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PharmVar GeneFocus: *CYP2C9*

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Abstract

The Pharmacogene Variation Consortium (PharmVar) catalogues star (*) allele nomenclature for the polymorphic human *CYP2C9* gene. Genetic variation within the *CYP2C9* gene locus

Conflicts of Interest:
Indiana University School of Medicine Pharmacogenomics Laboratory is a fee-for-service clinical laboratory that offers clinical pharmacogenetic testing. A.L.D. is a paid employee of Acadia Pharmaceuticals; S.A.S. is a paid consultant of Sema4. All other authors declared no competing interests for this work.

impacts the metabolism or bioactivation of many clinically important drugs including NSAIDs, phenytoin, anti-diabetic agents and angiotensin receptor blocker. Variable CYP2C9 activity is of particular importance regarding efficacy and safety of warfarin and siponimod as indicated in their package inserts. This GeneFocus provides a comprehensive overview and summary of *CYP2C9* and describes how haplotype information catalogued by PharmVar is utilized by the Pharmacogenomics Knowledgebase (PharmGKB) and the Clinical Pharmacogenetics Implementation Consortium (CPIC).

CYP2C9 Brief History

CYP2C9 is the most abundantly expressed human CYP2C isoform in the liver (1, 2). Data from the 1970s suggested that polymorphic expression affects metabolism of tolbutamide (3) but was not related to *CYP2D6* (4). A combination of protein purification and cDNA cloning approaches eventually identified CYP2C9 as the enzyme responsible for tolbutamide hydroxylation in humans (5, 6). A role for this enzyme in phenytoin hydroxylation was also demonstrated (7). Initially it was thought that the *CYP2C9* gene product was also responsible for mephenytoin metabolism, but this was refuted in 1991 after the discovery of *CYP2C19* (8). Subsequent studies on *S*-warfarin and diclofenac hydroxylation demonstrated that CYP2C9 was the main enzyme responsible for these reactions (9, 10). After that, many additional CYP2C9 substrates spanning a variety of drug classes, have been identified and are further discussed below.

The existence of *CYP2C9* variants became evident when comparing cDNA sequences that initially pointed to the existence of two common nonsynonymous variants (c.430C>T, p.R144C, rs1799853 and c.1075A>C, p.I359L, rs1057910), which are now defining variants of the haplotypes described as *CYP2C9*2* and *CYP2C9*3*, respectively (11). In the early studies of these variants, decreased metabolism of tolbutamide and *S*-warfarin *in vivo* and *in vitro* correlated with the presence of *CYP2C9*2* and **3* alleles (12, 13). Further sequencing during the early 1990s resulted in the discovery of additional variants, including the decreased function *CYP2C9*5* allele (c.1080C>G, p.D360E, rs28371686) (14) and the nonfunctional *CYP2C9*6* allele (c.818delA, L273frameshift, rs9332131) (15) among others (16).

Another milestone in the history of CYP2C9 is the US Food and Drug Administration (FDA) revision of labeling for two drugs, warfarin with a boxed warning added based in part on *CYP2C9* pharmacogenetics and siponimod (MAYZENT®) with testing required due to a contraindication for patients with a *CYP2C9*3/*3* genotype. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for various *CYP2C9* gene-drug pairs including warfarin, phenytoin and nonsteroidal anti-inflammatory drugs (NSAIDs) have been published (17–19). Guidelines have also been published by the Royal Dutch Pharmacogenetics Working Group (DPWG) and the Canadian Pharmacogenomics Network for Drug Safety (CPNDS) information for those can be accessed through PharmGKB (20).

Status of Nomenclature before PharmVar

CYP2C9 star allele nomenclature was maintained by the Human Cytochrome P450 Allele Nomenclature Database since 2000 until its transition to PharmVar in 2017 (21). The pharmacogenetics community accepted this as the central resource for cataloguing *CYP* variation and it was utilized by knowledge resources (e.g., PharmGKB), the pharmacogenetics testing and implementation communities, including clinical genetic testing laboratories and the CPIC, as well as to inform research.

A total of 60 *CYP2C9* haplotypes, *CYP2C9*1* through **60*, were submitted to this database before it was transitioned to the Pharmacogene Variation (PharmVar) Consortium in 2017 (22). Although only exons were required to be sequenced for submission, sequence variants in the upstream gene region or in flanking introns, may also have been submitted and included in haplotype definitions. These regions were, however, not consistently interrogated and captured by the database. Initially, so-called 'suballeles', e.g., *CYP2C9*1A-D*, were catalogued, but eventually no longer considered for independent naming. PharmVar has reviewed all transitioned star alleles and updated or revised their definitions as necessary to conform to PharmVar standards (23).

In this review, star (*) allele sequence variants are shown according to their relative position in the *CYP2C9* NM_000771.4 reference transcript sequence with the 'A' of the ATG translation start codon being +1; corresponding protein coordinates are also provided. For example, the *CYP2C9*2* allele-defining variant (rs1799853) is referred to as c.430C>T (p.R144C) indicating that this variant causes an arginine to cysteine change at amino acid position 144.

Resources cited throughout this review are summarized in Table 1.

Clinical Relevance

The CYP2C9 enzyme is part of the CYP450 superfamily contributing to the metabolism of many clinically used drugs, including warfarin, phenytoin, multiple NSAIDs (e.g. celecoxib, flurbiprofen, lornoxicam, ibuprofen, piroxicam, tenoxicam, and meloxicam), losartan, irbesartan, sulfonylureas (e.g. tolbutamide and gliclazide) and siponimod. Additional information on drugs metabolized by CYP2C9 can be found in the PharmGKB drug label annotations (24), the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling (25), the FDA Table of Pharmacogenetic Associations (26), and the CPIC drug-gene pairs (27). *CYP2C9* polymorphisms have significant consequences for narrow therapeutic index drugs, including warfarin and phenytoin (17, 19). In contrast, while *CYP2C9* variation influences the oral clearance of losartan (28) it is not clear whether this translates into clinically significant effects.

The most common clinical application of *CYP2C9* genotype information described to date is its use, together with *VKORC1* and possibly *CYP4F2*, to guide warfarin dosing (17). Individuals with one or two decreased or no function alleles have decreased metabolism of the more potent *S*-enantiomer of warfarin and increased risk of bleeding with usual warfarin doses (i.e., 5 mg per day) and thus, a lower warfarin dose is required to

achieve therapeutic anticoagulation (17, 29). Three multi-site clinical trials have examined the efficacy of *CYP2C9* plus *VKORC1* genotype-guided warfarin of which two trials demonstrated favorable effects of a genotype-guided approach on the outcome of improved anticoagulation control (30) or reduction in risk for bleeding, thromboembolism, death or supratherapeutic anticoagulation following total joint arthroplasty (31). The third trial, COAG, showed no improvement in anticoagulation control with a genotype-guided approach (32). However, in contrast to the other trials that were conducted predominantly in European ancestry populations, nearly 30% of participants in the COAG trial were of African ancestry, in whom genotype-guided dosing led to worse anticoagulation control compared to a non-genotype guided approach. All three trials limited genotyping to the *CYP2C9*2* and **3* alleles, which are the most common decreased or no function alleles in European ancestry patients but occur less often in those with African ancestry. The *CYP2C9*5, *6, *8* (c.449G>A, p.R150H, rs7900194) and **11* (c.1003C>T, p.R335W, rs28371685) alleles are predominantly found in people with African ancestry, and data show that not accounting for these alleles leads to significant over-estimation of warfarin dose requirements in African Americans (33). Failure to account for these alleles in the COAG trial may have contributed to the trial's negative outcomes. CPIC guidelines were revised in 2017 to state that genotype should only be used to guide warfarin dosing in African ancestral patients if the *CYP2C9*5, *6, *8*, and **11* alleles are tested (17). The Association for Molecular Pathology (AMP) includes these variants, in addition to the *CYP2C9*2* and **3* alleles, on their Tier 1 allele recommendations (34). *CYP2C9* testing options are discussed in detail elsewhere (35). Additional *CYP2C9* alleles may be common in other populations worldwide (36).

CYP2C9 decreased and no function alleles similarly lead to increased exposure to other CYP2C9 substrates, which may increase the risk for serious adverse effects, including neurotoxicity with phenytoin, gastrointestinal bleeding (37) and adverse cardiovascular effects with NSAIDs, and bradycardia with siponimod (38). Siponimod is contraindicated for poor metabolizers (PM) with the *CYP2C9* 3/*3* genotype, who are expected to have little to no enzyme activity. Thus, genotyping is required prior to siponimod initiation (39). Lower phenytoin maintenance doses are recommended for patients with genotypes associated with significant reductions in enzyme activity (19). For NSAIDs, the consequences of decreased CYP2C9-mediated metabolism are expected to be greatest for drugs with a long elimination half-life (e.g., piroxicam, tenoxicam, melocoxicam) and less significant for NSAIDs with shorter half-lives (e.g., celecoxib and ibuprofen). This is reflected by CPIC guidelines recommending that in PMs (e.g., *CYP2C9*2/*3* or **3/*3* genotypes), piroxicam, tenoxicam and melocoxicam be avoided, whereas celecoxib and ibuprofen may be used but should be started at lower than usual doses (18).

Other Factors that can Influence CYP2C9 activity

CYP2C9 is inhibited and induced by a wide range of drugs. Sulfamethoxazole, fluconazole, voriconazole, metronidazole, and amiodarone are examples of CYP2C9 inhibitors (40). Amiodarone represents a risk for anticoagulated patients since it is often prescribed concomitantly with warfarin. The drug-drug interaction between amiodarone and warfarin is well documented (41) and is associated with a 6–65% decrease in warfarin dose

requirement (42). On the other hand, known inducers of CYP2C9 are rifampicin, clotrimazole, nifedipine, hyperforin (an active component of the herbal drug St John's wort), phenobarbital, phenytoin, carbamazepine, dicloxacillin, flucloxacillin and tamoxifen (43–47). Although several mechanisms are known to be involved in CYP2C9 upregulation, the most prominent is by nuclear receptor binding to *cis*-regulatory elements in the promoter region (1, 48), such as the pregnane X receptor (PXR), constitutive androstane receptor (CAR), and the glucocorticoid, estrogen and vitamin D (VDR) receptors. Finally, cytochrome b5 has been shown to modulate catalytic activity of CYP2C9 (49), and it has been suggested that variation in its gene (*CYB5A*) might contribute to variable CYP2C9-mediated drug metabolism.

Age-related and sex differences

Sex-related differences in *CYP2C9* expression have been investigated to a limited extent and to date there is no evidence supporting meaningful differences between males and females (50). Data obtained in liver microsomes with tolbutamide as substrate (51, 52) and from phenotyping studies with losartan and tolbutamide as substrates (53, 54) did not reveal differences in activity between males and females, with the exception of one study that found slower metabolism of losartan in females compared with males (53). It has also been reported that females taking oral contraceptives show impairment of losartan metabolism (55), although this observation was not replicated using tolbutamide as substrate (56). However, considering that estrogens upregulate *CYP2C9* expression via the estrogen receptor (57), studies on sex differences should consider this possible confounder.

Age-related differences in *CYP2C9* expression have been reported. CYP2C9 metabolic activity is low in fetal livers and increases linearly immediately from birth up to 20 years (58, 59); the greatest increase in activity is observed in the first two years of life (60). Findings for the effect of age on *CYP2C9* contrast with *CYP2C19* where levels appear to be higher in children than in adults (61). Also, there are pediatric-specific recommendations for warfarin treatment based on *CYP2C9* and *VKORC1* genotype, but these only apply to patients of European ancestry and are limited to the *CYP2C9*2* and **3* alleles (17).

CYP2C9 and the Pharmacogenomics Knowledge Base (PharmGKB)

The PharmGKB collects, curates and disseminates knowledge about the impact of human genetic variation on drug response (62). The PharmGKB *CYP2C9* gene page allows structured access to gene-specific pharmacogenomic knowledge (20). Information is presented in sections including prescribing information, drug label annotations, clinical annotations, variant annotations, and curated pathways. Prescribing information encompasses: 1) annotations of clinical guidelines from sources such as CPIC, DPWG, CPNDS, 2) a list of drug labels that include prescribing information for a *CYP2C9* genotype or phenotype with respective excerpts and links to the label annotation (see below), and 3) "Rx study annotations" that provide genotype-based drug dosing reported in individual journal articles. Seven CPIC guideline annotations (one providing no dosing recommendations), nine DPWG guideline annotations (3 with recommendations and 6

providing no drug-specific recommendations) and 46 “Rx study annotations” are available as of March 2021 for *CYP2C9* with overlapping *CYP2C9*-drug pairs.

PharmGKB extracts pharmacogenomic-relevant information from agency-approved drug labels and applies a “pharmacogenetics (PGx) level” category, based on the level of action implied in each label (e.g., Testing required, Actionable PGx, Testing recommended or Informative PGx). On the *CYP2C9* page, annotations can be accessed (as of March 2021) for 15 US FDA approved labels, 3 European Medicines Agency (EMA) approved labels, 1 Pharmaceuticals and Medical Devices Agency, Japan (PMDA) approved label, 2 Health Canada (HCSC) approved labels, and 8 Swiss Agency of Therapeutic Products (Swissmedic) approved labels. Currently, PharmGKB contains 68 *CYP2C9* related clinical annotations, which are evidence-rated genotype level summaries for specific variant/allele-drug combinations based on curated literature (variant annotations). Pharmacokinetic pathways depicting CYP2C9 in drug metabolism are available for 36 drugs, although the significance for CYP2C9 involvement varies by drug. PharmGKB and CPIC work collaboratively to develop gene-specific resources that accompany each CPIC guideline, including allele definition mapping, allele functionality, allele frequency, and diplotype to phenotype mapping files in a standardized format. Gene-specific information tables for *CYP2C9* are available from PharmGKB (36). In addition, the Pharmacogenomics Clinical Annotation Tool (PharmCAT) is currently under development to facilitate the interpretation and reporting of pharmacogenomic-based dosing recommendations, including those for *CYP2C9* (63).

CYP2C9 and the Clinical Pharmacogenetics Implementation Consortium (CPIC)

The CPIC develops structured, evidence-based, clinical practice guidelines for drugs affected by pharmacogenetic variation (64, 65). Several *CYP2C9* gene-drug pairs have been prioritized through consideration of multiple factors such as the available body of literature, severity of clinical consequences, availability of alternative therapies and whether a prescribing change (drug choice or dose) is warranted. To date, three CPIC guidelines have been published that include CYP2C9: warfarin (17), phenytoin/fosphenytoin (19), and NSAIDs (18). Each guideline has multiple components, including CYP2C9 phenotype-specific therapeutic recommendations, a systematic evidence review, and implementation resources to support the translation of the guideline into electronic health records (EHRs) with example clinical decision support (CDS) text.

Genotype to Phenotype Translation

An individual has two *CYP2C9* haplotypes, one on each chromosome, which constitutes a diplotype. For example, a *CYP2C9*2/*3* diplotype assignment indicates that one chromosome (or allele) has a single nucleotide variation (SNV) defining the *CYP2C9*2* haplotype and the second chromosome (or allele) has a SNV defining the *CYP2C9*3* haplotype.

For CYP2C9 phenotypic classification, individuals are categorized into the following CPIC-recommended phenotype categories: poor (PM), intermediate (IM), and normal (NM) (formerly EM, extensive) metabolizers (66). To facilitate *CYP2C9* genotype to phenotype translation the Activity Score (AS) system first developed for the *CYP2D6* gene (67) has been adopted. Briefly, a value of 0, 0.5, or 1 is assigned to each allele reflecting no function, decreased function or normal function, respectively. The sum of the values assigned to each allele is a diplotype AS. For example, *CYP2C9*2/*3* has values of 0.5 (*CYP2C9*2,* decreased function allele) plus 0 (*CYP2C9*3*, no function allele) giving rise to an AS of 0.5. Individuals with activity scores of 0 and 0.5 are categorized as PMs, AS of 1 and 1.5 as IMs, and AS of 2 as NMs. By utilizing the AS system, different prescribing guidance can be provided within a phenotype category to tailor specific allele function combinations. For example, in the CPIC guideline for *CYP2C9* and nonsteroidal anti-inflammatory drugs (18), the recommendation differs between an AS of 1 (a combination of two decreased function alleles or one no function allele with a normal function allele) and an AS of 1.5 (a combination of a normal function allele with a decreased function) within the CYP2C9 IM category. The Diplotype-Phenotype-Table provided by the PharmGKB and CPIC serves as a reference for calculating the AS of each genotype (36).

Need for standardized genetic variation definitions and reporting of functional/clinical impact

In order to guide drug therapy, it is imperative to understand how *CYP2C9* allelic variation can impact CYP2C9 function. Although many alleles have been observed in phenotypic PMs and their causative genetic variations described (e.g., *CYP2C9*2, *3*, and **4*, etc.), some alleles have unknown or uncertain function. *In vitro* characterization of allelic variants often produces results that are inconsistent among test systems that may be attributed to differences in the substrates used and between the experimental variables (see CYP2C9 functionality table for a detailed summary (36)). Although *in silico* prediction tools are improving, *in vivo* validation is still the gold standard. Therefore, for any given allele (except for those shown to completely abolish activity) caution should be taken when extrapolating functional data from one drug or substrate to another. Ideally, one would be able to assess the *in vivo* function of each individual *CYP2C9* haplotype with each individual CYP2C9 substrate, which would refine the phenotype predicting capacity of *CYP2C9* genetic testing. In addition, co-medications (drug–drug interactions) may not affect all *CYP2C9* variants equally, and there is still limited or no information regarding genetic variability for many minority populations.

For many drug metabolizing enzymes, the combination of sequence variations that define haplotypes is critical to precisely predict enzyme function. A notable example is *CYP2C9*3*. Its defining variant (c.1075A>C, p.I359L, rs1057910) is also present in *CYP2C9*18*, which additionally harbors a missense variant (c.1190A>C, p.D397A, rs72558193).

As previously reported in the *CYP2D6* GeneFocus (68), clinical pharmacogenetic programs have successfully been implemented over the past years, but numerous challenges remain to accelerate adoption (69). Standardization is a key area that continues to represent an

opportunity for all pharmacogenetic stakeholders to improve upon, including for laboratory processes, test ordering, result reporting, and data representation. This is in alignment with recent reports, which emphasized that clinically actionable pharmacogenetic information must be accurately represented in electronic health records by using a harmonized system for genotype and phenotype information (70, 71). Although many pharmacogenetic laboratories utilize star nomenclature as recommended by PharmVar and CPIC, inter-laboratory differences in testing approaches and reporting remain. Clinical testing for *CYP2C9* can be performed on a variety of platforms using different methodologies and genotyping data can be reported in different ways, such as chromosomal or genomic position on reference sequence (RefSeq), amino acid change, dbSNP rsID, and/or using star (*) allele nomenclature (21, 72).

A study performed by the Genetic Testing Reference Material Program (GeT-RM) concluded that many pharmacogenetic variants interrogated were not consistent across commercial and laboratory platforms (73). Similar findings were reported by Moyer *et al.*, when they surveyed laboratories offering pharmacogenetic services for *CYP2D6* and *CYP2C19* genotyping (74). To address this, the AMP and College of American Pathologists (CAP) have recently published recommendations for alleles to be included in clinical *CYP2C9* testing (34). In brief, the recommendations are based on allele function, allele frequencies across populations and ethnicities, and the availability of reference materials. The AMP working group recommended Tier 1 alleles (minimum panel of variant alleles), which includes *CYP2C9*2, *3*, **5*, **6*, **8*, and **11*, and Tier 2 (extended panel of variant alleles) that includes *CYP2C9*12* (c.1465C>T, p.P489S, rs9332239), **13* (c.269T>C, p.L90P, rs72558187) and **15* (c.485C>A, p.S162X, rs72558190). The utilization of star allele nomenclature as provided by PharmVar (75) will not only ensure that each star allele represents a unique and fully defined haplotype but also minimize “mis-interpretation” of a genotype result and its clinical implication(s).

The two end-user groups benefiting the most from standardized allele designations are clinicians and patients. Standardized terms and language will help clinicians to convey and explain results and patients to understand what the results mean for them. Consistent nomenclature is critical for the integration of pharmacogenetics into EHR, as well as for the establishment of clinical decision support algorithms and the design of clinical support tools such as interruptive alerts (70, 71). For example, drug/allele combinations for alerts require systematic annotations within the EHR, using standardized nomenclature and terms. Finally, the analysis of pharmacogenetic clinical correlations will benefit from harmonized nomenclature of the gene variants, as well as support consistent test interpretation by providers across healthcare systems.

The *CYP2C9* Gene Locus

The *CYP2C9* gene is a member of the *CYP2C* family, has 9 exons and is translated into 490 amino acids. The gene is located on chromosome 10q23.33 spanning a region of approximately 390 kb. In addition to *CYP2C9* the locus also harbors *CYP2C8*, *CYP2C18* and *CYP2C19* (Figure 1). Genotyping assays need to employ *CYP2C9*-specific regions for primer design (e.g., using intronic sequences) to avoid amplification from any of the other

genes in the locus, in particular *CYP2C19*. Many commercial laboratories offer *CYP2C9* testing (76) using a variety of test platforms.

Although no copy number variation (CNV) alleles have been defined to date, *CYP2C9* can, in rare cases, be part of large CNV events affecting the *CYP2C* locus as described (77) and summarized by the *CYP2C19* GeneFocus (61). CNVs may implicate the entire *CYP2C9* gene, present as partial gene deletions or duplications, and often additional genes flanking the *CYP2*C gene locus are also involved. *CYP2C9* CNVs present at low frequency in the population (0.009%). CNVs affecting *CYP2C9* are not routinely tested and are most often detected as incidental findings by array platforms such as array comparative genomic hybridization (aCGH), performed for unrelated diagnostic purposes.

CYP2C9 allele, genotype, and phenotype frequencies across populations

The *CYP2C9* frequency table available at PharmGKB (36) summarizes population-based allele frequencies reported in the literature. Studies were considered for inclusion if 1) the ethnicity of the population was clearly indicated, 2) either allele frequencies or genotype frequencies were reported, 3) the methodology, by which the genes were genotyped was indicated, and 4) the study represented an original publication. The ethnicities/locations reported in the articles were mapped into seven geographically defined groups (American, Central/South Asian, East Asian, European, Near Eastern, Oceanian, and Sub-Saharan African) and two admixed groups (African American/Afro-Caribbean and Latino), using the biogeographical grouping system developed by PharmGKB (78). The *CYP2C9* frequency table is periodically updated and contains multiple tabs summarizing ‘allele frequencies by biogeographical group’, ‘diplotype frequencies by biogeographical group’, ‘phenotype frequency’, and ‘references’; the latter describes allele frequencies for each publication included in the listing, which also allows the user to customize allele frequencies as needed. There are, however, limitations regarding the accuracy of allele frequencies as follows: 1) frequencies are based on published allele data (limited or unavailable for some populations and many alleles), 2) most studies test for a limited number of allelic variants that may lead to an underestimation of certain alleles. For example, c.430C>T (p.R144C) is often defaulted to a *CYP2C9*2* assignment, although this SNV is also present on *CYP2C9*35* and *CYP2C9*61* (Figure 2). Likewise, if no SNVs are found, *CYP2C9*1* is assigned, which inflates the frequency of this allele. Therefore, all calculations based on allele frequencies are estimates at best and should be used with caution.

There is considerable variation among the estimated frequencies for individual alleles across and within the biogeographical groups. The decreased function *CYP2C9*2* allele has been found at higher frequencies in European (13%), Central/South Asian (11%), Near Eastern (13%), and Latino (8%) populations, but is less frequent in other populations (≤3%). Likewise, *CYP2C9*3*, a no function allele, is most frequent in Central/South Asian (11%), European (7.6%), and Near Eastern (8.3%), compared to other populations (≤4%). Other allelic variants impacting activity including *CYP2C9*5* (≤1.2%), *6 (≤0.9%), *8 (≤7.6%) and *11 (≤2.6%) are most often, but not exclusively, observed in individuals with African ancestry.

Frequency information from the literature is scarce for *CYP2C9* alleles across populations, especially for *CYP2C9*12* and higher, and limited to *CYP2C9*2* and *3* in Oceanians. Absence of a reported allele frequency does not necessarily indicate absence of an allele in that population and does not rule out that someone in that population might have the allele. It is important to keep in mind that the allele frequencies for each biogeographical group are averages of aggregated allele frequencies from multiple publications, each reporting on smaller, more specific study populations. The allele frequencies within each biogeographical group can range widely depending on the specific study population. For example, *CYP2C9*2* frequencies reportedly range from 0.5% (Ecuadorian Mestizos) to 19.6% (Brazilian admixed population), with both studies contributing to the allele frequency of the Latino biogeographical group (8%). Similarly, *CYP2C9*3* frequencies ranged from 1% in (Malay) to 15% (Vietnamese), both contributing to the allele frequency in the East Asian biogeographical group (4%). Therefore, reported allele frequencies for each biogeographical population is an estimate that cannot be applied to individual patients. Detailed allele frequency information for individual studies and across biogeographical groups can be accessed through the PharmGKB/CPIC Frequency table (36).

Considering *CYP2C9*1* through *71, hundreds of allele combinations are possible, and thus, the number of possible genotypes in a population or patient cohort can be quite large. The actual number of combinations that occur in a specific biogeographical population may be significantly less, however, depending on the number of alleles and their frequencies. Phenotype frequencies calculated from the averaged allele frequencies (i.e. not observed phenotype frequencies in populations) across populations are provided in the ‘Phenotype frequency’ tab in the PharmGKB/CPIC *CYP2C9* Frequency Table (36). We stress, however, to view all calculated phenotype group frequencies (including those shown in the *CYP2C9* gene-specific reference table) with caution due to the limitations regarding the accuracy of allele frequencies, as well as the method used to translate genotype into phenotype and inconsistencies in the classification of ‘population’, ‘ethnicity’, or ‘race’ (79).

CYP2C9 Allele function

PharmVar displays allele clinical function as determined by CPIC, using their respective terms (increased, normal, decreased, no, uncertain or unknown function) (66). Alleles that have not been assigned a function by CPIC are shown as ‘N/A’ (function not assigned). The filter option on the PharmVar *CYP2C9* gene page allows the user to sort alleles by functional status.

In 2019, CPIC drafted a standardized protocol that describes in more detail the criteria used for assigning clinical function to alleles that are part of CPIC guidelines to harmonize the process across guidelines (80). *CYP2C9* was the first gene for which the developed protocol was applied. A group of CYP2C9 experts discussed literature evidence for each *CYP2C9* allele and agreed upon consensus function assignments. It should be noted that CPIC’s primary focus is to assign allele function based on clinical actionability, not solely on molecular or biochemical function. Alleles with no clinical function may have some residual activity which is exemplified by *CYP2C9*3*. This allele is assigned no function under ‘allele clinical functional status’ but decreased function under ‘allele functional status’

given that its biochemical function is so minimal that, clinically, it is deemed a no function allele.

The expert consensus for allele functions is included in the supporting material for *CYP2C9*-based CPIC guidelines and can be accessed on PharmGKB (36). The table includes per allele the activity value, allele functional status, allele clinical functional status (displayed on PharmVar), and references that were reviewed during the assignment process.

PharmVar Nomenclature and *CYP2C9* allele designation

PharmVar stores and displays allelic data consistently across genes, relying on public standards and data sources wherever possible. The standardized nomenclature follows criteria developed by gene experts. The "Allele Designation and Evidence Level Criteria" document describes the nomenclature system and provides examples (81). For example, a new star (*) number is only issued if a haplotype contains a sequence variant that: 1) results in an amino acid change, e.g., *CYP2C9*2* harbors an arginine to cysteine change (c.430C>T, p.R144C); 2) causes a frameshift, e.g., *CYP2C9*6* harbors a frameshift at position p.L273 (c.818delA); 3) affects splicing; or 4) changes expression levels causing decreased or increased function. Importantly, new haplotypes that contain previously characterized variants that obliterate gene function are catalogued under the original star (*) allele number as a suballele. For example, any haplotype having a novel variant in addition to the *CYP2C9*15* defining c.485C>A (p.S162X) variant will be designated as a **15* suballele, and considered to have no function, regardless of the functional status of the novel variant.

The PharmVar *CYP2C9* gene expert panel

International experts representing research, clinical testing, and implementation interests were recruited from PharmVar members to serve on the *CYP2C9* expert panel. The panel also includes PharmGKB/CPIC representation to ensure that the nomenclature is consistent with CPIC guidelines and to facilitate dissemination to a greater audience through PharmGKB as well as other databases, such as ClinGen. The composition of the panel can be found on the PharmVar website (82). Table 2 summarizes the alleles reviewed and accepted by the panel to date.

The PharmVar *CYP2C9* gene page

The PharmVar *CYP2C9* gene details all allelic variants defined by PharmVar. Sequence variations are mapped to the latest genomic and cDNA reference sequences (RefSeqs), issued by the NCBI Reference Sequences database (83) and the GRCh37 (NC_000010.10) and GRCh38 (NC_000010.11) genome builds. For *CYP2C9*, the current genomic and transcript RefSeqs are NG_008385.2 and NM_000771.4, respectively. *CYP2C9* was transitioned from the original nomenclature site to PharmVar on Sept 26, 2017 (original content is available through the 'Archive' link on the PharmVar homepage).

A Locus Reference Genomic (LRG) record has been issued from the LRG Project, a NCBI (RefSeq) and EMBL-EBI (Ensembl/GENCODE) initiative (https://www.lrg-sequence.org/).

LRGs were created to serve as reference standards that 'never change' or version after their release. The *CYP2C9* LRG (LRG_1195) matches 100% with the NG_008385.2 RefSeq and was used by PharmVar instead of NG_008385.2. However, considering the ongoing Matched Annotation from NCBI and EMBL-EBI (MANE) project (https://www.ncbi.nlm.nih.gov/refseq/MANE/), genomic RefSeqs, derived from MANE Select transcripts, are viewed as the new gold standard. Thus, PharmVar will return to using the genomic RefSeq NG_008385.2.

On the PharmVar *CYP2C9* gene page, the user can easily cross-reference genomic and cDNA position(s) by choosing the respective reference sequence or genome build of interest; there is also the option of two count modes, i.e., counting from the first nucleotide in the reference sequence or the ATG translation start codon being +1. Variant annotations are now also provided according to HGVS along the more traditional PharmVar display format (84). Figure 3 provides an excerpt of the page illustrating *CYP2C9*2* and **8*. Additional details and an example are provided in the gene's Read Me document available through at the PharmVar *CYP2C9* gene page.

Each allele is listed in sequential order on the *CYP2C9* gene page and cross-references with its legacy name (if existing), variants (including core SNVs; see Core Allele section below), evidence level, and clinical function as assigned by CPIC. A 'Compare View' allows the viewer to toggle between the standard allele table and the Comparative Allele ViewEr (CAVE) tool. The *CYP2C9* gene page also includes 'Read Me' and 'Change Log,' documents, as well as links to other websites with CYP2C9 information including a link to PharmGKB's gene information.

CYP2C9 haplotype evidence levels

PharmVar designates the "Haplotype Evidence Level" for each of the star alleles reported on the *CYP2C9* gene page. Evidence levels are displayed as symbols indicating 'Def' (definitive), 'Mod' (moderate) or 'Lim' (limited) levels of support for a given haplotype reflecting the level of certainty that a haplotype exists in its reported form evidence (note that evidence levels in support of allele function can be found on the PharmGKB (36)). This three-tiered system represents a modified ClinVar classification system; more detailed information is provided in the 'Allele Designation and Evidence Criteria Level' document (81). This type of information (e.g., whether an allele was sequenced across the gene, how haplotype was determined) was not always systematically captured prior to PharmVar. For existing haplotype definitions, a literature review was conducted in order to assign evidence levels. Many alleles are currently labeled as 'Lim' because their definitions do not include any upstream region or do not extend 2 kb upstream, which is required by PharmVar allele designation requirements. This was the case for many allelic variants, including *CYP2C9*1.002* and **1.003*, as well as **4, *6, *21-*24* and **31-*60*. Other alleles, such as *CYP2C9*7, *10, *15-*20, *61* were labeled as 'Mod' despite being fully sequenced because the phase of the variants was computationally inferred and has not been validated. The value of evidence levels is centered on providing users with as much information on haplotype reliability as possible and enabling users to quickly parse haplotypes based on robust, high evidence as required for 'Def', versus other haplotypes with 'Lim' or 'Mod' evidence levels.

PharmVar solicits submissions for all alleles labeled ‘Lim’ and ‘Mod’ to ultimately raise their evidence levels to ‘Def’. Moreover, PharmVar also encourages encore submissions for alleles with single citations and shown as ‘Def’ to further corroborate a haplotype definition.

See the *CYP2C9* gene page (20) for current star allele definitions and their assigned evidence levels, including suballeles. Selected citations supporting respective haplotype definitions can also be found here.

PharmVar IDs

Each characterized haplotype receives a PharmVar ID (PVID). The PVID is a unique numeric identifier analogous to dbSNP rsIDs. Star allele names are driven by functional grouping, i.e., they are not guaranteed to be permanent and can be subject to change. Additional changes may be necessary in the future as more information becomes available. If an allele’s star designation is updated to a new star number, the PVID of the haplotype remains constant and does not change (no example for *CYP2C9*). In contrast, if a haplotype definition changes (e.g., through the addition or removal of variants) a new PVID will be assigned. Original PVIDs and their haplotype definitions can be tracked in the database via the PVID Lookup function. The CYP2C9 experts decided to not include intronic variants of unknown functional consequence as part of the required region to interrogate for a *CYP2C9* haplotype, therefore intronic variants were removed from *CYP2C9*26*, **27*, and **29* allele definitions and **26.001*, **27.001*, **29.001* received new PVIDs.

Core allele definitions

For already defined alleles, there is a growing number of suballeles that share one or more ‘core’ defining sequence variant(s). Although suballele information can be valuable for e.g., design of test platforms (sequence or genotype-based) and the interpretation of genotyping test results, there is no need to distinguish suballeles for phenotype prediction because all alleles under a star number are presumed to be functionally equivalent. Thus, even if a test is capable of distinguishing suballeles, from a functional standpoint, these can be simply reported using core allele definitions (e.g., *CYP2C9*1, *3, *8, etc.*).

A core allele is defined only by sequence variations that cause an amino acid change or impact function by changing expression levels or interfere with splicing and are present in all suballeles within an allele group (85). With this rule-based system, suballeles are collapsed into a single ‘core’ definition representing all suballeles categorized under a star (*) number. For example, *CYP2C9*2* suballeles share the c.430C>T (p.R144C) SNV that fulfills this rule. Thus, this SNV constitutes the *CYP2C9*2* core allele definition. Of importance, a sequence variant found in a core allele definition is not necessarily unique to that haplotype as illustrated in Figure 3.

One challenge with core allele definitions is that a definition may change over time as new information becomes available. However, this scenario is less likely for *CYP2C9* given that most core alleles are defined by a single variant.

The core alleles are the basis of the *CYP2C9* allele definition table used in CPIC guidelines and by PharmGKB (Table 1). The *CYP2C9* core allele definitions are also utilized for clinical annotations in PharmGKB.

The PharmVar Comparative Allele ViewEr

The *Comparative Allele ViewEr* (CAVE) tool was developed by PharmVar to easily compare core alleles (85). This tool can be accessed using the "Compare View" button on the *CYP2C9* gene page. Figure 2 not only exemplifies the utility of this tool on two sets of alleles, *CYP2C9*2, *35* and **61* and *CYP2C9*3, *18* and **68*, but also illustrates the pitfalls of allele identification if laboratories assign alleles based on few markers. In this display mode it is easy to see which core SNV(s) are shared among the selected haplotypes, whether they alter function and/or are unique to a haplotype. *CYP2C9*2, *35* and **61* harbor c.430C>T (p.R144C) while *CYP2C9*3, *18* and **68* have c.1075A>C (p.259L) in common. Additional unique variants are found on *CYP2C9*35* (c.374G>T, p.R125L, rs72558189) and **61* (c.1370A>G, p.N457S, rs202201137) and *CYP2C9*18* (c.1190A>C, p.D397A) and **68* (c.1149+1G>A, splice defect, rs542577750) which distinguishes these from *CYP2C9*2* and **3*, respectively.

Reporting genotype and translation into phenotype

PharmVar and PharmGKB have also collaboratively developed templates to facilitate more consistent and transparent reporting of genotype details and how genotype is translated into phenotype to be used by the community to include more detailed information as part of the submission of research findings to publishers. This information can be provided as supplemental materials of a publication to facilitate access to important data for subsequent curation. The first template file (Supplementary materials 1) collects information, including methods or platforms used for genotyping and which SNVs are interrogated; the template also provides a standardized set-up for reporting genotype results for individual subjects, as well as allele frequencies. The second template file (Supplementary materials 2) facilitates the reporting of how genotype is translated into phenotype, as well as genotype frequencies. Although it is recommended by CPIC, as well as other groups, to use their standardized translation method, not every investigator or laboratory adopts this method. Too often, papers reference previous work stating that 'genotyping was performed as previously described' or indicate that 'CYP2C9 phenotype was correlated with the metabolism of a drug' without specifying which SNVs or alleles were genotyped or how phenotype was assigned. The lack of such information can make it extremely difficult, if not impossible, for curators to compare findings or extract information for CPIC guideline development. Colleagues are therefore strongly encouraged to utilize the provided templates, or revised versions thereof, for publication of these types of information. These tables are available through the PharmVar *CYP2C9* gene page under 'More Documents' and at PharmGKB under 'PGx Publication Tips' (86).

CYP2C9 reference materials

The Genetic Testing Reference Material (GeT-RM) Coordination program is a combined effort among the Centers for Disease Control, Coriell Institute for Medical Research, and members of the pharmacogenetic testing community (87). Considering the growing use of pharmacogenetic testing, established sets of well-characterized reference materials are needed for assay development, validation, quality control, and proficiency testing. To address the increasing need for reference materials, a set of 137 genomic DNA samples were characterized for 28 pharmacogenes, including *CYP2C9* and "consensus" genotypes established (73). Although the most common variants were assayed, many rare alleles were not identified among the samples tested; additional samples to complement the existing materials for *CYP2C9* have been identified (88). Testing and research laboratories can acquire these materials from the Coriell Institute (Camden, NJ, USA), as they are publicly available. Information for testing materials is currently not included on the PharmVar *CYP2C9* gene page.

Curation efforts

Gene region mapped/required for allele definition:

CYP2C9 allele definitions include variants within the coding region, upstream region up to c.-1911 and downstream region. Intronic variants are generally not considered for allele definitions unless they affect enzyme activity. New submissions will be required to sequence the upstream region at least to −1950 bp (including c.-1911T>C) and the downstream region to 250 bp. Currently available allele definitions have some limitations however, including limited sequencing data for up- and downstream sequences and haplotypes that were computationally inferred but have not yet been independently confirmed.

Corrections, revisions, new alleles, and other updates:

Extensive curation efforts were part of the content transfer from the P450 nomenclature webpage into the PharmVar database to standardize the annotations to the above-mentioned conventions. A summary is provided in Table 3. The following sections describe general and specific efforts undertaken.

During the transition process into the PharmVar database, comments and footnotes were removed and errors corrected. References in support of allele definitions have been updated and those solely describing function removed. References for function are provided in the PharmGKB/CPIC CYP2C9 Allele Functionality table (36). Descriptors such as "*Existence of the CYP2C9*2 polymorphism 430C>T on the same allele cannot be excluded*" or "*linkage with c.-1188T>C cannot be excluded*" have been removed and thus, c.430C>T (p.R144C) and c.-1188T>C (rs4918758) are currently not part of the allele definition for *CYP2C9*24* and *CYP2C9*15*, respectively.

Both c.-1766T>C (rs9332094) and c.-1188T>C were included into the *CYP2C9*8.001* (former *CYP2C9*8*) haplotype definition since those variants were part of the original publication by Blaisdell et al (16) but were omitted when these alleles were first defined. The presence of c.-1766T>C and c.-1188T>C has been substantiated by new submissions to

PharmVar not only confirming the updated *CYP2C9*8.001* allele definition but also adding two novel suballeles, *CYP2C9*8.002* and *CYP2C9*8.003*. Similarly, c.1425A>T (p.G475G, rs1057911) was added to the *CYP2C9*3.001* and *CYP2C9*3.002* allele definitions. A review by the gene expert panel of the original literature revealed that this SNV had inadvertently been omitted from the haplotype definitions; the presence of c.1425A>T (p.G475G) was also corroborated by new submissions to PharmVar. Changes and revisions made are detailed in the 'Change Log' document on the *CYP2C9* gene page (20) and are summarized in Table 3.

As of April 2021, the *CYP2C9* expert panel has designated 11 novel alleles (**61* through **71*) and 17 new suballeles. In addition, seven alleles that were based on partial information are now fulfilling PharmVar allele definition requirements and their evidence level was raised to 'Def' (Table 2). There are still numerous allele definitions with 'Lim' and 'Mod' evidence levels for which PharmVar seeks submissions. The 'Change Log' document also tracks submissions and indicates the star alleles that have been updated.

Of the novel alleles, we would like to highlight *CYP2C9*71*. This allele has two core variants in *cis* (i.e. on the same allele), c.815A>G (p.E272G, rs9332130) and c.1465C>T (p.P489S, rs9332239), which are the core SNVs of *CYP2C9*10* and **12*, respectively. As shown in Figure 4b, long distance phasing using 10X Genomics Linked-Read technology revealed, however, that in this case, both SNVs are in *cis* and thus represents a novel haplotype. This finding raises concerns regarding the accuracy of the original *CYP2C9*10* and **12* allele definitions (16). Interestingly, c.815A>G and c.1465C>T were initially discovered in the same individual but presumed to be in *trans*, which resulted in the definition of two separate star alleles each characterized by a single SNV. Neither *CYP2C9*10* nor **12* have been independently confirmed to date.

The *CYP2C9*8* allele was initially defined by c.449G>T (p.R150H). After receiving submissions for this allele, its definition was revised in 2018 to include two variants in the upstream region, c.-1188T>C and c.-1766T>C and one in the 3'UTR (c.*67C>T, rs9332240). The former variants were noted in the allele's first report (16) but omitted when it was first defined. The presence of c.-1188T>C and c.-1766T>C on the *CYP2C9*8* haplotype was also described (89). Functional *in vitro* studies by this group suggested that c.-1766T>C impacts expression levels, and thus, c.-1766T>C was granted core SNV status. Recently, an allele was discovered which had c.449G>T but lacked c.-1766T>C; this allele would receive its own star number given the absence of the c.-1766T>C core SNV. Concerns were raised, however, whether there is indeed sufficient evidence supporting c.-1766T>C having a functional impact. The gene experts ultimately recommended to revert their initial decision and remove core SNV status from c.-1766T>C, which paved the way to designate the novel haplotype as a *CYP2C9*8* suballele. This case illustrates that allele designation is not always straightforward and underscores the need to develop more concrete criteria that need to be fulfilled for non-coding SNVs to receive core SNV status.

Methods for CYP2C9 allele characterization

CYP2C9 allele characterization presents the same challenges previously discussed in the *CYP2C19* PharmVar GeneFocus review (61). In this section we provide selected examples

of novel alleles submitted to PharmVar and describe how they were characterized, i.e., how it was determined of which SNVs are located on each chromosome.

Figure 4a illustrates a sample that was homozygous for c.-1188T>C and heterozygous for c.-29G>T. In this scenario each haplotype can be deduced without further experimental testing (the same is true if a sample is homozygous for all SNVs); the novel allele was designated as *CYP2C9*1.009* and received an evidence level of 'Def' indicating that the allele has been fully characterized and variants phased.

Several SNVs are often identified as heterozygous and further characterization is required to determine whether the variants are in *cis* (on the same chromosome) or in *trans* (on opposite chromosomes). WGS coupled with long-read sequencing is the most powerful and elegant approach to determine the phase of variants over long distances. As described above and shown in Figure 4b, the three SNVs found on the *CYP2C9*71* allele were phased to the same chromosome using 10X Genomic Linked Read technology (10X Genomics, Pleasanton, CA); this allele also received an evidence level of 'Def'. To characterize *CYP2C9*62* (90) (not shown), a combination of methods including long-range PCR, cloning and sequencing were used to determine that the new haplotype has two upstream region SNVs (c.-1565C>T and c.-1188T>C) in addition to a novel nonsynonymous variant (c.430C>T, p.R125C). Alternatively, allele-specific PCR or single molecule real-time sequencing (e.g., Pacific Biosciences, Menlo Park, CA or Oxford Nanopore Technologies, Oxford, UK) may be utilized to demonstrate that variants are on the same allele; these approaches are however, limited by the length of the PCR fragments that can be generated.

Haplotypes can also be inferred using statistical approaches such as PHASE software (91) or BEAGLE (92). PharmVar has recently updated requirements for allele definitions (81) to more readily accept haplotypes that are based on computational predictions. Alleles fulfilling the submission requirements receive an evidence level of 'Mod' or 'Lim' depending on the degree of uncertainty. Figure 4c details two examples of alleles submitted by Nizamudin et al. (93). The authors used data of 210 subjects and BEAGLE to infer haplotypes. The first example represents a subject who was heterozygous for two variants, which were phased in *trans* suggesting that c.431G>A (p.R144H) is the sole SNV on the novel haplotype designated as *CYP2C9*63*. This allele received an evidence level of 'Mod' because there were no other amino acid changing SNVs or other non-coding variants known to alter function. Although some uncertainty remains regarding the phase of c.-1188T>C, the function of *CYP2C9*63* would be the same because c.-1188T>C does not impact function according to current knowledge. The second example provided in Figure 4c received an evidence level of 'Lim'. As illustrated, seven SNVs were computationally inferred to represent the *CYP2C9*3.002* suballele while a novel variant, c.1297C>T (p.R433W, rs776908257), was predicted to be located on the opposite chromosome forming the novel *CYP2C9*67* haplotype. Due to the uncertainty of the phase of two nonsynonymous variants in this subject, the impact of the p.R433W amino acid change on enzyme activity remains to be established experimentally (*in silico* analyses predict p.R433W being deleterious or probably damaging (93)). Based on BEAGLE and *in silico* predictions, a *CYP2C9*3/*67* genotype may lead to PM status while a *CYP2C9*1/*3* genotype (with p.R433W being on

the **3* allele) would translate into IM status. A confirmatory submission for *CYP2C9*67* is needed to consolidate the definition of this allele definition.

Finally, as demonstrated in Figure 4d, haplotypes can also be delineated using inheritance information. The mother-father-child trio (data obtained from the Children's Mercy Data Warehouse) was utilized for confirmatory submissions for *CYP2C9*9.001* and **11.001*, which raised their respective evidence levels from 'Mod' and 'Lim', to 'Def'. The mother's *CYP2C9*8.002* allele was consistent with the allele's definition which already had 'Def' status at the time this pedigree was analyzed.

Conclusions

This PharmVar GeneFocus on *CYP2C9* provides essential information for the understanding of this highly polymorphic gene, complementing clinically relevant information provided by CPIC guidelines and other pharmacogenetic resources. We are summarizing PharmVar efforts of systematically cataloging *CYP2C9* allelic variation, as well as providing examples of submissions highlighting different approaches to fully characterize novel haplotypes. In addition, we stress our collaborative efforts with the PharmGKB to make the information useful and easily accessible to the entire pharmacogenetics community.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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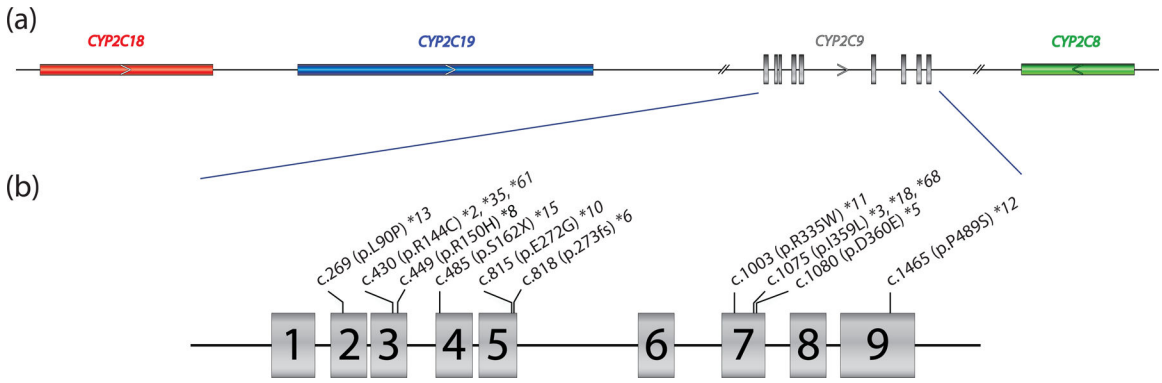


Figure 1. Overview of the gene locus and allelic variation

Panel (a) provides a graphical overview of the *CYP2C* gene locus containing *CYP2C18, CYP2C19, CYP2C9*, and *CYP2C8*. The latter is encoded on the reverse strand (arrow) while the other three genes are encoded on the forward strand. *CYP2C9* is composed of nine exons, covering 51.5 kb (GRCh37, 96,698,415 – 96,749,147; GRCh38, 94,938,658 – 94,990,091). **Panel (b)** shows the core sequence variations defining *CYP2C9*2,*3, *5, *6, *8, *10, *11, *12, *13* and **15* representing Tier 1 and Tier 2 alleles per AMP allele recommendation guidelines (34). p.R144C is also present on *CYP2C9*35* and **61* and is thus not unique to *CYP2C9*2*. Likewise, p.I359L is also part of several haplotypes, i.e., *CYP2C9*3, *18,* and **68*.

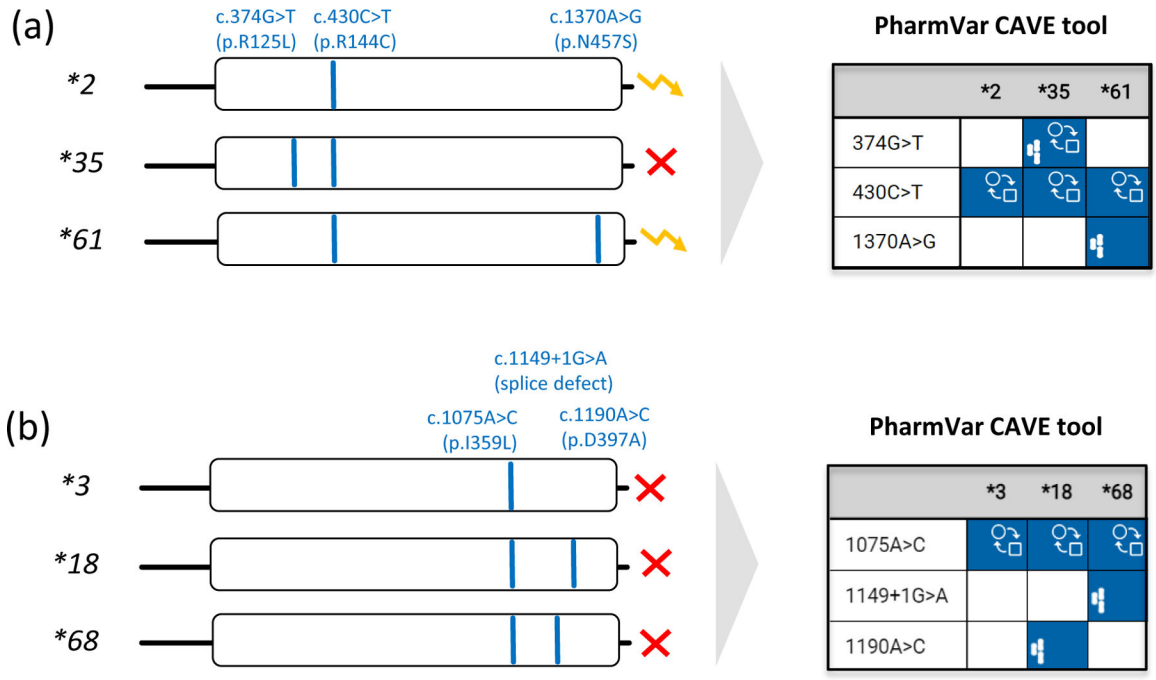


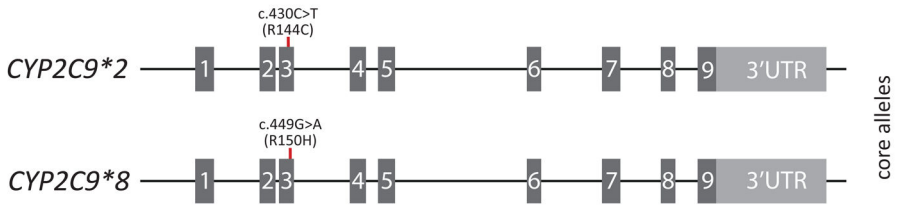
Figure 2. Allele assignment by 'default', a commonly used strategy

Pharmacogenetic test platforms typically comprise the more commonly observed SNVs and do not test for all star (*) alleles catalogued by PharmVar. Consequently, some alleles may not be identified or receive an assignment by 'default'. Therefore, it is important to understand which SNVs are tested and how alleles were called and translated into phenotype. **Panel (a)** shows that the relatively common SNV at c.430C>T (p.R144C) is not only a core SNV of *CYP2C9*2*, but also **35* and **61*. Unless c.374G>T and c.1370A>G are also tested, a *CYP2C9*35* or **61* allele will be called as a **2* potentially leading to an incorrect phenotype assignment. Since *CYP2C9*2* and **61* are both classified as decreased function by CPIC, defaulting *CYP2C9*61* as **2* will not impact the patient's phenotype call. **Panel (b)** demonstrates that c.1075A>C (p.I359L) is also present in *CYP2C9*18* and the recently discovered **68* alleles. c.1075A>C on its own causes severely decreased function and thus, *CYP2C9*3* is classified by CPIC as 'no function' allele. *CYP2C9*68* has an additional SNV interfering with splicing predicted to be deleterious/probably damaging (93). It remains unknown though whether p.D379A on *CYP2C9*18* also impacts function. The yellow 'zigzag' arrow and the red X denote decreased and no function, respectively. *Comparative Allele ViewEr* (CAVE) outputs are shown for both examples to demonstrate the utility of this tool. The graph visualizes which core alleles have the tested SNVs and may be not be identified by limited testing and default allele assignments. Blue boxes indicate the presence of core SNVs. The function ( ) symbol indicates that a core SNV alters function and the PharmVar ( ) symbol highlights that a core SNV is unique to a star allele. SNV positions refer to the transcript (NM_000771.4).

(a)

Allele Name	Legacy Label	PharmVar ID	Variants (Impact) variant = variants with dbSNP rsID	Allele Evidence Level	References
CYP2C9*2		PV00538	430C>T (R144H)		CPIC Clinical Function ↘
CYP2C9*2.001	CYP2C9*2A, CYP2C9*2B	PV00044	-1188T>C, -1096A>G, -620G>T, -485T>A, -484C>A, 430C>T (R144H)	Def	deposited by Gaedigk et al. Rettie et al. 1994 Crespi et al. 1997 Takahashi et al. 2004 King et al. 2004
CYP2C9*2.003	CYP2C9*2C	PV00045	-1096A>G, -620G>T, -485T>A, -484C>A, 430C>T (R144H)	Lim	King et al. 2004
CYP2C9*2.004		PV00417	-1188T>C, -1096A>G, -620G>T, 430C>T (R144H), 1581C>G	Moc	deposited by Campos et al.
CYP2C9*8		PV00544	449G>A (R150H)		CPIC Clinical Function ↘
CYP2C9*8.001	CYP2C9*8	PV00017	-1766T>C, -1188T>C, 449G>A (R150H), 1540C>T	Moc	deposited by Campos Blaisdell et al. 2004 Allabi et al. 2004
CYP2C9*8.002		PV00418	-1766T>C, -1188T>C, 449G>A (R150H), 1540C>T, 1628C>T	Def	deposited by Gaedigk et al.
CYP2C9*8.003		PV00416	-1766T>C, -1188T>C, 449G>A (R150H), 1561C>T	Def	deposited by Gaedigk et al.

(b)



(c)

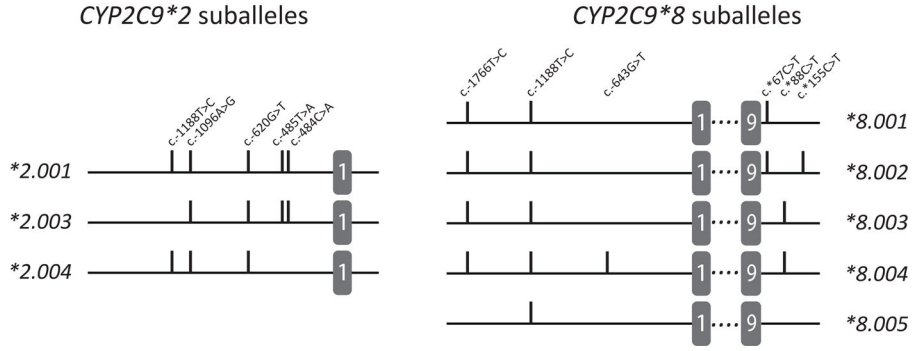


Figure 3. Overview of core allele and suballele categorization

Panel (a) shows the *CYP2C9*2* and **8* core allele definitions (gray bar) with NM_000771.4 as the reference sequence. Core SNVs, PharmVar ID (PVID), and evidence level is shown for each allele. All currently defined *CYP2C9*2* suballeles and selected *CYP2C9*8* suballeles are displayed underneath the core allele bar. Legacy allele designations are cross-referenced (e.g., **2.001* corresponds to **2A* and **2B* which have been merged). **Panel (b)** is a graphical representation of the *CYP2C9*2* and **8* core alleles. Each is characterized by a single core SNV is highlighted in red (*CYP2C9*2* by c.430C>T, p.R144C and *CYP2C9*8* by c.449G>A, R150H). While c.449G>A is unique to *CYP2C9*8*, c.430C>T is not only found in the *CYP2C9*2* haplotype (see Figure 2 for more details). Gray boxes represent the nine exons (scale is approximated); 3'UTR denotes the 3' untranslated region. **Panel (c)** shows the *CYP2C9*2* and **8* suballeles defined to date. As shown, suballeles of *CYP2C9*2* only differ in their upstream region (graph only shows this portion of the gene), while those

for *CYP2C9*8* also vary in their 3'UTR regions (graph showing respective regions). 'Lim', 'Mod' and 'Def' symbols denote 'Limited', 'Moderate' and 'Definitive' haplotype evidence levels.

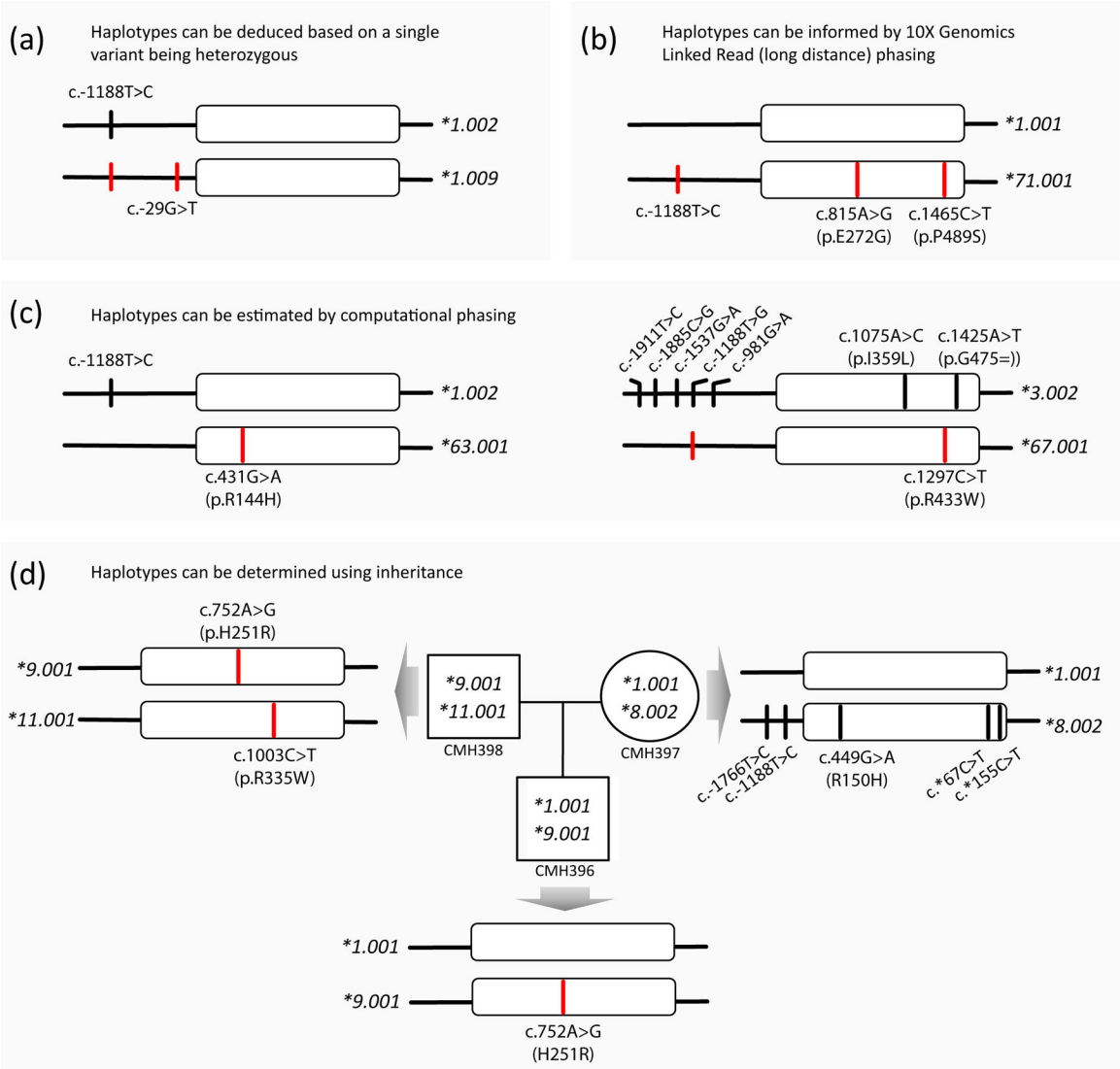


Figure 4. Characterization of novel allelic variants

Panels (a-d) provide examples of alleles submitted to PharmVar for naming or to confirm existing allele definitions. Variants of submitted alleles are highlighted by red lines. All submissions utilized WGS data which were either confirmed by WES or targeted NGS-based sequencing panels. **Panel (a)** exemplifies a subject whose haplotype can be unequivocally be deduced and **Panel (b)** shows a subject whose three heterozygous SNVs were placed on the same chromosome using 10X Genomics Linked-Read (long-distance) phasing technology; *CYP2C9*1.009* and **71* both received an evidence level of 'Definitive' ('Def'). **Panel (c)** depicts two examples for which haplotypes were computationally inferred. Due to uncertainty regarding the phase of the variants, *CYP2C9*63* and *CYP2C9*67* received evidence levels of 'Moderate' (Mod) and 'Limited' (Lim), respectively. **Panel (d)** illustrates how haplotype can be inferred using inheritance in a family trio.

Table 1

Online *CYP2C9* Resources - Links to Sites and Online Resources Referenced Throughout the Review

Sources and References	References
PharmVar	
CYP2C9 Gene Page • Read Me Document • Change Log Document • Structural Variation Document • Other Documents (Allele Frequency/Genotype Reporting Templates	(94)
Standards	(23)
Allele Designation and Evidence Level Criteria Document	(81)
CYP2C9 Gene Expert Panel Roster	(82)
P450 Nomenclature Site – Archive	(95)
PharmGKB	
CYP2C9 Gene Page	(20)
Gene-Specific Information Tables for *CYP2C9* • Allele Definition Table • Allele Functionality Table • Frequency Table • Diplotype-Phenotype Table • Gene Resource Mappings	(36)
CYP2C9 Drug Label Annotations	(24)
PGx Publication Tips	(86)
CPIC	
Guidelines Standard Operating Procedure (SOP) for Assigning Allele Function Standardized terms for clinical pharmacogenetic test results Gene/Drug Pairs • process for assigning CPIC levels • levels for gene/drug pairs • process for prioritizing CPIC guidelines	(96) (80) (66) (27)
FDA	
FDA Pharmacogenomic Biomarkers in Drug Labeling	(25, 40)
FDA Table of Pharmacogenetic Associations	(26)
Other Resources	
Drug Interactions Flockhart Table™	(40)
GTR:Genetic Testing Registry	(76)
Human Genome Variation Society (HGVS) Nomenclature	(97)
NCBI Reference Sequences Database	(83)
Locus Reference Genomic (LRG) Project	(98)

Sources and References	References
Genetic Testing Reference Materials Coordination Program (GeT-RM)	(87)

Table 2

Novel allele(s) and confirmed suballele(s)

Core Allele Designation	Novel alleles/suballeles
**1*	**1.005 – *1.013*
**2*	**2.004*
**8*	**8.002 – *8.005*
**61*	**61.001, *61.002*
**62 – *71*	**62.001 – *71.001*

Submissions for known alleles. Their original haplotype was confirmed, and/or evidence level raised from ‘Limited’ (Lim) or ‘Moderate’ (Mod) to ‘Definitive’ (Def):

*2.001, *3.002, *5.001, *8.001, *9.001, *11.001, *61.002

Table 3.

Summary of edits and changes during the transitioned into the PharmVar database and notable changes made thereafter

Reason	Change	SNVs and Affected Alleles
Standardization	Intronic SNVs were removed	**26, *27, *29*
	Comments removed	**1B−D, *2A−C, *3A+B, *11A+B, *15, *24, *26, *27, *29*
	Revised alleles after removing SNVs that are outside of the region used to define haplotypes	**2B, *11B, *27*
	Merged alleles after removing SNVs that are outside the region used to define haplotypes	**1.002 and *1.003; *2.001* and **2.002*
	Retired after its only defining SNV was removed due to being outside the region used to define haplotype	**1D*
	Revised	**3A, *3B, *8*
Other	Correction	**26* (c.-1565C>T was erroneously listed as 1565C>T)