Supplement to:

Clinical Pharmacogenetics Implementation Consortium Guideline for *CYP2B6* Genotype and Methadone Therapy

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GUIDELINE UPDATES

The Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for *CYP2B6* and methadone therapy is published in full on the CPIC website (https://cpicpgx.org/cpic-guideline-for-methadone-based-on-cyp2b6-genotype/) (1). Relevant information will be reviewed periodically, and updated guidelines published online.

LITERATURE REVIEW

The PubMed® database (1966 to December 2022) was searched for the following keywords: (CYP2B6 OR cytochrome P450 2B6) AND (methadone). The search was limited to studies conducted in humans and written in the English language, and review articles were excluded. Using these search terms, 103 publications were identified. Study inclusion criteria included publications that incorporated analyses for the association between *CYP2B6* genotype and methadone pharmacokinetic parameters or methadone-related clinical outcomes in patients. Following the application of these criteria, 46 publications were reviewed and included in the evidence tables (**Table S1**).

GENETIC TEST INTERPRETATION

The haplotype, or star (*) allele name, is determined by the presence of a specific single nucleotide variant (SNV) or a combination of SNVs that are interrogated in the genotyping analysis. In addition, two structural variants (2) have received star allele designation (*CYP2B6*29* and **30*) and appear to be rare (minor allele frequency [MAF] <1%) in the populations tested (~0.005% in African Americans and Asians, respectively).

The genotypes that constitute the haplotype, or star (*) alleles for *CYP2B6*, and the rsIDs for each of the specific genomic nucleotide alterations that define the alleles, are per PharmVar (https://www.pharmvar.org/gene/CYP2B6*)* and also described in the ***CYP2B6* Allele Definition Table** online (1, 3). The genotype results are generally reported as a diplotype, which includes one maternal and one paternal star allele (e.g., **1/*6*). The CYP2B6 function associated with each of the common star alleles is summarized in the ***CYP2B6* Allele Functionality Table** online (1, 3).

AVAILABLE GENETIC TEST OPTIONS

Commercially available genetic testing options change over time. The Genetic Testing Registry provides a central location for voluntary submission of genetic test information by providers and is available at: http://www.ncbi.nlm.nih.gov/gtr. Desirable characteristics of pharmacogenetic tests, including naming of alleles and test report contents, have been extensively reviewed by an international group, including CPIC members (4). CPIC recommends that clinical laboratories adhere to these test reporting standards. CPIC gene-specific tables adhere to these allele nomenclature standards. Moreover, these tables (***CYP2B6* Allele Definition Table**, ***CYP2B6* Allele Functionality Table**, and ***CYP2B6* Allele Frequency Table** (1, 3)) 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.

Because the genomic structure of the *CYP2B6* gene is complex, there are several factors that cause potential uncertainty in the genotyping results and phenotype predictions. 1) Since it is impractical to test for every variant in the *CYP2B6* gene, patients with rare variants may be

assigned a default genotype; this can happen when a patient has one or two rare allele(s) that are not included in the genotype test used. Several variants of the *CYP2B6* gene with potential functional consequences are rare (MAF <1%) in most populations, and thus sequencing-based approaches may be recommended in certain situations. 2) Structural variants containing a *CYP2B7::CYP2B6* hybrid (*CYP2B6*29*, switch in intron 4) or a *CYP2B6::CYP2B6* hybrid (*CYP2B6*30*, switch in intron 4) have been described; the *CYP2B7*-derived portions introduce numerous amino acid changes (see the PharmVar Structural Variation document available at https://www.pharmvar.org/gene/CYP2B6 for more details). *CYP2B6*29* harbors a singleton hybrid while that in *CYP2B6*30* is part of a duplication structure. Since the nature of the *CYP2B6* gene copy has not been determined (may be a **1, *4* or **6*), the function of this structure remains uncertain. Phenotype may not be accurately predicted in rare cases if these structural variants are not detected. Specifically, if copy number testing targets exons 1 through 4, *CYP2B6*30* could be misclassified as *CYP2B6*1x2*, which predicts an UM phenotype. Copy number testing should ideally query different gene regions and may also require long-range PCR-based amplification and sequencing to more fully characterize the hybrid genes and assess whether the allele has a singleton hybrid or a hybrid in addition to a *CYP2B6* gene copy (2). Given the rarity and complexity of detecting and characterizing such structural variants, these are typically not included in *CYP2B6* genotyping test platforms. 3) Some SNVs exist on multiple alleles (e.g., c.516G>T is found in combination with other variants in 16 other *CYP2B6* alleles [**6, *7, *13, *19, *20, *26, *29, *34, *36, *37, *38, *39, *40, *41, *42, *43*]) (**Figure S5**). If testing indicates heterozygosity for multiple SNVs, it may be difficult to accurately assign a specific genotype. For example, an individual heterozygous for the c.516G>T, c.785A>G, and c.1459C>T variants in the *CYP2B6* gene could be classified as *CYP2B6*1/*7* or **5/*6* unless

methods are available that allow distinguishing between these two genotypes on a routine basis (5). 4) Allele frequencies may vary considerably among patients of different biogeographical ancestry groups. For example, *CYP2B6*18* and other rare variants are relatively common in African ancestry populations and have a considerably lower prevalence, or are even absent (to date), in other ethnic groups such as those of European ancestry. Thus, the alleles that should be tested to predict phenotype for a given population may vary considerably. Given the limited numbers of individuals tested in many populations to date, and the heterogeneity of any given population, the ideal case would be to test for as many alleles as possible in all populations. 5) As described above, both *CYP2B6*29* and *CYP2B6*30* require complementary assays including sequencing to distinguish between the two variants. 6) The possibility that rare SNVs or pseudogenes may interfere with PCR amplification and/or detection on a particular platform or assay cannot be ruled out. (6). For example, testing for c.785A>G is challenging using the commercially available TaqMan assay. This SNV is located in a region that is identical to *CYP2B7*, a nonfunctional pseudogene. Small *CYP2B6*-specific PCR amplicons bracketing c.785A>G cannot be reliably generated. Thus, the genotype assay is often performed in two steps where exon 5 is first amplified with primers and then a pre-amplified *CYP2B6*-specific long-range PCR amplicon is used as a template for a custom TaqMan genotyping assay.

LEVELS OF EVIDENCE LINKING GENOTYPE TO PHENOTYPE

The evidence summarized in **Table S1** is graded on a scale of high, moderate, and weak based upon the level of evidence:

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 the 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 therapeutic recommendations are based on weighing the evidence from a combination of preclinical functional and clinical data, as well as on some existing disease-specific consensus guidelines. Some of the factors that are taken into account in evaluating the evidence supporting therapeutic recommendations include *in vivo* pharmacokinetic and pharmacodynamic data, *in vitro* enzyme activity of tissues expressing reference or non-reference CYP2B6, *in vitro* CYP2B6 enzyme activity from tissues isolated from individuals of known *CYP2B6* genotypes, and *in vivo* pre-clinical and clinical pharmacokinetic and pharmacodynamic studies.

Overall, the therapeutic recommendations are simplified to allow rapid interpretation by clinicians. CPIC uses a slight modification of a transparent and simple system for recommendations adopted from the rating scale for evidence-based guidelines on the use of antiretroviral agents (7):

- **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 effects 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.

TABLE S1. EVIDENCE LINKING CYP2B6 TO METHADONE PHENOTYPE

Type of Experimental Model	Major Findings	References	Level of Evidence[a]
Microsomes			
In vitro	Microsomes expressing *CYP2B6*6*, **7* and **9* had decreased racemic, R-, and S-methadone metabolism compared to microsomes expressing *CYP2B6*1*.	Gadel, *et al*. (2013) (8) Gadel, *et al*. (2015) (9) Wang, *et al*. (2022) (10)	High
In vitro	Microsomes expressing *CYP2B6*4* had increased R- and S-methadone metabolism compared to microsomes expressing *CYP2B6*1*.	Gadel, *et al*. (2015) (9) Wang, *et al*. (2022) (10)	Moderate
In vitro	Microsomes expressing *CYP2B6*18* had no methadone metabolism.	Gadel, *et al*. (2015) (9) Wang, *et al*. (2022) (10)	High
In vitro	Microsomes expressing *CYP2B6*5* had decreased methadone metabolism compared to microsomes expressing *CYP2B6*1*.	Gadel, *et al*. (2015) (9) Wang, *et al*. (2022) (10)	Weak
In vitro	Microsomes expressing *CYP2B6*17* had decreased methadone metabolism compared to microsomes expressing *CYP2B6*1* and similar metabolism to *CYP2B6*5*.	Wang, *et al*. (2022) (10)	Moderate
In vitro	Microsomes expressing *CYP2B6*19* and **26* had decreased methadone metabolism compared to microsomes expressing *CYP2B6*1* and *CYP2B6*5* and **17*.	Wang, *et al*. (2022) (10)	Weak
Plasma Concentrations			
Clinical	*CYP2B6* decreased function alleles were associated with higher dose-adjusted plasma R-methadone concentrations.	Crettol, *et al*. (2005) (11) Lotsch, *et al*. (2006) (12) Bogen, *et al*. (2013) (13) Lee, *et al*. (2013) (14) Victorri-Vigneau, *et al*. (2019) Talal, *et al*. (2020) (15)	Weak
Clinical	CYP2B6 poor metabolizers had higher plasma R-methadone concentrations than CYP2B6 normal metabolizers.	Crettol, *et al*. (2006) (16) Eap, *et al*. (2007) (17) Badhan, *et al*. (2021) (18)	Weak

Clinical	*CYP2B6* decreased function alleles were associated with higher dose-adjusted plasma S-methadone concentrations.	Crettol, *et al*. (2005) (11) Wang, *et al*. (2011) (19) Dobrinas, *et al*. (2013) (20) Lee, *et al*. (2013) (14) Victorri-Vigneau, *et al*. (2019) (21) Talal, *et al*. (2020) (15)	High
Clinical	CYP2B6 poor metabolizers had higher plasma S-methadone concentrations than CYP2B6 normal and intermediate metabolizers.	Crettol, *et al*. (2006) (16) Eap, *et al*. (2007) (17) Badhan, *et al*. (2021) (18) Chalabianloo, *et al*. (2023) (22)	Moderate
Clinical	CYP2B6 intermediate metabolizers had higher dose-normalized plasma S-methadone concentrations than CYP2B6 normal metabolizers.	Bogen, *et al*. (2013) (13)	Weak
Clinical	The noncoding *CYP2B6* SNVs rs10500282, rs10403955, and rs1038376 were associated with higher dose-normalized plasma S-methadone concentrations.	Wang, *et al*. (2011) (19)	Moderate
Clinical	The noncoding *CYP2B6* SNVs rs2279345 and rs707265 were associated with lower dose-normalized plasma S-methadone concentrations.	Wang, *et al*. (2011) (19)	Moderate
Clinical	*CYP2B6*11* was associated with increased plasma S-methadone concentrations.	Dobrinas, *et al*. (2013) (20)	Weak
Clinical	*CYP2B6*5* was associated with decreased plasma S-methadone concentrations.	Dobrinas, *et al*. (2013) (20) Ahmad, *et al*. (2017) (23)	Weak
Clinical	A significant interaction between PXR alleles and *CYP2B6* alleles increased plasma S-methadone concentrations, decreased S-methadone metabolism, and decreased S-methadone clearance.	Tsai, *et al*. (2013) (24)	Moderate
Clinical	Haplotypes of *CYP2B6* were associated with increased plasma S-methadone concentrations.	Yang, *et al*. (2016) (25)	Moderate
Clinical	CYP2B6 poor metabolizers had increased dose-normalized racemic plasma methadone concentrations compared to CYP2B6 normal metabolizers.	Eap, *et al*. (2007) (17) Kringen, *et al*. (2017) (26)	Moderate

Clinical	CYP2B6 intermediate metabolizers had increased dose-normalized plasma racemic methadone concentrations compared to CYP2B6 normal metabolizers.	Bogen, *et al*. (2013) (13) Kringen, *et al*. (2017) (26)	Weak
Clinical	Patients with methadone fatalities homozygous for *CYP2B6*5* had higher post-mortem plasma methadone concentrations than patients heterozygous or homozygous for the reference allele.	Ahmad, *et al*. (2017) (23)	Weak
Clinical	Patients with methadone fatalities heterozygous for *CYP2B6*2* had decreased methadone/EDDP ratio post-mortem than patients homozygous for the reference allele.	Ahmad, *et al*. (2017) (23)	Weak
Clinical	Children who are CYP2B6 poor metabolizers are predicted to have higher plasma methadone concentrations compared to children with non-*CYP2B6*6/*6* genotypes in physiological based pharmacokinetic modeling.	Gerhart, *et al*. (2022) (27)	Weak
Clearance/AUC			
Clinical	*CYP2B6* decreased function alleles were associated with decreased R-methadone clearance.	Lotsch, *et al*. (2006) (12) Wang, *et al*. (2011) (19) Bart, *et al*. (2014) (28) Csajka, *et al*. (2016) (29) Aruldhas, *et al*. (2021) (30) Wang, *et al*. (2022) (10)	Weak
Clinical	The noncoding *CYP2B6* SNV rs2279344 was associated with decreased S-methadone clearance but not R-methadone clearance.	Csajka, *et al*. (2016) (29)	Weak
Clinical	CYP2B6 poor metabolizers had decreased R-methadone oral clearance compared to CYP2B6 normal metabolizers, but there was no difference for IV dosing.	Kharasch, *et al*. (2015) (31)	Weak
Clinical	The noncoding *CYP2B6* SNVs rs10403955 and rs2279345 were associated with decreased R-methadone clearance.	Wang, *et al*. (2011) (19)	Weak
Clinical	*CYP2B6* decreased function alleles were associated with decreased S-methadone clearance.	Wang, *et al*. (2011) (19) Bart, *et al*. (2014) (28) Csajka, *et al*. (2016) (29) Aruldhas, *et al*. (2021) (30)	Moderate

		Wang, *et al*. (2022) (10)	
Clinical	CYP2B6 poor metabolizers had decreased S-methadone clearance compared to CYP2B6 normal metabolizers.	Kharasch, *et al*. (2015) (31)	Moderate
Clinical	CYP2B6 intermediate metabolizers had decreased S-methadone clearance compared to CYP2B6 normal metabolizers.	Kharasch, *et al*. (2015) (31)	Weak
Clinical	The noncoding *CYP2B6* SNV rs11882424 was associated with increased S-methadone fractional clearance in pediatric patients receiving intraoperative methadone.	Aruldhas, *et al*. (2021) (30)	Weak
Clinical	The noncoding *CYP2B6* SNVs rs10403955 and rs1038376 were associated with decreased S-methadone clearance.	Wang, *et al*. (2011) (19)	Weak
Clinical	Carriers of *CYP2B6*4* had increased R- and S-methadone oral clearance.	Kharasch, *et al*. (2015) (31) Bart, *et al*. (2021) (32)	Weak
Clinical	*CYP2B6*5* and **11* were not associated with altered S-methadone clearance.	Csajka, *et al*. (2016) (29)	Weak
Clinical	*CYP2B6*1/*18* was associated with the lowest methadone clearance in adolescent patients undergoing spine surgery.	Wang, *et al*. (2022) (10)	Weak
Metabolism			
Clinical	*CYP2B6* decreased function alleles were associated with decreased R-methadone metabolism.	Talal, *et al*. (2020) (15) Wang, *et al*. (2022) (10)	Weak
Clinical	CYP2B6 poor metabolizers had decreased R-methadone metabolism compared to CYP2B6 normal and rapid metabolizers.	Kharasch, *et al*. (2015) (31) Packiasabapathy, *et al*. (2021) (33)	Moderate
Clinical	CYP2B6 intermediate metabolizers had decreased R and S-methadone metabolism compared to CYP2B6 normal metabolizers.	Kharasch, *et al*. (2015) (31)	Weak
Clinical	The noncoding *CYP2B6* SNV rs8100458 was associated with R-and S-EDDP levels.	Wang, *et al*. (2011) (19)	Weak
Clinical	*CYP2B6* decreased function alleles were associated with decreased S-methadone metabolism.	Talal, *et al*. (2020) (15) Wang, *et al*. (2022) (10)	Moderate

Clinical	CYP2B6 poor metabolizers had decreased S-methadone metabolism compared to CYP2B6 normal and rapid metabolizers.	Kharasch, *et al*. (2015) (31) Packiasabapathy, *et al*. (2021) (33)	Moderate
Clinical	CYP2B6 intermediate metabolizers had decreased S-methadone metabolism compared to CYP2B6 normal metabolizers.	Kharasch, *et al*. (2015) (31)	Weak
Clinical	*CYP2B6*9* was not associated with altered racemic methadone metabolism.	Sutlovic, *et al*. (2020) (34)	Weak
Clinical	The noncoding *CYP2B6* SNV rs10500282 was associated with decreased racemic methadone metabolism.	Packiasabapathy, *et al*. (2021) (33)	Weak
Clinical	The noncoding *CYP2B6* SNV rs1038376 was associated with decreased racemic methadone metabolism.	Packiasabapathy, *et al*. (2021) (33)	Weak
Methadone Dose			
Clinical	CYP2B6 intermediate metabolizers required lower doses of methadone for opioid use disorder than CYP2B6 normal metabolizers.	Hung, *et al*. (2011) (35)	Weak
Clinical	CYP2B6 poor metabolizers required lower doses of methadone for opioid use disorder than CYP2B6 normal metabolizers.	Hung, *et al*. (2011) (35) Levran, *et al*. (2013) (36)	Moderate
Clinical	*CYP2B6* decreased or no function alleles were associated with lower doses of methadone for opioid use disorder.	Crettol, *et al*. (2005) (11) Eap, *et al*. (2007) (17) Fonseca, *et al*. (2011) (37) Lee, *et al*. (2013)(14) Mouly, *et al*. (2015) (38) Zahari, *et al*. (2016) (39) Crist, *et al*. (2018) (40) Victorri-Vigneau, *et al*. (2019) (21) Chawar, *et al*. (2021) (41) Chen, *et al*. (2022) (42)	Weak
Clinical	*CYP2B6*4* was not associated with methadone dose for opioid use disorder.	Chen, *et al*. (2022) (42)	Weak

Clinical	CYP2B6 poor metabolizers required lower doses of methadone for opioid use disorder than CYP2B6 intermediate or normal metabolizers.	Levran, *et al*. (2013) (36)	Weak
Clinical	The noncoding *CYP2B6* SNV rs16974799 was associated with lower maximum methadone dose for opioid use disorder.	Chiang, *et al*. (2017) (43) Chang, *et al*. (2020) (44)	Weak
Clinical	The noncoding *CYP2B6* SNV rs3760657 was not associated with methadone dose for opioid use disorder.	Chiang, *et al*. (2017) (43)	Weak
Clinical	Methadone dose for opioid use disorder was associated with a three gene matrix: *CYP2C19* x *CYP2B6* x *CYP3A4*.	Wang, *et al*. (2013) (45) Tsai, *et al*. (2014) (46)	Weak
Clinical	Children who are CYP2B6 poor metabolizers are predicted to need lower doses of methadone to achieve similar plasma concentrations compared to children with non-*CYP2B6*6/*6* genotypes based on physiological-based pharmacokinetic modeling.	Gerhart, *et al*. (2022) (27)	Weak
Clinical Outcomes			
Clinical	Carriers of either noncoding *CYP2B6* SNV rs10500282 or rs11882424 receiving perioperative methadone had increased pain scores compared to patients who were not carriers.	Packiasabapathy, *et al*. (2021) (33)	Weak
Clinical	Patients homozygous for the noncoding *CYP2B6* SNV rs4803419 reported lower pain scores while receiving perioperative methadone compared to patients with one or no copies of rs4803419.	Packiasabapathy, *et al*. (2021) (33)	Weak
Clinical	Patients homozygous for the noncoding *CYP2B6* SNV rs1038376 reported higher incidences of postoperative nausea/vomiting while receiving perioperative methadone compared to patients with at least one reference allele.	Packiasabapathy, *et al*. (2021) (33)	Weak
Clinical	*CYP2B6* decreased function alleles and the noncoding *CYP2B6* SNV rs8192719 were associated with methadone fatalities.	Bunten, *et al*. (2010) (47) Bunten, *et al*. (2011) (48) Bunten, *et al.* (2011) (49) Ahmad, *et al*. (2017) (23)	Weak
Clinical	*CYP2B6* decreased or no function alleles were associated with therapeutic methadone response for opioid use disorder.	Crettol, *et al*. (2005) (11) Fonseca, *et al*. (2011) (37) Hung, *et al*. (2011) (35)	Weak

		Lee, *et al*. (2013) (14) Mouly, *et al*. (2015) (38) Crist, *et al*. (2018) (40) Victorri-Vigneau, *et al*. (2019) (21) Chawar, *et al*. (2021) (41)	
Clinical	Infants with *CYP2B6* normal function alleles were more likely to require treatment for neonatal abstinence syndrome than infants with *CYP2B6* decreased function alleles.	Mactier, *et al*. (2017) (50)	Weak
Clinical	*CYP2B6* decreased function alleles may be associated with infant death from a breastfeeding mother on methadone.	Madadi, *et al*. (2016) (51)	Weak
Clinical	*CYP2B6* decreased function alleles were associated with lower pain threshold and lower pain tolerance for opioid use disorder.	Zahari, *et al*. (2016) (39)	Weak
Clinical	Patients homozygous for *CYP2B6*6* receiving methadone for opioid use disorder had a statistically higher (18 ms) median QTc interval than patients with non-*CYP2B6 *6/*6* genotypes.	Eap, *et al*. (2007) (17)	Moderate
Clinical	CYP2B6 poor metabolizers may be predicted to be higher risk of QTc prolongation than CYP2B6 normal metabolizers receiving high-dose methadone for opioid use disorder.	Csajka, *et al*. (2016) (29)	Weak

[a]Rating scheme described in the **Supplemental Material**

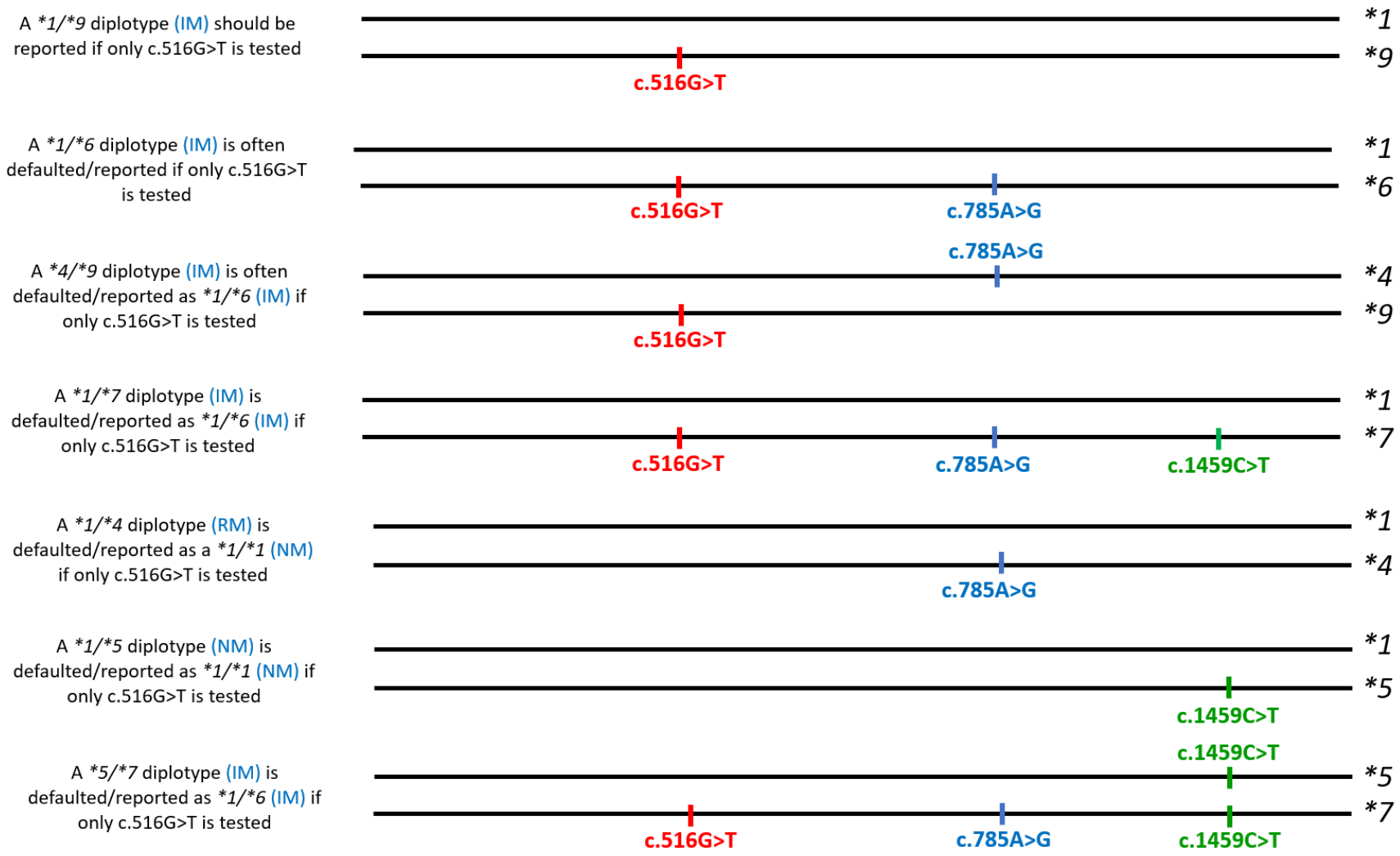

FIGURE S1. THE CHALLENGES WITH *CYP2B6* DIPLOTYPE CALLING

CYP2B6 genotyping often includes few variants besides c.516G>T, as the latter is viewed to be the single most important variant to predict CYP2B6 activity. Due to limited testing and uncertainty regarding the phase of variants (e.g., whether variants or in cis or

trans), alleles with c.516G>T are often defaulted to, and reported as, *CYP2B6*6.* This defaulting strategy is further driven by *CYP2B6*6* being the most common allele across populations with c.516G>T. The figure illustrates the impact of defaulting on phenotype assignments on selected diplotypes (specified on the right-hand side) and their respective default assignments if only c.516G>T is tested (detailed on the left-hand side).

Although defaulting practices may incorrectly assign diplotypes (i.e., misassign an allele as *CYP2B6*6)*, they often accurately predict intermediate (IM) metabolism. An example of an exception is *CYP2B6*1/*4* (RM) which would be reported as *CYP2B6*1/*1* (NM) if only c.516G>T is tested. It is also noted that two haplotypes (i.e., *CYP2B6*13* and **38,* not shown in figure) with c.516G>T have additional variant(s) which render these alleles nonfunctional rather than decreased function; not testing these variants may also lead to inaccurate phenotype predictions.

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