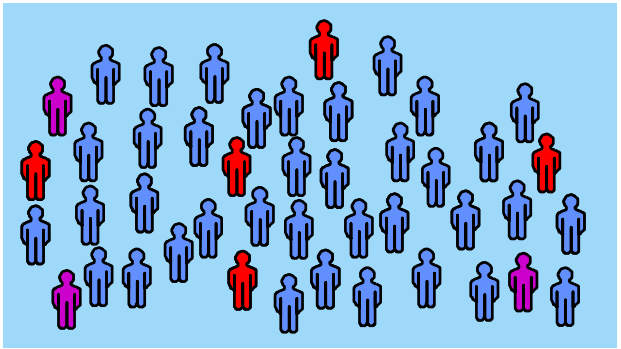
MEDCH 527

Pharmacogenomics of DMEs: PGEN II

CYP2D6, CYP2C19, CYP2C9



CPIC gene-drug pairs

https://cpicpgx.org/genes-drugs/

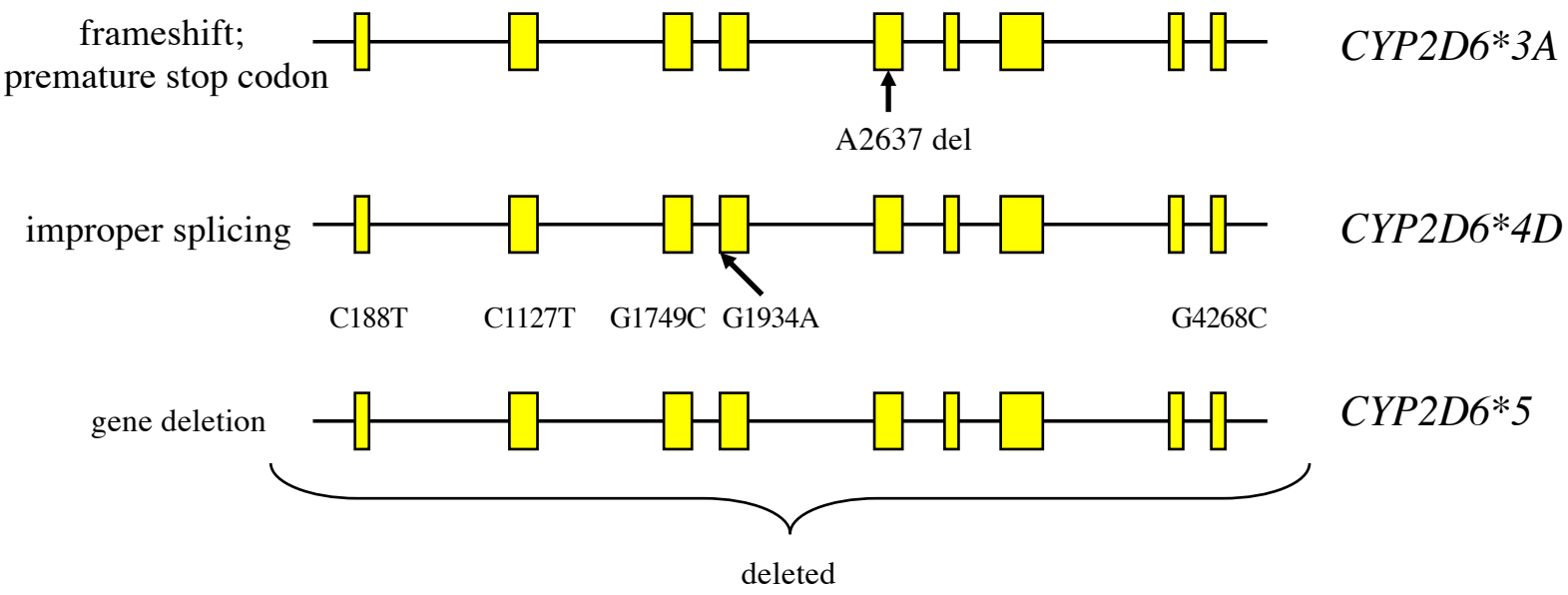
- CPIC level A denotes a situation where ‘genetic information should be used to change prescribing of the affected drug’.
- CPIC level B denotes a situation where ‘genetic information could be used to change prescribing of the affected drug because alternative therapies/dosing are extremely likely to be as effective and as safe as non-genetically based dosing’.
- 142 priority (level A or level B) gene-drug pairs, most of which are considered to be ‘clinically actionable’.
- ~50 of these gene-drug pairs involve **CYP2D6, CYP2C19 or CYP2C9!**

Selected CPIC drug-gene pairs

CYP2D6	tamoxifen	Guideline	A	1A	Testing required
CYP2D6	nortriptyline	Guideline	A	1A	Actionable PGx
CYP2D6	codeine	Guideline	A	1A	Actionable PGx
CYP2C19	clopidogrel	Guideline	A	1A	Actionable PGx
CYP2C9	warfarin	Guideline	A	1A	Actionable PGx
VKORC1	warfarin	Guideline	A	1A	Actionable PGx
CYP4F2	warfarin	Guideline	A	1A	

CYP2D6 Polymorphism

- At least 9 null mutations are known, but just three (*2D6*3*, *2D6*4* and *2D6*5*) account for most of the poor metabolizers – PM phenotype (phenotype = observable characteristic).

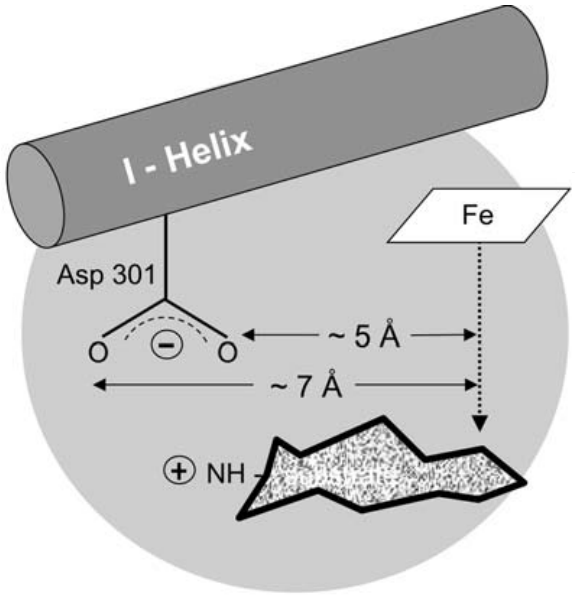


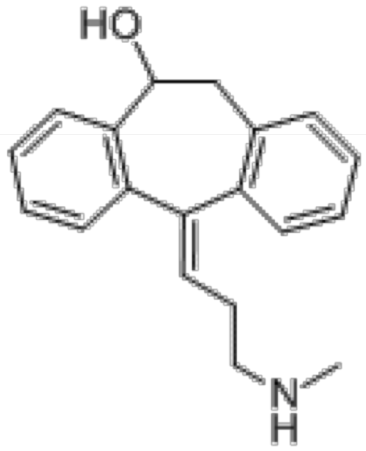
- 'Wild-type' genotype(s) correspond to extensive metabolizers (EMs).
- Other phenotypes: ultra-rapid metabolizers (UMs), intermediate metabolizers (IMs)

Common CYP2D6 Substrates

CYP2D6

Basic molecules with a protonatable nitrogen atom
4–7 Å from the metabolism site





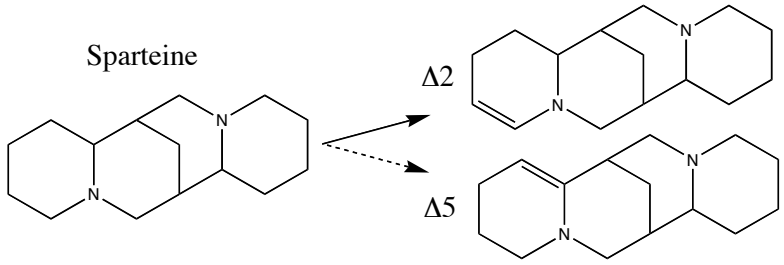
10-Hydroxy-Nortriptyline

Bufuralol
Dextromethorphan
Haloperidol
Metoprolol
Propafenone
Risperidone
Imipramine

Nortiptyline
- tricyclic antidepressant
– very low therapeutic index
- coma, convulsions, cardiotoxicity in overdose

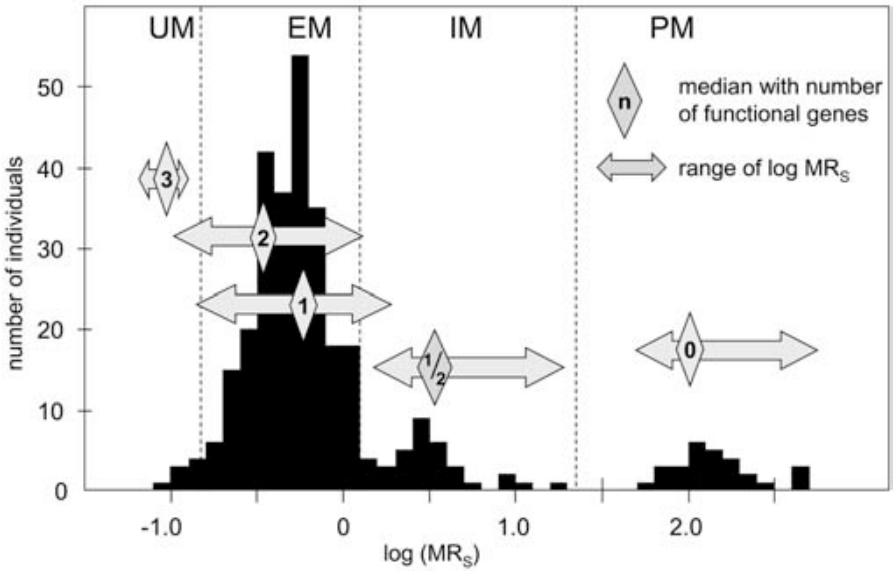
Polymorphism was discovered serendipitously (late 70s) as adverse drug reactions to the antihypertensive, debrisoquine and the oxytocic agent, sparteine.

Relating Genotype to (Metabolic Ratio) Phenotype for CYP2D6



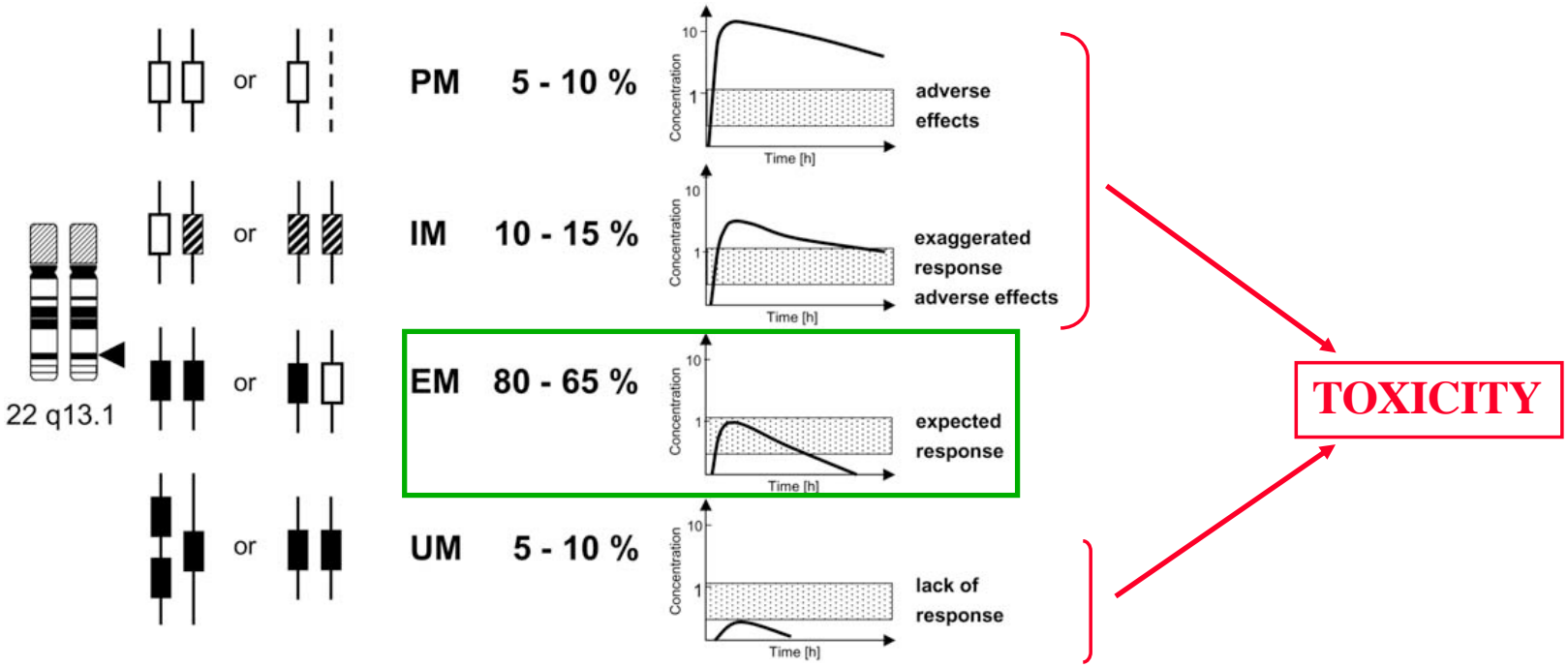
Sparteine Metabolic Ratio (MR) = $\frac{\text{Sparteine (in urine)}}{\Delta 2 + \Delta 5 \text{ metabolites}}$

- Sparteine is metabolized by CYP2D6 to the Δ2 and Δ5 dehydrosparteine metabolites
- The urinary ratio of parent drug to metabolites provides a quantitative index of CYP2D6 function
- The data in any given population reflects a multi-modal distribution of CYP2D6 activity due to the inheritance of 0 - 3+ functional genes.



Zanger et al., *Naunyn Schmied. Arch. Pharmacol.*, (2004)

Predicted Effect of CYP2D6 Allelic Variation on Pharmacodynamics

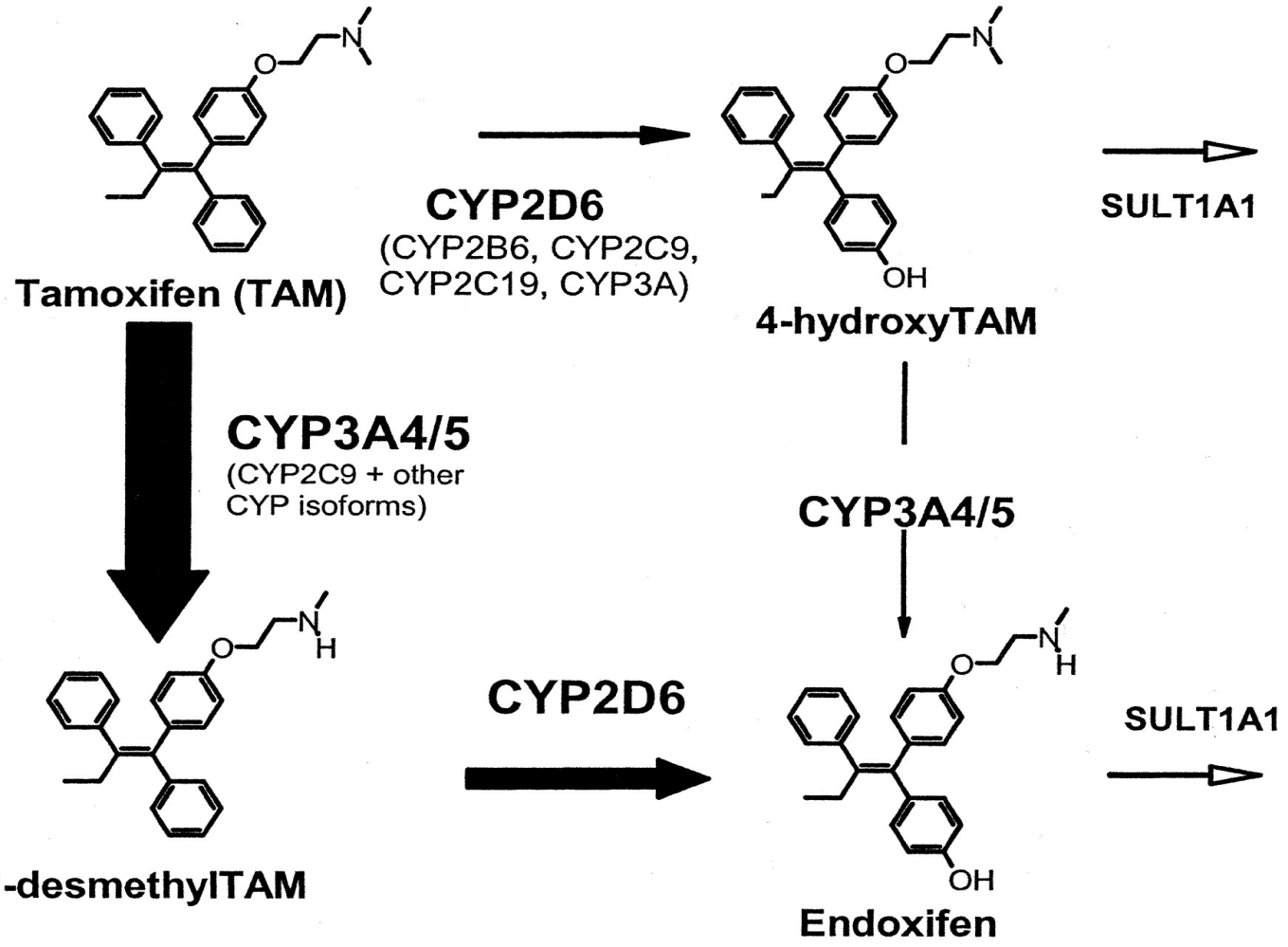


At 'standard' drug doses (ie those normalized for EMs);

- PMs and IMs might be expected to exhibit exaggerated or toxic drug responses

- UMs might be expected to exhibit loss of therapeutic benefit

Biotransformation of Tamoxifen and its Metabolites



Jin, Y. et al. J. Natl. Cancer Inst. 2005 97:30-39; doi:10.1093/jnci/dji005

- **4-OHT is 30-100-fold more potent than Tamoxifen**
- **Endoxifen similar in potency to 4-OHT and plasma levels are 6-10x higher**

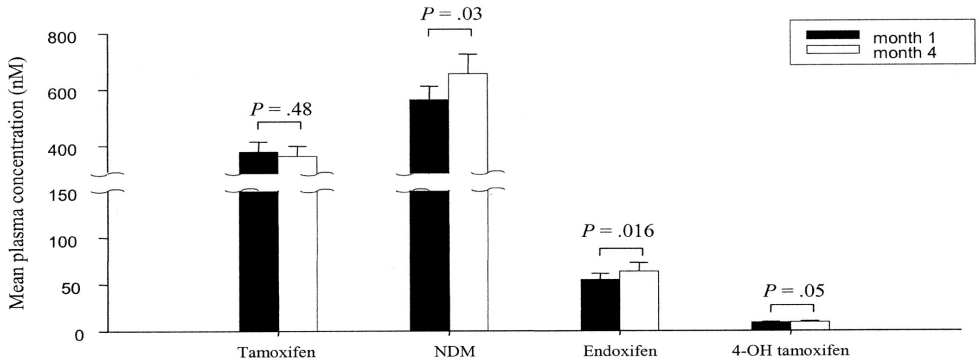
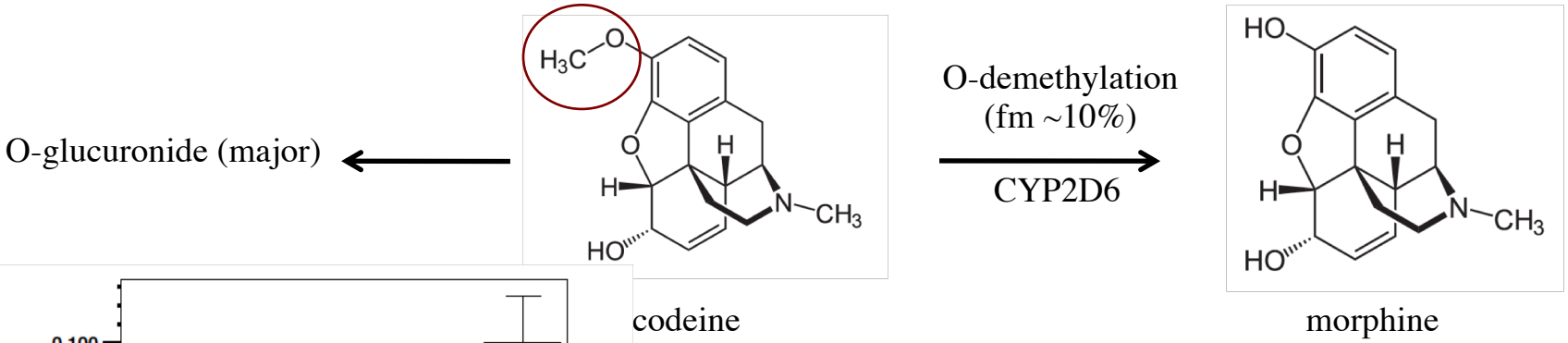


Table 2. Plasma concentrations of tamoxifen and its metabolites at 4 months of tamoxifen therapy in subjects with CYP2D6, CYP2C9, CYP3A5, and SULT1A1 genotype*

Genotype group	N	Mean concentration, nM (95% CI)			
		Endoxifen	4-Hydroxytamoxifen	N-desmethyltamoxifen	Tamoxifen
CYP2D6					
Wt/Wt	48	78.0 (65.9 to 90.1)	9.5 (8.4 to 10.6)	653.4 (562.5 to 744.3)	372.5 (321.2 to 423.8)
Wt/Vt†	29	43.1 (33.3 to 52.9)	8.3 (6.7 to 9.9)	687.3 (570.6 to 804.0)	353.3 (301.2 to 405.4)
Vt/Vt‡	3	20.0 (11.1 to 28.9)	7.1 (1.2 to 13.0)	664.1 (298.7 to 1029.5)	288.9 (172.9 to 404.9)
P		<.001	.86	.62	.92
CYP2C9					
Wt/Wt	55	63.4 (52.1 to 74.7)	8.9 (7.8 to 10.0)	648.3 (560.8 to 735.8)	349.8 (257.4 to 442.2)
Vt§	25	62.7 (47.6 to 77.8)	9.2 (7.4 to 11.0)	670.0 (555.7 to 784.3)	391.6 (335.2 to 448.0)
P		.87	.81	.90	.34
CYP3A5					
*1/**‖	17	82.0 (56.2 to 107.8)	9.7 (7.3 to 12.1)	655.3 (474.6 to 836.0)	402.3 (290.5 to 514.1)
*3/*3¶	63	58.1 (49.3 to 66.9)	8.7 (7.7 to 9.7)	654.8 (579.8 to 729.8)	352.4 (316.9 to 387.9)
P		.09	.57	.99	.98
SULT1A1					
*1*1	36	59.1 (46.4 to 71.8)	8.9 (7.4 to 10.4)	668.9 (572.2 to 765.6)	360.2 (307.1 to 413.3)
*1*2	38	65.1 (50.9 to 79.3)	8.8 (6.4 to 11.2)	667.6 (558.4 to 776.8)	377.0 (321.8 to 432.2)
*2*2	6	74.9 (47.2 to 102.6)	10.3 (5.7 to 14.9)	493.0 (37.1 to 948.9)	286.7 (177.6 to 395.8)
P		.73	.83	.58	.98

CYP2D6: Conversion of Codeine to Morphine



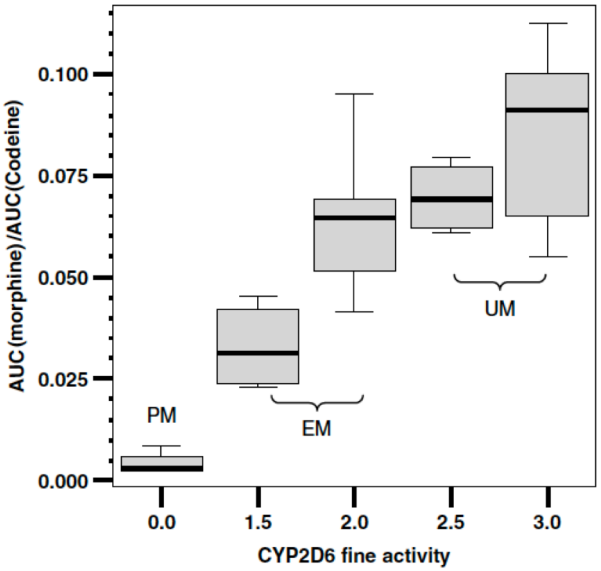
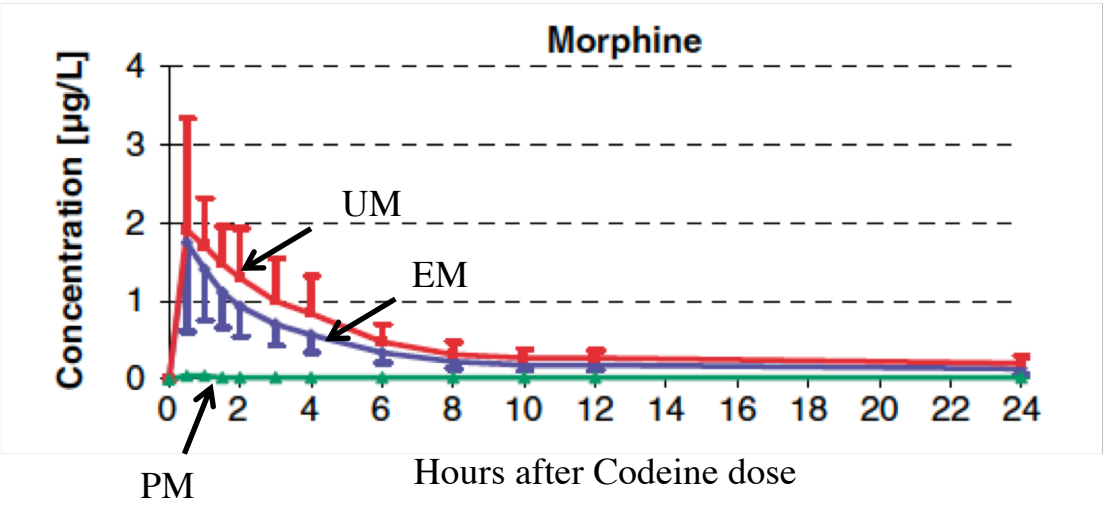


Figure 2 Ratio of plasma AUC of morphine over plasma AUC or codeine in relation to the CYP2D6 activity expressed by the number of active alleles differentiating between fully active alleles, which were considered with one arbitrary activity unit, and alleles with reduced activity, with were arbitrarily considered, which 0.5 activity units.



Kirchheiner et al, 2007

Ethnic Variation in *CYP2D6* Mutation Frequencies

Variant	Mutation	Phenotype	Allele Frequencies			
			White	*Asian*	*Black African*	*Ethiopean/ Saudi*
*2D6*2xN*	gene duplication	UM	1-5	0-2	2	**10-16**
*CYP2D6*4*	defective splicing	PM	**12-21**	1	2	1-4
*CYP2D6*5*	gene deletion	PM	2-7	6	4	1-4
*CYP2D6*10*	P34S, S486T	IM	1-2	**51**	6	3-9
*CYP2D6*17*	T107I, R296C, S486T	IM	0	ND	**34**	3-9

http://www.imm.ki.se/cypalleles/

CYP2C19 Polymorphism

- First detected from unusual response to anti-epileptic drug, mephenytoin (dysphoria/sedation)
- 3-6% of Whites and African Americans, but up to 25% of Chinese/Japanese/Koreans are PMs
- Common true null mutations leading to PM status arise from the **2* (681G>A) and **3* (636G>A) alleles (below)
- *CYP2C19*17* (-806C>T) is a common gain of function allele, associated with increased expression of enzyme

c.G681A Exon 5

Ile Cys
*CYP2C19*1* ….cttag ATA TGC…GGGAA

Glu
*CYP2C19*2* ….cttag atatgc……ag GAA

(new overriding acceptor site creates a 40 bp deletion from mRNA and premature stop 20 aa downstream in new exon-5)

c.G636A Exon 4

Pro Trp Ile Gln
*CYP2C19*1* ….CCC TGG ATC CAG gta…

Pro Stop
*CYP2C19*3* ….CCC TGA ATC CAG gta…

(Truncation of protein at aa 211 - loss of heme/substrate binding domains)

CYP2C19 Alleles: Phenotypes and Ethnic Differences

Table 1 Minor allele frequencies of common *CYP2C19* biomarkers (A) and associated metabolic phenotypes defined by Clinical Pharmacogenetics Implementation Consortium guidelines for individuals receiving clopidogrel [8] (B).

A

SNP	Minor allele frequency		
	Caucasian	African	Asian
*CYP2C19*2*	0.133	0.169	0.308
*CYP2C19*3*	0.00	0.021	0.058
*CYP2C19*17*	0.208	0.271	<0.02

B

	*CYP2C19*1*	*CYP2C19*2*	*CYP2C19*3*	*CYP2C19*17*
*CYP2C19*1*	**1/*1*(wt) Extensive metabolizer	**1/*2* Intermediate metabolizer	**1/*3* Intermediate metabolizer	**1/*17* Ultra-rapid metabolizer
*CYP2C19*2*		**2/*2* Poor metabolizer	**2/*3* Poor metabolizer	**2/*17* Unknown metabolizer
*CYP2C19*3*			**3/*3* Poor metabolizer	**2/*17* Unknown metabolizer
*CYP2C19*17*				**17/*17* Ultra-rapid metabolizer

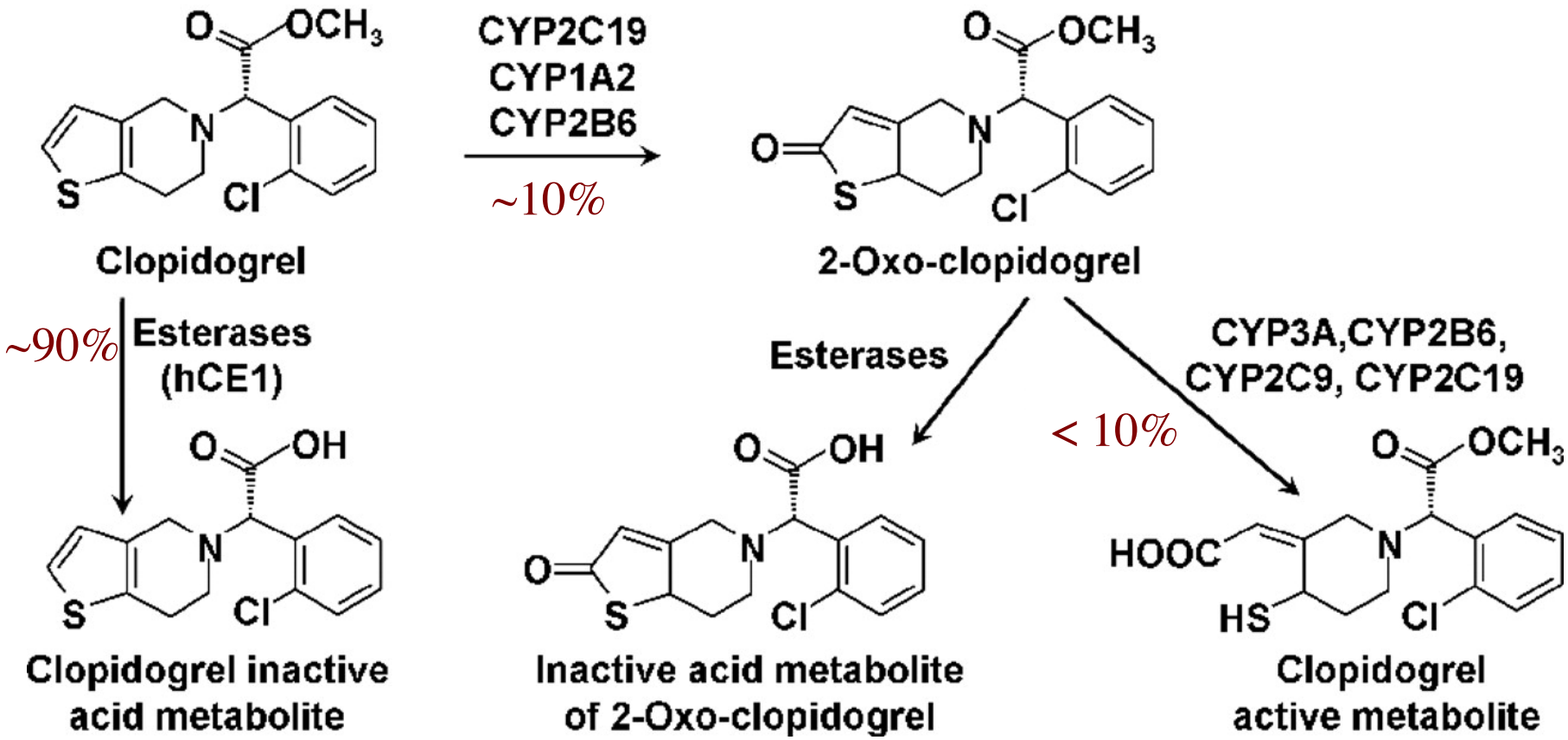
Frequencies are based on the 1000 Genomes Project at http://browser.1000genomes.org or HapMap at http://hapmap.ncbi.nlm.nih.gov/.

CYP2C19 Substrates

Drug	Drug class and therapeutic effect
Clopidogrel	Antiplatelet
Escitalopram	Antidepressant
Nelfinavir	Antiviral
Mephenytoin	Anticonvulsant (used as probe drug)
Omeprazole Lansoprazole	Proton pump inhibitor; antacid
Cyclophosphamide Teniposide	Cytotoxic agent
Amitriptyline Citalopram Clomipramine Moclobemide sertraline	Antidepressant
Tamoxifen	Anti-oestrogen
Voriconazole	Antifungal
Proguanil	Antimalarial
Propranolol	β-Blocker
Diazepam	Anxiolytic agent

Other enzymes may be more important than CYP2C19 in the metabolism of these substrates (e.g. CYP2D6 for tamoxifen).

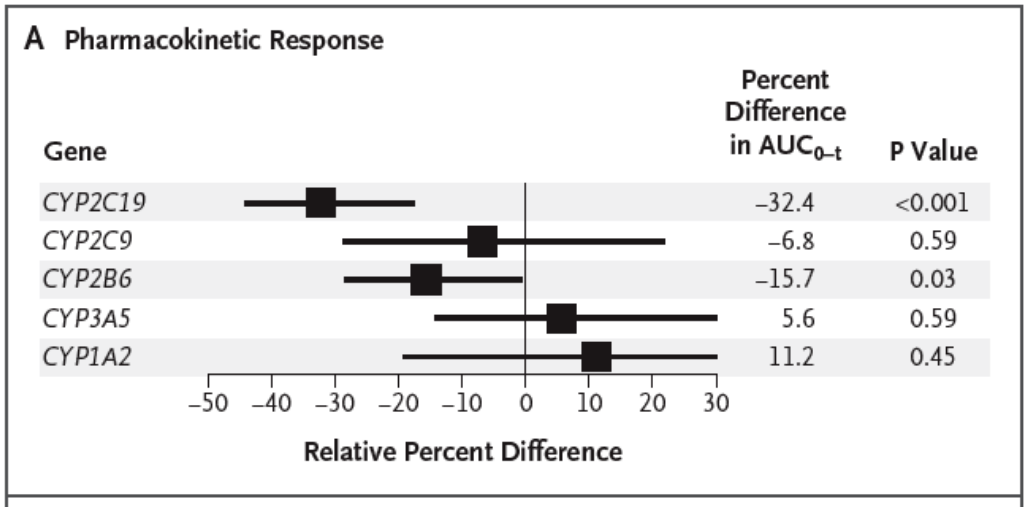
Li-Wan-Po et a/. *BJCP* 69:222-230 (2010)

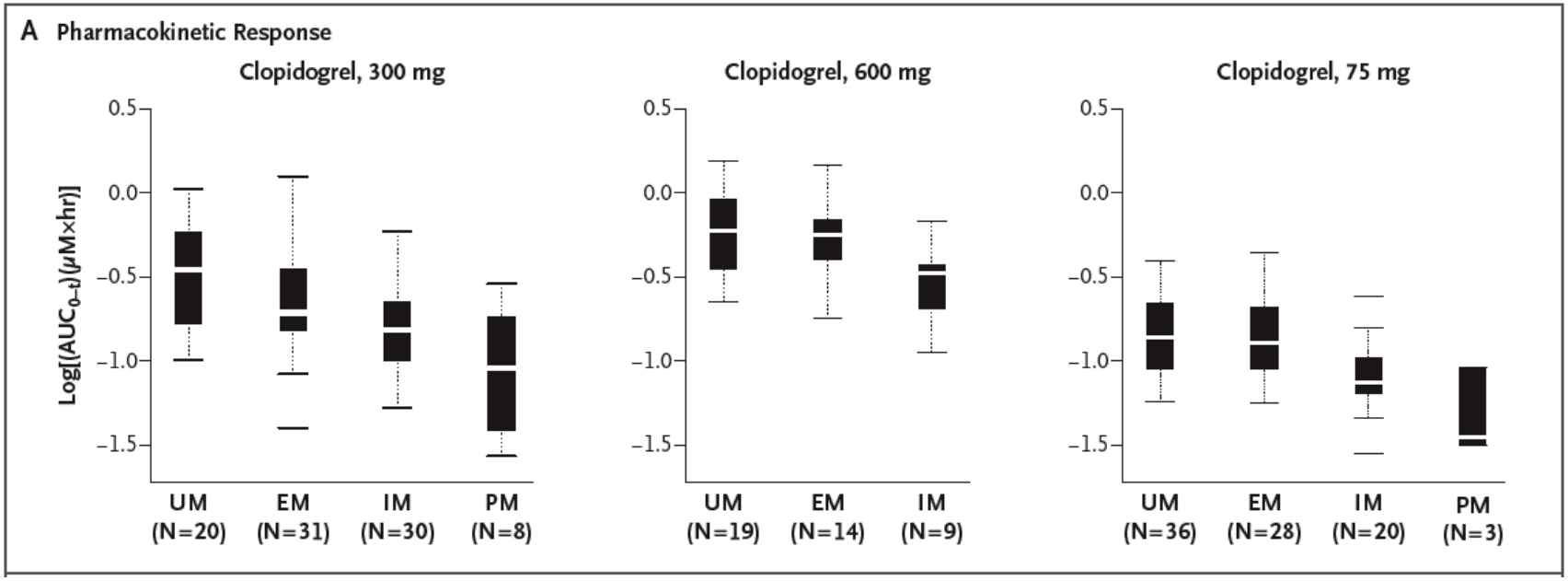


- Clopidogrel is well-absorbed, but undergoes extensive first-pass metabolism; primarily in the liver and the "inactivation pathway dominates.

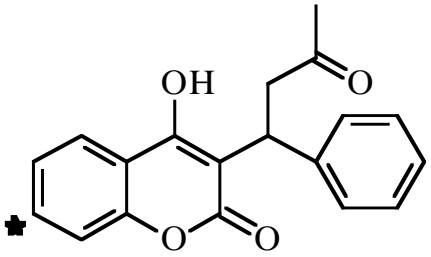
Farid et al, J Clin Pharmacol, 2010

Strong association with between clopidogrel response and CYP2C19 polymorphisms
plus graded response to gene-dose of inactivating alleles.
Mega et al. *NEJM* (2009)

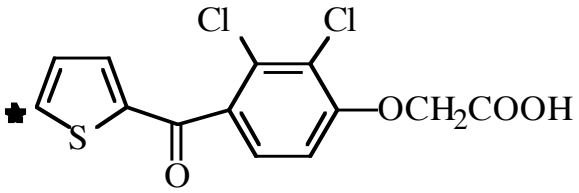




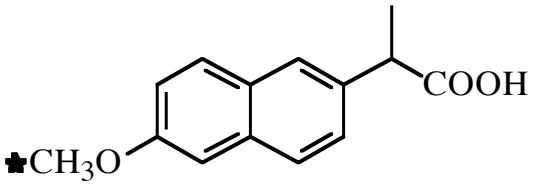
Common CYP2C9 Substrates



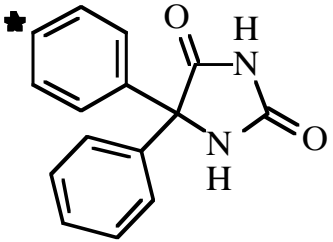
(S)-Warfarin



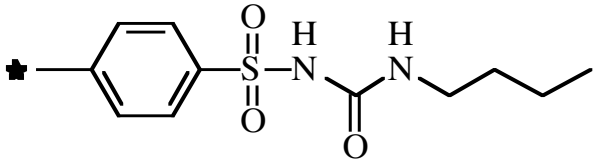
Tienilic acid



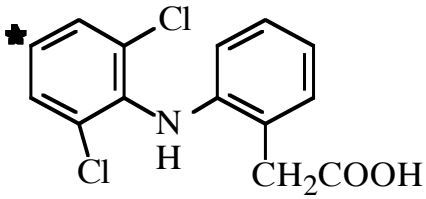
Naproxen



Phenytoin



Tolbutamide



Diclofenac

★ **Position metabolized**

CYP2C9 Alleles

- Currently, >30 coding-region variants of CYP2C9 are listed on the P450 Allele Website, about half of which appear to be functionally defective.

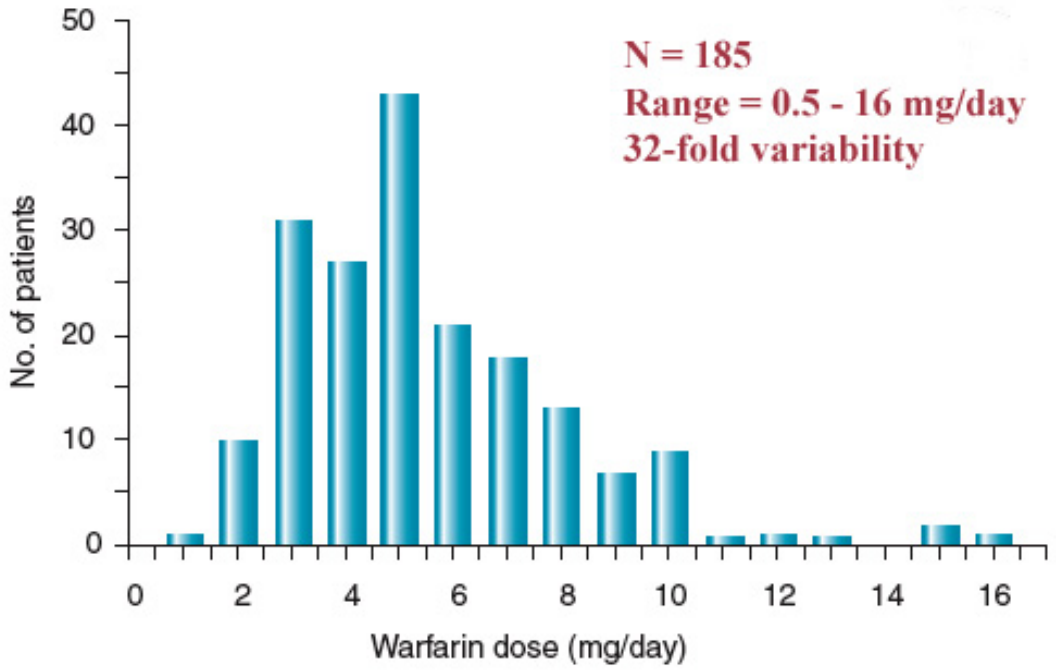
- In Caucasians, the most common functionally defective alleles are ***CYP2C9*2*** (R144C) and ***CYP2C9*3*** (I359L), with allele frequencies of ~ 12% and 8%, respectively.

- In African-Americans, ***CYP2C9*5*** *(*D360E*)* and ***CYP2C9*11*** (R335W) are the main functionally defective coding-region variants (2-3%), although both **2* and **3* are also found.

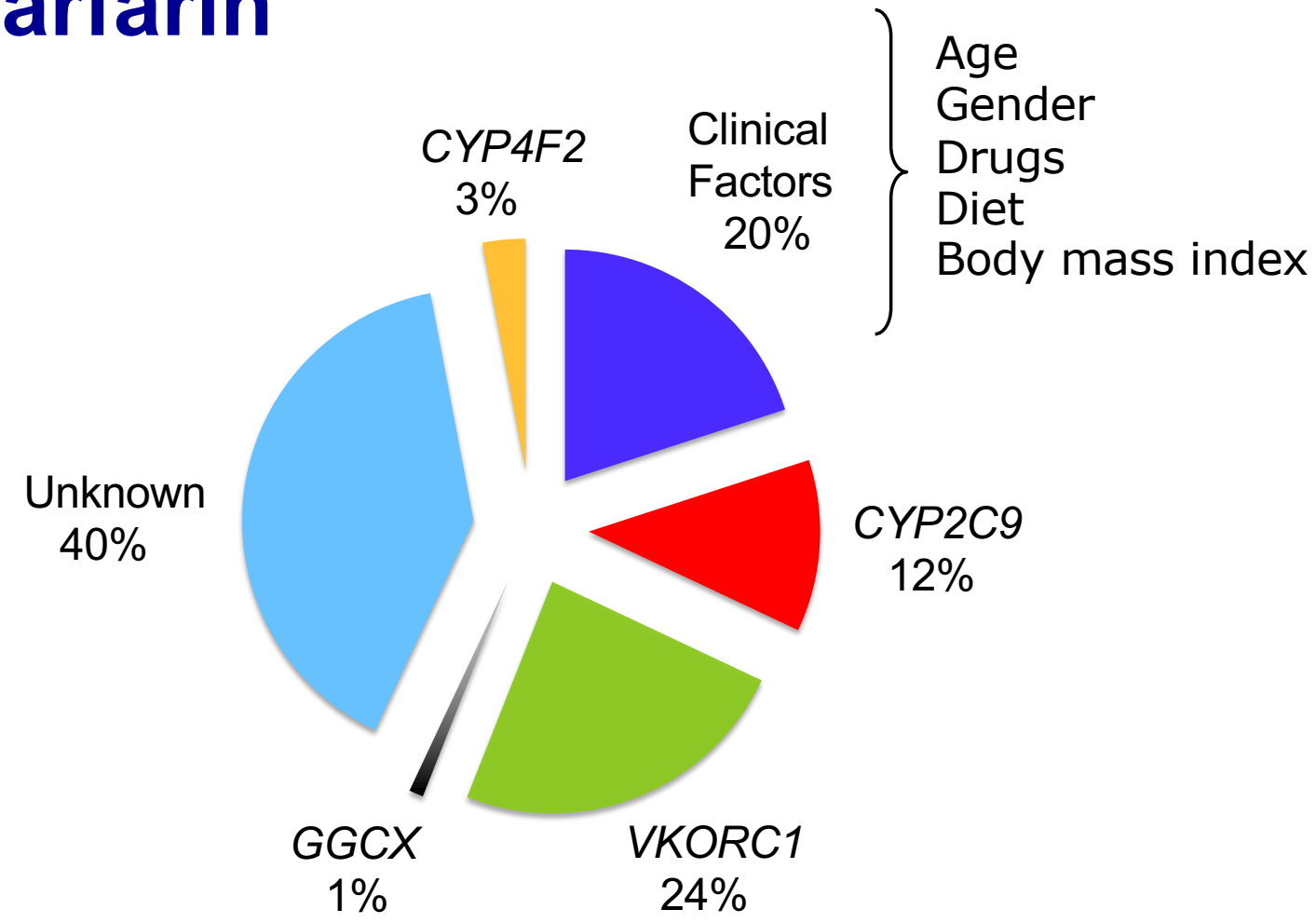
- In Asians, ***CYP2C9*3*** and ***CYP2C9*13*** are functionally defective coding-region variants (2-3%) and *CYP2C9*2* is absent.

Warfarin Therapy can be Difficult to Manage

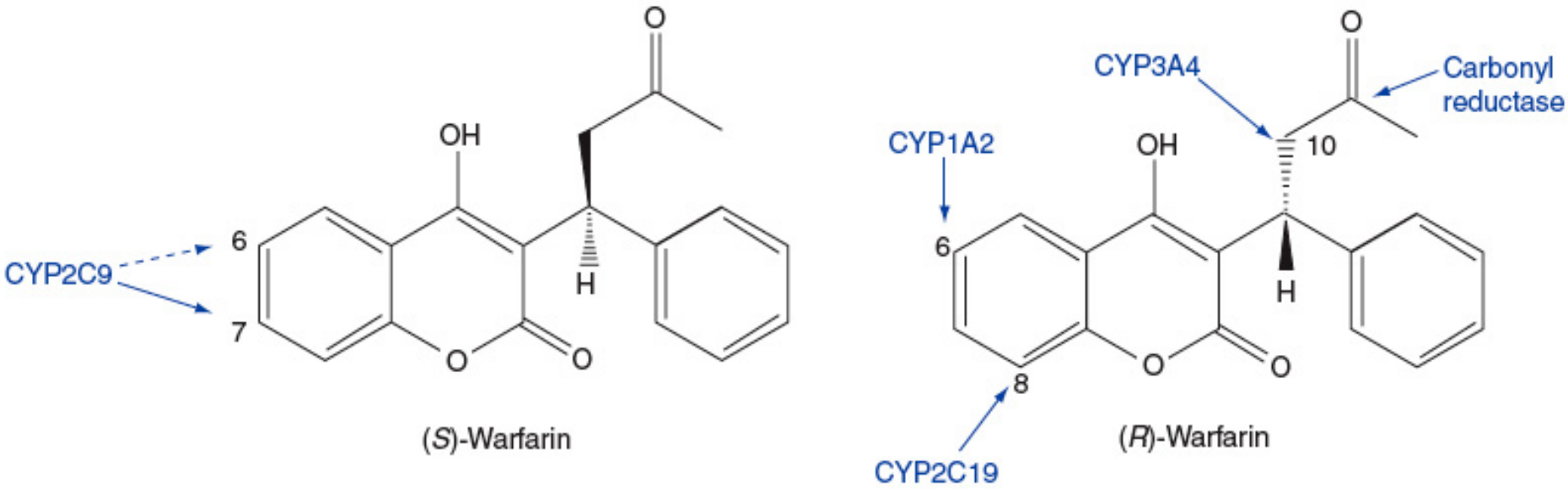
- Black Box warning: Warfarin can cause major or fatal bleeding.
- Narrow therapeutic range: INR > 4 *vs* INR 2-3
- Warfarin was the '**Culprit**' in 43,000 ER visits in US in 2004-05
- Drug-drug and drug-diet interactions
- Wide inter-individual variability in response



The Warfarin 'Pie'



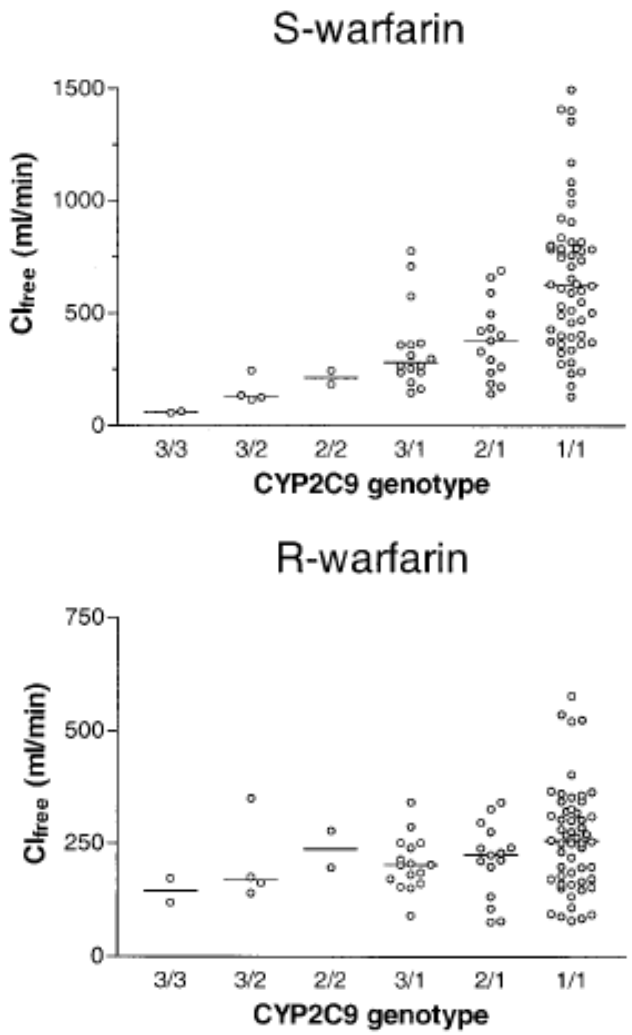
Enzymes Involved in the Metabolic Clearance of Warfarin

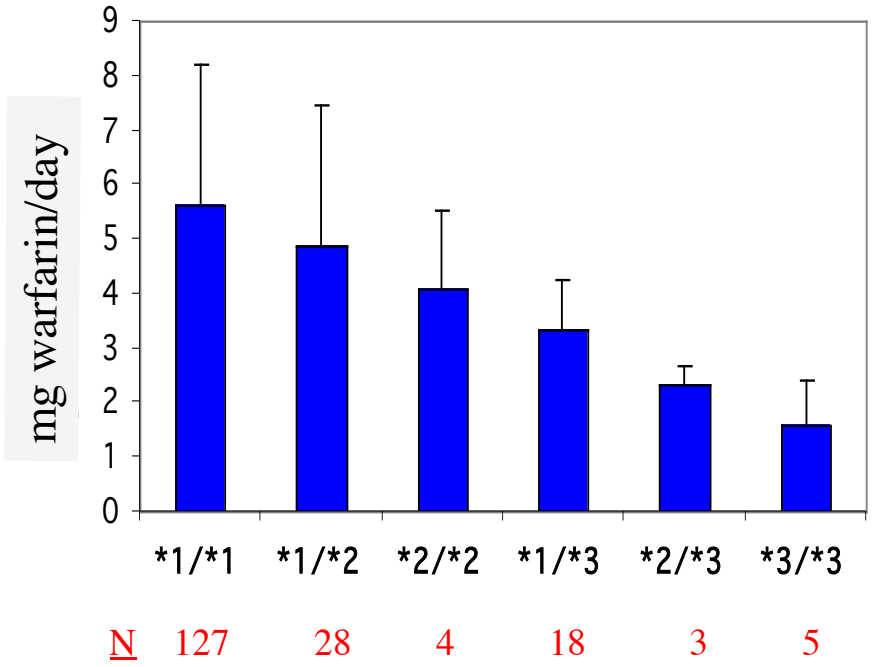


Thijjsen et al., 1988, Rettie et al.,. (1992), Kunze et al., 1996, Wienkers et al., 1996

Effect of CYP2C9 Genotype on Warfarin Dose and Clearance

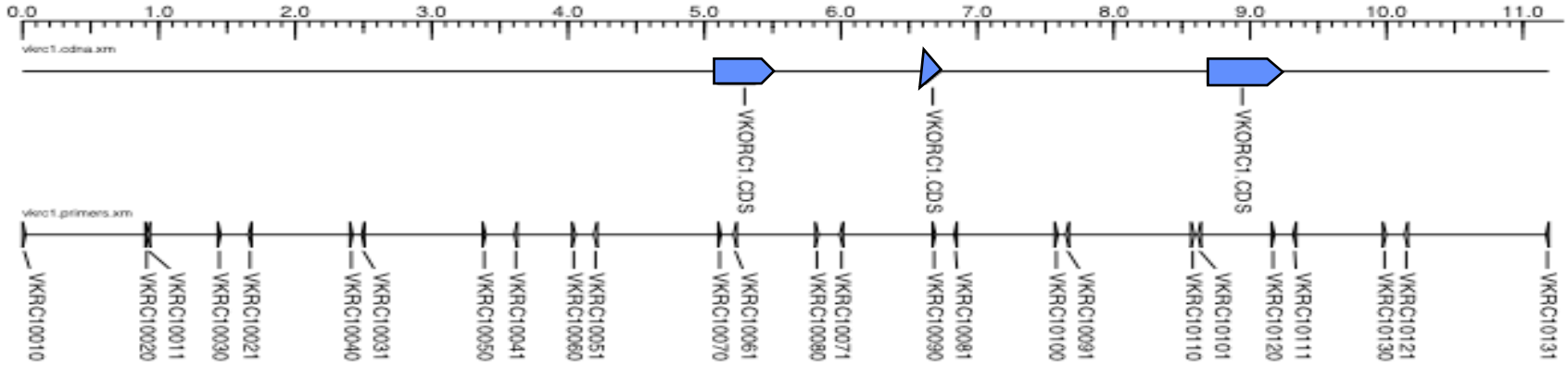
Scordo et al., *Clin.Pharm.Ther* (2002),
Higashi et al., *JAMA* (2002)





Common CYP2C9 variants account for ~12% of the variance in dose.

VKORC1 Re-Sequencing



- 27 regulatory SNPs > 5% MAF
- 5 common haplotypes defined by 10 SNPs

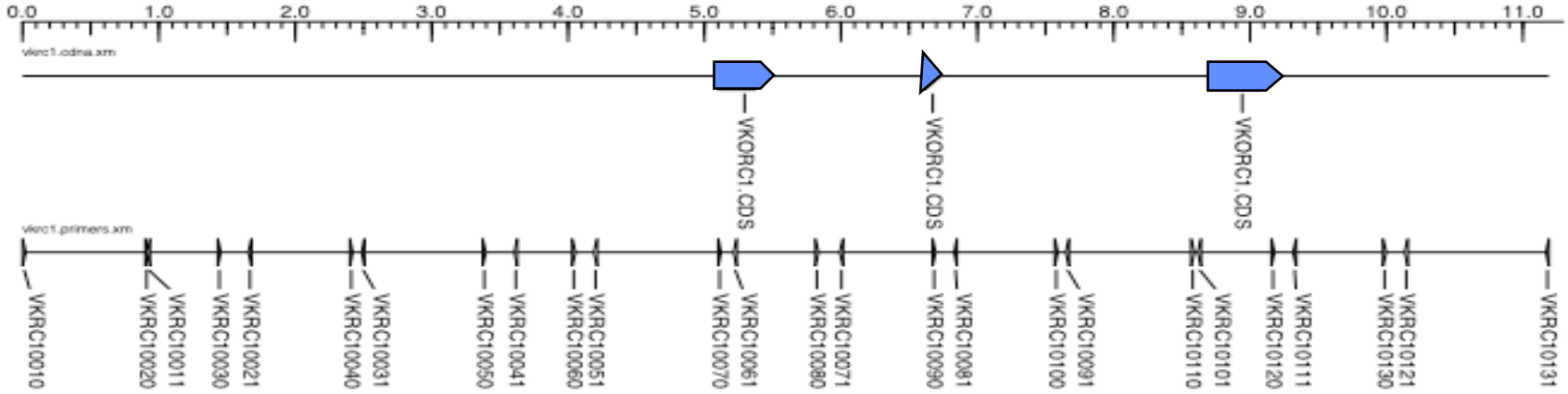
Rieder et al., NEJM (2005)

Rieder – coord.	Alleles (Rieder)	Standard-coordinate	Genome Coordinate (hg17-chr16)	rs# (dbSNP)	Gene location
381*	T/C	-4931	31018482	rs7196161	5' Flanking
861	C/A	-4451	31018002	rs17880887	5' Flanking
2653	G/C	-2659	31016210	rs17881535	5' Flanking
3673*	**G/A**	**-1639**	31015190	rs9923231	**5' Flanking**
5808	T/G	497	31013055	rs2884737	Intron 1
6009	C/T	698	31012854	rs17708472	Intron 1
6484*	**C/T**	**1173**	31012379	rs9934438	**Intron 1**
6853*	G/C	1542	31012010	rs8050894 (TaqMan-ABI)	Intron 2
7566*	C/T	2255	31011297	rs2359612 (TaqMan-ABI)	Intron 2
9041	G/A	3730	31009822	rs7294 (TaqMan-ABI)	3' UTR

* All in strong LD (r2 > 0.9) – These SNPs are the most important SNPs to test

VKORC1 Re-Sequencing

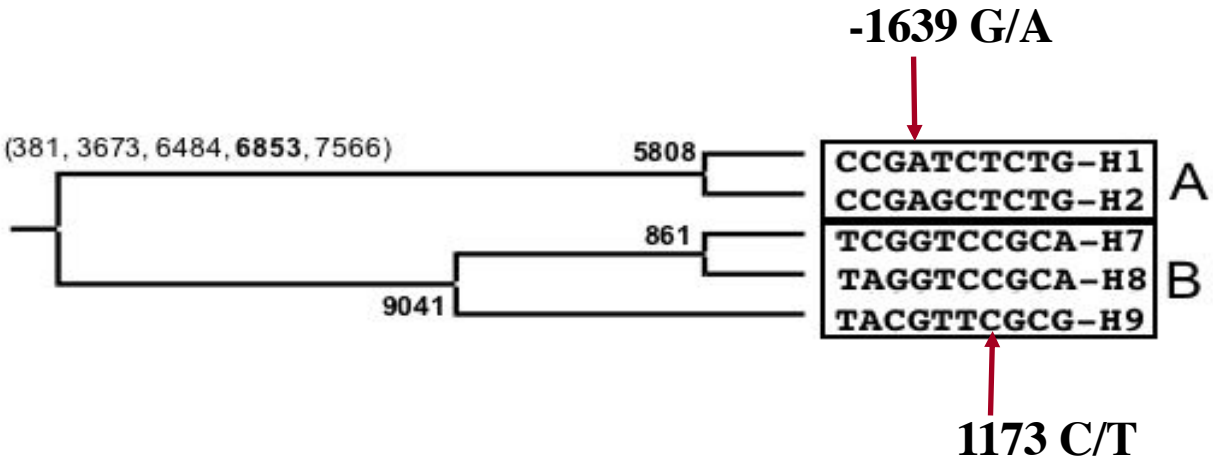
Rieder et al., *NEJM*, (2005)



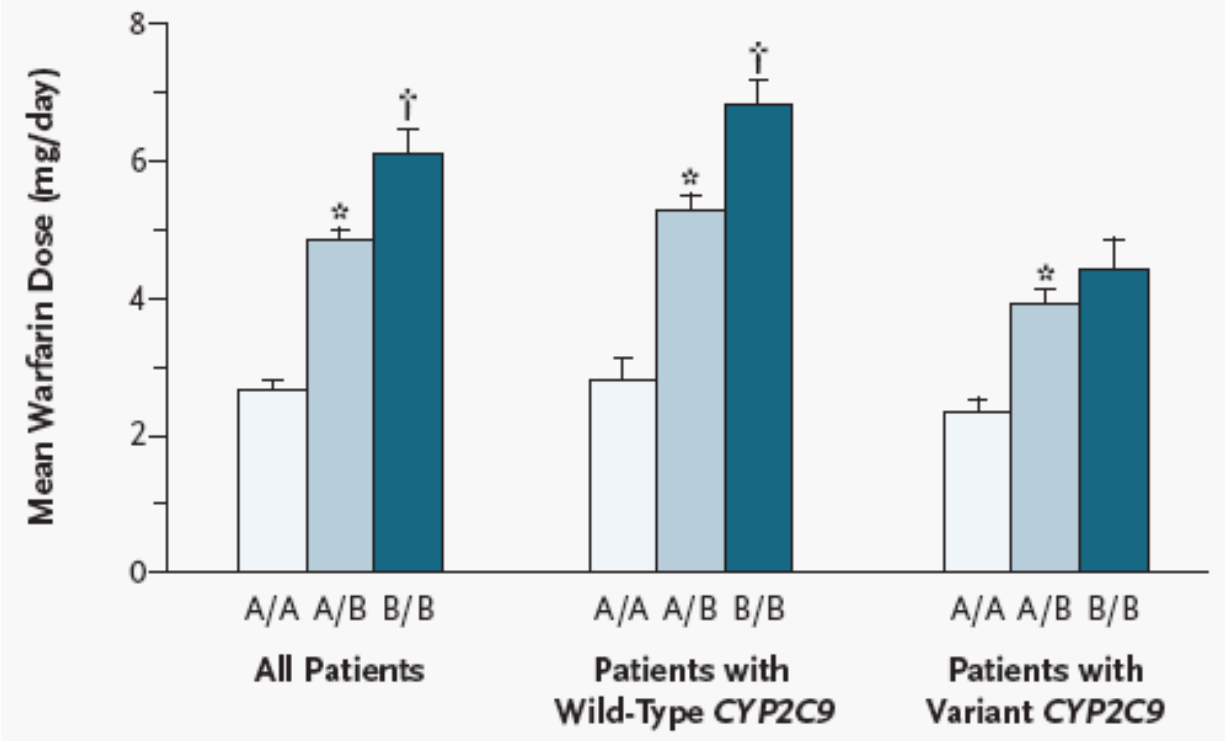
- **1** coding region SNP
  A41S

- **27** regulatory SNPs with > 5% MAF

- **5 common haplotypes defined by 10 SNPs**



VKORC1 Genotype Shows a Strong Association with Warfarin Dose

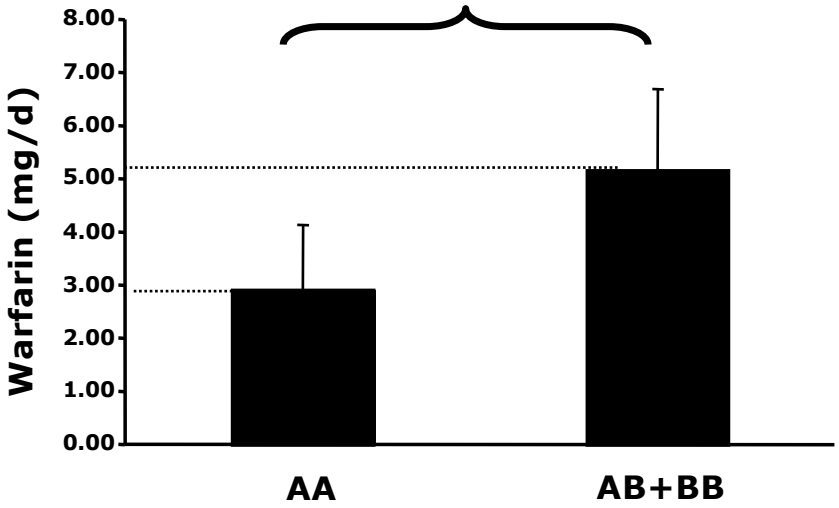


- Virtually identical data obtained in a replication cohort of European descent (n = 340)
- *VKORC1* genotype accounts for **~25%** of the variance in warfarin dose
- NB - much less of the variance accounted for in African-Americans by *VKORC1* (4-5%) - Schelleman et al., (2007); Limdi et al., (2008).

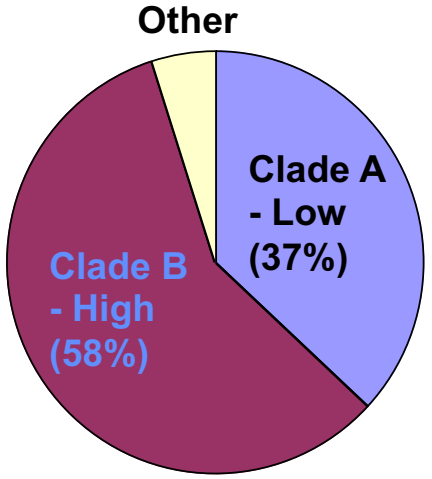
VKORC1 Effect on Warfarin Response in Asians

- Sixty nine warfarin patients recruited from Hong Kong
- Mean warfarin dose was **3.5 mg/day**
- ***VKORC1*** and *CYP2C9* genotypes explained **31%** and 8%, respectively, of the variance in warfarin dose

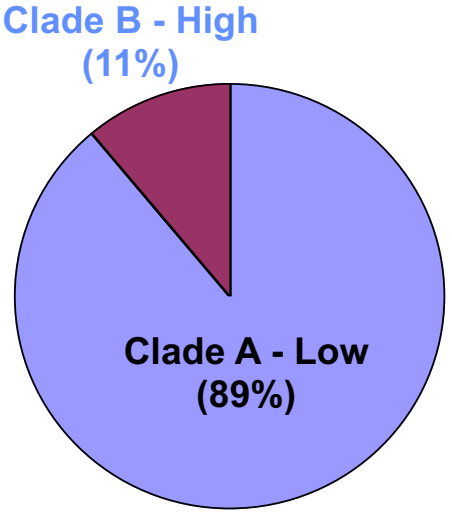
P<0.001



European (White)

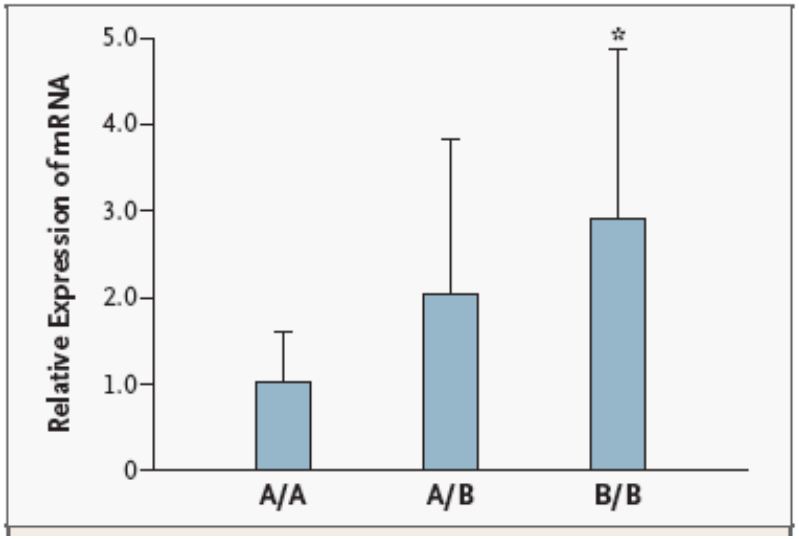


Asian



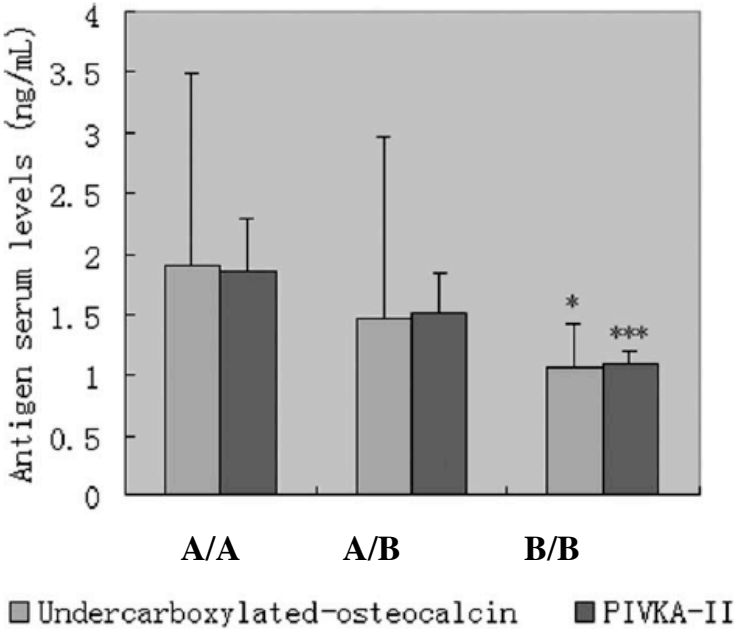
Veenstra et al., *Clin. Pharm. Ther.* (2005)

Correlation between VKORC1 Genotype and mRNA Expression



* P<0.05 for comparison between A/A and B/B groups

Correlation between VKORC1 Genotype and UC-Osteocalcin

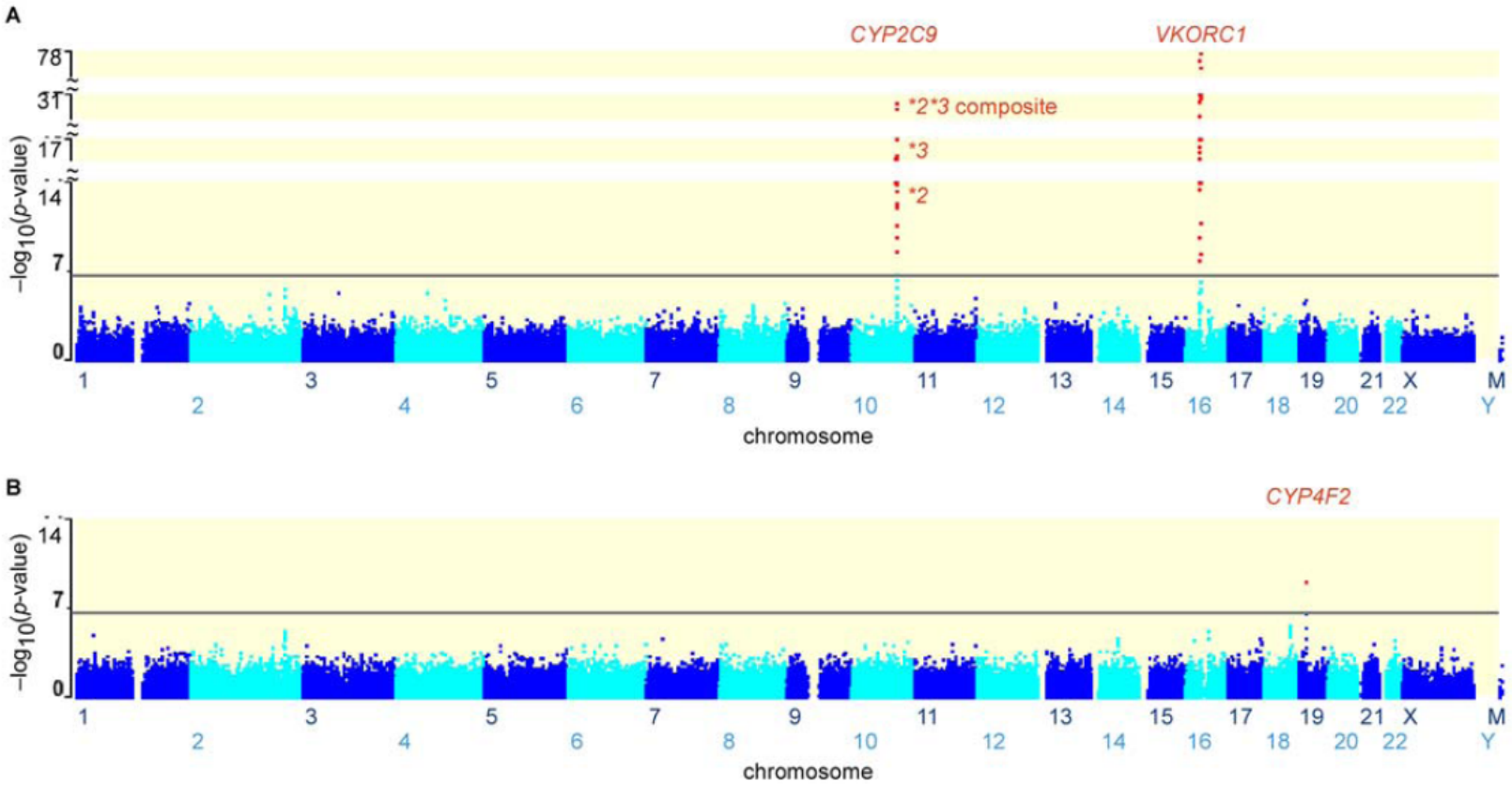


* P<0.05 for comparison between A/A and B/B groups

- VKORC1 haplotypes control mRNA expression of the warfarin target protein and correlate with the extent of Gla modification.

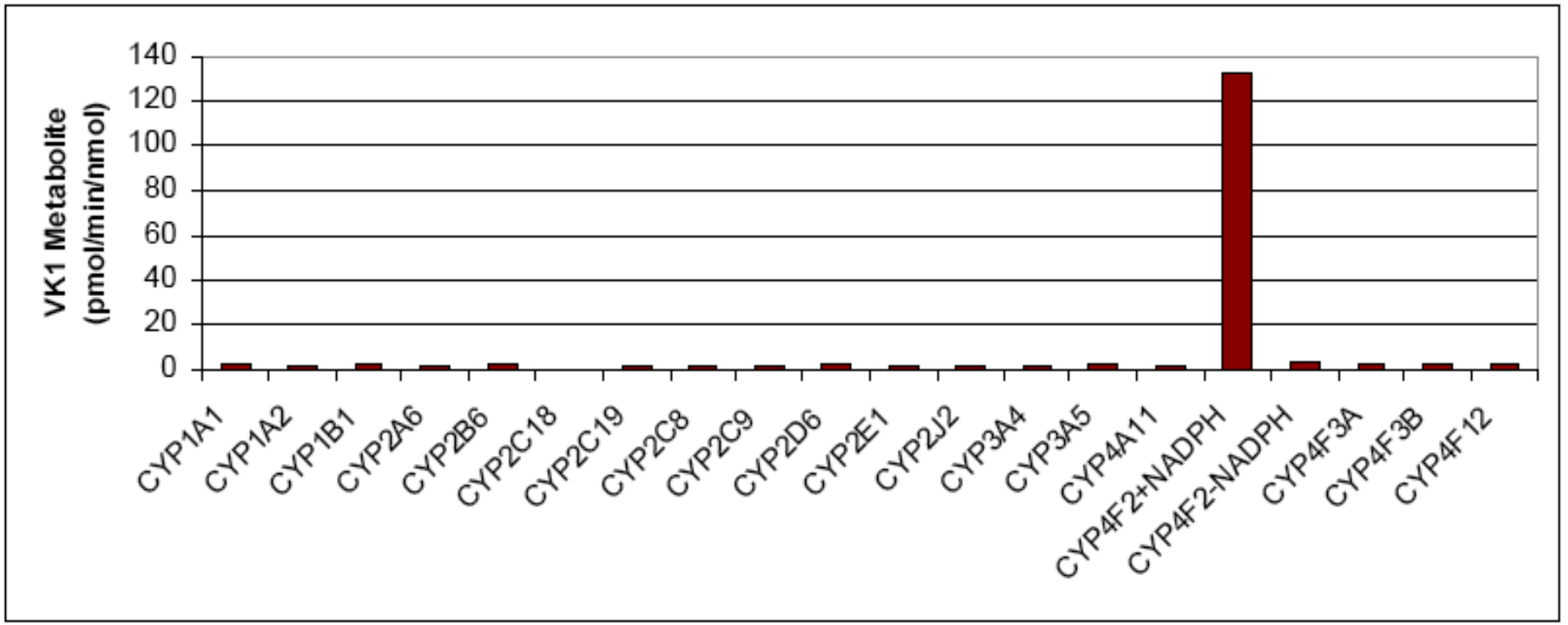
Rieder et al., *NEJM* (2005); Wang et al., *Circulation* (2005)

Genome Wide Association Studies Identify only VKORC1, CYP2C9 and CYP4F2 as Significant Contributors to Variability in Warfarin Dose



Takeuchi et al., *PLoS Genet.* (2009)

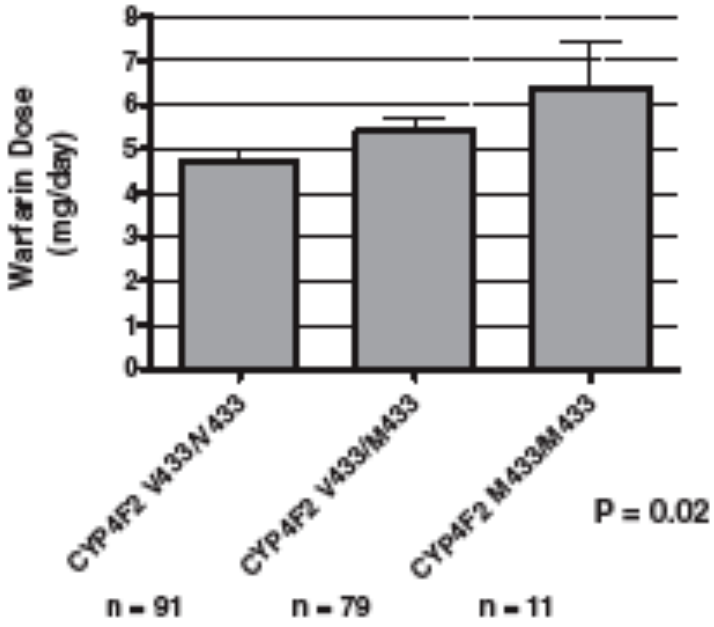
CYP4F2 Hydroxylates Vitamin K1

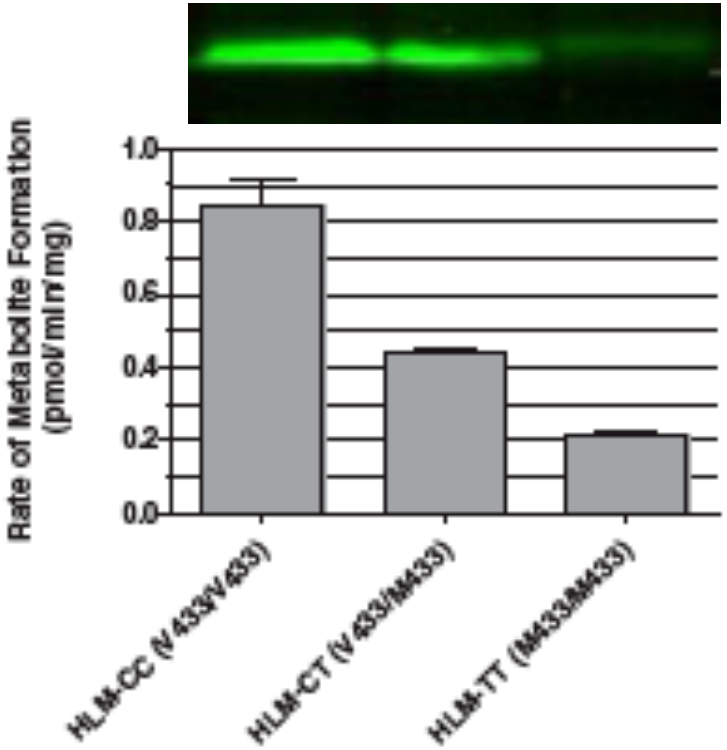


CYP4F11 is a second P450 that can metabolize vitamin K

McDonald et al., *Mol. Pharmacol.* (2009), Edson et al., unpublished

Effect of CYP4F2 V433M on Warfarin Dose, Microsomal Activity and Protein Expression





• The 433M allele increases warfarin dose by reducing microsomal vitamin K catabolism 2^{o} to altered protein stability.

McDonald et al., *Mol. Pharmacol.* (2009)

Vitamin K Cycle-Associated Genes that Impact Warfarin Dosing