

The Clinical Pharmacogenetics Implementation Consortium's consensus-based framework for assigning allele function.

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The Clinical Pharmacogenetics Implementation Consortium (CPIC) is a National Institutes of Health-funded international consortium of volunteers and a dedicated staff committed to integrating

pharmacogenetic (PGx) testing into patient care. 1 , 2 , 3 A major barrier to implementing PGx tests in clinical settings is the challenge of translating laboratory results into actionable prescribing decisions. CPIC aims to overcome this hurdle by developing, curating, and freely sharing peer-reviewed, evidence-based, and regularly updated gene-drug clinical practice guidelines. CPIC guidelines follow standardized formats, systematically grade evidence and clinical recommendations, use standardized terminology, 4 and undergo peer review. They are published in Clinical Pharmacology and Therapeutics and simultaneously posted on the ClinPGx website , 5 where updates are posted as needed and data can be downloaded. Each guideline provides therapeutic recommendations based on an individual's predicted drug phenotype (e.g., ultrarapid, rapid, normal, intermediate, or poor metabolizer) based on genotype. Thus, a key component of CPIC's work is translating genetic test results into clinically meaningful phenotypes upon which recommendations are based. This is achieved through the assignment of clinical function to individual pharmacogene variants or alleles by rigorous evidence review. For our purposes, "alleles" refer to either a single genetic variant or a defined combination of variants (a haplotype) present within a defined gene region, often referred to as "star alleles." PGx tests return either a genotype or a diplotype. For simplicity, we use the term genotype to refer to both, except when specifically discussing CPIC resources (e.g., diplotype-to-phenotype tables). An individual's genotype consists of two alleles, one inherited from each parent. In this report, we describe the framework and documentation used by CPIC to assign function, termed "allele clinical functional status," to PGx alleles. The process is modified from that used by the Clinical Genome Resource (ClinGen) for their gene-disease validity evaluation process. 6 , 7 , 8 , 9 While CPIC has always utilized a systematic process for guideline authors to assign allele clinical functional status, starting in 2024 the process was further formalized through the creation of gene-specific CPIC Pharmacogene Curation Expert Panels (PCEPs). The primary goal of CPIC's PCEPs is to assign an allele clinical functional status that leads to an interpretable phenotype assignment. These allele function assignments inform the clinical impact of pharmacogene variation across applicable CPIC guidelines and are generally drug or substrate agnostic. When supported by available data, the assignment of allele clinical function will take into account substrate specificity of alleles causing variable enzyme activity.

Assignment of allele clinical function and strength of evidence determines which genotypes drive actionable prescribing decisions. Allele clinical function assignments are used to create a mapping table, which determines how different combinations of alleles give rise to genotypes with specifically assigned phenotypes. Drug-specific data, such as the occurrence of drug-induced toxicity in individuals with a particular allele, may be considered during the functional assignment process. However, once an allele's clinical function is established, it is used to map phenotypes for all drugs metabolized by the enzyme. As such, the PCEP assigns clinical function to individual alleles with an understanding of how these assignments inform phenotype predictions, representing an essential step in translating PGx test results into clinical decision making. For instance, an individual with two decreased-function or no-function alleles of a given drug-metabolizing gene could have a "poor metabolizer" or an "intermediate metabolizer" phenotype, depending on the gene, highlighting the critical link between allele function assignments and phenotype interpretation. To cite an example, an individual with two CYP2B6 (MIM: 123930) decreased-function alleles is assigned a poor metabolizer phenotype, whereas an individual with two decreased-function CYP2D6 (MIM: 124030) alleles is assigned an intermediate metabolizer phenotype. In this example, the experts determined, based on the available data, that the enzymatic activity encoded by two decreased-function CYP2D6 alleles is more clinically appreciable than the activity encoded by two CYP2B6 decreased-function alleles. This example also demonstrates the variable functional effects of genetic variation across pharmacogenes. Consequently, the assignment of allele clinical function is an integral part of the development of guidelines for specific gene-drug pairs.

A PCEP is assembled for each gene curated by CPIC. The gene-specific work conducted by these panels will be utilized by the guideline author committees for all guidelines including that gene. While there is no predefined number of experts, panels generally have eight to ten members with at least six experts representing three or more institutions. Experts are identified through member- or self-nomination or by request of the CPIC Steering Committee. Generally, the expert panel is multidisciplinary, comprising a variety of scientists and clinicians from across the globe with a track

record of publication or expertise in the specific topic area of the gene/guideline, including senior individuals in the field. The committee is required to include at least two clinicians (defined as holding a clinical doctorate MD, DO, or PharmD) and two gene experts. Gene experts hold an advanced degree and demonstrate proficiency in the gene or guideline topic area (Table 1). Additional panel members include CPIC staff members with an advanced degree to support evidence review and maintain the allele functionality tables. Experts may be members of both the PCEP and the CPIC guideline author group for one or more genes. Figure 1 illustrates the collaborative nature of the various CPIC working groups and their roles in the overall guideline process. Table 1 PCEP expert selection process

MOU, memorandum of understanding; COI, conflict of interest.

Collaboration among CPIC expert panels, guideline authors, and members

Venn diagram illustrating the collaborative framework behind CPIC guidelines, highlighting the intersecting roles of guideline authors, pharmacogene experts, and CPIC members in developing and reviewing evidence-based pharmacogenetic clinical practice guidelines. Created with BioRender.com .

Importantly, to be considered for the panel, potential members also submit a signed conflict of interest (COI) disclosure (accessible on the CPIC website) and a signed CPIC publication memorandum of understanding (MOU). The final authorship plan documents the CPIC Steering Committee's decision to approve or exclude each potential expert. When evaluating COIs, the CPIC Steering Committee is guided by the following principles: (1) COIs must be transparent to all authors and readers; (2) the majority of the authorship team should not have financial COIs; (3) it is expected that pharmacogene curation panels will often have some members who are advocates for using PGx test information to inform prescribing decisions; (4) COIs due to employment by an entity in clear conflict will be considered high level and not consistent with guiding principles; and (5) the senior and first author should not be an individual with a COI. An important advantage of establishing PCEPs as distinct from the CPIC guideline author groups is the ability to include members affiliated with commercial entities, such as genetic testing companies. Because conflicts of interest are more directly relevant to clinical prescribing recommendations than to allele function assignment, individuals with valuable diagnostic and laboratory expertise can contribute meaningfully to the curation process. This structure allows CPIC to incorporate diverse perspectives from the academic and commercial testing community while maintaining transparency and integrity in guideline development.

There is also an extensive process for review and approval of experts who constitute CPIC guideline authors. The guideline authors depend upon the functional assertions made by the PCEP, and the PCEP members understand that their assertions will be used by guideline authors to serve as the basis for their clinical recommendations. In practice, there is substantial overlap between PCEP members and the guideline authors (Figure 1), and the groups work in concert. For guideline updates or subsequent guidelines including the same gene, the PCEP periodically reconvenes to reconsider its function assertions and update assignments for alleles with new evidence and/or newly described alleles. The PCEP and guideline author group will continue to work closely during guideline updates. This report will focus primarily on the work of the PCEP and how that work interfaces with that of the guideline authors.

Additionally, all PCEP members undergo variant curation and assessment training prior to their participation in compiling or reviewing evidence for assignment of allele clinical function. Initial training is conducted via a conference call wherein the CPIC Pharmacogene Curation standard operating procedure (SOP) is reviewed (available at CPIC Resources). 10 Experts receive the SOP prior to the initial conference call to allow review of the material. All aspects of the SOP are discussed on this call, including evaluation of evidence, assignment of allele clinical function, and how allele clinical function informs the translation of genotypes into phenotypes. Coaching by CPIC staff is offered throughout the allele function assignment process.

The assignment of a phenotype based on genotype is useful for minimizing the complexity involved in the interpretation of PGx test results, thus promoting clinical actionability. Although there may be thousands of possible genotypes for some pharmacogenes (e.g., >10,000 for CYP2D6), there is a small predefined number of possible phenotypes upon which dosing recommendations are made. Standardized terms for both allele function and PGx phenotypes have been established for a subset of genes through the Delphi method to achieve consensus among PGx experts. 4 The final consensus includes a unified set of terms to describe allele function for a subset of genes and three sets of terms for inferred phenotype, depending on the type of pharmacogene: drug-metabolizing enzymes, transporters, and high-risk genotypes (e.g., HLA-B [MIM: 142830] carrier status). For the first two types, allele function is categorized as increased, normal, decreased, no, unknown, or uncertain function (Table 3). Importantly, not all genes adhere to this framework. For example, some genes are not assigned an allele clinical function (e.g., HLA s, CFTR [MIM: 602421], VKORC1 [MIM: 608547], IFNL3 [MIM: 607402], and CYP4F2 [MIM: 604426]), and others use alternative terms to describe function (e.g., G6PD [MIM: 305900], RYR1 [MIM: 180901], CACNA1S [MIM: 114208], and MT-RNR1 [MIM: 561000]). Phenotype terms for drug-metabolizing enzymes include ultrarapid metabolizer, rapid metabolizer, normal metabolizer, intermediate metabolizer, and poor metabolizer; these are commonly abbreviated as UM, RM, NM, IM, and PM, respectively (Table 4). Some genes (e.g., TPMT [MIM: 187680] and CYP2C19 [MIM: 124020]) include modifiers to these phenotypes such as “possible” or “likely” to account for potentially high-risk genotypes with limited evidence, discussed in more detail below. Phenotype terms for transporter genes describe genotypes as increased function, normal function, decreased function, and poor function (Table 4). Lastly, phenotype terms to describe high-risk genotypes are positive, describing individuals homozygous or heterozygous for high-risk alleles; and negative, describing individuals who have no high-risk alleles.

These phenotype terms have been embraced by standardized vocabularies such as Logical Observation Identifiers Names and Codes (LOINC), enabling their use in electronic health records and facilitating implementation of PGx test results. Additionally, CPIC standard terminology is formally endorsed by the Association for Molecular Pathology, promoting widespread adoption by clinical genetic testing laboratories and professional organizations. Distilling genotypes into phenotypes facilitates interoperability of PGx results and allows for the use of both genotypic and direct phenotypic results (e.g., TPMT blood enzymatic activity) to guide pharmacogenomic decisions in the healthcare system. In addition, the use of standardized phenotype terms in CPIC guidelines facilitates the use of clinical decision support tools, which can be triggered based on specific PGx high-risk phenotypes. 11 , 12 Furthermore, these terms are useful for proficiency testing programs that are designed to improve quality assurance and uniform testing 13 , 14 and PGx interpretation among clinical genetic testing laboratories (e.g., College of American Pathologists).

Figure 2 provides an overview of CPIC's PCEP process of allele inclusion, evidence review, functional assignment to alleles, and updates. In brief, after the expert panel agrees on the inclusion of alleles, a CPIC staff member conducts a rigorous evidence review and the totality of evidence for an allele functional assignment is evaluated, a preliminary function is assigned using CPIC's standardized allele function terms, and an initial level of evidence to support that function is provided (Table 2). If applicable, activity values are also assigned at this time (see below for a discussion of activity scores and values). Assignments are documented in gene-specific allele functionality tables along with a written evidence summary. The draft is then disseminated to the PCEP, and each allele is reviewed by a minimum of two panel members. Reviewers note whether they agree with the function assignment as well as the strength of evidence provided. Additionally, reviewers provide feedback for the “summary of findings” to ensure the evidence is accurately summarized, including any conflicting studies (Figure 2). Next, the evidence and reviewer evaluations are disseminated to all expert panel members for review prior to a conference call so that all can contribute to the discussion and any potential disagreements between initial reviewers and/or panel members can be resolved. After all viewpoints and concerns have been discussed, experts verbally vote on allele function assignments during the conference call. Any remaining

disagreements are addressed and resolved by obtaining consensus among 70% of all panel members. If consensus cannot be reached among at least 70% of the panel members, the allele is assigned “uncertain function.” Additional details of these processes are provided below. Figure 2 Pharmacogene Curation Expert Panel workflow

Flowchart illustrating the systematic process conducted by CPIC Pharmacogene Curation Expert Panels (PCEPs). Alleles are primarily selected through PharmVar or other established resources, with additional nominations allowed by authors based on expertise—requiring 70% consensus among PCEP members. Evidence is collected primarily from PubMed, and additional databases¹⁵ are utilized to capture all relevant literature, particularly for rare variants. If allele function is well established, a de novo literature review may be bypassed using expert assessment and key references instead (e.g., a review and 1–2 primary research articles). Evidence is reviewed using accepted metrics,¹⁶ and any conflicting evidence is considered. Alleles are assigned a preliminary function and level of evidence by CPIC staff, which are then reviewed by at least two PCEP members. Feedback is synthesized and discussed in conference calls using a standardized process to resolve disagreements. Finally, updates to allele function assignments may occur due to outside inquiry regarding new data, through a structured guideline update, or when a new guideline is developed with the same pharmacogene as a previous guideline. Updates also include new alleles that were defined/published after the previous assessment. Created with BioRender.com .

Strength of evidence for assignment of allele clinical function

in vitro data (e.g., experimental data comparing the consequences of introducing the variant or correlative data showing concordance of genotype with phenotype) support the variant-drug phenotype association

computational activity predictions overall support in vivo and/or in vitro data

at least two individuals demonstrating drug phenotype causality

in vitro experimental data (e.g., engineered variant and effect measures support the variant-drug phenotype association)

at least one clinical study providing evidence for the allele's role in drug phenotype

in vitro data (e.g., experimental or correlative data) support the variant-drug phenotype association

computational activity predictions overall support in vivo and/or in vitro data

Summary of the evidence required to assign “increased,” “normal,” “decreased,” or “no function” opposed to “uncertain” or “unknown” function. The process is modified from that used by ClinGen for their gene-disease validity evaluation process.^{6, 7, 8, 9} Individual studies are evaluated using the criteria for evaluating the essential characteristics of pharmacogenetic studies.¹⁶

After a gene-drug pair is identified for a CPIC guideline, the PCEP identifies alleles to include for evidence curation and functional assignment using established gene allele definitions and variant nomenclature defined by PharmVar or other applicable resources (e.g., TPMT Allele Nomenclature Committee¹⁷ and UGT Official Nomenclature¹⁸). PharmVar is a centralized pharmacogene variation data repository that aims to standardize pharmacogene nomenclature through cataloging allelic variants of genes involved in drug response.^{19, 20, 21} In cases where PharmVar has not provided curations for the gene, other authoritative resources are utilized, such as ClinVar. ClinVar is a public archive of human genetic variations classified by disease association and drug responses.^{22, 23} Additionally, CPIC has expanded its collaboration with the former Pharmacogenomics Knowledgebase (PharmGKB) to create the Clinical Pharmacogenetics Resource (ClinPGx). ClinPGx is a comprehensive clinical pharmacogenomic resource that

supports and expands knowledge, implementation, and education of pharmacogenes and variants and their role in drug response. 24, 25 As such, CPIC has direct access to all curated gene and variant information in ClinPGx. Panel members can also nominate specific alleles for inclusion or exclusion based on their expert knowledge. In order for a variant or allele to officially be included or excluded, 70% consensus must be achieved among PCEP members.

Figure 3 details the evidence review process. Evidence to support a functional status for pharmacogene alleles is collected primarily from published peer-reviewed literature via PubMed. If requested by the expert panel, other databases such as Embase and Biosis can be utilized to ensure all relevant articles are captured. Generally, a PubMed search is conducted in the form of “[gene name] polymorphism” or “[gene name] variant.” Searches may be refined by including the Boolean operator “and” combined with known enzymatic substrates and/or drugs of interest. Additionally, LitVar, 15 an NCBI resource, is used to search for rare variants that may be included in studies but not described in the abstract. In addition to these searches, evidence may be attained from trusted resources, including PharmVar, ClinPGx, and ClinVar. Experts may propose the inclusion of additional publicly accessible curated resources for allele clinical function assignment. In these cases, the resource must have a publicly available description of their curation process, and at least 70% of experts must agree to using the newly proposed resource. Additionally, for some well-established pharmacogenes, there are conventional clinical functional assignments that are accepted within the field. For example, CYP2D6 * 4 and CYP2D6 * 5 represent a common splice defect and a gene deletion, respectively, which have been studied for more than 20 years and have been consistently assigned no function. For such cases, an extensive literature review is not necessary. Instead, PCEP members are responsible for noting their assessment of the allele function in the “summary of findings” column of the allele functionality table with reference to at least one primary article and one review that describes the function of the allele. In all other cases, a systematic de novo literature review is conducted, the details of which are described in the next section. Figure 3 Evidence review process

Diagram outlining the five-step evidence review process used by CPIC PCEPs, including evaluation by independent experts, dissemination and presentation of findings, resolution of disagreements, and finalization of evidence tables through structured review before, during, and after conference calls. Created with BioRender.com .

The literature search returns various types of articles, including clinical studies in which prescribing has been based on genotype and outcomes have been measured, clinical association studies, case reports, and experimental data (in vitro studies or in vivo preclinical studies using engineered variants and measures of drug-related phenotypes), as well as computational structural analysis and in silico predictions (Figure 4); reviews and articles that are not in English are excluded. In all cases, literature searches are well documented, including search terms, number of articles yielded, and number of articles included. Reasons for article exclusion vary from gene to gene but typically involve studies that did not experimentally determine genotypes, performed limited genetic testing, and/or focused solely on disease/treatment outcomes rather than assessing allele function. Individual studies are evaluated using established criteria for evaluating the essential characteristics of PGx studies. 16 In brief, essential PGx studies clearly and unambiguously identify the location of gene variants, especially for genes in which nomenclature has only recently been standardized. This includes explicitly stating the interrogated variants and how these variants contribute to the formation of haplotypes. These descriptions can be aided by the inclusion of standard allele definitions and rsIDs that help curators to cross-reference the data in other publicly available databases (i.e., ClinPGx, PharmVar, dbSNP, and ClinVar). Importantly, several pharmacogene alleles are characterized by multiple single-nucleotide variants (SNVs). As such, evidence for individual variants within an allele will be evaluated by experts in conjunction with any data existing for the full haplotype. Figure 4 Hierarchy of evidence

Pyramid diagram illustrating the hierarchy of evidence evaluated for allele function assignment, with increasing strength of evidence from computational data at the base to systematic reviews and meta-analyses at the top.

For each gene, the studies reviewed are cataloged in an allele function evidence spreadsheet maintained by a CPIC staff member. This file, included in the supplementary materials of all PCEP publications, is organized by PubMed ID and includes columns detailing the variants and alleles (haplotypes) tested, study type, population(s) studied, substrate(s)/drug(s) used, and relevant methods and results. This resource supports expert review and provides a transparent and comprehensive record of the data used to inform clinical allele function assignments. In addition to published literature, there is a plethora of computational tools that can aid in the determination of pharmacogene allele function. Some in silico prediction tools focus on the coding region of genes and often predict variant function as pathogenic, benign, or uncertain with regard to the variant's role in disease. In fact, many of the algorithms available are trained on known disease-causing variants and use evolutionary conservation as a key parameter. 26 , 27 However, pharmacogenes represent a special class of variant effect prediction, as guidelines are not disease specific and rather reflect the function of alleles in pharmacokinetic or pharmacodynamic processes. To address this, CPIC utilizes variant effect prediction (VEP) tools that have been specifically evaluated for their performance in predicting pharmacogene variant function. 28 , 29 , 30 These include PolyPhen-2, 31 , 32 AlphaMissense, 33 PROVEAN, 34 MutationAssessor, 35 VEST, 36 CADD, 37 , 38 , 39 SIFT, 40 , 41 REVEL, 42 Likelihood Ratio Test 43 (LRT), and PhD-SNPg. 44 , 45 Several of these tools are accessible in one location via the OpenCRAVAT web server, 46 which streamlines computational variant effect prediction for individual pharmacogenes. Of these, CADD and PhD-SNPg are capable of predicting function for nonsense and intronic variants, including those that impact mRNA splicing, as well as insertions and deletions (indels), providing additional support for alleles characterized by these types of genetic variation. Additionally, SpliceAI 47 and Pangolin 48 are used to predict effects on splicing. The Broad Institute manages a webpage that can be used to look up SpliceAI and Pangolin scores for SNVs in the genome. Additionally, PCEPs will follow American College of Medical Genetics and Genomics/Association for Molecular Pathology guidance for interpretation of splice variants. 49 , 50 Predictions (i.e., tolerated or damaging), along with scores from each tool, are recorded in the computational evidence tab of the allele function evidence file for each gene.

Predictions are generally interpreted as damaging (≥ 6 tools predict deleteriousness), conflicting (4–5 tools predict deleteriousness), and neutral (≤ 3 tools predict deleteriousness). During the initial training process, PCEP members discuss and reach consensus on how in silico evidence will be used for the assignment of allele clinical function. This includes discussion of the validity of VEPs for specific genes, as some fields have specific computational predictors, 51 , 52 and the extent to which in silico evidence will be weighed for assignment of clinical allele function.

Variants that result in the generation of a premature stop codon, disruption of a canonical splice site, or the loss of the translation initiation codon are often assigned “no function” due to the well-described detrimental effects on translation leading to loss of protein expression. In such cases, experts will follow established criteria to determine whether there are alternative start sites available or to predict whether a functional gene product may still be produced. 49 , 53 With no additional in vitro or in vivo evidence, in silico variant effect predictors aid in the final functional call in such cases with limited strength of evidence.

Essentially, the combined predictions from these ten tools are used to support the allele clinical function assignment for each variant, especially in cases where in vitro or clinical data may be limited. For example, NAT2 protein encoded by the NAT2 * 10 (MIM: 612182) allele exhibits decreased activity in limited in vitro studies; however, the majority of VEPs predict that the defining variant is neutral for protein function. With no additional in vitro evidence nor any clinical data, the NAT2 -PCEP agreed to assign “uncertain function” to NAT2 * 10 . In this manner, VEPs have the potential to influence whether an actionable function is assigned to an allele.

It is important to note that in silico VEPs assess function of individual variants and not haplotypes (i.e., the combination of non-synonymous SNVs that are found on many alleles). As such, the predictions for each individual SNV will be included in the summary of findings. It remains difficult to

predict combinatorial effects of two or more SNVs within the same allele. Accordingly, the PCEP relies more heavily on existing clinical and in vitro data for such alleles and will assign a limited strength of evidence or uncertain function when necessary.

Despite the supporting evidence provided by VEPs, CPIC cautiously interprets computational data. It is extremely rare for an allele to be assigned a clinical function based solely on in silico evidence. Although the tools utilized by CPIC have been shown to perform well for functional prediction of known pharmacogene variants, there is the potential for error and false predictions owing to the complexity of protein translation and the influence of individual variants on protein function across pharmacogenes. In many cases it is also impossible to predict with confidence whether a “damaging” variant causes decreased function or completely obliterates function, a distinction that may lead to differing phenotype translation. Ultimately, the extent to which in silico evidence is utilized for allele clinical function assignments is at the discretion of the PCEP, and these discussions will be made transparent in the PCEP publications for each gene.

The totality of evidence used to assign a clinical function is scored using a tiered system (Table 2 and Figure 3), with scores ranging from “no evidence” to “definitive” evidence. These scoring tiers represent the type, quality, and amount of evidence supporting the clinical functional assignment. Individual studies are evaluated using established criteria for evaluating the essential characteristics of PGx studies 16 as described above. The strongest evidence stems from meta-analyses, well-controlled clinical studies, cases of very rare alleles, case series, and case reports, although the amount of such evidence may be limited (Figure 4). Clinical studies provide direct evidence of allele function in individuals through pharmacokinetic data or other clinical outcomes (e.g., risk of drug-induced toxicity associated with specific alleles). When evaluating pharmacokinetic clinical studies, experts consider the comprehensiveness and quality of genotyping performed to ensure accurate reporting of PGx alleles. Experts evaluate the quality of evidence across various drugs or substrates, considering dosage, delivery methods, and measured outcomes, and determine whether the studies appropriately addressed potential confounding factors such as comorbidities, concurrent drug use, and organ function in the study population. Lastly, experts assess the significance level of individual studies by evaluating the statistical tests used and the contribution of observed population heterogeneity. It is not uncommon for a study to report the lack of an established association between an allele and particular phenotype due to small sample sizes. In such cases, experts will note the study and their reasons for excluding or downweighting the evidence in the summary of findings. To reach a moderate level of evidence, alleles must have data representing the observed drug-induced phenotype in at least two individuals with additional support from in vitro or computational evidence (Table 2). At times, experts may modify this threshold based on the severity of the phenotype and relative risk to the individual, such as for DPYD , discussed further below.

The next tier of evidence includes preclinical in vivo and in vitro studies. These types of studies provide direct functional evidence in animal models and cell systems that can be used to elucidate molecular mechanisms of the allele(s) of interest. Experts assess various aspects of each study, including the use of replicates, positive and negative controls, the suitability of model systems, statistical methods, and the substrates tested. For instance, in vitro activity measured in ex vivo systems (e.g., liver microsomes) may be given greater weight than data from heterologous expression systems (e.g., bacterial or insect cells), due to the influence of the native cellular environment on enzymatic function. In addition to evaluating experimental design, expert panels consider the location of the variant within the protein structure. Variants that occur in or near known functional domains, such as catalytic motifs, and substrate or cofactor binding sites, are interpreted in the context of available structural and biochemical data. In the absence of such data, the variant’s location is considered alongside in silico predictions, following established criteria. 50 , 53

Substrate specificity is also a critical consideration in the interpretation of in vitro functional data. When evaluating substrates used in preclinical and in vitro models, panel members assess whether the substrates are standard within the field and whether the variant’s impact may be substrate dependent. If evidence suggests that a variant’s function differs depending on the substrate, this is

reflected in the allele function assignment. The option to assign substrate-specific clinical function is included in the framework to accommodate future evidence that may necessitate such distinctions. Substrate specificity is discussed in greater detail in the following section.

The relative importance of different types of in vitro data can vary by gene and is determined by expert consensus during the evidence review process. These decisions, along with differing viewpoints, are clearly documented in the corresponding PCEP publication.

The lowest tier of evidence relies solely on computational predictions of function. In rare cases, in silico predictions may be sufficient for assigning function with a limited strength of evidence, particularly in the case of partial or entire gene deletions or if the sequence change predicts consequences such as a premature stop codon that will likely cause termination of translation, 50 , 53 as discussed above.

The tiered system establishes the threshold for the minimum evidence required to assign a clinical function other than “uncertain” or “unknown.” Therefore, this system distinguishes alleles with enough evidence to support assigning a clinical function from alleles with inadequate evidence. An allele clinical function must be assigned based on the totality of the evidence available. Thus, use of the tiered scoring system also takes into account the existence of conflicting evidence (Figure 2 and Table 2). Using this approach, allele function assignment can be based on drug-specific data; however, once a function is assigned, it applies universally to all drugs metabolized by the enzyme. In other words, while the data used to determine clinical function are drug specific, the resulting diplotype-to-phenotype mapping is drug agnostic.

Based on the evidence, the PCEP assigns allele clinical function as described in Table 3 . The assignment of discrete clinical functions allows phenotypes derived from the individual's genotype to be binned into categories that have actionable prescribing recommendations. To maximize clinical implementation, panel experts consider the likely impact of allele clinical function assignment on the downstream genotype and phenotype as well as the ultimate impact on prescribing guidelines. Alleles with uncertain or unknown function have inadequate, conflicting, or no evidence and are considered not to be actionable. Genotypes composed of two “uncertain-function” or “unknown-function” alleles, or a combination thereof, are assigned an “indeterminate phenotype,” which does not correspond to any prescribing recommendations. Genotypes comprising uncertain- or unknown-function alleles in combination with known function alleles are also assigned an indeterminate phenotype, with some exceptions, which are discussed further below. Table 3 Functional assignment to alleles

For pharmacogenes that do not fall under this category (e.g., CFTR), allele clinical function terms are agreed upon by the authors.

Function may differ from assigned biochemical function.

There are some genes (e.g., UGT1A1) for which the “normal metabolizer” phenotype encompasses both normal and very rapid metabolism, particularly when no drugs are known to require dosage adjustments compared to normal metabolizers. For others (e.g., NAT2), there is no normal metabolizer phenotype, owing to the distribution of NAT2 activity observed worldwide.

Table 2 provides a general framework for the evidence required to assign allele clinical function; however, specific expert panels may modify the criteria for a given gene, provided the criteria is well documented and an appropriate rationale is given for the modifications. Of particular interest in this regard are variants with a minor allele frequency <1%, which are more likely to have weak evidence but are considered to be clinically actionable. The rationale is to rely on sparse but non-conflicting data, as there is no additional evidence available for evaluation. In the case of weak evidence for an allele, the expert panel weighs the clinical importance of a type I error (acting on a genotype that may not have been actionable) versus a type II error (not acting on a genotype that should have

been actionable) and assesses the risk against benefit of any recommended alternative therapies. Immediate drug-induced death or permanent injury would be weighed against desired therapeutic effects. Experts then assess which error type results in a more dangerous consequence for an individual and weigh the potential harm to the individual for using the alternative therapy against not acting and continuing with the non-genetics-based standard of care. In this regard, experts minimize the possibility of committing the specified error type, which has the potential to cause more immediate harm based on their assessment of clinical consequences.

For example, in cases where the presence of a particular allele with known function confers a haploinsufficient phenotype that places an individual at risk of severe morbidity or mortality, experts may modify the threshold for clinical actionability to avoid making a type II error that could result in life-threatening consequences. In practice, this type of modification has been made for DPYD (MIM: 612779), for which DPYD intermediate and poor metabolizers are at an increased risk of severe or fatal fluoropyrimidine toxicity. In this example, the threshold of evidence for clinical actionability was modified to call an allele clinical function for decreased or no-function alleles in the setting of limited data. Additionally, experts may ultimately modify the clinical function assigned to an allele based on their clinical expertise. Modifications to the criteria for assigning clinical function must achieve at least 70% consensus among experts, including at least two practicing clinicians (e.g., MD or PharmD). The final summary of findings will note the experts' assessment of potentially conflicting or weak evidence as well as any modifications made to the evidence threshold. Practically, the summary may note evidence that conflicts with the assigned allele clinical function and will explicitly state why the experts chose to downweight or exclude the evidence. Reasons for exclusion may include lack of statistical power and influence of covariates, among other factors. In this manner, the summary of findings provides complete transparency for the rationale of the PCEP's final functional assignment for each allele.

The allele functionality table for each gene contains four required columns: "allele clinical functional status," "references," "strength of evidence," and "summary of findings" (Figure 5). In addition to these there are optional columns that provide additional information about alleles that may be helpful for clinicians to consider and/or is valuable for research efforts that further clinical implementation. First, "allele biochemical functional status" is an optional term that can be assigned to describe the biological function of an allele, which may differ from its clinical allele function assignment. Experts for each gene decide whether to use this column. Specifically, the allele biochemical functional status can accommodate expert evaluation of data on allele function that are of scientific interest but may not rise to a level supporting clinical actionability. For example, CYP2C9 * 3 (MIM: 601130) is assigned no function clinically but has an allele biochemical functional status of decreased. Although the protein encoded by the CYP2C9 * 3 allele retains a low level of enzymatic activity, the level is not clinically appreciable and, as such, CYP2C9 * 3 is assigned no function, clinically allowing it to be grouped with true non-functional alleles and translated as such for phenotype predictions to maximize benefit to individuals. The allele biochemical function is a designation separate from the mandatory CPIC "allele clinical function status." Allele biochemical status is not used for translating clinical genotypes into predicted phenotypes. Additionally, the biochemical functional status terms are not standardized across genes. Of note, the function information displayed by PharmVar corresponds to the CPIC allele clinical function (rather than the biochemical function) and is updated monthly. Figure 5 Example allele function and genotype-phenotype tables

Screenshot of the first six rows of the CYP2C19 (A) allele functionality table and (B) genotype-phenotype table. For full tables, see the CYP2C19 guideline on the CPIC website. CYP2C19 allele definitions are as defined by PharmVar at <https://www.pharmvar.org/gene/CYP2C19>.

Second, the "allele clinical function substrate specificity" column can be assigned to describe the clinical function of an allele toward a specific substrate, which may differ from the clinical function of the allele toward the majority of substrates. The substrate specificity term includes the affected drugs that differ from the majority, the function (e.g., metabolism or transport), and the direction of

specificity (e.g., “higher specific metabolism of amoxicillin”). Although there may be data suggesting that the function of a particular allele is substrate specific, 54 , 55 i.e., may vary from one substrate to another, the CPIC allele functionality table and diplotype-phenotype table are constructed to reflect function toward the majority of substrates (i.e., based on the field “allele clinical function status”). Only in instances where the experts deem, based on the totality of the substrate-specific evidence, that allele clinical function truly cannot be assigned without considering substrate specificity is this information and the expert rationale included in the “summary of findings” section of the allele functionality table. Whenever possible, panel members use drug-agnostic clinical function for translating genotypes to phenotypes. In the event that a clinical function could not be assigned without consideration of substrate-specific evidence, a substrate-specific function would be assigned to every allele of that gene. For example, an allele may be assigned “decreased function, [drug A]” and “normal function, [drug B].” Additionally, substrate specificity would be referenced in the CPIC guidelines’ prescribing recommendations for drugs that are affected, resulting in drug-specific genotype-to-phenotype mapping tables for that gene-drug pair. Currently, there are no examples of CPIC genes with substrate-specific functional assignments.

The conventions for translating genotype to phenotype are decided by the guideline authors in collaboration with the PCEP and may vary from gene to gene. Both guideline authors and expert panel members are responsible for documenting the conventions used based on their review of the evidence for assigning allele clinical function. As a part of the guideline manuscript process, a table is created based on these conventions that maps genotypes to phenotype terms based on combinations of allele functions (typically included as Table 1 in the CPIC guideline). This table, in combination with the allele functionality table, is used to automatically generate phenotypes from genotypes. For example, an individual with two increased-function CYP2C19 alleles will be assigned to the ultrarapid metabolizer phenotype. CPIC phenotype terms are found in Table 4 , along with functional definitions. The specifics of how alleles of different functions combine into phenotype groupings depend on the gene, and these specifics are addressed in the guideline table created by guideline authors. The gene-specific allele functionality and phenotype mapping tables are manually created by CPIC staff and experts and are deposited in the CPIC database. A diplotype-phenotype table containing every possible genotype mapped to a phenotype term is automatically generated from the aforementioned tables and is also stored in the database. Table 4 Phenotype terms and functional definitions

For pharmacogenes that do not fall under this category (e.g., CFTR), phenotype terms are agreed upon by the authors with input from CPIC members.

All terms should begin with the gene name (e.g., CYP2D6 poor metabolizer, TPMT normal metabolizer).

Based on the allele clinical function assignments included in the allele functionality table, all possible allele function combinations are identified in the “[gene] diplotype” column, and the assigned phenotype is described in the phenotype summary column (Figure 5). The phenotype assigned to each allele function combination reflects the totality of available evidence and is intended to represent the phenotype toward the majority of substrates. Importantly, the downstream genotype-to-phenotype translation partly drives the assignment of clinical allele function such that experts pay particular attention to these relationships as part of their allele clinical functional assignment process, as discussed previously. The collaborative nature of the CPIC guideline authors and PCEP members ensures that genotype-to-phenotype translations are clinically meaningful and grounded in both functional and phenotypic evidence.

Gene phenotypes can be modified using the terms “possible” or “likely.” A “possible” phenotype is assigned when the presence of one allele with known function confers a haploinsufficient phenotype that places an individual at risk of severe morbidity or mortality. Specifically, the “possible” modifier applies to individuals with a genotype consisting of a no-function allele combined with an uncertain- or unknown-function allele, even though the phenotype of these individuals cannot be determined unambiguously. The designation of that gene as representing a “possible”

high-risk genotype may be assigned by the experts if deemed to cause an actionable phenotype (e.g., TPMT possible intermediate metabolizer). The term “possible” is used as a modifier in electronic health record systems. In this scenario, the term “possible” is intended to prompt the prescribing clinician to pause and carefully consider the available information when prescribing medications associated with high-risk phenotypes. The “possible” modifier is used when more than one actionable phenotype (e.g., intermediate versus poor metabolizer) is possible; however, either phenotype is actionable. In other cases, the combination of a no-function allele with an unknown-function allele is not considered clinically actionable and would be assigned “indeterminate” (e.g., CYP2C9 * 6/ * 7).

The “likely” modifier is used when limited data exist to characterize a phenotype based on specific allele combinations and that phenotype may or may not be actionable. The “likely” modifier distinguishes the difference in levels of evidence between the allele functions characterizing the “likely” and the established phenotype. For example, due to limited data characterizing CYP2C19 decreased-function alleles, individuals with one decreased-function allele and one no-function allele (e.g., CYP2C19 * 2/ * 9) are classified as “likely poor metabolizers,” whereas individuals with two no-function alleles (e.g., CYP2C19 * 2/ * 3) are classified as “poor metabolizers,” reflecting the greater body of evidence supporting the functional impact of CYP2C19 no-function alleles compared to decreased-function alleles. “Likely” is a term accepted in several electronic healthcare record systems, although other modifier terms are also used.

Activity scores are used for drug-metabolizing enzymes or genes with products that have known quantitative effects to provide additional granularity to allele function assignment. The activity score is defined as the sum of activity values for each allele in a genotype. Activity values are discrete values used to fine-tune the function of alleles and are assigned at the time clinical function is assigned based on the totality of evidence. Importantly, activity values are assigned for binning purposes and do not necessarily reflect the actual percentage of activity compared to the function of the reference allele, which is assigned an activity value of 1. A larger activity value reflects greater enzyme activity of the allele toward the majority of substrates, and the lowest activity value of 0 reflects no clinically appreciable enzyme activity. Activity values in between (e.g., 0.25 and 0.5) represent different bins of decreased enzyme activity but do not indicate a given percentage reduction in activity (e.g., 25% and 50%). For alleles with multiple gene copies, the activity value of the allele is multiplied by the number of gene copies. 56 Although the idea of implementing a percent activity system in pharmacogenomics holds promise for greater precision, 57 , 58 several significant challenges currently limit its feasibility. Determining the activity of individual alleles is already complex, and assigning values in 10% increments is particularly difficult due to the lack of data for most alleles. Furthermore, substantial interindividual variability exists even among individuals with the same genotype, complicating efforts to standardize activity estimates. Even if such granular activity levels could be established, they would likely still need to be translated into a limited number of phenotype categories to ensure clinical practicality. Despite these obstacles, there is growing interest in advancing the field toward more precise activity quantification and in developing sophisticated dosing algorithms that incorporate genotype alongside other relevant clinical and pharmacological factors through population-based pharmacokinetic and pharmacodynamic modeling. 59 , 60

Activity scores are only applied to genotypes that include two alleles with actionable functional assignments. Specifically, alleles assigned uncertain or unknown function are not assigned an activity value and, thus, cannot be used for calculating activity scores and result in indeterminate phenotypes. Generally, an activity score system will be implemented at the discretion of the PCEP, particularly if doing so would ameliorate situations in which a phenotype group has more than one therapeutic recommendation or more than one classification of recommendation for a gene-drug pair. For example, although the genotype of a CYP2C9 intermediate metabolizer can be a combination of normal-, decreased-, or no-function alleles, the therapeutic recommendation for meloxicam differs for CYP2C9 intermediate metabolizers with a genotype comprising a normal-function allele plus a decreased-function allele compared to those with a normal-function allele plus a no-function allele. The introduction of an activity score in this example distinguishes

groups within the same phenotypic category of intermediate metabolizer and provides the necessary granularity needed to make therapeutic recommendations. Layering on activity scores, rather than creation of additional phenotypic categories, is preferable because the increased granularity in phenotypic groupings may be required for some, but not all, drugs. Additionally, use of activity scores promotes adherence to a limited number of standardized phenotypic terms, 4 which is desirable for purposes of phenotype interpretation, particularly when required in the pre-emptive (i.e., drug-agnostic) setting. To maintain consistency with the standardized phenotype terms, each phenotype corresponds to an activity score or to a range of activity scores if the phenotype can be defined by more than one combination of allele functions. For example, activity scores ranging from 0 to 0.5 and from 1 to 1.5 typically correspond to poor and intermediate metabolizer, respectively. For genes that implement an activity score system, therapeutic recommendations are individually tailored by distinct activity scores. Similar to the conventions for translating genotypes to phenotypes, activity score conventions are gene-specific assignments based on the activity values assigned in the allele functionality table and totality of the evidence. Assigning activity values to all alleles of drug-metabolizing enzymes is often not required, as additional granularity may not enhance the accuracy of phenotype interpretation. Furthermore, gene-specific differences exist in the levels of residual enzyme activity associated with particular allele combinations as well as in how that activity correlates with clinical activity. While allele function exists on a continuous spectrum from no function to increased function, clinical implementation typically involves categorizing alleles into discrete functional groups. This allows genotypes of individuals to be translated into actionable phenotype categories. For example, a CYP2D6 activity score of 0 corresponds to a poor metabolizer, whereas for DPYD both activity scores of 0.5 and 0 are classified as poor metabolizers. These are grouped into a single phenotype because the clinical recommendations are similar; in both cases fluoropyrimidines should be avoided, while for an intermediate metabolizer a dose reduction is advised. Current genes that use an activity score system include CYP2D6 , CYP2C9 , and DPYD . Gene-specific phenotype conventions, including activity scores when applicable to the gene, are documented in the phenotype mapping table.

Validation of CPIC's allele clinical functional assignments and prescribing recommendations is carried out through peer review and public input. Such validation has been ongoing since CPIC's inception, in that every guideline is discussed and available for review by the entire membership for at least 14 days prior to submission. Additionally, allele functionality tables are periodically updated in between guideline updates. CPIC is highly receptive and responsive to inquiries from its members and the general public. The CPIC website contains a contact page 61 to facilitate inquiries and comments from the community. CPIC members are regularly encouraged to submit inquiries and comments during monthly meetings and via communications from CPIC. Inquiries can be submitted through the CPIC website via an online survey tool to capture necessary and relevant information to address the inquiry.

Reassessment of allele clinical function status is triggered when CPIC becomes aware of or receives an inquiry that cites published literature supportive of evidence against an allele function assignment that has not already been assessed by experts. A trained CPIC staff member disseminates the evidence and an interim evidence table to all PCEP members and applicable guideline authors. Reassessment of allele clinical function status also occurs during subsequent guideline development, including guideline updates or new guidelines with the same pharmacogene as a previous guideline. Alleles that have been added to PharmVar since the previous assessment are also evaluated at this time. Reassessments follow the same evidence evaluation procedures as described above in " assigning allele clinical function and evidence score ," consider all evidence, and follow the same procedures to resolve disagreements as described herein, requiring consensus among at least 70% of the experts for the final function assignment and updated evidence summary.

Updates to genotype-phenotype conventions of a pharmacogene occur as a result of significant updates to allele function assignments based on new evidence and go through the same reassessment process as that described above. Updates to phenotype conventions may also occur to facilitate standardization across clinical laboratories and clinical practice guidelines. 62 The allele

functionality table and diplotype-phenotype table will display the most up-to-date information on the ClinPGx website and in the CPIC database.

While the CPIC PCEP framework provides a rigorous consensus-driven approach to allele function assignment, the process is inherently resource intensive and relies heavily on expert interpretation. This dependence on gene-specific expertise can limit scalability and slow the pace of pharmacogene variant classification, particularly as the number of identified variants continues to grow.

Another challenge to scalability is the need for ongoing updates. As the field evolves, activity scores are becoming increasingly granular. For example, the introduction of a 0.25 activity value reflects emerging evidence of clinically meaningful differences between alleles previously grouped together. It is likely that, over time, this system may naturally evolve into a more continuous scale. While this granularity may enhance clinical precision, it also increases the complexity of maintaining and updating allele function assignments.

To address these challenges, a future goal of the CPIC PCEP initiative is to develop a quantitative scoring system that could be applied by trained curators without requiring deep gene-specific expertise. Such a system would streamline the initial classification of pharmacogene variants but would still require expert discussion and consensus, particularly for rare alleles lacking robust clinical data.

Despite these limitations, the current framework and its application across multiple genes provide a foundation of evidence-based examples that can inform the design of a future scoring system. As more curated allele function assignments accumulate, these data will serve as a valuable training set for building and refining a scalable semi-automated approach to pharmacogene variant classification. Ultimately, the integration of such tools could enhance the efficiency, reproducibility, and accessibility of PGx implementation worldwide.

Adherence to the standards and procedures outlined in this document promotes the global adoption of CPIC guidelines in clinical practice. CPIC's standardized terminology is supported by respected professional organizations, including the Association for Molecular Pathology, the College of American Pathologists, the American Society of Health-System Pharmacists, and the American Society for Clinical Pharmacology and Therapeutics, highlighting the importance of consistent language in pharmacogenomics.

The overall process of CPIC's PCEPs has been thoroughly described to provide transparency in all aspects of CPIC's endeavors as well as to encourage participation and feedback from pharmacogenomics experts worldwide. As emphasized throughout this report, the efforts of CPIC PCEPs will be published alongside the clinical guidelines to promote community engagement, stimulate discussion, and support collaborative advancement in the field.

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