measure their phenotypic effect. *CYP2C9*9* is a normal function allele most frequent in African populations, with a minor allele frequency of up to 7.5% (Exome Aggregation Consortium, http://exac.broadinstitute.org, last accessed August 9, 2018); however, because this allele has no effect on enzymatic activity, it was not included in recommended clinical test panels because failure to detect it would have no clinical significance. *CYP2C9*18* is a recently described variant of unknown allele frequency that shares the *3* allele variants, with the addition of a NM_000771.3:c.1425A>T alteration resulting in a p.Asp397Ala amino acid change, and is likely a suballele of *CYP2C9*3*. Similarly, *CYP2C9*35* shares the *CYP2C9*2* variant as well as having NM_000771.3:c.374G>T (p.Arg125Leu).[19] More information is necessary regarding the functional significance and allele frequency of both *CYP2C9*18* and *CYP2C9*35* before they could be recommended by the Working Group for inclusion in clinical genotyping panels. Although *CYP2C9*25* is a frameshift deletion of 10 nucleotides resulting in loss of function, currently there is no allele frequency information available, and it is likely to be extremely rare. In addition, for many of these alleles (*CYP2C9*4*, *7*, *14*, *25*, and *31*), there are currently no publicly available RMs.

The AMP PGx Working Group identified seven commercially available platforms for *CYP2C9* genotyping at the time of this publication. All these platforms include *2 and *3; however, only two include all recommended tier 1

alleles, including the African alleles *5, *6, *8, and *11 and the tier 2 alleles [note that open platforms, such as OpenArray [Thermo Fisher Scientific, Waltham MA)] may be customized to include all tier 1 and tier 2 alleles). The PGx Working Group is aware that the recommendations to include the alleles more prevalent among African and African American populations may be difficult to implement with currently available genotyping platforms. However, the Working Group concluded that failure to include these alleles could lead to inaccurate CYP2C9 phenotype prediction among individuals with known or unknown African ancestry and may contribute to existing health care disparities in these populations. Implementation of this recommendation document is at the discretion of the laboratory. The tier 1 alleles are currently included in the available proficiency testing programs [eg, College of American Pathologists (*http://www.cap.org/web/home/lab/proficiency-testing?_adf.ctrl-state=8oixhrwfk_4&_afrLoop=116815371771304#*, last accessed August 15, 2018) and the North American Specialized Coagulation Laboratory Association (*https://www.nascola.com/AccessibleServices/Testing*, last accessed August 15, 2018)]. Most laboratories participating in these proficiency testing programs currently test for all the tier 1 alleles (CAP Biochemical Molecular Genetics Committee, PGX A, 2017 and PGXB, 2017 PT Surveys, College of American Pathologists, 2017). This indicates that many clinical laboratories are already testing the tier 1 alleles and that these recommendations would be practical to implement and reinforce standardization among laboratories. Of note, the FDA has recently approved a direct-to-consumer pharmacogenetics test that includes most of the *CYP2C9* tier 1 alleles (U.S. Food & Drug Administration, *https://www.accessdata.fda.gov/cdrh_docs/pdf18/DEN180028.pdf*, last accessed November 5, 2018) but does not include *CYP2C9* *8 and *11, which together are found in approximately 8% of individuals with African ancestry (PharmGKB, *https://www.pharmgkb.org/page/cyp2c9RefMaterials*, last accessed November 5, 2018).

This AMP document is limited to recommendations for clinical laboratory testing. It does not include, for example, mapping of genotypes to phenotype (metabolizer status), clinical interpretation of *CYP2C9* genotyping, or recommendations for changes to medication therapy based on *CYP2C9* genotype. Prediction of CYP2C9 metabolizer status based on genotype and recommendations for clinical actions based on CYP2C9 phenotype are covered by guidelines published by the CPIC and other professional groups. PGx is a rapidly changing field, and we intend to update these recommendation documents as new data and/or RMs become available. The AMP PGx Working Group recognizes that there are additional alleles that are not listed in this document; there are >60 *CYP2C9* alleles currently listed in the PharmVar database (*https://www.pharmvar.org/gene/CYP2C9*, last accessed August 15, 2018). Some of these may be updated to tier 2 or tier 1 recommended alleles in the future based on new data concerning functional impact, frequency, and availability of RMs.

In summary, this AMP document provides recommendations for a list of alleles that should be included in clinical *CYP2C9* genotyping tests. These recommendations are intended to facilitate *CYP2C9* genetic testing by clinical laboratories. In addition, these recommendations should help to standardize testing and genotyping concordance among laboratories.

Disclaimer

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