Supplement to: Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for *CYP2C9* and *HLA-B* Genotypes and Phenytoin Dosing: 2020 Update

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Literature Review

The PubMed database (April 2014 to August 2019) was searched for the keywords ([phenytoin OR fosphenytoin] AND [HLA-A OR HLA-B]), ([phenytoin OR fosphenytoin] AND [HLA]), ([phenytoin OR fosphenytoin] AND [cytochrome P450]) and ([phenytoin OR fosphenytoin] AND [CYP2C9]). Using these search terms, 112 publications were identified. Study inclusion criteria included publications that incorporated analyses for the association between *CYP2C9* or *HLA* genotypes and phenytoin pharmacokinetic and pharmacodynamic parameters or clinical outcomes. Non-English manuscripts and review articles were excluded. Following the application of these inclusion and exclusion criteria, 27 publications were reviewed and included in the updated evidence table (**Tables S1 and S2**).

Gene: *HLA-B*15:02*

Background

The exact biological interactions between *HLA-B*15:02* and phenytoin have not been established. Current hypotheses aim to explain the specific mechanisms by which small molecule drugs such as phenytoin activate T-cells. These include interactions between specific HLA molecules, T-cell receptors (TCRs) and the parent drug or a specific metabolite that can lead to an immune response, and in the case of *HLA-B*15:02,* lead to Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN). The hapten hypothesis proposes that T-lymphocytes recognize chemically reactive drug/metabolite bound covalently to a protein or major histocompatibility complex (MHC)-bound peptides, form a hapten-carrier complex, and this modified protein can induce an immune response. The "p-i concept" (direct pharmacological interaction of drugs with immune receptors) suggests that some drugs that lack hapten characteristics can activate T-cells by binding directly and reversibly (non-covalently) to TCRs in a concentration-dependent fashion (1). Studies using endogenous peptides of *HLA-B*15:02* in carbamazepine-induced SJS/TEN have shown a non-covalent interaction between drug/peptide/HLA and TCR, thus supporting the p-i concept model (2). However, whether phenytoin undergoes the same pathway needs to be further studied. Lastly, a recent hypothesis postulates that a drug can bind non-covalently to specific HLA molecules and alter its specificity for peptide binding. This has been shown for *HLA-B*57:01* and abacavir-induced hypersensitivity (3).

The literature evaluating the association of the *HLA-B* alleles and phenytoin-induced SJS/TEN is inconsistent with respect to the specific *HLA-B* allele responsible for this ADR. Interpretation of literature is complicated by the studies' small sample sizes, inconsistent definition of the SJS/TEN

phenotype, inconsistent genotyping methodologies and the variety of race/ethnic groups in which studies were performed. The strength of the association between *HLA-B*15:02* and phenytoin–induced SJS/TEN is weaker than the association between *HLA-B*15:02* and carbamazepine–induced SJS/TEN and the association between *HLA-B*57:01* and abacavir hypersensitivity syndrome. Even in the East Asian and Central/South Asian populations where *HLA-B*15:02* carriage is prevalent, the negative predictive value (NPV) of *HLA-B*15:02* falls significantly short of 100%. This lack of 100% NPV is illustrated by studies that have suggested that *HLA-B* alleles other than *HLA-B*15:02*, such as *HLA-B*13:01* and *HLA-B*13:15,* are associated with phenytoin-induced SJS/TEN (4, 5). Specific amino acid binding residues in *HLA-B* shared amongst risk alleles may be important and distinct drug-peptide-*HLA*-TCR interactions may also occur in patients with phenytoin SJS/TEN that do not carry *HLA-B*15:02.* However, this is only one of several non-mutually exclusive mechanisms to explain why different *HLA* alleles may be implicated in phenytoin-induced SJS/TEN (6). The observation of multiple *HLA* associations underscores the notion that the absence of these variants does not rule out the possibility of a patient developing phenytoin-induced SJS/TEN.

Additional Antiepileptics With An Aromatic Ring

Several additional drugs structurally and therapeutically similar to phenytoin have also been associated with drug-induced adverse cutaneous reactions and *HLA-B*15:02*.

Carbamazepine, an aromatic anticonvulsant related to the tricyclic antidepressants, is US Food and Drug Administration (FDA)-approved for the treatment of epilepsy, trigeminal neuralgia, and bipolar disorder. ***Oxcarbazepine*** is an analog of carbamazepine. As with carbamazepine, oxcarbazepine has been used in the treatment of partial seizures with and without generalization and in the treatment of neuropathic pain. CPIC guidelines are available for *HLA-B*15:02* and *HLA-A*31:01* and carbamazepine- and oxcarbazepine-induced SJS and TEN (7-9). Although the structural similarity between phenytoin and carbamazepine and the shared association of the *HLA-B*15:02* allele with SJS/TEN might suggest cross-reactivity with *HLA-A*31:01*, no association between *HLA-A*31:01* and phenytoin-induced SJS and TEN has been presently found.

Eslicarbazepine acetate is a prodrug that is activated to eslicarbazepine, an active metabolite of oxcarbazepine. To date, no cases of eslicarbazepine acetate-induced SJS/TEN have been reported; however, based on its structural similarity to oxcarbazepine, caution should be used in susceptible individuals positive for *HLA-B*15:02*.

Lamotrigine has been associated with SJS/TEN, particularly with rapid dose escalation or when used in combination with valproic acid. A possible trend of association between *HLA-B*15:02* and lamotrigine-induced SJS/TEN in Han Chinese has been reported in two studies (10, 11) but not in another (12). A recent meta-analysis (including these studies and original data) showed an association between *HLA-B*15:02* and lamotrigine-induced SJS/TEN (p=0.03) (13). One study identified a significant increase in frequency of *HLA-A*30:01* and *HLA-B*13:02* (p=0.013 and p=0.013, respectively) in Han Chinese patients with lamotrigine-induced maculopapular exanthema compared to a lamotrigine-tolerant group. This finding also suggests the presence of shared amino acid binding residues that contribute to the risk of cutaneous adverse reactions (14).

Available Genetic Test Options

Commercially available genetic testing options change over time. Below is some information that may assist in evaluating options.

Desirable characteristics of pharmacogenetic tests, including naming of alleles and test report contents, have been extensively reviewed by an international group, including CPIC members (15). CPIC recommends that clinical laboratories adhere to these test reporting standards. CPIC gene-specific tables (see **Allele Definition**, **Allele Functionality** and **Frequency Tables** (16, 17)) adhere to these allele nomenclature standards (15). Moreover, the **Allele Definition**, **Functionality**, and **Frequency Tables** may be used to assemble lists of known functional and actionable pharmacogenetic variants and their population frequencies, which may inform decisions as to whether tests are adequately comprehensive in interrogations of alleles. Furthermore, the Association for Molecular Pathology and College of American Pathologists have published a joint recommendation for the key attributes of alleles recommended for clinical testing and a minimum set of variants that should be included in clinical genotyping assays for *CYP2C9* (18).

The Genetic Testing Registry (GTR) provides a central location for voluntary submission of genetic test information by laboratories and is available at http://www.ncbi.nlm.nih.gov/gtr/.

Genetic Test Interpretation

CYP2C9 genetic variants are typically reported as haplotypes, which are defined by a specific combination of single nucleotide polymorphisms (SNPs) and/or other sequence variants including deletions of a small number of nucleotides that are interrogated during genotyping analysis. *CYP2C9* haplotypes are reported as star-alleles to allow for the standardization of genetic variation annotation (19-21). A complete list of *CYP2C9* star-allele nomenclature along with the genetic variants that define each star-allele is available at the PharmVar (https://www.pharmvar.org/), PharmGKB (https://www.pharmgkb.org) and CPIC (www.cpicpgx.org) websites (***CYP2C9* Allele Definition Table (16, 17)**). In any pharmacogenetic test, it is important to understand which SNPs or other genetic variants are interrogated by a particular test as the inclusion or exclusion of certain genetic variants in a pharmacogenetic test could affect the reported star-allele result. Reference laboratories usually report a diplotype, which is the summary of inherited maternal and paternal star-alleles (e.g., *CYP2C9*1/*3*, where an individual has inherited a **1* allele and a **3* allele). Commonly reported *CYP2C9* star-alleles are categorized into functional groups (i.e., normal function, decreased function or no function) based on the predicted clinical function of the encoded enzyme (***CYP2C9* Allele Functionality Table**). A patient's predicted CYP2C9 phenotype is determined by the expected clinical function of each reported allele in the diplotype (**Table 2**, main manuscript).

A *CYP2C9* genotype to phenotype translation table has been developed by CPIC and is updated on an ongoing basis on the CPIC website (www.cpicpgx.org). Of note, **Table 2 (**main manuscript**)** denotes a change to the previous (2014) genotype to phenotype translation tables for diplotypes containing *CYP2C9*2* and other decreased function alleles. The *CYP2C9*2/*2* diplotype (AS=1) is now translated into the IM phenotype group (originally translated to PM). This change is based on data for multiple substrates (flurbiprofen, celecoxib, phenytoin, and warfarin) showing a similar effect of *CYP2C9*1/*3* (AS=1) and *CYP2C9*2/*2* on metabolic ratio and dose requirements (warfarin) (22-24). Originally, *CYP2C9*2* was thought to have as compromised activity as *CYP2C9*3*, but a wealth of emerging evidence clearly shows that, for many substrates, *CYP2C9*2* has more activity than does *CYP2C9*3*. Furthermore, the *CYP2C9*3* allele is now classified as a 'no function' allele with a value of 0 for AS calculation. This is based on *CYP2C9*3/*3*, which represents the diplotype with the lowest metabolic activity; thus, the *CYP2C9*3* allele receives a 'no function' assignment. To accommodate the assignment of phenotype based on pre-emptive genotyping, it is necessary to use phenotype terms that are informative and yet drug-agnostic; to accommodate the fact that dosing recommendations may be different for particular CYP2C9 substrates (but not for other CYP2C9 substrates) for someone with an

AS of 1 vs an AS of 1.5, the activity score system allows each CPIC guideline's prescribing recommendations to be tailored based on the drug/AS score combination.

The dosing recommendations in this guideline are applicable to variant alleles that have sufficient data linking *CYP2C9* genotype to enzyme function, following CPIC's allele function assignment process. However, users should note that the strength of evidence linking each allele to its functional status can vary considerably between alleles and that the overall strength of evidence given in the ***CYP2C9* Allele Functionality Table** is not necessarily representative of the strength of evidence linking the allele specifically to phenytoin toxicity, which may be weaker. Furthermore, CYP2C9 functional metabolic activity may be substrate specific and extrapolations of function from one substrate to another may not be reliable. Consequently, some variants described in this phenytoin update have been categorized as "uncertain function" based on a lack of clinical studies linking these genotypes to enzyme function and/or conflicting data between different substrates, including phenytoin.

The *HLA-B*15:02* allele is a complex variant consisting of numerous nucleotide and resultant amino acid substitutions. Comparison of nucleotide sequences for a reference *HLA-B* allele with that of *HLA-B*15:02* reveals 42 differences within the open reading frame of the gene. These nucleotide sequence differences translate to a peptide exhibiting 27 amino acid substitutions in the variant allele (see CPIC's carbamazepine guideline online supplement (25)).

Many companies provide clinical testing services for the detection of *HLA-B*15:02*. They primarily employ two different detection methods. One is direct sequencing of the gene in which alleles are assigned by comparison of the sequence to the known variants that define *HLA-B*15:02* and reported as the diplotype of both *HLA-B* alleles. Genotyping is another common approach in which the sequence variants that define *HLA-B*15:02* are detected directly through a panel of DNA tests. Allele-specific polymerase chain reaction (PCR) is commonly employed where PCR primers specific for each nucleotide variant are used. The PCR products can then be detected using gel electrophoresis or other methods. A variety of other genotyping methods may also be used to directly detect each of the nucleotide variants for *HLA-B*15:02*. As the test is specific for *HLA-B*15:02*, the test will only report its presence or absence as opposed to the full diplotype available through sequencing.

Another option is the genotyping of one or more SNPs that are near the *HLA-B* locus and in linkage disequilibrium with the *HLA-B*15:02* allele (26). However, as this test is indirect and depends upon

linkage disequilibrium which may vary between different populations, it may have lower accuracy. It also requires genotyping and may not be any faster or less expensive than genotyping of the specific defining variants. These types of tests are not recommended because of the potential for false-positives and negatives and the need for confirmation. *HLA* alleles may also be imputed from genome-wide array data, which may be available for individuals through direct-to-consumer testing or participation in biorepositories. Overall imputation program concordance with *HLA* sequencing can be up to 98% or higher for *HLA* class I (27). While such programs are useful research tools, the potential for misclassification of *HLA* alleles in these algorithms, combined with relatively poor performance in populations with non-European ancestry, suggest that they should not be used for clinical decision making.

Drugs: Phenytoin and fosphenytoin

Background

In humans, phenytoin is metabolized to a putative arene epoxide with subsequent formation of phenytoin dihydrodiol, 5-(3'-hydroxyphenyl)-5-phenylhydantoin (*m*-HPPH) and mostly 5-(4'-hydroxyphenyl)-5-phenylhydantoin (*p*-HPPH) (**Supplementary Figure S1**). *In vivo,* 67-88% of an administered phenytoin dose is excreted as *p*-HPPH, which is conjugated mainly to glucuronic acid (28), with only a trace amount of *m*-HPPH formed (29). Phenytoin is a pro-chiral molecule and, besides this regio-selectivity of hydroxylation, metabolite formation is also highly stereoselective, as evidenced by the urinary (*S*)/(*R*) ratio for *p*-HPPH which is typically >40:1 (30-32).

Strong *in vitro* evidence from recombinant CYP2C9 studies recapitulates the high *in vivo* stereo-selectivity (>20:1) in the formation of (S)-*p*-HPPH, supporting CYP2C9 as the dominant phase I clearance pathway for phenytoin (33). In contrast, a second P450 enzyme - CYP2C19 – is much less stereoselective, forming only ~60% (S)-*p*-HPPH (33). CYP2C19 forms most of the (*R*)-*p*-HPPH encountered *in vivo* (34), but this enzyme assumes a minor role in phenytoin hydroxylation, unless the CYP2C9 pathway becomes saturated at higher doses of the drug (33). Because therapeutic plasma concentrations of phenytoin can be sufficiently high to saturate CYP2C9 and CYP2C19, the dosing of phenytoin is complicated by nonlinear pharmacokinetics; *i.e.* increases in phenytoin plasma concentrations are not proportional to dose increases (35).

Fosphenytoin is a water-soluble, phosphate ester pro-drug of phenytoin that was developed to overcome complications associated with parenteral phenytoin administration, including cardiac arrhythmias and

hypotension (36). Fosphenytoin is rapidly and completely metabolized to phenytoin by alkaline phosphatase (ALP) enzymes that, importantly for pro-drug activations, are found at high levels in plasma and the brush-border of the gastrointestinal tract (37). Metabolism by ALP forms a transient carbinolamine intermediate that spontaneously decomposes to phenytoin **(Supplementary Figure S2**). Effects of genetic variation of ALP enzymes in fosphenytoin bioactivation have not been described.

Linking genetic variability to variability in drug-related phenotypes

HLA-B. An increased risk of SJS/TEN has been associated with the *HLA-B*15:02* allele in East Asian and Central/South Asian populations (see **Supplemental Material; Table S1**). Cheung et al. conducted a meta-analysis of two studies in Taiwan (11) and Hong Kong (13), comprising 41 cases and 188 controls. An association of *HLA-B*15:02* with phenytoin-induced SJS/TEN (odds ratio 4.26 [95% CI 1.93–9.39], $p<3x10^{-4}$) was found using a fixed-effect model with statistically insignificant heterogeneity. By pooling data directly, the association had a sensitivity of 36.6% (95% CI 23.6–51.9) and specificity of 87.2% (95% CI 81.7–91.3). Therefore, the absence of this variant does not rule out the possibility of a patient developing phenytoin-induced SJS/TEN, including in populations at high risk for carriage of *HLA-B*15:02* such as East Asian and Central/South Asian populations. The strength of the association between *HLA-B*15:02* and phenytoin–induced SJS/TEN is weaker than that with carbamazepine-induced SJS/TEN. Significantly less than 1% of those who carry *HLA-B*15:02* when exposed to phenytoin will develop SJS or TEN. However, taken together with the known association of carbamazepine and SJS/TEN in carriers of *HLA-B*15:02*, this reproducible association supports the FDA recommendation to avoid phenytoin and potentially other aromatic anticonvulsants as substitutes for carbamazepine in individuals who test positive for *HLA-B*15:02* (9).

Other *HLA-B* alleles, such as *HLA-B*13:01,* have been associated with phenytoin-induced SJS/TEN in multiple studies, further affirming the lack of 100% negative predictive value (NPV) of *HLA-B*15:02* for phenytoin SJS/TEN (4-6). *HLA-B* alleles have also been associated with phenytoin-induced drug reactions with eosinophilia and systemic symptoms (DRESS) (38, 39). However, these associations are based on a small number of studies with insufficient evidence to currently consider clinical decisions based on test results. These associations reinforce the importance of continued monitoring for severe cutaneous ADRs during phenytoin treatment and avoiding false reassurance of a negative *HLA-B*15:02* result.

CYP2C9. Available model estimates predict that some variant *CYP2C9* alleles lower phenytoin intrinsic clearance. Several studies indicate that individuals with *CYP2C9*1/*3* and *CYP2C9*1/*2* genotypes have mildly-to-moderately reduced clearance values (**Table S2**) and so these subjects are classified as IMs. Individuals genotyped as *CYP2C9*2/*2* have been reclassified as IMs based on reduced phenytoin clearance values similar to *CYP2C9*1/*3* and *CYP2C9*1/*2* (**Table S2**) (40). Individuals with one decreased function allele and one no function allele or two no function alleles (*CYP2C9*2/*3* and *CYP2C9*3/*3*) have substantially reduced clearance of several drugs and are classified as CYP2C9 PMs. Phenytoin maintenance doses were reported to be reduced 23-38% in heterozygous individuals with one no or decreased function allele (41-43) and 31-52% for carriers with two no or decreased function alleles versus *CYP2C9*1/*1* (42, 43). Furthermore, multiple case studies have observed that CYP2C9 PMs are at increased risk for exposure-related phenytoin toxicities, and multiple studies have observed an association between the *CYP2C9*3* allele and SJS/TEN (5, 39, 44).

The majority of the evidence linking *CYP2C9* genotypes and phenytoin exposure and toxicity have been found in patients carrying *CYP2C9*2* and **3* and therefore, the effects of other decreased/no function alleles are extrapolated from the *CYP2C9*2* and **3* data. The ***CYP2C9* allele functionality table** (16) contains a level of evidence assignment for each allele function assigned as follows: 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) and 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 AND 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 two patient cases demonstrated drug phenotype causality and 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 one patient case and at least one of the following types of evidence: limited *in vitro* data (e.g. correlative data) support the variant-drug phenotype association and computational activity predictions overall support *in vivo* and/or *in vitro* data (45) AND no convincing evidence has emerged that contradicts the role of the variant in the noted drug phenotype.

Pediatrics

Special consideration should be taken with the pediatric population for *CYP2C9* genotype. Phenytoin is used in the treatment of neonatal seizures and subsequently after discharge from the neonatal intensive care unit. Maintaining therapeutic concentrations can be particularly problematic in this population. This may be due, at least in part, to the developmental expression of CYP proteins after birth including CYP2C9 (46). CYP2C9 activity levels are considerably lower in the fetus during the first trimester (1-2%) and at term (30%) compared to adult values. CYP2C9 activity increases during the first five months of life, approaching adult values between five months to two years of age (47). Other considerations include the clearance of phenytoin being twice that of adult values in children under six years of age. This is attributed to the finding that the maximal rate of phenytoin metabolism is inversely related to age. However, this varied significantly within age subgroups (48). For these reasons, phenytoin therapeutic recommendations based on *CYP2C9* genotype in this population are difficult. One study in a North Indian pediatric population found significantly higher serum phenytoin concentrations in *CYP2C9*2* and **3* carriers compared to NMs (p=0.009); however, there were no statistically significant differences in dose received and ADRs for *CYP2C9*2* and *CYP2C9*3* carriers compared to NMs (49). A study in Thai children with epilepsy found an association between *CYP2C9*3* and phenytoin-induced severe cutaneous ADRs (odds ratio = 14.52; p = 0.044) (50).

Levels of Evidence

The evidence summarized in **Supplemental Tables S1, S2 and S3** is graded using a scaled modified slightly from Valdes et al. (51):

> High: Evidence includes consistent results from well-designed, well-conducted studies.
>
> Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.
>
> Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information

Strength of Recommendations

CPIC's dosing recommendations are based on weighing the evidence from a combination of preclinical functional and clinical data (**Supplemental Tables S1-S2**) as well as on some existing disease-specific

consensus guidelines (52-54). Some of the factors that are taken into account in evaluating the evidence supporting dosage recommendations include: *in vivo* clinical outcome data for phenytoin, *in vivo* pharmacokinetic and pharmacodynamic data for phenytoin, *in vitro* enzyme activity of expressed wild-type or variant-containing *CYP2C9*, *in vitro* CYP2C9 enzyme activity from tissues isolated from individuals of known *CYP2C9* genotypes, *in vivo* pre-clinical pharmacokinetic and pharmacodynamic studies, and *in vitro* studies of CYP2C9 protein stability (55).

Overall, the therapeutic recommendations are simplified to allow rapid interpretation by clinicians. CPIC uses a slight modification of a transparent and simple system for just four categories for recommendations adopted from the rating scale for evidence-based recommendations on the use of antiretroviral agents (56):

Strong recommendation for the statement: "The evidence is high quality and the desirable effects clearly outweigh the undesirable effects."
Moderate recommendation for the statement: "There is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects."
Optional recommendation for the statement: "The desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action."
No recommendation: "There is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice at this time."

Resources to Incorporate Pharmacogenetics into an EHR with CDS

Clinical decision support (CDS) tools integrated within electronic health records (EHRs) can help guide clinical pharmacogenetics at the point of care (57-61). See https://cpicpgx.org/guidelines/guideline-for-phenytoin-and-cyp2c9-and-hla-b/ for resources to support the adoption of CPIC guidelines within an EHR. Based on the capabilities of various EHRs and local preferences, we recognize that approaches may vary across organizations. Our intent is to synthesize foundational knowledge that provides a common starting point for incorporating the use of *CYP2C9* genotype results to guide phenytoin use and use in an EHR.

Effectively incorporating pharmacogenetic information into an EHR to optimize drug therapy should have some key attributes. Pharmacogenetic results, an interpreted phenotype, and a concise

interpretation or summary of the result must be documented in the EHR (62, 63). To incorporate a phenotype in the EHR in a standardized manner, genotype test results provided by the laboratory must be consistently translated into an interpreted phenotype (**Tables 1 and 2, main manuscript**). Because clinicians must be able to easily find the information, the interpreted phenotype may be documented as a problem list entry or in a patient summary section; these phenotypes are best stored in the EHR at the "person level" rather than at the date-centric "encounter level". Additionally, results should be entered as standardized and discrete terms to facilitate using them to provide point-of-care CDS (57, 64).

Because pharmacogenetic results have lifetime implications and clinical significance, results should be placed into a section of the EHR that is accessible independent of the test result date to allow clinicians to quickly find the result at any time after it is initially placed in the EHR. To facilitate this process, CPIC is providing gene-specific information figures and tables that include full diplotype to phenotype tables, diagram(s) that illustrate how *CYP2C9* pharmacogenetic test results could be entered into an EHR, example EHR consultation/genetic test interpretation language and widely used nomenclature systems for genes relevant to the CPIC guideline (see https://www.pharmgkb.org/page/CYP2C9RefMaterials.

Point-of-care CDS should be designed to effectively notify clinicians of prescribing implications at any time after the test result is entered into the EHR. CPIC provides gene-drug specific tables that offer guidance to achieve these objectives with diagrams that illustrate how point-of-care CDS should be entered into the EHR, example pre- and post-test alert language, and widely used nomenclature systems for drugs relevant to the CPIC guideline (see https://cpicpgx.org/guidelines/guideline-for-phenytoin-and-cyp2c9-and-hla-b/).

Supplemental Table S1. Evidence linking *HLA* allele to phenytoin cutaneous adverse drug reaction phenotype.

Type of Experimental model (in vitro, in vivo, preclinical, or clinical)	Major Findings	References	Level of evidence*
Clinical	Significant association between *HLA-B*15:02* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients and/or healthy controls.	Man *et al*. (2007) (65) Hung *et al*. (2010) (11) Neuman *et al*. (2012) (66) Cheung *et al*. (2013) (13) Tassaneeyakul *et al.* (2016) (39) Chang *et al.* (2017) (67) Shi *et al.* (2017) (68) Yampayon *et al.* (2017) (5) Su *et al.* (2019) (4)	Moderate
Clinical	Association between *HLA-A*02:01/HLA-Cw*15:02* genotype and patients with phenytoin induced SJS/TEN compared to AED tolerant patients and population controls.	Ramirez *et al.* (2017) (69)	Weak
Clinical	Association between *HLA-A*02:01* and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant controls.	Shi *et al.* (2017) (68)	Weak
Clinical	Significant association between *HLA-B*13:01* genotype and patients with phenytoin induced DRESS/DHS compared to phenytoin tolerant patients.	Yampayon *et al.* (2017) (5) Su *et al.* (2019) (4)	Moderate
Clinical	Association between *HLA-B*13:01* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Association between *HLA-C*14:02* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Significant association between *HLA-B*15:13* genotype and patients with phenytoin induced SJS/TEN compared to	Chang *et al.* (2017) (67)	Moderate

	phenytoin tolerant patients and/or healthy controls.		
Clinical	Significant association between *HLA-B*15:13* genotype and patients with phenytoin induced DRESS compared to phenytoin tolerant patients and/or healthy controls.	Chang *et al.* (2017) (67)	Weak
Clinical	Association between *HLA-Cw*17:01* genotype and patients with phenytoin induced DRESS compared to population controls.	Ramirez *et al.* (2017) (69)	Weak
Clinical	Significant association between *HLA-A*24:02* and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant controls	Shi *et al.* (2017) (68)	Weak
Clinical	Association between *HLA-A*33:03* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Association between *HLA-B*38:02* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Association between *HLA-B*46:01* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Association between *HLA-B*51:01* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Association between *HLA-B*51:01* genotype and patients with phenytoin induced DRESS compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39) Ihtisham *et al.* (2019) (38)	Weak
Clinical	Association between *HLA-B*51:01* genotype and patients with any type of phenytoin induced reaction compared to phenytoin tolerant patients.	Su *et al.* (2019) (4)	Weak
Clinical	Significant association between *HLA-B*56:02/04* genotype and patients with phenytoin induced DRESS/DHS compared to phenytoin tolerant patients.	Harding *et al*.(2012) (70) Yampayon *et al.* (2017) (5) Somogyi *et al*. (2019) (71)	Weak
Clinical	Association between *HLA-B*56:02* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak

Clinical	Association between *HLA-B*58:01* genotype and patients with phenytoin induced SJS/TEN compared to phenytoin tolerant patients.	Tassaneeyakul *et al.* (2016) (39)	Weak
Clinical	Patient with urticaria with angioedema after phenytoin tested positive for *HLA-B*57:01* and *HLA-B*58:01*	Manoharan *et al.* (2019) (72)	Weak

***** High: Evidence includes consistent results from well-designed, well-conducted studies.

Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.

Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information

Supplemental Table S2. Evidence linking *CYP2C9* genotype to phenytoin metabolism and/or toxicities.

Type of Experimental model (in vitro, in vivo, preclinical, or clinical)	Major Findings	References	Level of evidence*
In vitro	*CYP2C9*2* results in a 29% reduction in phenytoin clearance as compared with *1.	Rettie *et al*. (1999) (73)	Moderate
In vitro	*CYP2C9*3* results in a 93-95% reduction in phenytoin clearance as compared with *1.	Rettie *et al*. (1999) (73) Takanashi *et al*. (2000) (74)	Moderate
Clinical	*CYP2C9*3* is associated with reduced (S)/(R) ratio of p-HPPH in urine samples.	Argikar *et al*. (2006) (31)	Moderate
Clinical	*CYP2C9*3* carriers have significantly increased serum concentrations of phenytoin after a single dose in healthy volunteers.	Aynacioglu *et al*. (1999) (75) Kerb *et al*. (2001) (76) Caraco *et al*. (2001) (77)	High
Clinical	*CYP2C9*3* carriers have significantly increased phenytoin serum concentrations under steady state conditions.	Odani *et al*. (1997) (78) Soga *et al*. (2004) (79) Ramasamy *et al*. (2010) (80) Phabphal *et al*. (2013) (81) Phabphal *et al*. (2013) (82) Chaudhary *et al.* (2016) (49) Tahker *et al.* (2017) (83) Fohner *et al.* (2019) (40)	High
Clinical	*CYP2C9*2* carriers have significantly increased serum concentrations of phenytoin after a single dose in healthy volunteers.	Aynacioglu *et al*. (1999) (75) Kerb *et al*. (2001)(76)	Moderate
Clinical	*CYP2C9*2* carriers have significantly increased phenytoin serum concentrations under steady state conditions	Ramasamy *et al*. (2010) (80) Fohner *et al.* (2019) (40)	Moderate
Clinical	*CYP2C9*3* carriers have significantly reduced serum p-HPPH/P ratio compared to wild-type carriers.	Aynacioglu *et al*. (1999) (75) Kerb *et al*. (2001) (76)	Moderate

Clinical	*CYP2C9*2* have significantly reduced serum p-HPPH/P ratio compared to wild-type carriers.	Aynacioglu *et al*. (1999) (75) Kerb *et al*. (2001) (76)	Moderate
Clinical	*CYP2C9*3* carriers have significantly lower maximal elimination rates than do compared to wild-type carriers.	Odani *et al*. (1997) (78) Mamiya *et al*. (1998) (84) Hung *et al*. (2004) (41) Yamamoto *et al*. (2011) (85)	High
Clinical	*CYP2C9*1/*3* associated with increased likelihood of ADR when treated with phenytoin in patients with epilepsy.	Lee *et al*. (2004) (86) Hennessy *et al*. (2009) (87) Kesavan *et al*. (2010) (88) Suvichapanich *et al.* (2015) (50) Su *et al.* (2019) (4)	High
Clinical	*CYP2C9*1/*2* is NOT associated with increased toxicity	Ramasamy *et al*. (2010) Fohner et al. (2019) (40)	Weak
Clinical	*CYP2C9*3* carriers associated with increased likelihood of phenytoin ADR	Ramasamy *et al*. (2010) (88) Fohner *et al.* (2019) (40)	Moderate
Clinical	*CYP2C9*2* and **3* associated with a significant reduction in cerebellar white matter volume but not in total cerebellar volume in patients receiving chronic phenytoin (>1 year).	Twardowschy *et al.* (2013) (89)	Moderate
Clinical	*CYP2C9*2/*2* associated with increased likelihood of ADR when treated with phenytoin in patients with epilepsy.	Hennessy *et al*. (2009) (87) Kevavan *et al*. (2010) (88)	Moderate
Clinical	*CYP2C9*2* and **3* associated with phenytoin toxicity.	Depondt *et al*. (2011) (90) Thakkar *et al*. (2012) (91)	Moderate
Clinical	*CYP2C9*3/*3* observed in patient with phenytoin toxicity.	Ramasamy *et al*. (2007) (92) Babu *et al*. (2013) (93) Chan *et al.* (2015) (94) Nissen *et al.* (2018) (95)	Moderate
Clinical	*CYP2C9*1/*3* observed in patient with phenytoin intoxication.	Ninomiya *et al*. (2000) (96) Citerio *et al*. (2003) (97) McCluggage *et al*. (2009) (98) Chan *et al.* (2015) (94) Veeravigrom *et al.* (2016) (99)	Moderate
Clinical	*CYP2C9*1/*3* observed in patient with DRESS after phenytoin	Somogyi *et al.* (2019) (71)	Weak

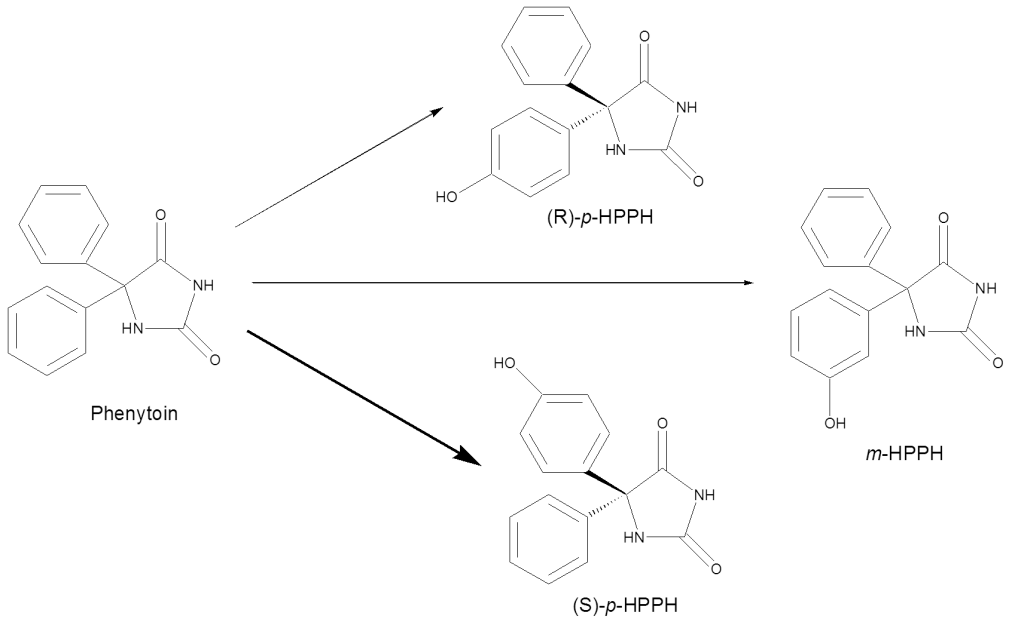
Clinical	*CYP2C9*2/*2* observed in patient with neurological phenytoin toxicity.	Dorado *et al*. (2012) (100)	Weak
Clinical	In epileptic patients receiving phenytoin, *CYP2C9*3* is associated with decreased maximum tolerable dose of phenytoin.	van der Weide *et al*. (2001) (42) Tate *et al*. (2005) (101)	High
Clinical	Epileptic patients who are *CYP2C9*3* carriers require significantly lower maintenance doses of phenytoin as compared to wild-type carriers.	Hung *et al.* (2012) (43)	Moderate
Clinical	In epileptic patients receiving phenytoin, *CYP2C9*2* is associated with decreased maximum tolerable dose of phenytoin.	van der Weide *et al*. (2001) (42)	Moderate
Clinical	CYP2C9 intermediate and poor metabolizers (**1/*2, *2/*2*, **1/*3*, and **2/*3*) prescribed phenytoin for seizure control are at increased risk of switching to an alternative anticonvulsant within 100 days after first phenytoin	Fohner *et al.* (2019) (40)	Moderate
Clinical	*CYP2C9*1/*3, *2/*2, *2/*3, *3/*3* associated with increased risk of having a lower dose by the end of the first year of phenytoin treatment	Fohner *et al.* (2019) (40)	Moderate
Clinical	*CYP2C9*5*, **6*, **8*, and **11* are associated with reduced urinary excretion of (S)-p-HPPH (8-hour urine collection after single dose).	Allabi *et al*. (2005) (102)	Moderate
Clinical	*CYP2C9*9* does NOT affect phenytoin metabolism.	Allabi *et al*. (2005) (102)	Moderate
Clinical	*CYP2C9*1/*3* is NOT associated increased likelihood of ADR when treated with phenytoin in patients with epilepsy.	Twardowschy *et al*. (2011) (103)	Weak
Clinical	*CYP2C9*2* is NOT associated with increased maximum dose of phenytoin.	Tate *et al*. (2005) (104) Chaudhary *et al.* (2016) (49)	Weak
Clinical	T allele of *CYP2C9 IVS8-109A>T* is associated with increased plasma concentrations of phenytoin	Oretega-Vazquez *et al.* (2016) (105)	Weak
Clinical	T allele of c.882G > T, p.L294F (rs544027339) in *CYP2C9* is observed in a patient with phenytoin toxicity	Guacci *et al*. (2016) (106)	Weak
Clinical	G allele of c.920G > A, p.R307K in CYP2C9 is observed in a patient with phenytoin toxicity	Guacci *et al.* (2016) (106)	Weak

Clinical	*CYP2C9*3* is associated with an increased risk of developing phenytoin-induced SJS/TEN	Tassaneeyakul *et al.* (2016) (39) Yampayon *et al.* (2017) (5)	Moderate
Clinical	*CYP2C9*3* is NOT associated with an increased risk of developing phenytoin-induced DRESS	Tassaneeyakul *et al.* (2016) (39)	Moderate
In vitro	*CYP2C9*2, *11, *23, *29, *34, *38, *44, *46* and *48 do NOT alter the rate of phenytoin clearance as compared to *CYP2C9*1*	Chen *et al.* (2016) (107)	Moderate
In vitro	*CYP2C9*27, *40, *41, *47, *49, *51, *53, *54, *56* and the *N418T* polymorphism are associated with a significantly increased rate of phenytoin clearance as compared to *CYP2C9*1*	Chen *et al.* (2016) (107)	Moderate
In vitro	*CYP2C9*3, *8, *13, *14, *16, *19, *31, *33, *36, *37, *39, *42, *43, *45, *50, *52* and **55* are associated with a significantly decreased rate of phenytoin clearance as compared to *CYP2C9*1*	Chen *et al.* (2016) (107)	Moderate
Clinical	*CYP2C9*3* is NOT associated with maintenance dose of phenytoin	Chaudhary *et al.* (2016) (49)	Weak
Clinical	*CYP2C9*2* and **3* are NOT associated with frequency of gum hypertrophy, ataxia or hirsutism side effects as a result of phenytoin treatment	Chaudhary *et al.* (2016) (49)	Weak
Clinical	*CYP2C9*2* is NOT associated with increased plasma levels of phenytoin in epilepsy patients	Chaudhary *et al.* (2016) (49)	Weak
Clinical	*CYP2C9*3* is significantly associated with an increased risk of phenytoin-induced severe adverse cutaneous reactions (DRESS and SJS/TEN)	Chung *et al.* (2014) (44)	Moderate

***** High: Evidence includes consistent results from well-designed, well-conducted studies.

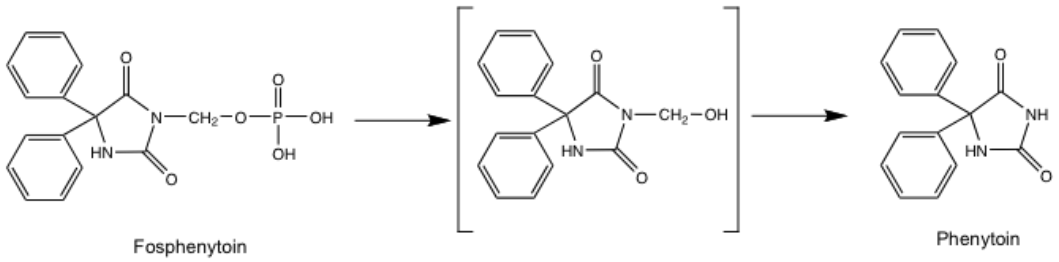
Moderate: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality, or consistency of the individual studies; generalizability to routine practice; or indirect nature of the evidence.

Weak: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information



Supplementary Figure S1. Metabolism of phenytoin

(R)-p-HPPH, R-isomer of 5-(4'-hydroxyphenyl)-5-phenylhydantoin (p-HPPH); (S)-p-HPPH, S-isomer of 5-(4'-hydroxyphenyl)-5-phenylhydantoin; m-HPPH 5-(3'-hydroxyphenyl)-5-phenylhydantoin



Supplementary Figure S2. Metabolism of fosphenytoin to phenytoin.

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