PharmVar GeneFocus: *CYP3A5*

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The Pharmacogene Variation Consortium (PharmVar) catalogs star (*) allele nomenclature for the polymorphic human *CYP3A5* gene. Genetic variation within the *CYP3A5* gene locus impacts the metabolism of several clinically important drugs, including the immunosuppressants tacrolimus, sirolimus, cyclosporine, and the benzodiazepine midazolam. Variable CYP3A5 activity is of clinical importance regarding tacrolimus metabolism. This GeneFocus provides a *CYP3A5* gene summary with a focus on aspects regarding standardized nomenclature. In addition, this review also summarizes recent changes and updates, including the retirement of several allelic variants and provides an overview of how PharmVar *CYP3A5* star allele nomenclature is utilized by the Pharmacogenomics Knowledgebase (PharmGKB) and the Clinical Pharmacogenetics Implementation Consortium (CPIC).

A BRIEF HISTORY OF *CYP3A5* AND ITS NOMENCLATURE

In 1989, a cytochrome P450 enzyme related to CYP3A4 was purified from human fetal liver samples.[1] In that same year, the full-length cDNA sequence for this enzyme (the gene now known as *CYP3A5*) was published.[2] These studies revealed that this newly described enzyme was only expressed in 10–20% of livers derived from European ancestry samples. In 1996, a study investigating the genetics underlying the variable expression of CYP3A5 suggested that a nonsynonymous variant in exon 11 (defined at the time as *CYP3A5*2*) segregated with the absence of the protein;[3] however, the role of this variant remained inconclusive. Finally, in 2001, two studies by Kuehl *et al*. and Hustert *et al*.[4,5] uncovered the presence of a variant in intron 3 of *CYP3A5* (rs776746),[5] which creates a cryptic splice site resulting in aberrant splicing and the creation of a premature stop codon that results in transcript degradation. This allele, now known as *CYP3A5*3*, explained the highly variable expression of CYP3A5 in the liver. Based on homozygosity for the *CYP3A5*3* allele, individuals were divided into CYP3A5 nonexpressors (*CYP3A5*3/*3*) and CYP3A5 expressors (*CYP3A5*1/*3* and *CYP3A5*1/*1*). *CYP3A5*3* is the most common allele in European and Asian populations, but it is the minor allele in people of African ancestry. In addition to *CYP3A5*3*, two additional variants abolishing the function of CYP3A5 were identified and subsequently designated as *CYP3A5*6*[5] and *CYP3A5*7*.[4] Differences in *CYP3A5*3*, **6*, and **7* across populations explained why CYP3A5 is only expressed in 10–30% of Europeans and Asians but in ~ 70% of people of African ancestry.[5]

CYP3A5 star (*) allele nomenclature was first devised by the Human Cytochrome P450 Allele Nomenclature Database and has since been widely utilized, including clinical genetic testing laboratories, the Pharmacogenomics Knowledgebase (PharmGKB), and the Clinical Pharmacogenetics Implementation Consortium (CPIC). *CYP3A5* was transitioned to the Pharmacogene Variation (PharmVar) Consortium in 2017[6] and introduced into the interactive PharmVar database in August 2020.

Nine major *CYP3A5* haplotypes, *CYP3A5*1* through **9*, and several so-called suballeles were cataloged before the gene was transitioned to PharmVar (the *CYP3A5* original content before it transitioned to PharmVar remains accessible through the "Archive" link on the PharmVar homepage[7]). Whereas the common *CYP3A5*3* allele has been well-defined, *CYP3A5*2* and **4* through **9* were based on ambiguous genetic information that raised concerns of

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[Correction added on 5 April 2022, after first online publication: the affiliation linked with the authors has been corrected in this version].

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Received December 10, 2021; accepted February 11, 2022. doi:10.1002/cpt.2563

whether their respective allele defining variants indeed occur by themselves or rather exist in cis (on the same haplotype) with the intron 3 variant that defines the nonfunctional *CYP3A5*3* allele. Furthermore, 5′ and 3′ flanking regions were not consistently included when a haplotype was first reported or inferred.

Resources cited throughout this review are summarized in **Table 1**.

CLINICAL RELEVANCE

CYP3A5 and its paralog CYP3A4 have 83% amino acid sequence similarity and overlapping substrate specificity (e.g., in the metabolism of steroid hormones, nifedipine,[8] cyclosporine,[9] and fentanyl[10,11]). The main differences between CYP3A4 and CYP3A5 are in their active centers and substrate access channels,[12] which may explain the observed differences in the kinetic parameters of catalysis and inhibition by various compounds[13,14] and the formation of alternative CYP3A5 metabolites for some substrates.[15,16] The CYP3A5 enzyme contributes to the metabolism of diverse clinical drugs, including tacrolimus,[17] cyclosporine,[9] sirolimus,[18] saquinavir,[19] maraviroc,[20] midazolam,[21] vincristine,[22] and statins.[23] CYP3A5 was also suggested to confer resistance to tyrosine kinase inhibitors and paclitaxel[24]; however, these associations have not been confirmed.[25] The most robust evidence supporting a clinical impact of *CYP3A5* genetic variation is for tacrolimus metabolism (CPIC guidelines[26]; PharmGKB level of evidence 1A[27,28]). Clinical implications of *CYP3A5* genetic variation for other drugs include the immunosuppressants cyclosporine and sirolimus (CPIC level C; PharmGKB level of evidence 3) and the sedative midazolam (CPIC level C/D; PharmGKB level of evidence 3).[27-29] Genetic variation has also been suggested to play a role in the metabolism of vinca-alkaloids, such as the anti-cancer drug vincristine.[22,30,31]

Tacrolimus, usually given in combination with mycophenolate mofetil and steroids to improve graft survival,[32] is the first-line immunosuppressant used to prevent graft rejection in solid organ transplantation. Due to its narrow therapeutic index and the large variability in patient drug plasma levels, therapeutic drug monitoring is routinely performed to individualize the tacrolimus dose.[33] Optimal dosing is crucial to decrease the risk of graft rejection when underexposed, or nephrotoxicity in cases of overexposure. *CYP3A5* genotyping can be used to optimize tacrolimus dosing to achieve target therapeutic blood concentrations.

Tacrolimus is extensively metabolized in the small intestine and liver predominantly by CYP3A5, with CYP3A4 and POR playing minor roles.[17,34,35] Thus, genetic variation of *CYP3A5* is highly relevant for tacrolimus dose requirements. Associations between *CYP3A5* genotype and tacrolimus plasma levels have been observed in adult and pediatric kidney, liver, heart, and lung transplant recipients, although *CYP3A5* variation has not been proven to cause acute rejection.[36] A randomized controlled clinical trial comparing the efficacy of *CYP3A5* genotype-based tacrolimus dosing against standard care demonstrated that dosing based on pre-emptive genotyping reduced the time required to reach optimal drug exposure.[37] Because tacrolimus is extensively metabolized in the liver, it has been suggested that both donor and recipient *CYP3A5* genotype may contribute to tacrolimus pharmacokinetics,[38,39] although there is not enough evidence to support this hypothesis fully.

Table 1 Online *CYP3A5* resources – links to sites and online resources referenced throughout the review

Sources	References
PharmVar	
CYP3A5 gene page	73
Read me document	
Change log document	
Structural variation document	
Other documents (allele frequency/genotype reporting templates)	
Standards	94
Allele designation and evidence level criteria document	78
CYP3A gene expert panel roster	83
P450 nomenclature site – archive	7
PharmGKB	
CYP3A5 gene page	28
Gene-specific information tables for *CYP3A5*	60
Allele definition table	
Allele functionality table	
Frequency table	
Diplotype-phenotype table	
Gene resource mappings	
CYP3A5 drug label annotations	95
PGx publication tips	63
CPIC	
Guidelines	64
SOP for assigning allele function	82
Gene/drug pairs	29
Process for assigning CPIC levels	
Levels for gene/drug pairs	
Process for prioritizing CPIC guidelines	
Other resources	
FDA Pharmacogenomic Biomarkers in Drug Labeling and FDA Table of Pharmacogenetic Associations	96,97
Drug interactions Flockhart Table	98
GTR Genetic Testing Registry	72
HGVS nomenclature	74
NCBI reference sequences database	99
LRG project	80
Database of genomic variants – catalogue of human genomic structural variation	75
PharmCAT	61

CPIC, Clinical Pharmacogenetics Implementation Consortium; GTR, Genetic Testing Registry; HGVS, Human Genome Variation Society; LRG, Locus Reference Genomic; NCBI, National Center for Biotechnology Information; PGx, pharmacogenomics; PharmCAT, Pharmacogenomics Clinical Annotation Tool; PharmGKB, Pharmacogenomics Knowledgebase; SOP, standard operating procedure.

There is conflicting evidence regarding the association between *CYP3A5* variation and drug plasma levels of the immunosuppressive drug cyclosporine.[40-45] Renal CYP3A5-mediated cyclosporine

metabolism may have clinical importance, as CYP3A5 expressors locally generate more nephrotoxic metabolites than nonexpressors, which may lead to an increased risk of nephrotoxicity.[46] Another study demonstrated significantly longer survival for kidney transplant recipients with a *CYP3A5*1* allele receiving cyclosporine treatment.[47] Despite these observations, the clinical relevance of *CYP3A5* pharmacogenetics for cyclosporine metabolism remains unclear.

CYP3A5 genotype was also reported to have a significant influence on sirolimus metabolism; however, this drug is metabolized by both CYP3A4 and CYP3A5, with CYP3A4 being the major metabolizing enzyme.[18]

FACTORS INFLUENCING CYP3A5 ACTIVITY

Similar to CYP3A4, CYP3A5 expression levels are inherently higher in women than men when assessed in liver tissue samples.[48] This sex dimorphism has also been observed via *in vivo* assessment of plasma metabolite levels.[49] It is believed that these differences are due to sex-dependent control of the transcription networks responsible for the hormone-induced expression of liver enzymes. Regarding developmental expression of *CYP3A5*, protein is detectable in liver samples at early gestational ages[50,51] and expression after that seems to remain constant and independent of age (from early gestation to 18 years).[50]

Cytochrome P450 oxidoreductase serves as the only electron donor for CYP3A5.[52] Monooxygenase catalysis by CYP3A5 depends on this electron donor,[53] thus the cellular level of oxidoreductase may impact CYP3A5 activity. Otherwise, the catalytic activity of CYP3A5 largely depends on the presence of the microsomal form of cytochrome *b* (CYB5A),[54] which is substrate dependent. There is also evidence that CYP3A5 forms protein-protein complexes with other CYPs, such as CYP3A4 and CYP2E1,[55] which may impact interindividual variability.

CYP3A5 activity can be impacted by inhibitors leading to phenoconversion,[56] or inducers that may increase CYP3A5 expression in individuals with a functional allele. Decreased activity may also be caused by inflammation.[57] Thus, patients with a *CYP3A5*1* allele and chronic kidney disease may require dose adjustments for drugs metabolized by CYP3A5.[58] Furthermore, the expression of CYP3A5 appears to be regulated, at least to some extent, by a long non-coding RNA, AC069294.1. This regulatory mechanism may account for additional variability in CYP3A4 and CYP3A5 expression.[59] Of note, the expression of AC069294.1 has been shown to be impacted by a *CYP3A4* intronic variant, rs2242480, that is part of the *CYP3A4*1G* haplotype.

CYP3A5 IN PHARMGKB AND CPIC

PharmGKB collects, curates, and disseminates knowledge about the impact of human genetic variation on drug response.[27] The PharmGKB *CYP3A5* gene page[28] allows structured access to gene-specific pharmacogenomic knowledge. Information is presented in sections, including prescribing information, drug label annotations, clinical annotations, variant annotations, and curated pathways. As of January 2022, the PharmGKB *CYP3A5* gene page includes 3 clinical guideline annotations for tacrolimus (CPIC,[18] Dutch Pharmacogenetics Working Group (DPWG) and French National Network of Pharmacogenetics (RNPGx)), and 5 drug label annotations for 3 drugs: Maraviroc (Swiss Agency of Therapeutic Products (Swissmedic)), Prasugrel (European Medicines Agency (EMA), US Food and Drug Administration (FDA), and Swissmedic), and Dolutegravir (Swissmedic). PharmGKB contains 67 CYP3A5-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 CYP3A5 in drug metabolism are available for 40 drugs, although the significance for CYP3A5 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 *CYP3A5* are available from PharmGKB.[60] In addition, the Pharmacogenomics Clinical Annotation Tool (PharmCAT) facilitates the interpretation and reporting of pharmacogenomic-based dosing recommendations, including those for *CYP3A5*.[61,62]

PharmVar and PharmGKB collaborated to develop templates to facilitate more consistent and transparent reporting of genotype details and how genotype is translated into phenotype. The two template files that have been developed may be adapted to individual needs and are available as supplemental materials (available through the PharmVar *CYP3A5* gene page under "More Documents" and at PharmGKB under "PGx Publication Tips)."[63] The first template collects information, including methods or platforms used for genotyping and which genetic variants are interrogated; the template also provides a standardized setup for reporting genotype results for individual subjects, as well as allele frequencies. The second template facilitates the reporting of how genotype is translated into phenotype and genotype frequencies. Publication of this information in a structured form greatly facilitates access to data for subsequent curation by PharmGKB and other groups.

The CPIC develops structured, evidence-based clinical practice guidelines for drugs affected by pharmacogenetic variation.[64] To date, one of the published CPIC guidelines is on *CYP3A5* and tacrolimus.[26] The guideline has multiple components, including CYP3A5 phenotype-specific therapeutic recommendations, a systematic evidence review, and implementation resources to support the translation of the guideline into electronic health records with examples of clinical decision support text.

For CYP3A5 phenotypic classification, individuals are categorized into the following CPIC-recommended phenotype categories: poor metabolizer, intermediate metabolizer, and normal metabolizer (formerly extensive metabolizers). Individuals are, however, often also described as "expressors" (those having one or two *CYP3A5*1* alleles) and "nonexpressors" (those having two nonfunctional alleles) in the literature. The diplotype-phenotype table provided by the PharmGKB and CPIC serves as a reference for translating *CYP3A5* genotype to phenotype.[60]

NEED FOR STANDARDIZED GENETIC VARIATION DEFINITIONS AND REPORTING OF FUNCTIONAL/CLINICAL IMPACT

To guide drug therapy, it is imperative to understand how *CYP3A5* allelic variation can impact CYP3A5 function and

utilize standardized reporting and data representation. This effort aligns with recent reports emphasizing that clinically actionable pharmacogenetic information must be accurately represented in electronic health records using a harmonized system for genotype and phenotype information.[65,66] Clinical testing for *CYP3A5* can be performed on a variety of platforms using different methodologies, and although genotyping data can be reported in different ways, such as chromosomal or genomic position on reference sequence (RefSeq), amino acid change, Single Nucleotide Polymorphism Database (dbSNP) rsID, and/or using star allele nomenclature,[6,66] many laboratories use PharmVar star allele nomenclature and the CPIC-recommended system to translate genotype to phenotype.

A study performed by the Genetic Testing Reference Material Program (GeT-RM) concluded that many pharmacogenetic variants were not interrogated consistently across commercial and laboratory platforms,[67] which was substantiated by Moyer *et al.* surveying reporting pharmacogenetic test results for *CYP2D6* and *CYP2C19*.[68] The use of nomenclature, as provided by PharmVar, will minimize inconsistent interpretations of pharmacogenetic test results. To guide pharmacogenetic testing, the Association of Molecular Pathology (AMP) and the College of American Pathologists (CAP) are publishing recommendations for clinical genotyping allele selection using PharmVar nomenclature, exemplifying its clinical utility. These recommendations also include information on test platforms. AMP/CAP recommendations have been published for *CYP2C9*,[69] *CYP2C19*,[70] and, most recently, *CYP2D6*[71] allele testing. Recommendations are planned for *CYP3A5*, which will include many commercially available pharmacogenetic test panels.[72]

Throughout this review, variants are denoted according to their relative position in the *CYP3A5* NM_000777.5 reference transcript sequence with the "A" of the ATG translation start codon being +1 (as shown on the PharmVar gene page[73]). For example, the *CYP3A5*3* allele-defining variant (rs776746) is referred to as c.219-237A>G (splice defect), indicating its intronic position. The *CYP3A5*6* defining variant (rs10264272) is located within the coding region and is thus shown as c.624G>A (splice defect). The *CYP3A5*7* allele-defining variant (rs41303343) has a 1-nucleotide insertion which is annotated by PharmVar as c.1035_1036insT (p.T346fs), denoting a frameshift at amino acid position 346. Per the Human Genome Variation Society (HGVS),[74] this insertion is described as NM_000777.5:c.1035dup.

CYP3A5 REFERENCE MATERIALS

The Centers for Disease Control's GeT-RM Coordination program is a combined effort with the Coriell Institute for Medical Research and members of the pharmacogenetic testing community. Established sets of well-characterized reference materials are needed for assay development, validation, quality control, and proficiency testing. The increasing need for reference materials based on the growing use of pharmacogenetic testing prompted the establishment of a set of 137 genomic DNA samples characterized for 28 pharmacogenes, including *CYP3A5* and "consensus" genotypes.[67] Although several platforms included testing of variants identifying the rare *CYP3A5*8*, and **9* alleles, only samples with *CYP3A5*3*, **6*, and **7* alleles were identified among the samples tested. Testing and research laboratories can acquire these materials from the Coriell Institute (Camden, NJ, USA), as they are publicly available.

THE *CYP3A5* GENE LOCUS

The *CYP3A5* gene is a member of the *CYP3A* family; it has 13 exons and is translated into a protein of 502 amino acids. The gene is located on the minus strand of chromosome 7q22.1 spanning a region of ~ 32 kilobases (kb). The *CYP3A* locus, in addition to *CYP3A5*, also harbors *CYP3A4*, *CYP3A7*, and *CYP3A43*, spanning 231 kb of genomic region (**Figure 1**). Genotyping assays need to employ *CYP3A5*-specific regions for primer design (e.g., using intronic sequences) to avoid amplifying any of the other genes in the locus. Numerous genotyping assays and tests are available commercially for *CYP3A5* testing.[72] Per the Database of Genomic Variants, a curated catalog of human genomic structural variation,[75] copy number variation appears to be exceedingly rare at the *CYP3A5* gene locus and thus is not routinely tested.

CYP3A5 ALLELE, GENOTYPE, AND PHENOTYPE FREQUENCIES ACROSS POPULATIONS

The *CYP3A5* frequency table available at PharmGKB[60] summarizes population-based allele frequencies reported in the literature. Studies were considered for inclusion if (1) the population's

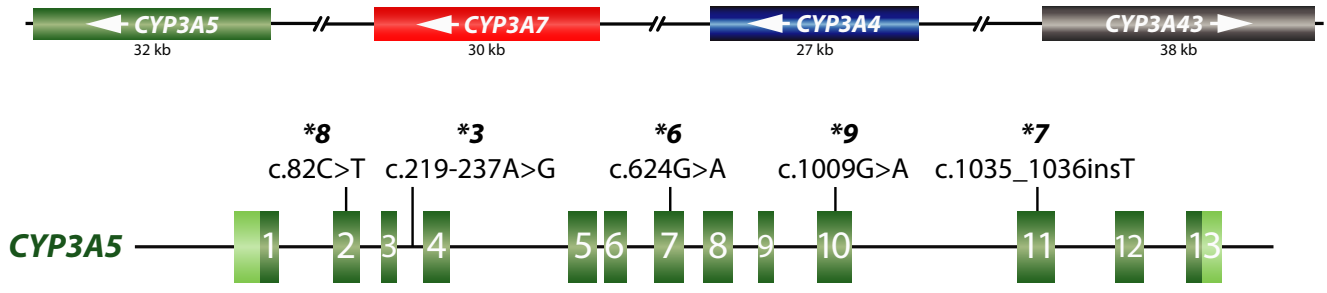


Figure 1 Overview of the gene locus and allelic variation. The top panel provides a graphical overview of the *CYP3A* gene locus containing the *CYP3A5*, *CYP3A7*, *CYP3A4*, and *CYP3A43* genes and their approximate length in kilobase pairs (kb). *CYP3A43* is encoded on the forward strand, whereas the other 3 genes are encoded on the minus strand as indicated by the white arrows. The bottom panel shows the *CYP3A5* gene in the forward orientation (5′ to 3′). *CYP3A5* comprises 13 exons as indicated by the green boxes; 5′ and 3′ untranslated regions (UTRs) are highlighted in light green. The core variants defining the *CYP3A5*3*, **6*, **7*, **8*, and **9* alleles are as shown.

ethnicity 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.[76] The *CYP3A5* 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 alleles); (2) most studies test for a limited number of allelic variants that may lead to an underestimation of certain alleles. For example, most tests only interrogate the c.219-237A>G splice defect and default to a *CYP3A5*1* assignment in its absence. This limitation may inflate the frequency of the *CYP3A5*1* allele and cause underreporting of other alleles. Therefore, all calculations based on allele frequencies are estimates.

There is considerable variation among the estimated frequencies for individual alleles across and within the biogeographical groups. The *CYP3A5*3* allele frequency is highest in Europeans and lowest in African American/Afro-Caribbeans and Sub-Saharan Africans, averaging 92%, 32%, and 24%, respectively. Its frequency is also high in Asians (67–75%), Latinos (77%), and Near-Eastern populations (84%). In contrast, *CYP3A5*6* and **7* are more readily observed in individuals with African ancestry (11–19% and 9–12%, respectively), although these alleles are less than 4% or rarely found in other ethnic groups.[60]

Considering the relatively small number of *CYP3A5* haplotypes and the low frequencies of most of the alleles, the actual number of genotypes found in a population or patient cohort is quite small compared with those seen for other *CYP* genes. Phenotype frequencies calculated from the averaged allele frequencies across populations are provided in the "Phenotype frequency" tab of the *CYP3A5* Frequency Table.[60] Calculated phenotype frequencies should be viewed with caution owing to the limitations mentioned above regarding the accuracy of allele frequencies, inconsistencies in the classification of "population," "'ethnicity," or "race,"[77] as well as the recent retirement of *CYP3A5*2*, **4*, and **5*, which is discussed in more detail below.

PHARMVAR NOMENCLATURE AND *CYP3A5* ALLELE DESIGNATION

PharmVar stores and displays allelic data consistently across genes, relying on public standards and data sources wherever possible. The "Allele Designation and Evidence Level Criteria" document describes the nomenclature system.[78] A new star number is only issued if a haplotype contains a sequence variant that: (1) results in an amino acid change (example: *CYP3A5*8* was defined based on having c.82C>T (rs55817950) causing a p.R28C change; (2) contains a nonsense variant (example: *CYP3A5*7* contains a 1-nucleotide insertion, c.1035_1036insT, causing a frameshift at amino acid position p.T346fs); or (3) changes expression levels or affects splicing (example: *CYP3A5*3* contains an intronic variant, c.219-237A>G, which causes a splice defect). Significantly, new haplotypes that contain previously characterized variants that lead to a nonfunctional protein are cataloged under the original star allele number as a suballele. For example, any haplotype having a novel variant in addition to c.219-237A>G will be designated as a *CYP3A5*3* suballele and considered to have no function, regardless of whether the novel variant impacts function on its own or not.

THE PHARMVAR *CYP3A5* GENE PAGE

The PharmVar *CYP3A5* gene page[73] details all currently defined star alleles. Each allele is listed in sequential order on the *CYP3A5* gene page and cross-referenced with its legacy name, if existing. Each allele contains information on variants, including core variants (see Core Allele section below), haplotype evidence level, and CPIC clinical allele function assignment. A "Compare View" allows the viewer to toggle between the standard allele table and the Comparative Allele ViewEr (CAVE) tool. The *CYP3A5* gene page also includes "Read Me," "Change Log," and "More documents" providing additional relevant information and resources, including examples and links to other websites, such as a link to PharmGKB's gene information. In addition, each characterized haplotype receives a PharmVar ID (PVID; i.e., a unique numeric identifier analogous to dbSNP rsIDs).[79] PVIDs and their haplotype definitions can be tracked in the database via the PVID Lookup function.

Variant mapping

Variants are mapped to the genomic (NG_007938.2) and transcript (NM_000777.5) reference sequences as well as to the genome builds GRCh37 (NC_000007.13) and GRCh38 (NC_000007.14). Of note, LRG_1431, the *CYP3A5* Locus Reference Genomic record[80] matches 100% with the RefSeq identifier NG_007938.2, and NM_000777.5 represents the Matched Annotation from National Center for Biotechnology Information (NCBI) and European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI; MANE) select transcript, which is representative of biology, is well-supported, expressed, and highly conserved.[81] This transcript matches GRCh38 and is 100% identical with its RefSeq for 5′UTR, CDS, splicing, and 3′UTR.

One rather crucial difference between GRCh37 and GRCh38 is that GRCh37 represents a *CYP3A5*3* allele (has a "G" at c.219-237 which corresponds to g.99270539C on GRCh37), whereas GRCh38 matches the *CYP3A5*1* allele (has an "A" at c.219-237 which corresponds to g.99270539T). Consequently, c.219-237A>G is not reported as a "variant" when sequences are compared to GRCh37. Therefore, when choosing GRCh37 as the reference setting on the *CYP3A5* gene page, the *CYP3A5*3* core allele does not have any variants displayed, whereas all other core alleles including their respective suballeles, have g.99270539C>T.

Lastly, g.99270539C>T is not annotated as "splice defect" when comparing to GRCh37 because this change represents the nucleotide allowing normal splicing. Also see illustration in the Read Me document available through the *CYP3A5* gene page on PharmVar.

On the PharmVar *CYP3A5* gene page, the user can easily cross-reference genomic coordinates and cDNA positions 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 also provided according to the HGVS[74] and the more traditional PharmVar display format. **Figure 2** provides an excerpt of the page illustrating *CYP3A5*3*, **6*, and **7*.

CYP3A5 allele function

PharmVar displays allele clinical function as determined by CPIC using their standardized protocol detailing the criteria for assigning clinical function to alleles to harmonize the process across guidelines.[82] It is important to realize that CPIC's primary focus is to assign allele function based on clinical actionability, not solely on molecular or biochemical function. The expert consensus for allele functions can be accessed on PharmGKB.[60] The table includes allele clinical functional status (displayed on PharmVar), and references reviewed during the assignment process. The filter option on the PharmVar *CYP3A5* gene page allows the user to sort alleles by functional status.

CYP3A5 haplotype evidence levels

PharmVar designates the "Haplotype Evidence Level" for each star allele reported on the *CYP3A5* gene page. Evidence levels are displayed as symbols indicating "definitive" (Def), "moderate" (Mod), or "limited" (Lim) levels of support for a given haplotype. This three-tiered system represents a modified ClinVar classification system; more detailed information is provided in the "Allele Designation and Evidence Criteria Level" document.[78] This type of information (e.g., whether an allele was sequenced across the gene, how haplotype was determined) was not systematically captured prior to PharmVar. For existing haplotype definitions, a literature review was conducted to assign evidence levels. Several *CYP3A5*3* suballeles are currently labeled as "Lim" and "Mod" because their definitions are not based on current PharmVar requirements, or the phase of the variants was computationally inferred. The value of evidence levels is centered on providing as much information on haplotype reliability as possible, enabling users to quickly parse haplotypes based on robust, high evidence as required for "Def" vs. other haplotypes with "Lim" or "Mod" evidence levels. PharmVar solicits submissions for all alleles labeled "Lim" and "Mod," but especially *CYP3A5*8* and **9*, to substantiate their current definitions and raise their evidence levels to "Def." PharmVar encourages encore submissions for alleles with single citations and shown as "Def" to further corroborate a haplotype definition.

Core allele definitions

Although many *CYP* genes have star alleles that share one or more "core" defining sequence variant(s), all *CYP3A5* haplotypes defined to date are characterized by a single unique variant. Suballele information may be valuable for designing assays or 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 can distinguish suballeles, these can be reported simply using their respective core allele definition.

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. This rule-based system allows all suballeles categorized under one star number to be collapsed into a single "core" definition. For example, all *CYP3A5*3* suballeles share the c.219-237A>G variant, causing a splice defect rendering this haplotype nonfunctional. Therefore, this variant constitutes the *CYP3A5*3* core allele definition (**Figure 2**).

The core alleles are the basis of the *CYP3A5* allele definition table used in CPIC guidelines and PharmGKB (**Table 1**). The *CYP3A5* core allele definitions are also utilized for clinical annotations in PharmGKB.

The PharmVar Comparative Allele ViewEr (CAVE) tool

PharmVar developed the CAVE tool to compare core alleles easily. This tool can be accessed using the "Compare View" button on the *CYP3A5* gene page. **Figure 2c** visualizes the utility of this tool by comparing the *CYP3A5*3*, **6*, **7*, **8*, and **9* alleles. In this display mode, it is easy to see that no core variants are shared among the currently defined star alleles and which genetic variants are known to alter function.

CURATION EFFORTS

CYP3A5 nomenclature was curated by a panel of international experts representing research, clinical testing, implementation interests, and PharmGKB/CPIC representatives to ensure that the nomenclature is consistent with CPIC guidelines and to facilitate dissemination to a greater audience through PharmGKB and other resources, such as ClinGen. The composition of the panel can be found on the PharmVar website.[83]

Gene region mapped/required for allele definition

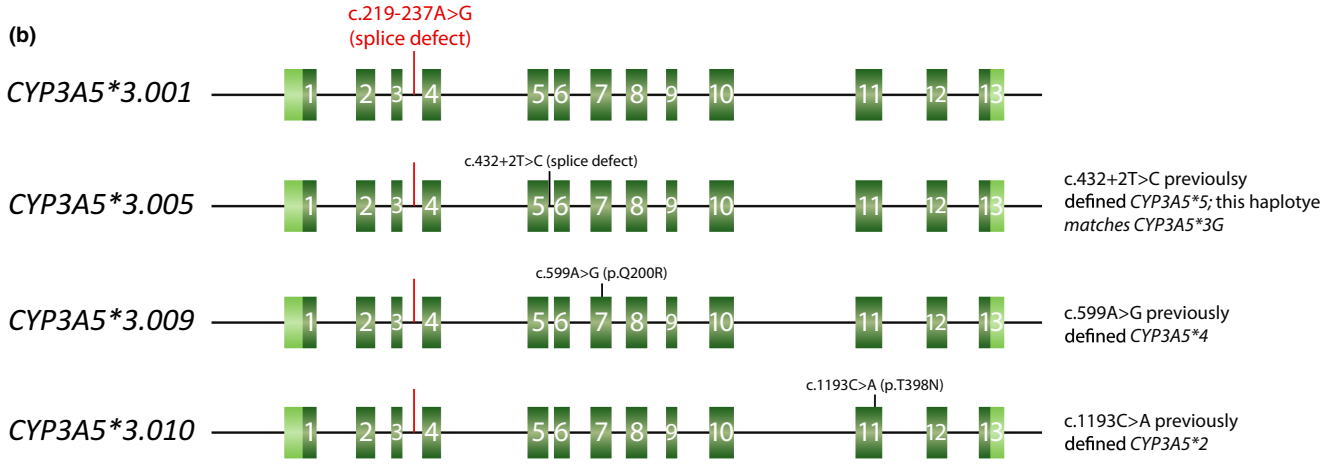
CYP3A5 allele definitions include variants within the coding region, 1000 bp upstream of the ATG translation start codon, and 250 bp of the 3′ untranslated region. Intronic variants are not considered for allele definitions unless they affect enzyme activity. Consequently, the *CYP3A5*3E*, **3H*, and **3I* suballeles were not transitioned into the PharmVar database. New submissions require covering the regions mentioned above and information for the intronic c.219-237A>G splice variant. Some of the alleles defined before PharmVar are based on limited sequencing data (e.g., lack information for up- and downstream regions) or were computationally inferred.

Revisions and corrections

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)

Allele Name	Legacy Label	PharmVar ID	Variants (Impact) variant = variants with dbSNP rsID	Haplotype Evidence Level	References and finction
CYP3A5*3		PV01325	6981A>G (splice defect)		CPIC Clinical Function ✕
CYP3A5*3.001	CYP3A5*3A	PV01340	6981A>G (splice defect)	Def	deposited by Gaedigk et al. Kuehl et al. 2001
CYP3A5*3.002	CYP3A5*3B	PV01338	3705C>T (H30Y), 3709_3710insG (G31fs), 6981A>G (splice defect)	Def	deposited by Gaedigk & Wagner Hustert et al. 2001
CYP3A5*3.003	CYP3A5*3D	PV01353	6981A>G (splice defect), 7244T>G (L82R), 31606T>C	Mod	Lee et al. 2003
CYP3A5*6		PV01328	14685G>A (splice defect)		CPIC Clinical Function ✕
CYP3A5*6.001	CYP3A5*6	PV01337	14685G>A (splice defect), 31606T>C	Def	deposited by Gaedigk et al. Kuehl et al. 2001
CYP3A5*7		PV01329	27126_27127insT (T346fs)		CPIC Clinical Function ✕
CYP3A5*7.001	CYP3A5*7	PV01339	27126_27127insT (T346fs), 31606T>C	Def	deposited by Gaedigk et al. Hustert et al. 2001



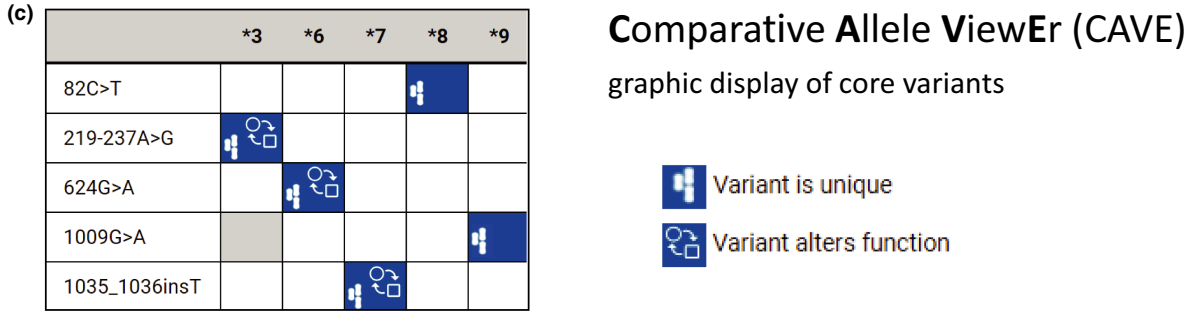


Figure 2 Overview of core allele and suballele categorization. Panel (**a**) is an excerpt of the *CYP3A5* gene page showing **3*, **6*, and **7* allele definitions with NM_000777.5 as the reference sequence; their respective core allele definitions are depicted by gray bars. Core variants, PharmVar ID (PVID), and haplotype evidence levels are shown for each allele. Selected *CYP3A5*3* suballeles are displayed underneath the core allele bar; currently, *CYP3A5*6* and **7* have only one suballele. Legacy allele designations are cross-referenced (e.g., CYP3A5**3.001* corresponds to **3A* and **3.003* corresponds **3D*). Panel (**b**) is a graphical representation of the *CYP3A5*3* suballeles containing the variants that previously defined the *CYP3A5*2*, **4*, and **5* alleles; these are now cataloged as *CYP3A5*3* suballeles due to having the intron 3 c.219-237A>G splice variant (highlighted in red). Green boxes represent exons; the 5′ and 3′ untranslated regions are shown in light green. Panel (**c**) represents the graphical output of the Comparative Allele ViewEr (CAVE) to when comparing the five currently existing star alleles. The PharmVar symbols indicate that the variant is unique to the allele and the function symbol signifies that the variant alters function. CPIC, Clinical Pharmacogenetics Implementation Consortium.

Revisions made are detailed in the "Change Log" document on the *CYP3A5* gene page[73] and are summarized in **Table 2**; this document also records submissions received by PharmVar. The following sections highlight selected efforts undertaken.

During the transition process into the PharmVar database, comments and footnotes were removed and errors corrected. References that support allele definitions were updated and those solely describing function removed (note that references describing function are summarized in the PharmGKB/CPIC *CYP3A5* Allele Functionality table[60]). As detailed in **Table 2**, several suballeles (*CYP3A5*1B*, **1C*, **1E*, and **3C*) were not transitioned due to insufficient evidence supporting the haplotypes as initially defined. Finally, a variant in the 3′ untranslated region (rs15524, c.1523T>C) was annotated as "C>T" on the legacy page[7] but is now shown as "T>C." Lifting allele definitions to the current RefSeq NG_007938 caused a switch in annotations for this variant (i.e., haplotypes shown on the legacy page as having this variant no longer do, whereas all others gained c.1523T>C; note that this variant is annotated by HGVS as c.*14T>C). *CYP3A5*1E* and **3C* were not transitioned, as it was uncertain whether these haplotypes have c.1523T>C or not.

Allele retirements and novel haplotypes

PharmVar has retired 3 star alleles, namely *CYP3A5*2* (defined before the *CYP3A5*3* allele was discovered[3]), **4*, and **5*. As detailed in **Table 3**, it was uncertain whether the variants defining the latter indeed occurred on their own or are part of *CYP3A5*3* haplotypes. To address these concerns, the PharmVar team utilized data available through the 1000 Genomes Project to show that c.1193C>A (*CYP3A5*2* core variant), c.599A>G (CYP3A5**4* core variant), and c.432+2T>C (CYP3A5**5* core variant) do not occur by themselves, as projected by their original star allele definitions, but are part of *CYP3A5*3* haplotypes. Several samples heterozygous for each, c.1193C>A, c.599A>G, or c.432+2T>C, were homozygous for the *CYP3A5*3* c.219-237A>G splice variant unequivocally informing both haplotypes (**Figure 3a**). Unpublished genotype data further corroborated this observation as c.1193C>A, c.599A>G, or c.432+2T>C, when tested, were only found in samples that were either homozygous or heterozygous for the *CYP3A5*3* splice variant.[84] The c.1193C>A and c.599A>G variants are now part of the newly created *CYP3A5*3.010* and *CYP3A5*3.009* suballeles; regarding c.432+2T>C, there already was a suballele, *CYP3A5*005* (formerly **3G*) accurately representing this haplotype. Of note, *CYP3A5*2*, **4*, and **5* are reported as *CYP3A5*3* if a pharmacogenetic test does not interrogate their respective identifying variants. For example, a patient who tested homozygous for the *CYP3A5*3* c.219-237A>G splice variant and heterozygous for the *CYP3A5*2* c.1193C>A variant may previously have been reported as "*CYP3A5*2+*3/*3*" or "*CYP3A5*3/*3 +*2*." Moving forward, these should now simply be reported as *CYP3A5*3/*3*.

*CYP3A5*8* was initially found in a subject that did not have the *CYP3A5*3* splice variant, suggesting that its core variant (c.82C>T, p.R28C) does indeed occur by itself as initially published.[85] Diplotypes of two subjects submitted by Twesigomwe and Hazelhurst (**Figure 3c**) further substantiate the initial findings and current *CYP3A5*8* allele definition. Uncertainty remains, however, regarding *CYP3A5*9*. The sentinel subject was heterozygous for c.1009G>A (p.A337T) and c.219-237A>G, and thus it remained uncertain whether c.1009G>A indeed occurs on its own.[85] One sample in the St. Jude database,[86] heterozygous for c.1009G>A and negative for *CYP3A5*3*, supports the current allele definition and, thus, the *CYP3A5*3.011* suballele that was created to reflect this uncertainty, was retracted (**Table 3**). Because c.1009G>A is exceedingly rare, it may be difficult to identify additional subjects for further analysis.

Last, although no novel haplotypes have been identified, PharmVar has expanded its catalog of *CYP3A5*3* suballeles (**Table 2**) and raised evidence levels for several alleles to "Def."

CYP3A5 ALLELE CHARACTERIZATION: METHODS AND APPROACHES

Whereas of considerably lower genetic complexity than many other pharmacogenetically relevant *CYP* genes, the determination of variant linkage is nevertheless essential for the complete characterization of *CYP3A5* haplotypes. This section offers examples from *CYP3A5* submissions to PharmVar to illustrate how alleles can be characterized. Additional information

Table 2 Novel suballeles, confirmed alleles and alleles not transitioned into the PharmVar database

Core allele designation	Novel suballeles	Confirmed existing allele definitions (legacy name)	Other revisions
**1*	**1.003*, **1.004*, **1.005*	**1.002* (**1D*)	**1B*, **1C* were not transitioned due to uncertainty of having c.-86G>A and c.-74C>T with c.219-237A as originally published **1E* not transitioned due to uncertainty of having c.1523T>C
**3*	**3.012* – **3.017*	**3.001* (**3A*), **3.002* (**3B*), **3.005* (**3G*) **3.009* **3.010*	**3C* was not transitioned due to uncertainty of having c.1523T>C **3E*, **3H*, and **3I* were not transitioned; these matched **3.001* after removing intronic variants
**6*, **7*, and **8*	None	**6.001* (**6*), **7.001* (**7*), and **8.001* (**8*)	None

All novel suballeles received a haplotype evidence level of "definitive" (Def); the haplotype evidence level of those confirmed with new data was raised from "limited" (Lim) to "definitive" (Def).
PharmVar, Pharmacogene Variation Consortium.

Table 3 Retirement of *CYP3A5*2*, **4*, and **5*. Variants that defined these star alleles are now part of *CYP3A5* suballeles

Allele	Notes
*CYP3A5*2*	Jounaïdi *et al*. 1996[3] found c.1193C>A (p.T398N) in the cDNA of 2 subjects; the **3* intronic variant c.219-237A>G was unknown at the time this variant was first described. Data available through the 1000 Genomes Project showed unequivocally that c.1193C>A is part of a *CYP3A5*3* haplotype and does not occur on its own. Therefore, the *CYP3A5*2* allele was retired; c.1193C>A is now part of the **3.010* suballele.
*CYP3A5*4*	Chou *et al*. 2001[100] first identified c.599A>G (p.Q200R) but did not provide information regarding the subject's **3* genotype (c.219-237A>G). Shih *et al*.[101] identified a subject heterozygous for c.599A>G and homozygous for c.219-237A>G. Data available through the 1000 Genomes Project showed unequivocally that c.599A>G is part of a *CYP3A5*3* haplotype and does not occur on its own. Therefore, the *CYP3A5*4* allele was retired; c.599A>G is now part of the **3.009* suballele.
*CYP3A5*5*	Chou *et al*. 2001[100] first identified c.432+2T>C (splice defect) but did not provide information regarding the subject's **3* genotype (c.219-237A>G). Saeki *et al*. 2003[102] identified a subject who was heterozygous for c.432+2T>C and homozygous for c.219-237A>G; this allele was defined as *CYP3A5*3G* (now **3.005*). Data available through the 1000 Genomes Project showed unequivocally that c.432+2T>C is part of the *CYP3A5*3.005* haplotype and does not occur on its own. Therefore, the *CYP3A5*5* allele was retired; c.599A>G was already part of the **3.005* suballele.
*CYP3A5*3.011*	Lee *et al*. 2003[85] found c.1009G>A (p.A337T) in a subject who was heterozygous for **3*; it was uncertain, however, whether c.1009G>A is on the **3* allele or indeed on the newly defined **9* allele. The **3.011* was created to reflect this uncertainty. New data supports the current **9* definition and thus, **3.011* was retracted.

regarding this topic can also be found in previous GeneFocus articles.[87,88]

Haplotypes can unequivocally be determined using deductive logic. Homozygosity of any number of variants indicates that all define the haplotype; the haplotype is also evident if all variants but one are homozygous, as shown in **Figure 3a**. Homozygosity of c.219-237A>G and heterozygosity of c.608T>G informs that the new suballele, designated as *CYP3A5*3.017*, has both variants. Likewise, subjects homozygous for the *CYP3A5*3* splice variant (c.219-237A>G) and heterozygous for c.1193C>A, c.599A>G or c.432+2T>C, the core variants that were initially used to define *CYP3A5*2*, **4*, and **5*, respectively, classifies these as *CYP3A5*3* suballeles. As described above in the section on curation, all available diplotypes containing c.1193C>A, c.599A>G, or c.432+2T>C were either homozygous or heterozygous for c.219-237A>G and thus warranted their retirement as individual star alleles and reclassification as *CYP3A5*3.010*, **3.009*, and **3.005*, respectively, as shown in **Figure 3a**.

Variant phases can, in some instances, be informed by short-read next-generation sequencing data. As shown in **Figure 3b**, a subject homozygous for the splice variant has 2 additional variants, c.88C>T and c.92_93insG, which were found on the same next-generation sequencing short reads indicating that both are on the same allele. This haplotype confirmed *CYP3A5*3.002* as it was originally defined. Phasing variants via short reads is, however, limited to variants that are in close proximity. Other approaches, such as single molecule sequencing (e.g., PacBio single-molecule real-time sequencing and Oxford Nanopore sequencing) can be used to resolve the phase of distant variants (exceeding the length of short reads).[89,90] Although there are no examples to demonstrate the utility of these methods for *CYP3A5* allele characterization to date, these methods have successfully been utilized for other highly polymorphic pharmacogenomic loci, including *CYP2C9*, *CYP2C19*, and *CYP2B6*.

Variants may also be phased using statistical approaches that leverage population-specific reference genomes. However, the reliability of such computationally inferred haplotypes critically depends on the quality of the reference genome in question and the number of samples in the data set. Thus, this method may only provide limited or moderate evidence of whether a set of variants occurs in *cis* or *trans*.

In some cases, such as that illustrated in **Figure 3c**, the phase of the variants found in subjects of interest cannot be determined with certainty (the shown diplotypes were manually inferred). Although these data on their own would not be sufficient to define a novel haplotype, they are supportive of the current *CYP3A5*8* allele definition.

Last, haplotypes may also be determined using inheritance. This approach has been successfully utilized to infer haplotypes of other *CYP* genes, including *CYP2B6*, *CYP2C8*, *CYPC9*, and *CYP2C19*.[87,88,91,92]

CONCLUSIONS

This PharmVar GeneFocus on *CYP3A5* provides essential information for understanding this important pharmacogene, complementing clinically relevant information provided by clinical guidelines and other pharmacogenetic resources. We summarize PharmVar's efforts systematically cataloging *CYP3A5* allelic variation, including the retirement of 3 star alleles: *CYP3A5*2*, **4*, and **5*. This review also offers examples of submissions highlighting different approaches to characterize novel haplotypes fully. In addition, we stress our collaborative efforts to make the information useful and easily accessible to the entire pharmacogenetics community through the PharmGKB.

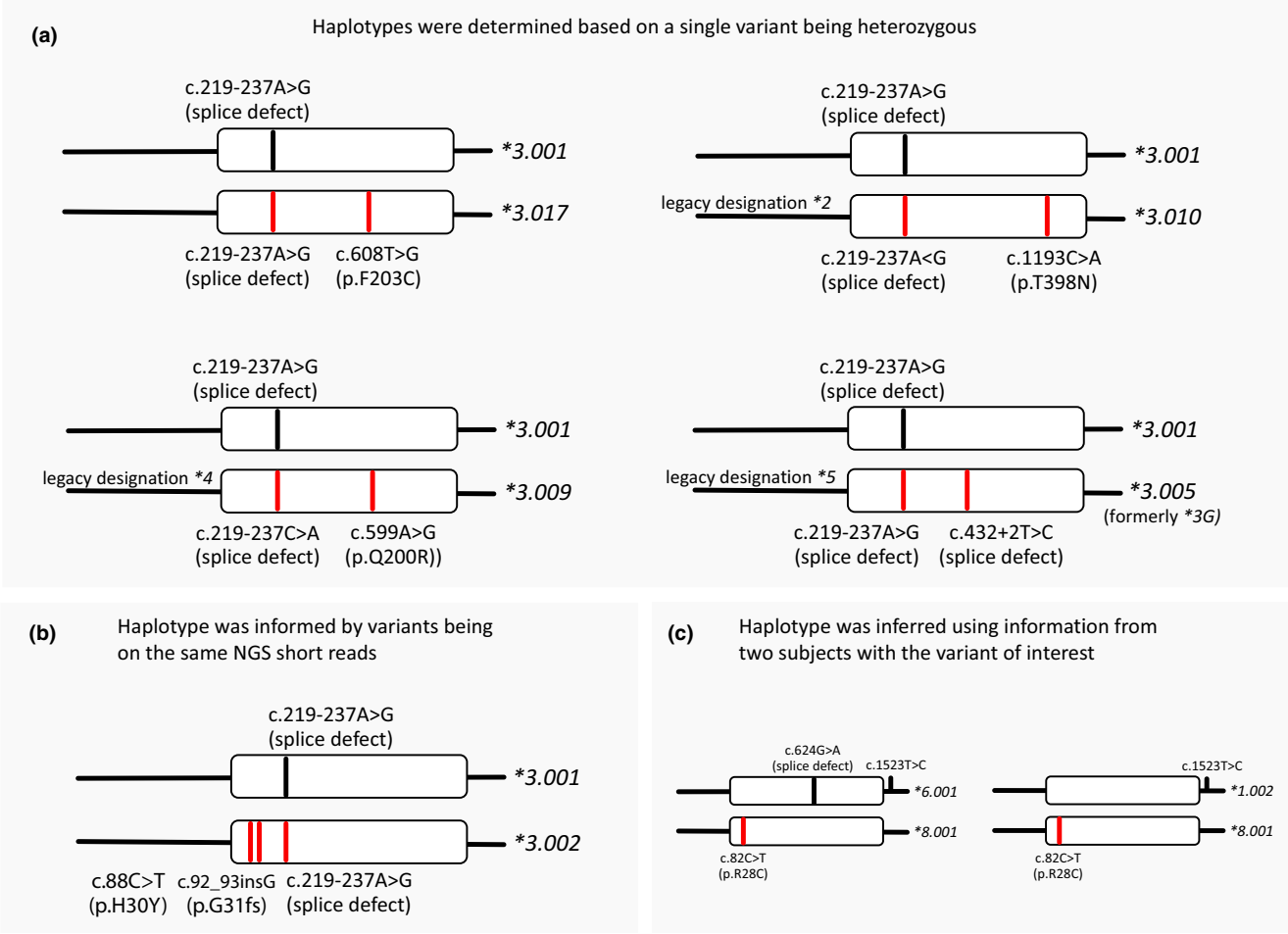


Figure 3 Methods and approaches to characterize *CYP3A5* allelic variants. Panels (**a–c**) provide examples of alleles submitted to Pharmacogene Variation Consortium (PharmVar) to name or confirm existing allele definitions. Red lines highlight variants found on the submitted alleles. Haplotypes shown in panels **a** and **b** utilized whole-genome sequencing (WGS) data either confirmed by whole exome sequencing (WES) or targeted next-generation sequencing (NGS)-based sequencing panels; haplotypes in panel **c** represent two subjects of the Simons Genome Diversity Project[93] who underwent WGS. Haplotypes are inferred; variants were not independently confirmed. Panel **a** provides examples of haplotypes that can unequivocally be deduced as only a single variant is heterozygous. These include selected submissions supporting the reclassification of *CYP3A5*2*, **4*, and **5* as *CYP3A5*3* suballeles as shown. Each subject was homozygous for the *CYP3A5*3* splice variant c.219-237A>G and heterozygous for c.1193C>A, c.599A>G, or c.432+2T>C. Thus, the latter variants are part of *CYP3A5*3* haplotypes and do not occur independently. Panel **b** shows a subject whose 3 heterozygous variants were placed on the same chromosome because c.88C>T and c.92_93insG were found on the same short reads. Thus, variants in close proximity may be deduced directly from short-read data. Panel **c** illustrates 2 samples with the *CYP3A5*8* core variant c.82C>T. Although the phase of their respective haplotypes could not be inferred with certainty, the data support that c.82C>T is not on a *CYP3A5*3* haplotype.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Yoshiro Saito of the National Institute of Health Sciences, Kawasaki, Japan, for his expertise and serving on the expert panel and Drs. Cyrine Haidar (St. Jude Children's Research Hospital, Memphis, TN), Ann Moyer (Mayo Clinic, Rochester, MN), and Charity Nofziger (Pharmgenetix GmBH, Salzburg, Austria) for sharing genotype data and Drs. David Twesigomwe and Scott Hazelhurst (University of the Witwatersrand, Johannesburg, SA) for collaborating on *CYP3A5*8* and submitting their data to PharmVar. [Correction added on 30 July 2022, after first online publication: In the Acknowledgments section, David Hazelhurst has been corrected to Scott Hazelhurst.]

FUNDING

PharmVar is supported by the Children's Mercy Research Institute. PharmGKB is supported by the National Institutes of Health (U24 HG010615; PI, T.E.K.) and D.W. by R35GM140845. V.M.L. acknowledges support from the Swedish Research Council (grant agreement numbers: 2016-01153, 2016-01154, and 2019-01837), the EU/EFPIA/OICR/McGill/KTH/Diamond Innovative Medicines Initiative 2 Joint Undertaking (EUbOPEN grant number 875510) and the Robert Bosch Foundation, Stuttgart, Germany. B.I.D. is supported by a Canadian Institute of Health Research Tier 2 Canada Research Chair in Pharmacogenomics and Precision Medicine. G.A.A. acknowledges support from the Russian Federation Fundamental Research Program for the long-term period for 2021–2030. V.M.P. acknowledges partial support from National Human Genome Research Institute (U01 HG010245).

CONFLICTS OF INTEREST

Indiana University School of Medicine Pharmacogenomics Laboratory is a fee-for-service clinical laboratory that offers clinical pharmacogenetic testing. V.M.L. is co-founder and shareholder of PersoMedix AB, CEO and shareholder of HepaPredict AB, and discloses consultancy work for Enginzyme AB. Sema4 is a fee-for-service clinical laboratory that offers clinical pharmacogenetic testing. B.E.R. is a paid employee of Sema4 and the founder and CEO of Phoenix Laboratory Consulting, LLC. All other authors declared no competing interests for this work.

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