CPIC PHARMACOGENE CURATION

STANDARD OPERATING PROCEDURE

Overview of Pharmacogenetic Variant Curation for

Assigning CPIC Allele Clinical Function

and

Translating Diplotypes to Phenotypes

Clinical Pharmacogenetics Implementation Consortium (CPIC)

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Version 1.0

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Introduction

Using published evidence to link specific genes with specific drug effects has been undertaken by groups such as Pharmacogenomics Knowledgebase (PharmGKB) and Clinical Pharmacogenetics Implementation Consortium (CPIC) (1-4). Some of the criteria (e.g. rarity of allele frequency in the population, familial segregation) used for assigning function to disease risk genes do not apply to pharmacogenes. Likewise, the terminology applied to disease risk variants (pathogenic, benign) do not apply to pharmacogenetic variants (5), which generally do not confer a phenotype unless the individual is challenged with an affected drug. For a subset of pharmacogenes, consensus allele function terms have been developed (6) and assigned to specific pharmacogenetic alleles, and these assignments serve as part of the basis for clinical pharmacogenetic guidelines (7-27). The same process to develop consensus terms will be expanded to include additional pharmacogenes.

The purpose of this document is twofold. First, it describes the framework and documentation CPIC uses to assign function, termed the "allele clinical functional status," to pharmacogenetic variants, and these assertions can be used to determine which diplotypes drive potentially actionable prescribing decisions. Second, the document also describes processes for documenting how diplotypes are translated into pharmacogenetic phenotypes.

Although the processes for assigning function and translating diplotypes to phenotypes have been similar for CPIC guidelines up until 2020, the processes for documentation of evidence review, training, and other related processes are being formalized in 2020-2021 and are described in this document.

A unique contribution of CPIC is to assign an allele function that leads to a phenotype assignment that can drive clinical prescribing actionability. It is clear that there are cases for which a "biochemical" functional assignment can be made for an allele, but it does not completely correspond to clinical actionability (e.g. *RYR1* alleles with increased and decreased activity both correspond to the same clinical recommendation of withholding inhaled anesthetics). Therefore, it is important to note that the primary goal of the CPIC guideline authorship committees is to assign an "Allele clinical functional status" that leads to an interpretable phenotype assignment, as described in detail below. In this standard operating procedure (SOP) document we describe a process modified from that used by ClinGen for their gene-disease validity evaluation process (28). The purpose is to evaluate relevant clinical and experimental evidence supporting or contradicting a functional assertion for allele-drug relationships. As part of the CPIC guideline development process, allele functions are assigned based on expertise of appropriate gene and drug experts (i.e. guideline authors and CPIC staff).

Definitions

Activity Score: For some genes, an "activity value" is used to describe a graded activity for alleles, that, when summed (maternal + paternal allele) give a composite diplotype "activity score". An activity score system is a numeric system where a larger activity value and activity score reflect greater enzyme activity of an allele and genotype, respectively, towards a majority of substrates. This allows for additional characterization of phenotypes based on genotypes, and is used by CPIC when widely used in peer-reviewed literature (e.g. for CYP2D6) or when experts deem the increased granularity will facilitate translation of diplotypes to phenotypes or of phenotypes to prescribing actionability. In an activity score system, an activity value, a discrete value describing relative function, is assigned to each allele and an activity score is calculated for each diplotype. Activity values are documented in the allele functionality table and activity scores are documented in the diplotype-phenotype translations.

Allele Biochemical Functional status: Allele Biochemical Functional status is an optional term that can be assigned to describe the biologic function of the allele, which may differ from the clinical function. Expert authorship groups have the option of assigning "Allele biochemical functional status" (not allele clinical function status) to alleles. This is to accommodate their evaluation of data on allele function that are of scientific interest, but which do NOT rise to a level supporting clinical actionability. This is a separate designation from the mandatory CPIC "Allele Clinical Function Status." Thus, allele biochemical status will NOT be used for purposes of deciding on actionability of alleles in CPIC guidelines. The biochemical functional status terms are not standardized across genes.

Allele Clinical Function Substrate Specificity: Allele Clinical Function Substrate Specificity can be assigned to describe the clinical function of an allele towards a specific substrate which may differ from the clinical function of the allele towards the majority of substrates. The term will include the affected drugs (that differ from the majority), the function (e.g. metabolism, transport), and the direction of specificity (e.g. "higher specific metabolism of amoxicillin"). Although there are sometimes data suggesting that the function of a particular allelic variant may be substrate-specific, i.e. may vary from one substrate to another, the CPIC Allele Functionality table and Diplotype-Phenotype table are constructed to reflect function towards the majority of substrates (i.e. based on the field "Allele Clinical Function Status"). Only if the expert authors deem that based on the totality of the substrate specific evidence that allele clinical function truly cannot be assigned without considering substrate specificity will this information and the expert rationale be included in the evidence summary section of the allele functionality table. Authors are encouraged to use drug-agnostic clinical function for purposes of assigning allele function and translating diplotypes to phenotypes; where relevant, substrate specificity will be referenced in the CPIC guidelines prescribing recommendations for drugs that are affected, likely resulting in drug-specific tables for prescribing recommendations for that gene/drug pair.

Allele Clinical Functional Status: Allele Clinical Functional Status is assigned to all alleles in the allele functionality table to describe the clinically relevant function (not biochemical function) of the allele and distinguish clinically actionable alleles from non-actionable alleles (usually the normal function alleles). The goal is to assign "Allele Clinical Functional Status" to

pharmacogenetic allelic variants (including haplotypes) using a standardized procedure and standardized terms, and to document how assignments are made. CPIC's process for assignments of standardized allele function terms has been supported by the Association for Molecular Pathology (AMP). Allele clinical function assignments should be informed by the likelihood that for most pharmacogenes, it is the diplotype (and the inferred phenotype) that will drive clinical actionability. This differs from many disease-risk genes or HLA high-risk variants, where the presence of a single high-risk variant (even if mono-allelic) is actionable. Therefore, experts are encouraged to consider the consequence of the variant allele when combined with another allele in a diplotype, and the different phenotypic groupings likely to be needed for the gene (using the standardized terms) that will drive prescribing actions. Experts will review published and publicly available evidence and assign an Allele Clinical Function Status to each allele (along with a strength of evidence) or indicate that such an assignment is not yet possible based on the evidence (see below, Evidence Review and Table 1). CPIC Allele Clinical Function Status may be used to generate lists of which alleles or variants of a gene may be clinically actionable.

Clinically actionable: A clinically actionable variant is a variant that if present in the right gene dosage (e.g. homozygosity, in compound heterozygosity with another actionable variant, or combined with a normal function allele for a gene for which haploinsufficiency is actionable), prescribing decisions will be altered from normal (i.e. standard starting dose) prescribing actions. Although CPIC recognizes that some consider variants assigned normal function as "clinically actionable" because their normality indicates a lack of changes to prescribing, the term clinically actionable is used to indicate variants whose presence may actively change prescribing from the non-genetically informed recommendations.

CPIC: The Clinical Pharmacogenetics Implementation Consortium (CPIC) is an international consortium of individual volunteers and a small dedicated staff who are interested in facilitating use of pharmacogenetic tests for patient care. One barrier to implementation of pharmacogenetic testing in the clinic is the difficulty in translating genetic laboratory test results into actionable prescribing decisions for affected drugs. CPIC's goal is to address this barrier to clinical implementation of pharmacogenetic tests by creating, curating, and posting freely available, peer-reviewed, evidence-based, updatable, and detailed gene/drug clinical practice guidelines.

CPIC Facilitator: The CPIC facilitator is a trained CPIC staff member responsible for managing and coordinating a CPIC guideline.

CPIC Scientific Advisory Board: The CPIC Scientific Advisory Board offers strategic guidance on program initiatives. Members of the scientific advisory board include distinguished leaders in genomic medicine and translational science. Membership is by invitation from CPIC leadership and approved by NHGRI program officers. Specific responsibilities include providing feedback and strategic advice on current and planned CPIC gene/drug pairs and CPIC levels, CPIC guideline format, and interactions with external groups and users. The CPIC Scientific Advisory Board was formed in 2015 and convenes twice a year or more frequently by email.

CPIC Steering Committee: The CPIC Steering Committee is responsible for oversight of CPIC processes, projects, and outcomes. Members of the Steering Committee are leading researchers

and clinicians in the field of pharmacogenomics. Membership is by invitation from CPIC leadership and approved by NHGRI program officers. Specific responsibilities include approval of experts for a guideline and evaluation and management of experts' conflict of interests. The CPIC Steering Committee was formed in 2009 and convenes quarterly or more frequently by email.

Expert: Experts are responsible for gene/drug clinical practice guideline development, including allele clinical function and phenotype assignment. Experts are primarily volunteer CPIC authors but also include CPIC staff. The process for selection of experts is described in the "Experts" section of this document.

Phenotype: Phenotype refers to the observable characteristics of an individual resulting from the interaction of the diplotype for each gene with its affected drug; it is inferred based on the diplotype as described in the "Translating diplotype to phenotype" section of this document.

Strength of Evidence: A descriptor for the strength of the evidence underlying the assignment of allele clinical function will be provided for every allele in the allele functionality table. As described in Table 1 the strength of evidence criteria distinguishes alleles with enough evidence (definitive, strong, moderate, limited) to support assigning a function from alleles with inadequate evidence (none, inadequate) that therefore do not inform prescribing.

Summary of Findings: Brief text summary of the findings from the literature that support or refute the evidence underlying each allele's clinical function assignment, as described in the "Summarizing the evidence" section of this document.

Experts

Experts are identified through self-nomination or by request of the CPIC Steering Committee to be a guideline author.

The expert committee should be multidisciplinary, comprising a variety of scientists and clinicians. Experts should have a track record of publication or expertise in the specific topic area of the guideline. At a minimum, experts are required to have an advanced degree and demonstrate proficiency in at least one aspect of the guideline topic area. Proficiency can be demonstrated by a peer-reviewed publication or professional experience (e.g. researcher, clinician, or implementer) related to the gene, drug, or disease state relevant to the guideline. A brief description of expertise and interest in the guideline topic area is submitted to the CPIC Steering Committee for review. Desirable characteristics for experts include involvement in the specific CPIC topic that will lend credibility to the prescribing recommendations; international representation; and adequate representation of senior individuals. Potential authors must submit a signed conflict of interest disclosure and signed CPIC Publication MOU to be considered for the expert committee. For those who have been experts for a previous CPIC guideline, past responsiveness, and adherence to CPIC guideline procedures will also be considered by the CPIC

Steering Committee. There is no predefined number of experts, however, there are generally at least 8-10 authors per guideline. Each guideline should have at least 6 experts and represent 3 or more institutions. The expert group should be multidisciplinary but must include at least 2 clinicians and 2 gene experts on each guideline.

CPIC staff members with an advanced degree that have completed training as described in the "Professional Training" section of this document are considered experts and may assist with guideline development, even if not co-authors on the published guideline. For transparency, participating CPIC staff, if not co-authors, are acknowledged in the manuscript. All CPIC staff participating in guidelines (whether authors or not) undergo the same training as experts, and they are required to keep their conflict of interests current on https://cpicpgx.org/about-us/.

If a gene is the subject of more than 1 guideline, the author experts of one of the guidelines may update the allele functional assignment (or diplotype-to-phenotype) tables for that gene; in that case, the author experts for all other guidelines including that gene must evaluate and agree to any updates to allele or phenotype tables. All authors for that gene's guidelines will have a chance to comment on updates, participate in deliberations over changes, and will be asked for their approval of changes. Approvals by author experts are documented via an on-line survey tool before updated materials are made publicly available as described in the "Re-evaluations and Updates" section.

Management of Conflict of Interests

CPIC is guided by the Institute of Medicine Standards for Developing Trustworthy Clinical Practice Guidelines to minimize conflicts of interest (COI) of its authors and staff (1).

COIs of CPIC staff are disclosed on the CPIC website. CPIC staff (even if not an author on a guideline) are required to complete the CPIC conflict of interest disclosure form and submit an updated form with any new COIs within 30 days to the CPIC Director.

Potential authors must complete and sign the CPIC conflicts of interest disclosure form before they can be considered for involvement in a guideline. All authors should declare all current interests and activities potentially resulting in a COI by written disclosure to the CPIC Steering Committee before the approval of the authorship plan. Potential authors must include all possible conflicts, including NIH funding, that could be interpreted to indicate that authors are "advocates" of the enclosed recommendations, as well as any sources of revenue from patents, stock ownership, etc. They must include spouses/family members in declarations. All COIs that could be interpreted to indicate that authors are "advocates" of the enclosed recommendations will be reported in the guideline manuscript. Each author with an established or possible COI should explain how their relationship(s) could (or does not) influence the guideline development process or specific recommendations.

As shown in Figure 1, in evaluating COIs the CPIC Steering Committee will be guided by the principles that (a) COIs must be transparent to all authors and readers (b) the majority of the authorship team should not have financial COIs (c) it is expected that CPIC guidelines will often

have some authors who are advocates for using test information to inform prescribing (d) COIs due to employment by an entity in clear conflict will be considered high-level and not consistent with guiding principles (e) the senior and first author should not be a person with a COI. COIs due to employment by an entity in clear conflict or major financial interest are deemed high-level and should result in recusal from participating in guideline development. The final authorship plan documents the CPIC Steering Committee's decision to approve or exclude each potential expert. Before submission for publication, each guideline (including any documented COIs) is available for a minimum of 14 days for review by the CPIC membership, which will include evaluations of language, tone, and conclusions of the recommendation in light of an author(s)' conflicts. CPIC currently has over 400 members (membership list available upon request). CPIC members are not considered part of the authorship team, and CPIC members are not routinely evaluated for COIs. In addition, each CPIC guideline undergoes expert peer review, with all COIs documented for peer reviewers to evaluate.

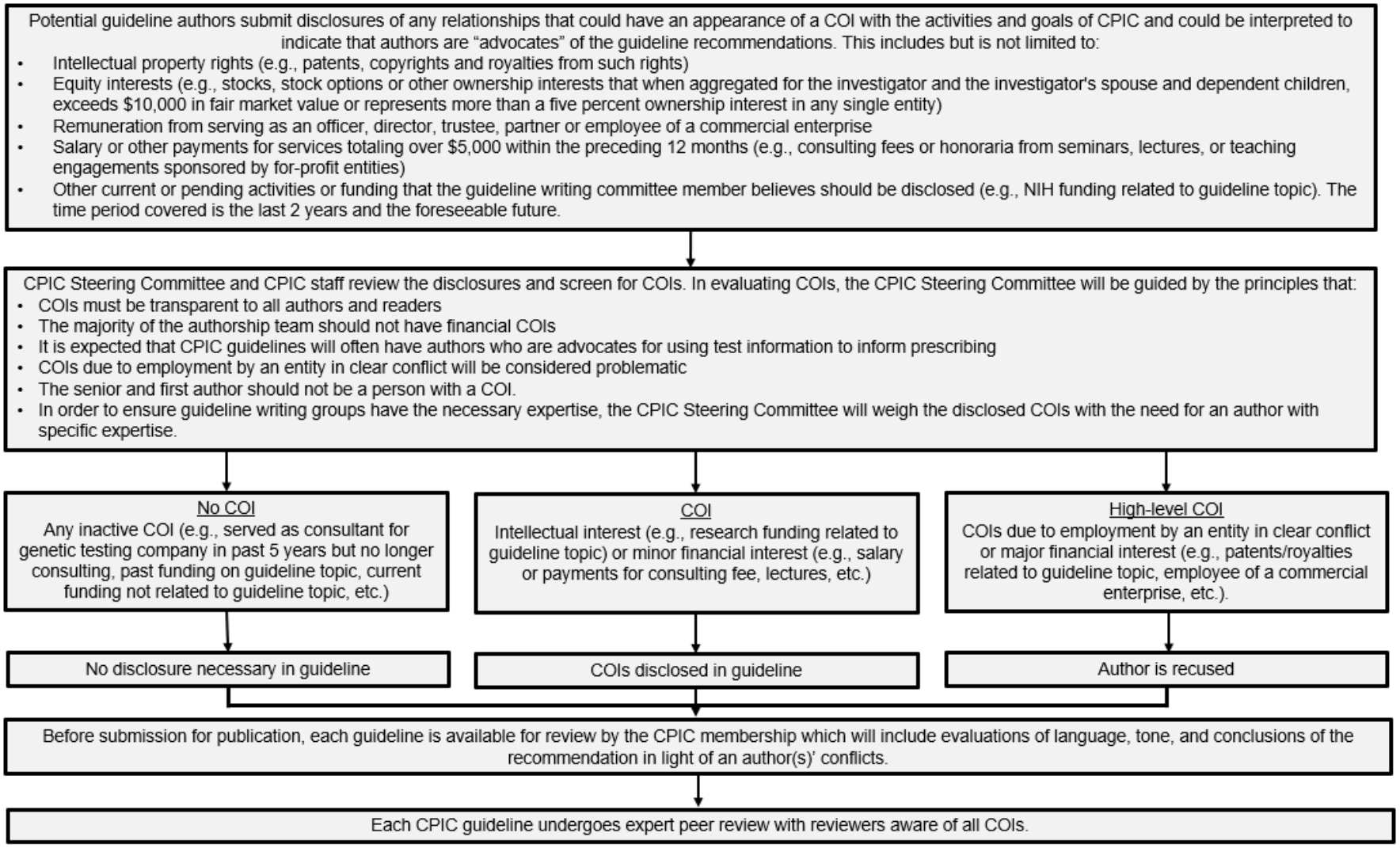

Figure 1: Conflict of Interest Management

Professional Training

Variant curation and assessment training is offered to all experts prior to their participation in compiling or reviewing evidence to assign allele clinical function. Training consists of a conference call with SOP training, a post-training assessment, and coaching if necessary. This CPIC Pharmacogene Curation SOP is disseminated prior to a conference call where procedures are reviewed, and questions are answered. The training provided during the conference call pertains to all aspects of this SOP including evaluation of evidence and assignment of allele clinical function as described in this document and is applicable to evidence obtained from all approved resources (primarily original peer-reviewed publications and PharmGKB, but occasionally ClinVar and PharmVar). Following the conference call, a recording of the call and an assessment are disseminated. Experts are encouraged to complete the assessment prior to the next conference call, and those scoring <80% on the assessment receive coaching from the CPIC facilitator and retake the assessment. During a coaching session the expert is given the opportunity to ask questions and provide rationale for the answers submitted in the assessment to assist the CPIC guideline facilitator in addressing aspects of the SOP requiring clarification. The CPIC facilitator will identify incorrectly answered questions and review the applicable section of the SOP with the expert. Additional coaching is provided by request or at the discretion of the CPIC facilitator. To maintain proficiency, a recording similar to the initial training which reviews procedures related to variant curation and assessment is disseminated to experts for their review prior to participating in re-evaluations and updates. Experts attest to reviewing training videos and materials via an on-line survey tool. Experts are exempt from this if they have successfully completed an assessment or attested to reviewing the training video within the past year as part of their involvement in other guidelines or updates. Training achievements and deficiencies are monitored using an on-line survey tool and documented for every expert in an excel file.

Assigning Allele Clinical Function

Inclusion of variants/alleles

Evidence is summarized on a variant, or an allele, if there are peer-reviewed publications or approved authoritative resources on the function or the clinical actionability of the variant or allele. Whenever available, allele definitions match those in PharmVar or other legacy gene authorities (e.g. TPMT Allele Nomenclature Committee, UGT Official Nomenclature). Guideline authors may nominate alleles for inclusion or exclusion based on their expert knowledge of the gene by submitting in writing prior to a conference call or verbally discussing the allele and rationale for inclusion or exclusion during a conference call. Objections to the inclusion or exclusion of an allele may be brought up during the conference call or submitted in writing to the CPIC facilitator within 10 days of dissemination of the meeting summary. Approved authoritative resources include PharmGKB (https://www.pharmgkb.org/), PharmVar (https://www.pharmvar.org/), and ClinVar 3- and 4-star submissions (https://www.ncbi.nlm.nih.gov/clinvar/). To add an authoritative resource beyond these three, the new authoritative reference must have been developed by a professional society, non-profit

organization, or other non-commercial working group with expertise in the gene, drug, or disease state. At least 70% of experts must agree to the use of the authoritative resource and document rationale on the conference call; all new resources are declared in written summaries of the call and experts are required to note any objections to the new resource using the on-line survey tool within 10 days of dissemination of the meeting summary, or by writing to the CPIC facilitator. If there are objections to the inclusion or exclusion of an allele or authoritative resource, objections are discussed on a conference call. After all viewpoints are made consensus among at least 70% of experts must be achieved on the conference call. If consensus cannot be achieved the allele will not be included/excluded or the authoritative resource will not be added. The outcomes of such disagreements are declared in written summaries of the call.

Evidence Review

The evidence is collected primarily from published peer-reviewed literature via PubMed (https://pubmed.ncbi.nlm.nih.gov/) but may also be retrieved from approved publicly accessible curated resources, such as ClinVar's 3-star and expert panel submissions (29) (https://www.ncbi.nlm.nih.gov/clinvar/), PharmVar (30) (https://www.pharmvar.org/) and PharmGKB (4) (https://www.pharmgkb.org/). These 3 resources have clearly delineated variant curation processes, the details of which will not be reproduced here. To use other publicly accessible curated resources for allele clinical function assignment, the resource must make their curation process available for review by the experts. At least 70% of experts must agree to the use of the publicly accessible curated resource during the conference call. All new resources are declared in written summaries of the call and experts are required to note any objections to the new resource using the on-line survey tool within 10 days of dissemination of the meeting summary, or by writing to the CPIC facilitator. Objections are discussed on a conference call. After all viewpoints are made, consensus among at least 70% of experts must be achieved on the conference call. If consensus cannot be achieved the resource will not be used. The outcomes of such disagreements are declared in written summaries of the call. For well-established canonically accepted allele clinical functional assignments (e.g. *CYP2D6*5*, representing a gene deletion and studied for > 20 years) do not require a *de novo* literature review as they have sufficient evidence to meet the criteria for definitive strength of evidence and have been studied for >20 years. These well-established alleles that do not undergo a primary literature review must be identified by the guideline gene experts and note their assessment in the Summary of Findings column of the Allele functionality table with supporting references, including one primary publication to describe the allele and at least one review article. For other non-canonical variants or alleles, literature searches will be conducted.

Compiling the evidence

The procedure used for evidence review includes the following. Keywords and inclusion criteria for evidence review are initially developed by trained CPIC staff and will generally include the genomic identifiers (e.g. dbSNP rs number, star alleles, additional common names). PubMed is

the preferred resource, but literature searches can be conducted in Embase, Biosis, or other databases as needed for cross reference to ensure all relevant articles are captured. Evidence can include clinical studies in which prescribing has been based on genotype and outcomes have been measured, clinical association studies, case reports, experimental data (in vitro studies or in vivo preclinical studies using engineered variants and measures of drug-related phenotypes), computational and in silico predictions. The approach to evidence review may be iterative, as initial searches may yield additional informative search terms and require additional evidence review. The expertise of the authors is leveraged to refine and describe the procedure used for literature review, to ensure that no critical publications are overlooked. Experts review keywords, inclusion criteria, and compiled evidence and the iterative process continues until all experts agree verbally or in writing that the compiled evidence is sufficient. The process used for literature review is transparent, available to users, and documented in the allele functionality table in the tab "methods".

Scoring the evidence used to assign allele clinical function

The strength of evidence for a finding uses a tiered system (Table 1), with scores ranging from no evidence to definitive evidence which reflect the type and amount of evidence supporting the clinical functional assignment (e.g. whether it stems from well-controlled clinical studies, meta-analyses of multiple well controlled clinical studies, small case series, case reports, preclinical *in vivo* studies, preclinical *in vitro* studies, or is solely based on computational predictions of function) (5, 28). The tiered system establishes the threshold for the minimum evidence required to assign a clinical function (function other than uncertain or unknown) and therefore distinguishes alleles with enough evidence to support assigning a function from alleles with inadequate evidence. Individual studies are evaluated using the criteria for evaluating the essential characteristics of pharmacogenetic studies (31). *In silico* predictions may rarely be sufficient for assigning function with a limited strength of evidence, particularly in the case of gene deletions or if the sequence change predicts consequences such as a premature stop codon that will likely cause termination of translation (5). Experts generally organize the evidence based on findings, and each finding may have supportive and conflicting citations. The authors must assign an Allele Clinical Function Status to each allele based on the totality of the available evidence. Authors should consider the likely impact of allele clinical function assignment on the downstream diplotype and phenotype, and the impact on prescribing implications that are based on the resulting phenotype. The process used by the authors should include evaluating the evidence for the allele, including the possible extent of altered function (e.g. highly increased activity or "no" function being weighed more than moderate functional effects), as well as allele frequency (rare alleles of < 1% being more likely to have weak evidence and yet be actionable than common alleles), using their clinical expertise to decide if prescribing would be affected based on the presence or absence of this allele by assessing if the phenotypes containing this allele would be clinically distinct from phenotypes comprised of normal function alleles. If so, then the allele should be assigned a clinical function, even if the strength of evidence is weak (see Table 1). Their process will include evaluating the clinical consequences (efficacy or toxicity) of acting on the finding, including dosage changes or drug changes, vs the

consequences of not acting on the finding (because clinical allele function is not assigned, i.e. is deemed inadequate or no evidence). There are no specific quantitative thresholds for such assignments.

Table 1: Assignment of Allele Clinical Function and Strength of evidence.

Summary of the evidence required to assign increased, normal, decreased or no function opposed to "uncertain function". The process is modified from that used by ClinGen for their gene-disease validity evaluation process (28). Individual studies are evaluated using the criteria for evaluating the essential characteristics of pharmacogenetic studies (31).

Supportive Evidence needed to assign function vs uncertain	**DEFINITIVE**	The causal role of this allele in this particular drug phenotype has been repeatedly demonstrated in independent clinical studies, and has been upheld over time (in general, at least 3 years). No convincing, adequately powered evidence has emerged that contradicts the role of the allele in the
	STRONG	There is strong evidence to support a causal role for this allele in this drug phenotype, including at least two independent clinical studies providing evidence for the allele's role in drug phenotype in addition to at least one of the following types of evidence: • Case reports • *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 (5) AND no convincing, adequately powered evidence has emerged that contradicts the role of the allele in the noted drug phenotype.
	MODERATE	There is **moderate** evidence to support a causal role for this allele in this drug phenotype, including at least two of the following types of independent evidence: • At least 2 patient cases 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 AND no convincing, adequately powered evidence has emerged that contradicts the role of the allele in the noted drug phenotype.

	LIMITED	There is limited evidence to support a causal role for this allele in this drug phenotype, including at least two independent studies based on the following types of evidence: • A case report • *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 (5) Computational activity predictions may be sufficient in unequivocal cases of early stop codon or complete gene deletions. AND no convincing, adequately powered evidence has emerged that contradicts the role of the allele in the noted drug phenotype. Function assignment based on limited data should only be made for genes whose resulting drug phenotype dictates changes to prescribing that are much more likely to result in improved clinical outcomes than not changing prescribing based on genetic test results, including consideration of life-threatening consequences if not considered.
Inadequate EVIDENCE = uncertain function		Fewer than 2 patient cases with no convincing *in vitro* experimental data, with extremely limited or conflicting *in vitro* data. This designation should be used when the evidence is not sufficiently strong to support a clinical functional status that can inform prescribing actionability. The threshold for what evidence is sufficient to inform actionability may differ among genes.
No EVIDENCE = unknown function		There is no literature describing function.

A summary of the evidence required to assign Allele Clinical Function is detailed in Table 1. The process is modified from that used by ClinGen for their disease-gene validity evaluation process (28). This is a general framework and experts may modify criteria for a specific gene provided the criteria used and rationale for modification is documented and publicly available. As noted in Table 1, one possible modification to the strength of evidence framework is the threshold for clinical actionability. For pharmacogenes where the presence of one allele with known function confers a haplo-insufficient phenotype which places them at risk of severe morbidity or mortality (e.g. *DPYD*/5FU), the experts may modify the threshold for clinical actionability to reduce the probability of making a type II error which could result in life threatening consequences. Additionally, authors may ultimately modify the clinical function assigned to an allele based on clinical expertise provided the rationale for assigning a different function is documented in the Summary of Findings column of the allele functionality table. Such rationale could include relying on sparse but non-conflicting data, particularly for rare alleles (<1% allele frequency), because more common alleles should have more evidence available for evaluation. Modifications to clinical actionability and clinical function must achieve 70% consensus among the expert group which includes at least 2 clinicians (MD or PharmD). Disagreement with modifications are submitted and addressed as previously described. Modifications are declared in written summaries of the relevant guideline conference call and experts are required to note any objections to the new resource using the on-line survey tool within 10 days of dissemination of the meeting summary, or by writing to the CPIC facilitator. Objections are discussed on a conference call and after all viewpoints are made consensus among at least 70% of experts must be achieved on the conference call. If consensus cannot be achieved the proposed modification will not be implemented. The outcomes of such disagreements are declared in written summaries of the call.

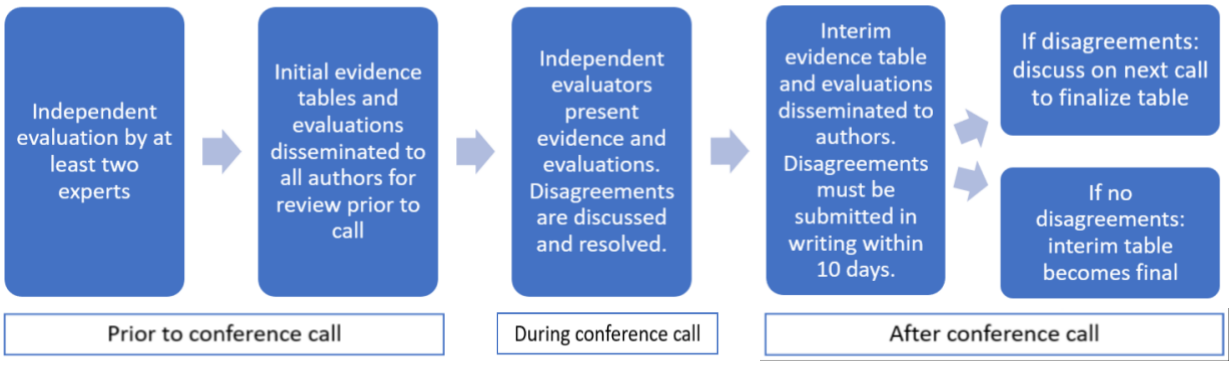

Figure 2: Initial evidence review process

Initially, a minimum of two authors will independently evaluate the literature as described in greater detail below. These authors are responsible for summarizing findings, providing an initial level of evidence (definitive, strong, moderate, limited, inadequate, or none) in support of a clinical function assignment, providing an initial allele clinical function assignment, and drafting the "Summary of Findings" content for their assigned alleles. For pharmacogenes using activity

scores as described in greater detail below, these authors will assign an activity value in addition to the allele clinical function and strength of evidence. The working draft of the Allele Functionality table will include individual experts' assignments; the final Allele Functionality table will summarize these individual assessments into a single "Summary of Findings" column, which will include a discussion of conflicts. Evidence and author evaluations are disseminated prior to an author conference call and all authors are expected to review the material prior to the call to contribute to discussion and subsequent resolution of disagreements between initial reviewers or among authors. Authors may submit disagreements and/or propose resolutions for disagreements between initial reviewers by e-mail prior to the conference call or verbally during the conference call (Figure 3). Written disagreements are submitted to the CPIC facilitator and the CPIC facilitator keeps a record of written and verbal disagreements brought up on the conference call. Discussion and further analysis of the evidence are encouraged. After all viewpoints are expressed, authors verbally vote on each disagreement during the conference call. All disagreements are addressed and resolved by obtaining consensus among 70% of all authors. If consensus cannot be reached among at least 70% of the authors, then the allele functional assignment should be "uncertain function." An interim evidence table updated with the outcomes of each discussed disagreement is disseminated to authors after the conference call. Authors must submit disagreements with assertions made in the interim table within 10 days of dissemination of the interim table and meeting summary of each conference call. Any disagreements will be discussed on the next scheduled conference call (generally within 1-3 months pending author group availability) and resolved as previously described. If no disagreements are submitted after 10 days, the assigned functions and evidence strength in the interim table become final. Experts must attest to their agreement or disagreement with the assertions made in the tables via an online survey tool, which serves as the official record of expert consensus.

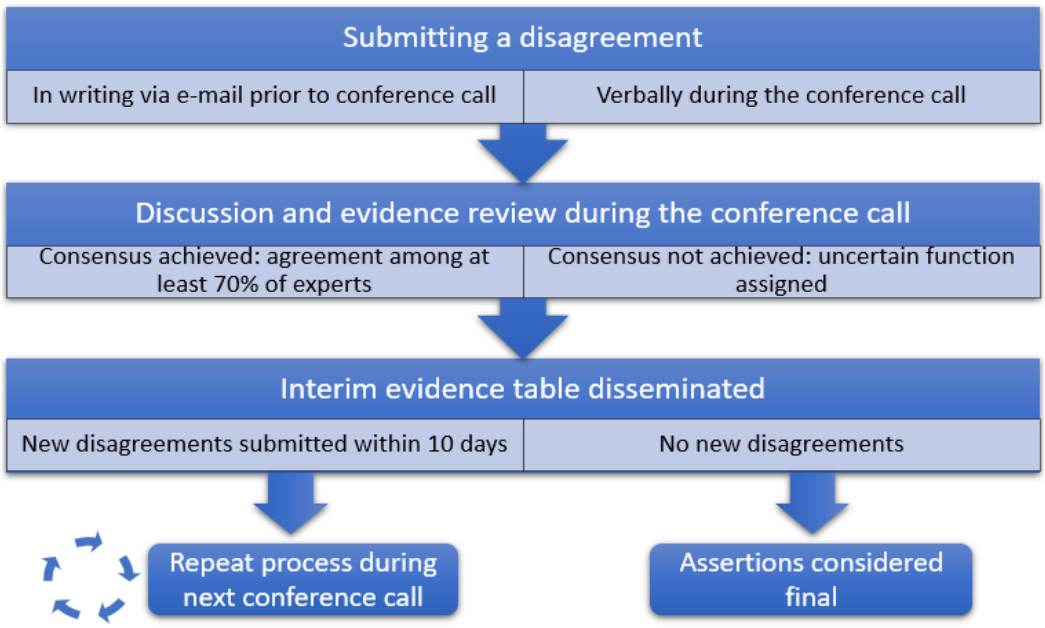

Figure 3: Resolving disagreements

Many clinical studies reporting the lack of an association between an allele and a phenotype are underpowered. Such studies must be down-weighted when considering them, such that some low-power studies may not be considered "conflicting" at all. When critically evaluating evidence from clinical studies, factors experts should consider include those described as essential characteristics of pharmacogenetic studies (31) including overall sample size, sample sizes for allelic groups, phenotypic effect size, comprehensiveness and quality of genotyping or phenotyping, significance level, drug substrates used, outcomes measured, population heterogeneity, and extent of ascertainment of confounding factors (diseases, drug use, organ function). For preclinical studies, replicates, positive and negative controls, appropriateness of model systems (animal, expression systems), appropriate statistical analysis, and substrates used will be evaluated.

The evidence for allele functional assignment is then assessed in light of all available evidence. If at least 70% of the experts do not find the evidence convincing after discussion and verbal voting on the conference call, expert consensus and rationale is documented in the evidence summary. If consensus among 70% experts cannot be achieved uncertain function should be assigned and rationale documented in the evidence summary. In other cases, both supporting and conflicting data may be equally convincing. The authors will weigh the clinical importance of a type I error (acting on a variant which may not have been actionable) vs a type II error (not acting on a variant which should have been actionable), and will assess the risk-benefit of any recommended alternative therapies. Immediate drug-induced death or permanent injury would be weighed against desired therapeutic effects; thus, therapy of an immediately life-threatening disease (e.g. myocardial infarction) would be considered more important than therapy of a less immediately life-threatening disease (e.g. depression). Experts should then assess which error type results in a more dangerous consequence for a patient and weigh the potential patient harm for using the alternative therapy vs not acting and continuing with the non-genetics based original therapy. Experts should 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. A summary of the authors' assessment of potentially conflicting evidence is included in Summary of Findings column of the allele functionality table and documented using an on-line survey tool.

Alleles for which the evidence is not sufficient to support clinical function assignment are categorized as "uncertain function" (Table 1). Alleles will be assigned "unknown function" if no evidence regarding their functional status can be found during the literature review process. Such alleles are re-assessed during guideline development or guideline updates and may be re-classified as more evidence becomes available as described in the "Re-evaluations and updates" section.

Allele clinical function and phenotype (based on the diplotype of those alleles) are assigned as described in Tables 2 and 3. Although recognizing that allele function ranges on a continuous scale from no function to increased function, clinically, allele function is categorized into groupings such that the phenotype from the patient diplotype can be binned into actionability categories. Alleles with uncertain or unknown function have inadequate, conflicting, or absent

evidence such that the experts deem that based on those alleles a phenotype of "indeterminate" should be assigned. A guideline will not provide a recommendation based on genetic testing results for individuals with diplotypes composed of two "uncertain function" or "unknown function" alleles or a combination thereof. For some genes, an individual carrying one "uncertain function" or "unknown function" allele in combination with a known function actionable allele will result in assignment of a "possible" phenotype to these individuals, so that prescribers are alerted that an abnormal phenotype is possible. Additional drug-specific actionability may apply to these "possibly" high-risk phenotype on a guideline-specific basis. For example, for *TPMT*, an individual carrying one no-function allele plus one uncertain function allele would be assigned a "possible" intermediate metabolizer phenotype, which is actionable, rather than designate the individual as "indeterminate" phenotype, because the evidence is so strong that the presence of at least one no-function allele confers a haplo-insufficient phenotype and requires a dosage decrease of the thiopurine.

Table 2: Functional assignment to alleles (see PMID: 27441996)

Allele function is assigned using CPIC standardized terms when possible.

Term/Gene Category[+]	**Allele functional Term***	**Functional Definition**	**Considerations for diplotype/phenotype that may inform the assignment of function to an allele**	**Example**
Allele Clinical Functional Status- pharmaco-genes based on enzymes, transporters, or gene products with known quantitative effects	Increased Function	Function greater than normal function	When both alleles have increased function, the phenotype of the patient is considerably higher than a normal metabolizer warranting different categorization (e.g. "ultrarapid metabolizer"). 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.	*CYP2C19*17* *CYP2D6*2x2*
	Normal Function	Fully functional	When both alleles have normal function, the phenotype of the patient is "normal metabolizer."	*CYP2C19*1* *CYP2D6*1*
	Decreased Function	Function less than normal function but greater than no function	When patients are homozygous for decreased function alleles, or when patients have one decreased function allele and one normal function or no function allele, the phenotype of such a person is different compared	*CYP2C19*9* *CYP2D6*10*

			to a patient who is homozygous for a normal function or homozygous for a no function allele.	
	No Function	Non-functional or expected non-functional based on allele definition (e.g. early stop codon, frameshift, complete gene deletion)	When both alleles have no function, the phenotype of the patient is "poor metabolizer". The patient with this phenotype may have no metabolic activity or very low activity. Many "no function" alleles have some residual activity. Patients with one "no function" allele and one "normal function" allele are intermediate metabolizers.	*CYP2C19*2* *CYP2D6*4*
	Unknown Function	No literature describing function, or the allele is novel	N/A	*CYP2D6*58* *CYP2C9*57*
	Uncertain Function	Literature supporting function is insufficient, conflicting, or weak	N/A	*CYP2C19*12* *CYP2D6*22*
+ For pharmacogenes that do not fall under this category (e.g. *CFTR*), allele clinical function terms are agreed upon by the authors. *All terms should begin with the gene name (e.g., CYP2D6 Poor metabolizer, TPMT Normal metabolizer, SLCO1B1 Decreased Function)				

Summarizing the evidence

The working draft of the Allele Functionality table to summarize the evidence for and against assigning an allele clinical function (or deeming it uncertain) will be made by the authors initially assigned a number of alleles for evidence evaluation (see "Scoring the evidence used to assign allele clinical function" section), requiring review by all authors. The working draft will include a putative functional assignment for each allele, and how the literature supports or refutes that assignment, with an eye toward impact on phenotype assignment and thus on downstream prescribing recommendations.

A written evidence summary will be included in the final Allele Functionality table for each allele. The Summary of Findings column will (a) summarize the evidence supporting the assigned allele function- must note if it is a well-established association that did not require a primary literature review; in this case this field may point to an authoritative resource for allele function (b) note conflicting evidence and summarize the experts' assessment of this evidence – including consensus and rationale for final assertion if there was disagreement among experts (c)

note if an assertion was modified based on clinical expertise- must include the original assertion and the experts' rationale for modification.

Attestation of expert review and agreement with the assertions in the evidence table is required before it is submitted for publication and made publicly available and will be documented using an on-line survey tool.

Translating Diplotypes to Phenotypes

CPIC guideline authors are responsible for documenting the conventions for translating diplotypes to phenotypes. These conventions are defined by authors, based on their review of evidence for assigning allele function, in the table that maps allele function to phenotype terms that is created as part of the guideline manuscript process. That mapping table, plus the allele functionality table discussed above, are used to automatically generate phenotypes from diplotypes. Authors are ultimately responsible for approving the translation of diplotypes to phenotypes, and their approval will be documented via an on-line survey tool prior to finalizing the guideline.

Activity scores may be used for drug metabolizing enzymes or genes with products with known quantitative effects to provide additional grading of allele activity using discrete values. For genes with alleles that have activity values as well as function assignments in the functionality table, the sum of the activity values for each allele in a diplotype is called an activity score. Activity scores will be used for genes where there is already published literature using such a scoring system that has been accepted in the community, and in addition, CPIC experts may implement an activity score system for genes if the absence of additional grading of function would result in a phenotype group having 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. Distinguishing these groups is therefore necessary to provide the granularity needed for therapeutic recommendations, and this could be achieved by introduction of an activity score. Layering on of activity scores, rather than creation of additional phenotypic categories, is preferable because the increased granularity in phenotypic groupings may only be required for some but not all drugs, and adherence to a limited number of standardized phenotypic terms (6) is desirable for purposes of phenotype interpretation, particularly when phenotype interpretation is required in the pre-emptive (i.e. drug-agnostic) setting. Consensus among at least 70% of all experts must be achieved for an author group to implement an activity score system, and documentation of expert approval is obtained via an on-line survey tool before the guideline is published. Activity values are discrete values to describe relative function and are assigned at the time clinical function is assigned based on the totality of evidence. A larger activity value reflects greater enzyme activity of the allele towards the majority of substrates. For example, the lowest activity value of 0 reflects little to no enzyme activity and an activity value of 1 reflects full enzyme activity. An activity value of 0.5 reflects enzyme activity between no and full function; it does not indicate a 50% reduction in activity. For alleles with multiple gene copies the activity value of the allele is multiplied by the number of gene copies (32). Alleles assigned uncertain or unknown function are not assigned an activity value. To maintain consistency with use of the standardized phenotype terms, each phenotype corresponds to an activity score or to a range of activity scores if the phenotype is made up of more than one combination of allele

functions. For example, an activity score of 0, 1, 2, corresponds to poor metabolizer, intermediate metabolizer, and normal metabolizer, respectively. Additional activity score conventions are gene-specific assignments based on the activity values assigned in the allele functionality table and totality of the evidence. Gene-specific phenotype conventions, including activity scores when applicable to the gene, are documented in the phenotype mapping table. Expert approval is obtained via an on-line survey tool.

The assignment of a phenotype is useful for minimizing the complexity involved in interpretation of genetic test results and eventual clinical actionability. Whereas there may be many thousands of diplotypes for each pharmacogene, there are a relatively small number of possible phenotypes. Thus, standardized terms for many pharmacogenetic phenotypes have been established (6). These phenotype terms have been embraced by standardized vocabularies such as Logical Observation Identifiers Names and Codes (LOINC); distilling diplotypes into phenotypes facilitates interoperability of pharmacogenetic results, and facilitates use of both genotypic and direct phenotypic results (e.g. G6PD or TPMT blood enzymatic activity) to guide pharmacogenomic decisions in the health care system (6). In addition, the use of standardized phenotype terms in CPIC guidelines will facilitate the use of clinical decision support tools, which can be triggered based on specific pharmacogenetic high-risk phenotypes (33, 34).

CPIC's phenotype terms have been supported by the Association for Molecular Pathology. Additionally, these terms may be useful for proficiency testing programs that are designed to improve quality assurance and uniform testing (35, 36) and pharmacogenetic interpretation among clinical genetic testing laboratories (e.g., College of American Pathologists (CAP-PGX)). CPIC phenotype terms are found in Table 3, along with functional definitions. The specifics of how alleles of different functions combine into phenotype groupings depends on the gene, and these specifics are addressed in the mapping table created by authors. Based on the allele clinical function assignments included in the allele functionality table all possible allele function combinations are identified in the Allele 1 function and Allele 2 function columns and described in the Description column. The phenotype of each allele function combination is assigned based on the totality of the evidence and reflect the phenotype towards the majority of substrates. The evidence and procedures used to assign allele clinical function are used to determine if the phenotype resulting from the diplotype of each combination of alleles is "normal," or would be clinically distinct from the normal phenotype. The downstream diplotype/phenotype assignment is partly driving the assignment of clinical allele functional assignment, and this is utmost in mind of the experts as part of their allele clinical functional assignment, as described above.

Table 3. Phenotype terms and functional definitions (see PMID: 27441996)

Phenotype is assigned using CPIC standardized terms when possible.

Term/Gene Category[+]	Final Term*[#]	Functional Definition	Example diplotypes
Phenotype-Drug Metabolizing Enzymes (*CYP2C19, CYP2D6, CYP3A5, CYP2C9, TPMT, DPYD, UGT1A1*)	Ultrarapid Metabolizer	Increased enzyme activity compared to rapid metabolizers	*CYP2C19*17/*17* *CYP2D6*1/*1XN* (where N is ≥ 2)
	Rapid Metabolizer	Increased enzyme activity compared to normal metabolizers but less than ultra-rapid metabolizers	*CYP2C19*1/*17*
	Normal Metabolizer	Fully functional enzyme activity	*CYP2C19*1/*1* *CYP2D6*1/*2*
	Possible Intermediate Metabolizer	At least decreased enzyme activity (activity between normal and poor metabolizer) as this individual should be treated with "at least" the same precautions as would apply to an intermediate metabolizer	*TPMT*2/*8* *CYP3A5*1/*2*
	Intermediate Metabolizer	Decreased enzyme activity (activity between normal and poor metabolizer)	*CYP2C19*1/*2* *CYP2D6*10/*41* *TPMT*1/*2*
	Poor Metabolizer	Little to no enzyme activity	*CYP2C19*2/*2* *CYP2D6*4/*5* *TPMT*2/*3A*
	Indeterminate	Uncertain enzyme activity	*CYP2C19 *1/*12* *CYP2C9 *7/*17*
Phenotype-Transporters (*SLCO1B1*)	Increased Function	Increased transporter function compared to normal function	*SLCO1B1*1/*14*
	Normal Function	Fully functional transporter function	*SLCO1B1*1/*1*
	Decreased Function	Decreased transporter function (function between normal and poor function)	*SLCO1B1*1/*5*
	Possible Decreased Function	At least decreased transporter activity (activity between normal and poor metabolizer) as this individual should be treated with	No examples to date

		"at least" the same precautions as would apply to and individual with decreased function	
	Poor Function	Little to no transporter function	*SLCO1B1*5/*5*
	Indeterminate	Uncertain transporter function	*SLCO1B1 *1/*4*
Phenotype-High risk genotype status (*HLA-B*)	Positive	Detection of high-risk allele	*HLA-B*15:02*
	Negative	High risk-allele not detected	

A "possible" phenotype may be assigned when the presence of one allele with known function, based on at least strong evidence according to Table 1, confers a haplo-insufficient phenotype which places a patient at risk of severe morbidity or mortality. For example, if an individual has a diplotype consisting of a known function allele combined with an unknown function allele, even though the phenotype of that individual cannot be determined unambiguously, the designation of that gene as representing a "possible" high risk diplotype may be assigned by the experts, if they deem the possible phenotype actionable (e.g. *TPMT*). The term "possible" is widely used as a modifier in ubiquitous systematized nomenclature systems such as SNOMED, which defines terms used in clinical documentation and reporting. In other cases, the combination of a known function allele with an unknown function allele is not considered clinically actionable and would be assigned "indeterminate" (e.g. *CYP2C9*). Thus, it is important that CPIC authors participate in both the allele clinical function evidence review and designation as well as the allele function to phenotype mappings used to generate the diplotype-phenotype table so that they fully understand the consequences of their designations.

Additionally, if all alleles of a specific function have limited evidence, the phenotypes containing that specific allele function may be designated as "likely" phenotypes. The "likely" 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" due to significantly more evidence characterizing *CYP2C19* no function alleles. "Likely" is a term accepted in several electronic health care record systems, although other modifier terms are also used.

The gene-specific allele functionality table and phenotype mapping table are manually created by experts and deposited in the CPIC database. A file with the mappings for every possible diplotype to phenotype term is automatically generated from the database resulting in the diplotype-phenotype table. This file is automatically generated using the information contained in the allele functionality table and phenotype mapping table. All possible allele combinations

for every allele in the allele functionality file is generated in the diplotype column, and the allele functions for each combination are automatically looked up in the allele function to phenotype mapping table to determine the phenotype term listed in phenotype column for each diplotype. Authors are responsible for reviewing the diplotype-to-phenotype translations for each gene and approval is documented via an on-line survey tool.

Re-evaluations and updates

Validation of CPIC's assertions is via 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. Its members include leaders within PharmGKB, ClinGen, FDA, and PharmVar, diagnostic laboratories such as ARUP and LabCorp, and clinicians and researchers from institutions across the globe. CPIC's processes are supported by the Association for Molecular Pathology (AMP) and College of American Pathologists (CAP). CPIC guidelines are peer reviewed and endorsed by American Society of Health System Pharmacists (ASHP) and American Society for Clinical Pharmacology & Therapeutics (ASCPT). CPIC is highly receptive and responsive to inquiries from its members and general public. The CPIC website contains a Contact CPIC page to facilitate inquiries and comments from the general public. 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 on-line survey tool to capture necessary and relevant information to address the inquiry. CPIC staff receive an automatic e-mail when an inquiry is submitted via the on-line survey tool. The information contained in the inquiry is exported into a document where CPIC staff track and document the review and response to inquiries. All inquiries are reviewed by the CPIC Director within 30 days of submission and based on the nature of the inquiry are assigned a timeline and pathway to address the inquiry according to Table 4. Updates are prominently posted on each guideline page.

Table 4. Addressing Inquiries

Nature of Inquiry	How to Address Inquiry	Timeline
Clinical study with potentially conflicting evidence which may change phenotype resulting in altered prescribing recommendations	Expert review, following disagreement and consensus process as previously described	Evidence and interim table disseminated to experts within 7 days of review by CPIC Director. Updated materials posted on CPIC website, uploaded to database, and written acknowledgement sent to submitter of inquiry within 60 days of review by CPIC Director.

New potentially conflicting evidence which contradicts the role of the allele in the specified drug phenotype	Expert review, following disagreement and consensus process as previously described	Evidence and interim table disseminated to experts within 30 days of review by CPIC Director. Updated materials posted on CPIC website, uploaded to database, and written acknowledgement sent to submitter of inquiry within 90 days of review by CPIC Director.
New evidence to support current assertions	Expert review, following disagreement and consensus process as previously described	Evidence reviewed by experts when pharmacogene is topic of new guideline or guideline update Written acknowledgement sent to submitter of inquiry within 30 days of review by CPIC Director.
New evidence to support assigning a clinical function to an unknown or uncertain function allele	Expert review, following disagreement and consensus process as previously described	Evidence reviewed by experts when pharmacogene is topic of new guideline or guideline update Written acknowledgement sent to submitter of inquiry within 30 days of review by CPIC Director.
Data entry error	CPIC staff to correct error	Updated materials posted on CPIC website, uploaded to database, and written acknowledgement sent to submitter of inquiry within 60 days of review by CPIC Director.
Other inquires (clarification, question, etc.)	CPIC staff to address	Written acknowledgement sent to submitter of inquiry within 90 days of review by CPIC Director.

Reassessment of allele clinical function status is triggered when CPIC becomes aware of or receives an inquiry which cites published literature supportive of evidence against an allele function assignment and was not already assessed by experts. A trained CPIC staff member disseminates the evidence and an interim evidence table to all experts of the pharmacogene according to the timeline in Table 4. Reassessments follow the same evidence evaluation procedures as described previously in the "Scoring the evidence" section taking into account all

evidence and follow the same procedures to resolve disagreements as described previously, requiring consensus among at least 70% of all experts of the final function assignment and updated evidence summary. Documentation of expert approval of updates to a table is obtained via an on-line survey tool before the updated materials are made publicly available.

Reassessment of allele clinical function status also occurs during subsequent guideline development (guideline update or new guideline with same pharmacogene as a previous guideline). The authors of the subsequent guideline will focus on reassessment of alleles assigned uncertain or unknown function. Updates to allele function assignment, strength of evidence, and evidence summary will require the approval of all experts (i.e. authors from all guidelines which include the pharmacogene). Disagreement among experts will be handled as described previously in the "Scoring the evidence" section of this document. Expert approval of updates to a table is documented via an on-line survey tool before the updated materials are made publicly available. Reassessment of alleles with unknown or uncertain function does not occur on a scheduled basis as such reassessments are driven by new evidence, new guidelines and guideline updates.

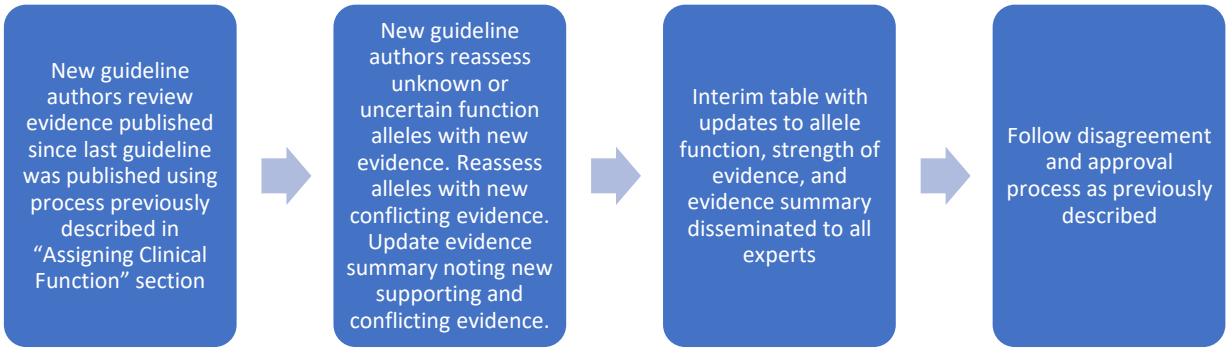

Figure 4: Reassessment due to new guideline

Updates to diplotype-phenotype conventions of a pharmacogene may occur as a result of significant updates to allele function assignments based on new evidence. Updates to diplotype-phenotype conventions will then go through the same reassessment process as described above for allele function requiring review and consensus among 70% of all experts to resolve disagreements. Expert approval of updates to a table is documented via an on-line survey tool before the updated materials are made publicly available. Updates to phenotype conventions may also occur to facilitate standardization across clinical laboratories and clinical practice guidelines (37).

The Allele Functionality table and Diplotype-Phenotype table will display the most up to date information on the CPIC website and in the CPIC database. Version control mechanisms are in place through the CPIC API and repository where all CPIC documents are stored as described in the CPIC Database SOP.

Pharmacogene Table Examples

GENE: CYP2C9							
Allele/cDNA/rsID	**Activity Value (Optional)**	**Allele Biochemical Functional Status (Optional)**	**Allele Clinical Functional Status (Required)**	**Allele Clinical Function Substrate Specificity (Optional)**	**PMID (Required)**	**Strength of Evidence (Required)**	**Summary of Findings (Required)**
*5	0.5	Decreased function	Decreased function		15289788; 16220110; 17504998; 11455026; 11901091; 23752738	Strong	CYP2C9*5 is assigned decreased function based on strong evidence in heterozygous patients. Three subjects carrying the CYP2C9*5 allele (CYP2C9*1/*5, CYP2C9*5/*6, CYP2C9*5/*8 genotype) had decreased losartan metabolism compared to wildtype based on urinary metabolites after a single 25mg dose (15289788). Two CYP2C9 *1/*5 subjects, one CYP2C9 *5/*6 subject, and one CYP2C9*5/*8 subject had lower phenytoin metabolism compared to wildtype based on urinary excretion of a phenytoin metabolite after a single 300mg dose. Additionally, the CYP2C9 *5/*6 subject demonstrated the lowest phenytoin metabolism as the only subject of the 109 subject cohort to have a urinary metabolite concentration below the limit of quantification (16220110). A CYP2C9*1/*5 subject demonstrated decreased indisulam metabolism with an AUC nearly two times greater than wildtype subjects (17504998). These results correlated with in vitro pharmacokinetic studies which demonstrated decreased clearance of warfarin, diclofenac, flurbiprofen, naproxen, and piroxicam compared to wildtype (11455026, 11901091). A separate group also found in an in vitro study with warfarin and tolbutamide substrates that CYP2C9*5 has decreased enzyme activity compared to wildtype (23752738). Therefore, consensus among experts was decreased function with an activity value of 0.5 based on strong evidence.
*6	0	No Function	No Function		11740344; 15094935; 17895500; 15289788; 16220110; 23752738; 21811894	Strong	CYP2C9*6 is assigned no function based on strong supporting evidence in heterozygous and homozygous patients. This allele was first identified in a CYP2C9*6/*6 patient experiencing phenytoin toxicity after recently starting the medication. Based on serial phenytoin plasma concentrations after discontinuing phenytoin, the patient had an estimated 17% phenytoin clearance compared to wildtype (11740344). In a separate case report, a patient with CYP2C9*6/*6 genotype stable on a low weekly warfarin dose demonstrated impaired warfarin metabolism with an S:R warfarin ratio 6x greater than expected (15094935). The S:R warfarin ratio of this patient was the highest among 52 patients on a stable dose of warfarin with a CYP2C9 genotype of *1/*1, *1/*2, *1/*3, *2/*2, or *2/*3 (17895500). Two subjects (CYP2C9*1/*6 and CYP2C9*5/*6 genotype, respectively) had decreased losartan metabolism compared to wildtype subjects based on urinary metabolites after a single 25mg dose (15289788). Three CYP2C9*1/*6 subjects and one CYP2C9*5/*6 subjects had lower phenytoin metabolism compared to wildtype based on urinary excretion of a phenytoin metabolite after a single 300mg dose. Additionally, the CYP2C9*5/*6 subject demonstrated the lowest phenytoin metabolism as

Figure 5: Allele functionality table – CYP2C9 example

CYP2C9 Diplotype	Activity score	CYP2C9 Phenotype
*1/*1	2	CYP2C9 Normal Metabolizer
*1/*10	N/A	Indeterminate
*1/*13	1	CYP2C9 Intermediate Metabolizer
*1/*15	1	CYP2C9 Intermediate Metabolizer
*1/*17	N/A	Indeterminate
*1/*18	N/A	Indeterminate
*1/*2	1.5	CYP2C9 Intermediate Metabolizer
*1/*25	1	CYP2C9 Intermediate Metabolizer
*1/*3	1	CYP2C9 Intermediate Metabolizer
*1/*5	1.5	CYP2C9 Intermediate Metabolizer
*1/*6	1	CYP2C9 Intermediate Metabolizer
*1/*7	N/A	Indeterminate
*1/*8	1.5	CYP2C9 Intermediate Metabolizer
*10/*10	N/A	Indeterminate
*10/*13	N/A	Indeterminate
*10/*15	N/A	Indeterminate
*10/*17	N/A	Indeterminate
*10/*18	N/A	Indeterminate
*10/*25	N/A	Indeterminate
*13/*13	0	CYP2C9 Poor Metabolizer
*13/*15	0	CYP2C9 Poor Metabolizer
*13/*17	N/A	Indeterminate
*13/*18	N/A	Indeterminate
*13/*25	0	CYP2C9 Poor Metabolizer
*15/*15	0	CYP2C9 Poor Metabolizer
*15/*17	N/A	Indeterminate
*15/*18	N/A	Indeterminate
*15/*25	0	CYP2C9 Poor Metabolizer
*17/*17	N/A	Indeterminate
*17/*18	N/A	Indeterminate
*17/*25	N/A	Indeterminate

Figure 6: Diplotype to Phenotype table – CYP2C9 example

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