# Assigning level of evidence to PharmGKB clinical annotations

Rachel Huddart Ph.D.

Scientific Curator, PharmGKB

# Introduction to PharmGKB

- The Pharmacogenomics Knowledgebase (PharmGKB) www.pharmgkb.org
- Collect, curate and disseminate knowledge about the impact of human genetic variation on drug response.
- Active collaborator in pharmacogenomics

CPIC

Pharmcat

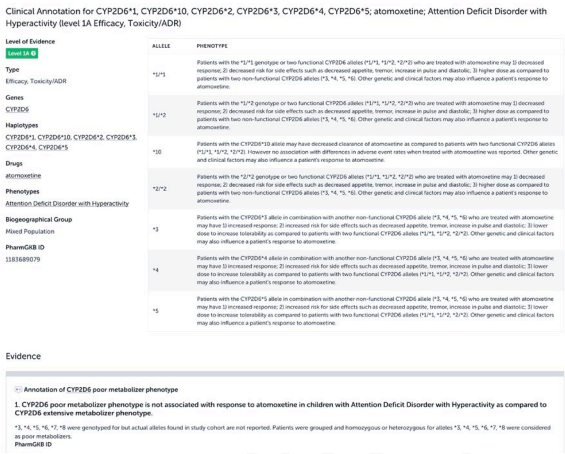
PharmVar
Pharmacogene Variation Consortium

# PharmGKB curation

Variant Annotation - Single sentence recording a single finding from a single paper.

Clinical Annotation – Summaries of all curated information on a variant-drug pair.

| | VARIANT | PMID | MOLECULES | ASSOCIATION |
|---|---|---|---|---|
| Read Now | CYP2D6*1, CYP2D6*17 | 17470523 | bufuralol | CYP2D6 *17 is associated with decreased clearance of bufuralol as compared to CYP2D6 *1. |
| Read Now | CYP2D6*1, CYP2D6*17 | 17470523 | atomoxetine | CYP2D6 *17 is associated with decreased clearance of atomoxetine as compared to CYP2D6 *1. |
| Read Now | CYP2D6*1, CYP2D6*17 | 17470523 | fluoxetine | CYP2D6 *17 is associated with decreased clearance of fluoxetine as compared to CYP2D6 *1. |



# PharmGKB clinical annotations

- Started providing clinical annotations in 2010
- PharmGKB curators assign a Level of Evidence to represent the strength of the evidence base
- New evidence is added as it is curated into PharmGKB
- One of the most popular features on PharmGKB
  - Part of CPIC's considerations when assigning CPIC levels to gene-drug pairs

# Clinical Annotation for CYP2D6*1, CYP2D6*10, CYP2D6*17, CYP2D6*1xN, CYP2D6*2, CYP2D6*2xN, CYP2D6*3, CYP2D6*4, CYP2D6*40, CYP2D6*41, CYP2D6*5, CYP2D6*6 related to codeine - efficacy/toxicity (1A)

**Level of Evidence**

Level 1A

**Type**

efficacy, toxicity

**Genes**

CYP2D6

**Haplotypes**

CYP2D6*1, CYP2D6*10, CYP2D6*17, CYP2D6*1xN, CYP2D6*2, CYP2D6*2xN, CYP2D6*3, CYP2D6*4, CYP2D6*40, CYP2D6*41, CYP2D6*5, CYP2D6*6

**Drugs**

codeine

**Phenotypes**

Pain

| ALLELE | PHENOTYPE |
|---|---|
| *1/*1 | Patients with the *1/*1 genotype who are treated with codeine may have 1) increased metabolism/clearance of codeine, 2) increased likelihood of response to codeine and 3) decreased, but not absent risk for side effects as compared to patients with non-functional (*3, *4, *5, *6, *40) or reduced function (*10, *17, *41) alleles. Other genetic and clinical factors may also influence a patient's response to codeine. |
| *1/*1xN | Patients with more than two copies of CYP2D6 functional alleles (*1, *2) who are CYP2D6 ultrarapid metabolizers (i.e., a CYP2D6 activity score of >2.0) may have 1) increased formation of morphine following codeine administration and 2) higher risk of toxicity as compared to patients with *1/*1 genotype, who are CYP2D6 normal metabolizers. Patients who are CYP2D6 ultrarapid metabolizers should avoid codeine use due to potential for toxicity. Alternative analgesics such as morphine or a non-opioid should be considered. Other genetic and clinical factors may also influence a patient's response to codeine. |
| *1/*2xN | Patients with more than two copies of CYP2D6 functional alleles (*1, *2) who are CYP2D6 ultrarapid metabolizers (i.e., a CYP2D6 activity score of >2.0) may have 1) increased formation of morphine following codeine administration and 2) higher risk of toxicity as compared to patients with *1/*1 genotype, who are CYP2D6 normal metabolizers. Patients who are CYP2D6 ultrarapid metabolizers should avoid codeine use due to potential for toxicity. Alternative analgesics such as morphine or a non-opioid should be considered. Other genetic and clinical factors may also influence a patient's response to codeine. |
| *10 | Patients with the *10 reduced functional allele in combination with a non-functional (*3, *4, *5, *6, *40) allele may have 1) decreased metabolism/clearance of codeine, 2) decreased likelihood of response to codeine as compared to patients with *1/*1 genotype, who carry two functional alleles. Patients who are CYP2D6 intermediate metabolizers should be monitored closely for less than optimal response and should be offered an alternative analgesic if required. Other genetic and clinical factors may also influence a patient's response to codeine. |
| *17 | Patients with the *17 reduced functional allele in combination |

Overview
PGx Prescribing Info
Clinical Annotations
Variant Annotations
Haplotypes
Literature
Related To
Links & Downloads

Annotation of rs1799971 in OPRM1

**1. Genotype AA is associated with increased reduction in pain when treated with morphine in people with Neoplasms as compared to genotypes AG + GG.**

Not Available

**from publication:**

Association of ABCB1/MDR1 and OPRM1 gene polymorphisms with morphine pain relief *Clinical pharmacology and therapeutics*. 2008. Campa D et al. PubMed 17898703

Paper discusses: efficacy

**Study Parameters**

| | SIZE | ALLELE | FREQUENCY |
|---|---|---|---|
| CASE | 138 | - | - |
| CONTROL | - | - | - |

**P-value:**
< 1.0E-4
**Type:**
cohort
**Race:**
european
**Population Characteristics:**
Disease: opioid-based cancer pain relief therapy

Annotation of rs1799971 in OPRM1

**2. Genotype GG is not associated with increased dose of methadone in people with Heroin Dependence as compared to genotype AA.**

Not Available

**from publication:**

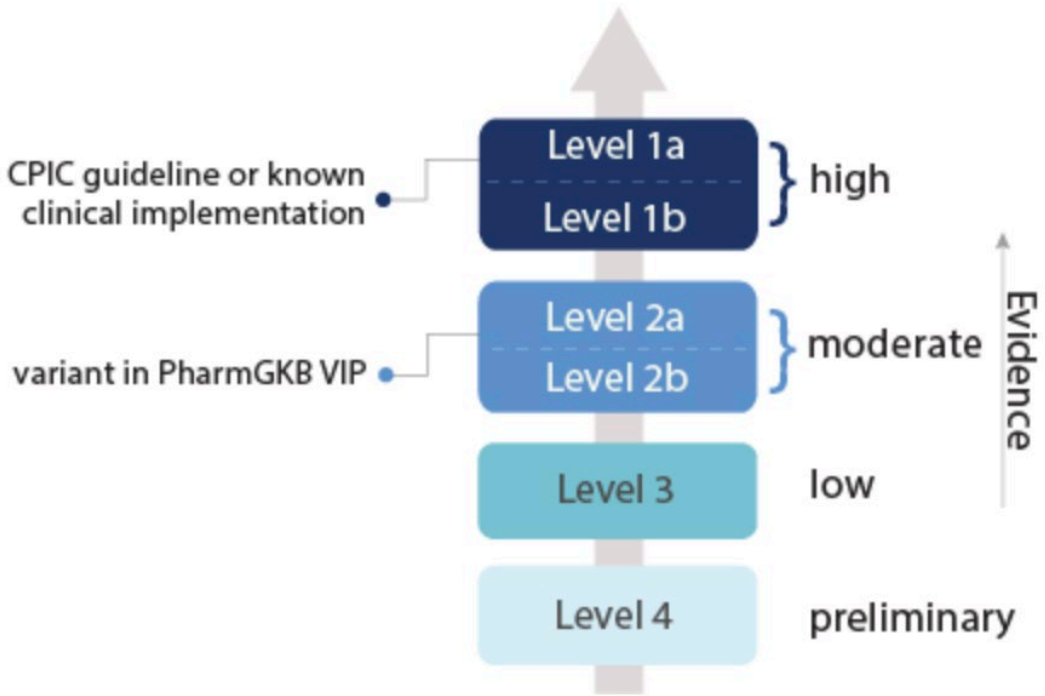
Impact of genetic polymorphisms in ABCB1, CYP2B6, OPRM1, ANKK1 and DRD2 genes on methadone therapy in Han Chinese patients *Pharmacogenomics*. 2011. Hung Chin-Chuan et al. PubMed 21902500

Paper discusses: dosage, metabolism/PK

**Study Parameters**

| | SIZE | ALLELE | FREQUENCY |
|---|---|---|---|
| CASE | 321 | G | 0.33 |
| CONTROL | - | - | - |

**Type:**
cohort
**Race:**
asian
**Population Characteristics:**
Study Cohort: patients undergoing methadone maintenance therapy




## Level 1A

Annotation for a variant-drug combination in a CPIC or medical society-endorsed PGx guideline, or implemented at a PGRN site or in another major health system.

## Level 1B

Annotation for a variant-drug combination where the preponderance of evidence shows an association. The association must be replicated in more than one cohort with significant p-values, and preferably will have a strong effect size.

## Level 2A

Annotation for a variant-drug combination that qualifies for level 2B where the variant is within a VIP (Very Important Pharmacogene) as defined by PharmGKB. The variants in level 2A are in known pharmacogenes, so functional significance is more likely.

## Level 2B

Annotation for a variant-drug combination with moderate evidence of an association. The association must be replicated but there may be some studies that do not show statistical significance, and/or the effect size may be small.

## Level 3

Annotation for a variant-drug combination based on a single significant (not yet replicated) study or annotation for a variant-drug combination evaluated in multiple studies but lacking clear evidence of an association.

## Level 4

Annotation based on a case report, non-significant study or in vitro, molecular or functional assay evidence only.

24,535 variant annotations

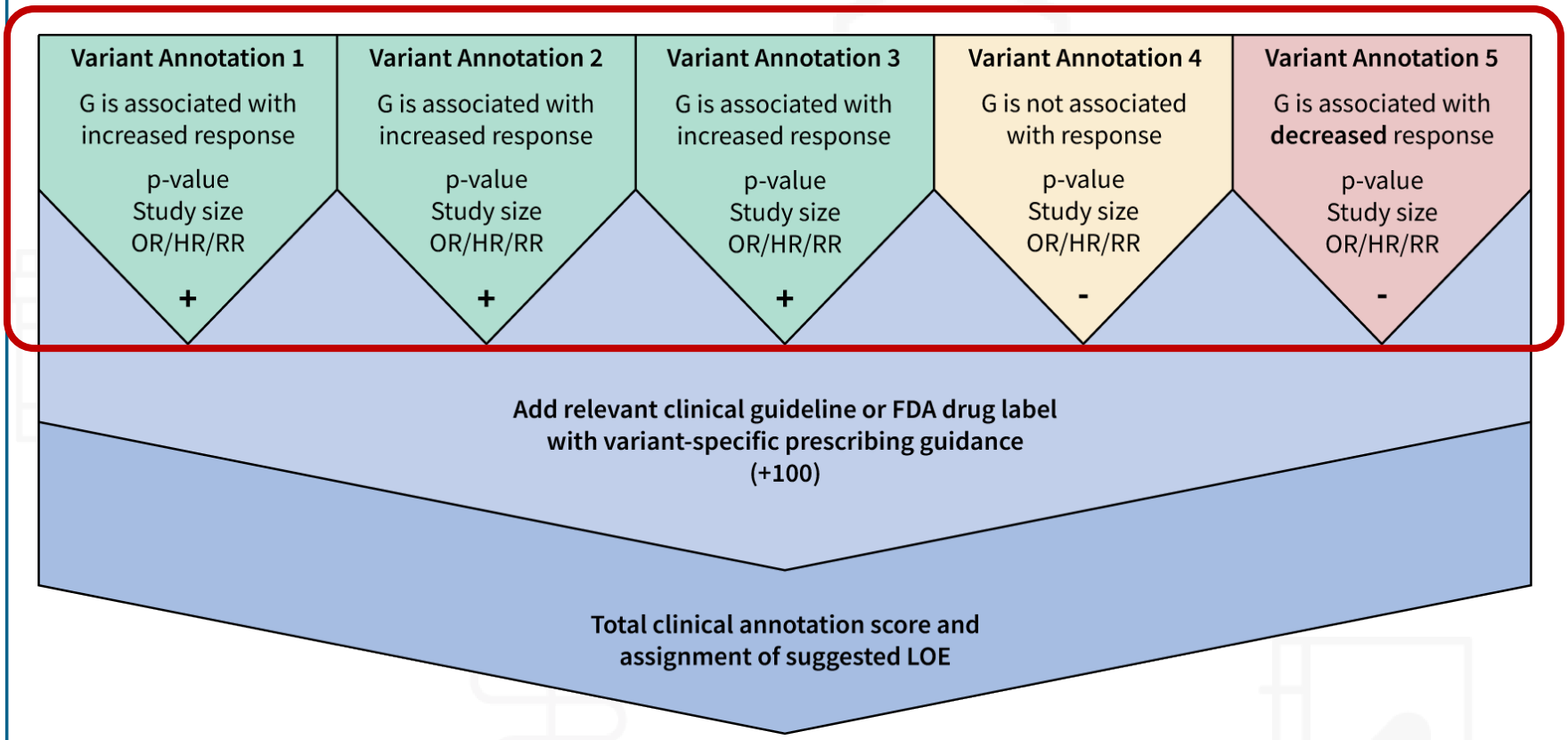
4,723 clinical annotations

| TYPE | N | POSITIVE | MIXED | NEGATIVE |
|---|---|---|---|---|
| VAR ANNS | 26 | 9 | N/A | 17 |
| LITERATURE | 25 | 8 | 1 | 16 |

How can we maintain consistency in assigning level of evidence (LOE) to clinical annotations between curators and over time?

**Automate it!**

# Annotation Scoring System



# Variant Annotation Scoring Algorithm

Process which assigns points to certain attributes of a variant annotation.

(Step 1 + Step 2 + Step 3 + Step 4) * (Step 5A * Step 5B)

**Step 1** – Phenotype category

**Step 2** – p-value

**Step 3** – Cohort size

**Step 4** – Effect size

**Step 5A** – Study type

**Step 5B** – Association and significance

**Scoring of variant annotations is not a judgement of study quality.**

It is a metric used by PharmGKB curators when comparing variant annotations against each other as part of the process of creating and updating clinical annotations.

# Variant Annotation Anatomy

3. Annotation of CYP2D6 poor and ultrarapid metabolizers

CYP2D6 poor and ultrarapid metabolizers is associated with increased likelihood of discontinuation when treated with codeine or tramadol in people with Pain as compared to CYP2D6 normal metabolizer.

**From Publication**

Drug-gene and drug-drug interactions associated with tramadol and codeine therapy in the INGENIOUS trial. *Pharmacogenomics.* 2019. Fulton Cathy R et al.

PMID:30784356 DOI:10.2217/pgs-2018-0205

**Gene :** CYP2D6

**Phenotype Category :** Efficacy, Toxicity, Metabolism/PK

**Association Significance :** The study reports this association is significant

**PharmGKB ID :** 1450376063

**Score :** 1.625

## Study Parameters

1. Used For Scoring

**Study type :** clinical trial

**Study size :** 70

**Association p-value :** = 0.002

**Statistical analysis :** OR: 24.7

**Confidence interval :** 3.6 - 503.1

**Biogeographical group :** Multiple groups

**Population description :**

men; women

Study Cohort: Discontinuation PM/UM vs NM/IM, INGENIOUS trial NCT02297126

2. **Study type :** clinical trial

**Study size :** 70

**Association p-value :** = 0.01

**Statistical analysis :** OR: 19

**Confidence interval :** 2.8 - 160.4

**Biogeographical group :** Multiple groups

**Population description :**

men; women

Study Cohort: Alternate opioid prescribed by 60 days PM/UM vs NM/IM, INGENIOUS trial NCT02297126

# Step 1 – Phenotype category

Studies of clinical outcomes (dosage, efficacy, toxicity) score higher than PK or PD studies

PHARMGKB

Search PharmGKB

Menu Help

## Annotation of rs4680

Allele G is not associated with increased risk of Hyperprolactinemia when treated with risperidone in people with Schizophrenia as compared to allele A.

**From Publication**

Association between dopamine-related polymorphisms and plasma concentrations of prolactin during risperidone treatment in schizophrenic patients. *Progress in neuro-psychopharmacology & biological psychiatry.* 2008. Yasui-Furukori Norio et al.

PMID:18579277 DOI:10.1016/j.pnpbp.2008.05.006

**Variant**

rs4680

**Phenotype Category**

Toxicity

**Association Significance**

The study reports this association is not significant

# Step 2 – p-value

Lowest, most significant p-value is used for scoring here and in subsequent steps. Lower p-values are given higher scores.

Algorithm can check whether GWAS results show genome-wide significance

Study Parameters

1. **Study type :** meta-analysis
   **Study size :** 7027
   **Association p-value :** < 0.001
   **Biogeographical group :** Multiple groups
   **Population description :**
   Study Cohort: P-value given for analysis across all pain types

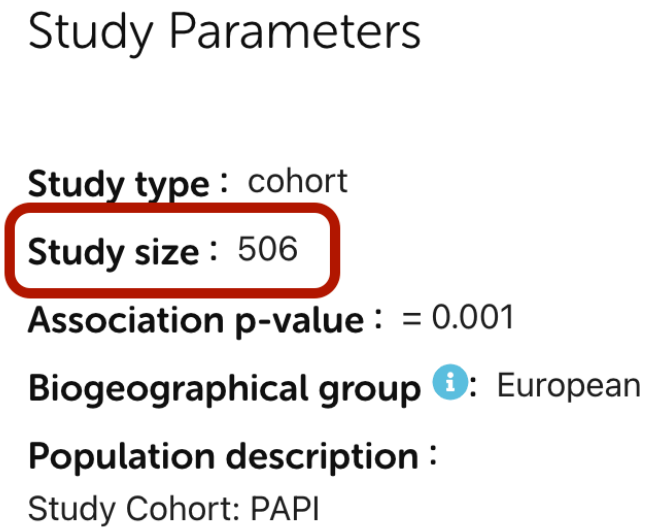
2. **Study type :** meta-analysis
   **Study size :** 507
   **Association p-value :** = 0.143
   **Biogeographical group :** Multiple groups
   **Population description :**
   Study Cohort: P-value given for subanalysis in cancer pain

3. **Study type :** meta-analysis
   **Study size :** 6017
   **Association p-value :** = 0.005
   **Biogeographical group :** Multiple groups
   **Population description :**
   Study Cohort: P-value given for subanalysis in postoperative pain

# Step 3 – Cohort size

Larger cohort sizes increase study power and reduce Type II errors, so are given a higher score.

Study parameters identified in Step 2 are used again here.



# Step 4 – Effect size

Points are awarded to variant annotations with a set of study parameters which meets all the following criteria:

- Reports a OR/HR/RR ≥2 or ≤0.5
- The confidence interval does not cross 1
- Has an associated p-value ≤0.05

**Study type :** cohort

**Study size :** 71 (cases=51; controls=20)

**Association p-value :** = 0.004

**Statistical analysis :** OR: 8.62

**Confidence interval :** 1.96 - 37.57

**Allele frequency :** A: case=0.71, control=0.43

**Biogeographical group :** East Asian

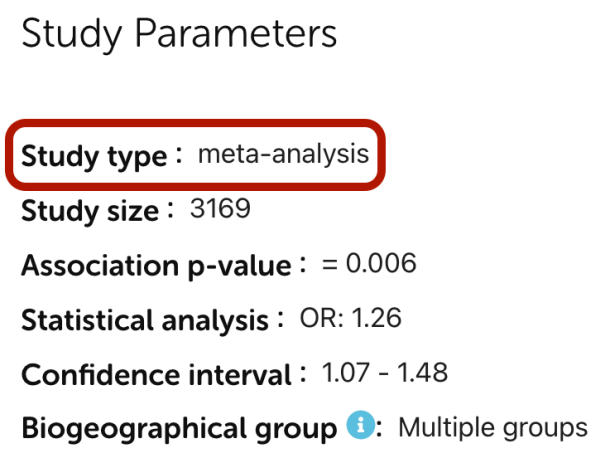
**Population description :**

men; women

Study Cohort: Statistics here for association with hepatic toxicity. Here, cases = grade 1-4 toxicity / controls = grade 0.

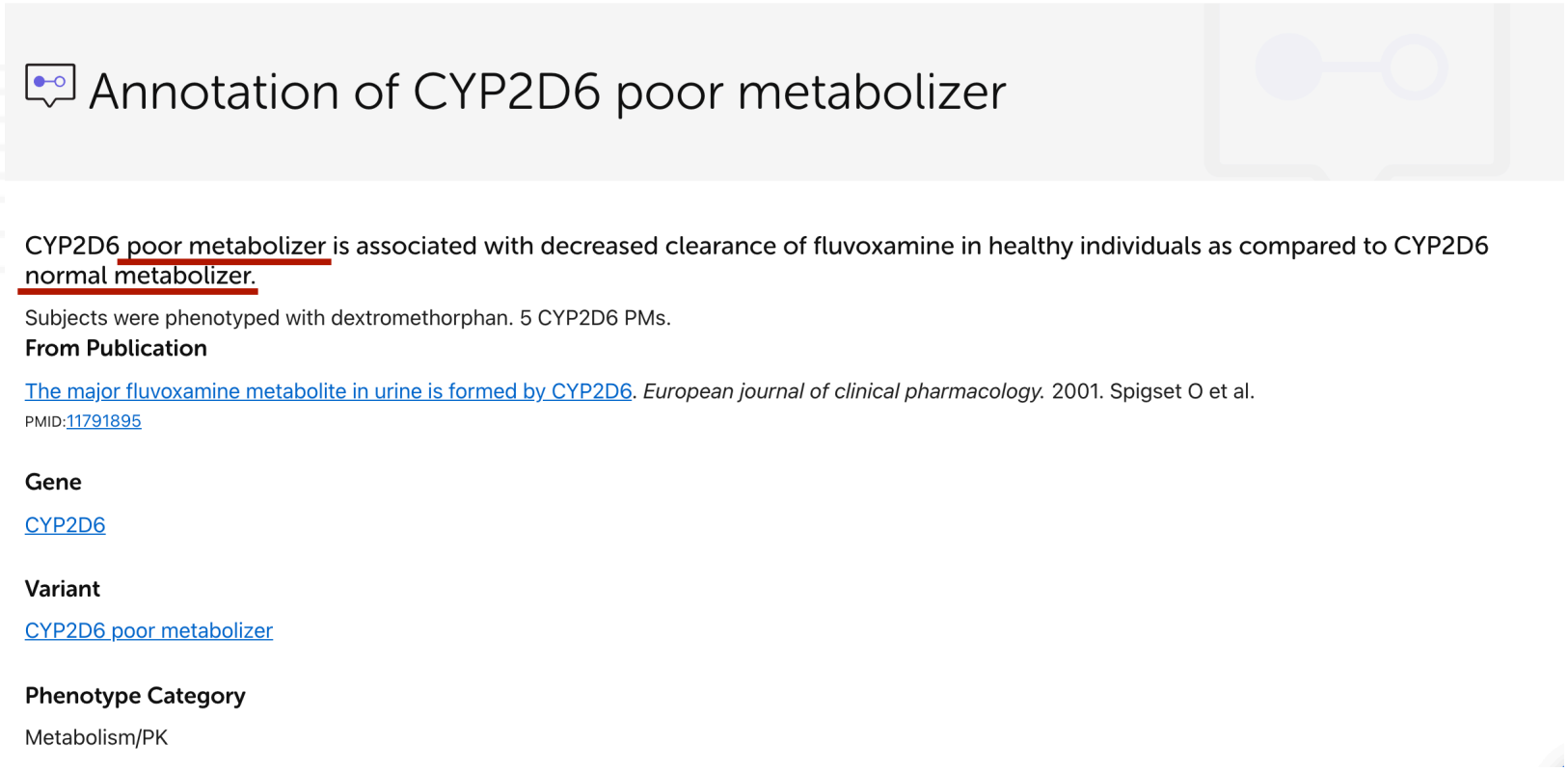
# Step 5A – Study type

Variant annotations from *in vitro* studies and meta-analyses are given a reduced score.



# Step 5A – Study type

Variant annotations from studies which only report on phenotype groups and don't give information about specific diplotypes found in the study cohort are also given a reduced score.



Annotation of CYP2D6 poor metabolizer

CYP2D6 poor metabolizer is associated with decreased clearance of fluvoxamine in healthy individuals as compared to CYP2D6 normal metabolizer.

Subjects were phenotyped with dextromethorphan. 5 CYP2D6 PMs.

**From Publication**

The major fluvoxamine metabolite in urine is formed by CYP2D6. *European journal of clinical pharmacology.* 2001. Spigset O et al.

PMID:11791895

**Gene**

CYP2D6

**Variant**

CYP2D6 poor metabolizer

**Phenotype Category**

Metabolism/PK

# Step 5B – Association and significance

Variant annotations reporting a significant association between a variant-drug pair are given a positive score.

## Annotation of CYP2C19*1, CYP2C19*2

CYP2C19 *1/*2 is associated with metabolism of clobazam in people with Epilepsy as compared to CYP2C19 *1/*1.

The N-desmethylclobazam (N-CLB)/clobazam (CLB) plasma metabolic ratio was significantly higher in subjects carrying a *2 allele as compared to those with the wild-type genotype. N-CLB is the major metabolite of CLB. The authors also note that CYP3A4 and CYP2C19 are the main enzymes involved in the N-demethylation of CLB to N-CLB and that CYP2C19 is the major contributor to the hydroxylation of N-CLB to OH-N-CLB.

**From Publication**

In vitro characterization of clobazam metabolism by recombinant cytochrome P450 enzymes: importance of CYP2C19. *Drug metabolism and disposition: the biological fate of chemicals.* 2004. Giraud Carole et al.

PMID:15483195

**Gene :** CYP2C19

**Haplotype :** CYP2C19*1, CYP2C19*2

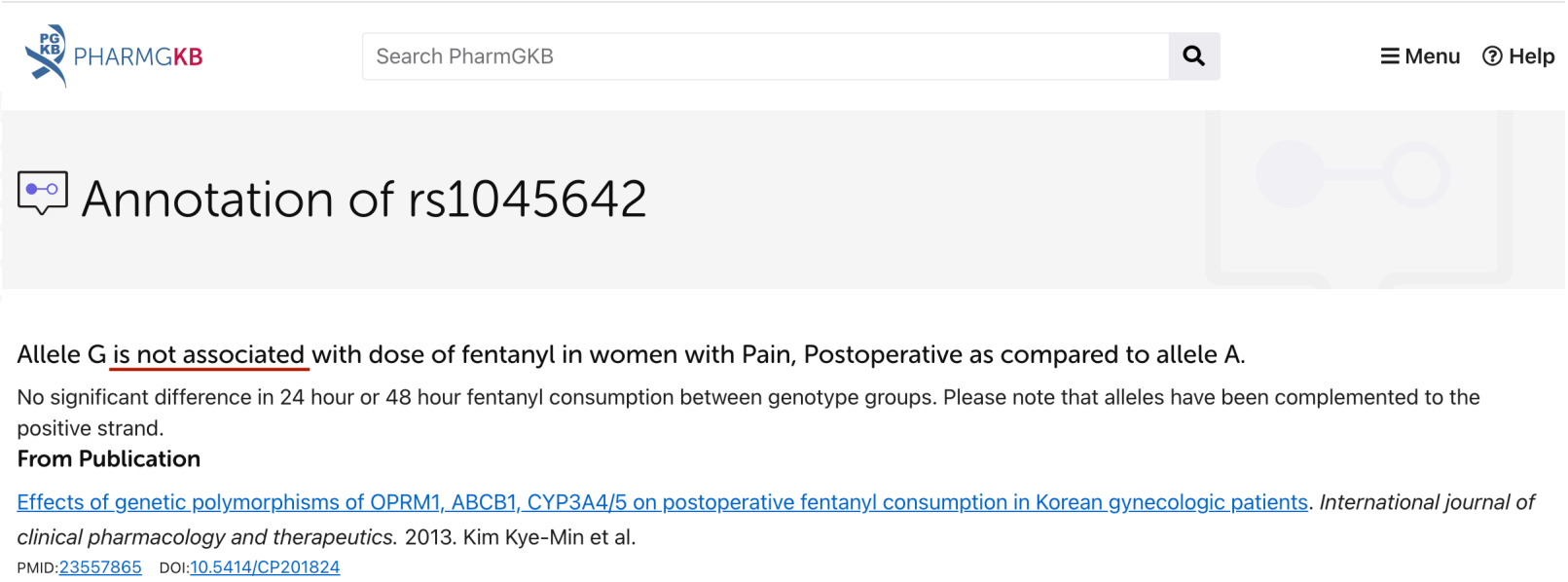
**Phenotype Category :** Metabolism/PK

**Association Significance :** The study reports this association is significant

**PharmGKB ID :** 1444695013

# Step 5B – Association and significance

Variant annotations reporting no association between a variant-drug pair are given a negative score.



# Step 5B – Association and significance

Variant annotations reporting a non-significant association or where the authors don't provide any information about the significance are generally scored 0* and don't contribute to the total clinical annotation score.

* Exception for variant annotations reporting a small (<50) cohort size.

Annotation of CYP2D6 haplotype

CYP2D6 ultrarapid metabolizer is associated with increased metabolism of risperidone in people with Schizophrenia as compared to CYP2D6 poor metabolizers.

Receiver under the operator curve (ROC) method and area under curve (AUC) were calculated and then used to predict metabolic ratio (risperidine/9-OH-risperidone) for individual CYP2D6 genotypes. To evaluate the proposed cutoff of metabolic ratio of <0.01 to predict whether an individual is an ultra rapid metabolizers the sensitivity (80%) specificity (77%), positive predictive value (18%), and negative predictive value (98%) were calculated.

**From Publication**

Risperidone metabolic ratio as a biomarker of individual CYP2D6 genotype in schizophrenic patients. *European journal of clinical pharmacology.* 2014. Mannheimer Buster et al.
PMID:24643635 DOI:10.1007/s00228-014-1664-3

**Gene :** CYP2D6

**Variant :** CYP2D6 ultrarapid metabolizer

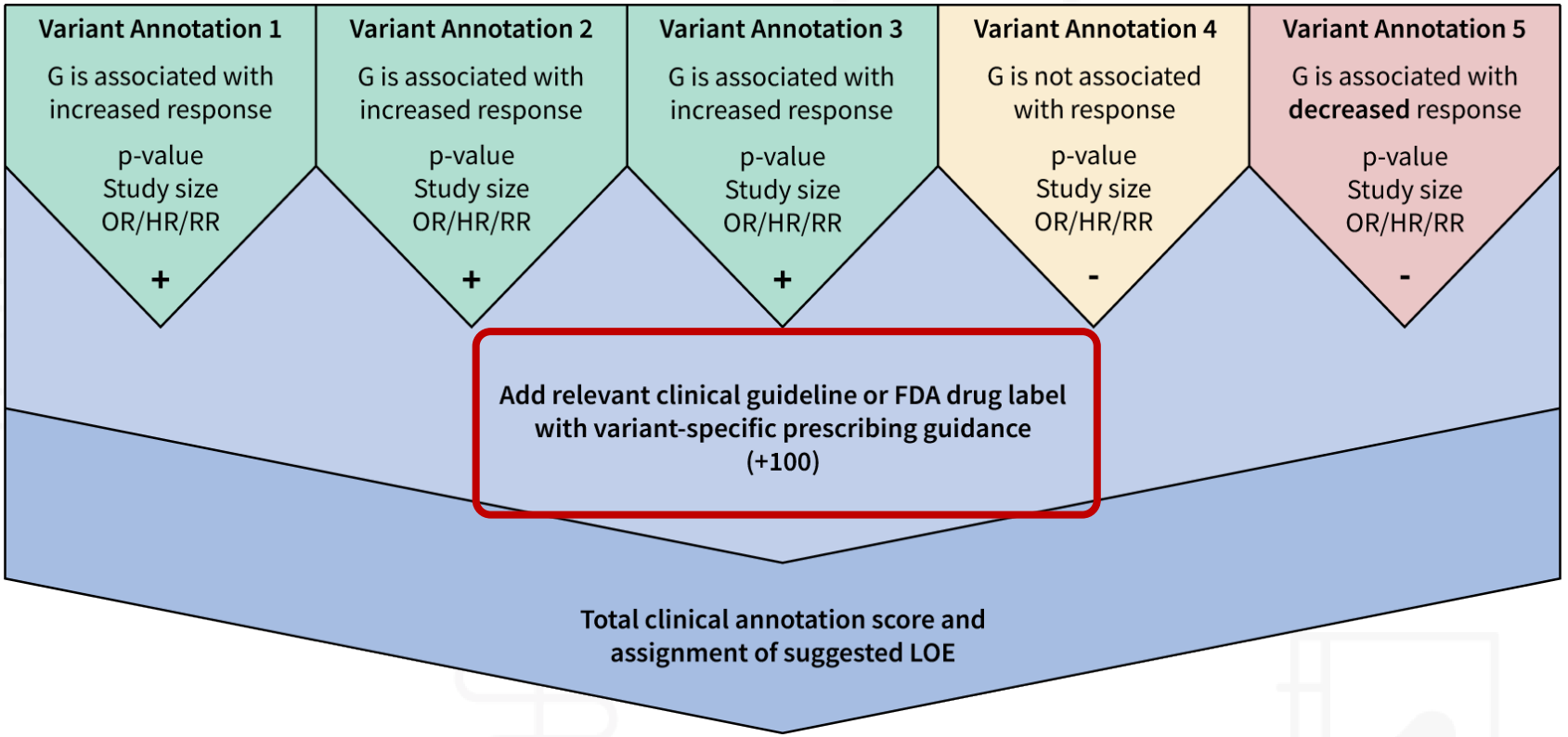
**Phenotype Category :** Metabolism/PK

**Association Significance :** The study does not report on the significance of this association

**PharmGKB ID :** 1184754471

All relevant variant annotations are added to a clinical annotation, regardless of score.

# Annotation Scoring System

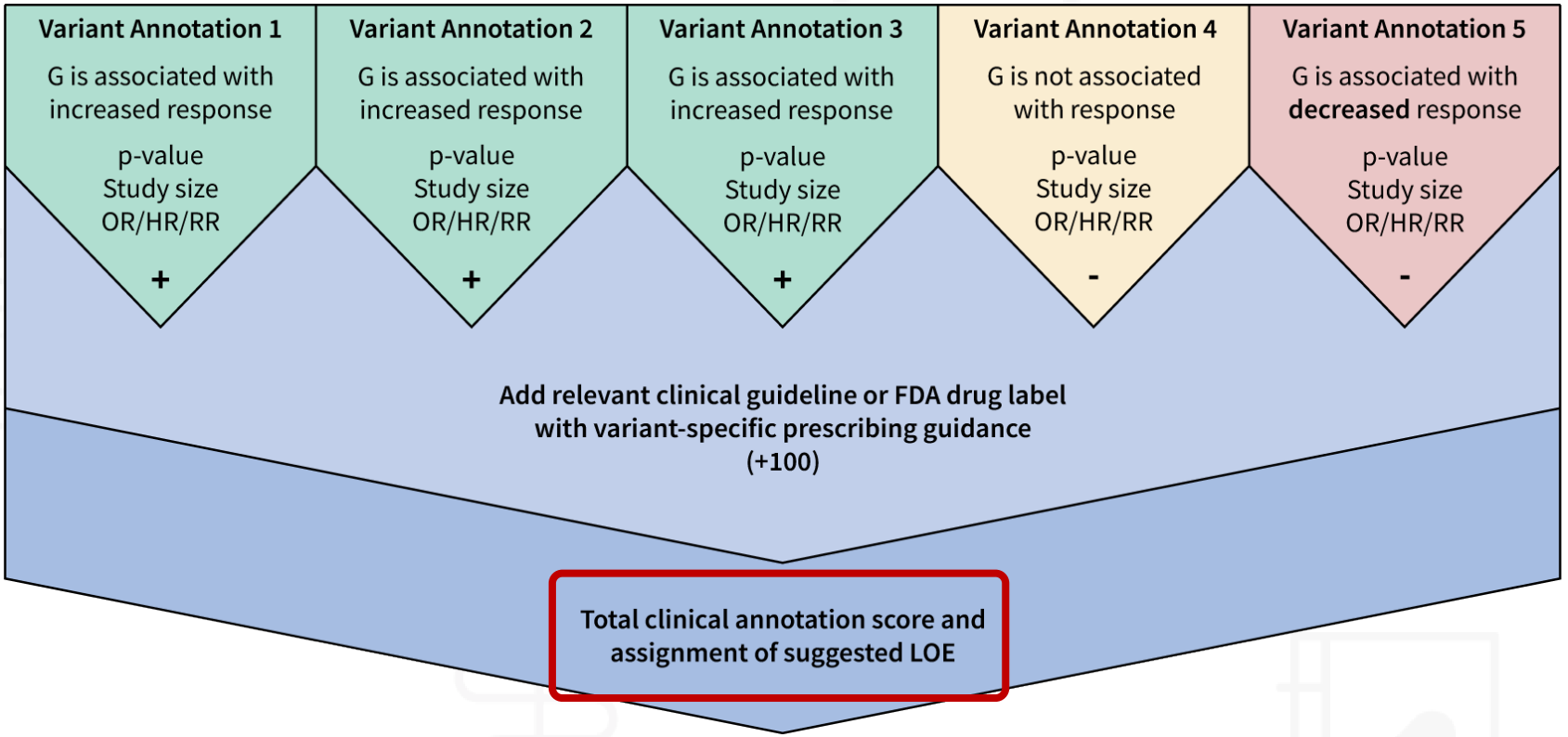


# Clinical guideline annotation & drug label annotation scoring

Annotations on the following types of guidelines and drug labels can be added as supporting evidence:

- a CPIC guideline with recommendations for the gene, drug and phenotype that the clinical annotation is written on.
- a DPWG guideline that provides recommendations for a **specific allele or genotype** for the gene and drug that the clinical annotation is written on.
- an FDA drug label with a Prescribing Info tag and recommendations for a **specific allele or genotype** for the gene and drug that the clinical annotation is written on.

# Annotation Scoring System



Score of all attached annotations (variant, clinical guideline and/or drug label) are summed to give the clinical annotation score.

Curators make final adjustments to the score by marking variant annotations which are conflicting.

7. Annotation of rs1799971 in OPRM1

Genotype AA is associated with decreased risk of Opioid-Related Disorders due to opioids in men as compared to genotypes AG + GG.

**From Publication**

Haplotype-Based Association and In Silico Studies of OPRM1 Gene Variants with Susceptibility to Opioid Dependence Among Addicted Iranians Undergoing Methadone Treatment. *Journal of molecular neuroscience : MN.* 2020. Tolami Hedyeh Fazel et al.

PMID:31853823 DOI:10.1007/s12031-019-01443-4

**Gene :** OPRM1

**Variant :** rs1799971

**Phenotype Category :** Toxicity

**Association Significance :** The study reports this association is significant

**PharmGKB ID :** 1451137681

**Score :** -2.25 Conflicting Evidence

# Clinical annotation score is used to automatically set a Level of Evidence.

| Level of Evidence | Standard scoring range | Rare variant scoring range |
|---|---|---|
| 1A | ≥80 and supported by guideline/drug label | ≥80 and supported by guideline/drug label |
| 1B | 25-79.9375 | 10-79.9375 |
| 2A | 8-24.9375 and variant in a Tier 1 VIP | 3-9.9375 and variant in a Tier 1 VIP |
| 2B | 8-24.9375 | 3-9.9375 |
| 3 | 0-7.9375 | 0-2.9375 |
| 4 | < 0 | < 0 |

## Level of Evidence Calculation

**Total Score :** 13.75

**Score Breakdown :**

- Variant Annotations = 13.75
- Dosing Guideline Annotations = 0
- Drug Label Annotations = 0

**Level Modifiers :** Tier 1 VIP

**Calculated Level from Score :** 2A

## Evidence

- 0 Dosing Guideline Annotations
- 0 Drug Label Annotations
- 9 Variant Annotations from 9 Publications

# If the curation team feels that the calculated level of evidence isn't representative of the evidence base, the level can be manually overridden.

## Clinical Annotation for rs9934438 (VKORC1); warfarin (level 1B Dosage)

**Level of Evidence**

Level 1B

**Phenotype Category**

Dosage

**Genes**

VKORC1

**Variant**

rs9934438

**Drugs**

warfarin

**Specialty Population**

Pediatric

**PharmGKB ID**

655385392

| ALLELE | PHENOTYPE |
|---|---|
| AA | Patients with the rs9934438 AA genotype may require a lower dose of warfarin as compared to patients with the AG or GG genotype. However, conflicting evidence has been reported. Other genetic and clinical factors may also influence warfarin dose requirements. |
| AG | Patients with the rs9934438 AG genotype may require a lower dose of warfarin as compared to patients with the GG genotype, and a higher dose as compared to patients with the AA genotype. However, conflicting evidence has been reported. Other clinical and genetic factors may also influence warfarin dose requirements. |
| GG | Patients with the rs9934438 GG genotype may require higher dose of warfarin as compared to patients with the AG or AA genotype. However, conflicting evidence has been reported. Other genetic and clinical factors may also influence warfarin dose requirements. |

### Level of Evidence Calculation

**Total Score :** 107.375

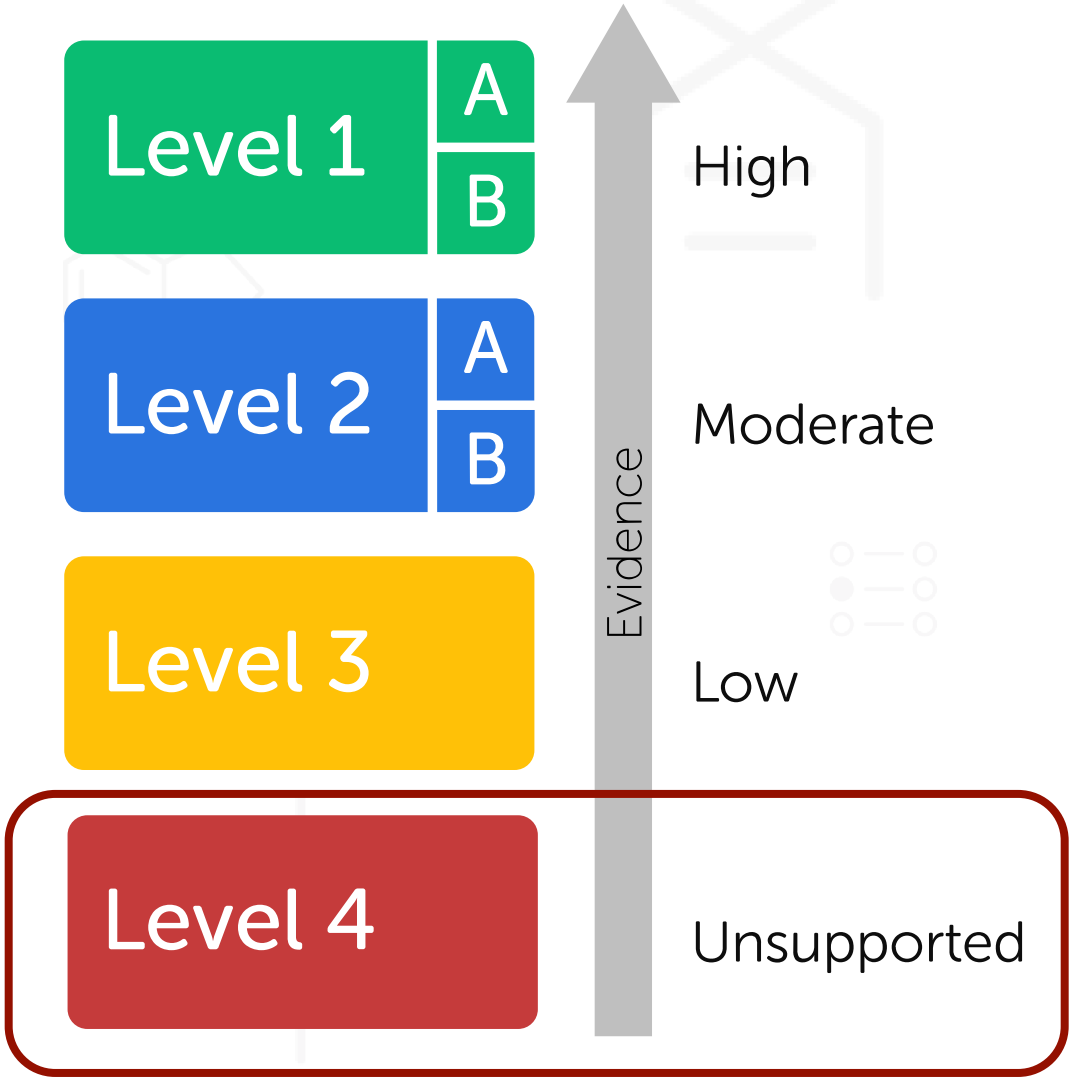
**Score Breakdown :**

- Variant Annotations = 107.375
- Dosing Guideline Annotations = 0
- Drug Label Annotations = 0

**Level Modifiers :** Tier 1 VIP

**Level Override :** The level of evidence was set by a curator instead of by the scoring system.

**Reason for Override :** Assigned level 1B because variant is not specifically mentioned in clinical guidelines or labels, in high LD with rs9923231


Level 1
A
B
Level 2
A
B
Level 3
Level 4
Evidence
High
Moderate
Low
Unsupported

| ALLELE | PHENOTYPE |
| --- | --- |
| *1<br>Normal function | The CYP2D6*1 allele is assigned as a normal function allele by CPIC. Patients carrying the *1 allele in combination with alleles that result in a normal metabolizer phenotype may have decreased metabolism of paroxetine as compared to patients with two increased function alleles or an increased function allele in combination with a normal function allele or a decreased function allele with an activity value of 0.5. Patients carrying the *1 allele in combination with alleles that result in a normal metabolizer phenotype may also have decreased metabolism of paroxetine as compared to patients with an increased function allele with an activity value of 3 or greater in combination with a no function allele or a decreased function allele with an activity value of 0.25 but increased metabolism of paroxetine as compared to patients with a no function allele in combination with a decreased or normal function allele or two decreased or no function alleles. However, conflicting evidence has been reported. This annotation only covers the pharmacokinetic relationship between CYP2D6 and paroxetine and does not include evidence about clinical outcomes. Other genetic and clinical factors may also influence paroxetine metabolism. |
| *9<br>Decreased function | Limited Evidence<br>The CYP2D6*9 allele is assigned as a decreased function allele with an activity value of 0.5 by CPIC. Patients carrying the *9 allele in combination with a decreased or no function allele may have decreased metabolism of paroxetine as compared to patients with alleles that result in a normal metabolizer phenotype, while patients carrying the *9 allele in combination with an increased function allele may have increased metabolism of paroxetine as compared to patients with alleles that result in a normal metabolizer phenotype. This annotation only covers the pharmacokinetic relationship between CYP2D6 and paroxetine and does not include evidence about clinical outcomes. Other genetic and clinical factors may also influence paroxetine metabolism. |

All clinical annotations moving into or out of levels 1 and 2 are independently reviewed by a second curator

# Summary

- Developed a scoring system to automate and standardize assigning Level of Evidence to clinical annotations.
- Assigning Levels of Evidence is now more consistent, reproducible and transparent to users.
- Scoring only used to assign of Levels of Evidence. Not used anywhere else on PharmGKB or by any other group.
- Score not used to rank or order CAs within a given LOE.

## The Team

Teri E. Klein, PhD
Principal Investigator

Russ B. Altman, MD, PhD
Co-Principal Investigator

Michelle Whirl-Carrillo, PhD
Director, PharmGKB

Caroline F. Thorn, PhD
Scientific Curator

Mark Woon, BSE
Co-Technical Lead

Ryan Whaley, BS
Co-Technical Lead

Li Gong, PhD
Senior Scientific Curator

Katrin Sangkuhl, PhD
Senior Scientific Curator

Rachel Huddart, PhD
Scientific Curator

Tiffany Murray, BA
Outreach Coordinator/Technical Editor

Clarissa Klein, BA
FDA Recognition Curator

Reid Barber, BS
Software Developer

Rachel Dalton, PharmD
Scientific Curator

Binglan Li, PhD
Post-doc (PharmCAT)

Thanks to Dr. Andrea Gaedigk and Dr. Houda Hachad for valuable feedback

Funded by NIH/NHGRI/NICHD (U24 HG010615)

feedback@pharmgkb.org

@PharmGKB