**Pharmacogenomics Consensus Group: OVERVIEW OF PHARMACOGENETIC VARIANT CURATION**

CPIC has been assigning “function” to pharmacogenetic alleles since its inception, and this led to a Delphi process and consensus publication on standardizing terms used for allele function and pharmacogenetic phenotypes for some genes. The unique contribution of CPIC is to assign an allele function that leads to a phenotype assignment that can drive clinical prescribing actionability. Now that CPIC has tackled several types of genes (pharmacokinetic and pharmacodynamic), it is timely to establish written SOPs that describe in more detail the criteria used for assigning function. In addition, it is clear that there are cases for which a “biochemical” functional assignment does not completely correspond to clinical function, and therefore we are distinguishing that the primary goal of the CPIC guideline authorship committees is to assign Allele Clinical Functional status, as described in detail below.

The goal is to assign “Allele Clinical Function Status” to allelic variants (i.e. haplotypes) as defined by PharmVar or other gene authorities (e.g. TPMT Allele Nomenclature Committee, UGT Official Nomenclature) or to sequence variations such as single nucleotide polymorphisms (SNPs) or insertions/deletions (indels), using a standardized protocol and terms. 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 spectrum of frequency and variety of alleles, 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) when creating phenotypic groupings that will drive prescribing actions.

\* Inclusion of variants/alleles: evidence should be summarized on a variant, or on an allele, if there are peer-reviewed publications on the function or on the clinical actionability of the variant or allele, and in some cases, if clinical labs are already routinely testing for the variant or allele (even if function is uncertain or unknown).

\* CPIC Allele Clinical Function Status will be used to evaluate which alleles or variants of a gene may be clinically actionable. By clinically actionable, we mean that if that variant is 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 staring dose) prescribing actions.

\* The decision as to how each pharmacogene must be minimally interrogated to adequately rule out actionable variants is a complex one, and beyond the scope of this document on Pharmacogenetic Variant Curation. Other groups, such as Association for Molecular Pathology (AMP), have taken on the task of developing minimum performance standards for pharmacogenetic tests (1, 2).

\* Moreover, expert panels have the option of assigning “Allele Function Status (not clinical)” to alleles. This is to accommodate data on allele function that are of scientific interest, but which do not rise to such the level that clinical actionability can be ascribed. This is a separate designation from the mandatory CPIC “Allele Clinical Function Status” (which is to be used to translate diplotypes into phenotypes and prescribing actionability), and this status will not be used for purposes of actionability in CPIC guidelines.

\* Allele Clinical Function Substrate Specificity: 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 Allele Definition, Allele Functionality, and Diplotype-to-Phenotype tables are constructed to reflect function towards the majority of substrates (i.e. based on the field “Allele Clinical Function Status”). The allele function table, however, will allow for indicating which alleles have “strong” substrate-specific effects toward specific drugs. “Strong” substrate specific effects are those that would change the allele functional assignment (e.g. normal vs decreased function or decreased vs no function) such that phenotype would change from the default, and therefore prescribing recommendations for drug A vs drug B would differ. This should be rare but may occur. This drug substrate specificity will be referenced in the CPIC guidelines for the affected drugs, likely resulting in drug-specific tables for that gene.

**Strength of Evidence:** The evidence is collected primarily from published peer-reviewed literature, but may also be retrieved from publicly accessible curated resources, such as variant databases including ClinVar, PharmVar, and PharmGKB, which can be used with discretion and if their variant curation process is approved by the guideline gene experts. Literature searches will be conducted using PubMed ([http://www.ncbi.nlm.nih.gov/pubmed)](http://www.ncbi.nlm.nih.gov/pubmed%29) and/or Google Scholar ([http://scholar.google.com/)](http://scholar.google.com/%29) (which has a full-text search feature). Advanced searches are generally more informative but are not guaranteed to be a comprehensive survey of all of PubMed. As only abstracts are indexed, allele information only referenced in the full text may not be found. Additionally, alleles and variants may be referred to by a variety of names; multiple names will be searched but it is possible for publications using alternate terms to be missed. Finally, many alleles may only have published functional data in combination with other alleles (i.e. diplotype metabolizer status) which may or may not be useful for allele function assignment. As there is no way to guarantee all literature that may contain functional information about an allele can be found in a standard search, the expertise of the guideline authorship committee will be leveraged to ensure no critical publications are overlooked. The Strength of Evidence assignment must support the Allele Clinical Function Status, and it is recommended but optional to summarize the strength of this evidence for each allele. The Strength of Evidence assignment refers only to the Allele Clinical Function Status and is not linked to the Allele Function Status.

For well-established associations, one need not comprehensively curate all evidence for a gene variant-drug phenotype pair. The authors may focus on curating and evaluating the relevant pieces of evidence for newly-discovered variants or for variants with conflicting data.

**Classifying different evidence types used to assign Allele Clinical Function to alleles:** The evidence will be classified as to the type of evidence supporting the functional assignment (e.g. whether 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)(3, 4). In silico predictions may be considered, particularly if the sequence change predicts consequences such as early termination (3). The authors can choose to organize the assignment of strength of evidence based on individual findings (which might have supportive and conflicting citations), or to assign the strength of the evidence in single citations, or both. In the end, the authors must decide on an Allele Clinical Function to assign to each allele, based on the totality of the evidence and the likely impact on assignment of phenotype and the impact downstream prescribing implications. Authors should decide if they would ever change prescribing based on the presence of this allele. In the case of insufficient or conflicting evidence, the variant will be assigned an Allele Clinical Function of “uncertain function” (see table below). Alleles will be assigned “unknown function” if no evidence regarding their functional status can be found during the literature review process and the discovery article will be used as reference to have the allele supported in the functionality material available through CPIC. An individual carrying two “uncertain function” or “unknown function” alleles will mean that the resulting guideline will not provide a recommendation for that individual. In a number of guidelines, an individual carrying one “uncertain function” or “unknown function” allele will result in no recommendation for that individual regardless of the function of the other allele.

**Table: Assignment of Allele Clinical Function.** This Table summarizes the evidence required to assign an allele as increased, normal, decreased or no function opposed to “uncertain function” and uses a process modified slightly from ClinGen’s gene-disease validity evaluation process (4). Allele function is assigned using CPIC standardized terms when possible.

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| --- | --- | --- |
| **Supportive Evidence needed to assign function vs uncertain** | **DEFINITIVE** | The causal role of this allelic variant in this particular drug phenotype has been repeatedly demonstrated, and has been upheld over time (in general, at least 3 years). No convincing evidence has emerged that contradicts the role of the allele in the specified drug phenotype. |
|  |  |
| **STRONG** | The causal role of this allelic variant in the drug phenotype has been independently demonstrated in at least two separate clinical studies providing **strong** supporting evidence for this allele’s role in drug phenotype; there is compelling variant-level evidence from different types of supporting experimental data. In addition, no convincing 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 variant in this drug phenotype, including both of the following types of evidence:* At least 2 patient cases demonstrated drug phenotype causality
* Some *in vitro* experimental data (e.g. engineered variant and effect measures support the variant-drug phenotype association

AND no convincing evidence has emerged that contradicts the role of the variant in the noted drug phenotype. |
|  |  |
| **LIMITED** | There is **limited** evidence to support a causal role for this allelic variant in this drug phenotype, including at least two of the following types of evidence:Fewer than 2 patient cases * Limited *in vitro* data (e.g. correlative data) support the variant-drug phenotype association
* Computational activity predictions overall support *in vivo* and/or *in vitro* data (3)

AND no convincing evidence has emerged that contradicts the role of thevariant in the noted drug phenotype. |
|  |  |
| **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 strong enough to support a clinical functional status that can inform prescribing actionability. The threshold for what evidence is enough to inform actionability may differ for different genes. |
| **No EVIDENCE = unknown function** | No literature describing function  |

Notes on conflicting evidence.

1. Many clinical studies reporting the lack of an association between a variant 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.
2. In cases in which supporting and conflicting data are both convincing, the authors need to 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). For these reasons, CPIC may choose to assess Allele Clinical Function differently than non-clinical functional assignments. Alleles for which the evidence supporting a particular function is so uncertain that no clinical actionability can be recommended for diplotypes including that allele should be categorized as “uncertain function.”

Summary Matrix for functional assignment to alleles

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| --- | --- | --- | --- | --- |
| **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. *TPMT, 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 to a patient who is homozygous for a normal function or homozygous for a no function allele  | *CYP2C19\*9**CYP2D6\*10* |
| No Function | Non-functional | 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 | *CYP2C19\*29**CYP2D6\*58* |
| Uncertain Function | Literature supporting function is insufficient, conflicting or weak | N/A | *CYP2C19\*12**CYP2D6\*22* |
| Allele Functional status for high-risk genes with unclear mechanism (*HLA-B*) | Positive | Detection of high-risk allele | Homozygous or heterozygous for high-risk allele | *HLA-B\*15:02* |
|  | Negative | High risk-allele not detected | No copies of high-risk allele |  |
| \*All terms should begin with the gene name (e.g., CYP2D6 Poor metabolizer, TPMT Normal metabolizer, SLCO1B1 Decreased Function) |

Phenotype terms and relation to functional alleles

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| --- | --- | --- | --- | --- |
| **Term/Gene Category** | **Final Term\*** | **Functional Definition** | **Genetic Definition** | **Example diplotypes** |
| Phenotype-Drug Metabolizing Enzymes (*CYP2C19, CYP2D6, CYP3A5, CYP2C9, TPMT, DPYD, UGT1A1*)  | Ultrarapid Metabolizer | Increased enzyme activity compared to rapid metabolizers. | Two increased function alleles, or more than 2 normal function alleles | *CYP2C19\*17/\*17**CYP2D6\*1/\*1XN* |
| Rapid Metabolizer | Increased enzyme activity compared to normal metabolizers but less than ultra-rapid metabolizers. | Combinations of normal function and increased function alleles | *CYP2C19\*1/\*17* |
| Normal Metabolizer | Fully functional enzyme activity | Combinations of normal function and decreased function alleles | *CYP2C19\*1/\*1**CYP2D6\*1/\*2* |
| Intermediate Metabolizer | Decreased enzyme activity (activity between normal and poor metabolizer) | Combinations of normal function, decreased function, and/or no function alleles | *CYP2C19\*1/\*2**CYP2D6\*10/\*41**TPMT\*1/\*2* |
| Poor Metabolizer | Little to no enzyme activity | Combination of no function alleles and/or decreased function alleles | *CYP2C19\*2/\*2**CYP2D6\*4/\*5**TPMT\*2/\*3A* |
| Phenotype-Transporters (*SLCO1B1*) | Increased Function | Increased transporter function compared to normal function. | One or more increased function alleles | *SLCO1B1\*1/\*14* |
| Normal Function | Fully functional transporter function | Combinations of normal function and/or decreased function alleles | *SLCO1B1\*1/\*1* |
| Decreased Function | Decreased transporter function (function between normal and poor function) | Combinations of normal function, decreased function, and/or no function alleles | *SLCO1B1\*1/\*5* |
| Poor Function | Little to no transporter function | Combination of no function alleles and/or decreased function alleles | *SLCO1B1\*5/\*5* |
| Phenotype-High risk genotype status (*HLA-B*) | Positive | Detection of high-risk allele | Homozygous or heterozygous for high-risk allele | *HLA-B\*15:02* |
|  | Negative | High risk-allele not detected | No copies of high-risk allele |  |
| \*All terms should begin with the gene name (e.g., CYP2D6 Poor metabolizer, TPMT Normal metabolizer, SLCO1B1 Decreased Function) |

Example of variant curation columns for a pharmacogenetic variant:



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