MUNI
MED

Genetics in Dentistry

Pharmacogenetics

Pharmacogenetics & Pharmacogenomics

- Pharmacogenetics: The role of genetics in drug responses.
  - F. Vogel. 1959

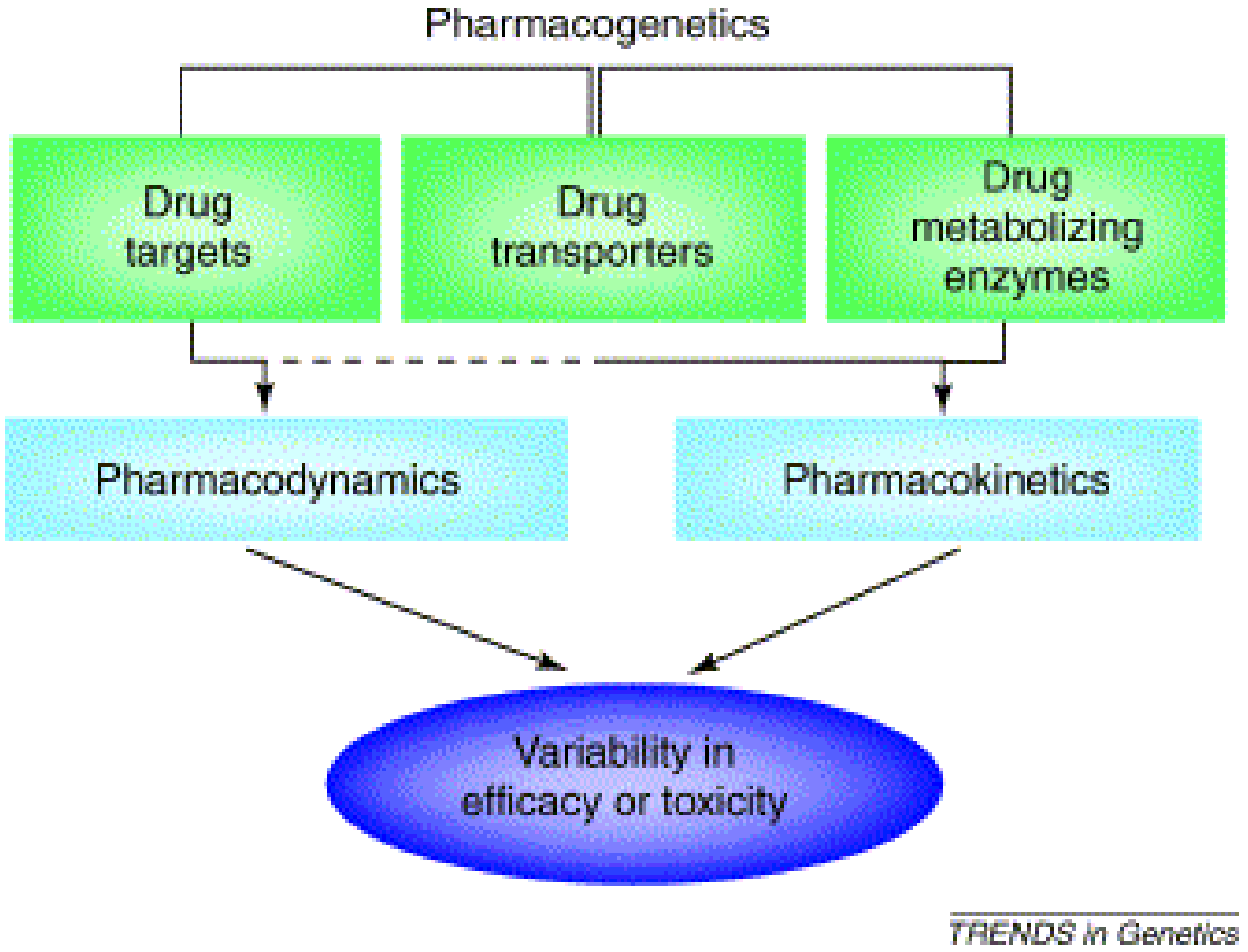
- Pharmacogenomics: The science that allows us to predict a response to drugs based on an individuals genetic makeup.
  - Felix Frueh, Associate Director of Genomics, FDA

Courtesy Felix W. Frueh

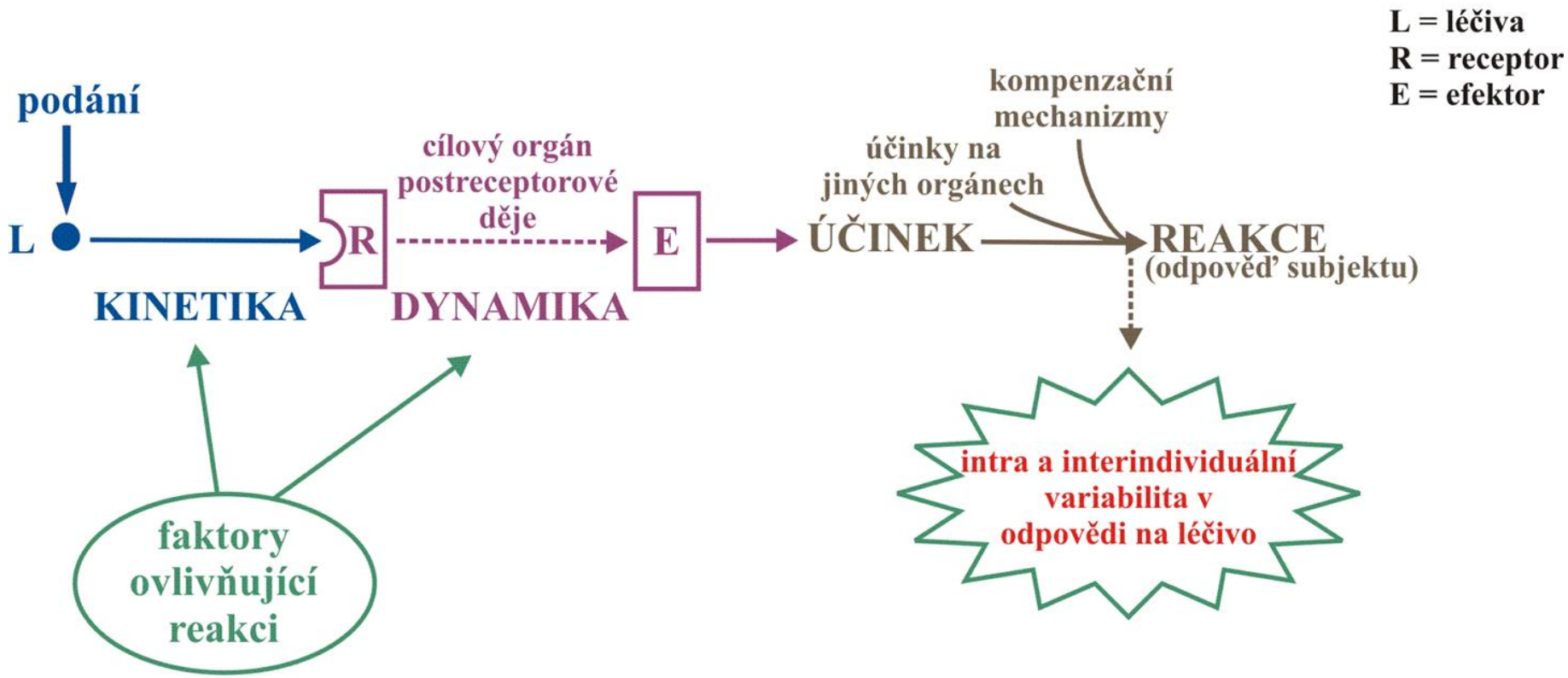
Pharmacogenetics & Pharmacogenomics

- Pharmacogenetics: study of individual gene-drug interactions, usually one or two genes that have dominant effect on a drug response (SIMPLE relationship)

- Pharmacogenomics: study of genomic influence on drug response, often using high-throughput data (sequencing, SNP chip, expression, proteomics - COMPLEX interactions)



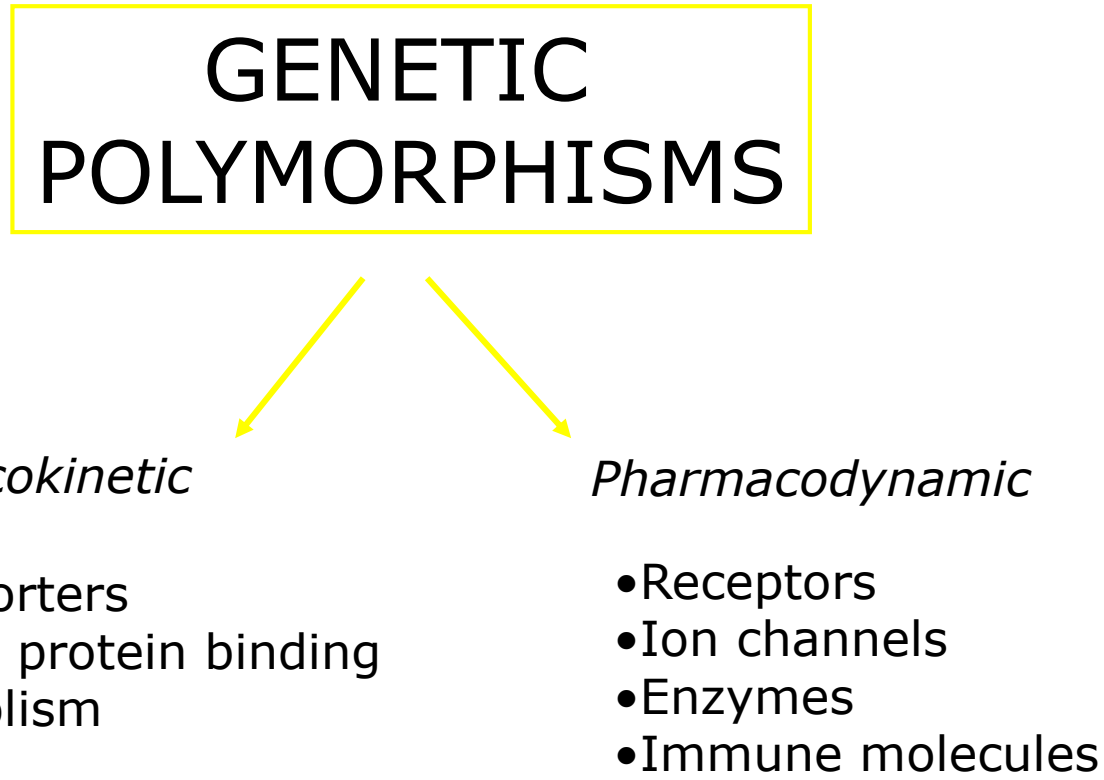
Fáze a faktory rozhodující o reakci subjektu na léčivo



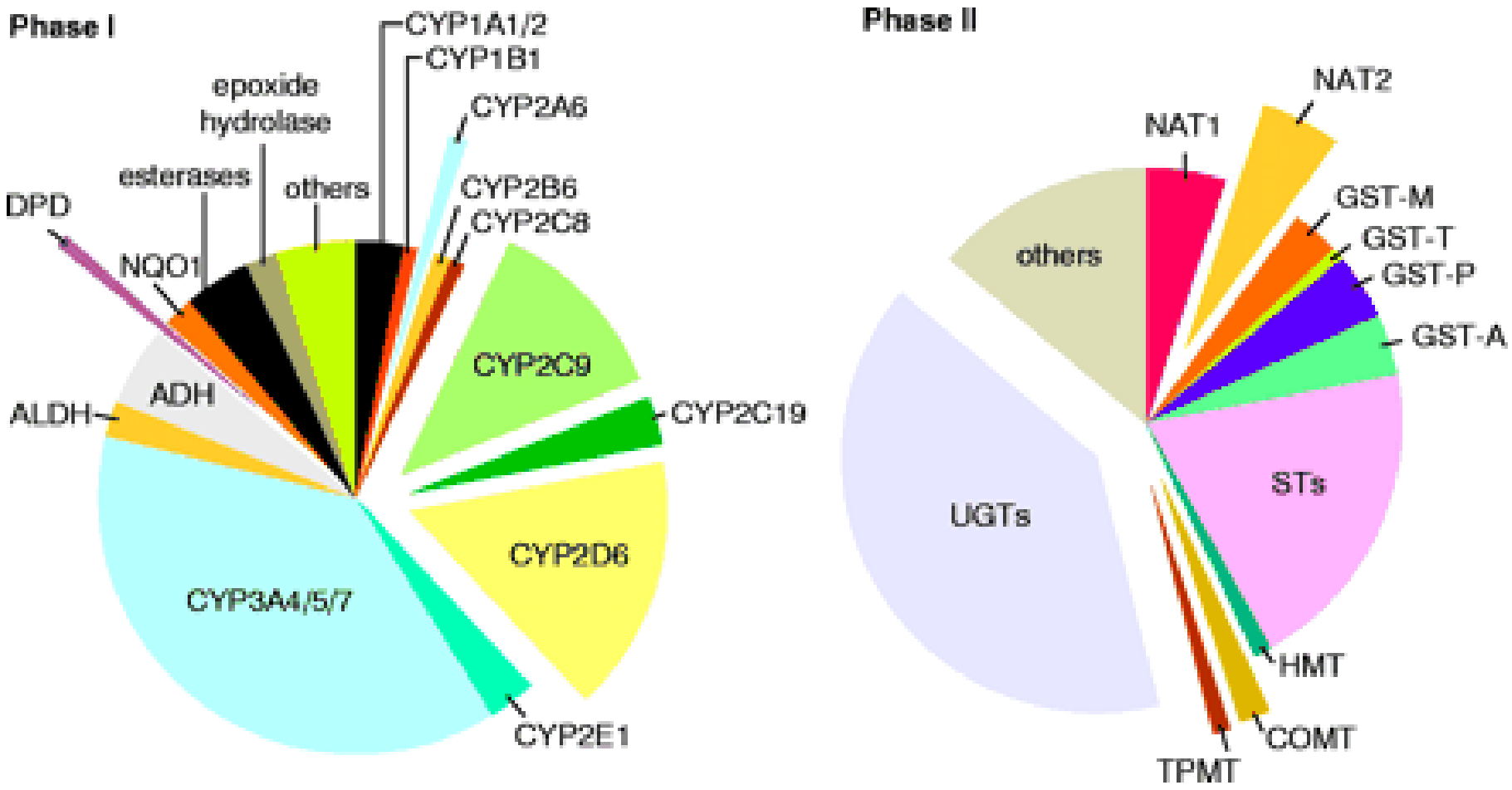
Relation to genes

- Almost every pathway of drug metabolism, its transport or activation is influenced by genetic variability.

  - Clinical variability in the response
  - The risk of side effects
  - Genotype specific dosage
  - Polymorphic targets drug



Genetic polymorphisms in drug metabolizing enzymes



From: Evans WE, Relling MV. Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science* 286:487-491, 1999.

10 questions in polygenic disorders

- ✓ How important are genetic influences in the most common forms of multigene diseases?
- ✓ What is the influence of the environment on the onset of the disease?
- ✓ Which are the most promising approaches to the determination of genetic factors leading to the onset of disease?
- ✓ Which genes have already been selected as candidate genes?
- ✓ Which paths contribute to genetic susceptibility for the disease?
- ✓ How many genes are involved in susceptibility to disease?
- ✓ Are the most common forms of polygenic diseases associated with frequent or rare genetic variability in the population? (hypothesis frequent variations / frequent genetic disease vs. heterogeneous model)
- ✓ Why alleles that are associated with the disease were not eliminated from the population?
- ✓ The importance for the disease-environment interaction genes and genes-genes? What are the implications for pharmacogenetics?

Candidate genes - Association

- with the intermediate phenotype
- with clinical manifestation of disease
- with clinical severity of disease
- with responsiveness to treatment of disease

Pharmacogenetics: A Case Study

Individuals respond differently to the anti-leukemia drug 6-mercaptopurine.

Most people metabolize the drug quickly. Doses need to be high enough to treat leukemia and prevent relapses.

Others metabolize the drug slowly and need lower doses to avoid toxic side effects of the drug.

A small portion of people metabolize the drug so poorly that its effects can be fatal.

Pharmacogenetics: A Case Study

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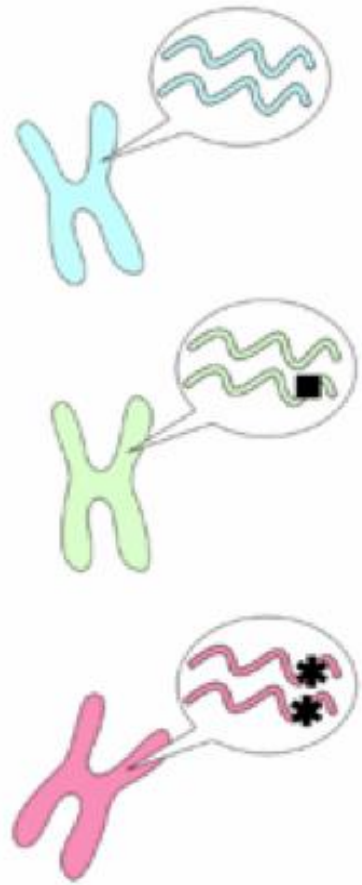
The diversity in responses is due to variations in the gene for an enzyme called TPMT, or thiopurine methyltransferase.



Pharmacogenetics: A Case Study

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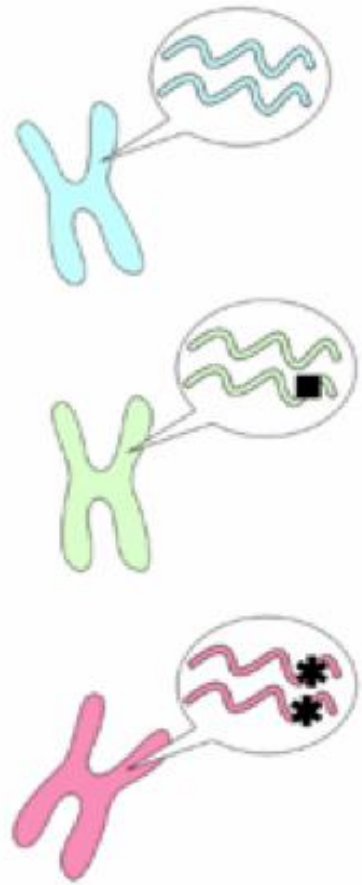
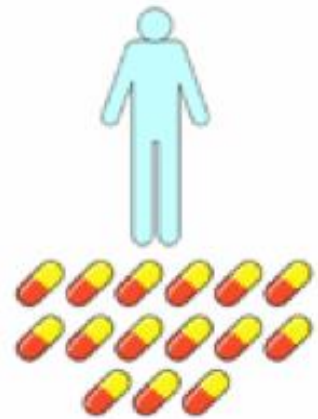


After a simple blood test, individuals can be given doses of medication that are tailored to their genetic profile.



Normal dose

Dose for an extra slow metabolizer (TPMT deficient)

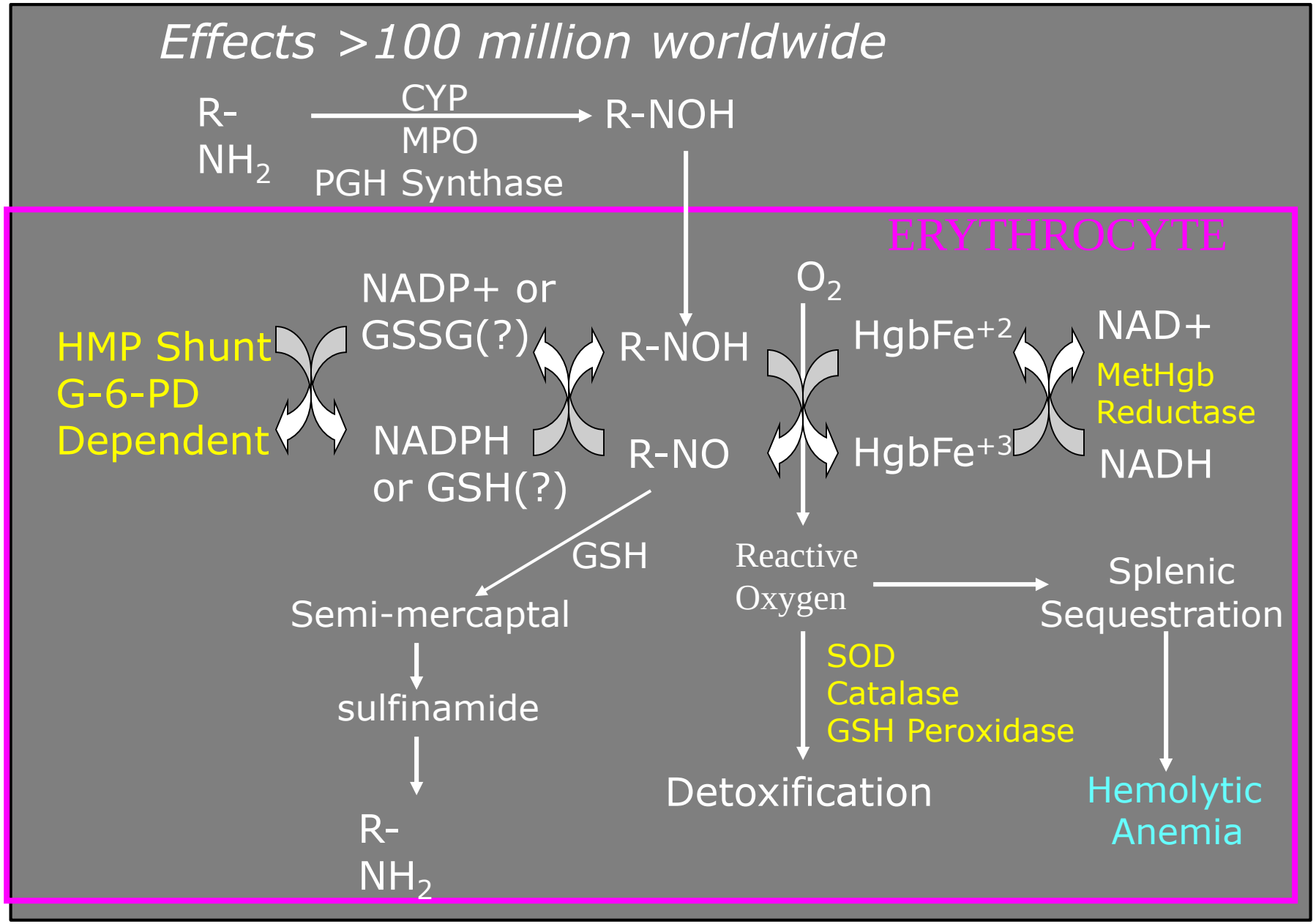
Clinically relevant genetic polymorphisms in relation to side effects of drugs

Gene[a]	Polymorphism	Minor allele frequency[b]	Drug(s)	Genetic association	Refs
Drug metabolizing enzymes					
TPMT	Multiple	0.3% of Caucasian population carry two nonfunctional alleles	Thiopurines	Hematological toxicities	[19–22]
CYP2D6	Multiple	1–2% of Asians and African descent and 6–8% of Caucasians carry two nonfunctional alleles	Numerous cardiovascular drugs, antidepressants antipsychotics Codeine	Enhanced drug effect and increased toxicity	[19,23–25]
				Decreased drug efficacy	[26,27]
CYP2C9	*2 (Arg144Cys)	0.02–0.10	Warfarin	Increased bleeding risk, decreased dose requirements	[28–30]
	*3 (Ile359Leu)	0.02–0.08			
Drug Transporter					
ABCB1	3435C→T (Ile1145Ile)	0.10–0.50	Numerous, including anticonvulsants, protease inhibitors, digoxin and others	Differences in plasma drug concentration and efficacy	[3,31–34]
Drug-targets or pharmacological response proteins					
ADRB1	Ser49Gly	0.15–0.30	β-blockers	Blood pressure lowering by β-blockers	[35,36]
	Arg389Gly	0.25–0.47			
ADRB2	Arg16Gly	0.41–0.54	β-agonists	Bronchodilation and cardiovascular responses to β-agonists	[37,38]
	Gln27Glu	0.07–0.35			
DRD3	Ser9Gly	0.30–0.70	Antipsychotics	Differential antipsychotic efficacy, antipsychotic-induced tardive dyskinesia and acute akathisia	[39–41]
ADD1	Gly460Trp	0.06–0.60	Diuretics	Differential antihypertensive response and differences in degree of reduction in risk for myocardial infarction and stroke in hypertensives	[42–44]
GNB3	C825T (creates splice variant)	0.32–0.76	Diuretics, antidepressants	Differential drug efficacy	[45,46]
APOE	ε2 Cys130 and Cys176	0.04–0.16	Tacrine, statins	Differential drug efficacy	[47–49]
	ε3 Cys130 and Arg176	0.60–0.85			
	ε4 Arg130 and Arg176	0.09–0.25			
F5	Arg506Gln (Factor V Leiden)	Absent to 0.04	Estrogen, oral contraceptives	Increased venous thromboembolism risk	[50,51]

Clinically relevant genetic polymorphisms in relation to the effectiveness of drugs

Disease	Treatment	Comment	Reference
M3-AML	all *trans*-retinoic acid	Patients with PLZF/RARA fusion are not responsive to retinoids.	[4]
Glioma	carmustine, BCNU	Only tumors with CpG methylation of the promoter of the O^6-methylguanine-DNA-methyltransferase gene respond to treatment with alkylating substances.	[22]
Asthma	5-lipoxygenase inhibitors	ALOX5 promoter genotype influences response to treatment; individuals with two non-wild type alleles show no response to 5-LOH inhibitors.	[23]
	β_2 adrenergic agents	Gly16-allele of β_2 adrenergic receptor is associated with much stronger bronchodilator desensitisation than Arg16.	[24]
Depression	imipramine	Fast metabolisers do not reach therapeutic drug levels with normal dosage.	[19]

Glucose-6-phosphate dehydrogenase activity



Drugs and Chemicals Unequivocally Demonstrated to Precipitate Hemolytic Anemia in Subjects with G6PD Deficiency

Acetanilide
Methylene Blue
Naphthalene
Sulfamethoxazole
Nitrofurantoin
Sulfacetamide
Sulfanilamide
Primaquine
Nalidixic Acid
Sulfapyridine

INCIDENCE OF G6PD DEFICIENCY IN DIFFERENT ETHNIC POPULATIONS

Ethnic Group	*Incidence(%)*
Asiatics	
Chinese	2
Filipinos	13
Indians-Parsees	16
Javanese	13
Micronesians	<1
Iranians	8
Greeks	0.7-3
Persia	15

Cytochrome Oxidase P450 Enzymes

- 57 Different active genes
- 17 Different families
- CYP1, CYP2 and CYP3 are primarily involved in drug metabolism.
- CYP2A6, CYP2B6, CYP2C9 ,CYP2C19, CYP2D6, CYP2E1 and CYP3A4 are responsible for metabolizing most clinically important drugs

Polymorphic Cytochrome P-450s

CYP2B6

Selected Substrates	Location	Poor Metabolizer Incidence
bupropion cyclophosphamide efavirenz methadone ifosfamide	Chromosome 19	3-4% of Caucasians

CYP2C9

Selected Substrates	Location	Poor Metabolizer Incidence
NSAIDs celecoxib diclofenac ibuprofen naproxen piroxicam Oral Hypoglycemic Agents tolbutamide glipizide ARBs irbesartan losartan fluvastatin warfarin phenytoin	Chromosome 10	1-3% Caucasians

CYP2C19

Selected Substrates	Location	Poor Metabolizer Incidence
Proton pump (-) amitriptyline cyclophosphamide diazepam indomethacin phenytoin phenobarbital progesterone voriconazole	Chromosome 10	2-4% African-Americans 3-5% Caucasians 15-20% Asians

CYP2D6

Selected Substrates	Location	Poor Metabolizer Incidence
antidepressants beta-blockers antipsychotics chlorpheniramine codeine dextromethorphan ondansetron lidocaine promethazine tamoxifen tramadol	Chromosome 22	5-10% Caucasians

Effect of Metabolic Rate on Drug Dosage

Drug	Poor Metabolizer Phenotype
Prodrug, needs metabolism to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Poor efficacy Possible accumulation of prodrug
Active drug, inactivated by metabolism (example is omeprazole)	Good efficacy Accumulation of active drug can produce adverse reactions May need lower dose

Drug	Ultra-rapid Metabolizer Phenotype
Prodrug, needs metabolism to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Good efficacy, rapid effect
Active drug, inactivated by metabolism (example is omeprazole)	Poor efficacy Need greater dose or slow release formulation

Genotype	# of Subjects	Metabolic Ratio
CYP2D6wt/$(CYP2D6L)_2$	9	0.33
CYP2D6wt/CYP2D6wt	12	1.50
CYP2D6wt/CYP2D6(A or B)	9	2.14
CYP2D6B/CYP2D6B	6	48.84

Data from: Agundez JG et al. *Clin Pharmacol Ther* 57:265, 1995.

Codeine and Cytochrome P450 CYP2D6

- Codeine is a commonly used opioid
  - Codeine is a prodrug
  - It must be metabolized into morphine for activity
- Cytochrome P450 allele CYP2D6 is the metabolizing enzyme in the liver
- 7% of Caucasians are missing one copy of the Cytochrome P450 CYP2D6 gene
  - codeine does not work effectively in these individuals

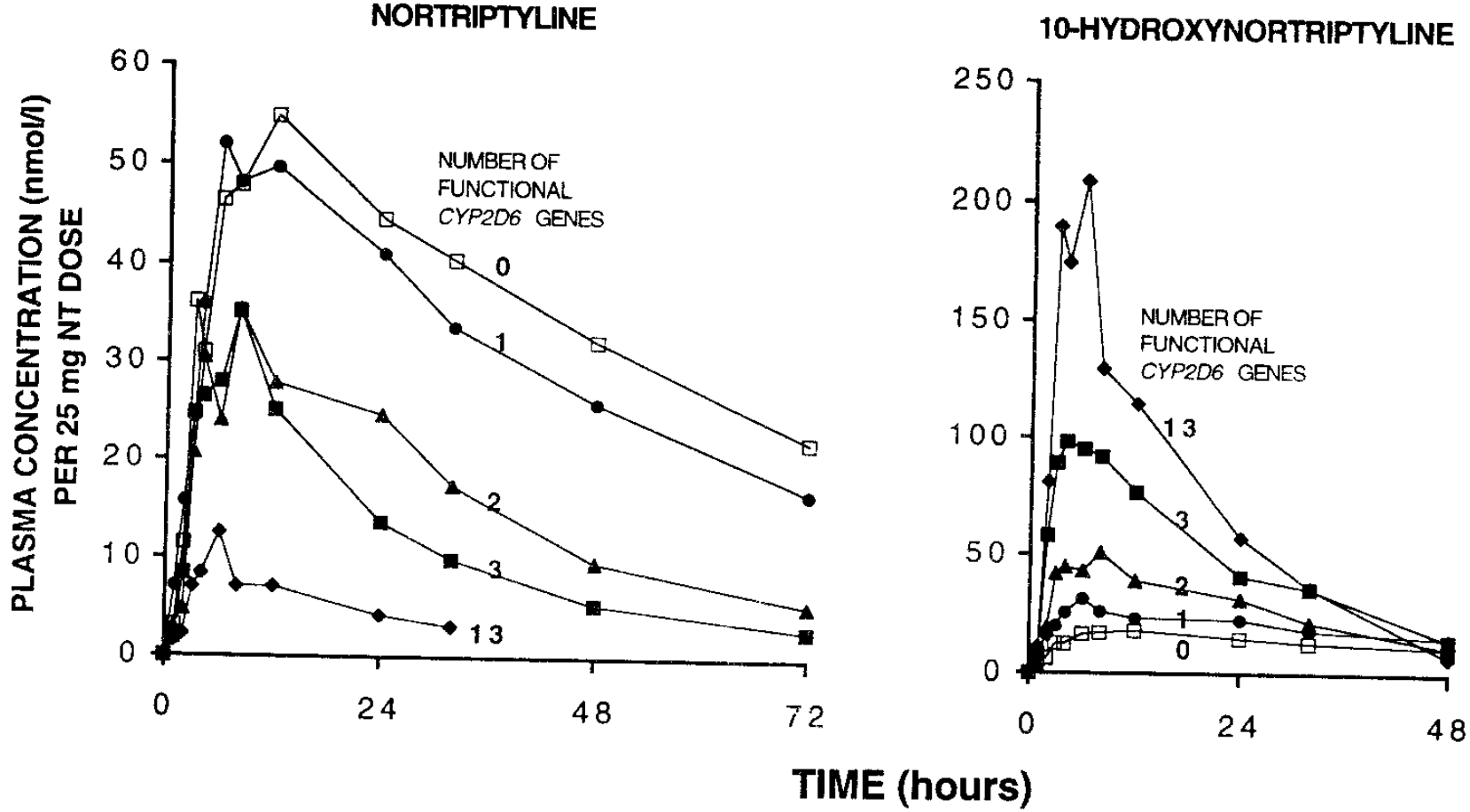
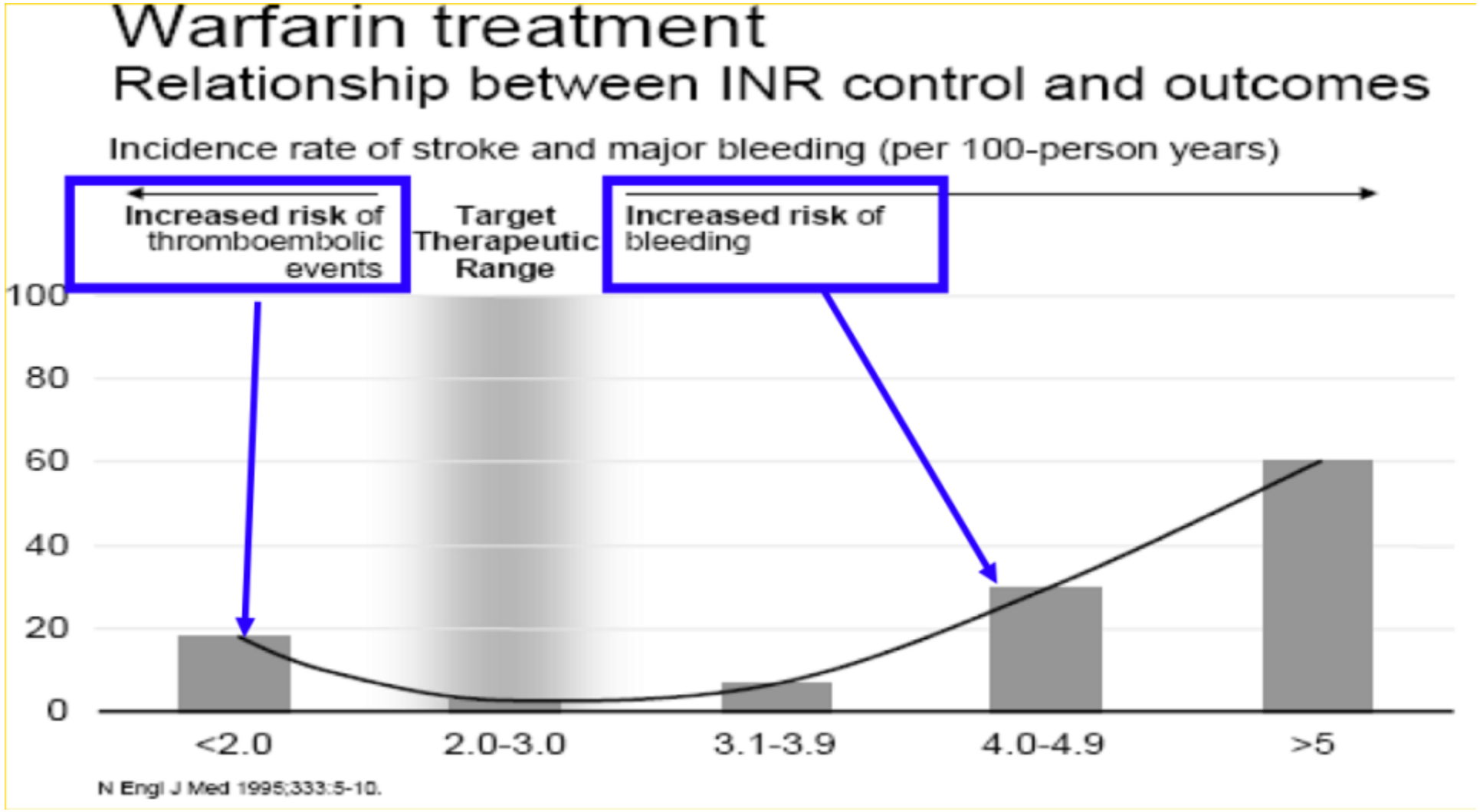


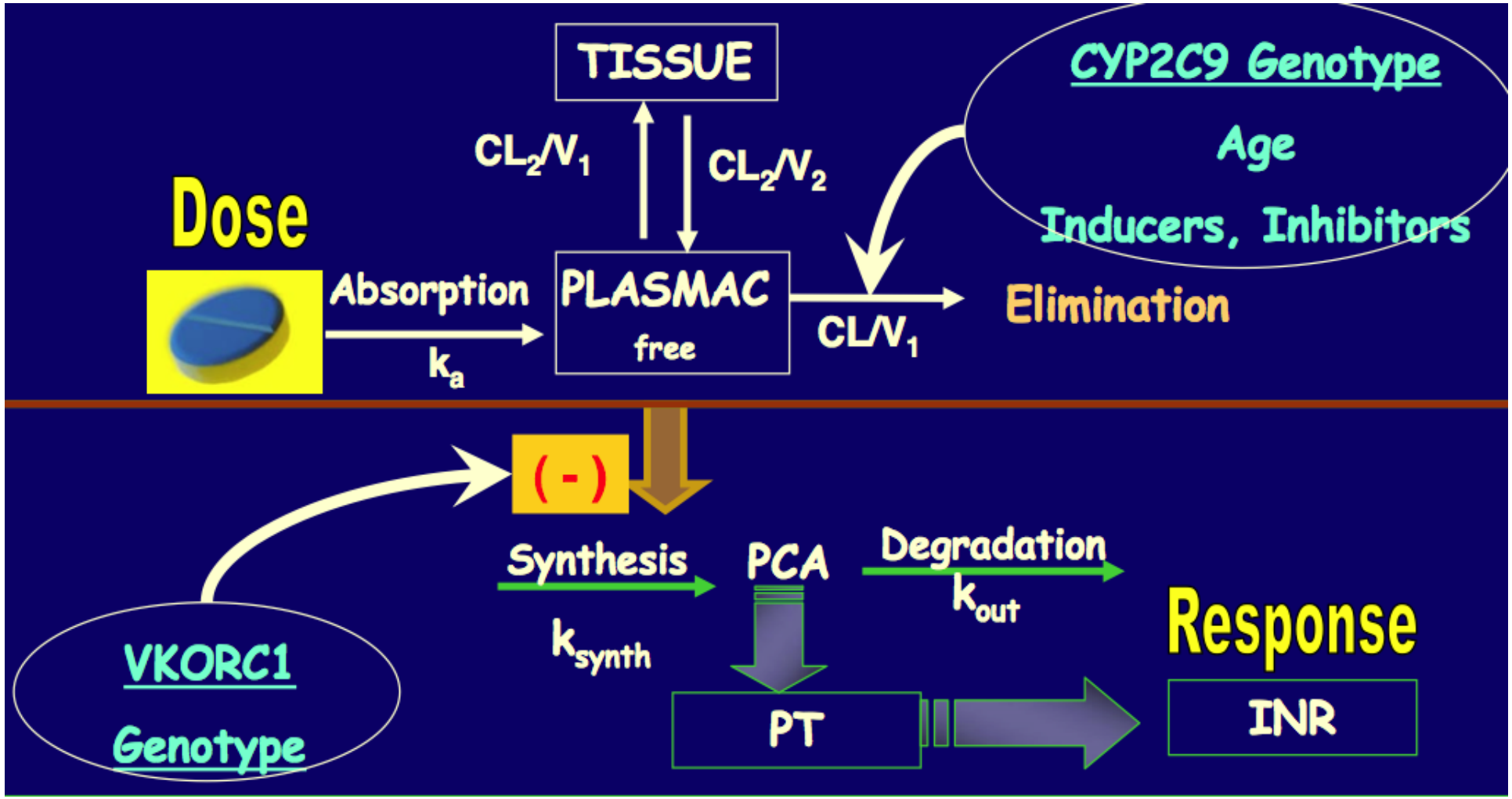
Fig. 1. Mean plasma concentrations of nortriptyline and 10-hydroxynortriptyline in different genotype groups after a single oral dose of nortriptyline. For subjects with 3 and 13 functional genes, plasma concentrations are adjusted to the 25 mg dose by means of division of the values by 2. The numerals close to the curves represent the number of functional *CYP2D6* genes in each genotype group.

Why Maintaining Warfarin Therapeutic Range is Critical

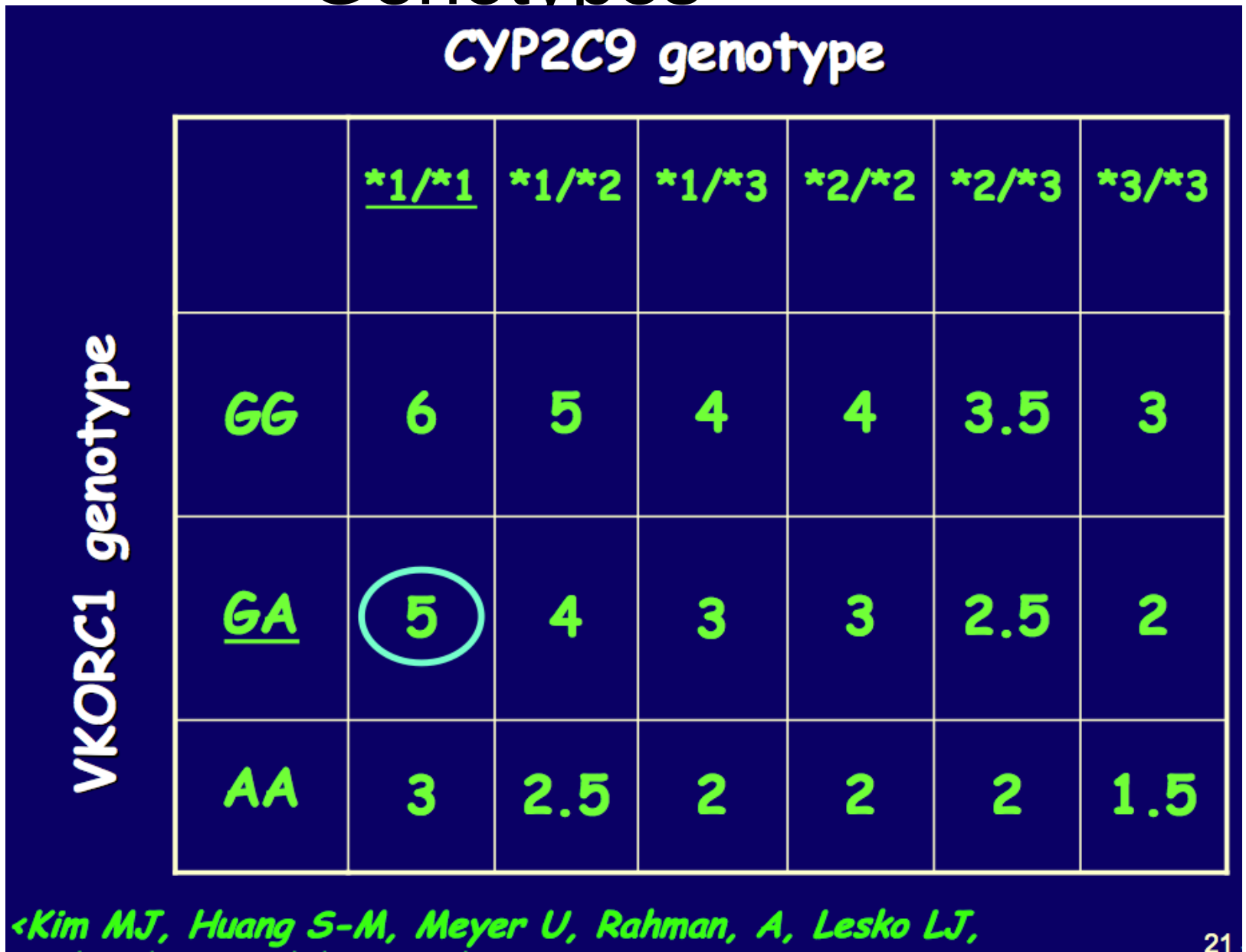


European Atrial Fibrillation Trial Study Group, N Engl J Med 1995;333:5-10.

Warfarin Levels Depend on Two Enzymes – CYP2C9 & VKORC1



Estimated Warfarin Dose (mg/day) Based on Genotypes



CYP2C9 ACTIVITA

Warfarin Dose*	Genotype
5.63 (2.56)	*1/*1
4.88 (2.57)	*1/*2
3.32 (0.94)	*1/*3
4.07 (1.48)	*2/*2
2.34 (0.35)	*2/*3
1.60 (0.81)	*3/*3

From: Higashi MK, et al. Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy. *JAMA* 287:1690-1698, 2002.

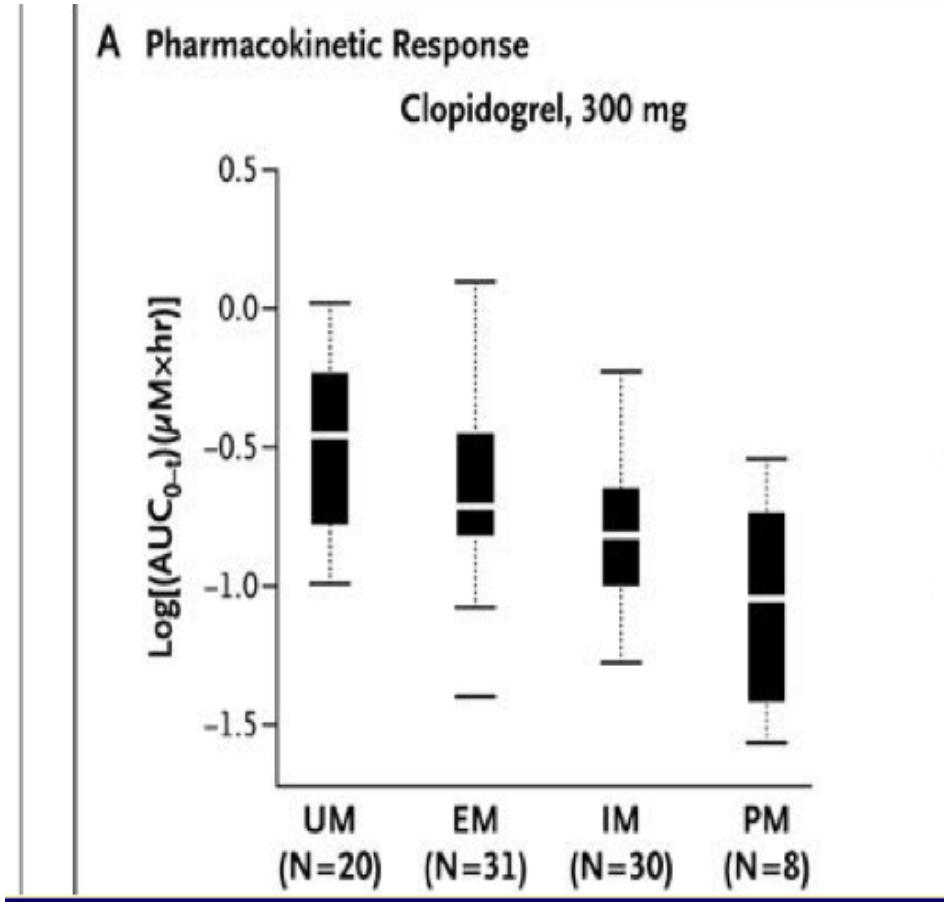
Frequency of VKORC1 Alleles in Various Populations

-1639 G>A	AA	AG	GG
Caucasians (N=297)	19%	56%	25%
Spanish (N=105)	32%	40%	28%
Chinese (N=104)	80%	18%	2%
African Americans (N=159)	0%	21%	79%

Asians may need a lower dose

Sconce et al. Blood 2005, Yuan et al. Human Mol Genetics 2005, Schelleman et al. Clin Pharmacol Ther 2007, Montes et al Br J Haemat 2006

Another Anticoagulant Clopidogrel (Plavix) and CYP2C19 Alleles



PM: with two reduced function alleles
IM: one reduced function allele
EM: no variant alleles;
UM: one or two *17

Interaction with drogs metabolized and/or reactingi with CYP2C9

17, 20, 21

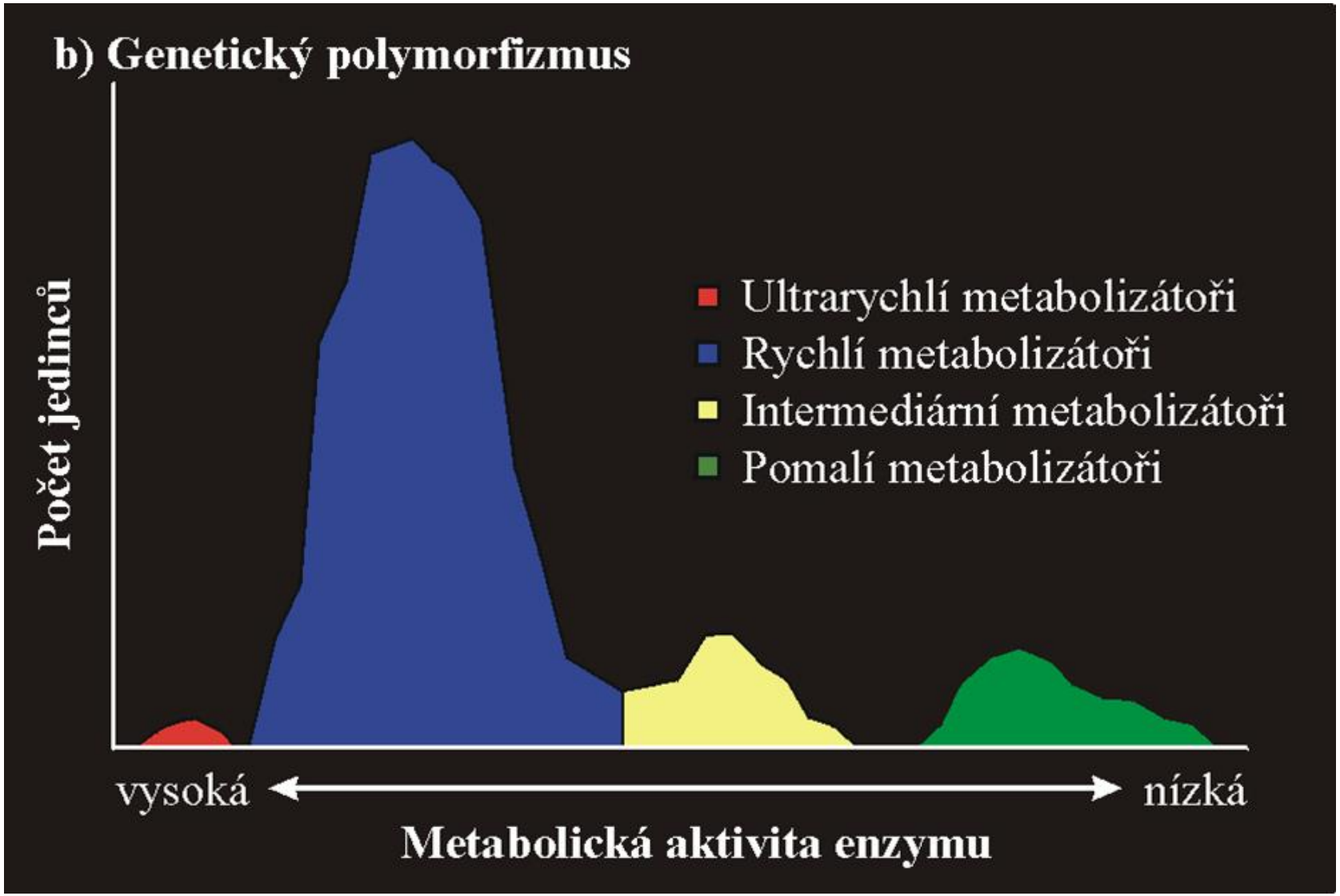
Competition	Enzyme inductor	Enzyme inhibitor
ASA a většina NSAID	rifampicin	fluvoxamin (ostatní SSRI slabí)
fenobarbital, fenytoin	fenobarbital, fenytoin	omeprazol
S-warfarin	karbamazepin	inhibitory HMG-CoA reduktázy
losartan		tolbutamid
tolbutamid		cimetidin (slabý)
sulfonamidy, dapson		azolová antimykotika (slabá)
diazepam, tenazepam		ritonavir
fluoxetin, moclobemid		desethylamiodaron
zidovudin		

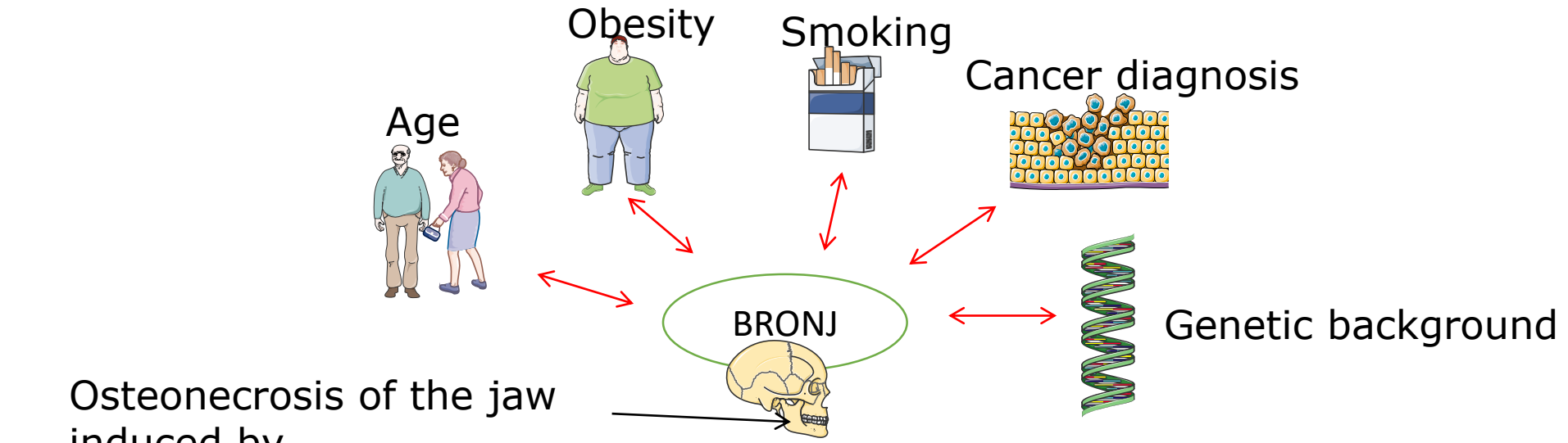
20. Topinková E et al: Postgrad Med 2002; 5:477-82

21. Naganuma M et al: J Cardiovasc Pharmacol Ther 2001; 6:636-7

Metabolic rate

- According to the activity of the enzyme may be a population divided into four main groups - poor metabolisers (PM), intermediate metabolizers (IM), efficient metabolizers (EM), and ultra-fast metabolizers (UM).
- Most individuals among the white population - extensive metabolizers (EM) - the drugs are metabolized by the expected rate.
- 5-10% of individuals are genetically determined poor metabolisers (PM) - the slow degradation of substances metabolised and are at a higher incidence of adverse events.
- Intermediate metabolizers (IM) are represented in 10-15% and in long term treatment in response – comparable to PM.
- Ultra-fast metabolizers (UM) – metabolization is intensive; clinically unresponsive to the usual doses of drugs - 5-10%.


b) Genetický polymorfizmus
Počet jedinců
Ultrarychlí metabolizátoři
Rychlí metabolizátoři
Intermediární metabolizátoři
Pomalí metabolizátoři
vysoká
nízká
Metabolická aktivita enzymu



Osteonecrosis of the jaw induced by bisphosphonates (BP)

DNA

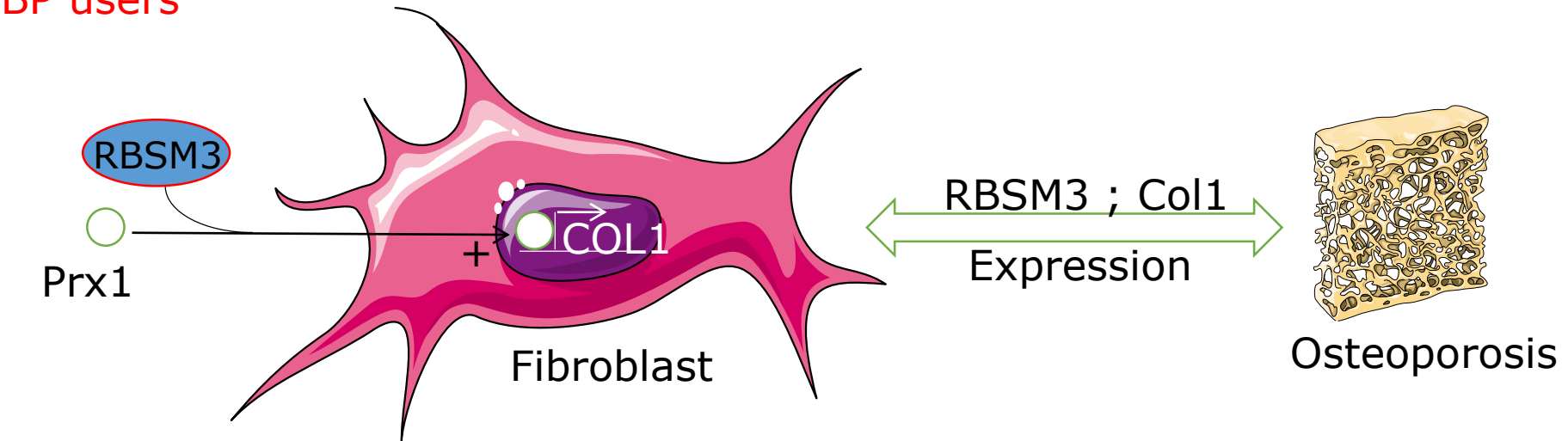
Genome-wide Sreening

SNPs in *RBSM3*

RBSM3

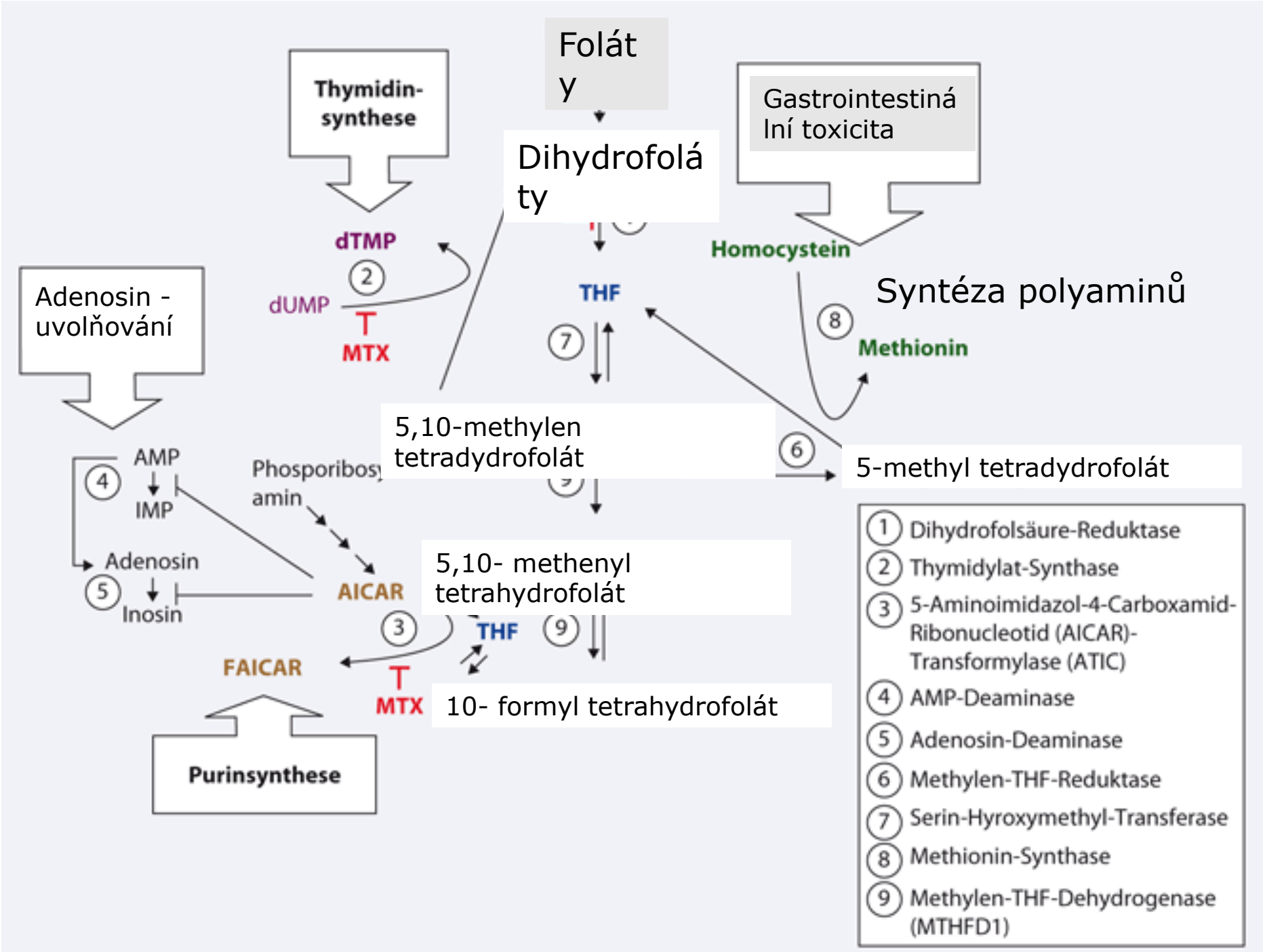
DNA

BRONJ
BP users



Methotrexate in RA

- Effectiveness of treatment of rheumatoid arthritis (RA) by methotrexate (MTX) 46% - 65% (ACR20)
- During treatment with MTX side effects may occur. At least one in 72.9% of patients, severe in 30% of patients.
  - gastrointestinal toxicity (nausea, vomiting, diarrhea, 20% - 65%
  - Hepatotoxicity 10% - 43%
  - oral ulceration 37%
  - alopecia to 4%
  - pulmonary toxicity 2.1% - 8%
  - Bone marrow suppression light 12%
  - pancytopenia 0.8%


Folát y
Thymidin-synthese
Dihydrofolá ty
Gastrointestiná lní toxicita
dTMP
2
dUMP
MTX
THF
Homocystein
Syntéza polyaminů
Adenosin - uvolňování
8
Methionin
7
5,10-methylen tetradydrofolát
6
5-methyl tetradydrofolát
AMP
4
IMP
Phosporibosy amin
Adenosin
5
Inosin
AICAR
5,10- methenyl tetrahydrofolát
3
THF
9
FAICAR
MTX
10- formyl tetrahydrofolát
Purinsynthese
1 Dihydrofolsäure-Reduktase
2 Thymidylat-Synthase
3 5-Aminoimidazol-4-Carboxamid-Ribonucleotid (AICAR)-Transformylase (ATIC)
4 AMP-Deaminase
5 Adenosin-Deaminase
6 Methylen-THF-Reduktase
7 Serin-Hyroxymethyl-Transferase
8 Methionin-Synthase
9 Methylen-THF-Dehydrogenase (MTHFD1)

Methotrexate

Table 1. Pharmacogenetics of MTX transporters*

Gene	Polymorphism	Amino acid substitution in enzyme	Biochemical effects	Clinical effects	Reference
RFC-1	G80A	Histidine to arginine at codon 27	May affect transcriptional activity of RFC1 gene and MTX entry into cell	May affect response to MTX	48, 72
ABCB1	C3435T	No amino acid substitution	May affect MTX entry into cell	May affect response to MTX	55

* MTX = methotrexate; RFC-1 = reduced folate carrier 1; ABCB1 = ATP binding casette transporter B1.

Table 2. Pharmacogenetics of MTHFR*

Gene	Polymorphism	Amino acid substitution in enzyme	Biochemical effects	Clinical effects (ref.)
MTHFR	C677T	Alanine to valine	Thermolabile MTHFR with decreased activity; increased plasma homocysteine	May increase the following: GI toxicity (60); hepatic and GI toxicity, alopecia, stomatitis, and rash (62,63); headache, lethargy (74). No effect on toxicity (62); no effect on efficacy or toxicity (71)
MTHFR	A1298C	Glutamine to alanine	May decrease MTHFR activity and increase plasma homocysteine	May affect MTX efficacy (63); may increase susceptibility to RA and decrease MTX toxicity (62). No effect on efficacy or toxicity (71)

* MTHFR = methylenetetrahydrofolate reductase; GI = gastrointestinal; MTX = methotrexate; RA = rheumatoid arthritis.

Ranganathan P, McLeod HL. A&R 2006

Methotrexate

Table 3. MTX pathway pharmacogenetics

Gene	Role in MTX pathway	Polymorphism	Effects on gene product/enzyme	Clinical effects	Reference
ATIC	Conversion of AICAR to 10-formyl-AICAR; target of MTX	C347G	May decrease ATIC activity and affect AICAR accumulation and adenosine release	May affect MTX efficacy and toxicity	72, 74
TYMS	Conversion of dUMP to dTMP; target of MTX	5′-UTR 28-bp repeat	May increase TYMS enzyme activity	May affect MTX efficacy and toxicity	71, 74
		3′-UTR 6-bp deletion	May decrease TYMS mRNA stability and expression	May affect MTX efficacy	71

* MTX = methotrexate; ATIC = aminoimidazole carboxamide ribonucleotide transformylase; AICAR = aminoimidazole carboxamide ribonucleotide; TYMS = thymidylate synthase; 5′-UTR = 5′-untranslated region.

Table 4. Other genes with potential pharmacogenetic implications in the MTX pathway*

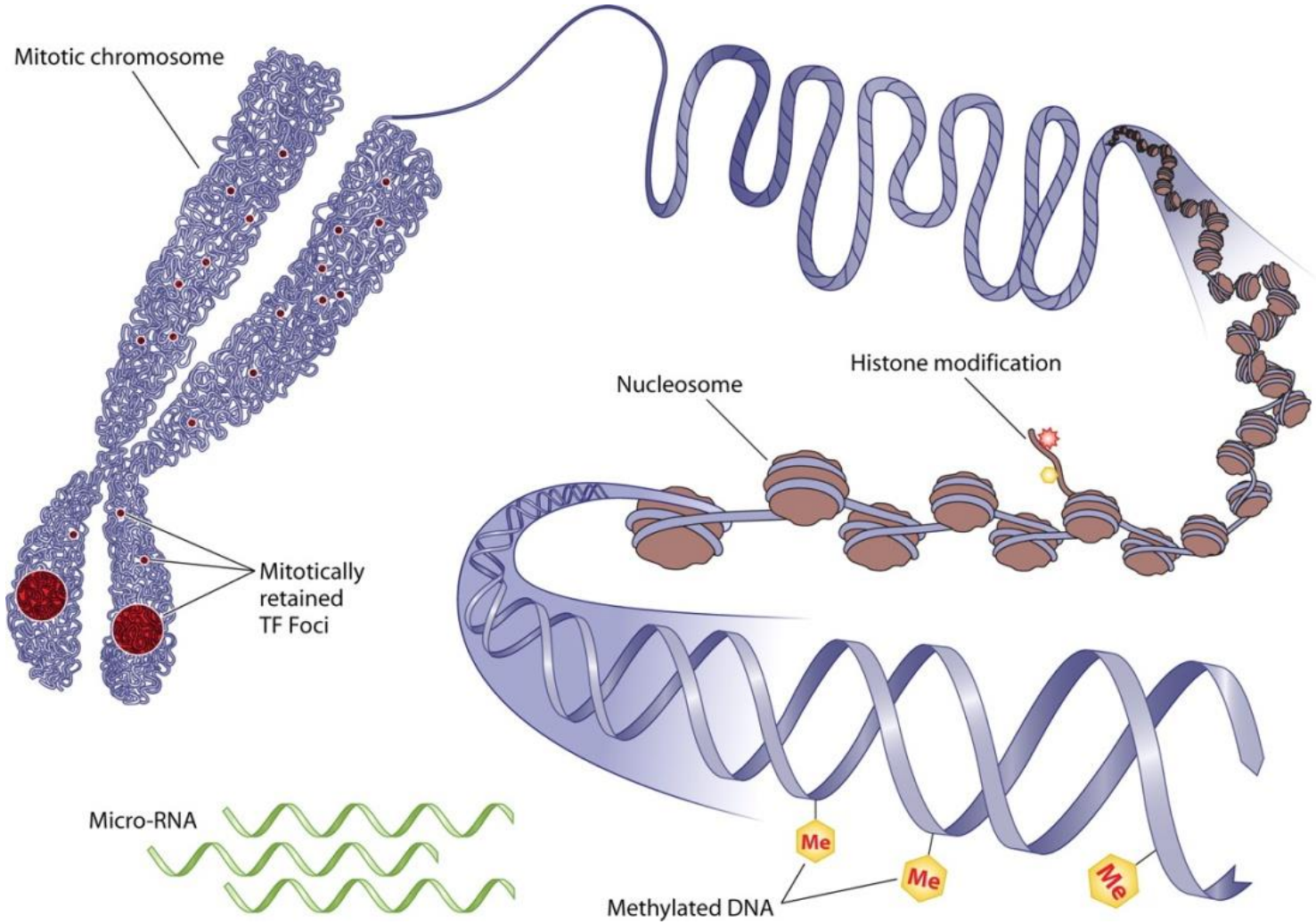
Gene	Role in MTX pathway	Polymorphism	Effects on gene product/enzyme	Postulated clinical effects	Reference
GGH	Conversion of long-chain MTXPGs to short-chain MTXPGs by removal of glutamates	C452T	Decreased binding affinity of GGH for MTXPGs	May affect MTX efficacy	76
		C401T	Affects MTXPG levels	–	47
DHFR	Reduction of DHF to THF; target of MTX	3′-UTR T721A and C829T	May increase DHFR expression	May affect MTX efficacy	77
MS	Methylation of homocysteine to methionine	A2756G	May decrease MS activity; increase homocysteine levels	May affect MTX toxicity	79, 80
MTRR	Methylation of cobalamin cofactor required for the action of MS	A66G	May decrease MTRR activity; increase homocysteine levels	May affect MTX toxicity	81, 82

* MTX = methotrexate; GGH = γ-glutamyl hydrolase; MTXPGs = methotrexate polyglutamates; DHFR = dihydrofolate reductase; DHF = dihydrofolate; THF = tetrahydrofolate; 3′-UTR = 3′-untranslated region; MS = methionine synthase; MTRR = methionine synthase reductase.

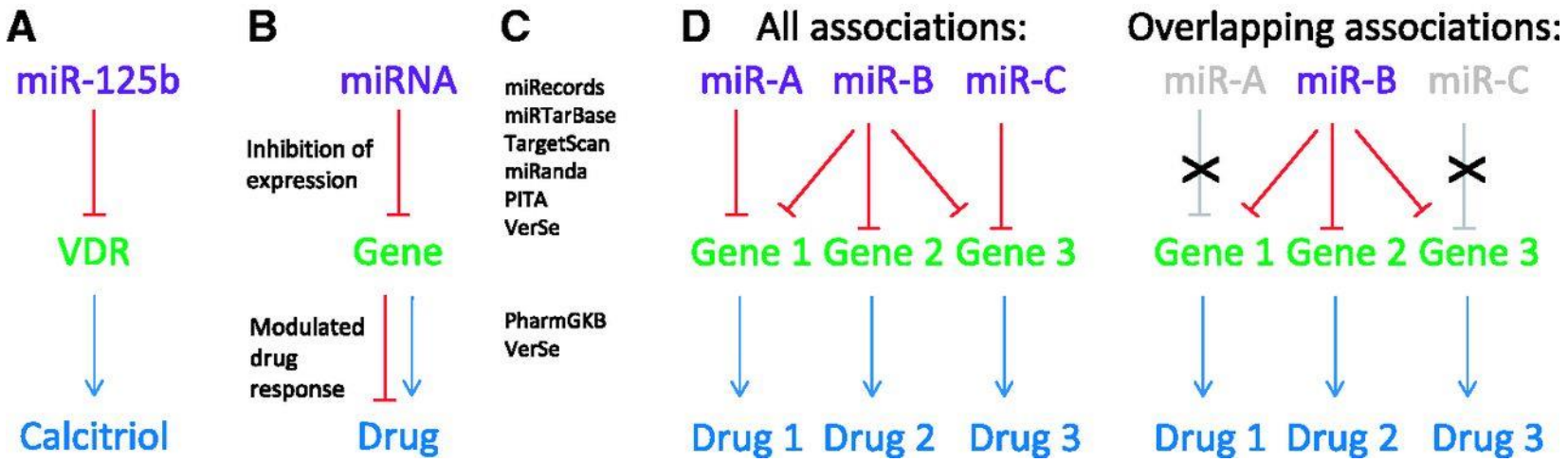
Ranganathan P, McLeod HL. A&R 2006

MDR1

- MDR1 (ATP-binding cassette B1/multidrug resistance 1) is an efflux pump that transports toxic endogenous substances, drugs and xenobiotics out of cells.
- It is known to affect susceptibility to many hematopoietic malignancies.
- ABCB1/MDR1 polymorphisms may either change the protein expression or alter its function, suggesting a possible association between ABCB1/MDR1 single nucleotide polymorphisms (SNP) and clinical aspects of T-cell lymphoma.
- Therefore, association of two polymorphisms in the gene with clinical staging and therapy was evaluated.

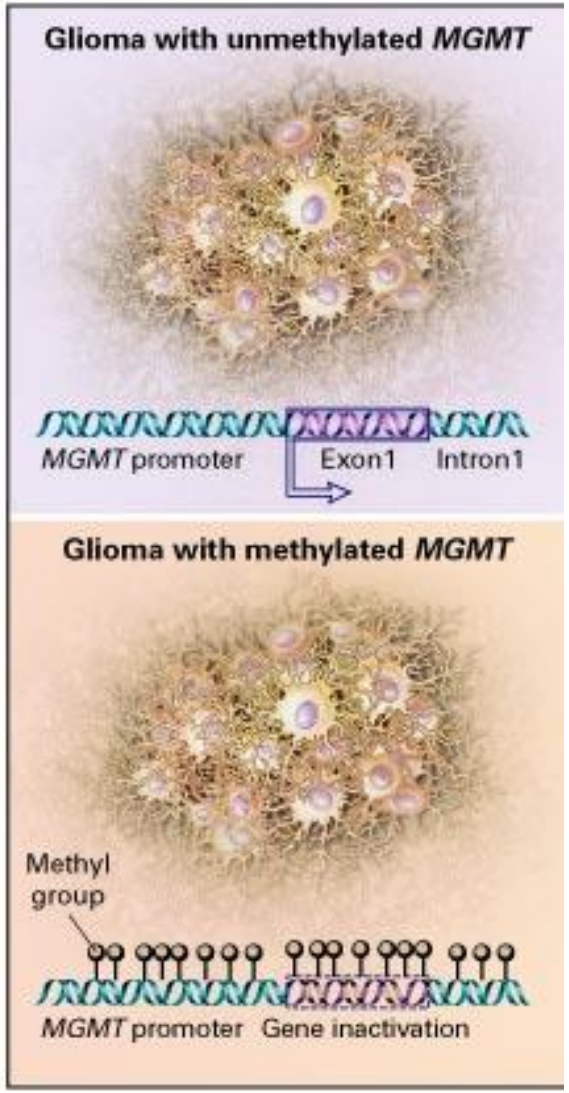

Mitotic chromosome
Mitotically
retained
TF Foci
Nucleosome
Histone modification
Micro-RNA
Me
Me
Me
Methylated DNA

(A) An example of an experimentally verified miRNA pharmacogenomic set. miR-125 b inhibits vitamin D receptor (VDR) expression.



Rukov J L et al. Brief Bioinform 2013;bib.bbs082

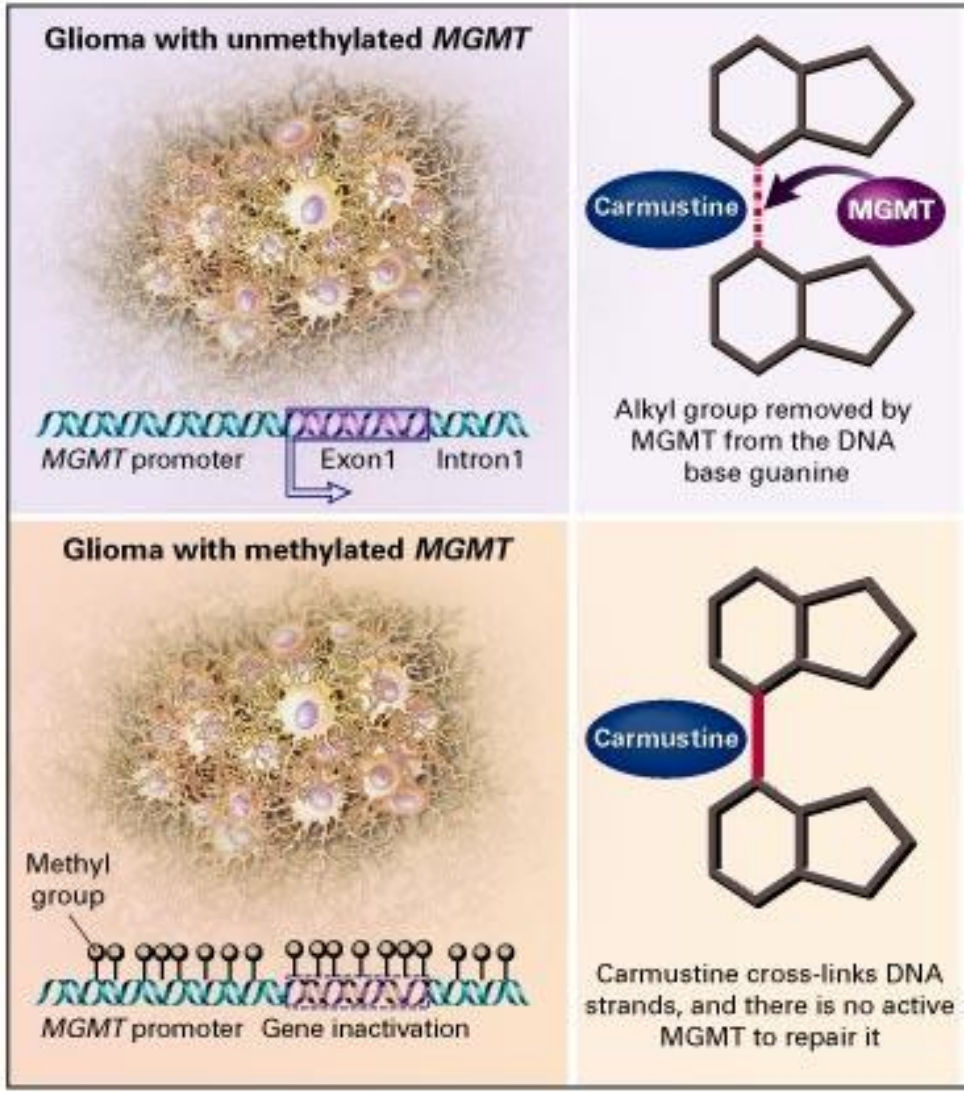
Briefings in Bioinformatics



Evidence suggests this may be the result of an epigenetic phenomenon – one that does not involve a change in DNA sequence.

MGMT – methylguanine-DNA methyltransferase
Methylation of the promoter region of MGMT may silence the gene

From: Esteller M, et al. Inactivation of the DNA-repair gene *MGMT* and the clinical response of gliomas to alkylating agents. *NEJM* 243:1350-1354, 2000.



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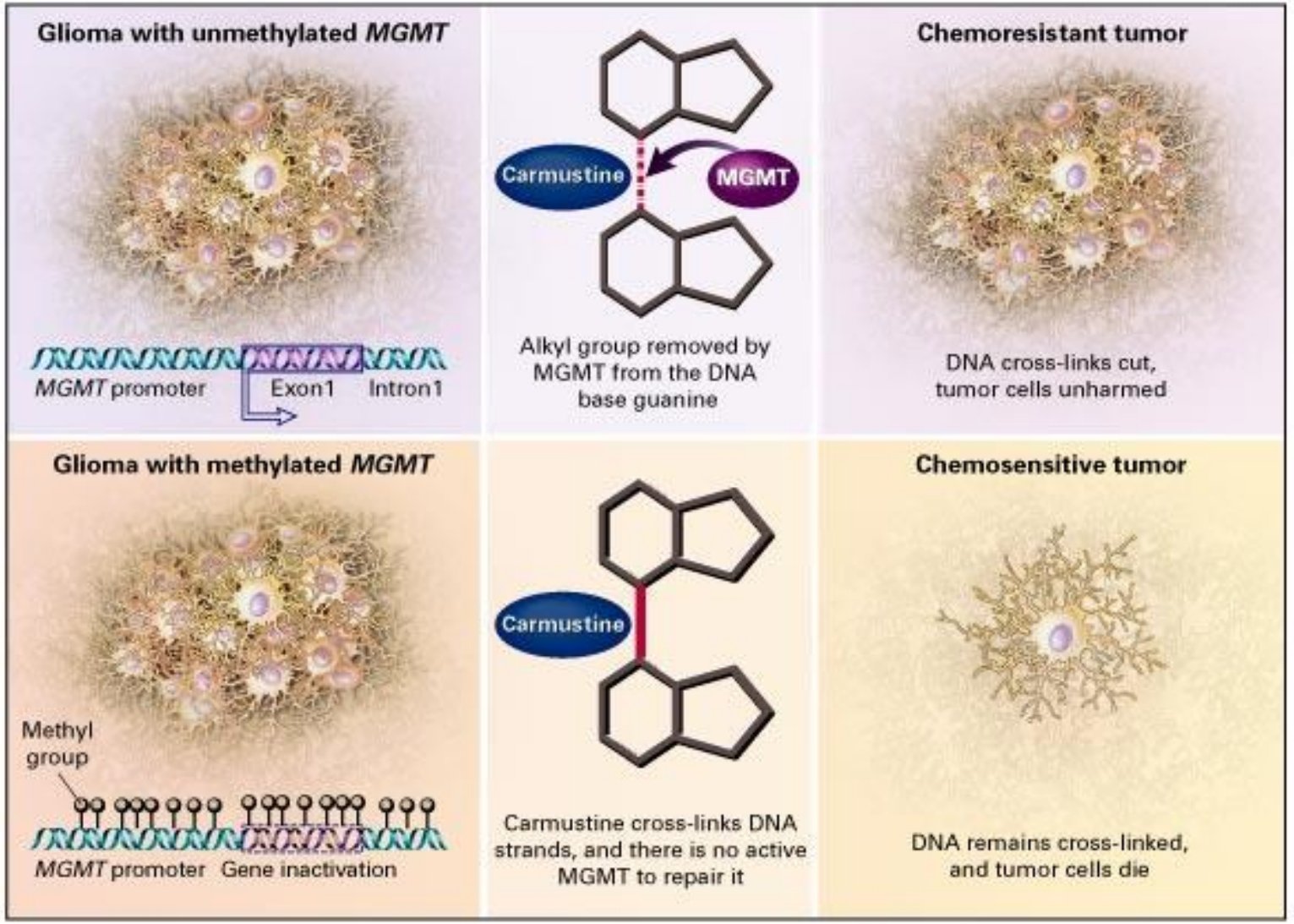

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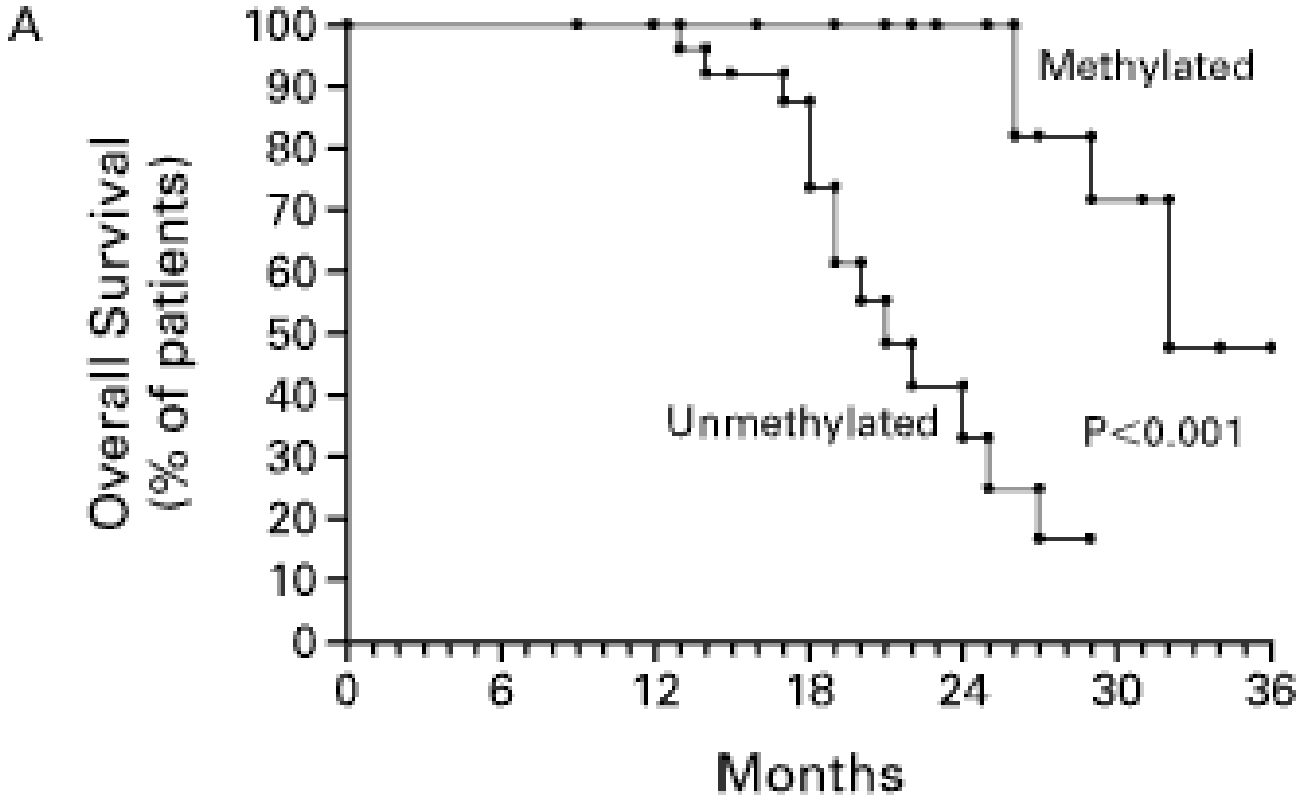


No. of Subjects					
Methylated	19	19	19	18	13
Unmethylated	28	28	26	19	5

Personalized Drugs

- Herceptin (breast cancer, target: Her2/neu)
- Erbitux (colorectal cancer, target: EGFR)
- Tarceva (lung cancer, target: EGFR)
- Strattera (attention-deficit/hyperactivity disorder, Metabolism: P4502D6)
- 6-MP (leukemia, Metabolism: TPMT)
- Antivirals (i.e. resistance based on form of HIV)

- etc. and the list is growing rapidly ...

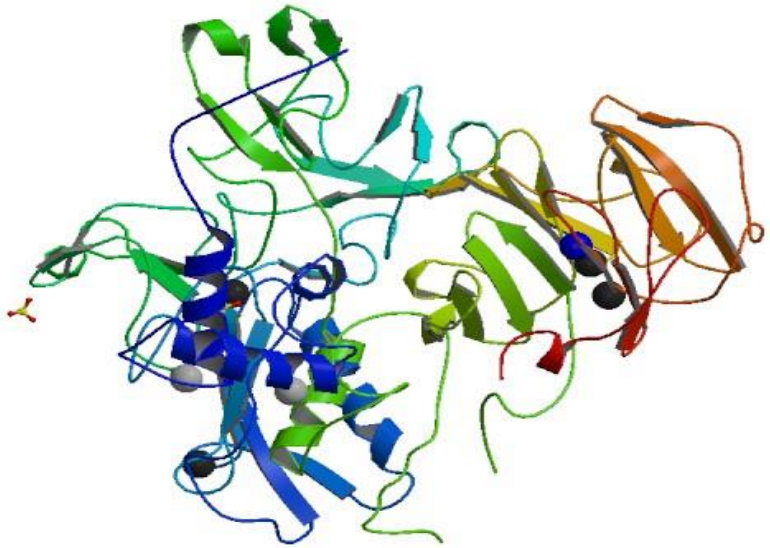
FDA Requires Genetic Tests for Certain Therapies

List of FDA Required or Recommended Biomarker Tests in Drug Labels

Biomarker	Test[13]	Drug Example	User Prevalence (%) (n=36.1 million)
CYP2C9	Recommended	Warfarin	2.0896
EGFR	Required	Cetuximab	0.0001
G6PD deficiency	Recommended	Dapsone	0.0257
G6PD deficiency	Recommended	Rasburicase	0.0000
HER2/neu overexpression	Required	Trastuzumab	0.0003
TPMT variants	Recommended	Azathioprine	0.1168
TPMT variants	Recommended	Mercaptopurine	0.0541
TPMT variants	Recommended	Thioguanine	0.0012
UGT1A1 variants	Recommended	Irinotecan	0.0002
Urea cycle enzyme deficiency	Recommended	Valproic acid	0.48
Total			2.768

CYP = cytochrome P450; EGFR = human epidermal growth factor receptor; G6PD = glucose-6-phosphate dehydrogenase; HER2/neu = human epidermal growth factor receptor 2; TPMT = thiopurine S-methyltransferase.

Thank you for your attention



„I just need a closer look...“