CPIC Guideline Update on PharmGKB

For: Clinical Pharmacogenetics Implementation Consortium Guidelines for Cytochrome P450 2D6 Genotype and Codeine Therapy: 2014 Update

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Description:

Supplemental table S3 (Association between allelic variants and CYP2D6 enzyme activity) was updated with Supplemental table S2 from the Clinical Pharmacogenetics Implementation Consortium (CPIC) Guideline for CYP2D6 and CYP2C19 Genotypes and Dosing of Selective Serotonin Reuptake Inhibitors (2015 Supplement) PMID: 25974703. The table is an updated version including additions to functional status and additional alleles.

Please see the updated guideline supplement at:

(https://www.pharmgkb.org/guideline/PA166104996)

Supplemental Material

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for cytochrome P450 2D6 (CYP2D6) genotype and codeine therapy: 2014 Update

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CPIC Updates

Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines are published in full on the PharmGKB website (www.pharmgkb.org). Relevant information will be periodically reviewed and updated guidelines will be published online.

CPIC Updates in Supplement v2.0:

- **Updated literature review from February 2011 to August 2013.**
- **Updated *CYP2D6* genetic testing interpretation.**
- **Updated frequencies of *CYP2D6* alleles in major race/ethnic groups**
- **Updated evidence linking *CYP2D6* genotype to phenotype.**

Literature Review:

We searched the PubMed® database (1966 to August 2013) and Ovid MEDLINE (1950 to August 2013) for keywords (cytochrome P450 2D6) OR (CYP2D6) AND (codeine OR morphine) for the association between *CYP2D6* genotype and codeine metabolism or codeine-related adverse drug event (ADE) or outcome. For additional reviews, see references.(1, 2)

To construct a *CYP2D6* minor allele frequency table based on ethnicity, the PubMed® database (1966 to August 2013) and Ovid MEDLINE (1950 to August 2013) were searched using the following criteria: ((CYP2D6 or 2D6) AND (genotype OR allele OR frequency OR minor allele OR variant OR ethnic OR race OR racial OR ethnicity)). Studies were considered for inclusion if: (1) the ethnicity of the population was clearly indicated, (2) either allele frequencies or minor allele percentages for *CYP2D6* genotypes were reported, (3) the method by which *CYP2D6* was genotyped was reliable and proven (no proof-of-principle experiments), (4) the sample population consisted of at least 50 patients (with few exceptions), and (5) the study represented an original publication (no reviews or meta-analyses).

Gene: *CYP2D6*

Genetic test interpretation

The haplotype, or star (*) allele name, is determined by the combination of single nucleotide polymorphisms (SNPs) and other sequence variations including insertions and deletions that are interrogated in the genotyping analysis. In addition, large rearrangements including an entire gene deletion (i.e. *CYP2D6*5*), duplications or multiplications of functional, reduced function and non-functional genes, e.g. *CYP2D6*1xN, *2xN, *41xN* and *4xN* can be observed. Also, non-functional hybrid genes composed of *CYP2D7* and *CYP2D6* have been described. *CYP2D7* carries an additional 'T' in exon 1 that causes a frameshift and renders the gene nonfunctional. Hybrid genes that have such a *CYP2D7*-derived exon 1 and switch to *CYP2D6* downstream of the additional 'T' are now consolidated under the *CYP2D6*13* allele designation.(3) *CYP2D6*13*-like hybrid genes are typically not tested by reference laboratories and/or assay platforms. Depending on the particular structure of a *CYP2D7/6* hybrid and the platform used for testing, such alleles may not be detected (i.e., no amplification products are formed from hybrid genes); in such cases the genotype may appear to be homozygous for the allele that is detected. For example, a *CYP2D6*2/*13* will appear as a *CYP2D6*2/*2*. On some platforms the *CYP2D6*-portion of a hybrid may, however, support the generation of some amplification products that may suggest the presence of a functional variant. Also, some alleles carry a combination of non-functional and functional genes which may be misinterpreted as functional gene duplications unless a test identifies such tandem gene structures.(4) Specifically, *CYP2D6*13*-like hybrid genes have been found in tandem with a functional *CYP2D6*1* or *2. Such tandems may present as duplications in some XL-PCR-based as well as quantitative copy number (CNV) assays. For example, *CYP2D6*13+*2* tandems such as the one originally published as *CYP2D6*77+*2* (5) will support amplification of a ~3.5 kb long XL-PCR product from its duplication-specific intergenic gene region as well as a 2-copy signal from both gene units with all TaqMan-based CNV assays targeting gene regions downstream of the switch to *CYP2D6* in intron 1; this includes the most popular TaqMan-based copy number assays targeting the intron 6 and exon 9 regions, respectively (assay id #'s Hs04502391_cn and Hs00010001_cn) (6). Unless complementary assays are performed the true nature of the non-functional hybrid may not be revealed and the allele incorrectly assigned as *CYP2D6*2x2*. It is therefore not only important to

know which SNPs a particular test includes and how alleles are defined, but also to know which gene rearrangements a platform is capable of detecting. Furthermore, not every genotyping test necessarily discriminates between functional and non-functional gene duplications. For example, a *CYP2D6*2/*4* subject who is duplication-positive may be defaulted to *CYP2D6*2xN/*4*. *CYP2D6*2xN* duplications are more commonly observed compared to *CYP2D6*4N* in Caucasians and other populations, however, the latter is about as frequent as *CYP2D6*1xN* and **2xN* combined in African Americans (see supplemental Table S1 and citation) (7). Consequently, CYP2D6 activity may be over-estimated in some individuals carrying duplications and/or other rearranged allelic variants if they elude detection or alleles are assigned by default. The complexities of *CYP2D6* gene analysis and interpretation have been summarized by Gaedigk. (Gaedigk, Complexities of *CYP2D6* Gene Analysis and Interpretation. International Review of Psychiatry, in press)

Each star (*) allele is defined by the presence of specific sequence variations. The genotypes that constitute the most common haplotype, or star (*) alleles for *CYP2D6* and the rs# for each of the specific genomic nucleotide alterations that define the alleles, are described in Supplemental Table S2. Tools for *CYP2D6* allele calling, genotype assignment and phenotype predicting are being developed by PharmGKB and can be accessed at www.pharmgkb.org.

Challenges of CYP2D6 *genotyping*

Because the genomic structure of the *CYP2D6* 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 variation in the *CYP2D6* gene, patients with rare variants may be assigned a default genotype; this can happen when a patient's one or two rare allele(s) are not included in the genotype test used. 2) There are multiple gene units involved in duplication and other major rearrangements. These may be functional, reduced function, or nonfunctional. If the specific gene units involved in the duplication or other rearrangements are not specifically tested for, the phenotype prediction may be inaccurate (see previous paragraph)(4). 3) Some SNPs exist on multiple alleles (e.g. rs1065852 100C>T exists on *CYP2D6*4, *10* and **36* alleles; another example is *CYP2D6*69* which carries the 'key' SNPs for *CYP2D6*10* and **41*. If testing indicates heterozygosity for these 2 SNPs (in the absence of 1846G>A), a *CYP2D6*10/*41* genotype is typically assigned, because this is the most likely genotype. However, a *CYP2D6*1/*69* genotype cannot be excluded with certainty.) Therefore to

unequivocally determine the presence of certain alleles, testing for multiple SNPs may be required. 4) Allele frequencies may vary considerably among patients of different populations and ethnic backgrounds. *CYP2D6*10,* for instance, is very common in Asian populations, and *CYP2D6*17* is common in people of Sub-Saharan African descent. These alleles, however, have a considerably lower prevalence, or are even absent, in other ethnic groups such as Caucasians of European ancestry. Another example is *CYP2D6*14A:* unless the *CYP2D6*14A* 'key' SNP 1758G>A is tested, heterozygosity of 100C>T and 2850G>A may lead to an assignment of *CYP2D6 *2/*10* and not the correct *CYP2D6*1/*14A* assignment. *CYP2D6*14* is present in Asian populations and therefore has been incorporated in Asian genotyping panels.(8) Thus, the alleles that should be tested for a given population may vary considerably. 5) Certain alleles carry genes in tandem arrangements. One such example is *CYP2D6*36+*10* (one copy of the non-functional *CYP2D6*36* and one copy of the reduced function *CYP2D6*10*). This tandem is predominantly detected in Asians and is typically reported as a default assignment of *CYP2D6*10*. Lastly, 6) rare or private SNPs may interfere with PCR amplification and/or detection on a particular platform or assay as exemplified by the drop-out of a rare *CYP2D6*6* allele using TaqMan assay technology (9) or a *CYP2D6*4* subvariant that also eludes detection using the commercially available TaqMan assay (Gaedigk, unpublished observations).

Effect of enhancer sequences on CYP2D6 gene expression

A recent study identified two SNPs that appear to impact the transcription of the *CYP2D6* gene (10). These completely linked SNPs (rs5758550 and rs133333, MAF 13-42%) are located in an enhancer region over 100 kb downstream of the *CYP2D6* gene. In vitro experiments demonstrated an increase in transcription levels of up to 2.5-fold when these SNPs were present suggesting that this enhancer interacts with the *CYP2D6* promoter. Furthermore, the effect of these SNPs were demonstrated in a pediatric cohort that was phenotyped with the CYP2D6 probe substrate dextromethorphan (urinary metabolic ratio of dextromethorphan/dextrorphan) and extensively genotyped. These findings, however, need to be substantiated in other population samples and the effect of the enhancer SNPs further evaluated for allelic variants that are in LD with rs5758550 and rs133333. Adjusting activity score values based on the absence or presence of these SNPs may fine-tune phenotype prediction in the future. These SNPs are currently not available for testing by reference laboratories.

Available Genetic Test Options

Commercially available genetic testing options change over time. Additional updated information can be found at: http://www.pharmgkb.org/resources/forScientificUsers/pharmacogenomic_tests.jsp. Furthermore, the Genetic Testing Registry (GTR) provides a central location for voluntary submission of genetic test information by providers and is available at http://www.ncbi.nlm.nih.gov/gtr/tests/?term=cyp2d6.

Other Considerations

Other genes affecting codeine metabolism and response

Glucuronidation of codeine and of morphine is mediated by the polymorphic UGT2B7 enzyme.(11) Although the production of morphine-6-glucuronide is almost exclusively catalyzed by UGT2B7, several isoforms of the UGT1A subfamily are also involved in the formation of morphine-3-glucuronide. Conflicting evidence exists regarding the impact of the *UGT2B7*2* variant on the glucuronidation of codeine.(12) Polymorphisms in the *ABCB1* transporter (*MDR1*) gene also appear to have a modest association with opioid dose requirements.(13) The response to codeine may also be influenced by polymorphisms in drug response genes including, but not limited to, the opioid receptor μ_1 gene *OPRM1,* although the importance of this gene on clinical outcome is not yet fully appreciated.(13)

Effect of pregnancy on CYP2D6

Wadelius et al. demonstrated an increase in CYP2D6 activity by measuring dextromethorphan/dextrorphan metabolic ratio that was decreased by 53% in pregnancy, while Heikkinen et al. demonstrated that the norfluoxetine/fluoxetine metabolic ratio increased 2.4-fold.(14, 15) The apparent oral clearance of metoprolol was shown to increase by 4-5-fold during pregnancy.(16) Although mean CYP2D6 activity appears to increase during pregnancy, the large interindividual variability in the increase and the limited number of subjects studied make it difficult to recommend how to adjust the activity scores of functional alleles during pregnancy. The CYP2D6 activity scores of nonfunctional alleles are not affected by pregnancy.

Levels of Evidence

The evidence summarized in Supplemental Table S6 is graded using a scale based on previously published criteria(17) and applied to other CPIC guidelines:(18-20)

- **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.

Every effort was made to present evidence from high-quality studies, which provided the framework for the strength of therapeutic recommendations.

Strength of Therapeutic Recommendations

CPIC's therapeutic recommendations are based on weighting the evidence from a combination of preclinical functional and clinical data. Some of the factors that are taken into account in evaluating the evidence supporting therapeutic recommendations include: *in vivo* pharmacokinetic and pharmacodynamic data for codeine, *in vitro* enzyme activity of tissues expressing wild-type or variant-containing CYP2D6, *in vitro* CYP2D6 enzyme activity from tissues isolated from individuals of known *CYP2D6* 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 just three categories for recommendations adopted from the rating scale for evidence-based recommendations on the use of retroviral agents (http://aidsinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf): 'strong', where "the evidence is high quality and the desirable effects clearly outweigh the undesirable effects"; 'moderate', in

which “there is a close or uncertain balance” as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects; and ‘optional’, in which the desirable effects are closely balanced with undesirable effects and there is room for differences in opinion as to the need for the recommended course of action (18, 21).

- ‘Strong’ recommendation for the statement
- ‘Moderate’ recommendation for the statement
- ‘Optional’ recommendation for the statement

Supplemental Table S1. Frequencies[1] of *CYP2D6* alleles in major race/ethnic groups[2]

Allele	African	African American	Caucasian (European + North American)	Middle Eastern	East Asian	South/Central Asian	Americas	Oceanian
1[3]	39.23	40.60	53.63	58.04	34.17	53.70	64.28	70.15
2[4]	20.12	14..15	26.91	21.72	12.82	31.90	23.48	1.20
3	0.03	0.31	1.32	0.10	0.00	0.00	0.73	0.00
4	3.36	6.23	18.50	7.80	0.42	6.56	11.28	1.13
5	6.07	6.14	2.69	2.34	5.61	2.54	1.88	4.95
6	3.05	0.24	0.95	0.72	0.02	0.00	0.43	0.00
7	0.00	0.00	0.11	0.00	0.00	ND	0.00	0.00
8	0.00	0.00	0.02	0.00	0.00	ND	0.07	0.00
9	0.10	0.48	2.14	0.00	0.07	1.43	1.32	0.00
10	6.77	4.18	3.16	3.49	42.31	19.76	3.37	1.60
14	0.26	0.00	0.00	0.00	0.86	0.00	0.33	0.00

17[5]	19.98	18.22	0.32	1.58	0.01	0.38	3.0	0.05
36	0.00	0.56	0.00	0.00	1.58	ND	0.25	0.00
41[6]	10.94	9.41	8.56	20.37	1.97	10.50	5.93	0.00
1xN[7]	1.47	0.44	0.80	3.07	0.28	0.50	0.73	11.83
2xN[7]	1.56	1.61	1.27	3.87	0.38	0.5	2.38	0.00
4xN[7]	1.40	2.07	0.25	0.00	0.00	0.00	0.60	0.00

ND: not determined.

[1]Average frequencies are based on actual numbers of subjects with each allele reported in multiple studies. For full details and references please see http://www.pharmgkb.org/download.action?filename=CYP2D6_Literature_Table_and_Legend.pdf.

[2]Worldwide race/ethnic designations correspond to the Human Genome Diversity Project-Centre Etude Polymorphism Humain (HGDP-CEPH).(22, 23)

[3]Note that because *CYP2D6*1* is not genotyped directly, all alleles testing negative for a sequence variation are defaulted to a *CYP2D6*1* assignment. Likewise, sequence variations of alleles that were not tested for also default to a *CYP2D6*1* assignment and hence contribute to the frequencies reported for this allele. Its inferred frequency is calculated as: 100% - (sum of variant allele frequencies reported in %).

[4]*CYP2D6*2* is a 'default' assignment and, unless tested and discriminated, *CYP2D6*8, *11, *17, *35, *41* among others are defaulted to a *CYP2D6*2* assignment. Its frequency as shown here may therefore be over-estimated.

[5]*CYP2D6*17* is a 'default' assignment and, unless tested and discriminated, includes the rare *CYP2D6*40* and *58* variants.

[6]Note that *CYP2D6*41* has not consistently been determined by its key SNP 2988G>A across studies; some platforms still use the -1584C>G SNP to discriminate between *CYP2D6*2* and **41*. This may lead to an overestimation of the *CYP2D6*41* frequency especially in Africans and their descendents.

[7]*CYP2D6*1xN*. **2xN* and **4xN* frequencies shown here represent those from studies that discriminated allele duplications. Duplications/multiplications that were defaulted to a *CYP2D6*2xN* assignment, i.e. the test determined the presence of a duplication, but did not determine the nature of the duplicated gene, were excluded as they may inflate the actual frequency of *CYP2D6*2xN*.

Supplemental Table S2. Commonly tested polymorphisms defining *CYP2D6* variant alleles and their effect on CYP2D6 protein.

Allele[a]	Major Nucleotide Variation[b,c]	dbSNP Number[d]	Effect on CYP2D6 Protein
1 [e]	-	-	-
1xN	Gene duplication or multiplication	-	Increased protein expression
2[f]	2850C>T 4180G>C[g]	rs16947, rs1135840	R296C S486T
2xN	Gene duplication or multiplication	-	Increased protein expression
3	2549delA	rs35742686	Frameshift
4	100C>T, 1846G>A [4180G>C[g]]	rs1065852, rs3892097 rs1135840	P34S, splicing defect [S486T]
4xN	Gene duplication or multiplication	-	P34S, splicing defect
5	Gene deletion	N/A	Gene deletion
6	1707delT	rs5030655	Frameshift
10	100C>T 4180G>C [g]	rs1065852, rs1135840	P34S S486T
17	1023C>T 2850C>T 4180G>C [g]	rs28371706, rs16947, rs1135840	T107I R296C S486T
41	2850C>T 2988G>A 4180G>C [g]	rs16947, rs28371725, rs1135840	R296C Splicing defect S486T

[a]See Human Cytochrome P450 Allele Nomenclature Committee website (http://www.cypalleles.ki.se) for comprehensive haplotype definitions of *CYP2D6* variant alleles and updated allele information.

[b]Based on accession # M33388.

[c]Some of the alleles may carry multiple nucleotide variations. More specific details on the combinations of SNPs present in each allele can be found at http://www.cypalleles.ki.se or http://www.pharmgkb.org/gene/PA128#tabview=tab4. In addition, the specific SNPs included in the genotyping assays can be found in the assays' product inserts.

[d]RefSNP accession ID number (*http://www.ncbi.nlm.nih.gov/snp/*).

[e]The *CYP2D6*1* allele is characterized by the absence of any sequence variations. Consequently, this allele cannot be identified by a SNP; rather *CYP2D6*1* is assigned by default when no SNPs are detected during testing.

[f]The *CYP2D6*2* allele is characterized by two amino acid changes; both, however also occur in many other alleles. Therefore, if an allele carries these two SNPs exclusively, it is designated *CYP2D6*2*. This is the only way to truly distinguish *CYP2D6*2* from other alleles (e.g., *CYP2D6*17* and **41*).

[g]This SNP is present on many allelic variants including functional and non-functional variants. Specifically, it has been found on some *CYP2D6*4* subvariants. While some tests include this SNP, it cannot be utilized to identify an allelic variant with certainty.

SUPPLEMENTAL TABLE S3. ASSOCIATION BETWEEN ALLELIC VARIANTS[A] AND CYP2D6 ENZYME ACTIVITY

Functional Status	Activity Value[c,d]	Alleles
Increased function	>1	**1xN, *2xN, *35xN, *45*[g]*xN*
Normal or Increased function	1 or >1[h]	**9xN, *10xN, *17xN, *29xN, *41xN*
Normal function[b]	1	**1*[e]*, *2, *27, *33, *34*[f]*, *35, *39*[f]*, *45*[g]*, *46*[g]*, *48, *53*
Decreased function	0.5	**9, *10*[i]*, *14B,*17, *29, *41, *49, *50, *54, *55, *59, *72*
No-function	0	**3, *3xN, *4, *4xN, *5, *6, *6xN, *7, *8, *11, *12, *13, *14A, *15, *18, *19, *20, *21, *31, *36, *36xN, *38, *40, *42, *44, *47, *51, *56, *57, *62,*68, *69, *92, *100, *101*
Unknown	N/A	**22, *23, *24, *25, *26, *28, *30, *32, *37, *43, *43xN, *52, *58, *60, *61, *63, *64, *65, *70, *71, *73, *74, *75, *81, *82, *83, *84, *85, *86, *87, *88, *89, *90, *91, *93, *94, *95, *96, *97, *98, *102, *103, *104, *105*

[a]See http://www.cypalleles.ki.se/cyp2d6.htm for updates on *CYP2D6* allelic variants and nomenclature.

[b]An important caveat for all genotyping tests is that the decision to assign an allele a wild-type status is based upon a genotyping test that interrogates only the most common and already-proven sites of functional variation. It is always possible that a new, previously undiscovered (and therefore un-interrogated) site of variation is defaulted to a functional allele assignment

(wild-type). There is a rare possibility that such variation confers reduced or no activity in an individual and that the person's CYP2D6 function is not accurately predicted.

[c]For some allelic variants there is no or sparse information regarding their activity; therefore no value can be assigned and no CYP2D6 activity score can be calculated. In such cases, the activity score may be estimated based on the second/known allele. A recent in vitro investigation using tamoxifen as substrate provides preliminary information for alleles listed here as unknown (PMID: 24647041).

[d]For certain *CYP2D6* alleles *in vivo* data are lacking to unambiguously assign an activity value. For instance, the *CYP2D6*10* and **17* activity values may be substrate dependent, and for particular drugs the activity value could be closer to 1 (normal function) or 0 (no function). It should be noted that the CYP2D6 activity score is a nominal scale. An allele with an activity score of 0.5 does not necessarily have half the metabolic activity of an allele with an activity score of 1. Rather the score of 0.5 indicates the allele has decreased metabolic activity when compared to the *CYP2D6*1* reference allele.

[e]*CYP2D6*1* serves as reference and is defined as wild-type.

[f] Function of *CYP2D6*34* and **39* is extrapolated from **2*. Both star alleles have SNP(s) that are part of the **2* haplotype.

[g]Limited data are available to determine the predicted activity value of *CYP2D6*45* and **46*. Although an activity value of 1 (functional) is assigned to *CYP2D6*45* and **46* in this guideline, others may assign an activity value of 0.5 (reduced function).

[h]Activity value is dependent on the number of duplications/multiplications present.

[i] Although *CYP2D6*10* has been associated with a marked reduction in enzyme activity, an activity score of 0.5 is assigned to this allele as well as all other allelic variants conferring reduced activity. Consequently, *CYP2D6*10/*10* genotypes receive an activity score of 1.0, which leads to an extensive metabolizer classification of subjects with this genotype (and genotypes consisting of two reduced function alleles). This classification is, however, particularly controversial for *CYP2D6*10/*10.* To evaluate whether a revision of the value assigned to *CYP2D6*10* is warranted, a systematic literature search was performed and assessed as described in more detail in a review article (PMID: 24524666). The available body of literature revealed strong evidence for some drugs in support for assigning a reduced value of e.g. 0.25 to the *CYP2D6*10* allele, a change that would classify *CYP2D6*10/*10* as intermediate metabolizers in this guideline. However, there were only sparse data for codeine and tramadol and the three available reports were rated as providing moderate and moderate/weak evidence, respectively.

Supplemental Table S4. Examples of *CYP2D6* genotypes with resulting activity scores and phenotype classification.

Allele 1	Allele 2	CYP2D6 Diplotype	CYP2D6 Activity Score	Phenotype
**1*	**1xN*[a]	**1/*1xN*	≥3.0	UM
**2x2*[b]	**41*	**2x2/*41*	2.5	UM
**1*	**2*	**1/*2*	2.0	EM
**1*	**17*	**1/*17*	1.5	EM
**2*	**3*	**2/*3*	1.0	EM
**1*	**4x2*	**1/*4x2*[c]	1.0	EM
**10*	**10*	**10/*10*	1.0	EM[e]
**4*	**10*	**4/*10*	0.5	IM
**5*	**6*	**5/*6*[d]	0	PM

EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer; UM: ultrarapid metabolizer. Extensive metabolizers with an activity score of 2.0 are expected to exhibit higher CYP2D6 enzyme activity versus individuals with activity scores of 1.5 and 1.0, respectively.

See www.pharmgkb.org and http://www.cypalleles.ki.se/cyp2d6.htm for updates on *CYP2D6* alleles and nomenclature

[a] **1xN* denotes that the allele carries 2 or more copies of a normal activity *CYP2D6*1* gene. In case of a duplication (2 copies), an activity score value of 2 will be assigned; in case of 3 gene copies, a value of 3 will be assigned, etc. Therefore, if paired with a second functional allele, the activity score is ≥3 depending on the number of genes present.

[b] **2x2* denotes an allele that carries two functional gene copies. In this example the gene duplication is paired with a *CYP2D6*41* allele that carries one copy of a reduced function allele.

[c] Regardless of the number of copies present, *CYP2D6*4* and **4xN* are always non-functional.

[d] The 1707delT variation will present as homozygous in a test due to the absence of a gene copy on the second allele. If no test is performed for the *CYP2D6*5* gene deletion, the genotype will be assigned as homozygous *CYP2D6*6/*6* which is technically inaccurate, but correctly predicts a PM phenotype. The same may occur in the presence of *CYP2D7/2D6* hybrid genes.

[e] Note that some investigators may define patients with a *CYP2D6*10/*10* genotype as intermediate metabolizers. The classification used in this guideline is based on data specific for formation of morphine from codeine.(25, 26). Also see footnote [e] in Supplemental Table S3.

Supplemental Table S5. Predicted metabolizer phenotypes based on *CYP2D6* diplotypes (allele combinations).

		Predicted Metabolizer Phenotype (Range Multi-Ethnic Frequency[a])								
Allele	***1***	***2***	***1xN or *2xN***	***3***	***4 or *4xN***	***5***	***6***	***10***	***17***	***41***
1	**EM**	**EM**	**UM**	**EM**	**EM**	**EM**	**EM**	**EM**	**EM**	**EM**
2		**EM**	**UM**	**EM**	**EM**	**EM**	**EM**	**EM**	**EM**	**EM**
1xN or *2xN			**UM**	**EM or UM**	**EM or UM**	**EM or UM**	**EM or UM**	**UM**	**UM**	**UM**
3				**PM**	**PM**	**PM**	**PM**	**IM**	**IM**	**IM**
4					**PM**	**PM**	**PM**	**IM**	**IM**	**IM**
5						**PM**	**PM**	**IM**	**IM**	**IM**
6							**PM**	**IM**	**IM**	**IM**
10								**EM[b]**	**EM[b]**	**EM[b]**
17									**EM[b]**	**EM[b]**
41										**EM[b]**

EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer; UM: ultrarapid metabolizer

[a] Frequencies of predicted metabolizer phenotypes can be estimated based on the frequencies provided in Table S1.

[b] Note that some investigators may define patients with these diplotypes as intermediate metabolizers. The classification used in this guideline is based on data specific for formation of morphine from codeine.(25, 26) Also see footnote [e] in Supplemental Table S3.

Supplemental Table S6. Evidence linking CYP2D6 phenotype or genotype with codeine metabolism or response.

Type of experimental model (in vitro, in vivo preclinical, or clinical)	Major findings	References	Level of evidence*
In Vitro	Decreased Vmax and higher apparent Km for codeine *O-demethylation* to morphine in human liver microsomes with PM phenotype by dextromethorphan metabolism versus EM phenotype	Dayer *et al.* 1988 (27)	High
In Vitro	Less morphine formation from codeine *O*- demethylation in human liver microsomes with PM phenotype by dextromethorphan versus EM phenotype	Mortimer *et al.* 1990 (28)	High
In Vitro	Higher apparent Km for codeine *O*- demethylation to morphine in microsomes prepared from yeast cells expressing human CYP2D6 with PM genotype versus EM genotype	Oscarson *et al.* 1997 (29)	High
In Vitro	Decreased Vmax for codeine *O-demethylation* to morphine in microsomes prepared from insect cells expressing human CYP2D6 reduced-function alleles versus *1 alleles	Yu *et al.* 2002 (30) Shen *et al.* 2007 (31) Zhang *et al.* 2009 (32)	High
Preclinical	No analgesia observed in rats deficient for CYP2D1, a homolog for *CYP2D6* in humans, after codeine administration	Cleary et al. 1994 (33)	High
Clinical	CYP2D6 IM phenotype by drug metabolism assay associated with lower formation or excretion of morphine and related metabolites following codeine administration versus EM phenotype	Chen *et al.* 1988 (34)	High

Clinical	CYP2D6 PM phenotype by drug metabolism assay associated with lower formation or excretion of morphine and related metabolites following codeine administration versus EM phenotype	Yue *et al.* 1989 (35) Chen *et al.* 1988 (34) Sindrup *et al.* 1990 (36) Chen *et al.* 1991 (37) Desmeules et al. 1991(38) Caraco *et al.* 1996 (39) Poulsen *et al.* 1996 (40) Caraco *et al.* 1997 (41) Hasselström *et al.* 1997 (42) Hedenmalm *et al.* 1997 (43) Mikus *et al.* 1997 (44) Poulsen *et al.* 1998 (45) Eckhardt *et al.* 1998 (46) Lötsch *et al.* 2006 (47)	High
Clinical	Reduced or no analgesia observed in CYP2D6 PM phenotype by drug metabolism assay	Sindrup *et al.* 1990 (36) Desmeules et al. 1991 (38) Poulsen *et al.* 1996 (40) Eckhardt *et al.* 1998 (46)	High
Clinical	CYP2D6 PM phenotype by drug metabolism assay associated with reduced opioid associated adverse effects following codeine administration versus EM phenotype	Caraco *et al.* 1996 (39) Mikus *et al.* 1997 (44)	High
Clinical	CYP2D6 PM genotype associated with reduced formation or excretion of morphine and related metabolites following codeine administration	Tseng et al. 1996 (48) Eckhardt *et al.* 1998 (46) Williams *et al.* 2002 (49) Lötsch *et al.* 2009 (13) Molanaei *et al.* 2010 (50)	High

Clinical	Rifampin induced codeine metabolism to morphine in EM but not PM phenotype by drug metabolism assay	Caraco *et al.* 1997 (41)	High
Clinical	CYP2D6 PM phenotype by drug metabolism assay no difference in adverse effect profile in PM versus EM following codeine administration	Hasselström *et al.* 1997 (42) Eckhardt *et al.* 1998 (46)	High
Clinical	No association between *CYP2D6* genotype and analgesia after codeine administration	Vree *et al.* 2000 (51) Williams *et al.* 2002 (49)	High
Clinical	No significant difference in plasma concentration of morphine and related metabolites in IM genotypes versus EM genotype	Williams *et al.* 2002 (49) Lötsch *et al.* 2006 (47)	High
Clinical	Higher plasma concentrations of morphine and related metabolites following codeine administration in healthy volunteers with *CYP2D6* gene duplication (> 2 functional alleles) than in carriers of 2 functional *CYP2D6* alleles; greater incidence of sedation in UM versus EM	Kirchheiner *et al.* 2007 (52)	High
Clinical	Low morphine formation following codeine administration in PM predicted by *CYP2D6* genotyping or dextromethorphan-based phenotyping; high morphine formation in UM predicted by combining dextromethorphan- based phenotyping and *CYP2D6* genotyping	Lötsch *et al.* 2009 (13)	High
Clinical	African-American patients with variant *CYP2D6* alleles (*7, *29, *41) had significantly lower excretion of morphine and related metabolites after codeine vs those without variant alleles	Shord *et al.* 2009 (53)	High

Clinical	Heterozygous EMs (*1/*4) associated with lower urinary excretion of morphine and related metabolites following codeine and paracetamol or levomepromazine with codeine and paracetamol administration versus homozygous EMs (*1/*1)	Vevelstad *et al.* 2009 (25)	High
Clinical	Decreased analgesia from codeine observed in CYP2D6 PMs by genotype	Persson *et al.* 1995 (54) Fagerlund *et al.* 2001 (55) Foster *et al.* 2007 (56) vanderVaart *et al*. 2011(57)	Moderate
Clinical	Increased opioid related adverse events, including fatal toxicity, observed in CYP2D6 UMs by genotype following normal doses of codeine	Dalen *et al.* 1997 (58) Gasche *et al.* 2004 (59) vanderVaart *et al*. 2011 (57) Ciszkowski *et al.* 2009 (60) Kelly *et al*. 2012 (61)	Moderate
Clinical	Increased opioid-related adverse events, including fatal toxicity, in infants breastfed by a CYP2D6 UM mother	Koren *et al.* 2006 (62) Madadi et al. 2009 (63) Sistonen *et al.* 2012 (64)	Moderate
Clinical	Severe opioid related adverse events, including respiratory depression and hypoxia, observed in children with EM genotype after receiving codeine	Kelly *et al*. 2012 (61) Friedrichsdorf *et al.* 2013 (65) Voronov *et al.* 2007 (66)	Weak
Clinical	CYP2D6 genotype was not a predictor of changes in respiratory parameters in pediatric patients receiving codeine	Khetani *et al*. 2012 (67)	Weak

EM: extensive metabolizer; IM: intermediate metabolizer; PM: poor metabolizer; UM: ultrarapid metabolizer

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