Phase 2 polymorphisms

(+ drug activation, receptors)

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

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Learning objectives

- … continued from Phase 1 polymorphism lecture
- Phase 2 polymorphisms
  - N-AcetylTransferases (NAT)
  - Thiopurine S Methyltransferase (TPMT)
  - Glutathione S Transferases (GST), UDP Glucuronosyl-Transferases (UGT)
- Other polymorphisms
- Other sources of variability

Phase 2 polymorphisms

Drug metabolism

Phase I

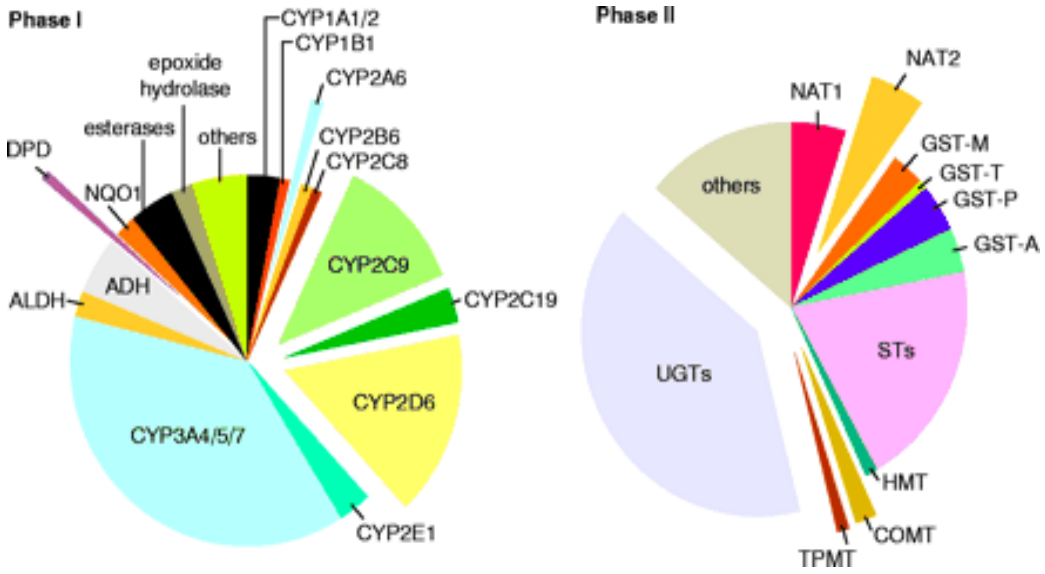
- Oxidation
- Reduction
- Hydrolysis
- Hydration
- Dethioacetylation
- Isomerization

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

Phase II

- Glucuronidation
- Sulphation
- 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.

Pharmacogenetics

Implications of polymorphisms on Pharmacokinetics

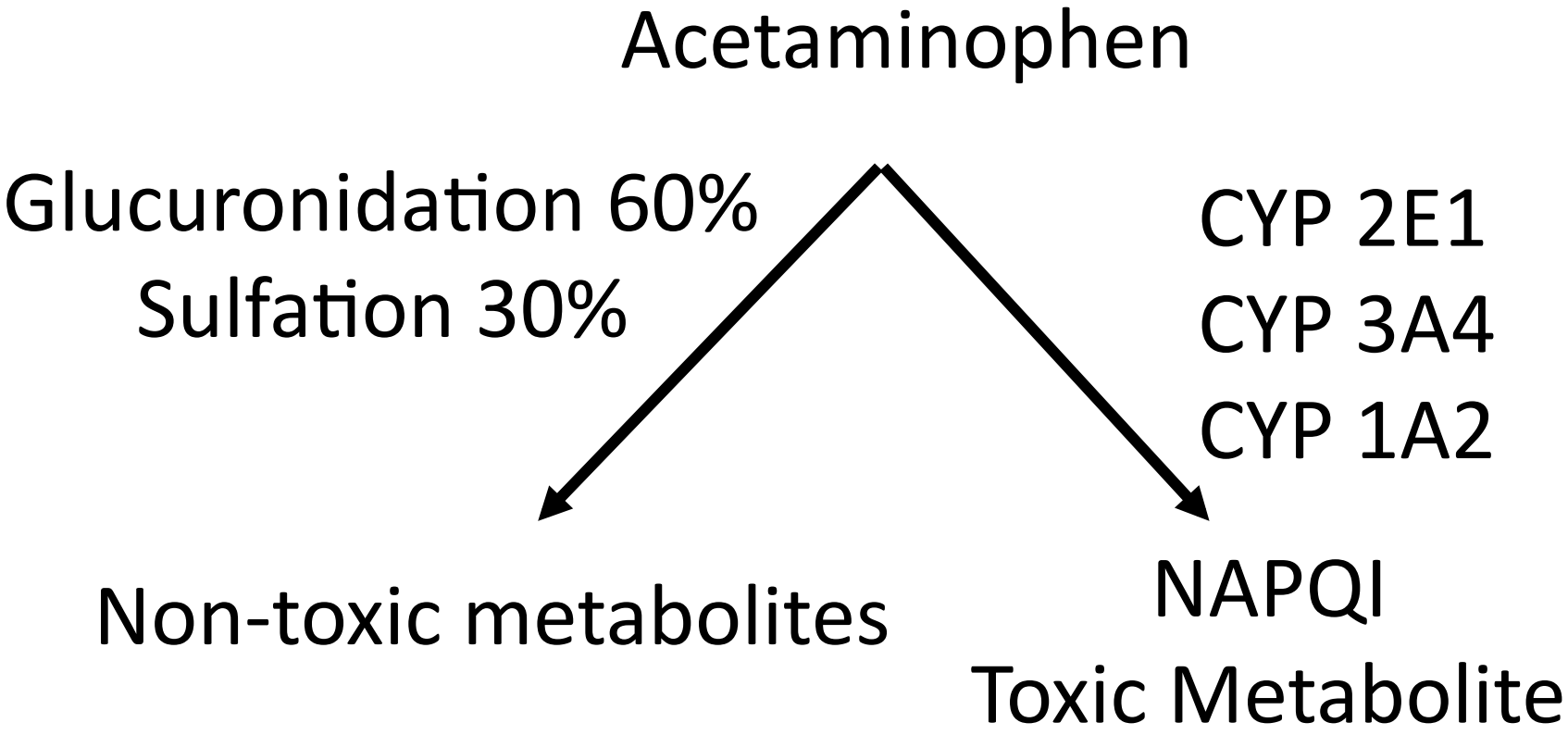
- **Drug Absorption**
- **Drug Distribution**
- **Drug Elimination**
- **Drug Metabolism**
- **Drug Activation**

Implications of polymorphisms on Drug Effect

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

Polymorphism of phase II metabolism: conjugation I.

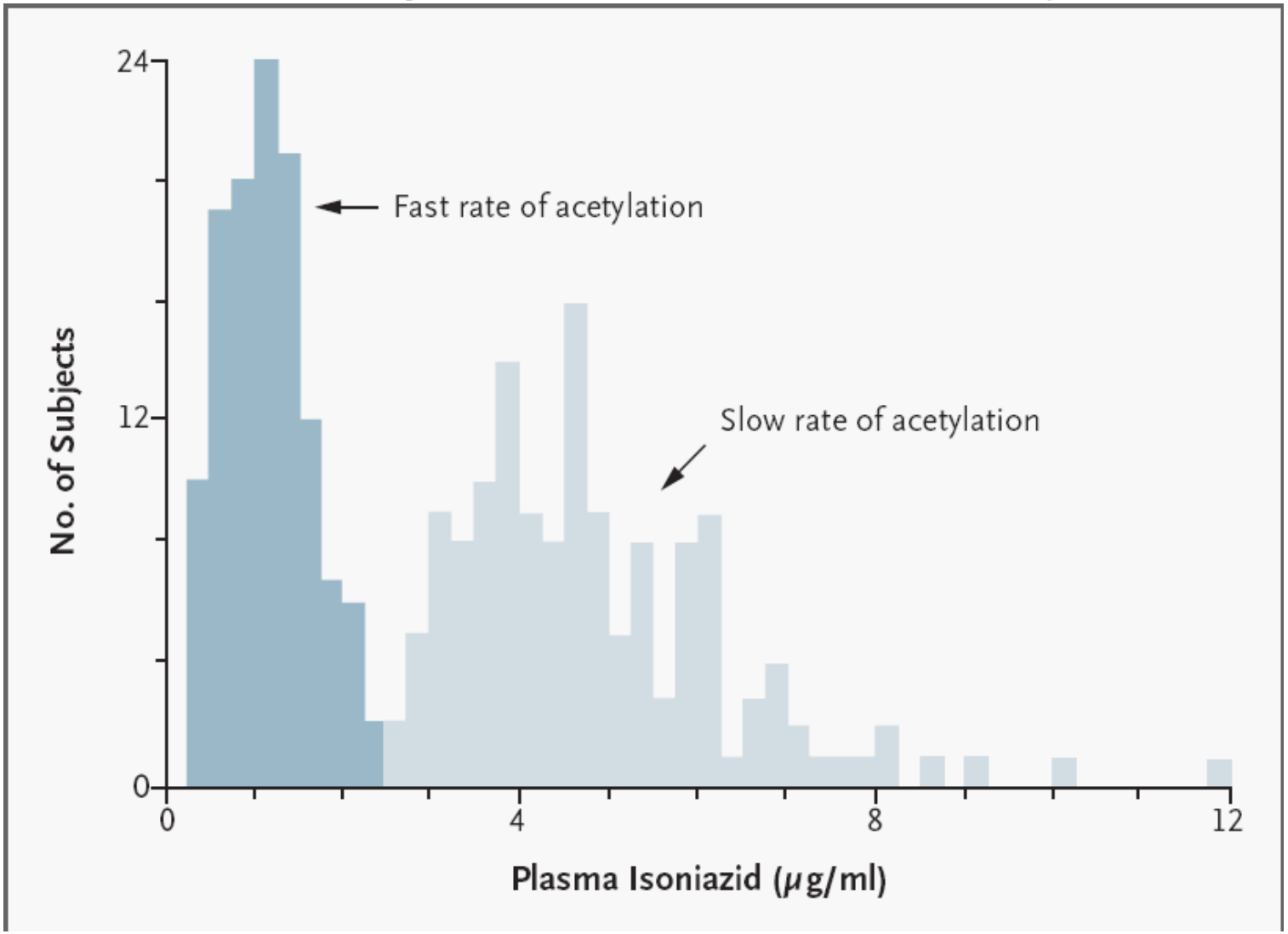
- Paracetamol: conjugated with glucuronide (55-60%) and sulphate (35%), can be used for testing of polymorphism of phase II reactions
  - UDPGT: uridinglucuronyltransferase
  - PST: sulphotransferase, both under monogenic control, genetic deficiency is important in Parkinson's disease, Gilbert syndrome, Crigler-Najjar syndrome


Acetaminophen
Glucuronidation 60%
Sulfation 30%
CYP 2E1
CYP 3A4
CYP 1A2
Non-toxic metabolites
NAPQI
Toxic Metabolite

Polymorphism of phase II metabolism: conjugation II.

- Acetylation
  - INH (isoniazid) is acetylated by N-acetyltransferase (NAT)
  - Speed of acetylation is genetically determined: bimodal distribution, slow and fast acetylators
  - Autosomal recessive inheritance
  - The rate of slow acetylators increases with age
  - Rate of slow acetylators is higher: Gilbert syndrome, rheumatoid arthritis, ischaemic heart disease
  - N-substituted arylamines are less carcinogenic after acetylations

Pharmacogenetics of Acetylation



Weinshilboum NEJM 2003

N-acetyl-transferase-2
NAT-2

Inducers

- Disulfuram
- Prednisone

Inhibitors

- Cimetidine
- Ketoconazole

Substrates

- Caffeine
- Hydralazine
- Isoniazid
- Amrinone
- Procainamide

NAT2 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, %
NAT2	50-59, White; 41, African American; 20, Chinese; 8-10, Japanese; 92, Egyptian	*NAT2*5A*	1-4 White
		*NAT2*5B*	38-45 White
		*NAT2*5C*	1-4 White
		*NAT2*6A*	24-30 White
		*NAT2*7A*	1 White
		*NAT2*7B*	1 White
		*NAT2*13*	2 White
		*NAT2*14A*	<0.6 White
		*NAT2*14B*	No prevalence data

Frequency of fast acetylators in different populations:

Population	Frequency %
Canadian Eskimos	95-100
Polynesians	93
Koreans, Japanese	90
Germans	43
Czechs & Slovakians	40
Egyptians	18
Hungarians	43

Xenobiotics subject to polymorphic acetylation in man

Hydrazines

isoniazid
hydralazine
phenylzine
acetylhydrazine
hydrazine

Arylamines

dapsone
procainamide
sulfamethazine
sulfapyridine
aminoglutethimide

Carcinogenic Arylamines

benzidine
β-naphthylamine
4-aminobiphenyl

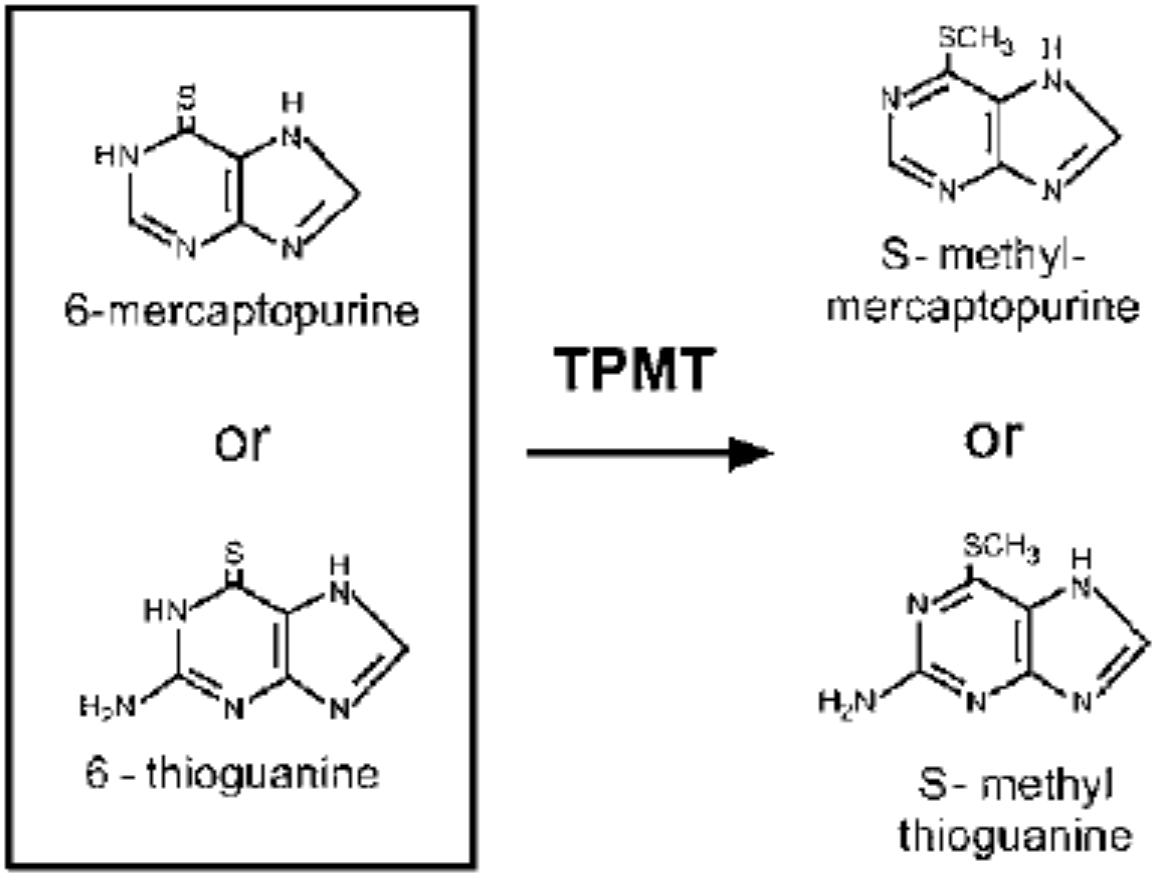
Drugs metabolized to amines

sulfasalazine nitrazepam
clonazepam caffeine

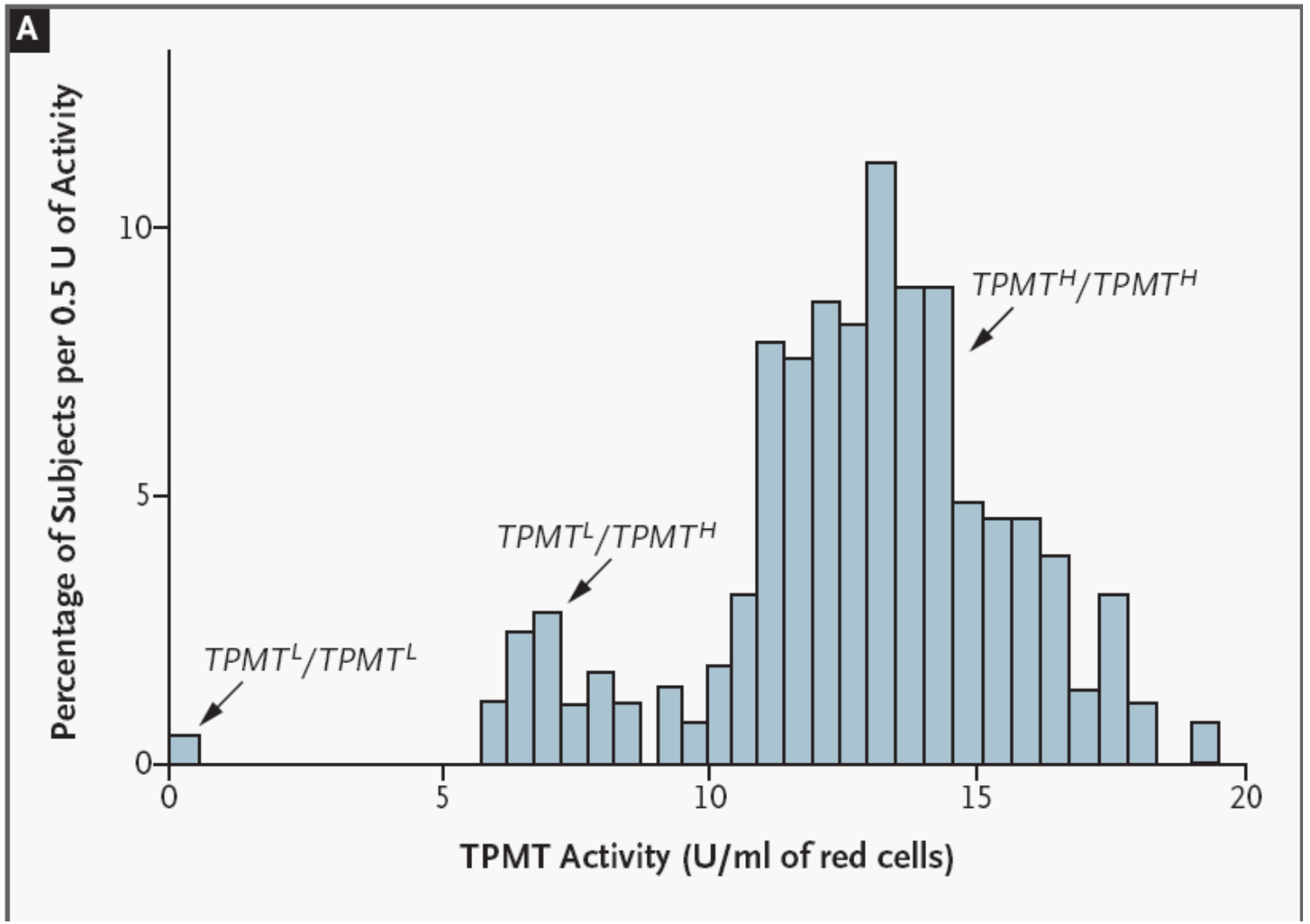
Thiopurine S-methyltransferase (TPMT)

- Drugs:
  - 6-mercaptopurine
  - azathiopurine
- Diseases:
  - Acute lymphoblastic leukemia
  - Inflammatory bowel disease
- Toxicity:
  - Fatal myelosuppression
  - Hematopoietic toxicity

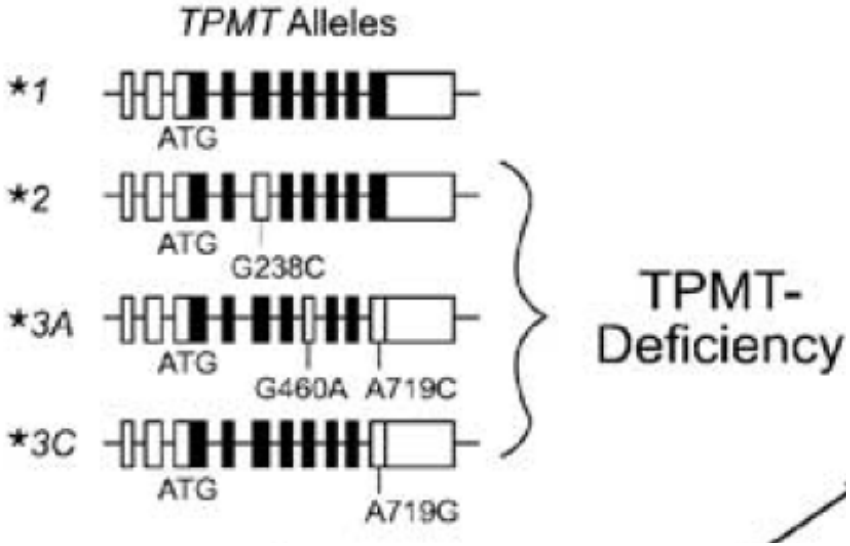
TPMT and 6-mercaptopurine

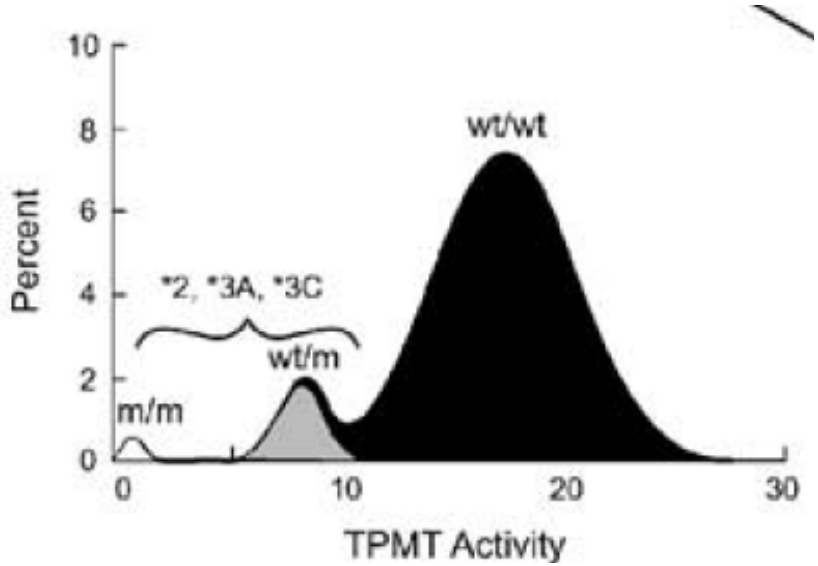


Pharmacogenetics of TPMT

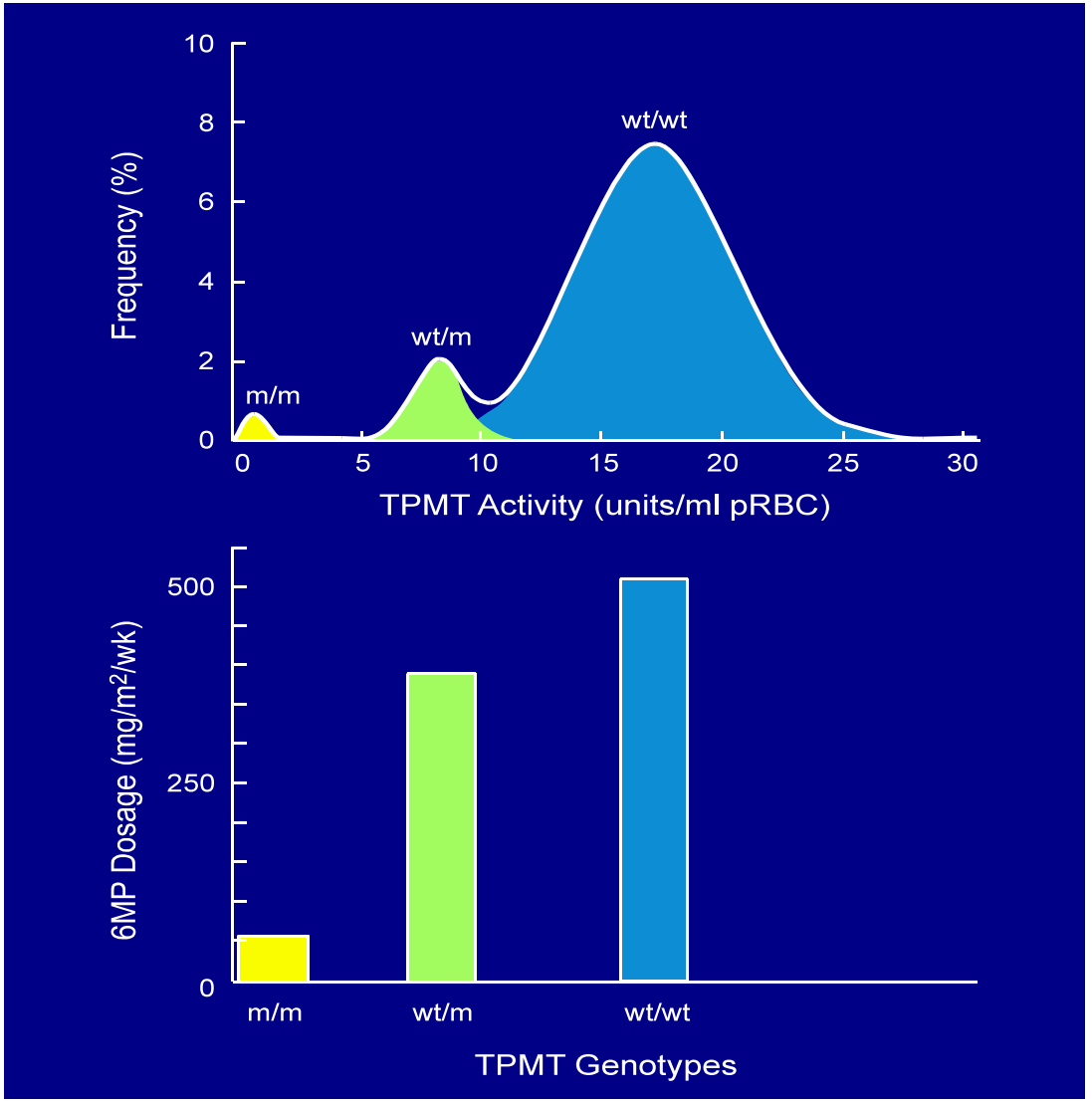


TPMT Haplotypes and Activity





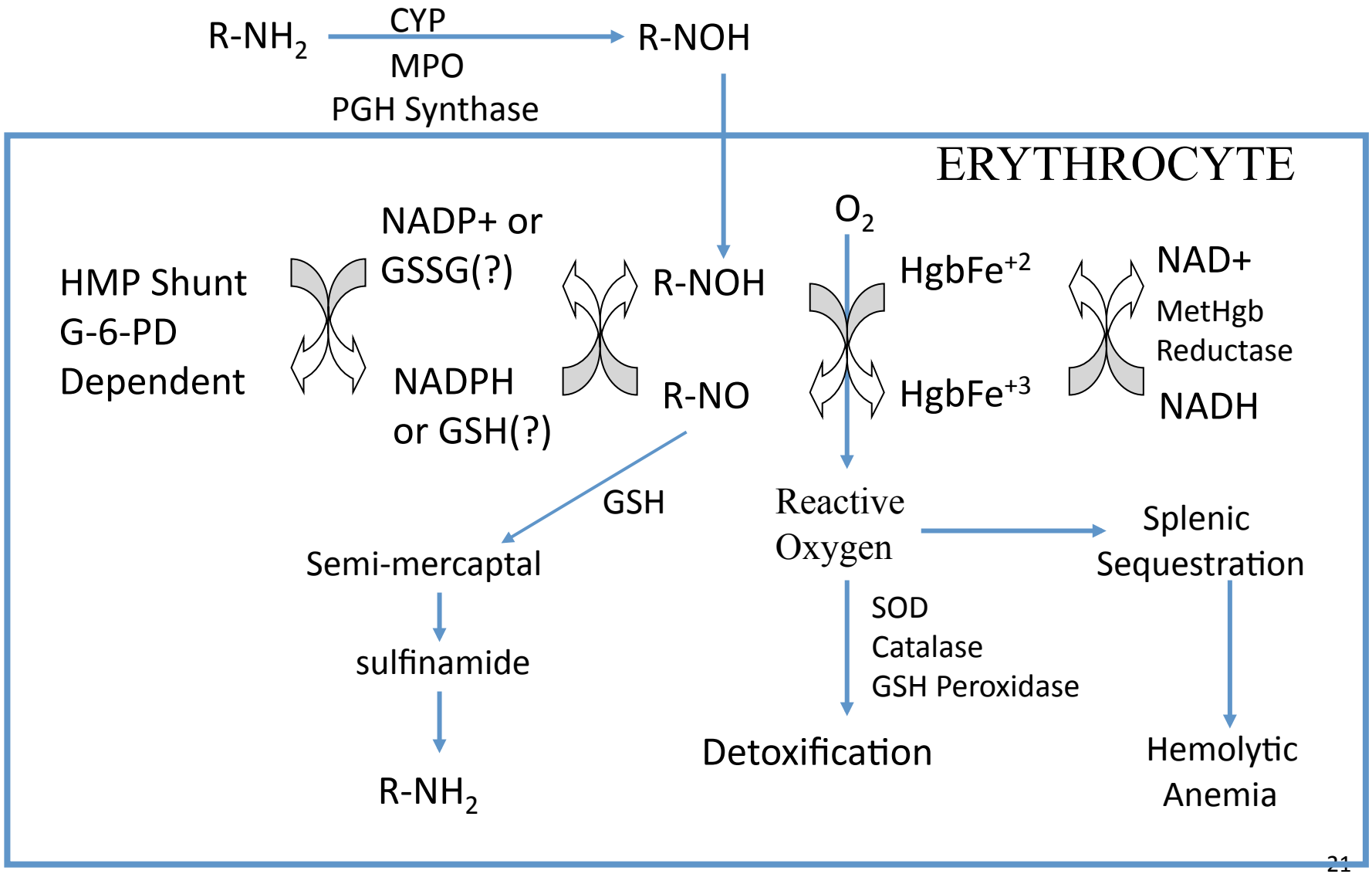
Genotype Specific TPMT Dosing



Other important pharmacogenetic polymorphisms I.

- Glucose-6-phosphate dehydrogenase
  - Most frequent pharmacogenetic enzymopathy
  - Affects 100 million people worldwide
  - 130 enzym variants, only some are abnormal
    - Antimalaria drugs (primaquine), antibiotics (sulfonamides, chloramphenicol, nitrofurantoine), other medicines (quinine, quinidine, phenylhydrazine, dapson) cause fatal haemolysis in some patients
    - Favism: haemolysis after consumption of legumes, gooseberry, blackcurrant

Glucose-6-phosphate dehydrogenase activity



Other important pharmacogenetic polymorphisms II.

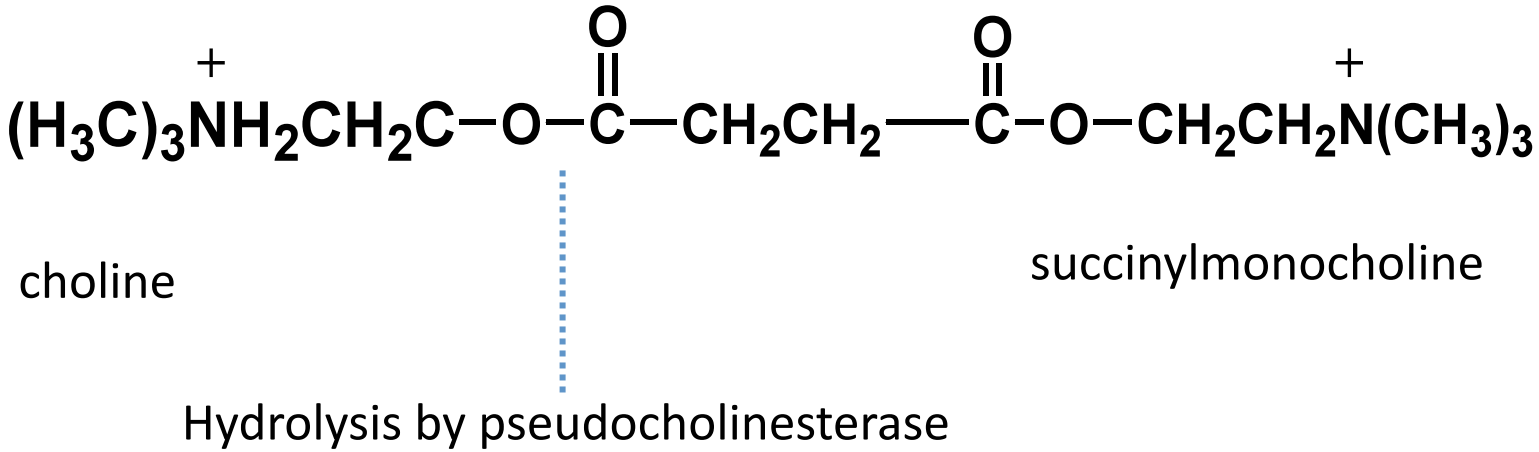
- Alcohol dehydrogenase (ADH)
  - Speed of ethanol ⇒ acetaldehyde reaction is increased
  - Acetaldehyde dehydrogenase activity is unaffected, so acetaldehyde is not metabolised at a sufficient rate
  - Acetaldehyde is accumulated causing flushing and tachycardia
  - Frequency: 5-20% in caucasians, 90% among Chinese

Other important pharmacogenetic polymorphisms III.

- Serum cholinesterase
  - Activity of serum cholinesterase is reduced 1/100 in some people (1/25000)
  - Atypical cholinesterase
  - Administration of succinylcholine causes paralysis of breathing muscles

Atypical Plasma Cholinesterase

SUCCINYLCHOLINE



- a rapid acting, rapid recovery muscle relaxant - 1951
- usual paralysis lasted 2 to 6 min in patients
- occasional pt exhibited paralysis lasting hrs
- cause identified as an “atypical” plasma cholinesterase

Implication of polymorphisms on absorption, distribution and elimination of drugs

Pharmacogenetics

Implications of polymorphisms on Pharmacokinetics

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Implications of polymorphisms on Drug Effect

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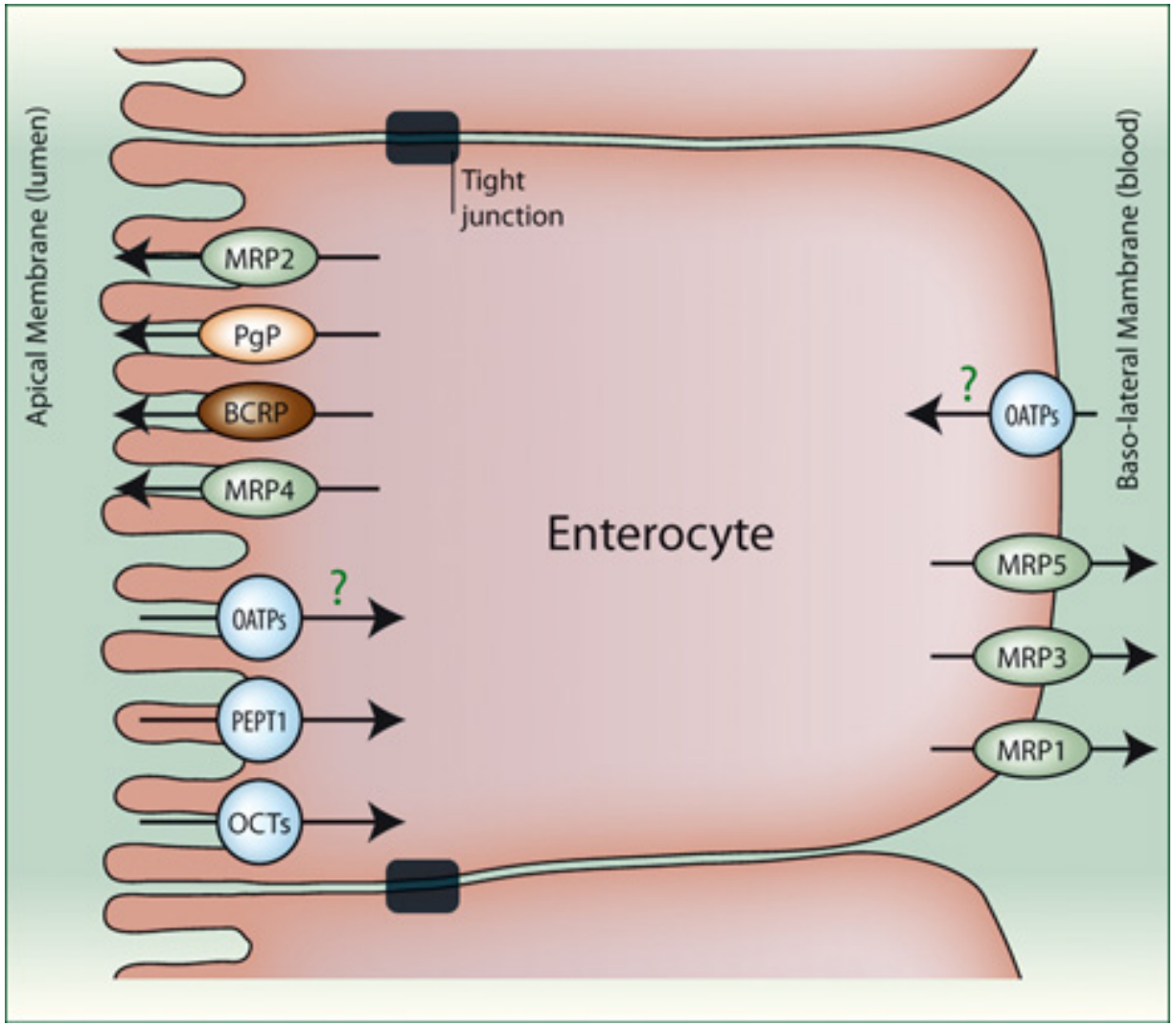
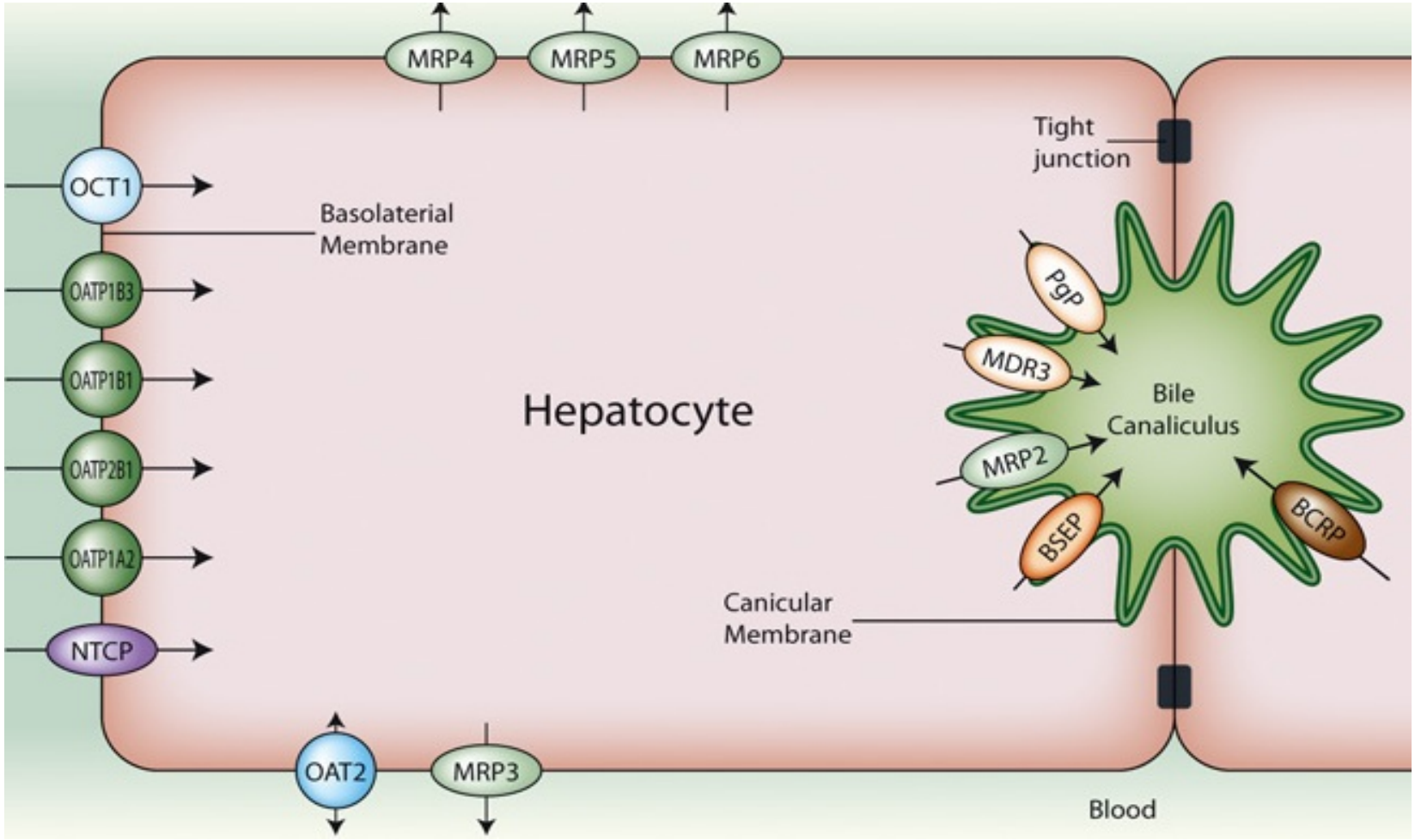
- **Target Proteins**

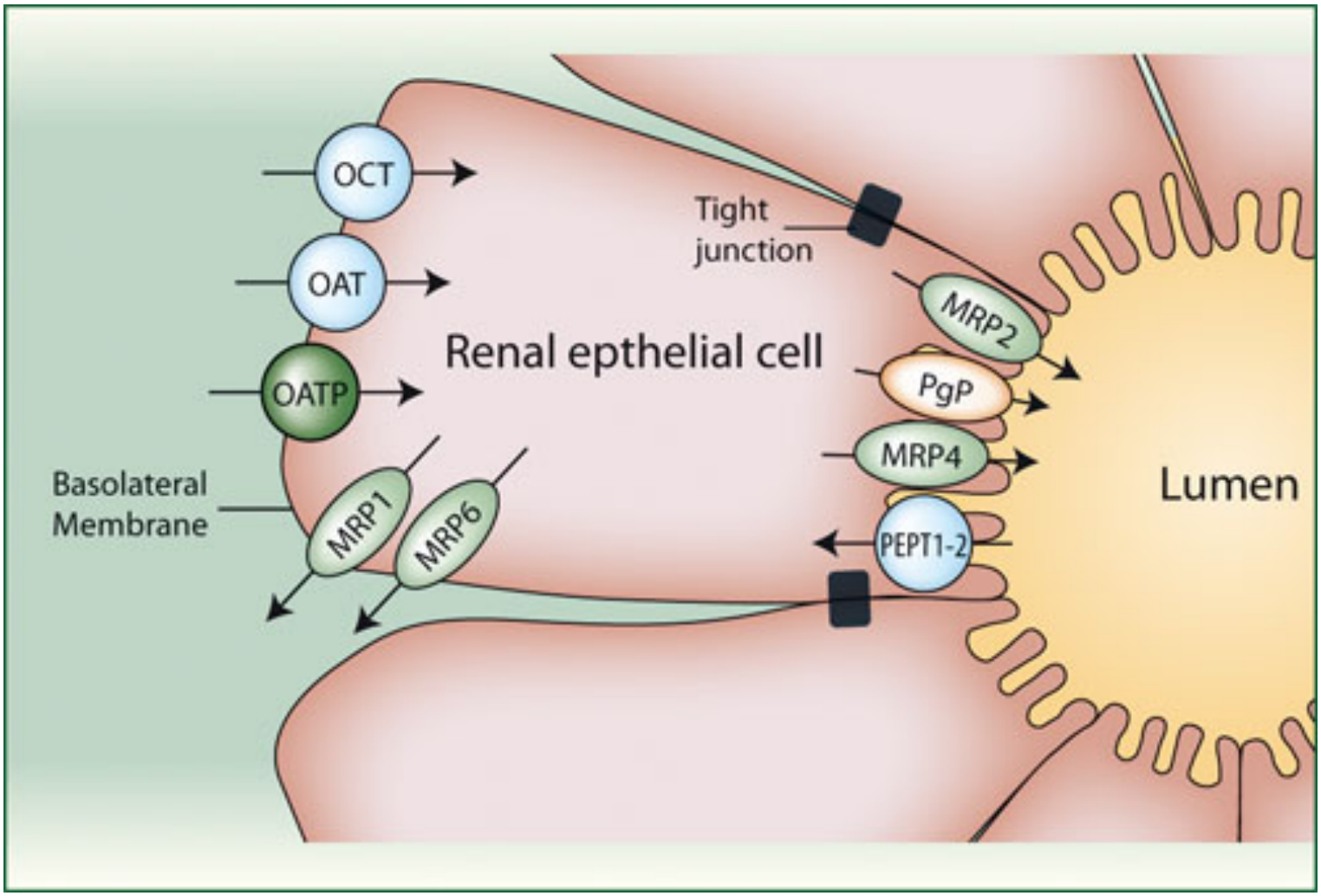
Transporters Mediate Absorption



Transporters Mediate Bile Elimination



Transporters Mediate Renal Elimination



Different types of transporters

- MRP: Multidrug-Resistance like Proteins
  - ATP-binding cassette (ABC) transporters
  - members include: MRP1, MRP2, MRP3, MRP4, MRP5, MRP6, permeability glycoprotein (PgP)

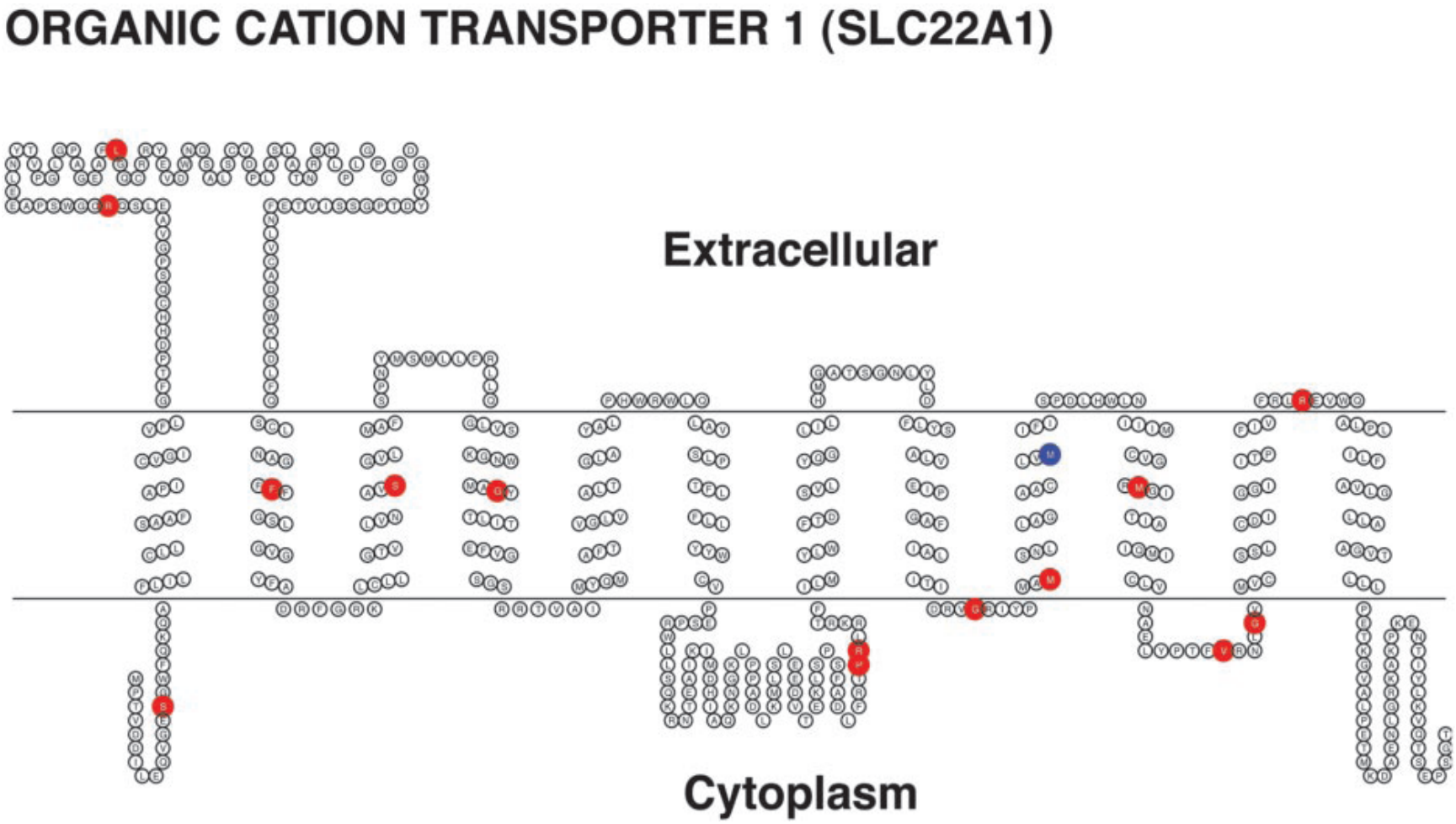
- Organic anion transporters (OAT, for negative ions)
  - Organic anion transporting polypeptides

- Organic cation transporters (OCT, for positive ions)

- Peptide transporters (PEPT)

- All members of the solute carrier family (SLC)
- Numerous polymorphisms.

Genetic Variation in OCT1



Shu et al PNAS 2003

Pharmacogenetics

Implications of polymorphisms on Pharmacokinetics

- Drug Absorption
- Drug Distribution
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Implications of polymorphisms on Drug Effect

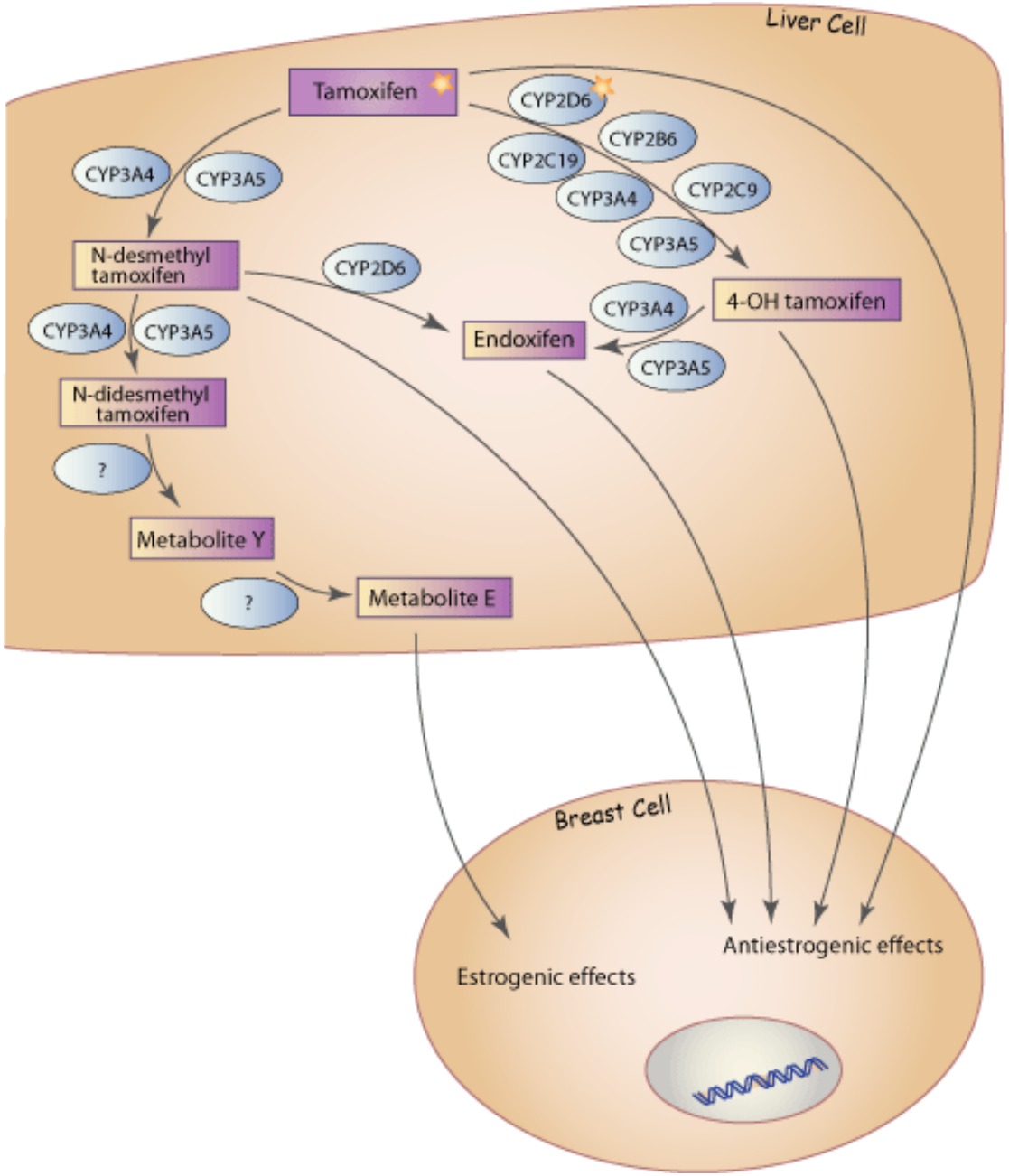
- Receptors
- Target Proteins

Tamoxifen

- SERM
- Used for adjuvant therapy in ER/PR+ breast CA
- Metabolite Endoxifen is 100x more effective than parent compound
- CYP2D6 mediates activation of Tamoxifen to Endoxifen

Tamoxifen

Importance of CYP2D6



Pharmacogenetics

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Implications of polymorphisms on Drug Effect

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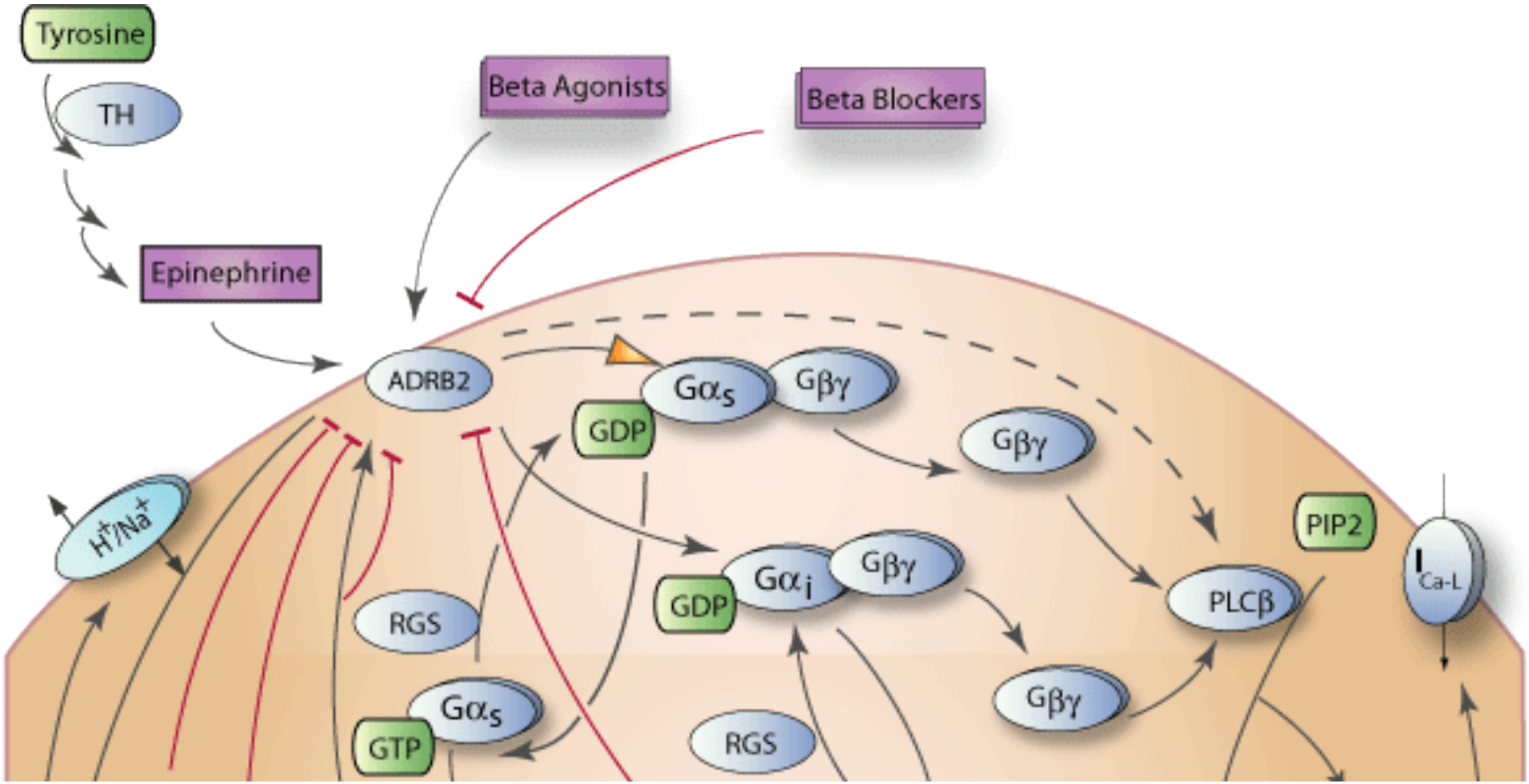
Beta Receptor and HTN/CHF

- Beta blockers – well named
- ADRB1 and ADRB2
- In ADRB1, 2 common functional polymorphisms (Ser49Gly and Gly389Arg).
- In HTN, pts treated with Metoprolol, Gly389:
  - WT/WT: 10.4% drop in SBP
  - WT/Variant: 2.8%
  - Variant/Variant: 1.1%
  - Similar differences found in HR and SBP at rest and with exercise
- In CHF, WT patients need more medications/dosages

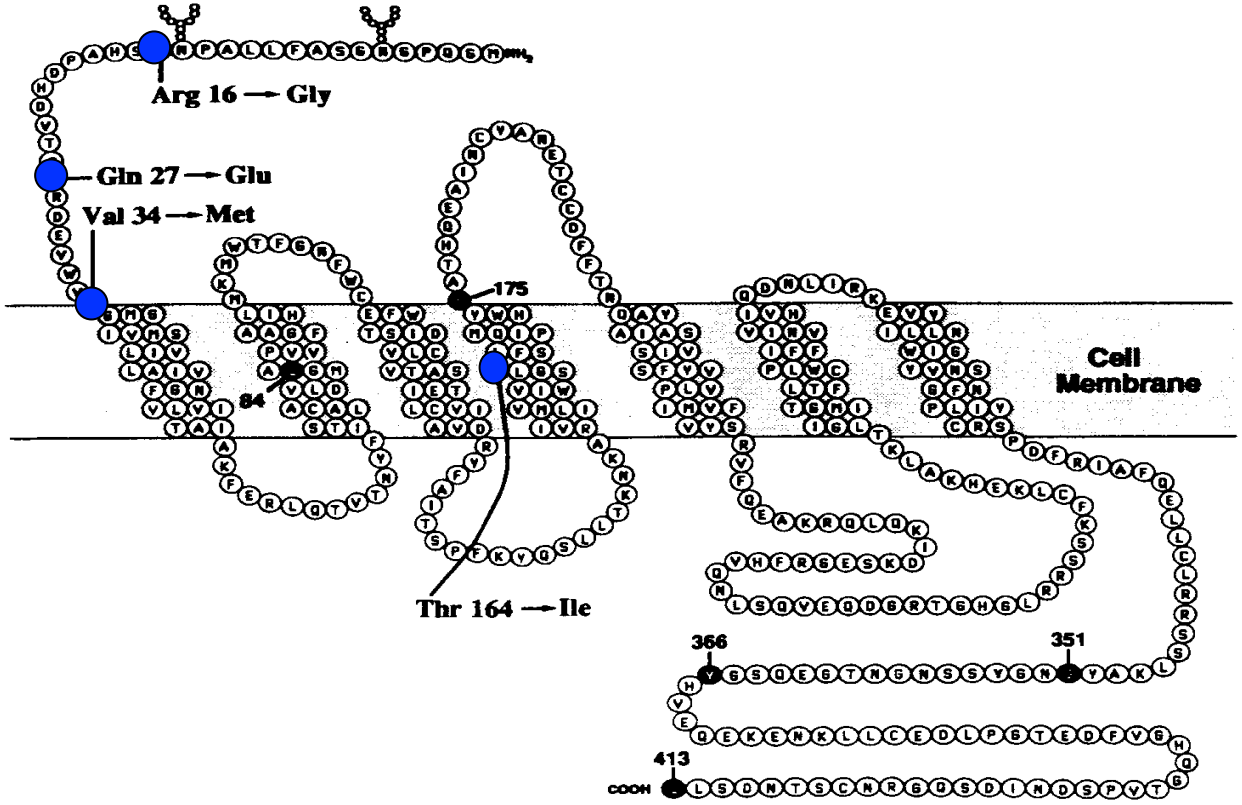
Liu J et al. Clin Pharmacol Ther 2003, 2006

Terra SJ, et al. Clin Pharmacol Ther 2006

Beta Receptor and ACS



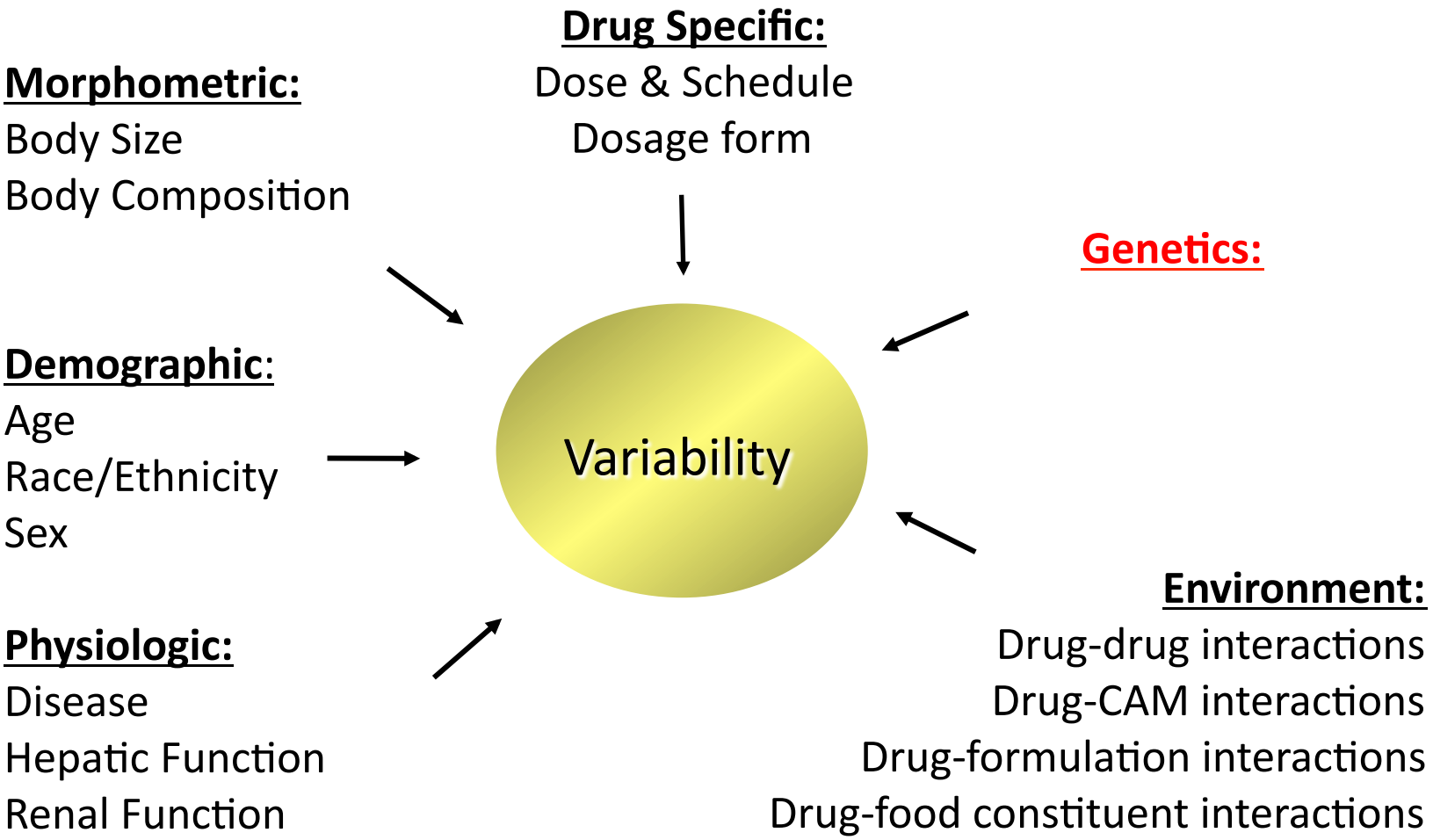
β_2-Adrenergic Receptor Polymorphisms



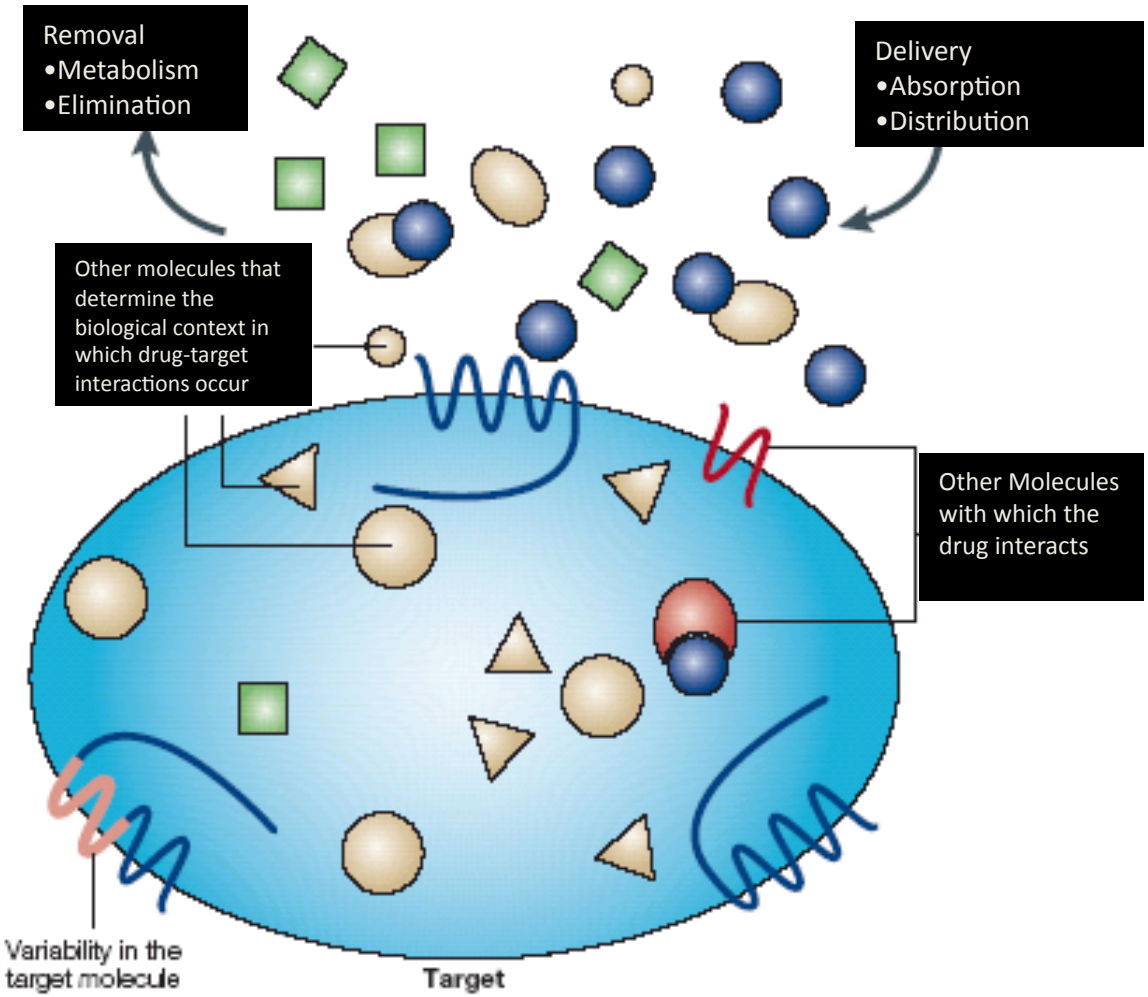
Reihsaus etal. *Am J Respir Cell Mol Biol.* 1993;8:334-339.

Other sources of variability

Sources of Pharmacokinetic and Pharmacodynamic Variability



Sources of Drug Variability at the Target



DM Roden et al: Nature Reviews, Drug Discovery, 1, 37-43, 2002

Gene-environment interactions: intraindividual variability I.

- Diet: may alter hepatic cytochrome P 450 activity
  - Smoked foods (polycyclic aromatic hydrocarbons) increase CYP1A activity (Kall & Clausen 1995)
  - Cruciferous vegetables (brussels sprouts, cabbage, broccoli): alter activity of selected CYP isoenzymes
    - Indole-containing vegetables (cabbage, cauliflower) upregulate CYP1A (Pantuck et al., 1989)
    - Isothyocyanate-containing vegetables (watercress) inhibit CYP2E1 (Kim & Wilkinson 1996)
  - Organosulfur compounds (garlic) inhibit CYP2E1 and induce CYP1A, CYP3A and phase II enzymes
  - Grapefruit juice phytochemicals influence CYP3A activity
  - Vitamins, spices

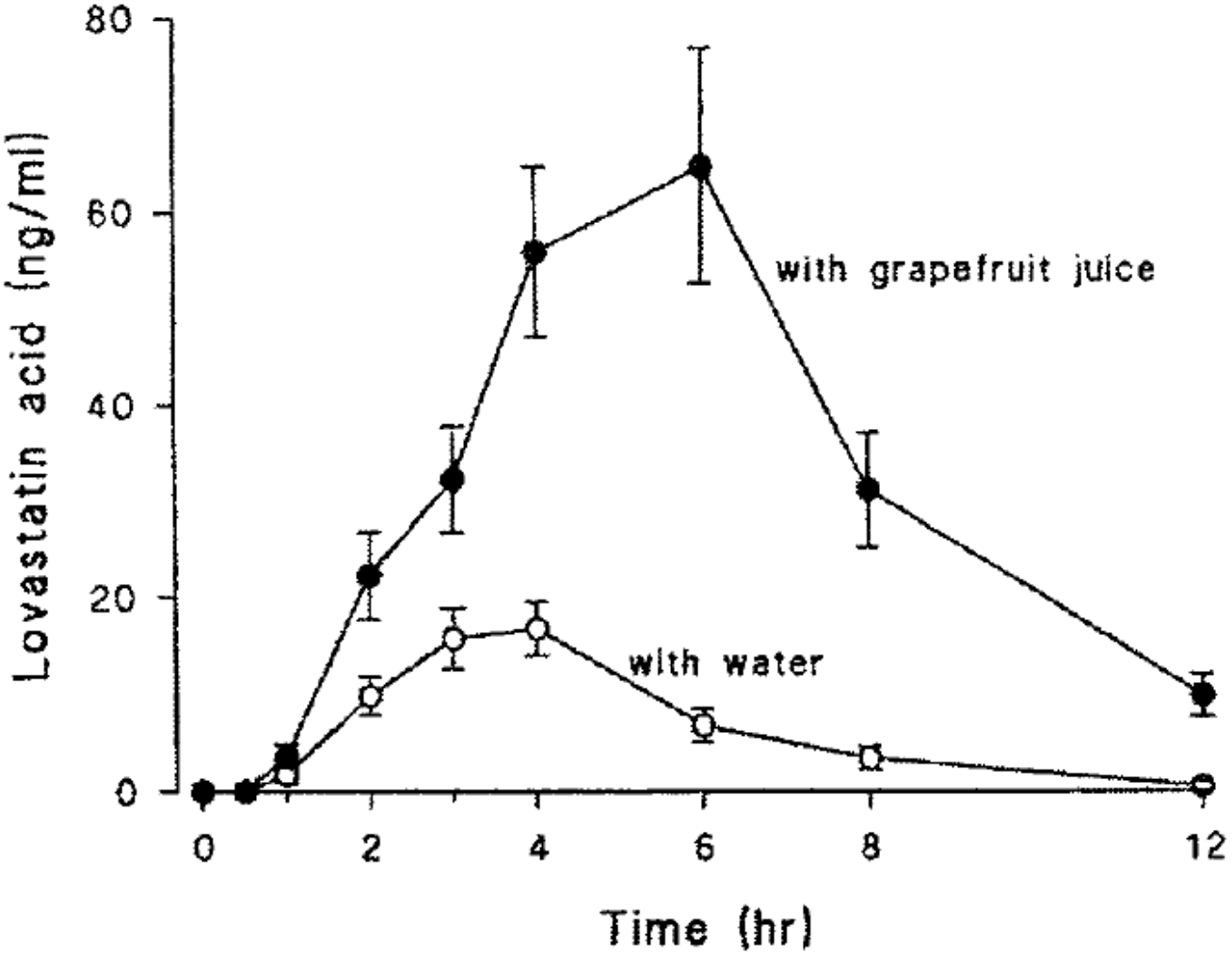
Gene-environment interactions: intraindividual variability II.

- Drug-drug interactions:
  - Enzyme inductors or inhibitors: rifamycins, anticonvulsants, macrolide antibiotics, azole antifungal drugs, nefazodone, certain SSRIs
- Nutraceutical influences: herbs and dietary compounds
  - St. John's wort (Hypericum peforatum) CYP3A inductor
- Aging: lower blood flow and liver volume decreases from the third decade, but the effect on enzymes is moderate
- Disease
  - Acute inflammation and infection affect drug metabolism
  - Liver disease modifies blood flow and reduces enzyme activity

Pharmacoenvironment

- Grapefruit juice-felodipine interaction
- bergamottin inhibition of CYP3A4 in the small intestine

Drug levels



Dahan and Altman EJCN 2004

Grapefruit juice drug interactions

Table 1 Summary of drug interaction with grapefruit juice

Drug	*Grapefruit juice influence*	*Potential risk*	*Recommandation*
Calcium channel antagonists			
Felodipine	Increased bioavailability	Hypotension, tachicardia	Avoid combination
Nisoldipine	Increased bioavailability	Hypotension, tachicardia	Avoid combination
Nicardipine	Increased bioavailability	Hypotension, tachicardia	Avoid combination
Nitrendipine	Increased bioavailability	Hypotension, tachicardia	Avoid combination
Pranidipine	Increased bioavailability	Hypotension, tachicardia	Avoid combination
Nimoldipine	Increased bioavailability	Hypotension, tachicardia	Avoid combination
Nifedipine	No influence		None
Amlodipine	No influence		None
Verapamil	Increased bioavailability	Hypotension, thchicardia	Avoid combination
Diltiazem	No influence		None
CNS modulators			
Diazepam	Increased bioavailability	Increased CNS depression	Avoid combination
Triazolam	Increased bioavailability	Increased CNS depression	Avoid combination
Midazolam	Increased bioavailability	Increased CNS depression	Avoid combination
Alprazolam	No influence		None
Carbamazepine	Increased bioavailability	Increased adverse effects	Avoid combination
Buspirone	Increased bioavailability	Increased adverse effects	Avoid combination
Sertraline	Increased bioavailability	Increased adverse effects	Avoid combination
HMG coA reductase inhibitors			
Simvastatin	Increased bioavailability	Rhabdomyolysis, acute renal failure	Avoid combination
Lovastatin	Increased bioavailability	Rhabdomyolysis, acute renal failure	Avoid combination
Atorvastatin	Increased bioavailability	Rhabdomyolysis, acute renal failure	Avoid combination
Pravastatin	No influence		None

Dahan and Altman EJCN 2004

Conclusions

- Phase 1 & 2 polymorphisms v. important in explaining drug metabolism variation
- Other polymorphisms in transport
- Certain drug metabolising enzyme activities are determined by environment
- Inherent complexity
  - multiple pathways for each drug
  - direct and indirect consequences
- Everybody is different