Clinical Functional Assignment of *NAT2* Alleles by the CPIC Pharmacogene Curation Expert Panel

Bailey Tibben, PhD

CPIC Meeting

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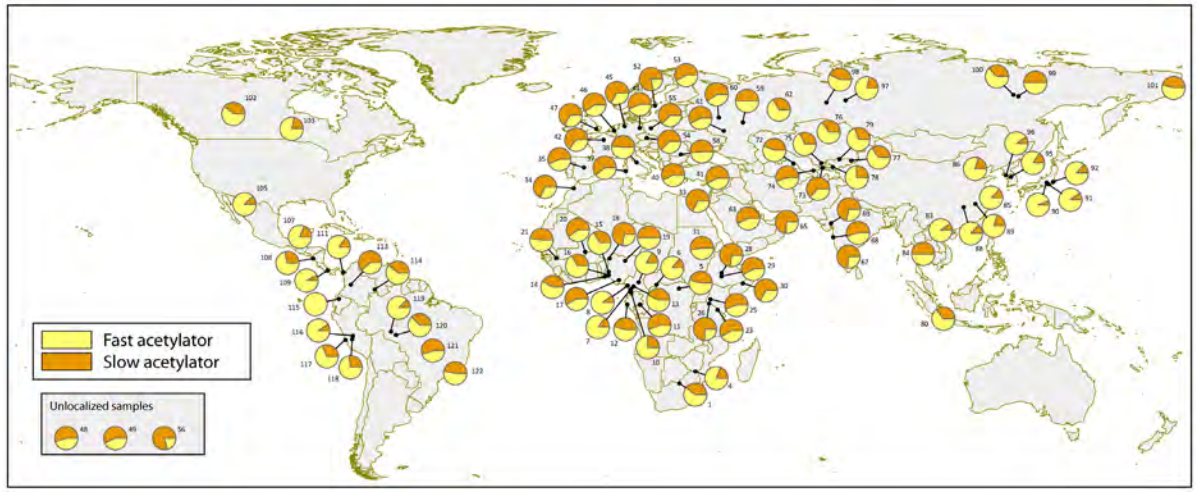
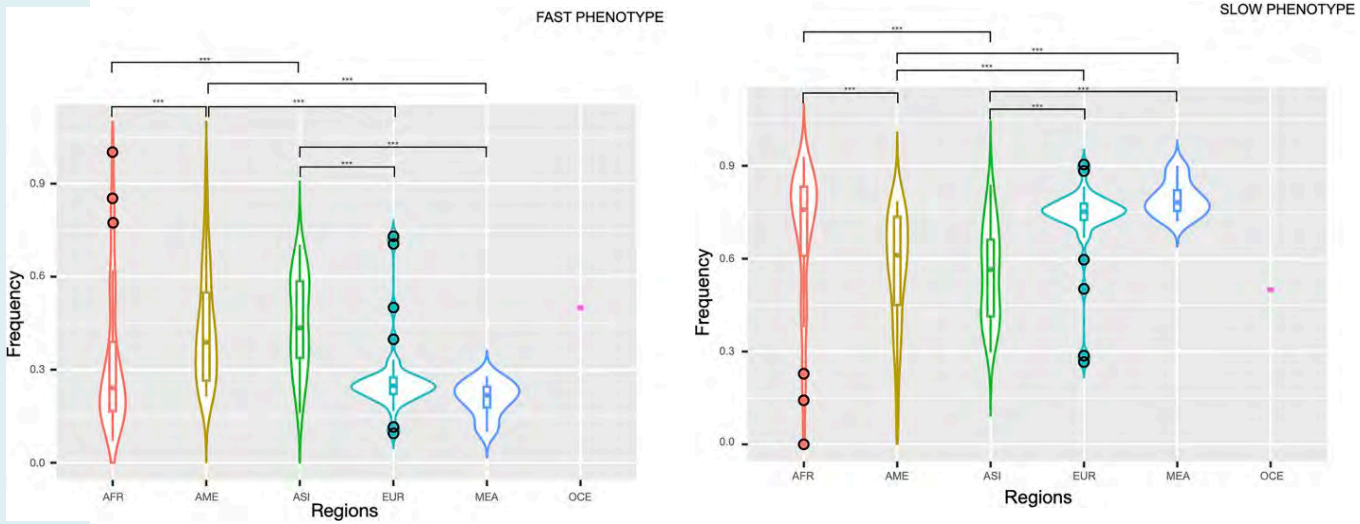
NAT2 Overview

- *NAT2* encodes arylamine N-acetyltransferase 2
  - phase 2 hepatic metabolism of arylamines and arylhydrazines
  - hydralazine, isoniazid, dapsone, and sulfamethazine
- *NAT2* is highly polymorphic
  - Bimodal distribution of rapid and poor metabolizers
- High degree of concordance b/w *NAT2* genotype and phenotype
- Highly expressed in the liver, small intestine, and colon
- NAT2 acetylation can create active or inactive metabolites



Sabbagh *et al.* 2011, *PLoS One*



Gutiérrez-Virgen *et al.* 2023, *PLoS One*

NAT2 Pharmacogene Curation Expert Panel (PCEP)

José A. G. Agúndez, MD, PhD
University of Extremadura
Chair

Michael Eadon, MD
Indiana University
School of Medicine

David W. Hein, PhD, FAAPE
University of Louisville
School of Medicine

Sotiria Boukouvala, D.Phil.
Democritus University of Thrace

Rod Minchin, PhD
The University of Queensland
School of Biomedical Sciences

Adalberto Rezende Santos, PhD
Oswaldo Cruz Foundation

Michelle Whirl-Carrillo, PhD
Stanford University

Andrea Gaedigk, PhD
Children's Mercy Research Institute

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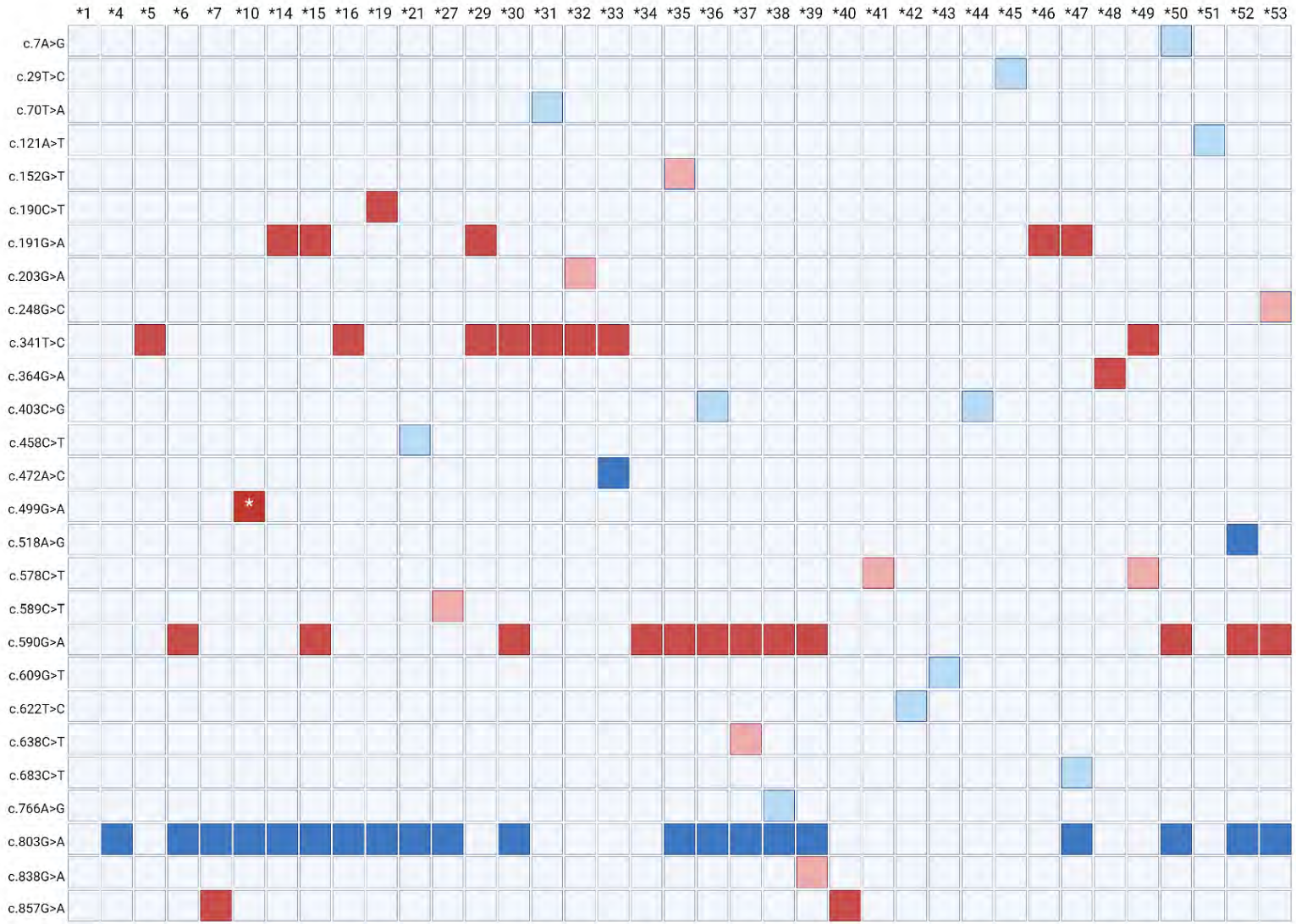
Inclusion of Alleles

- *NAT2* nomenclature transferred to PharmVar in March 2024
- 48 alleles currently catalogued in PharmVar
- RefSeq NG_012246.1 – corresponds to legacy allele, *NAT2*12A* (now *NAT2*1*)
  - Variant switching at c.803
  - Several alleles lost c.803A>G and several gained c.803G>A
- Legacy *NAT2* clusters broken into separate core alleles
  - *NAT2*5A* and *NAT2*5D* now *NAT2*16*
  - *NAT2*5E* now *NAT2*30*

NAT2 Allele Comparison



LEGEND

- Variant Present. Variant does not alter function
- Variant Present. Variant has uncertain or unknown function.
- Variant Present. Variant associated with decreased function based on experimental evidence.
- Variant Present. Variant associated with decreased function based on *in silico* evidence.

*c.499G>A was shown to reduce NAT2 activity *in vitro*, however *in silico* predictions indicate that the variant is neutral for protein function.

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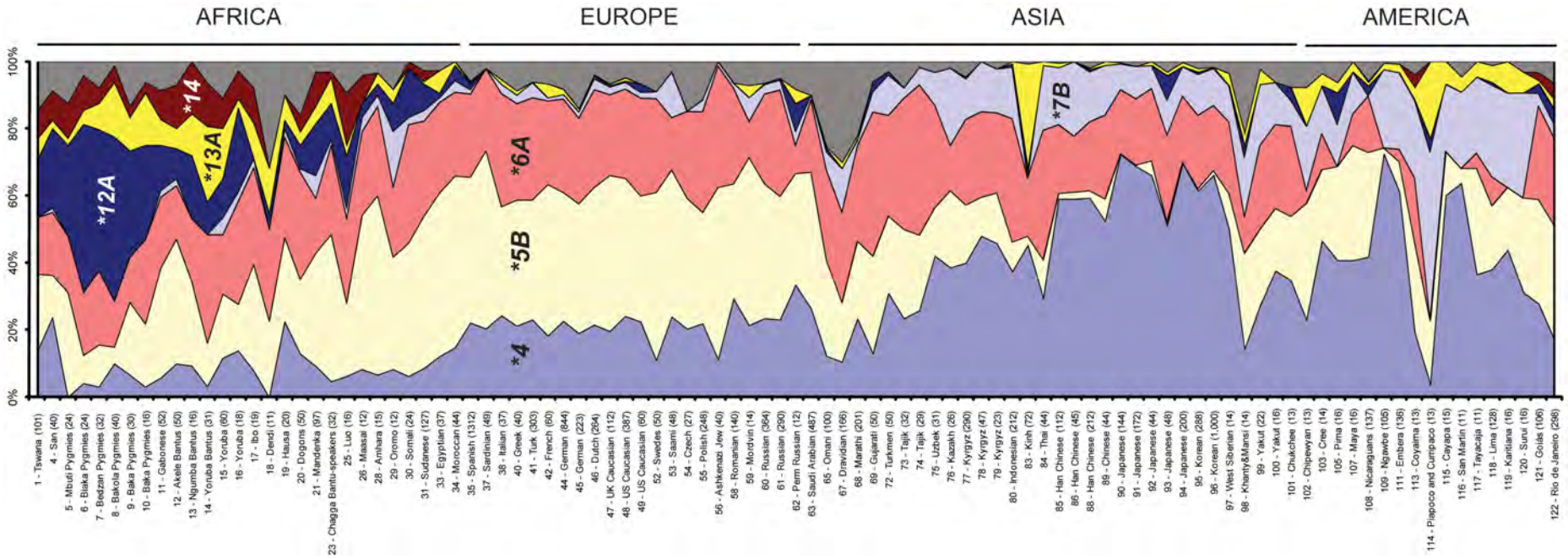
PharmVar
Pharmacogene Variation Consortium

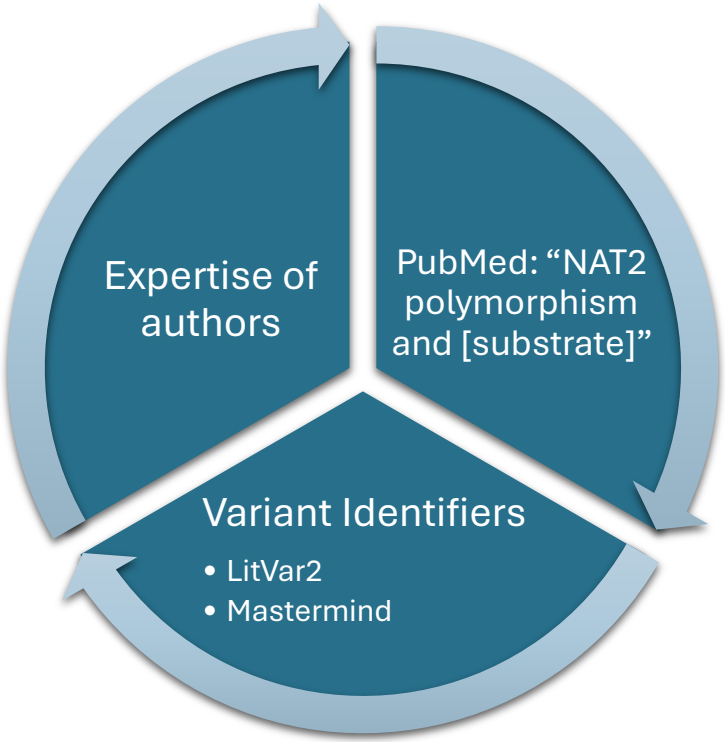
Allele Frequency

- c.191G>A (defining **14*) is present in 10% of African and American populations
- c.341T>C (defining **5*) is present in 50% of Europeans
- c.590G>A (defining **6*) is most common in Europeans, but is also prevalent in the Americas and Asia
- c.857G>A (defining **7*) is present in 20% of individuals with East Asian ancestry



Sabbagh *et al.* 2011, *PLoS One*

Evidence Review



I HAVE A QUESTION OR COMMENT ABOUT ALLELE FUNCTION OR DIPLOTYPE-PHENOTYPE TABLES

Contact us with questions specifically about CPIC alleles

Contact about functions or diplotype-phenotypes

https://cpicpgx.org/contact/

Evidence Review

In Vitro

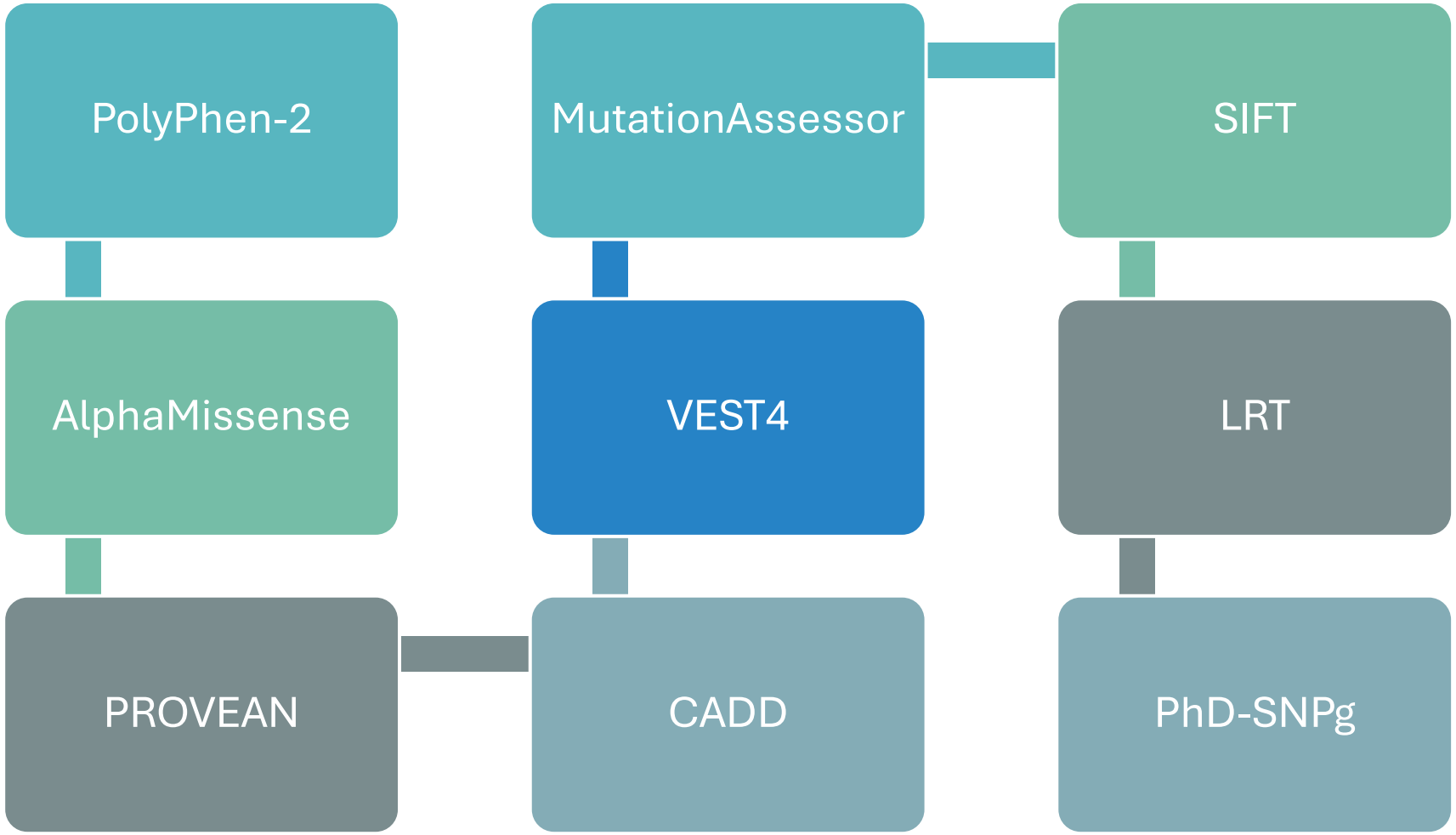
- NAT2 variants expressed in bacterial or mammalian cells
- Commonly tested substrates include sulfamethazine, *p*-aminosalicylic acid, 2-aminofluorene, 4-aminobiphenyl, and procainamide

Clinical Phenotyping

- Early clinical studies use caffeine as a probe drug
- Sulfonamides – sulfamethazine, sulfasalazine. dapsone
- Hydralazine
- Isoniazid

***NAT2*4* is used as the reference allele in these studies**

In Silico Variant Effect Prediction (VEP)



Genotype to Phenotype Determination

Predicted Phenotype	Acetylator Phenotype	Genotype	Examples of *NAT2* diplotypes
NAT2 Rapid Metabolizer (RM)	Rapid acetylator	An individual carrying two *increased* function alleles	**1/*1, *1/*4*
NAT2 intermediate metabolizer (IM)	Intermediate acetylator	An individual carrying one *increased* function allele and one *decreased* function allele	**1/*5, *4/*6*
NAT2 poor metabolizer (PM)	Slow acetylator	An individual carrying two *decreased* function alleles	**5/*5, *6/*6, *6/*7*
NAT2 indeterminate	NA	An individual carrying one or two uncertain or unknown function alleles	**1/*27, *4/*10*

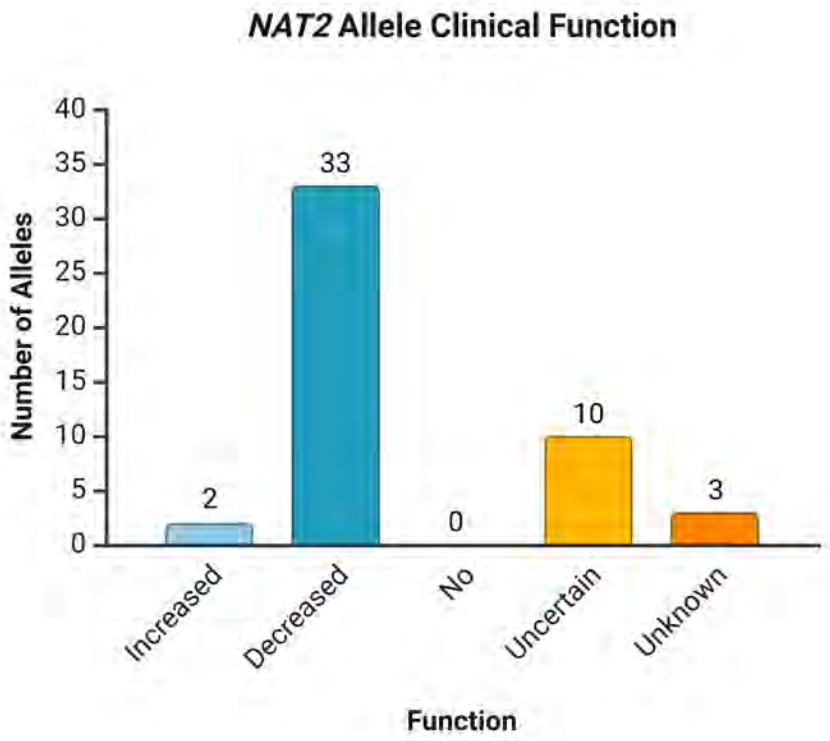
As always, tables will be available on the CPIC website as soon as the guideline is published.

GENE: NAT2					
Allele/cDNA/rsID	Allele Biochemical Functional Status (Optional)	Allele Clinical Functional Status (Required)	References (Required)	Strength of Evidence (Required)	
*1	Rapid	Increased Function	9154882, 30564082, 15558239, 10744128, 22092036, 2	Definitive	*NAT2*1* is assigned increa
*4	Rapid	Increased Function	9154882, 8781741, 28653770, 8102597, 30564082, 15	Definitive	*NAT2*4* is assigned increa
*5	Slow	Decreased function	9154882,8781741, 28653770, 8102597, 30564082, 155	Definitive	*NAT2*5* is assigned decrea
*6	Slow	Decreased function	9154882, 8781741, 29589062, 28653770, 8102597, 30	Definitive	*NAT2*6* is assigned decrea
*7	Slow	Decreased function	9154882, 29589062, 28653770, 8102597, 30564082, 15	Definitive	*NAT2*7* is assigned decrea
*10		Uncertain function	17434923, 18680467	Inadequate	*NAT2*10* is assigned unce
*14	Slow	Decreased function	8102597, 9154882, 28653770, 7668286, 22092036, 91	Definitive	*NAT2*14* is assigned decre
*15	Slow	Decreased function	28523442	Moderate	*NAT2*15* is assigned decre
*16	Slow	Decreased function	9154882, 28653770, 30564082, 15558239, 7668286, 10	Definitive	*NAT2*16* is assigned decre
*19	Slow	Decreased function	18680467, 12222688, 9173883	Limited	*NAT2*19* is assigned decre
*21		Uncertain function	19909761	Inadequate	*NAT2*21* is assigned unce
*27		Uncertain function	19164093	Inadequate	*NAT2*27* is assigned unce
*29	Slow	Decreased function	10762005, 28523442, 15564878	Moderate	*NAT2*29* is assigned decre
*30	Slow	Decreased function	15558239, 15564878	Moderate	*NAT2*30* is assigned decre
*31	Slow	Decreased function	19909761, 15564878	Moderate	*NAT2*31* is assigned decre
*32	Slow	Decreased function	1559981, 9173883, 19909761, 15564878	Moderate	*NAT2*32* is assigned decre
*33	Slow	Decreased function	19164093, 19909761, 15564878	Moderate	*NAT2*33* is assigned decre
*34	Slow	Decreased function	28523442, 9154882, 15558239, 17011540, 28959610	Definitive	*NAT2*34* is assigned decre
*35	Slow	Decreased function	19909761	Moderate	*NAT2*35* is assigned decre
*36	Slow	Decreased function		Moderate	*NAT2*36* is assigned decre
*37	Slow	Decreased function	29273096	Moderate	*NAT2*37* is assigned decre
*38	Slow	Decreased function	19909761	Moderate	*NAT2*38* is assigned decre
*39	Slow	Decreased function	24533708, 19909761	Moderate	*NAT2*39* is assigned decre
*40	Slow	Decreased function	28959610, 17434923, 18680467, 11337936	Definitive	*NAT2*40* is assigned decre
*41		Uncertain function	19909761	Inadequate	*NAT2*41* is assigned unce
*42		Uncertain function	19909761	Inadequate	*NAT2*42* is assigned unce
*43		Uncertain function	19909761, 24444407	Inadequate	*NAT2*43* is assigned unce
*44		Uncertain function	19909761, 28959610, 24444407	Inadequate	*NAT2*44* is assigned unce
*45		Uncertain function	19909761, 24444407	Inadequate	*NAT2*45* is assigned unce
*46	Slow	Decreased function	28523442, 28959610, 18680467, 10574910	Definitive	*NAT2*46* is assigned decre
*47	Slow	Decreased function	18680467, 19909761, 24533708	Limited	*NAT2*47* is assigned decre
*48	Slow	Decreaaed function	17434923, 18680467, 17264801	Limited	*NAT2*48* is assigned decre

Strength of Evidence Determination

Strength of Evidence	Evidence Criteria	Examples
Definitive	Alleles characterized by variants with **well-established function** that have been extensively studied over the last 25+ years	*NAT2*1, NAT2*4, NAT2*5, NAT2*6, NAT2*7, NAT2*14*
Strong		NA
Moderate	Alleles characterized by two or more variants with **well-established function** Alleles characterized by one variant with **well-established function** and one with concordant VEPs	*NAT2*15, NAT2*29, NAT2*33, NAT2*50*
Limited	Alleles characterized by one variant with **well-established function** and one variant with unknown or uncertain function, particularly when VEPs were conflicting	*NAT2*19, NAT2*47, NAT2*48*
Inadequate – Uncertain function	No *in vivo* data Conflicting *in vitro* and/or *in silico* data	*NAT2*10, NAT2*21, NAT2*43, NAT2*51*
No evidence – Unknown function	Alleles with a lack of literature describing function	*NAT2*55, NAT2*60, NAT2*61*

Clinical Function Assignment

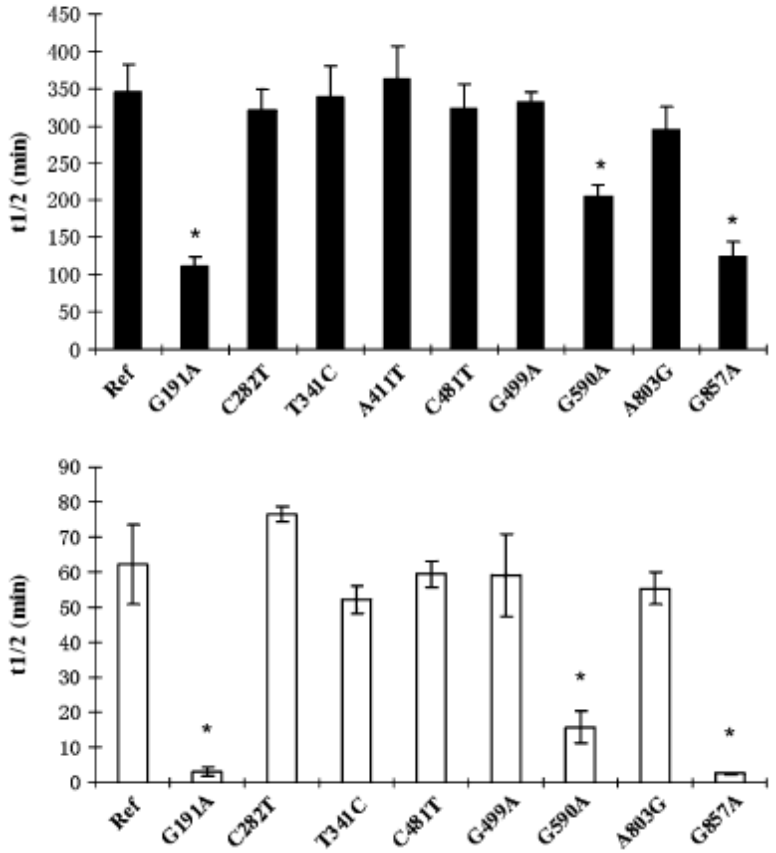


Allele Clinical Function	Strength of Evidence	NAT2 Alleles
Increased	Definitive	*1, *4
Decreased	Definitive	*5, *6, *7, *14, *16, *34, *40, *46
Decreased	Moderate	*15, *29, *30, *31, *32, *33, *35, *36, *37, *38, *39, *49, *50, *52, *53, *56, *58, *59, *62, *63, *64
Decreased	Limited	*19, *47, *48
Uncertain	Inadequate	*10, *21, *27, *41, *42, *43, *44, *45, *51, *54, *57
Unknown	No evidence	*55, *60, *61

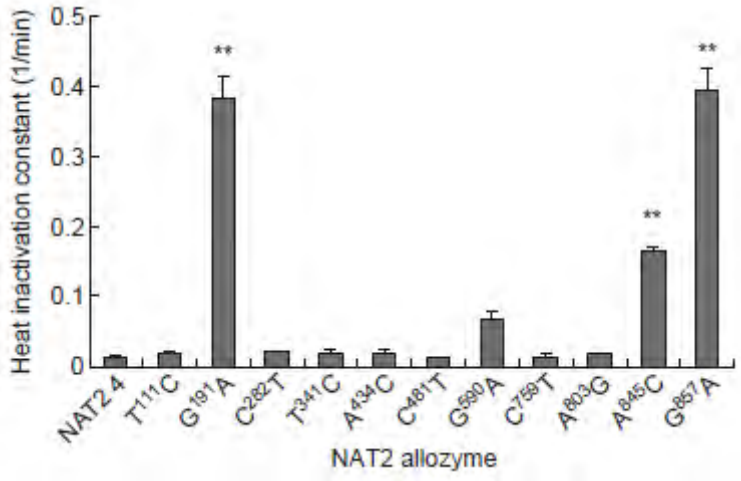
Molecular Mechanisms and Biochemical Function

- Mechanisms of decreased function
- *NAT2*7* substrate specificity
- Alleles considered for “no function” assignment

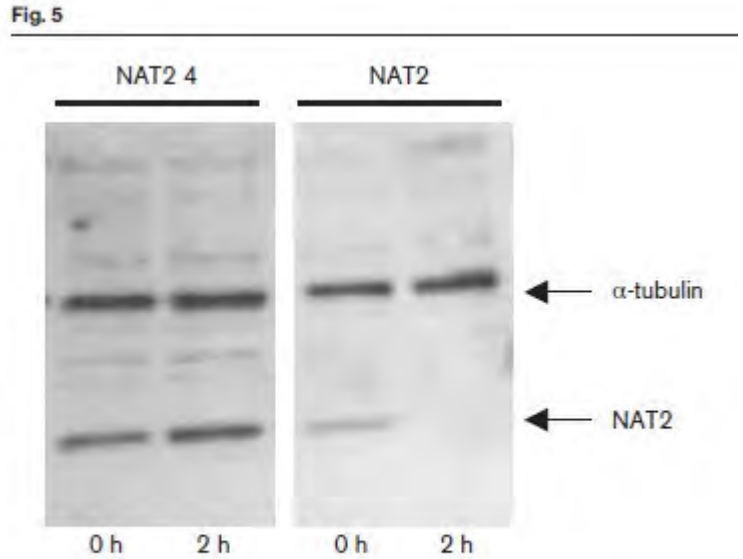
Different molecular mechanisms lead to decreased function



Zang 2007, *Carcinogenesis*



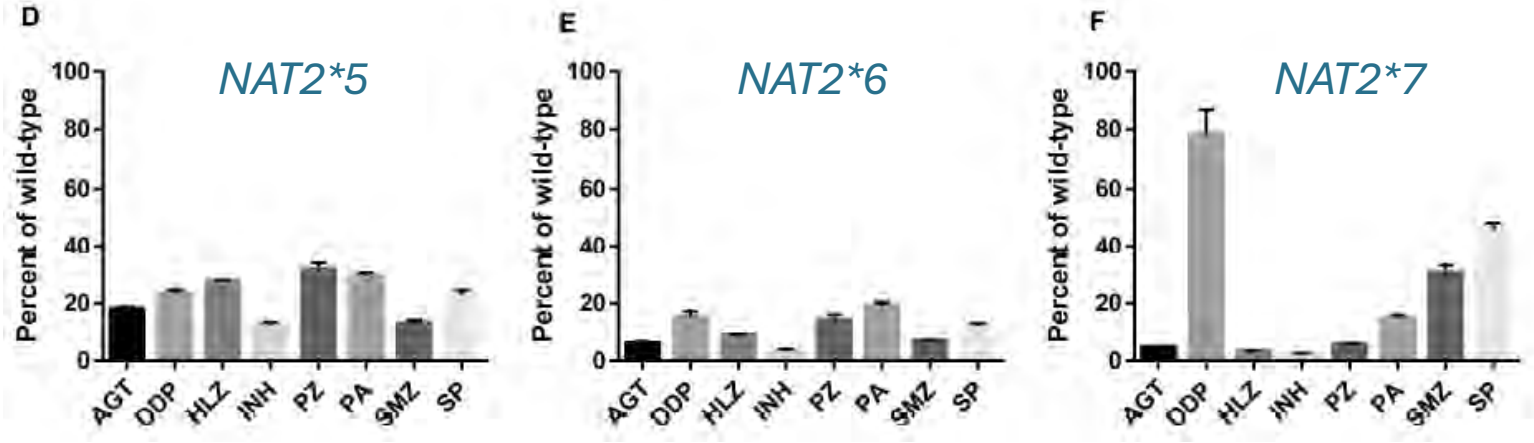
Fretland 2001, *Pharmacogenetics*



Western blot of NAT2 in COS-1 cells expressing NAT2 4 (Ile114) or NAT2 5D (Thr114) incubated with cycloheximide (100 μg/ml) for 2 h. Control treatment (0 h).

Zang 2004, *Pharmacogenetics*

*NAT2*7* substrate specificity



Fukunaga *et. al.* 2021, *Frontiers in Genetics*

Alleles considered for “no function” assignment

*NAT2*27*

- c.589C>T; p.R197X
- Missing 1/3 of the coding sequence, but retains active site residues
- Lack of data to call function
- Called uncertain function

Matimba 2009, *Human Genomics*

*NAT2*32*

- c.203G>A; p.C68Y
- Alters key active site residue
- p.C68G and p.C68A abolish enzymatic activity
- Called decreased function based on c.341T>C (*5)

Dupret 1992, *JBC*

Deloménie 1997, *The Biochemical Journal*

*NAT2*48*

- c.364G>A; p.D122N
- Protein expression undetectable in cells
- Undetectable enzymatic activity *in vitro*
- Lack of *in vivo* data
- Called decreased function

Zang 2007, *Pharmacogenetics and Genomics*

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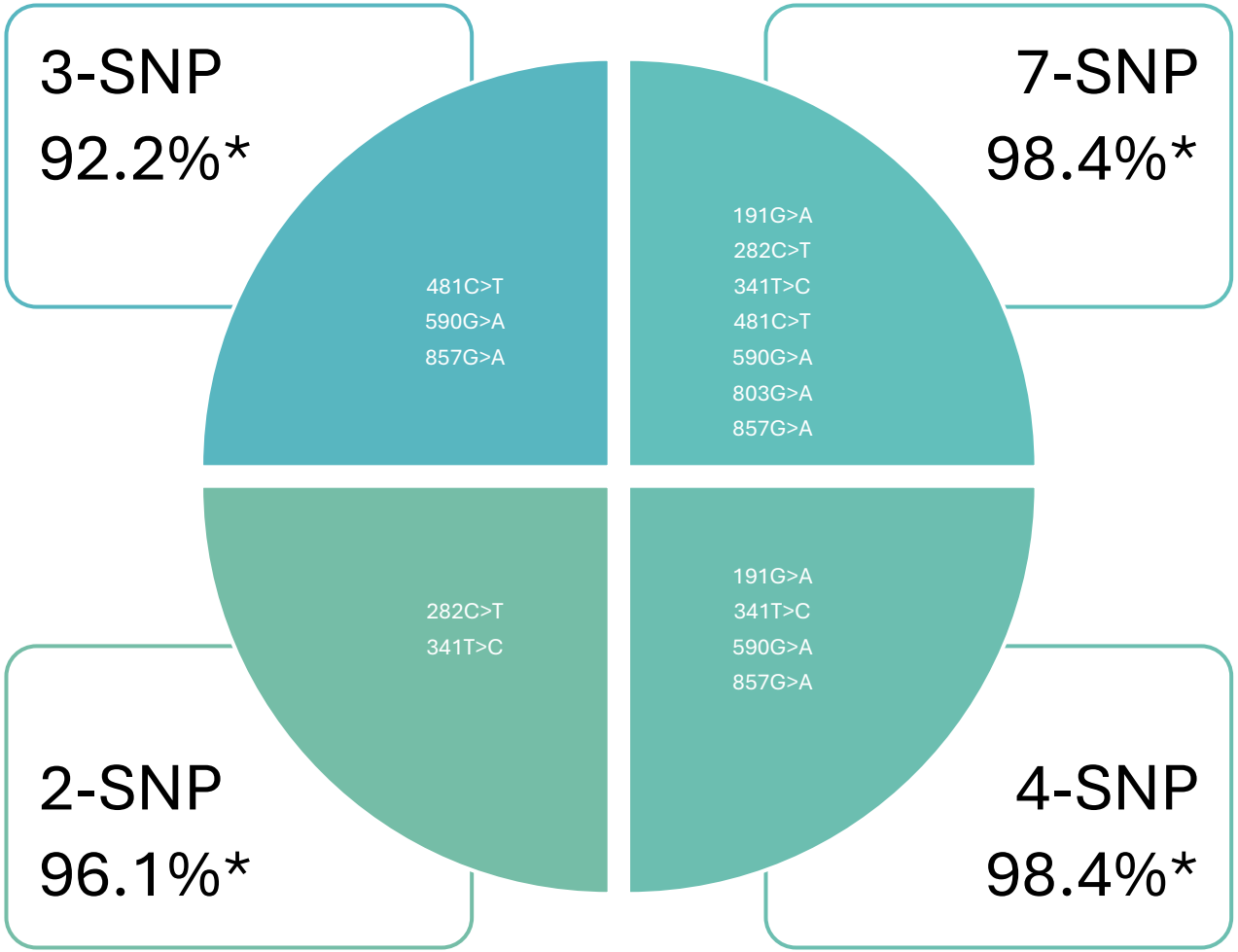
NAT2 Genotyping and Clinical Relevance

- *NAT2* not currently on the AMP recommended test list
- 10 testing laboratories worldwide reporting *NAT2* genotype using 13 available tests



*Accuracy of predicting NAT2 phenotype determined in cryopreserved human hepatocytes
Hein and Doll 2012, *Pharmacogenomics*

Conclusion and Future Directions

- Major changes arose from nomenclature transition to PharmVar. *NAT2* PCEP considered these changes when assigning function.
  - Clusters / sub-families annotated similarly despite new * allele designations
  - c.803G>A distinguishes *NAT2*1* and *NAT2*4*
- Use of CPIC standardized terms “increased” and “decreased” function promotes clinical implementation while avoiding confusion with legacy terms
  - No “normal” function
- NAT2 function determined agnostic of drug/substrate
  - Functional terms will apply to potential future guidelines, i.e., isoniazid

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Questions

Abstract submitted for poster

at PGRN – ClinPGx 2025