Phase 1 polymorphisms

B.Sc. Pharmacology & Translational Medical Science, yr 2

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Learning objectives

- Different sources of variability
- Implication of polymorphisms on absorption, distribution and elimination of drugs
- Phase 1 and phase 2 metabolism
- Cytochrome P450 (CYP) -facts
- CYP polymorphisms

Different sources of variability

Pharmacogenetics

Implications of polymorphisms on Pharmacokinetics

- **Drug Absorption**
- **Drug Distribution**
- **Drug Elimination**
- **Drug Metabolism**
- **Drug Activation**

Implications of polymorphisms on Drug Effect

- **Receptors**
- **Target Proteins**

Phase 1 and phase 2 metabolism

Drug metabolism

Phase I

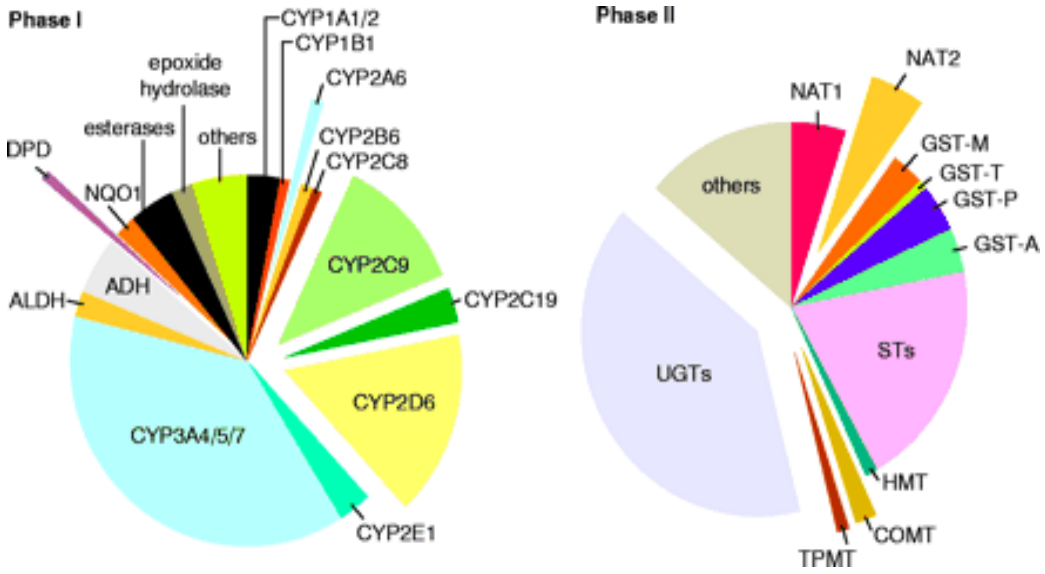
- Oxidation
- Reduction
- Hydrolysis
- Hydration
- Dethioacetylation
- Isomerization

- Aim: introduce a new functional group
- Cytochrome P450 enzymes in hepatocytes

Phase II

- Glucuronidation
- Sulfation
- Methylation
- Acetylation
- Amino Acid Conjugation
- Glutathione Conjugation
- Fatty acid conjugation
- Aim: to increase water solubility
- Ususally in the cytosol

Drug Metabolizing Enzymes



Phase I: CYP and nonCYP involved with modification of functional groups (such as oxidation). See CYP P450 dominate over the others.

Phase II: Conjugative process for enhancing elimination of drug/metabolites out of the body. Note: UGT's (UDP Glucoronyl transferases) are the most predominant phase II enzyme.

Cytochrome P450 (CYP) -facts

Cytochrome p450 enzymes

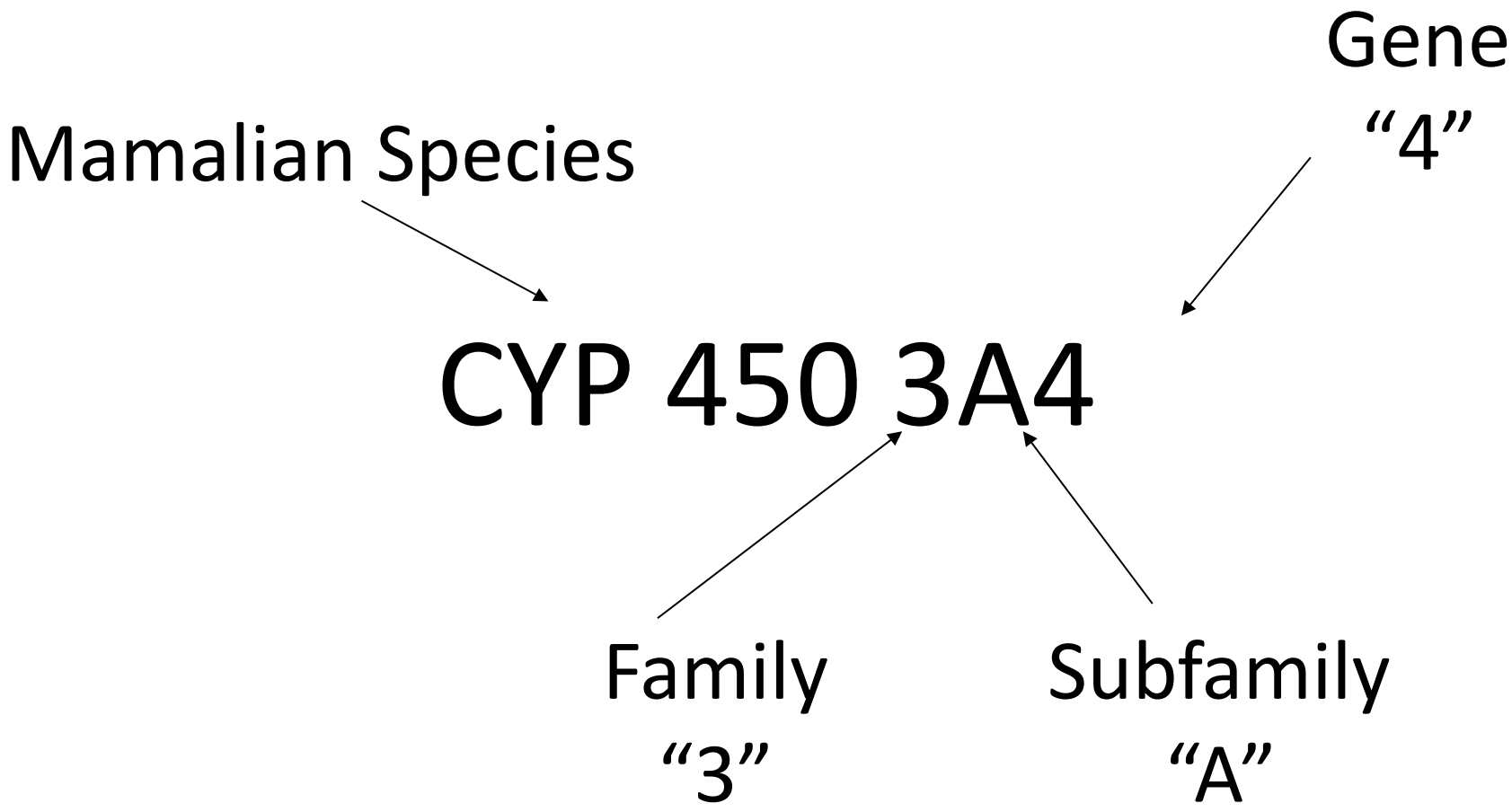
- Cytochrome P450 enzyme family typically involved in oxidations (cf phase 1 drug metabolism)

- CYP enzymes of different gene families have a 40% or more homology in their amino acid sequences, but enzymes within one subfamily may have different substrates, regulation, etc.

- Over 70 % of total CYP content of the human liver is shared by seven subfamilies: CYP1A2, CYP2A6, CYP2B6, CYP2C, CYP2D6, CYP2E1, CYP3A

Cytochrome P450

Nomenclature



Polymorphism of phase I metabolism

- Extent of metabolism is determined by
  - Affinity of substrate-enzyme complex
  - Relative abundance of a given CYP enzyme relative to the total CYP content

- Sample reactions:
  - Debrisoquine ⇒ 4-OH-debrisoquine CYP2D6
  - Dextrometorphan ⇒ dextorphan CYP2D6
  - Dextrometorphan ⇒ methoxymorphinan CYP3A
  - Sparteine ⇒ 2-dehydrosparteine CYP2D6
  - Mephenytoin ⇒ 4-hydroxy-mephenytoin CYP2C9

Major P450 Isoforms

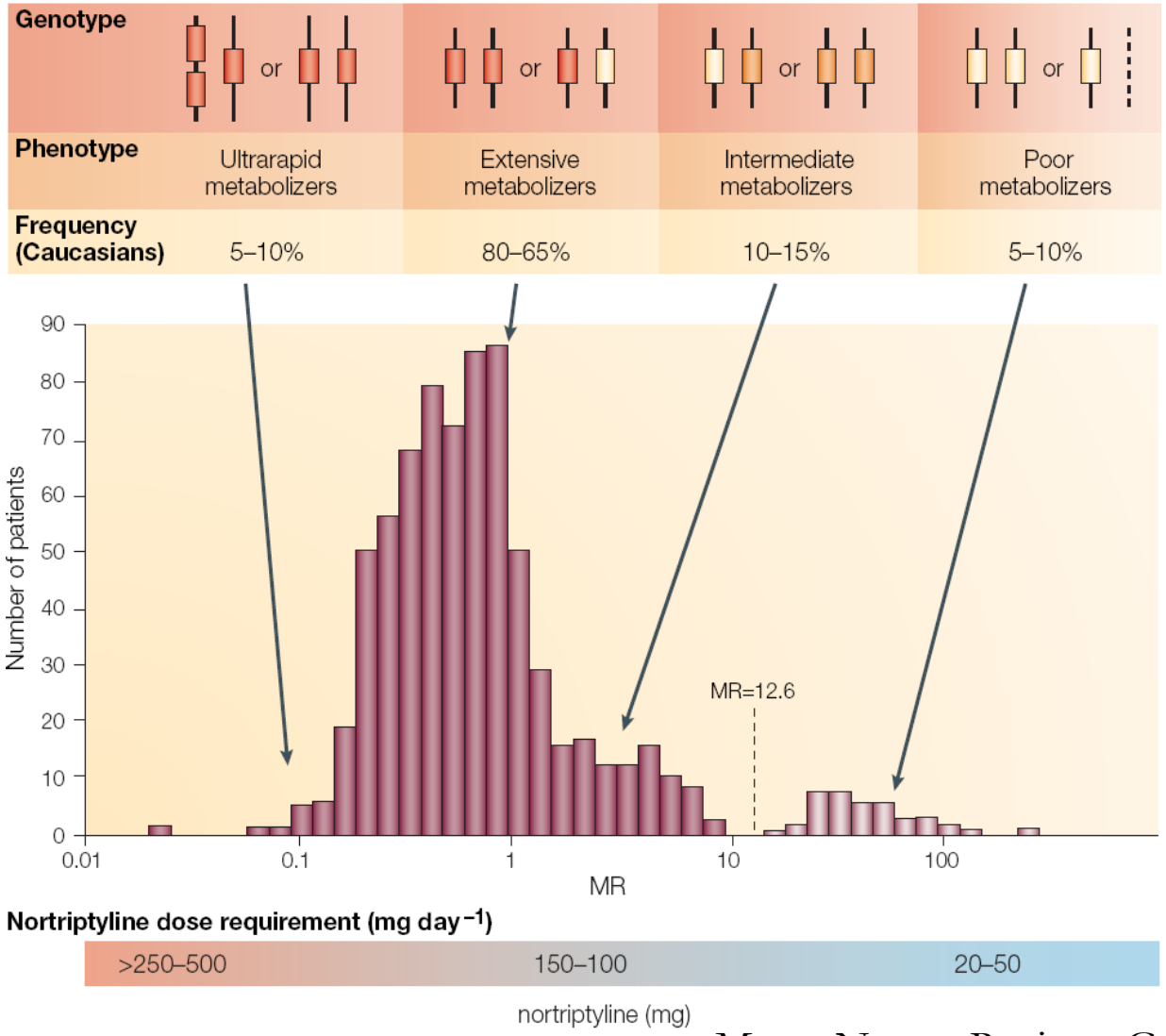
- CYP3A4 –
- CYP2D6 - Polymorphism
- CYP2C19 - Polymorphism
- CYP2C9 - Polymorphism
- CYP1A2
- CYP2E1

Few CYP polymorphisms

CYP2D6 Polymorphism

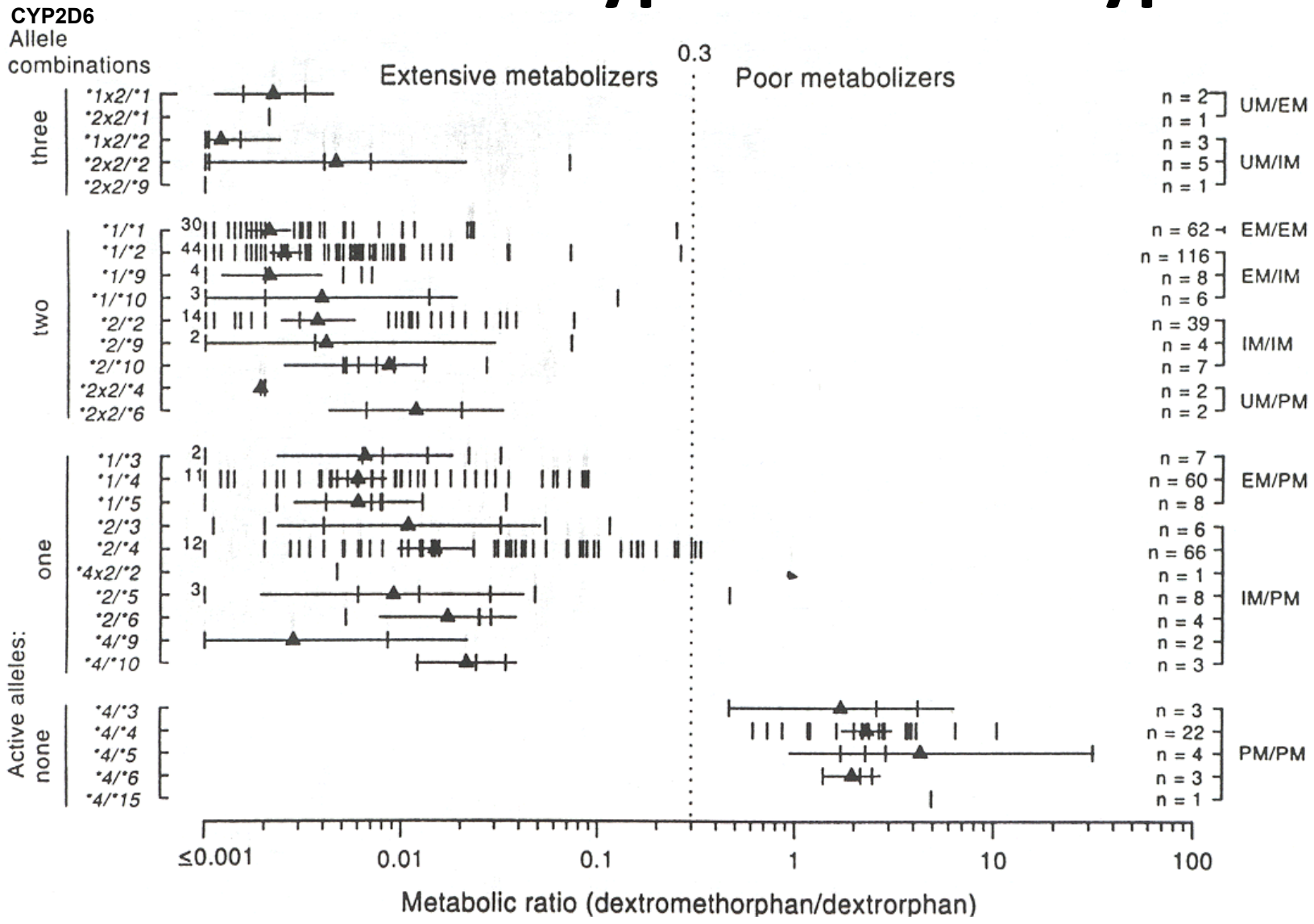
- Discovered in the 1970s
- One of the most widely studied polymorphisms in drug metabolism
- 2% of total liver CYP content
- More than 50 alleles
- Up to 1,000 fold variation in the population
- Trimodal distribution:
  - Poor Metabolizers (PM)
  - Ultraextensive metabolizers (UEM)
  - Extensive Metabolizers (EM)
- Example: nortriptyline (tricyclic antidepressant)

Pharmacogenetics of nortriptyline



Meyer Nature Reviews Genetics 2004

CYP2D6 Genotype vs Phenotype



Sachse et al. Am J Hum Genet 60:284 1997

CYP2D6 Poor Metabolizers (PM)

- Inheritance of two mutant *CYP2D6 alleles,* due to nucleotide substitutions, deletions, insertions or gene conversions

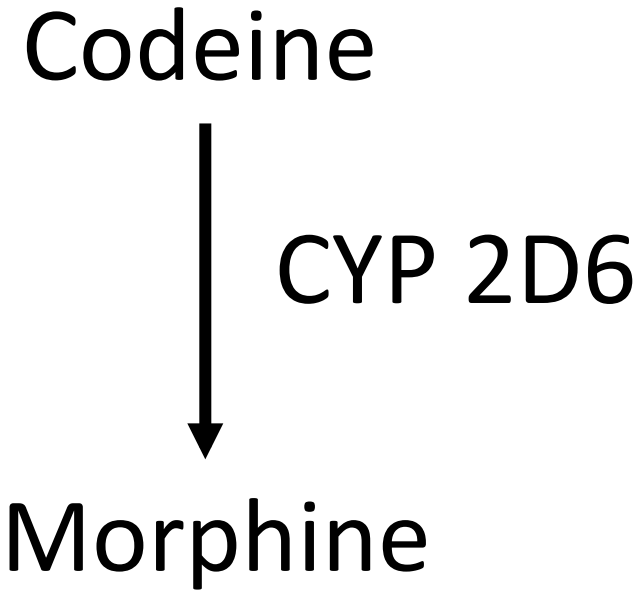
- No enzyme protein or very poor enzyme activity; impaired metabolism of CYP2D6 substrates

- Frequencies:
  - Caucasians 8 – 10%
  - American Blacks 1 – 3%
  - Japanese / Chinese < 1%

- Clinical considerations: higher plasma drug level due to decreased drug clearance; exaggerated clinical outcome and increased risk of dose-dependent side effects; may have to lower drug dose
  - PMs are at risk of drug toxicity even at standard doses, resulting in poor compliance
  - PMs may also present with treatment resistance to prodrugs that require activation (codeine)

Poor Metabolizer

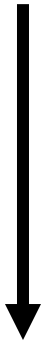
Failure of Therapy



Poor Metabolizer

Toxicity

Phenformin



CYP 2D6

Oxidative Metabolite

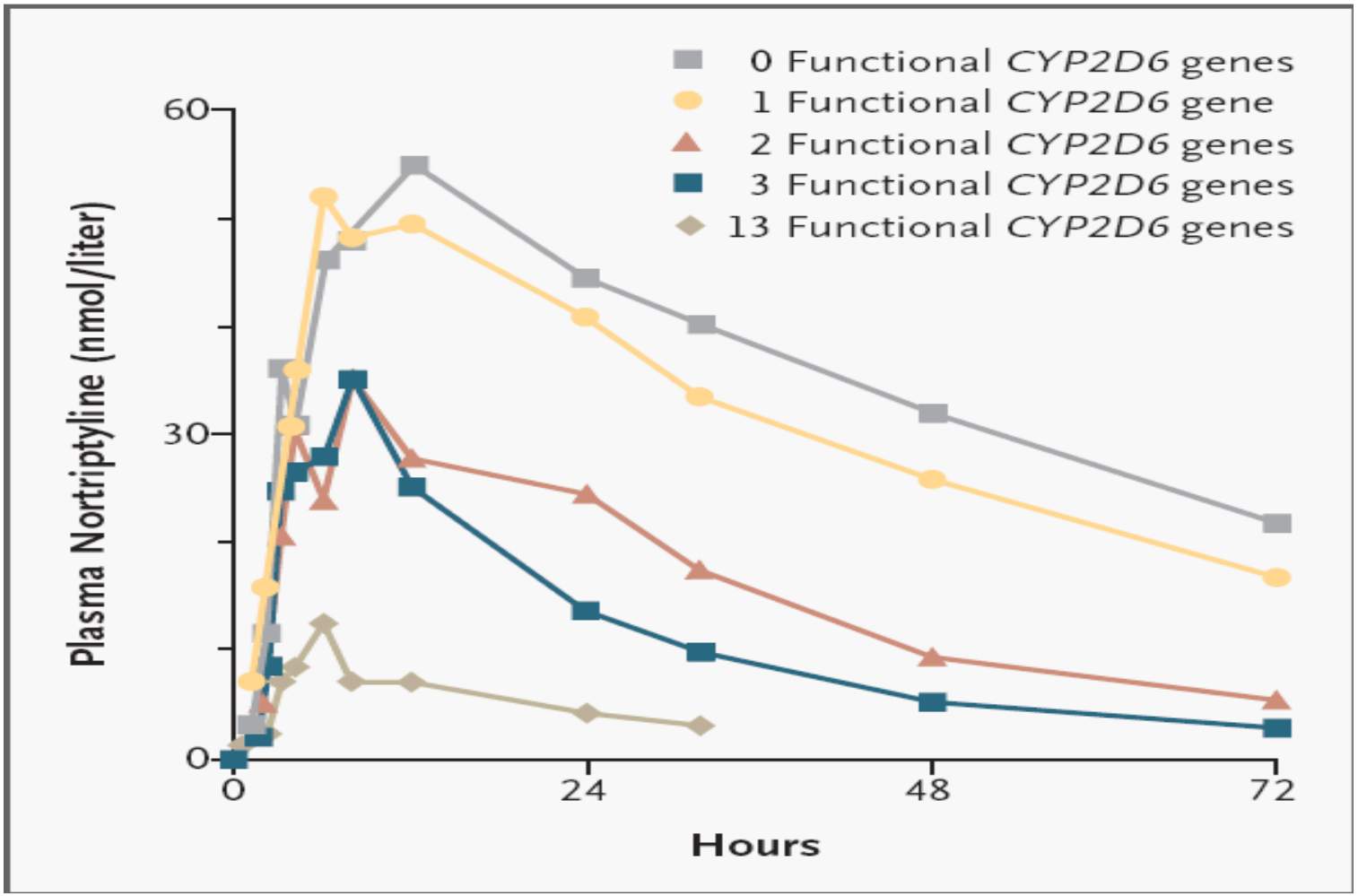
CYP2D6 Ultraextensive Metabolizers (UEM)

- Inheritance of alleles with duplication or amplification (up to 13 copies) of functional *CYP2D6 genes*
- Excessive amount of enzyme expressed, high metabolic capacity

- Frequency: from 2% in Swedish population to 29% in Ethiopian Population

- Clinical considerations: Possibly higher than normal drug dose required for efficacy; side effects if metabolites are toxic
- UEM present delayed therapeutic response or treatment resistance (29% of Ethiopians carry multiplicated functional CYP2D6 alleles)

Number of copies



Weinshilboum NEJM 2003

CYP2D6 Extensive Metabolizers (EM)

- Individuals who are either homozygous for the normal-functioning alleles or functional mutant alleles, or heterozygous with one active and one mutant allele

- Largest, but most diverse population, can have wide range of metabolic capacity

- Clinical considerations: high or low end of the group may need drug dose adjustment for acceptable efficacy and safety

Drugs Metabolized by CYP2D6

- Psychotropic medications: tricyclic antidepressants (nortriptyline), serotonin-norepinephrine reuptake inhibitor - SNRIs (venlafaxine), classical and atypical antipsychotics (haloperidol, perphenazine risperidone, atomoxetine)
- β-receptor antagonists (beta-blockers): metoprolol, propranolol, timolol
- Analgesic: phenacetine
- Antitussive: Dextromethorphan
- Chelator: D-penicillamine
- Opioids: Codeine, hydroxycodone, Oxycodone, Tramadol
- Abused drugs

CYP2D6 and Race/Ethnicity

Variant Alleles With Known Poor Metabolism for Enzymes That Metabolize Adverse Drug Reaction–Implicated Drugs*

Enzymes	Prevalence of Poor Metabolizers, Race, %	Variant Alleles	Prevalence of Variant Alleles, Race, %
CYP2D6	3-10, White; <2 Chinese, Japanese, African American	*CYP2D6*2A*	28-30, White; 20, Chinese; 12, Japanese
		*CYP2D6*3A*	21, White
		*CYP2D6*3B*	2, White
		*CYP2D6*4A, B*	20-23, White; 7-9, African American; 9, African
		*CYP2D6*5*	2-5 White; 10-13 Japanese
		*CYP2D6*6A*	2 White
		*CYP2D6*7*	<1-2 White
		*CYP2D6*8*	<1 White
		*CYP2D6*9*	2 White
		CYP2D610 (no further designation)	5 White; 50 Asian
		*CYP2D6*10A, B*	2-5 White, 43-51 Chinese; 33-60 Japanese
		*CYP2D6*11*	<1 White
		*CYP2D6*12*	<1 White
		*CYP2D6*17*	0 White, 26 African American; 9-34 African; 19 Korean
		*CYP2D6*36*	9 Korean; 31 Chinese and Japanese
		*CYP2D6*4C, D, K, 4X2, 6B, 6C*	No prevalence data
		*CYP2D6*13, 14, 15, 16, 18, 20, 38*	No prevalence data

Distribution of CYP2D6 enzymes in different populations

Variant alleles	Enzyme function	Allele frequency %			
		Caucasians	Asians	Black Africans	Ethiopians and Saudi Arabians
CYP2D6*2xN	Increased	1-5	0-2	2	10-16
CYP2D6*4	Inactive	12-21	1	2	1-4
CYP2D6*5	No enzyme	2-7	6	4	1-3
CYP2D6*10	Unstable	1-2	51	6	3-9
CYP2D6*17	Reduced affinity	0	ND	34	3-9

Ingelman-Sundberg et al., 1999

CYP2D6: polymorphism of debrisoquine metabolism

- Debrisoquine is the most frequently used test substrate in studies of the polymorphism of drug metabolism
- Frequency of phenotypes

Population	PM frequency %
Hungary	7.74
Caucasians	3-10
Cuna Indians	0
Bushmen	19

Tailored dosing

Recommend dosage adjustment to Atomoxetine in CYP2D6 PM and those taking strong 2D6 inhibitors

– Individual > 70 kg: start at 40 mg/day

– Individual ≤ 70 kg: start at 0.5 mg/kg/day.

*Increase to the usual target dose of 80 mg/day and 1.2 mg/kg/day, respectively, only if treatment fails to improve symptoms after 4 weeks and the initial doses are well tolerated.

More facts about CYP2D6

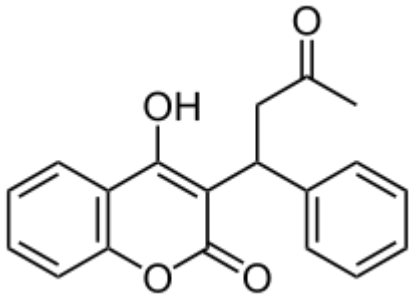
- **CYP 2D6** also present in **brain**
- Functionally associated with **dopamine transporter**
- Might have a role in **dopaminergic transmission**

- Some studies have suggested **differences** in **personality traits** between **PMs** and **EMs**:

– Type A vs Type B personality

– Higher levels of anxiety / impulsivity (PMs)

Another example: Warfarin (Coumadin)



- Used for **chronic anticoagulation**
- **Prevention** of **thrombosis** (blood clots) and embolism (abnormal circulation of blood clots)
- Discovered after hemorrhaging in cattle fed with mouldy silage made from sweet clover.
- Two enantiomers (R- and S-), cleared by different pathways

- Very successful drug… but:
- Interactions with Vitamin K present in plant-based food
- Adverse reactions include:
  - Hemorrhage
  - Necrosis
  - Osteoporosis

Warfarin Metabolism

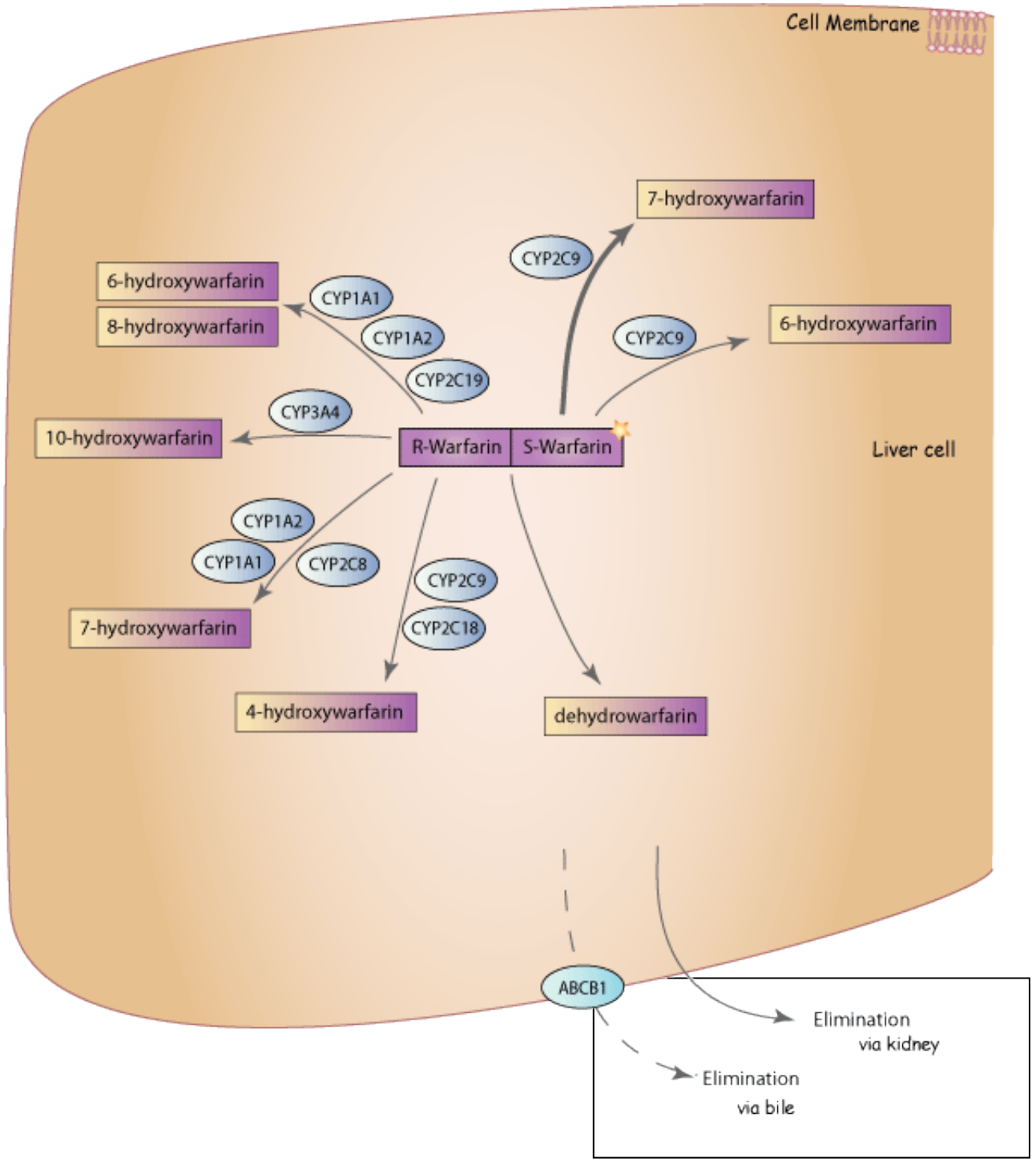
2 enantiomers

= 2 pathways

Importance of

CYP2C9 (S)

CYP2C19 (R)

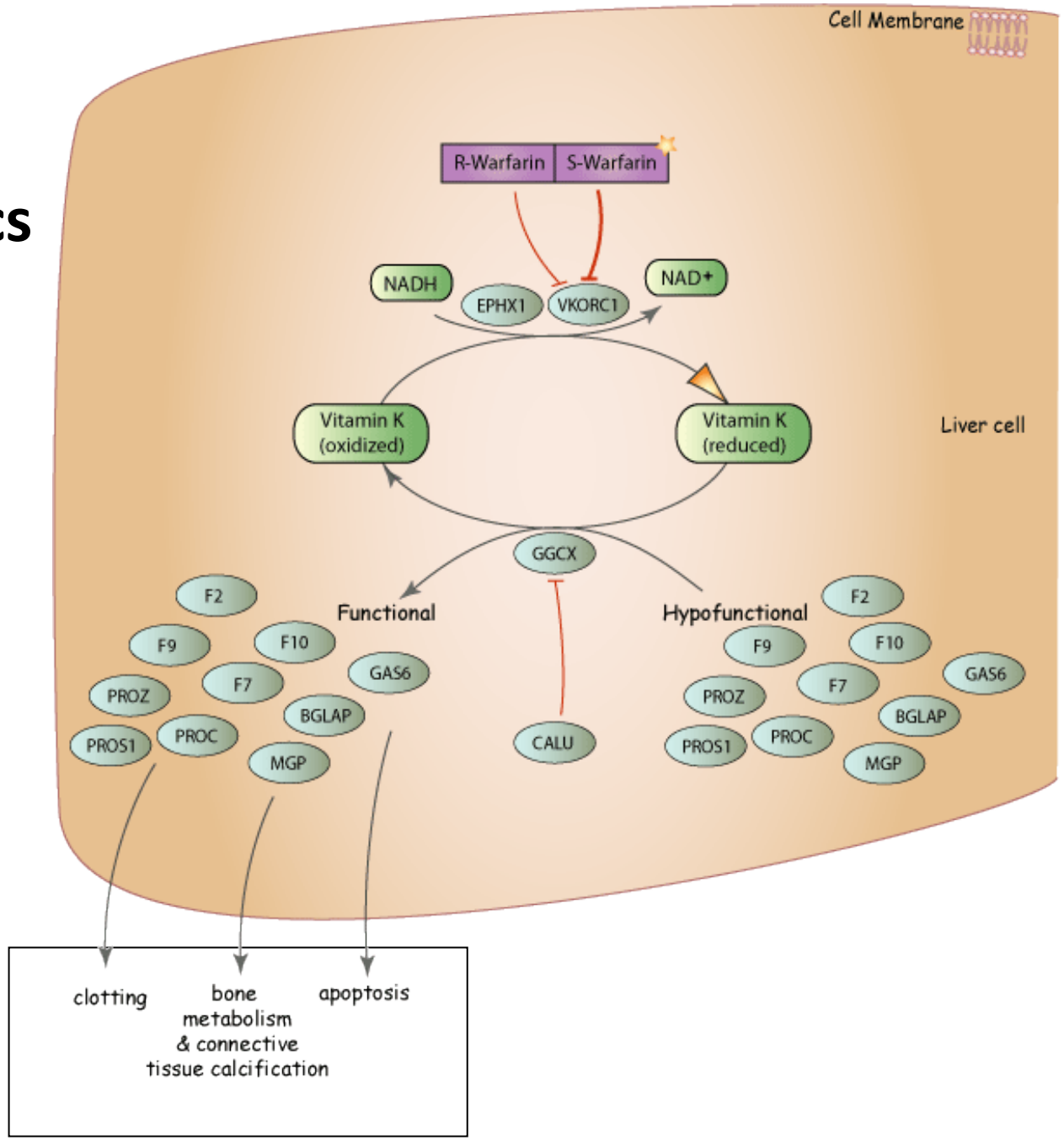


Warfarin Pharmacodynamics

Importance of

VKORC1

(interaction with Vitamin K)



CYP2C19 Polymorphism

- Poor Metabolizers
  - 3–5% of Caucasian
  - 15–25% of Asians (Chinese, Japanese, Koreans, Indians, etc)

- May affect clearance of:

amitriptyline, **diazepam, clomipramine, phenytoin, progesterone, propranolol, PPIs (lansoprazole, omeprazole,** pantoprazole, rabeprazole, etc),

warafarin

CYP2C19 Polymorphism and Diazepam

- **Why diazepam metabolism is slower in Asians compared to Caucasians?**
- Effect on diazepam:

Genotype	Allele	Diazepam $t_{½}$
EM	CYP2C19 *1/*1	20 hours
PM	CYP2C19 *2/*2	84 hours

- **About 15 – 25% of Asians have high frequency of mutant alleles CYP2C19**

CYP2C19 Polymorphism and H.pylori

- **H.pylori: bacteria from the gut, responsible for gut ulcers**

Genotype	Allele	Cure rate
Wild type	CYP2C19 *1/*1	**29 %**
Htz	**CYP2C19 *1/*2**	**60 %**
HMz variant	**CYP2C19 *2/*2**	**100 %**

- **Higher cure rates in variant HMz due to higher concentrations and longer duration of omeprazole dose**

  = CYP2C19 *2 variants have a beneficial effect in this case

CYP 2C19

Inducers

- Rifampin

Inhibitors

- Fluvoxamine
- Ticlopidine
- Fluoxetine

Substrates

- Omeprazole
- Diazepam
- TCAs
- Clomipramine
- Phenytoin

CYP2C9 Polymorphism

- More than 50 SNPs have been described in the regulatory and coding regions of the CYP2C9 gene
- Some of them are associated with reduced enzyme activity
- 10–35% of Caucasians are poor metabolizers
- May affect clearance of:

– **Phenytoin, *S-warfarin***

– losartan, valsartan, glipizide, glyburide

Frequency of CYP2C9 Phenotype in Various Populations

Groups	*1/*1 (%)	*1/*2 (%)	*1/*3 (%)	*2/*2 (%)	*3/*3 (%)
Caucasians	65	20	12	1	0.4
African american	97	2	1	0	0
Chinese	97	0	4	0	0
Japanese	96	0	4	0	0
Korean	98	0	2	0	0
Turkish	62	18	17	1	1
Spanish	50	16	24	2	0

CYP1A2: nonpolymorphic drug metabolism with polygenic control

- 13% of total liver CYP content
- Varies up to 130fold in individuals and in populations
- Important in disposition of several important psychotropic medications: clozapine, olanzapine

CYP 3A4

- Most abundant P450 in the liver (40 % by mass and metabolizes 60% of drugs)
- Liver, small bowel wall
- Not Polymorphic

CYP 3A4

<u>Inducers</u>

- Phenobarbital
- Rifampin
- Prednisone
- Carbemazepine
- Phenytoin

<u>Substrates</u>

- Steroids
- Macrolides
- CCB
- Hormones
- Antihistamines
- Taxol, Vinblastine
- Cisapride

Causes of Variability

- 80% of the variability of 2D6 is due to genetic factors
- 3A4, no genetic variability- variability is probably due to induction (rifampin increases 3A4 activity 20 fold)

Conclusions

- Phase 1 polymorphisms
- Mostly CYP

- To be continued with next lecture in pharmacogenetics:
- Phase 2 polymorphisms + ADME