# *CYP3A4* and *CYP3A5* Genotyping Recommendations:

A Joint Consensus Recommendation of the Association for Molecular Pathology, Clinical Pharmacogenetics Implementation Consortium, College of American Pathologists, Dutch Pharmacogenetics Working Group of the Royal Dutch Pharmacists Association, European Society for Pharmacogenomics and Personalized Therapy, and Pharmacogenomics Knowledgebase

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*Providing global expertise in molecular testing that drives patient care.*

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# Learning Objectives

- Introduce the AMP PGx Working Group, including a review on consensus recommendation/opinion development
- Highlight the importance of standardization
- Describe the AMP PGx Working Group *CYP3A4* and *CYP3A5* Tier 1 and Tier 2 alleles

# AMP PGx Working Group

- **Victoria M. Pratt** (Co-Chair), Agena Bioscience and Indiana University School of Medicine
- **Karen E. Weck** (Co-Chair), University of North Carolina
- **Larisa H. Cavallari,** University of Florida
- **Makenzie L. Fulmer,** ARUP Laboratories and University of Utah School of Medicine
- **Andrea Gaedigk,** PharmVar and Children's Mercy Kansas City
- **Houda Hachad**, AccessDx Laboratory
- **Yuan Ji**, ARUP Laboratories and University of Utah School of Medicine
- **Lisa V. Kalman**, Division of Laboratory Systems, Centers for Disease Control and Prevention
- **Reynold C. Ly,** Indiana University
- **Ann M. Moyer,** Mayo Clinic, CAP representative
- **Stuart A. Scott**, Stanford University Medical Center
- **Ron van Schaik,** Erasmus MC University Medical Center, ESPT and DPWG representative
- **Michelle Whirl-Carrillo,** Stanford University, CPIC/PharmGKB

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# AMP PGx Working Group

## Goals

- To develop recommendations defining a minimum set of variants (a “Must-Test” list) that should be included in clinical genotyping assays
- Used by the clinical PGx testing community as a reference for test development

## Members

- Subject matter expert representatives from the clinical PGx testing community (US and Europe) including organizational representation from CAP, CPIC, PharmGKB, PharmVar, DPWG, and ESPT

## Previous Projects – Allele Selection for Clinical Genotyping

- *CYP2C19* - **Pratt VM, et al. JMD, 2018;20:269-276**
- *CYP2C9* - **Pratt VM, et al. JMD, 2019;21:746-755**
- Warfarin-Related Genes - **Pratt VM, et al. JMD, 2020;22:847-859**
- *CYP2D6* – **Pratt VM, et al. JMD, 2021; 23:1047-1064**
- *TPMT/NUDT15* –**Pratt VM, et al. JMD, 2022; 24:1079-1088**
- ***Recommendations for additional genes will be forthcoming***

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# Considerations in PGx Test Interpretation

- Many pharmacogenes are reported using a “star-allele” (*) system to represent haplotypes
  - Variants are typically named in the order of discovery
  - *1 indicates that no variants were identified (considered normal or wildtype allele)
- For most genes, an individual will have 2 alleles, each with 1 copy of the gene
  - Reported as a diplotype (e.g. *1/*2)
  - Some genes may have a deletion or duplication/multiplication involving one allele
- Diplotype is used to predict the phenotype (metabolizer status)
  - Ultrarapid, rapid, normal, intermediate, poor metabolizer
- **If no variants are detected, the diplotype is often reported as *1/*1 and the phenotype as normal metabolizer**

# PGx genotyping recommendations are needed

*GOAL: To promote standardization of PGx allele testing across clinical laboratories*

Inconsistent test design can lead to discordant interpretation and therapeutic recommendations

Test 1:
Detects *2, *5, *9

Test 2:
Detects *2, *3, *4, *5

Patient's Genotype is *1/*4

Test 1:
Reports *1/*1
Normal Metabolizer

Test 2:
Reports *1/*4
Intermediate Metabolizer

- Both tests performed as expected and produced accurate results.
- Different genotypes reported, different phenotypes reported, different recommendations

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# PGx genotyping recommendations are needed

*GOAL: To promote standardization of PGx allele testing across clinical laboratories*

- Inconsistent interpretation can lead to discordant therapeutic recommendations
- Publications show lack of consistency in alleles included in commercial platforms and clinical tests
- **Genomic Medicine X: Research Directions in Pharmacogenomics Implementation**
  - **NHGRI Meeting in 2017 - Call for assay standardization**

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# PGx genotyping recommendations are needed

- Option 1: Test all known alleles
  - Not practical – for example - *CYP2D6*, there are currently >145 alleles/sub-alleles!!!
- Option 2: Sequence instead of targeted genotyping
  - Current state: pharmacogenes are technically challenging by short-read NGS chemistry
  - Challenges in reporting genotypes and interpreting rare variants/alleles
- **Option 3: Why not use a similar approach to ACMG recommendations for *CFTR* testing?**
  - Define a minimum set of variants based on multiethnic allele frequency in order to optimize diagnostic test rate

# AMP PGx Working Group Genotyping Recommendations

These recommendations are intended to:

- Promote standardization of PGx testing across different laboratories
- Inform clinical laboratory professionals when designing and validating clinical PGx assays
- Complement other clinical guidelines, such as those issued by CPIC and DPWG, *which primarily focus on the interpretation of genotyping results and therapeutic recommendations for specific drugs*

Future work:

- Other PGx genes with clinical relevance planned/ in progress
- International collaboration/ European PGx groups

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# AMP PGx Working Group:

## Expert consensus recommendation/opinion development

- **Tier 1 -** Minimum "must-test" alleles
  - Known effect on protein function and/or gene expression
  - Appreciable minor allele frequency in a patient population
  - Available reference materials
  - Technical feasibility to detect variant in a clinical laboratory
- **Tier 2 -** Extended panel
  - Meet at least one but not all the criteria for inclusion in Tier 1
- **Other**
  - Variants with unknown or uncertain function are not recommended for inclusion in clinical test panels

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# AMP PGx Working Group: *CYP3A4/CYP3A5*

- 6th deliverable
- Challenges:
  - Limited reference materials for *CYP3A4* and *CYP3A5*
    - Co-project with CDC GeT-RM to characterize additional DNAs from Coriell Cell Repository, Camden NJ
  - CPIC has not curated *CYP3A4* (no CPIC guidelines)
  - *CYP3A4/5* genes are oriented on the negative strand of the chromosome, and therefore the coding DNA (cDNA) sequence is the reverse complement of the human reference genome sequence.
    - using GRCh37 as reference, the *CYP3A5*3* allele is considered the reference sequence and is not reported as variant

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# Existing Clinical Guidelines and Recommendations

- *CYP3A4*/quetiapine
  - DPWG has developed recommendations for *CYP3A4* genotype-based dosing for quetiapine (https://www.g-standaard.nl/risicoanalyse/B0005991.PDF)
- *CYP3A5*/tacrolimus
  - CPIC - https://cpicpgx.org/guidelines/guideline-for-tacrolimus-and-cyp3a5/
  - FDA - lists *CYP3A5*/tacrolimus gene-drug pair in their pharmacogenetic associations table (https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations)

# Testing Platforms

- Selection of a molecular platform for testing PGx variants depends on many factors, including:
  - Technical feasibility
  - Cost
  - Laboratory workflow
  - Test turnaround time
- Standard molecular techniques may be used (targeted genotyping or sequencing)
  - Many standard techniques do not allow for phasing of variants
  - Diplotypes from raw genotyping or sequencing data is typically empirical or inferred

# AMP Guidelines for *CYP3A4/CYP3A5* clinical testing – Tier 1

| Allele | Allele Functional Status† | Defining Functional Variant | RefSeqGene | HGVS cDNA Nomenclature | HGVS Protein Nomenclature | Reference Material Available | Multiethnic Allele Frequency |
|---|---|---|---|---|---|---|---|
| *CYP3A4*22* | Decreased function | rs35599367 | NG_008421.1: g.20493C>T | NM_017460.6: c.522-191C>T | Splicing defect | Yes | 0-9% |
| *CYP3A5*3* | No function† | rs776746 | NG_007938.2: g.12083A>G (GRCh38) | NM_000777.5: c.219-237A>G | Splicing defect | Yes | 24-92% |
| *CYP3A5*6* | No function† | rs10264272 | NG_007938.2: g.19787G>A | NM_000777.5: c.624G>A | Splicing defect NP_000768.1: p.Lys208= | Yes | 0-19% |
| *CYP3A5*7* | No function† | rs41303343 | NG_007938.2: g.32228dup | NM_000777.5:c.1035dup | NP_000768.1: p.Thr346TyrfsTer3 | Yes | 0-12% |

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# AMP Guidelines for *CYP3A4/CYP3A5* clinical testing – Tier 2

| Allele | Allele Functional Status† | Defining Functional Variant | RefSeqGene | HGVS cDNA Nomenclature | HGVS Protein Nomenclature | Reference Material Available | Multiethnic Allele Frequency |
|---|---|---|---|---|---|---|---|
| *CYP3A4*20* | Probably no function | rs67666821 | NG_008421.1: g.31002dup | NM_017460.6: c.1461dup | NP_059488.2: p.Pro488ThrfsTer7 | Yes | 0-0.12% |

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# Alleles considered, but not included in a Tier

- Likely no function, but rare
  - *CYP3A4*6* (NM_017460.6:c.830dup, p.Asp277GlyfsTer9, rs4646438)
  - *CYP3A4*26* (NM_017460.6:c.802C>T, p.Arg268Ter, rs138105638)
  - *CYP3A4*30* (NM_017460.6:c.388C>T, p.Arg130Ter, rs778013004)
- Uncertain function, but meets allele frequency cut-offs
  - *CYP3A4*2* (NM_017460.6:c.664T>C, p.Ser222Pro, rs55785340)
  - *CYP3A4*3* (NM_017460.6:c.1334T>C, p.Met445Thr, rs4986910)
  - *CYP3A4*4* (NM_017460.6:c.352A>G, p.Ile118Val, rs55951658)
  - *CYP3A4*10* (NM_017460.6:c.520G>C, p.Asp174His, rs4986908)
  - *CYP3A4*15* (NM_017460.6:c.485G>A, p.Arg162Gln, rs4986907)
  - *CYP3A4*18* (NM_017460.6:c.878T>C, p.Leu293Pro, rs28371759)
  - *CYP3A4*23* (NM_017460.6:c.484C>T, p.Arg162Trp, rs57409622)

# Alleles that should NOT be tested

- Normal function
  - *CYP3A4*1B* (*CYP3A4*1.001*) corresponds to the G nucleotide in the current reference sequences NM_017460.6 and NG_008421.1 (GRCh38) but is the minor allele for rs2740574
  - *CYP3A4*1.002* (formerly *CYP3A4*1A*, c.-392G>A; NG_008421.1:g.4713G; rs2740574) also occurs on numerous other haplotypes

# Proficiency Testing/External Quality Assessment

- Several proficiency testing (PT) or external quality assessment programs are available for *CYP3A4/CYP3A5* genotyping
- Aside from CAP, programs that include *CYP3A4* are currently not widely available
  - Note that EMQN and RfB,  only include the *CYP3A4*22* allele

# AMP PGx Working Group Genotyping Recommendations

These recommendations are intended to:

- Promote standardization of PGx testing across different laboratories
- Inform clinical laboratory professionals when designing and validating clinical PGx assays
- Complement other clinical guidelines, such as those issued by CPIC, *which primarily focus on the interpretation of genotyping results and therapeutic recommendations for specific drugs*

# Limitations of the AMP PGx Working Group Recommendations

- The guidelines only focus on allele recommendations for clinical laboratory genotyping assays that include *CYP3A4* and *CYP3A5*
- The guidelines do not include:
  - Genotype-phenotype correlation
  - Clinical interpretation of test results
  - Recommendations for medication management based on genotype
- There are no recommendations or endorsements for any specific molecular methodology or testing platform

# Acknowledgement


- **AMP Clinical Practice Committee**
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- **AMP PGx Working Group**

**Please email Dr. Victoria Pratt (vicky.pratt@agenabio.com), Co-Chair of the AMP PGx Working Group, for feedback and suggestions!**


Thank you!

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