WASHINGTON STATE UNIVERSITY

Fundamentals of Pharmacogenomics (PGx), Genetic Variation in Drug Metabolism, and Interpretation of PGx Testing Results- Part 2

Rustin D. Crutchley, PharmD, AAHIVP

Scholarly Associate Professor

Program Director, Interprofessional Clinical Pharmacogenomics Certificate Program

Department of Pharmacotherapy

Conflict of Interest Disclosure

- Nothing to disclose

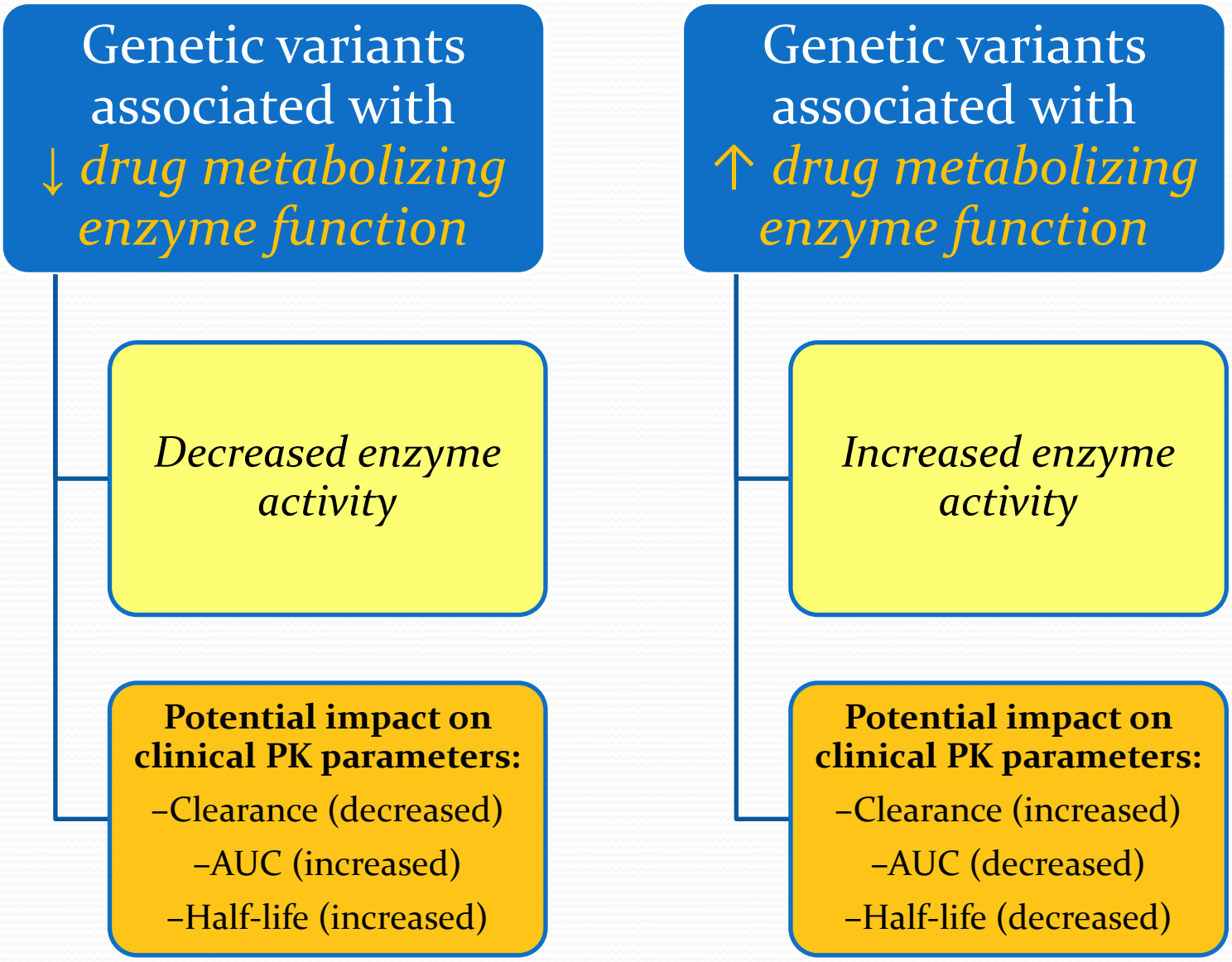
LEARNING OBJECTIVES

- Describe fundamental principles of pharmacogenomics (PGx)
- Discuss how to navigate PGx database resources such as Clinical Pharmacogenetics Implementation Consortium (CPIC) and Pharmacogenomics Knowledge Database (PharmGKB)

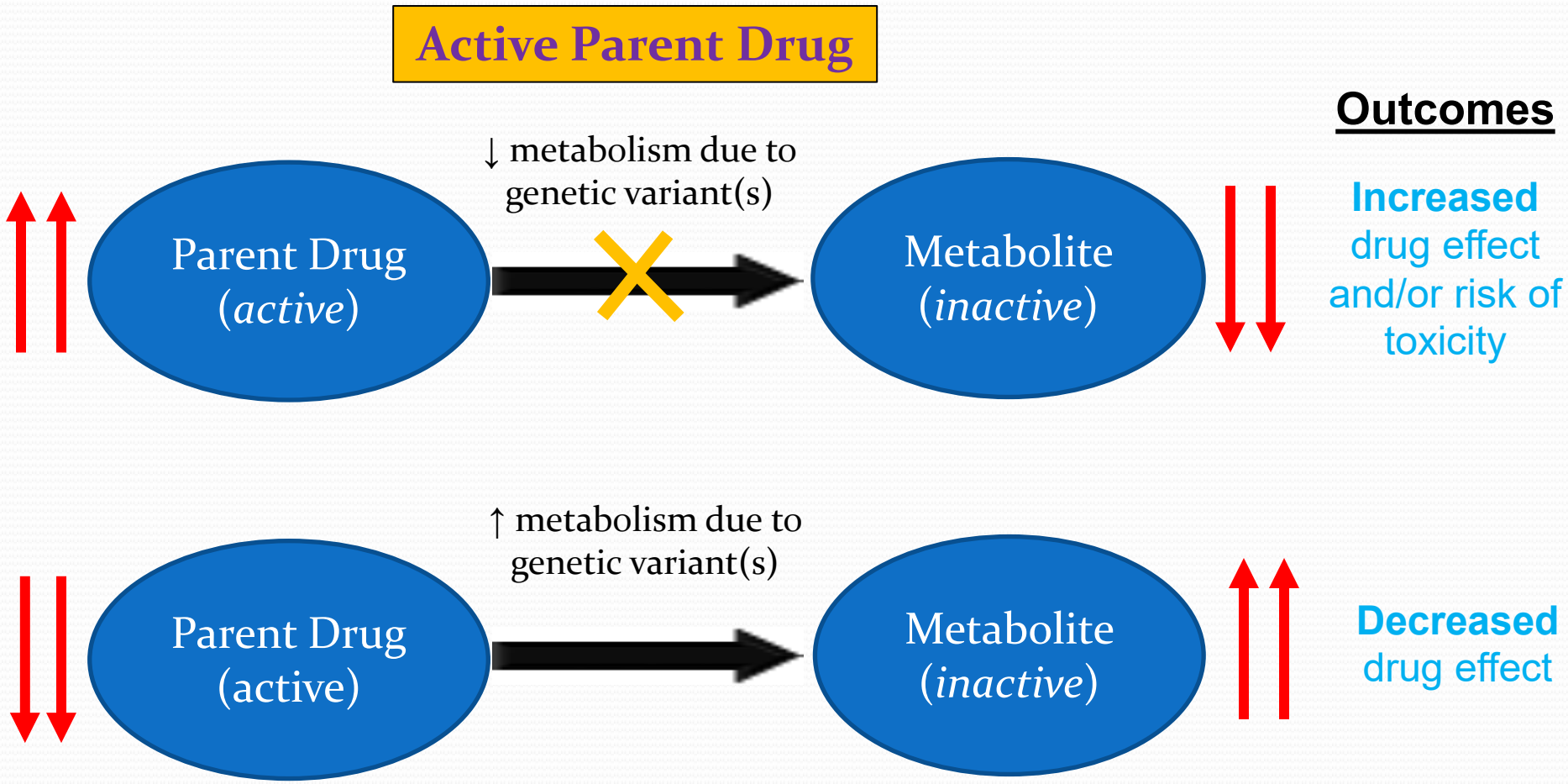
- Illustrate general implications of genetic variants in drug metabolism in context of active parent drug versus inactive prodrug
- Outline function of major genetic variants associated with Phase I (CYP2C9, CYP2C19, CYP2D6) and Phase II (UGT1A1, TPMT) drug metabolism and describe potential impact on pharmacokinetics (PK) and drug response
- Define phenoconversion and predict CYP2D6 phenotypes by calculation of total activity scores
- Describe relevant FDA resources used for disseminating PGx-related drug-gene pair information

Illustrate general implications of genetic variants in drug metabolism in context of active parent drug versus inactive prodrug

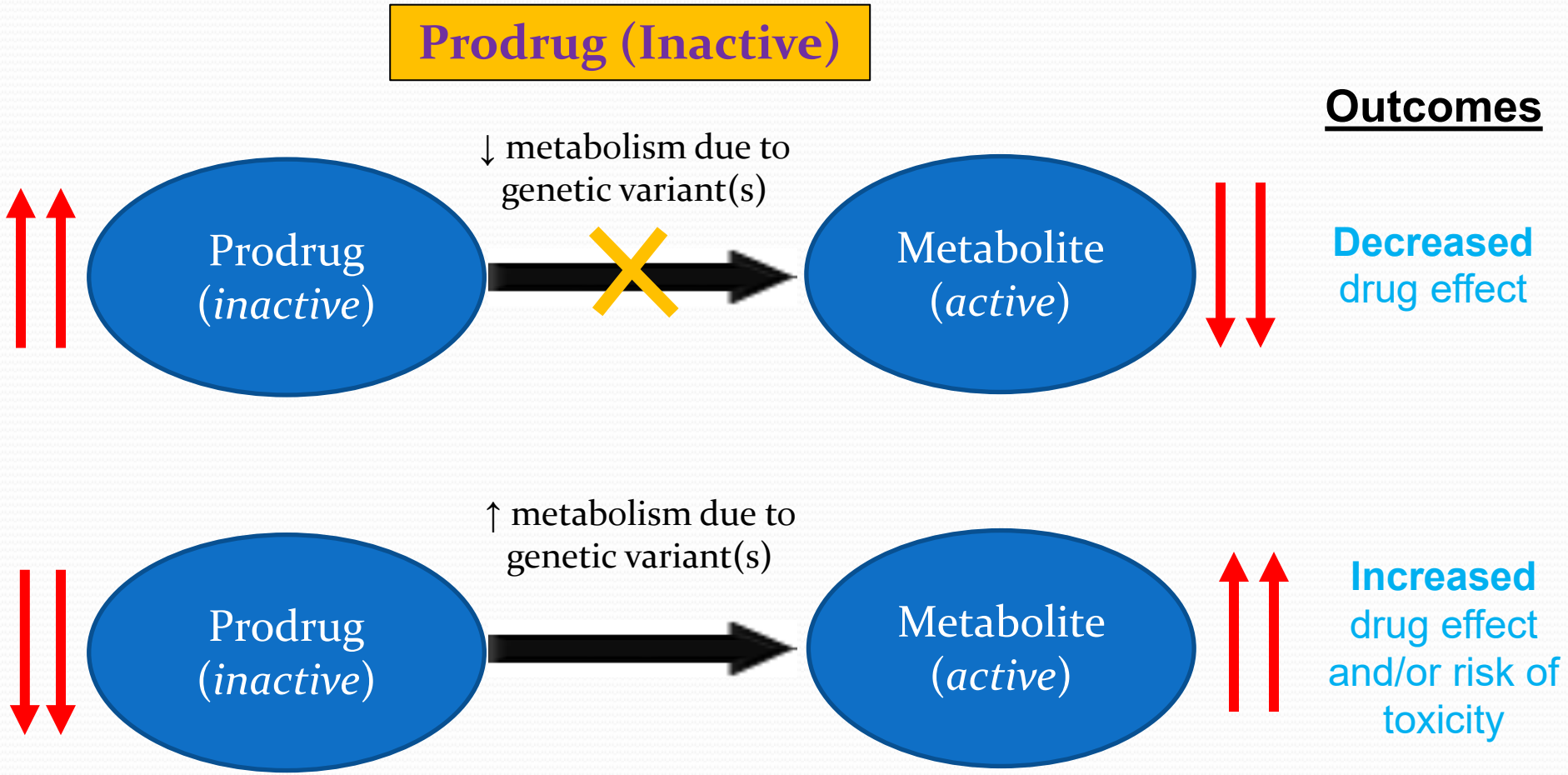
General Overview Schematic of Genetic Variants on Drug Metabolism



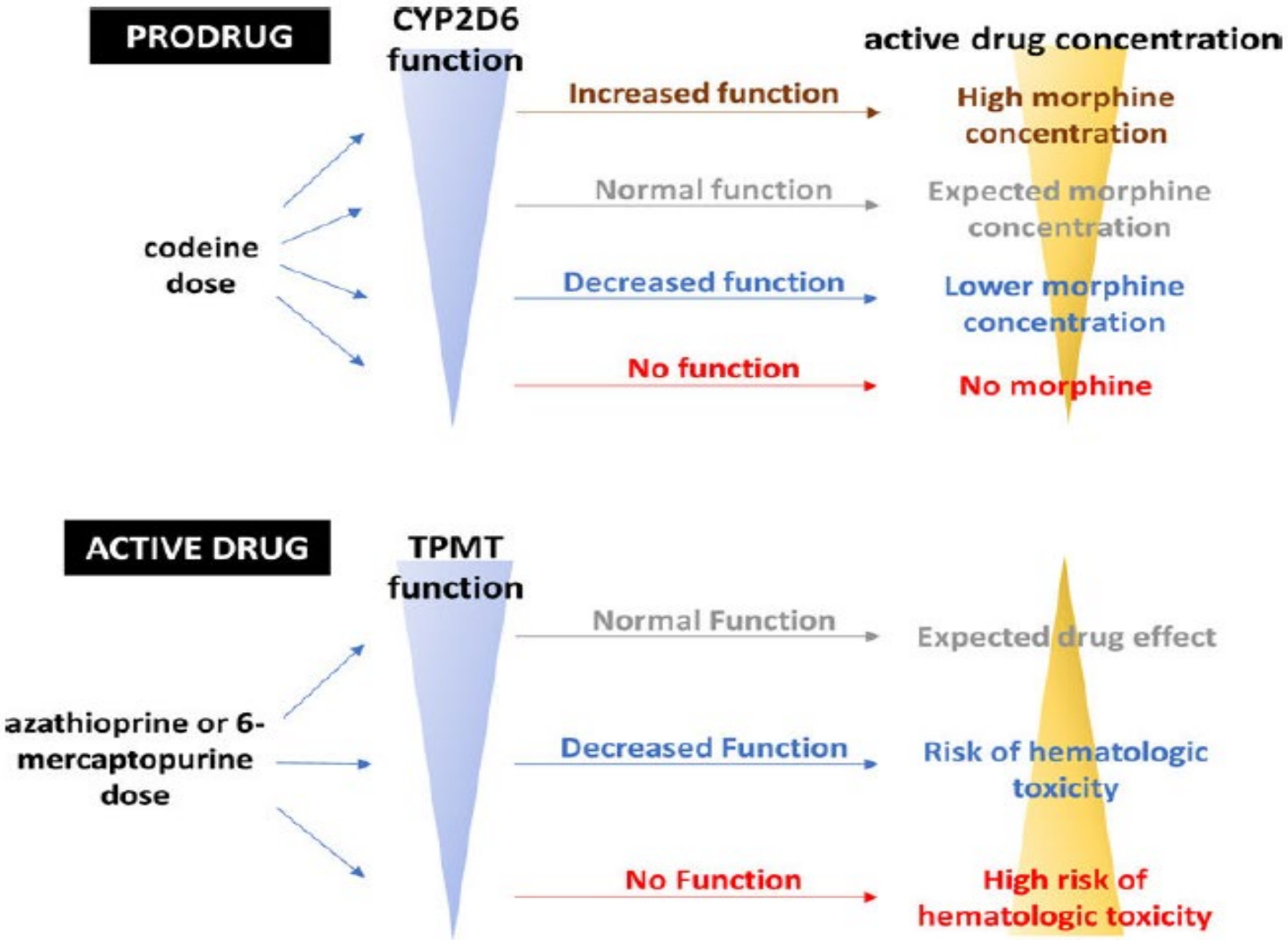
Implications of Genetic Variants in Drug Metabolism in the Context of Active Parent Drug VERSUS Inactive Prodrug



Implications of Genetic Variants in Drug Metabolism in the Context of Active Parent Drug VERSUS Inactive Prodrug



Examples of prodrugs: *clopidogrel, codeine*



Roden DM et al. *Lancet*. 2019; 394(10197): 521–532

Outline function of major genetic variants associated with Phase I (CYP2C9, CYP2C19, CYP2D6) and Phase II (UGT1A1, TPMT) drug metabolism and describe potential impact on pharmacokinetics (PK) and drug response

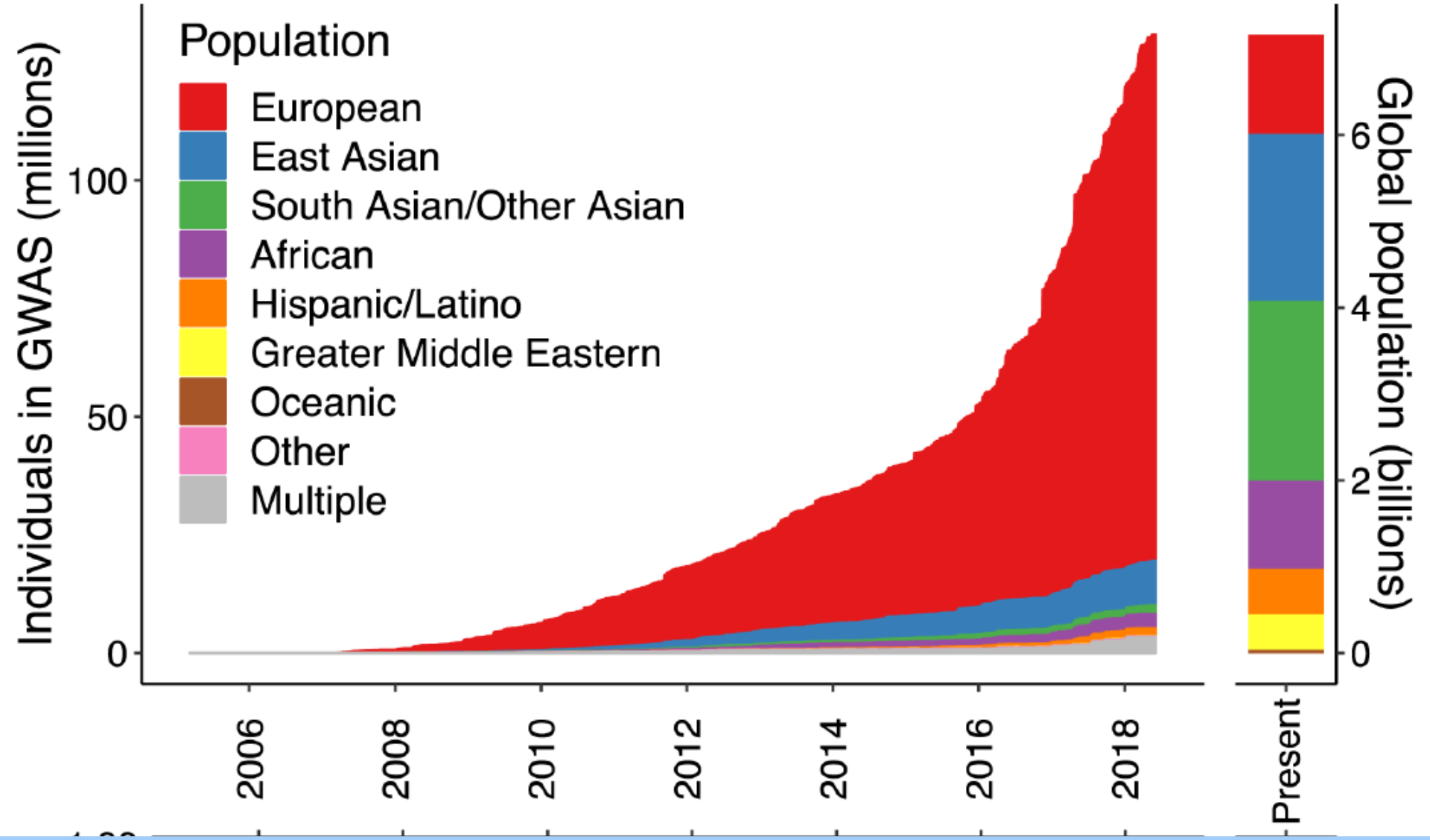
Table 1. Selection of P450 allelic variation across populations, highlighting variability within and among populations.

Allelic variant	Function	Africa	African American	Caucasian	East Asia	Americas	Middle East	South Central Asia	Oceania
CYP2C9									
**2*	Decreased	0–9%	1–4%	8–16%	0–1%	0.3–14%	5–27%	2–26%	0–3%
**3*	None	0–3%	0.5–2%	4–11%	1–5%	0–6%	2–19%	6–13%	1–4%
**5*	Decreased	0–3%	0.7–2.5%	0%	0%	0–2%	0–0.1%	0%	n/a
**6*	None	0–2%	0–1.3%	0%	0%	0–1%	0%	0%	n/a
**8*	Decreased	2–8%	3–12%	0–1%	0%	0–2%	0–1%	0–1%	n/a
**11*	Decreased	1–5%	1–2%	0–1%	0–0.2%	0–1%	0%	0–1%	n/a
CYP2C19									
**2*	None	4–22%	12–25%	8–27%	6–49%	2–31%	6–24%	9–51%	20–78%
**3*	None	0–7%	0–1%	0–6.8%	0–21%	0–4%	0–20%	0–6%	2–33%
**4*	None	0%	0%	0–1%	0–0.5%	0–0.2%	n/a	0%	0%
**17*	Increased	10–18%	18–22%	11–33%	0–6.2%	1–25%	22–26%	12–18%	3–6%
CYP2D6									
**4*	None	1–7%	4–8%	8–33%	0–4%	0.2–43%	4–13%	3–18%	0–8%
**5*	Gene deletion	1–17%	3–9%	0–9%	0–10%	0–5%	1–4%	0–16%	1–8%
**10*	Decreased	3–19%	3–8%	0.4–15%	9–64%	0–12%	1–9%	4–55%	0–6%
**17*	Decreased	9–34%	14–26%	0–2.2%	0–0.2%	0–18%	0–3%	0–1%	0–0.2%
**29*	Decreased	4–20%	5–8%	0–0.3%	0%	0–11%	0–2%	0–0.2%	0%

Allele frequencies for *CYP2C9*, *CYP2C19* and *CYP2D6* are from tables compiled for the Clinical Pharmacology Implementation Consortium (CPIC) and available through PharmGKB. Frequencies are rounded and might slightly deviate from those posted as new literature is added.
n/a, no frequencies are available.

Rashmi R. Shah and Andrea Gaedigk. *Ther Adv Drug Saf*. 2018; 9(1) 45–62

Significant need to include underrepresented groups in future PGx clinical studies



Martin AR et al. *Nat Genet*. 2019; 51(4): 584–591

PGx of Major Drug-Metabolizing Enzyme Genes [Phase I]

- CYP1A2
- CYP2A6
- CYP2B6
- CYP2C9
- CYP2C19
- CYP2D6
- CYP3A4/5
- Dihydropyrimidine dehydrogenase (DPYD)
- Butyrylcholinesterase (BChE)

** We will focus on CYP2C9, CYP2C19 and CYP2D6 as noted in LEARNING OBJECTIVES**

Drug Metabolism-Phase I (CYP2C9)

- CYP2C9 metabolizes **15-20%** of all medications
- 2 major non-synonymous SNPs that can result in poor metabolizer phenotype
  - **CYP2C9*2** (Arg144Cys); **[REDUCED FUNCTION]**
  - **CYP2C9*3** (Ile359Leu); **[NO FUNCTION]**
- **CYP2C9*6** (no function allele in African Americans)
- **CYP2C9*5, *8 and *11** (reduced function alleles in African Americans)

Substrates	Inhibitors	Inducers
Celecoxib	Etravirine	Rifampin
Phenytoin	Amiodarone	Phenobarbital
Warfarin	Fluconazole	Carbamazepine
Sulfonylureas	Voriconazole	St John's Wort

https://www.pharmgkb.org/vips Accessed December 20, 2021
https://www.pharmvar.org/gene/CYP2C9 Accessed Jan 6,2022

Drug-Gene Pairs of Clinical Interest for CYP2C9

- Warfarin
- NSAIDs
- Phenytoin

Drug Metabolism-Phase I (CYP2C19)

- Comprises ~12% metabolism of all drugs
- Major CYP2C19 SNPs include the following:
  - **CYP2C19*2** (681 G>A); NO function
  - **CYP2C19*3** (636G>A); NO function
  - **CYP2C19*17**; INCREASED function

Substrates	Inhibitors	Inducers
Clopidogrel	Fluvoxamine	Rifampin
Diazepam	Omeprazole	Carbamazepine
Omeprazole	Fluconazole	Phenobarbital
Voriconazole	Voriconazole	St. John's Wort

https://www.pharmgkb.org/vips Accessed December 20, 2021
https://www.pharmvar.org/gene/CYP2C19 Accessed Jan6,2022

Drug-Gene Pairs of Clinical Interest- CYP2C19

- Clopidogrel
- Proton pump inhibitors
- SSRIs (citalopram, escitalopram, sertraline)
- Tricyclic antidepressants (amitriptyline)
- Voriconazole

Drug Metabolism-Phase I (CYP2D6)

- Formerly known as debrisoquine/sparteine hydroxylase
- **1st human drug metabolic enzyme** identified as polymorphic
- Metabolizes **25%** of therapeutic drugs
- **Major CYP2D6 SNPs include the following:**
  - *Non-functional alleles* (**CYP2D6*3, *4, *5 and *6**)
  - *Reduced function alleles* (**CYP2D6*10, *17 and *41**)
  - *Functional alleles* (**CYP2D6*1xN and *2xN duplications**)

Substrates	Inhibitors	Inducers
Codeine	Bupropion	Non-inducible
Ondansetron	SSRIs (fluoxetine, paroxetine)	
Tramadol	Quinidine	
Tamoxifen	Ritonavir	

https://www.pharmgkb.org/vips Accessed December 20, 2021
https://www.pharmvar.org/gene/CYP2D6 Accessed Jan 6,2022

Drug-Gene Pairs of Clinical Interest- CYP2D6

- Codeine/tramadol
- SSRIs (paroxetine, fluvoxamine)
- SNRIs (venlafaxine)
- TCAs* (amitriptyline, nortriptyline)
- Tamoxifen

Define phenoconversion and predict CYP2D6 phenotypes by calculation of total activity scores

What is Phenoconversion?

"Mismatch between the individual's genotype-based prediction of drug metabolism and true capacity to metabolize drugs due to non-genetic factors"

Hahn, M.; Roll, S.C. *Pharmaceuticals* 2021, 14, 487. https://doi.org/10.3390/ph140504

Calculation of Activity Scores

- Pertinent to very polymorphic **CYP2D6** enzyme
- An individual's **activity score** is calculated by:
  - Assigning an activity score to EACH allele
  - Adding together the activity scores for BOTH alleles
    - total score typically ranges between 0-2

- *Numeric allelic activity scoring system is categorized as*:
  - **1 = Functional allele**
  - **0.5 = Reduced-function allele**
  - **0 = Non-functional allele**

- Concurrent medications can MODIFY activity scores (PHENOCOPYING)
  - For example- with *strong inhibitors*, multiply by zero, resulting in activity score of zero (i.e. poor metabolizer)

Gaedigk A et al. *Clin Pharmacol Ther*. 2008;83(2):234-42

Standardizing *CYP2D6* Genotype to Phenotype Translation: Consensus Recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group

Kelly E. Caudle[1,*], Katrin Sangkuhl[2], Michelle Whirl-Carrillo[2], Jesse J. Swen[3], Cyrine E. Haidar[1], Teri E. Klein[2], Roseann S. Gammal[1,4], Mary V. Relling[1], Stuart A. Scott[5,6], Daniel L. Hertz[7], Henk-Jan Guchelaar[3] and Andrea Gaedigk[8,9]

Translating *CYP2D6* genotype to metabolizer phenotype is not standardized across clinical laboratories offering pharmacogenetic (PGx) testing and PGx clinical practice guidelines, such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG). The genotype to phenotype translation discordance between laboratories and guidelines can cause discordant cytochrome P450 2D6 (CYP2D6) phenotype assignments and, thus lead to inconsistent therapeutic recommendations and confusion among patients and clinicians. A modified-Delphi method was used to obtain consensus for a uniform system for translating *CYP2D6* genotype to phenotype among a panel of international CYP2D6 experts. Experts with diverse involvement in CYP2D6 interpretation (clinicians, researchers, genetic testing laboratorians, and PGx implementers; $n = 37$) participated in conference calls and surveys. After completion of 7 surveys, a consensus (> 70%) was reached with 82% of the CYP2D6 experts agreeing to the final *CYP2D6* genotype to phenotype translation method. Broad adoption of the proposed *CYP2D6* genotype to phenotype translation method by guideline developers, such as CPIC and DPWG, and clinical laboratories as well as researchers will result in more consistent interpretation of *CYP*2D6 genotype.

Caudle KE et al. *Clin Transl Sci* (2020) 13, 116–124.

Standardization of CYP2D6 Genotype to Phenotype

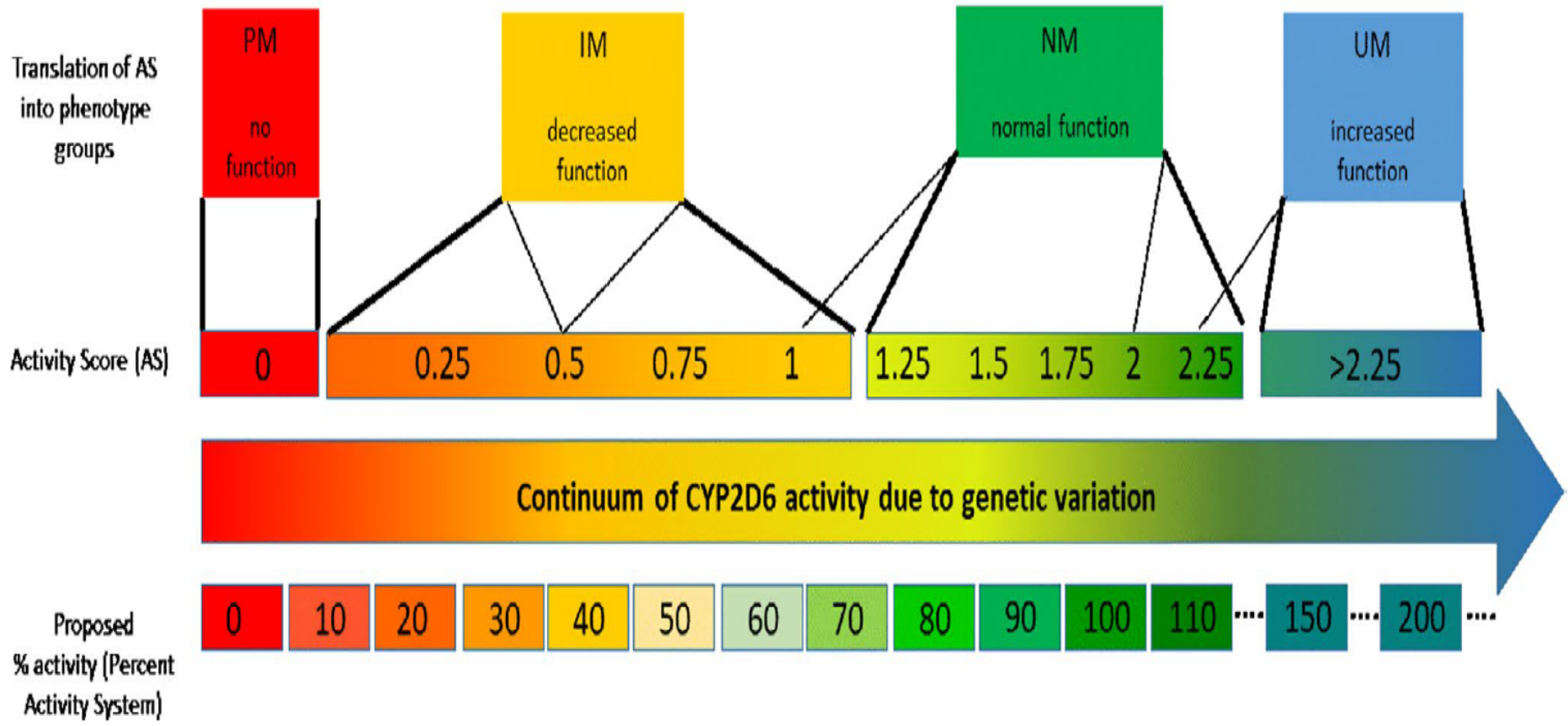


Figure 3 Comparison of the Clinical Pharmacogenetics Implementation Consortium method and percentage activity method for translating *CYP2D6* genotype to phenotype. Thin lines represent different ways to translate activity score (AS) into phenotype and the bold lines represent the recommended *CYP*2D6 genotype to phenotype translation consensus system. IM, intermediate metabolizer; NM, normal metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

Caudle KE et al. *Clin Transl Sci* (2020) 13, 116–124.

Example of Calculation of Total Activity Scores to Determine CYP2D6 Phenotypes

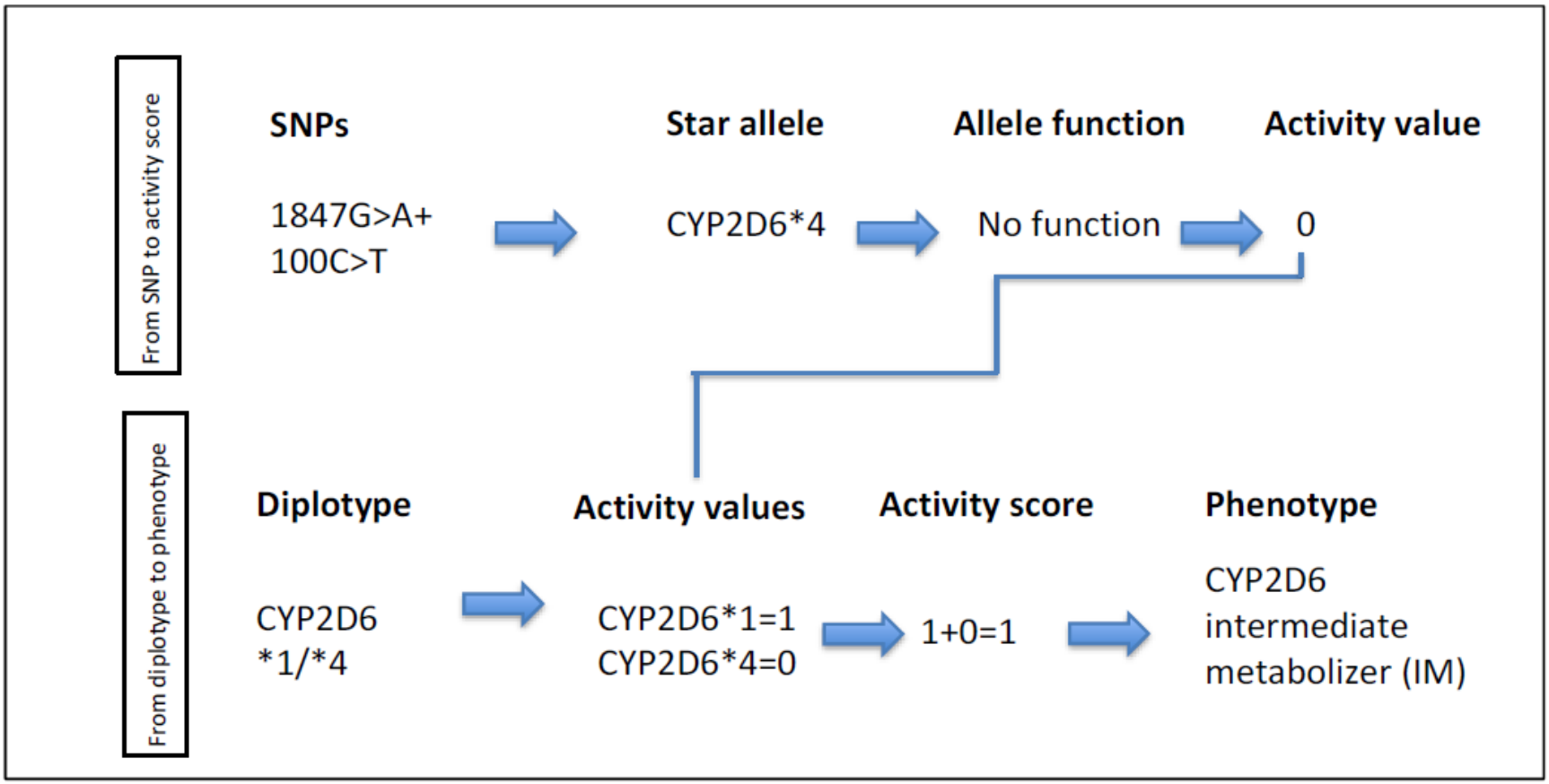


Figure 1. Explanation how to transfer a SNP to an activity score (first line) and how to calculate the phenotype from the diplotype (second line).

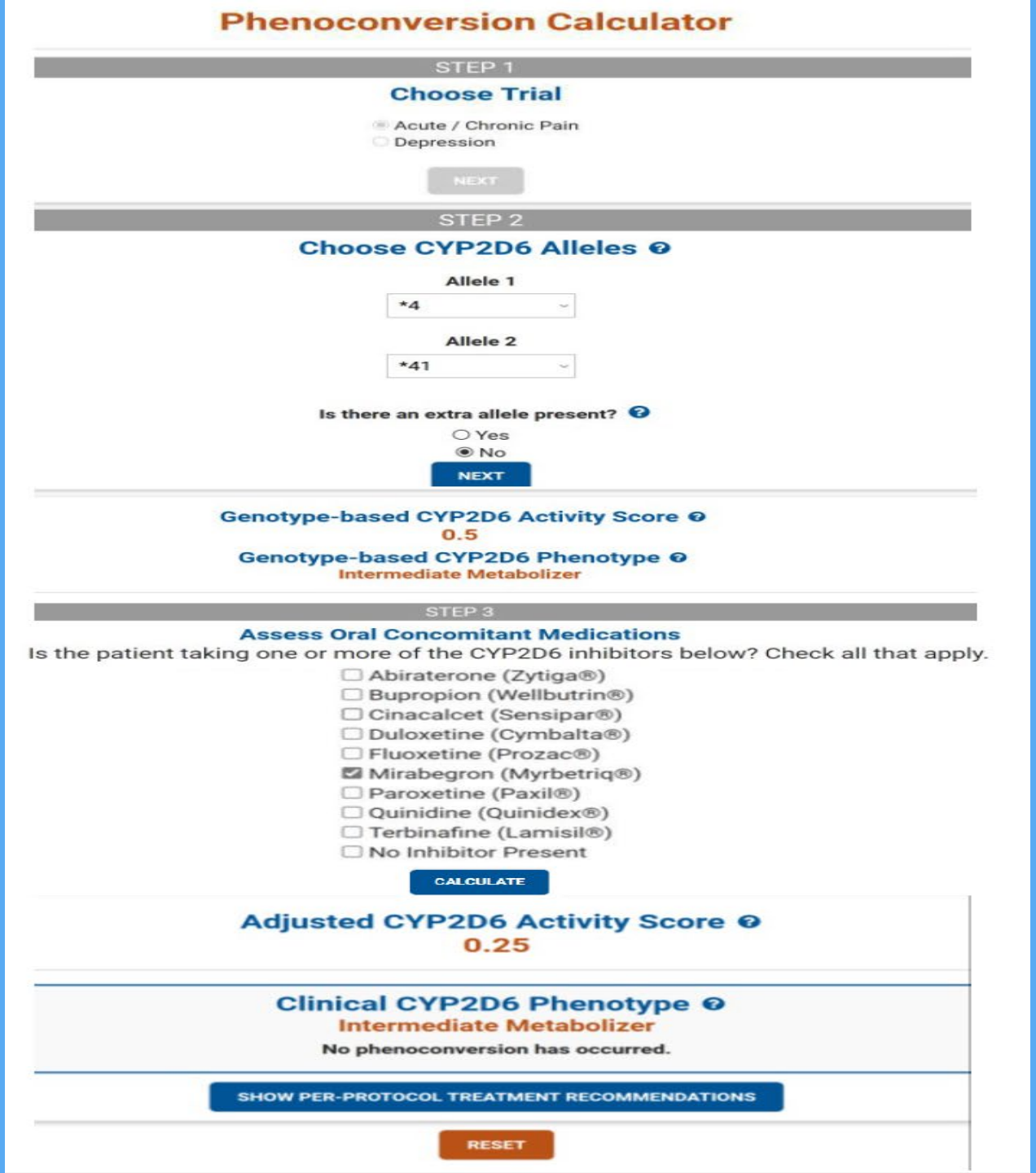
Hahn, M.; Roll, S.C. *Pharmaceuticals* 2021, 14, 487. https://doi.org/10.3390/ph140504

CYP2D6 Phenoconversion Calculator

The PROP™ Pharmacogenetics Calculator is intended to help clinicians integrate a standardized method of assessing CYP2D6 phenoconversion into practice when a CYP2D6 genotype is available. The CYP2D6 drug metabolizing enzyme is susceptible to inhibition by concomitant drugs, which can lead to a clinical phenotype that is different from the genotype-based phenotype, a process referred to as phenoconversion. Phenoconversion is highly prevalent but not widely integrated into practice because of either limited experience on how to integrate or lack of knowledge that it has occurred.

Use this calculator to enter a patient's CYP2D6 genotype and choose any interacting medications and the patient's activity score and clinical phenotype will be displayed. This calculator uses updated recommendations for translating CYP2D6 genotype to phenotype from the Clinical Pharmacogenetics Implementation Consortium (CPIC) and includes drugs classified as strong or moderate CYP2D6 inhibitors by the Food and Drug Administration. For complete information on how the calculator was developed, see Cicali EJ, et al., How to Integrate CYP2D6 Phenoconversion into Clinical Pharmacogenetics: A Tutorial. *Clin Pharmacol Ther*. 2021 Jul 7. doi: 10.1002/cpt.2354. Epub ahead of print. PMID: 34231197.

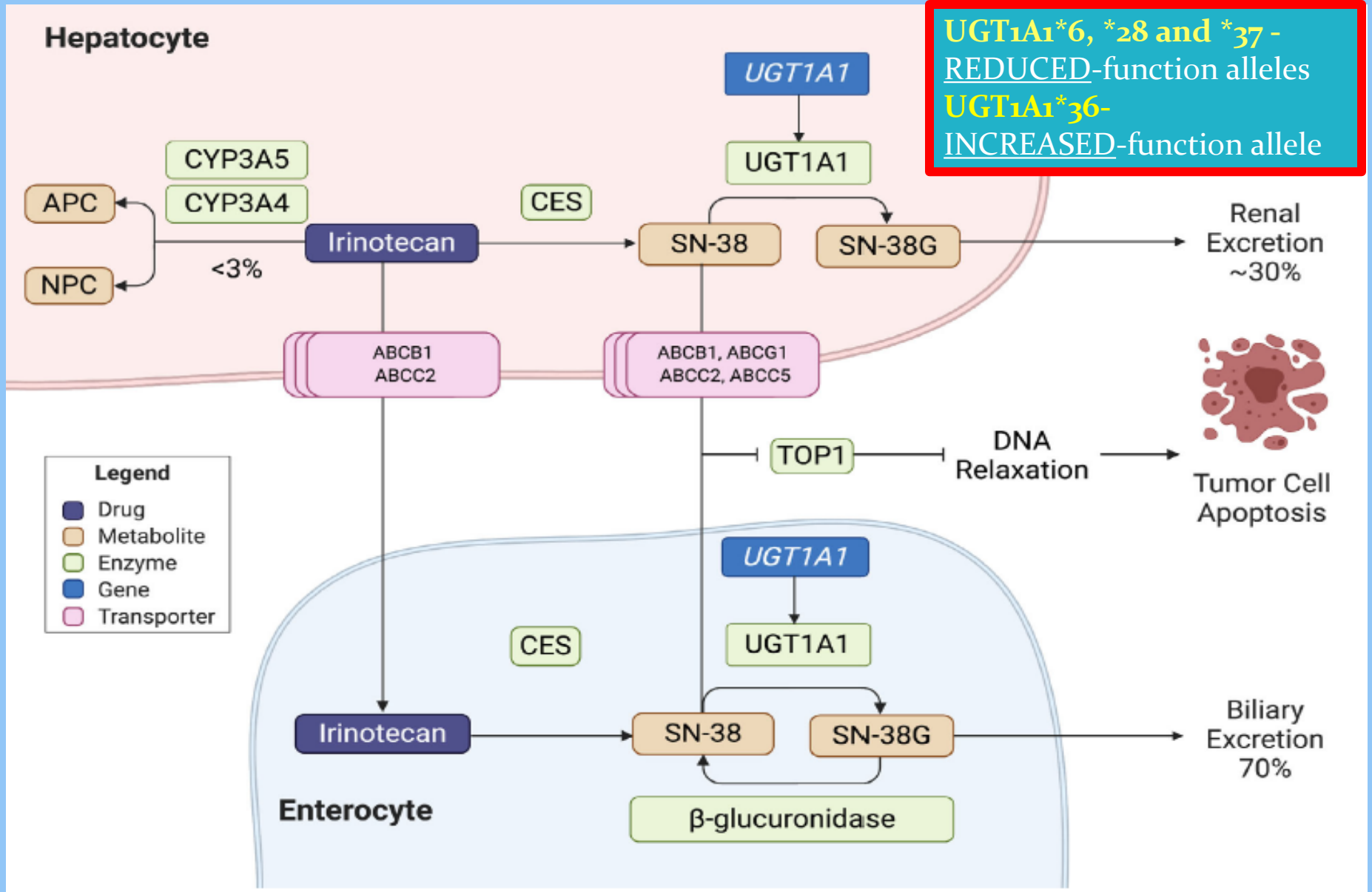
https://precisionmedicine.ufhealth.org/phenoconversion-calculator/ Accessed January 23, 2022



Cicali E et al.
Clinical Pharmacology and Therapeutics.
2021; 110(3): 677-687

Outline function of major genetic variants associated with Phase I (CYP2C9, CYP2C19, CYP2D6) and Phase II (UGT1A1, TPMT) drug metabolism and describe potential impact on pharmacokinetics (PK) and drug response

PGx of Phase II Enzymes



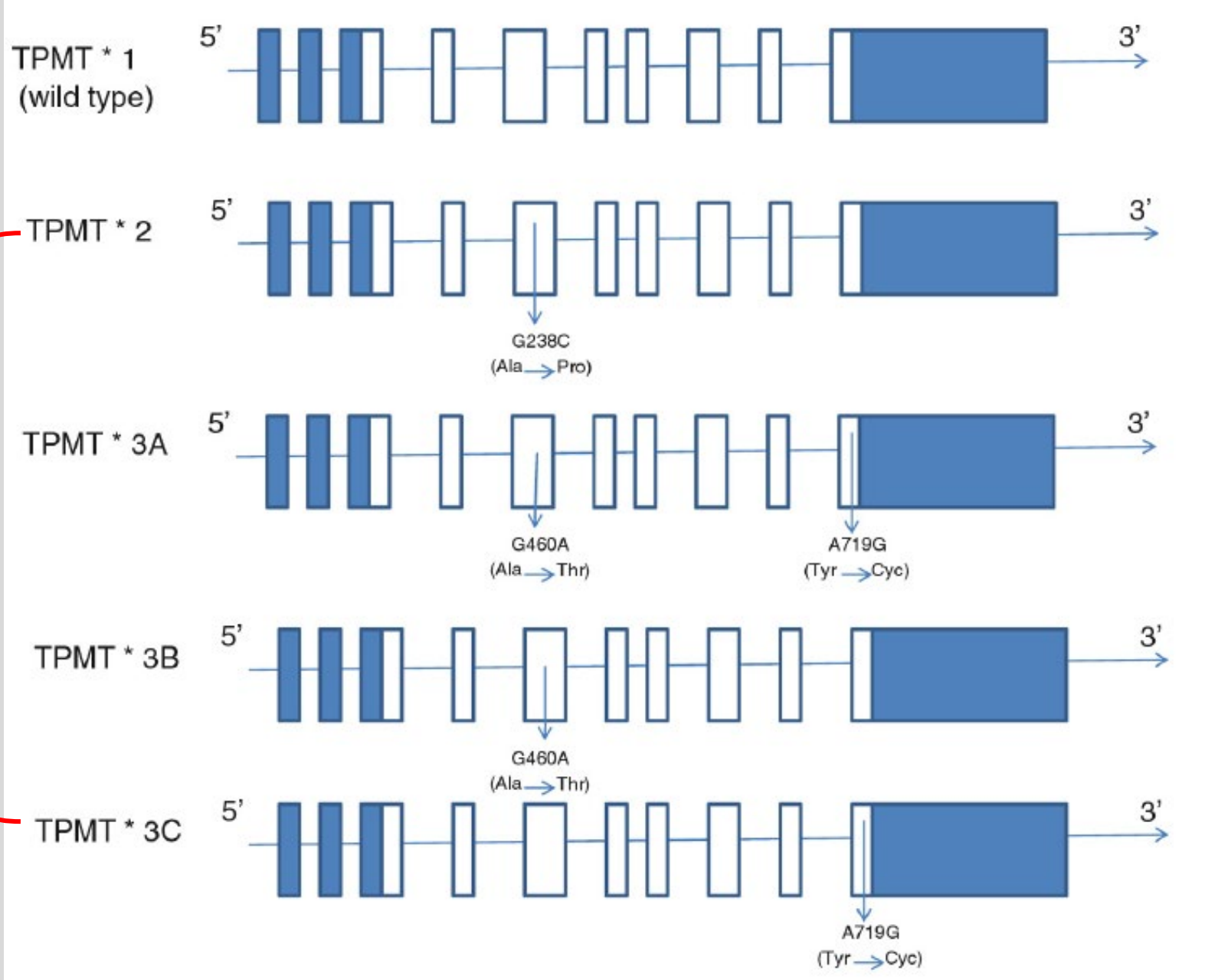
UGT1A1*6, *28 and *37 - REDUCED-function alleles
UGT1A1*36- INCREASED-function allele

Varughese LA et al. *Pharmacotherapy* 2020;40(11):1108–1129

Drug-Gene Pairs of Clinical Interest- UGT1A1

- Atazanavir
- Irinotecan
- Belinostat

No function alleles in TPMT gene



Zeglam HB et al. *Libyan Journal of Medicine. 2015;* 10:27053 DOI:10.3402/ljm.v10.27053

Drug-Gene Pairs of Clinical Interest- TPMT

- Azathioprine
- Mercaptopurine
- Thioguanine

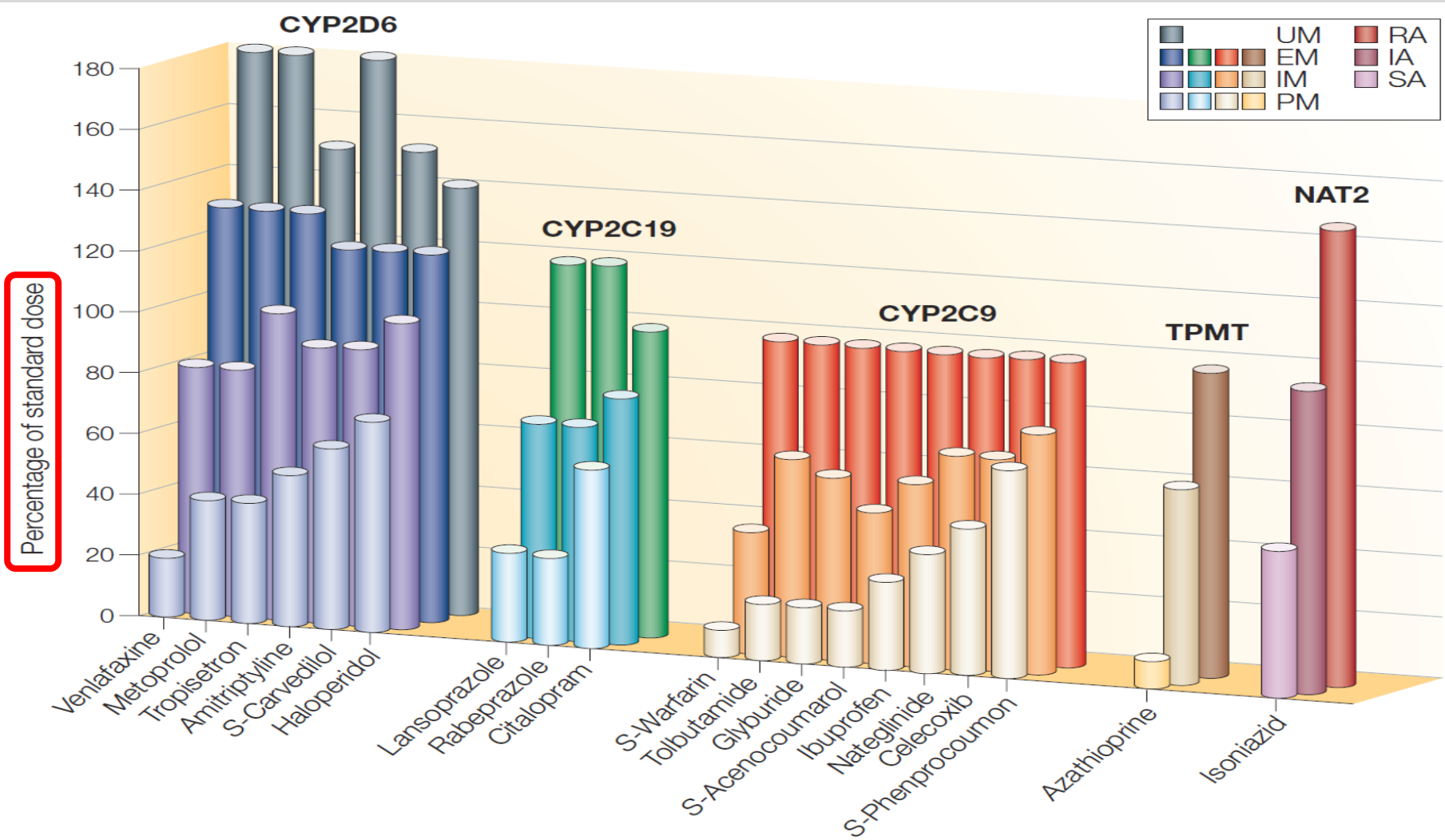
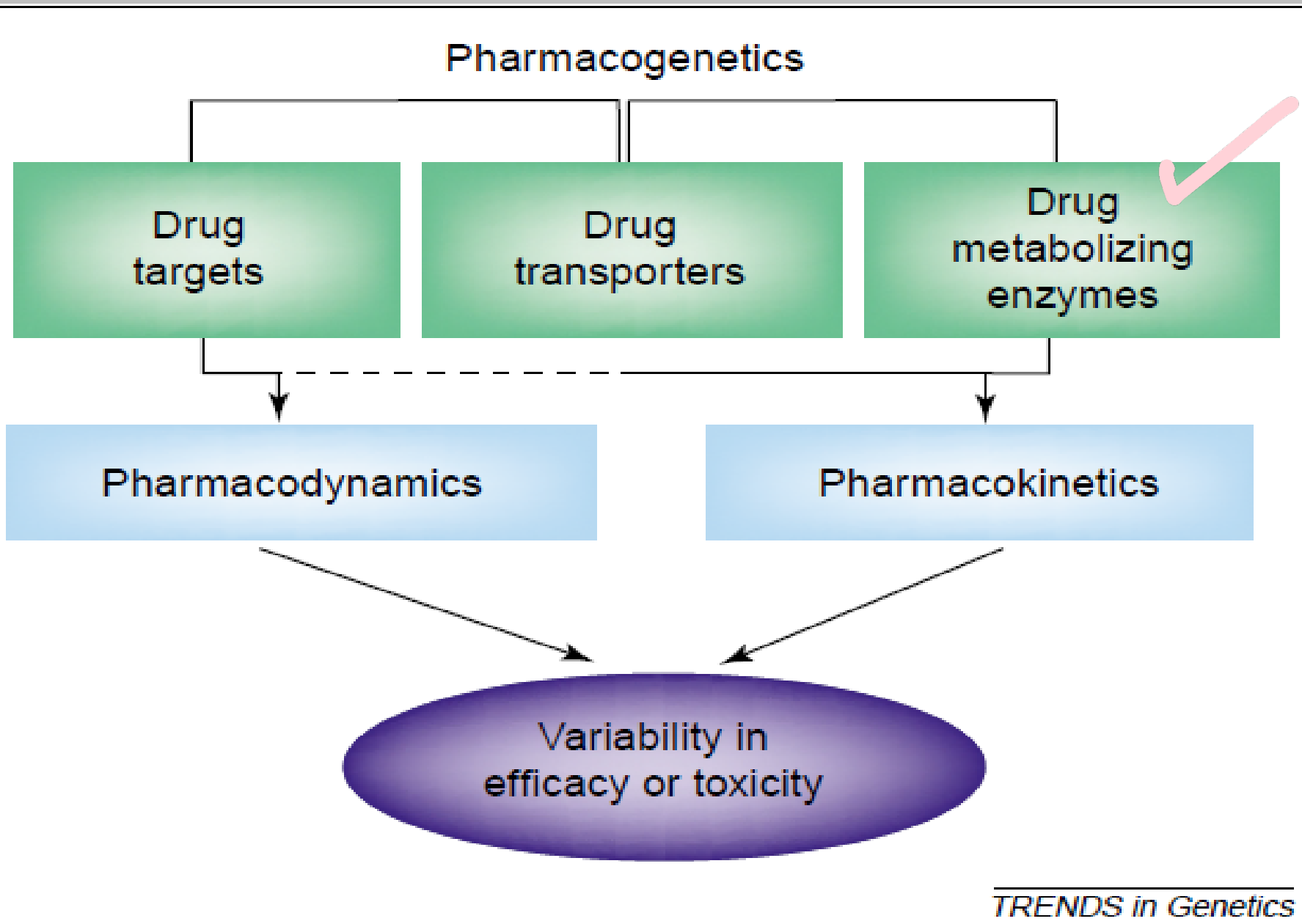


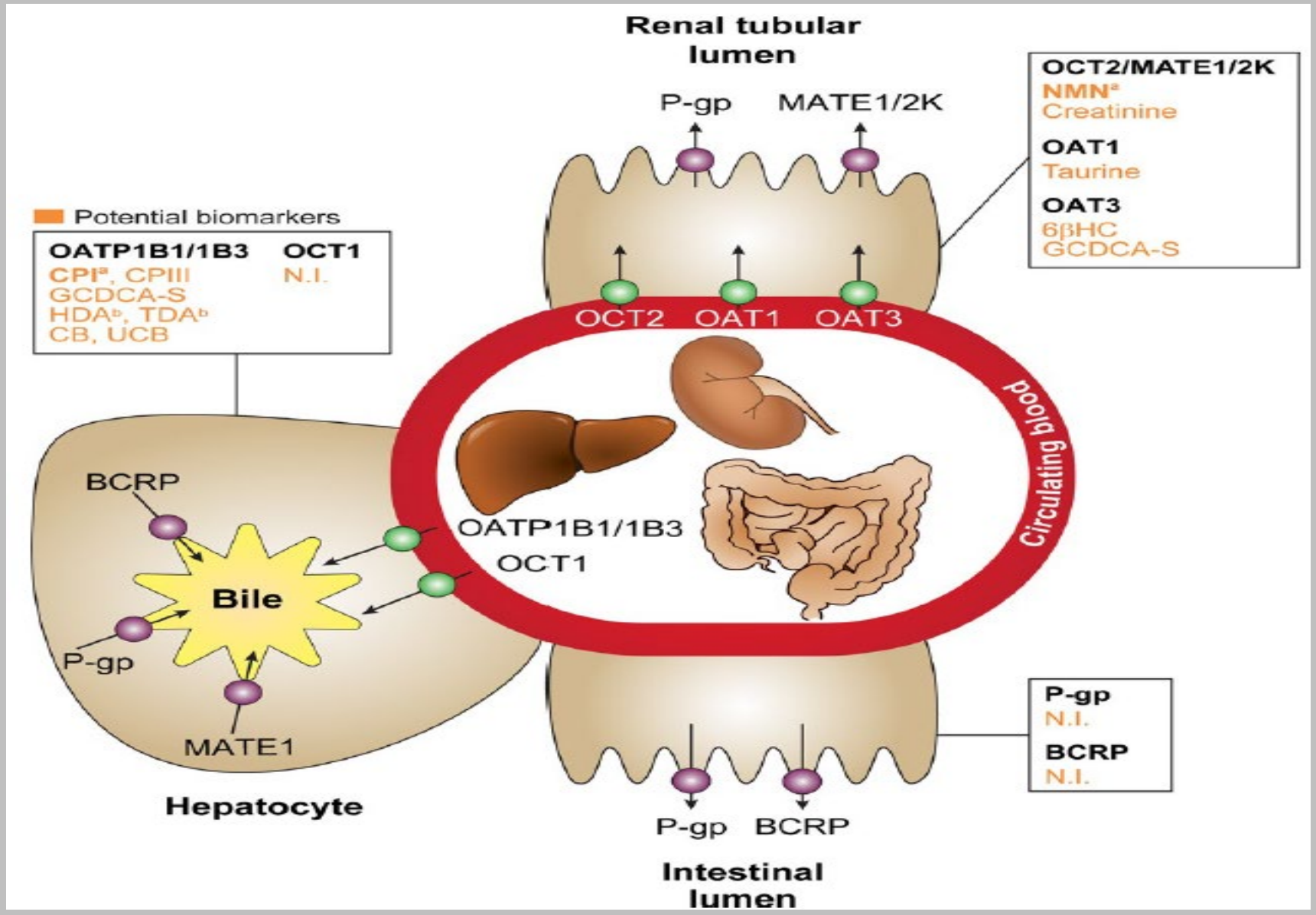
Figure 2 | **Examples of dose adjustments based on PGDx.** The influence of genetic polymorphisms in cytochrome P450 enzymes CYP2D6, CYP2C19 and CYP2C9, thiopurine *S*-methyltransferase (TPMT) and *N*-acetyltransferase type 2 (NAT2) is expressed as subpopulation-specific dosages, according to the difference in pharmacokinetic parameters from clinical studies[1,12,14,32,33]. The dose adjustments illustrated by the bars in this graph are based on differences in dose-related pharmacokinetic parameters (clearance, AUC, STEADY-STATE CONCENTRATION) caused by particular genotypes and are calculated using the methods described earlier[12]. Substantial adjustments need to be made to drug dose to achieve the same level of drug exposure in individuals with different genotypes. AUC, area under the

Kirchheiner J, Fuhr U, Brockmöller J. *Nat Rev Drug Discov*. 2005; 4(8):639-47



Johnson JA. *Trends Genet* 2003; 19(11): 660-666

PGx of Drug Transporters



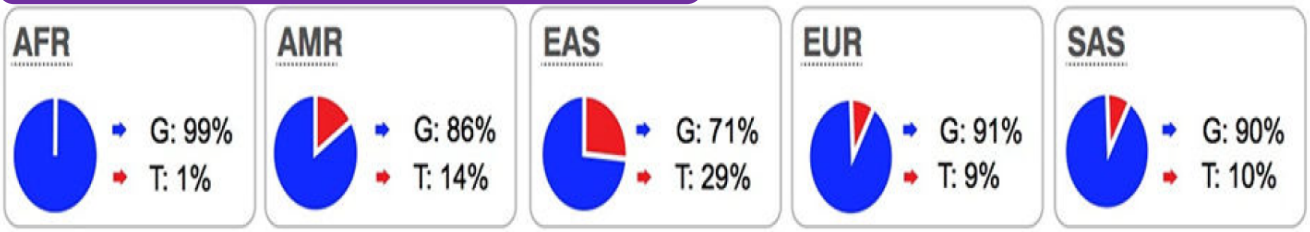
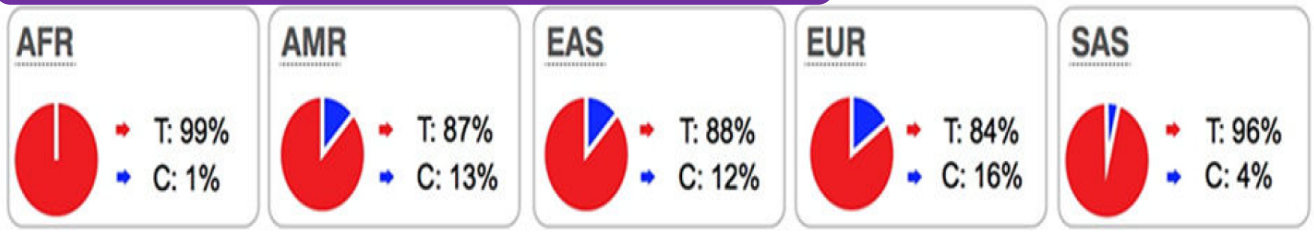
Giacomini KM et al. *Clin Pharmacol Ther*. 2018;104(5):766-771

Drug Transporters of Emerging Clinical Interest

- P-glycoprotein (P-gp; ABCB1/MDR-1)
- Breast cancer resistance protein (BCRP; ABCG2)
- Organic anion transporting polypeptide (OATP) 1B1 (SLCO1B1) and OATP1B3 (SLCO1B3)
- Organic anion transporter (OAT)1 (SLC22A6) and OAT3 (SLC22A8)
- Organic cation transporter (OCT) 1 (SLC22A1) and (OCT) 2 (SLC22A2)
- Multidrug and toxin extrusion protein (MATE) 1 (SLC47A1)

Giacomini KM et al. *Clin Pharmacol Ther*. 2018;104(5):766-771
Yee SW et al. *Clin Pharmacol Ther.* 2018; 104(5): 803–817

Major PGx Variants of Drug Transporters

Transporter variants and allele frequencies in different populations[1]	Supporting evidence	*In vitro* effect	**In vivo* effect
ABCG2: rs2231142, BCRP-Q141K, c. 421C>A 	KO mice: Yes DDI: Yes GWAS: Yes	Reduced expression levels and reduced transporter activity.	Increased plasma levels of drug substrate (e.g. atorvastatin, tenofovir) due to reduced efflux of drug from the intestine. Increased efficacy of drug substrate (e.g. rosuvastatin) due to reduced efflux of drug in the liver.
SLCO1B1: rs4149056, OATP1B1-V174A, c. 521T>C 	KO mice: Yes DDI: Yes GWAS: Yes	Reduced transporter activity (see Supplemental Table 1)	Increased plasma levels of substrates (e.g. pravastatin, atorvastatin). Increased toxicity of drug substrates (e.g. simvastatin, cerivastatin) due to increased plasma levels.

Yee SW et al. *Clin Pharmacol Ther.* 2018; 104(5): 803–817

Major PGx Variants of Drug Transporters

Transporter variants and allele frequencies in different populations[1]	Supporting evidence	*In vitro* effect	**In vivo* effect
SLC22A1: rs34130495, OCT1-G401S, c.1201G>A AFR: G: 100%, A: 0% AMR: G: 99%, A: 1% EAS: G: 100% EUR: G: 98%, A: 2% SAS: G: 100%, A: 0%	KO mice: Yes DDI: Yes GWAS: Yes (metabolite levels)	Reduced transporter activity and some with reduced protein expressions on the membrane (see Supplemental Table 1)	Increased plasma levels of drug, which could lead to increased drug toxicity (see Table 2).
SLC22A1: rs12208357, OCT1-R61C, c.181C>T AFR: C: 100%, T: 0% AMR: C: 98%, T: 2% EAS: C: 100% EUR: C: 94%, T: 6% SAS: C: 98%, T: 2%			
SLC22A1: rs34059508, OCT1-G465R, c.1393G>A AFR: G: 100% AMR: G: 98%, A: 2% EAS: G: 100% EUR: G: 98%, A: 2% SAS: G: 100%			
SLC22A1: rs202220802, OCT1-420Del, c.1258_1260ATG>deletion AFR: ATG: 95%, -: 5% AMR: ATG: 71%, -: 29% EAS: ATG: 100%, 0: 0% EUR: ATG: 82%, -: 18% SAS: ATG: 85%, -: 15%			

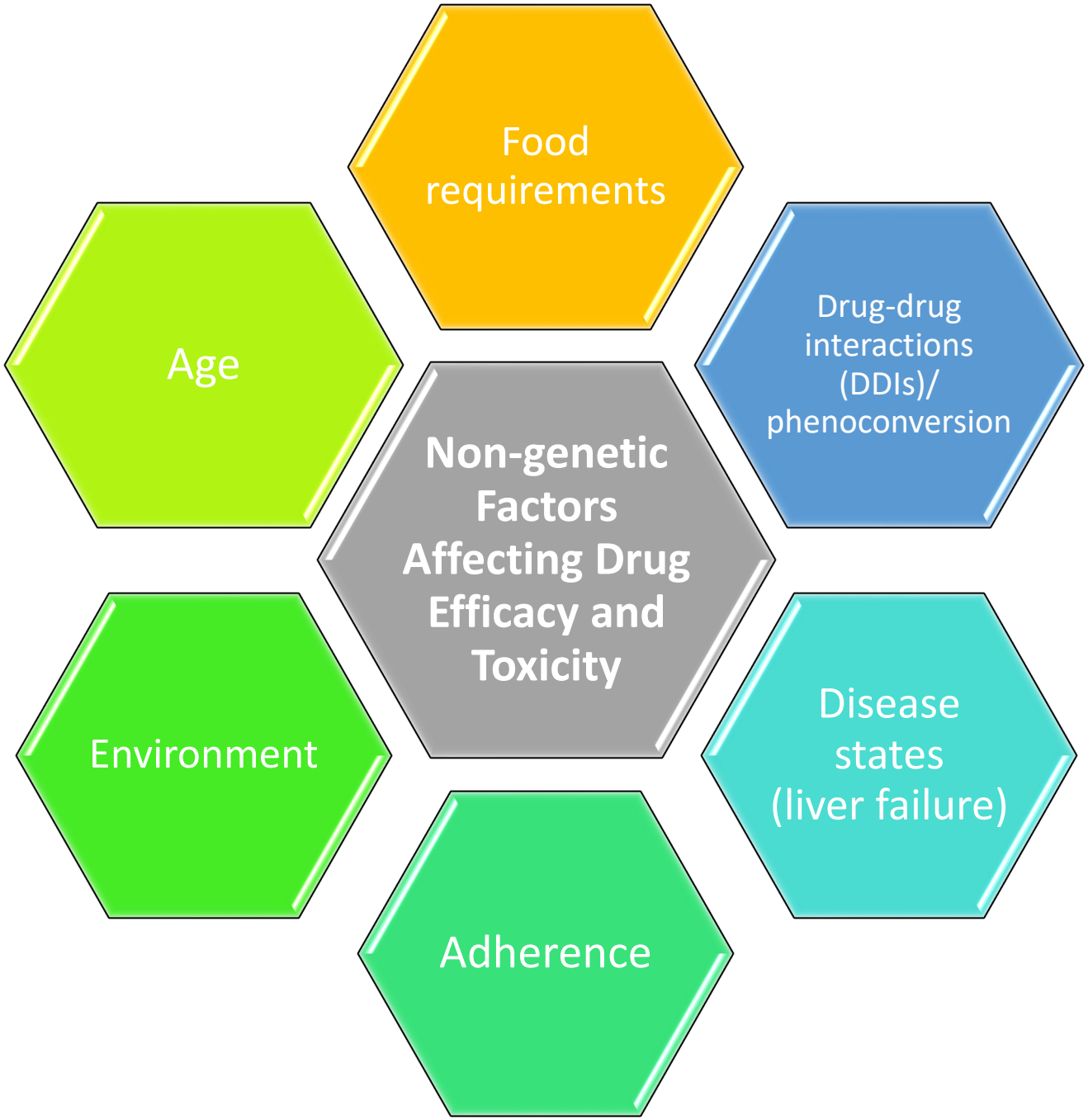
Yee SW et al. *Clin Pharmacol Ther.* 2018; 104(5): 803–817

Drug-Gene Pairs of Clinical Interest/ Drug Transporters

- Simvastatin (SLCO1B1)
- Rosuvastatin (ABCG2)

Drug-Gene Pairs of Clinical Interest/ Pharmacodynamic Markers (Drug Targets)

- Warfarin (VKORC1)
- SSRIs (5HTR2A and SLC6A4)
- Abacavir/allopurinol/carbamazepine/ oxcarbazepine/phenytoin (HLA-B)
- Carbamazepine (HLA-A)


Food requirements
Age
Drug-drug interactions (DDIs)/ phenoconversion
Non-genetic Factors Affecting Drug Efficacy and Toxicity
Environment
Disease states (liver failure)
Adherence

Describe relevant FDA resources used for disseminating PGx-related drug-gene pair information

https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations Accessed February 22, 2022
https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling Accessed February 22, 2022

FDA Resources for PGx-related Information

PGx Information in FDA Drug Labeling	FDA Table of PGx Associations (introduced Feb 2020)
❑ Subsection 12.5 in drug labeling (i.e. can include specific dosing recommendations)	❑ Not all inclusive and updated periodically with evolving evidence
❑ Summary of essential scientific information needed for safe and effective use of prescription drug	❑ Reports associations supporting therapeutic management recommendations/potential impact on safety/response + PK
❑ Focuses on somatic/germline mutations	❑ Focuses only on germline mutations
❑ Reports positive and negative associations	❑ Reports positive associations only
❑ May suggest testing recommendations	❑ Does not report testing recommendations

PGx Level

Testing required The label states or implies that some sort of gene, protein or chromosomal testing, including genetic testing, functional protein assays, cytogenetic studies, etc., should be conducted before using this drug. This requirement may only be for a particular subset of patients. PharmGKB considers labels that state the variant is an indication for the drug, as implying a test requirement. If the label states a test "should be" performed, this is also interpreted as a requirement.

Testing recommended The label states or implies that some sort of gene, protein or chromosomal testing, including genetic testing, functional protein assays, cytogenetic studies, etc., is recommended before using this drug. This recommendation may only be for a particular subset of patients. PharmGKB considers labels that say testing "should be considered" or "Consider genotyping or phenotyping" to be recommending testing.

Actionable PGx The label may contain information about changes in efficacy, dosage, metabolism or toxicity due to gene/protein /chromosomal variants or phenotypes (e.g. "poor metabolizers"). Or the label may mention contraindication of the drug in a particular subset of patients with particular variants/genotypes/phenotypes. However, the label does not require or recommend gene, protein or chromosomal testing.

Informative PGx

1. The label contains information stating that particular gene/protein/chromosomal variants or metabolizer phenotypes do not affect a drug's efficacy, dosage, metabolism or toxicity. Or, the label states that particular variants or phenotypes affect a drug's efficacy, dosage, metabolism or toxicity, but this effect is not "clinically" significant.

OR

2. The label appears or appeared on the FDA Biomarker List but does not currently meet the requirements to be assigned as "Testing required", "Testing recommended" or "Actionable PGx". PharmGKB annotates every label that appears on the FDA Biomarker list, regardless of whether we would otherwise annotate the label.

M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine" *Clinical Pharmacology & Therapeutics* (2012) 92(4): 414-417
https://www.pharmgkb.org/page/drugLabelLegend#pgx-level Accessed December 20, 2021

PharmGKB also annotates drug labels from other agencies. See the full list of PharmGKB annotated labels.

LABEL FOR	ALLELES	FDA BIOMARKER LIST	FDA PGX ASSOCIATION	PGX LEVEL	TAGS
abacavir		All	All	All	All
abacavir and HLA-B	HLA-B*57:01:01	On FDA Biomarker List	Recommendations	Testing required	Alternate Drug, Prescribing Info

Testing required | Alternate Drug | Prescribing Info

PharmGKB ID : PA166104833

Variants Discussed on Label

HLA-B*57:01:01

Summary

The FDA-approved label for abacavir (ZIAGEN) states that genetic testing for the HLA-B*5701 allele is required prior to initiating or reinitiating treatment with abacavir in patients of unknown HLA-B*5701 status. Abacavir is contraindicated in patients with the HLA-B*5701 allele due to risk for abacavir hypersensitivity reactions.

FDA Table of Pharmacogenetic Associations

From the FDA Table of Pharmacogenetic Associations:

CATEGORY	DRUG	GENE	AFFECTED SUBGROUP	INTERACTION DESCRIPTION
Recommendations	abacavir	HLA-B	*57:01 allele positive	Results in higher adverse reaction risk (hypersensitivity reactions). Do not use abacavir in patients positive for HLA-B*57:01.

M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine" *Clinical Pharmacology & Therapeutics* (2012) 92(4): 414-417
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