Not Just One Gene: Medications Affected by Variability in Multiple Genes

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Disclosure

- I declare no conflicts of interest, real or apparent, and no financial interests in any company, product, or service mentioned in this program, including grants, employment, gifts, stock holdings, and honoraria.

- The University of Florida College of Pharmacy is accredited by the Accreditation Council for Pharmacy Education as a provider of continuing pharmacy education.

Objectives

- Identify relevant medications and potential clinical effects of individual variability in multiple genes affecting drug metabolism.
- Develop strategies for integrating pharmacogenetic test results for multiple genes into clinical decision making.

How we thought Pgx would work…

"Here's my DNA sequence."

The Reality…

- More than one gene can impact a drug in an actionable way, but not as complex as we thought it would be
- 1 or 2 genes account for most of a drug's response variation
- PK + PD or PK + PK

Examples

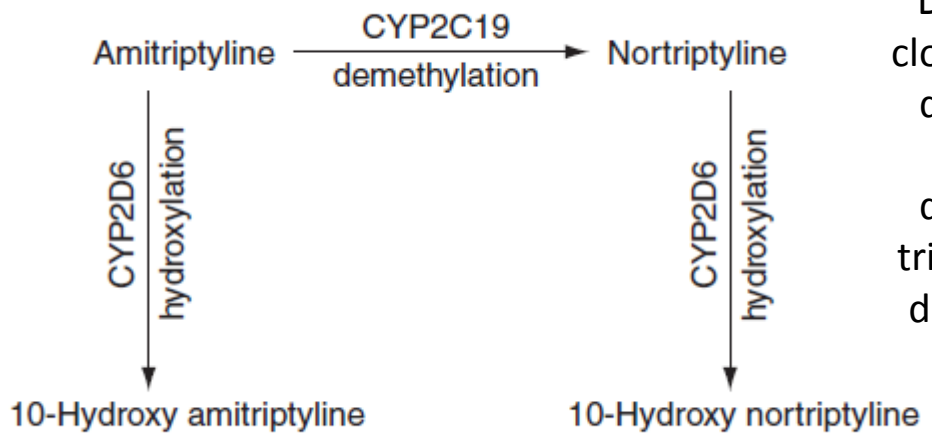
- Tri-cyclic antidepressants (amitriptyline, clomipramine, doxepin, imipramine, and trimipramine)
  - *CYP2D6* and *CYP2C19*
- Antidepressants/Antipsychotics combinatorial approach
- Phenytoin
  - *HLA-B* and *CYP2C9*
- Warfarin
  - *VKORC1* and *CYP2C9*
- Thiopurines
  - *TPMT* and *NUDT15*

Tricyclic Antidepressants

- Mixed serotonin and norepinepherine reuptake inhibitors
- Used to treat:
  - Depression
  - Obsessive-compulsive disorder
  - Neuropathic pain
  - Migraine prophylaxis

Metabolic Pathway

Clomipramine, doxepin, imipramine, trimipramine



Desmethyl-clomipramine, desmethyl-doxepin, desmethyl-trimipramine, desipramine

Hicks JK, et al. Clin Pharmacol Ther 2013;

Tricyclic Pharmacokinetics (con't)

Parent drug	CYP2C19 metabolite[a]	CYP2D6 metabolite[b]	Therapeutic drug monitoring[c]
Amitriptyline	nortriptyline[d]	hydroxy-amitriptyline	amitriptyline + nortriptyline
Clomipramine	desmethyl-clomipramine	hydroxy-clomipramine	clomipramine + desmethyl-clomipramine
Desipramine[d]	--------	hydroxy-desipramine	desipramine
Doxepin	desmethyl-doxepin	hydroxy-doxepin	doxepin + desmethyl-doxepin
Imipramine	desipramine[d]	hydroxy-imipramine	imipramine + desmethyl-imipramine
Nortriptyline[d]	--------	hydroxy-nortriptyline	nortriptyline
Trimipramine	desmethyl-trimipramine	hydroxy-trimipramine	trimipramine + desmethyl-trimipramine

[a]The pharmacologically active CYP2C19 metabolites are hydroxylated by CYP2D6 to less active compounds.
[b]The hydroxylated metabolites are glucuronidated, rendering the lipophilic drugs to water-soluble compounds that are renally eliminated (34).
[c]The parent drug and CYP2C19 metabolite are both pharmacologically active compounds. As a part of therapeutic drug monitoring the plasma concentrations of both are monitored (88-90).
[d]Desipramine and nortriptyline are the CYP2C19 metabolites of imipramine and amitriptyline respectively. Both are also FDA approved drugs.

Hicks JK et al. 2016 update

Prevalence of *CYP2D6* and *CYP2C19* Phenotypes

Likely phenotype	Activity score[a]	Genotypes	Examples of diplotypes
Assignment of CYP2D6 phenotype			
Ultrarapid metabolizer (~1–2% of patients)[b]	>2.0	An individual carrying duplications of functional alleles	*(*1/*1)xN, (*1/*2)xN, (*2/*2)xN*[c]
Extensive metabolizer (~77–92% of patients)	1.0–2.0[d]	An individual carrying two functional alleles or two reduced function alleles or one functional and nonfunctional allele or one functional and reduced function allele	**1/*1, *1/*2, *2/*2, *1/*9, *1/*41, *41/*41, *1/*5, *1/*4*
Intermediate metabolizer (~2–11% of patients)	0.5	An individual carrying one reduced function and one nonfunctional allele	**4/*41, *5/*9, *4/*10*
Poor metabolizers (~5–10% of patients)	0	An individual carrying only nonfunctional alleles	**4/*4, *3/*4, *5/*5, *5/*6*
Assignment of CYP2C19 phenotype			
Ultrarapid metabolizer (~5–30% of patients)[e]		An individual carrying two gain-of-function alleles or one functional allele and one gain-of-function allele	**17/*17, *1/*17*
Extensive metabolizer (~35–50% of patients)		An individual carrying two functional alleles	**1/*1*
Intermediate metabolizer (~18–45% of patients)		An individual carrying one functional allele and one loss-of-function allele	**1/*2, *1/*3*
Poor metabolizers (~2–15% of patients)		An individual carrying two loss-of-function alleles	**2/*2, *2/*3, *3/*3*

Tricyclic Treatment Recommendations with CYP2D6 and CYP2C19

Phenotype	CYP2D6 Ultrarapid metabolizer	CYP2D6 Normal metabolizer	CYP2D6 Intermediate metabolizer	CYP2D6 Poor metabolizer
CYP2C19 Ultrarapid or Rapid metabolizer	Avoid amitriptyline use.[c] Classification of recommendation[d]: Optional	Consider alternative drug not metabolized by CYP2C19.[c, e] Classification of recommendation[d]: Optional	Consider alternative drug not metabolized by CYP2C19.[c, e] Classification of recommendation[d]: Optional	Avoid amitriptyline use.[c] Classification of recommendation[d]: Optional
CYP2C19 Normal metabolizer	Avoid amitriptyline use. If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers).[f, g] Classification of recommendation[d]: Strong	Initiate therapy with recommended starting dose.[h] Classification of recommendation[d]: Strong	Consider 25% reduction of recommended starting dose.[f, h] Classification of recommendation[d]: Moderate	Avoid amitriptyline use. If amitriptyline is warranted, consider 50% reduction of recommended starting dose.[f, h] Classification of recommendation[d]: Strong
CYP2C19 Intermediate metabolizer	Avoid amitriptyline use.[c] Classification of recommendation[d]: Optional	Initiate therapy with recommended starting dose.[h] Classification of recommendation[d]: Strong	Consider 25% reduction of recommended starting dose.[f, h] Classification of recommendation[d]: Optional	Avoid amitriptyline use. If amitriptyline is warranted, consider 50% reduction of recommended starting dose.[f, h] Classification of recommendation[d]: Optional
CYP2C19 Poor metabolizer	Avoid amitriptyline use.[c] Classification of recommendation[d]: Optional	Avoid amitriptyline use.[c] If amitriptyline is warranted, consider a 50% reduction of recommended starting dose.[f, h] Classification of recommendation[d]: Moderate	Avoid amitriptyline use.[c] Classification of recommendation[d]: Optional	Avoid amitriptyline use.[c] Classification of recommendation[d]: Optional

Hicks JK et al. Clin Pharmacol Ther 2016; epub ahead of print

Clinical Dilemma

- You have CYP2C19 genotype information available at your health system in patients who have undergone percutaneous coronary intervention, but not CYP2D6 genotype
- You would like to implement best practice alerts for antidepressants
- Which antidepressants should you target?

Answers

A. Amitriptyline

B. Nortriptyline

C. Citalopram/escitalopram

D. A and C

E. All of the above

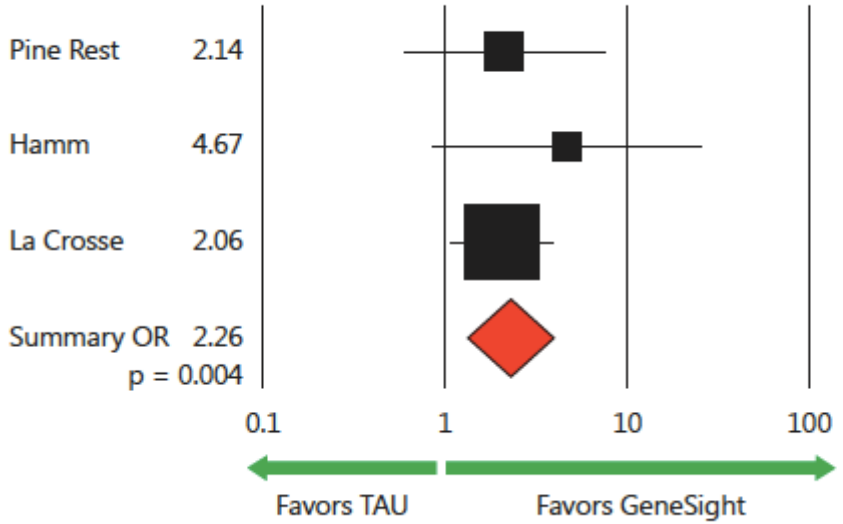
Other Antidepressants and Antipsychotics

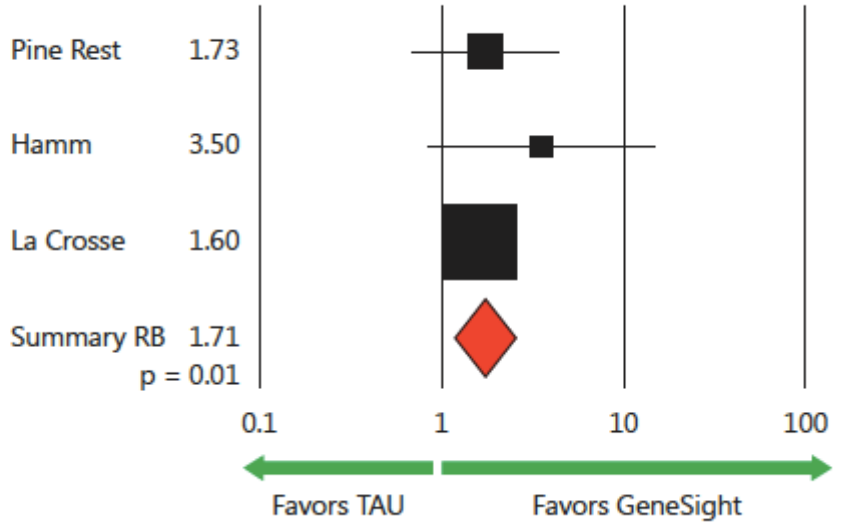
- Multiple proprietary algorithms exist for assigning drug response phenotype categories based on multiple pgx genes
- One example – a company detects 77 variants in 8 genes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2B6, CYP3A4, HLA-B, HTR2A, SLC6A4, UGT1A4, UGT2B15)

ANTIDEPRESSANTS

USE AS DIRECTED	MODERATE GENE-DRUG INTERACTION		SIGNIFICANT GENE-DRUG INTERACTION	
desvenlafaxine (Pristiq®)	**trazodone** (Desyrel®)	1	**bupropion** (Wellbutrin®)	1,6
levomilnacipran (Fetzima®)	**venlafaxine** (Effexor®)	1	**mirtazapine** (Remeron®)	1,6
vilazodone (Viibryd®)	**selegiline** (Emsam®)	2	**amitriptyline** (Elavil®)	3,8
	fluoxetine (Prozac®)	1,4	**clomipramine** (Anafranil®)	1,6,8
	citalopram (Celexa®)	3,4	**desipramine** (Norpramin®)	1,6,8
	escitalopram (Lexapro®)	3,4	**doxepin** (Sinequan®)	1,6,8
	sertraline (Zoloft®)	3,4	**duloxetine** (Cymbalta®)	1,6,8
			imipramine (Tofranil®)	1,6,8
			nortriptyline (Pamelor®)	1,6,8
			vortioxetine (Trintellix®)	1,6,8
			fluvoxamine (Luvox®)	1,4,6,8
			paroxetine (Paxil®)	1,4,6,8

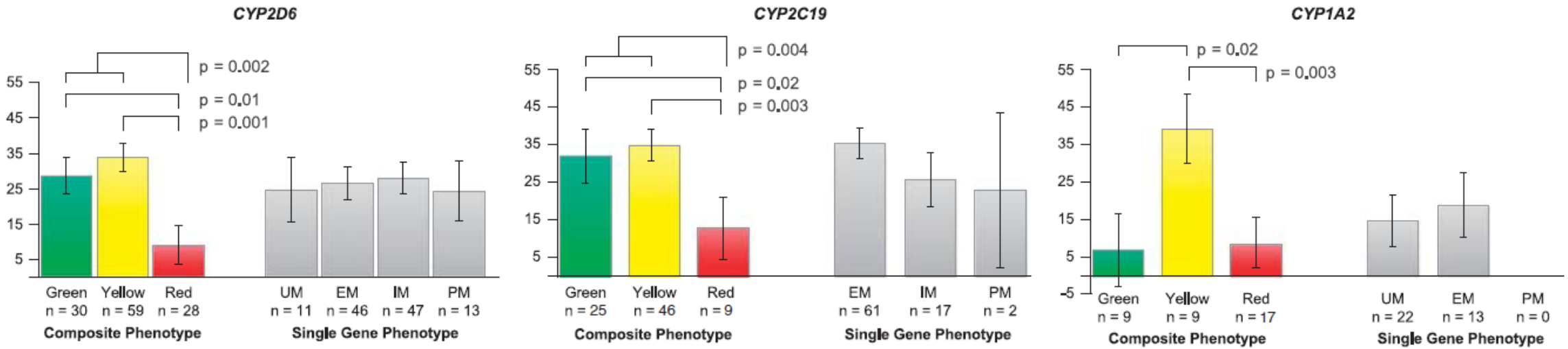
Clinical Utility of Combinatorial PGx in Depression





Hall-Flavin DK, et al. Pharmacogenet Genomics 2013;23:535.

Combinatorial Approach vs Single Gene



Altar CA, et al. Pharmacogenomics J; 2015;15:443.

Combinatorial Approach Summary

- Interesting
- Compelling RCT data
- Difficult to fully evaluate or implement outside given the proprietary nature of the algorithm

Clinical Case

- You work in a retail pharmacy. A patient presents with major depressive disorder. He has a genotype report and prescriptions for aripiprazole 5 mg daily and clomipramine 25 mg daily.
- His genotype report has the following information:
  - CYP2D6 Poor Metabolizer; CYP2C19 Ultrarapid Metabolizer; CYP3A4 Normal Metabolizer

CYP1A2 **Extensive (Normal) Metabolizer**
*1/*1

This genotype is most consistent with the extensive (normal) metabolizer phenotype.

CYP2B6 **Intermediate Metabolizer**
*1/*6

CYP2B6*1 allele enzyme activity: Normal
CYP2B6*6 allele enzyme activity: Reduced

This genotype is most consistent with the intermediate metabolizer phenotype. This patient may have reduced enzyme activity as compared to individuals with the normal phenotype.

CYP2C19 **Ultrarapid Metabolizer**
*17/*17

CYP2C19*17 allele enzyme activity: Increased
CYP2C19*17 allele enzyme activity: Increased

This genotype is most consistent with the ultrarapid metabolizer phenotype. This patient may have increased enzyme activity as compared to individuals with the normal phenotype.

CYP2C9 **Intermediate Metabolizer**
*1/*2

CYP2C9*1 allele enzyme activity: Normal
CYP2C9*2 allele enzyme activity: Reduced

This genotype is most consistent with the intermediate metabolizer phenotype. This patient may have reduced enzyme activity as compared to individuals with the normal phenotype.

CYP3A4 **Extensive (Normal) Metabolizer**
*1/*1

CYP3A4*1 allele enzyme activity: Normal

CYP2D6 **Poor Metabolizer**
*4/*4 (Duplication)

CYP2D6*4 allele enzyme activity: None
CYP2D6*4 allele enzyme activity: None

This genotype is most consistent with the poor metabolizer phenotype. This patient may have reduced enzyme activity as compared to individuals with the normal phenotype.

A duplication of the gene CYP2D6 has been detected in this patient. While current genotyping techniques allow for the detection of this duplication, in the case of heterozygosity, such techniques do not allow for the identification of the allele that has been duplicated. This duplication, depending on the allele duplicated, can result in increased expression of CYP2D6.

UGT1A4 **Extensive (Normal) Metabolizer**
*1/*1

UGT1A4*1 allele enzyme activity: Normal
UGT1A4*1 allele enzyme activity: Normal

This genotype is most consistent with the extensive (normal) metabolizer phenotype. The patient is expected to have normal enzyme activity.

UGT2B15 **Intermediate Metabolizer**
*2/*2

UGT2B15*2 allele enzyme activity: Reduced
UGT2B15*2 allele enzyme activity: Reduced

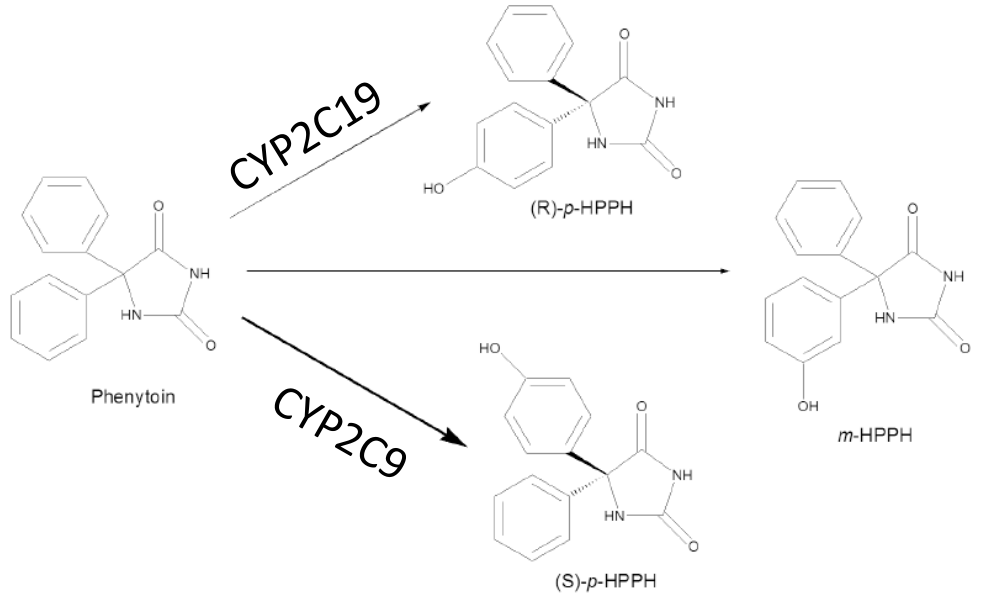
This genotype is most consistent with the intermediate metabolizer phenotype. This patient may have reduced enzyme activity as compared to individuals with the normal phenotype.

Clinical Case: What would you do?

A. Fill the prescriptions as ordered
B. Fill the prescriptions as ordered and make note of his genotypes into your database
C. Call the physician to recommend a decreased dose of clomipramine and aripiprazole
D. Call the physician to recommend alternative treatments

Phenytoin

- Most widely prescribed epileptic drug with a narrow therapeutic index
- Therapeutic drug monitoring often necessary
  - Complex, non-linear PK
- Adverse effects include:
  - Sedation, nystagmus, nausea, and cognitive impairment
  - Highly allergenic- mild rash to life-threatening



HLA-B*15:02 and Phenytoin-induced SJS/TEN

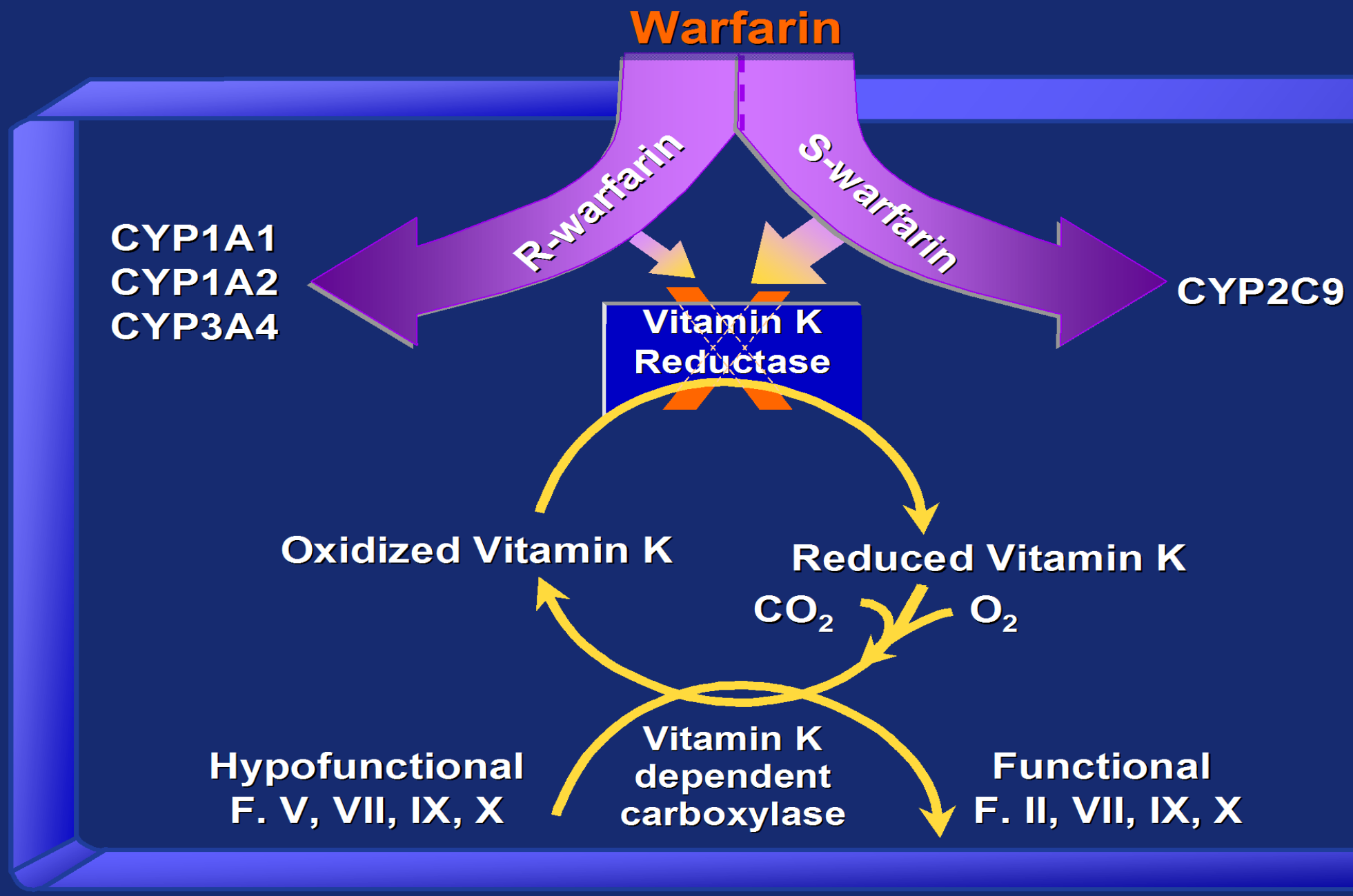
- Association between HLA-B*15:02 and SJS/TEN is weaker than with carbamazepine (fewer studies)
- OR for phenytoin-induced SJS/TEN with HLA-B*15:02 = 4.25 (95% CI 1.93-9.39)
- Sensitivity of 36.6% (95% CI 23.6-51.9) and Specificity of 87.2% (95% CI 81.7-91.3)
  - Absence of variants does not rule out SJS/TEN
- **FDA recommends avoiding phenytoin/fosphenytoin as substitutes for carbamazepine in HLA-B*15:02 carriers**

Phenytoin Treatment Recommendations

	*HLA-B*15:02* carrier			*HLA-B*15:02* noncarrier		
Phenotype/ genotype	Implication	Therapeutic recommendation	Classification of recommendation[a]	Implication	Therapeutic recommendation	Classification of recommendation[a]
CYP2C9 extensive metabolizer	Increased risk of phenytoin-induced SJS/ TEN	If patient is phenytoin naive,[b] do not use phenytoin/ fosphenytoin[c]	Strong	Normal phenytoin metabolism	Initiate therapy with recommended maintenance dosed	Strong
CYP2C9 intermediate metabolizer	Increased risk of phenytoin-induced SJS/ TEN	If patient is phenytoin naive,[b] do not use phenytoin/ fosphenytoin[c]	Strong	Reduced phenytoin metabolism. Higher plasma concentrations will increase probability of toxicities	Consider 25% reduction of recommended starting maintenance dose.[d] Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response	Moderate
CYP2C9 poor metabolizer	Increased risk of phenytoin-induced SJS/ TEN	If patient is phenytoin naive,[b] do not use phenytoin/ fosphenytoin[c]	Strong	Reduced phenytoin metabolism. Higher plasma concentrations will increase probability of toxicities	Consider 50% reduction of recommended starting maintenance dose.[d] Subsequent maintenance doses should be adjusted according to therapeutic drug monitoring and response	Strong

Frequencies:
CYP2C9 IMs ~ 8%
CYP2C9 PMs ~ 1%
HLA-B *15:02 carriers ~1.4%

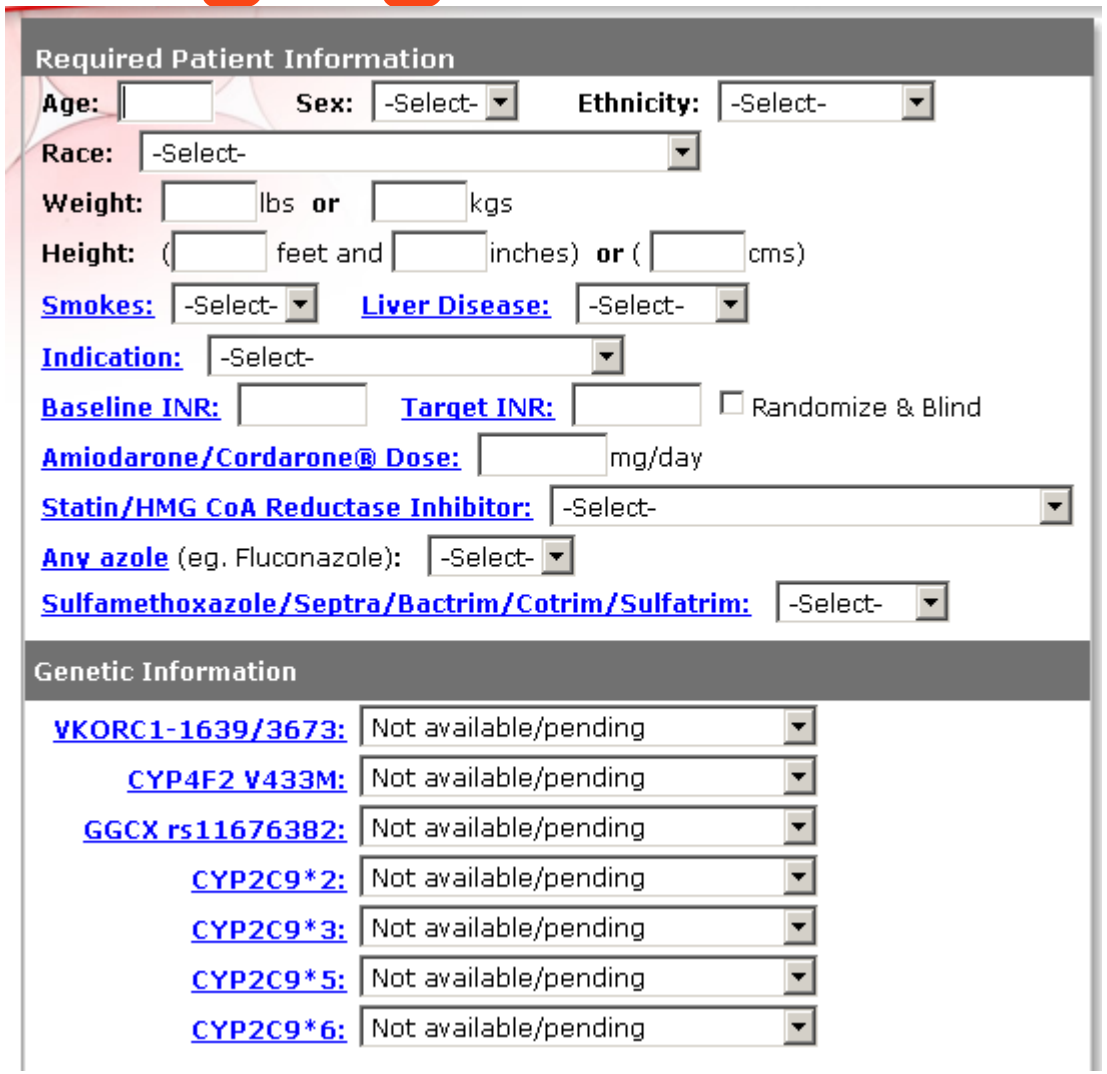
Caudle KE, et al. Clin Pharmacol Ther 2014;96:542.

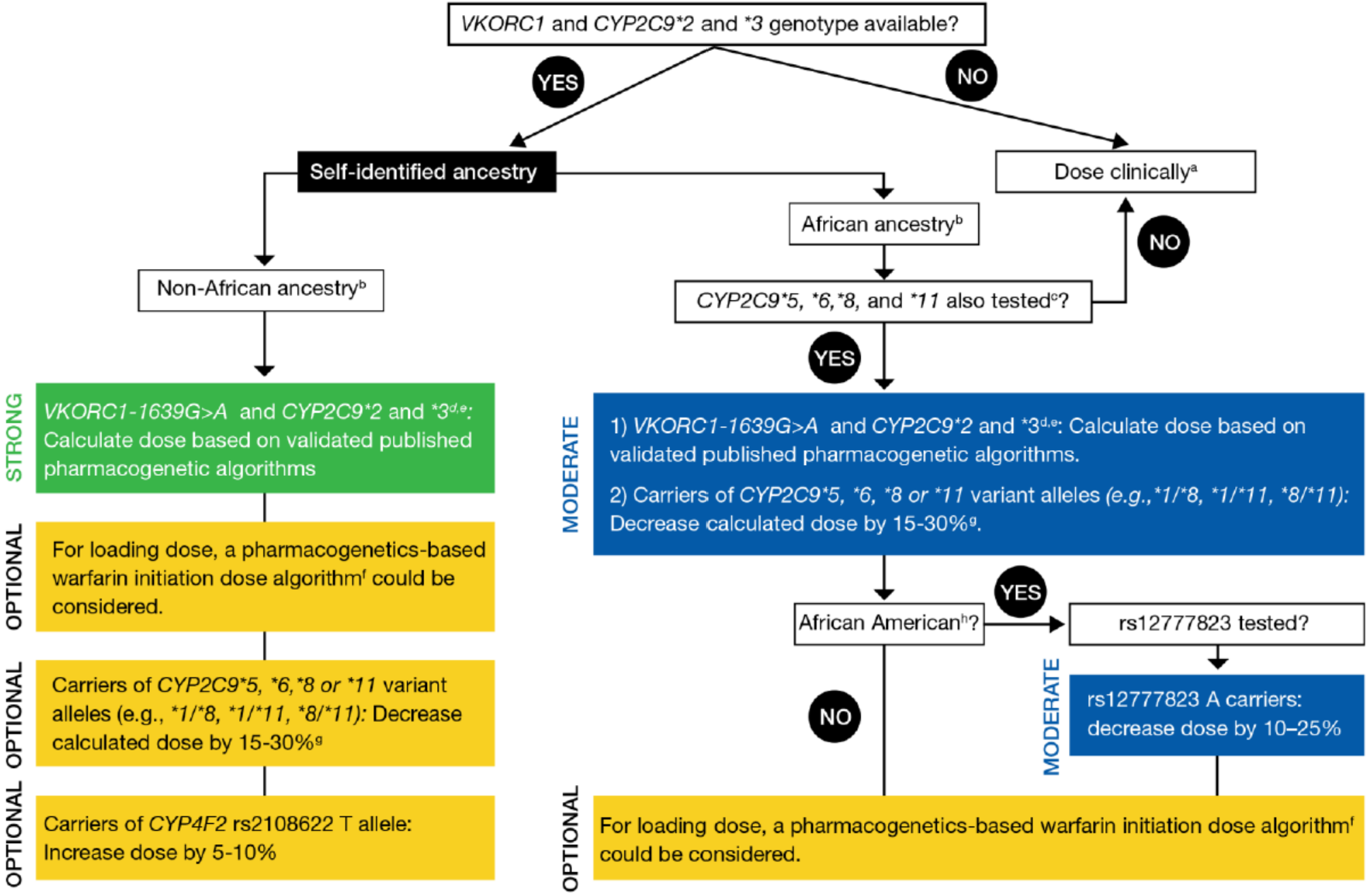

Warfarin
R-warfarin
S-warfarin
CYP1A1
CYP1A2
CYP3A4
CYP2C9
Vitamin K Reductase
Oxidized Vitamin K
Reduced Vitamin K
CO_2
O_2
Vitamin K dependent carboxylase
Hypofunctional F. V, VII, IX, X
Functional F. II, VII, IX, X

Warfarin Labeling

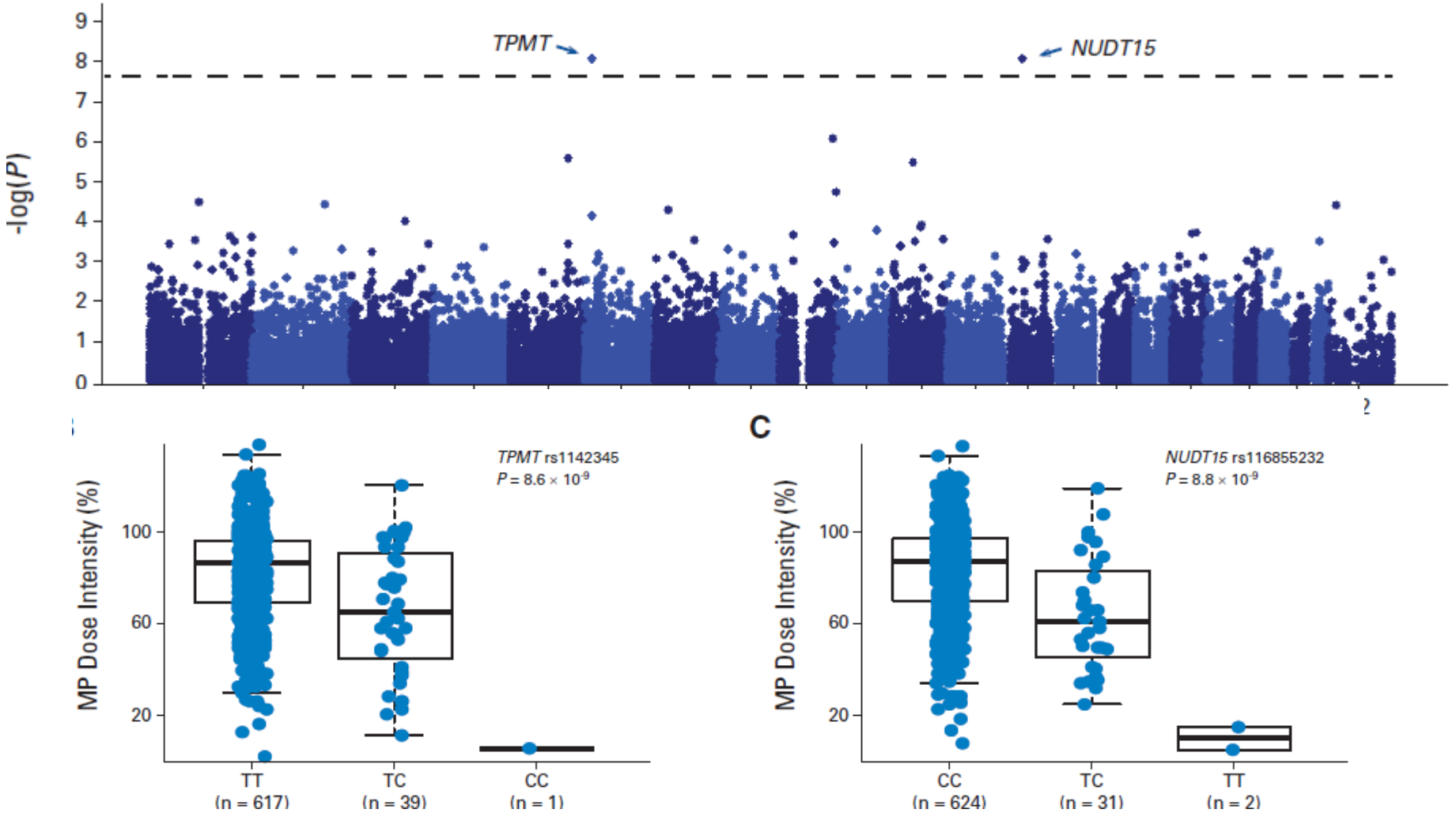
VKORC1	CYP2C9					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
GG	5-7 mg	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg
AG	5-7 mg	3-4 mg	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg
AA	3-4 mg	3-4 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg	0.5-2 mg

Warfarindosing.org



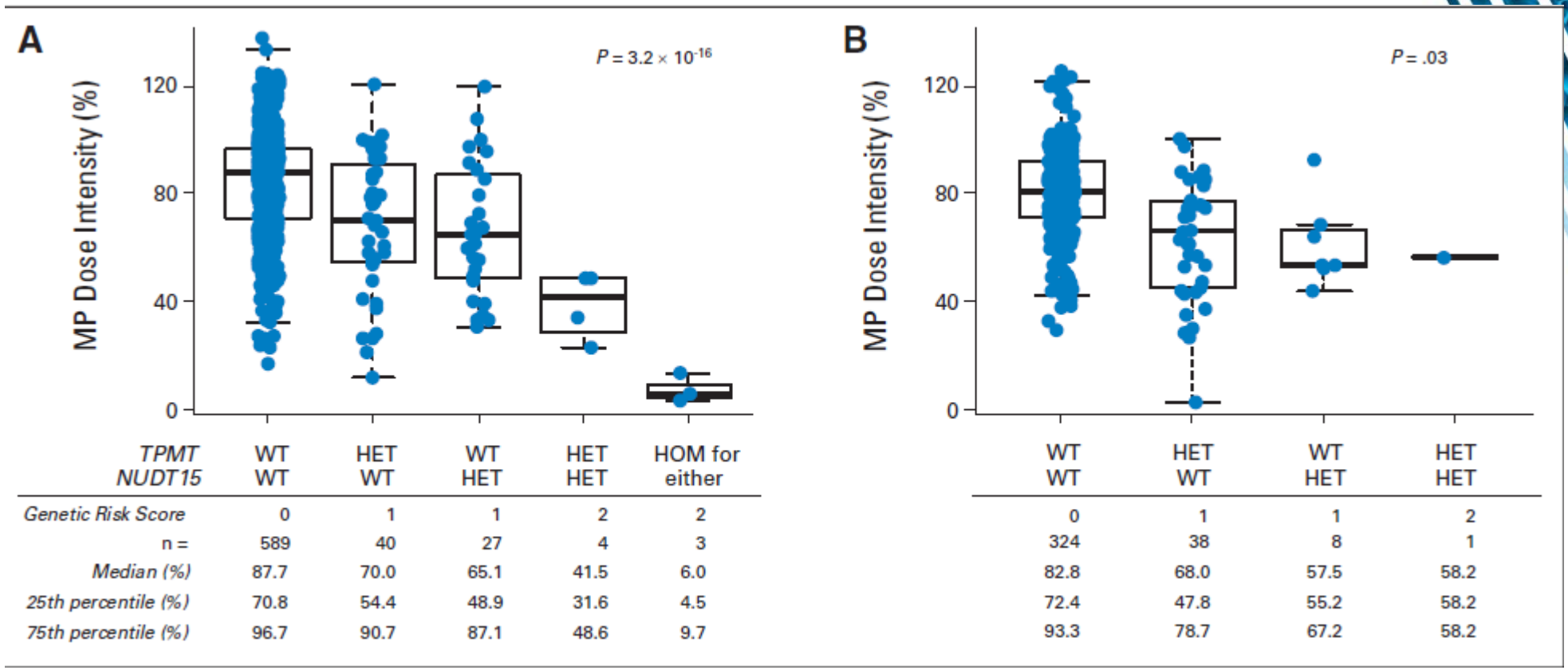

VKORC1 and *CYP2C9*2* and *3 genotype available?
YES
NO
Self-identified ancestry
Dose clinically[a]
African ancestry[b]
Non-African ancestry[b]
NO
*CYP2C9*5, *6,*8, and *11* also tested[c]?
YES
STRONG
VKORC1-1639G>A and *CYP2C9*2* and *3[d,e]: Calculate dose based on validated published pharmacogenetic algorithms
MODERATE
1) *VKORC1-1639G>A* and *CYP2C9*2* and *3[d,e]: Calculate dose based on validated published pharmacogenetic algorithms.
2) Carriers of *CYP2C9*5, *6, *8 or *11* variant alleles *(e.g.,*1/*8, *1/*11, *8/*11)*: Decrease calculated dose by 15-30%[g].
OPTIONAL
For loading dose, a pharmacogenetics-based warfarin initiation dose algorithm[f] could be considered.
African American[h]?
YES
rs12777823 tested?
OPTIONAL
Carriers of *CYP2C9*5, *6,*8 or *11* variant alleles (e.g., *1/*8, *1/*11, *8/*11):* Decrease calculated dose by 15-30%[g]
NO
MODERATE
rs12777823 A carriers: decrease dose by 10–25%
OPTIONAL
Carriers of *CYP4F2* rs2108622 T allele: Increase dose by 5-10%
OPTIONAL
For loading dose, a pharmacogenetics-based warfarin initiation dose algorithm[f] could be considered.

Thiopurines



Yang JJ, et al. J Clin Oncol 2015;33:1235

Combined Effect of TPMT and NUDT15



TPMT / *NUDT15*	WT WT	HET WT	WT HET	HET HET	HOM for either
Genetic Risk Score	0	1	1	2	2
n =	589	40	27	4	3
Median (%)	87.7	70.0	65.1	41.5	6.0
25th percentile (%)	70.8	54.4	48.9	31.6	4.5
75th percentile (%)	96.7	90.7	87.1	48.6	9.7

WT WT	HET WT	WT HET	HET HET
0	1	1	2
324	38	8	1
82.8	68.0	57.5	58.2
72.4	47.8	55.2	58.2
93.3	78.7	67.2	58.2

Yang JJ, et al. J Clin Oncol 2015;33:1235

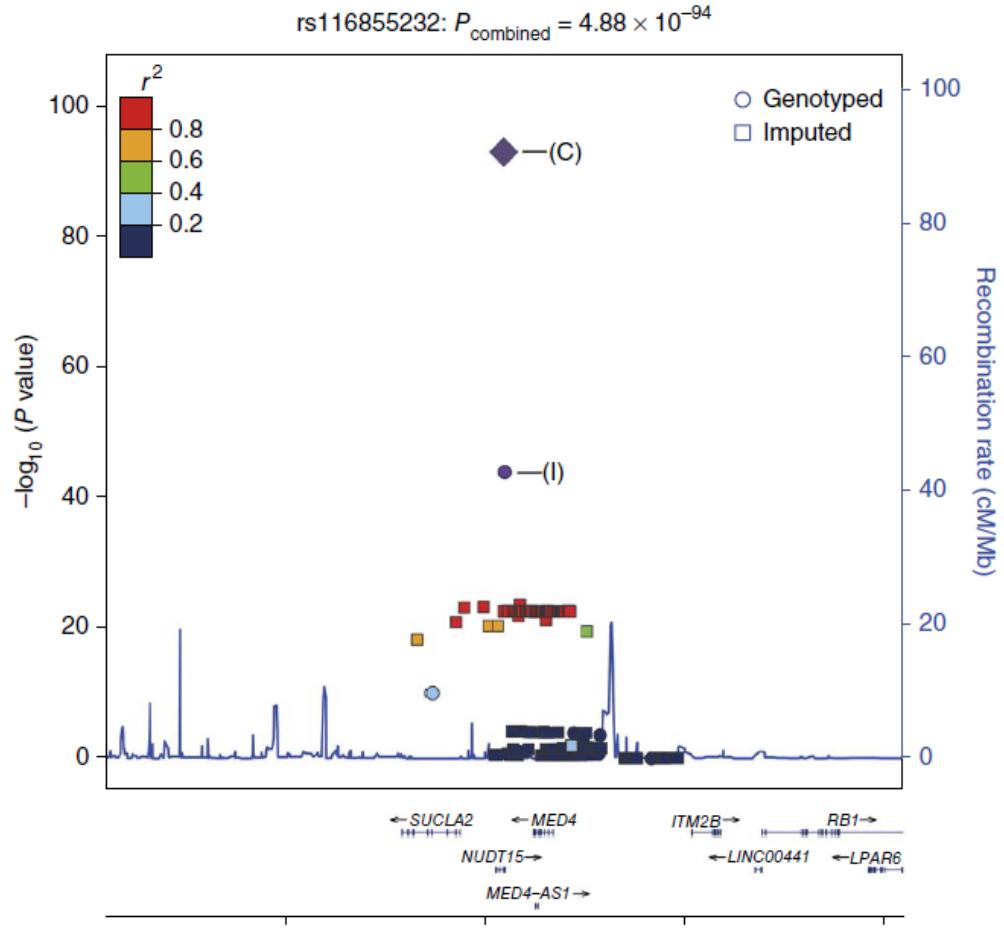


Table 1 Association of *NUDT15* rs116855232 (p.Arg139Cys) with thiopurine-induced early leukopenia in the discovery, replication and combined samples

	Cases					Controls		
Study group	*n*	RAF	OR (95% CI)	*P* value[a]	P_{BD}[b]	*n*	RAF	OR (95% CI)
Discovery	33	0.530	39.65 (20.03–78.47)	2.79×10^{-48}		307	0.028	1.00
Replication	33	0.576	32.57 (17.41–60.93)	7.18×10^{-48}		325	0.040	1.00
Combined	66	0.553	35.63 (22.47–56.51)	4.88×10^{-94}	0.677	632	0.034	1.00

RAF, risk allele frequency; OR, odds ratio; CI, confidence interval.
[a]*P* values were calculated using allelic association tests. Combined *P* values were calculated using the Cochran-Mantel-Haenszel test. [b]Asymptotic *P* value of the Breslow-Day test for heterogeneity in the OR.

Yang SK, et al. Nature Genet 2014;46:1017.

Clinical Case

- You work at a children's hospital where TPMT genotyping is performed prior to thiopurine dosing in ALL. A 2 yo girl of Asian ancestry presents for maintenance treatment. Her *TPMT* genotype is *1/*1. What would be your treatment plan?

  - Start 6-MP at usual dose
  - Reduce 6-MP dose by 30%
  - Obtain a *NUDT15* genotype

TPMT and *NUDT15* Contribution to Thiopurine Dose

- NUDT15 explains in part the higher prevalence of thiopurine toxicity in Asian populations despite lower frequency of TPMT mutations
- TPMT and NUDT15 each independently contribute to thiopurine intolerance and each explains about 20% of the variability in thiopurine dose requirements
- 5.7% of patients who required >50% dose reductions were wild type for both TPMT and NUDT15 suggesting other genetic and non-genetic factors are still to be discovered

Summary

- More than 1 gene can matter
- BUT it is usually a small number of genes that impact a drug response in an actionable way
- Very important for drugs with narrow therapeutic index
- Ideally, clinical decision support can help in these situations
  - Does make things more complex