FARMACOGENETICA: verso la personalizzazione della terapia

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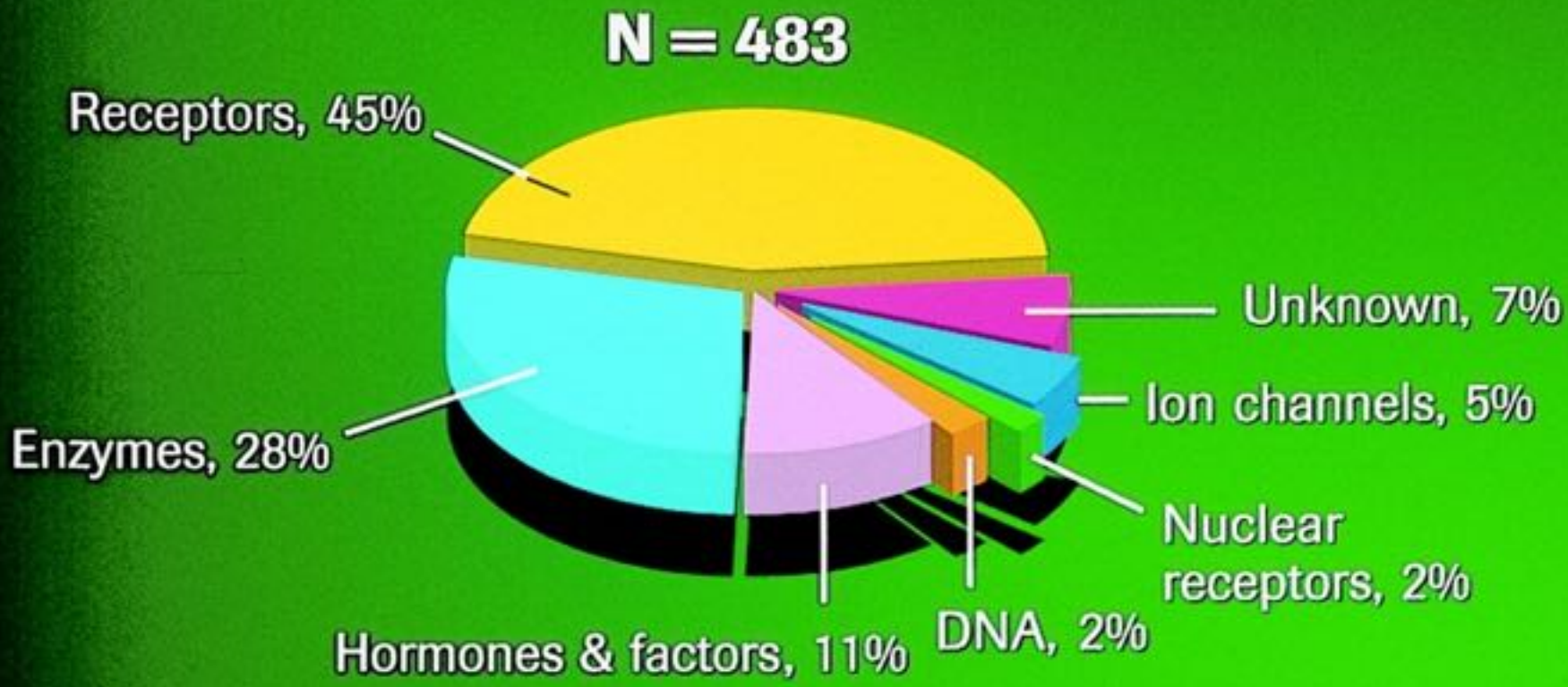
Sezione di Farmacologia Cellulare e Molecolare
Milano

Pesaro, 22/09/2007

Biochemical Classes of Drug Targets of Current Therapies



Le reazioni avverse ai farmaci

(ADR=ADVERSE DRUG REACTION)

costituiscono la quarta causa di morte negli Usa

dopo l'infarto del miocardio, i tumori e l'ictus

LAZAROU J. *et al.* **JAMA (1998) 279: 1200-5**

La maggior parte di queste imprevedibili morti è dovuta a variabilità individuale nelle risposte ai farmaci

L'aspetto speculare di questa variabilità è *la mancata efficacia di un determinato trattamento farmacologico* con esposizione del paziente ai soli effetti collaterali o tossici del trattamento stesso

senza alcun beneficio terapeutico

Il 90% degli individui con più di 65 anni di età assume almeno un farmaco al giorno

Molti anziani assumono più farmaci contemporaneamente, aumentando le probabilità di reazioni avverse, interazioni tra farmaci e interazioni sfavorevoli tra farmaci e patologie.

La frequenza delle reazioni avverse negli anziani oscilla nei diversi studi tra il 10 e il 35%

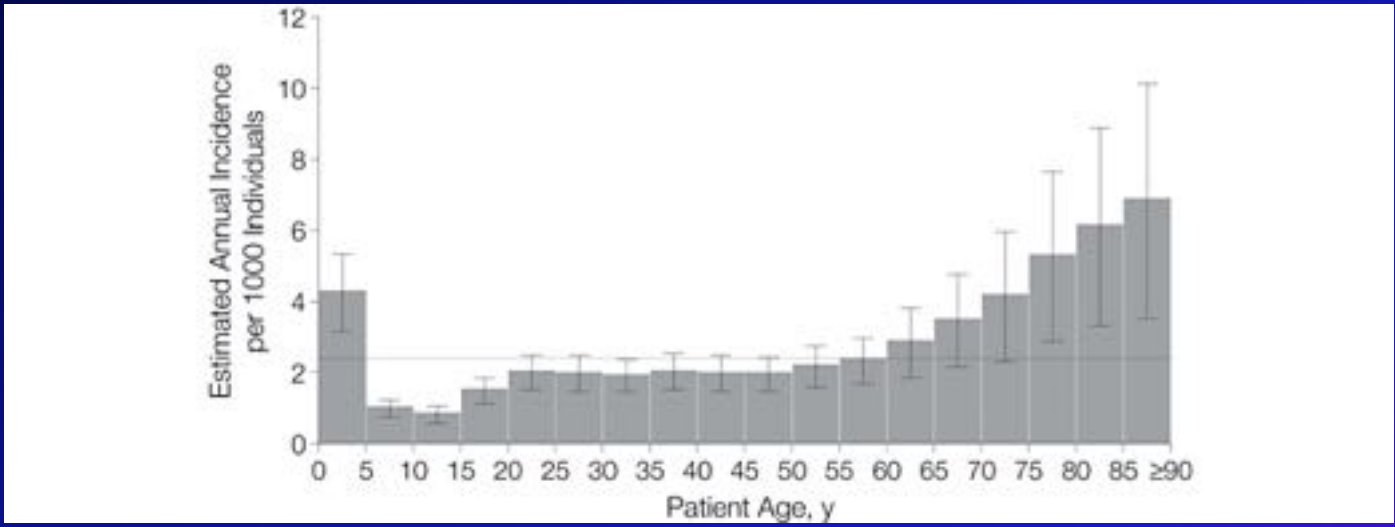
Higashi T et al. Ann.Int.Med. (2004)140:714-20

National Surveillance of Emergency Department Visits for Outpatient Adverse Drug Events

Daniel S. Budnitz, MD, MPH; Daniel A. Pollock, MD; Kelly N. Weidenbach, MPH; Aaron B. Mendelsohn, PhD, MPH; Thomas J. Schroeder, MS; Joseph L. Annest, PhD

JAMA. 2006;296:1858-1866.

Estimated Annual Incidence of Adverse Drug Events Treated in US Emergency Departments



Budnitz, D. S. et al. JAMA 2006;296:1858-1866.

JAMA

Number of Cases and Annual Estimate of Individuals With Adverse Drug Events Treated in Emergency Departments by Condition--United States, 2004-2005

Table 2. Number of Cases and Annual Estimate of Individuals With Adverse Drug Events Treated in Emergency Departments by Condition—United States, 2004-2005

Condition	Adverse Drug Events	
	Cases, No.	Annual Estimate, No. (%)*
Dermatologic	5323	184 208 (26.3)
Gastrointestinal	2865	99 944 (14.2)
Neurological	2829	97 699 (13.9)
Metabolic/endocrine	1999	73 533 (10.5)
Bleeding/coagulation dysfunction	1800	68 545 (9.8)
Altered mental status	1898	68 075 (9.7)
Facial edema	1636	55 079 (7.9)
Respiratory	1557	54 089 (7.7)
Syncope/dizziness	1542	53 610 (7.6)
Cardiovascular	996	35 884 (5.1)
Psychological	859	29 048 (4.1)
Musculoskeletal	714	22 772 (3.2)
Injection site injury	552	16 274 (2.3)
Renal/genitourinary	417	17 101 (2.4)†
Peripheral edema	425	15 388 (2.2)
Ophthalmologic	413	14 013 (2.0)
Nonspecific symptoms	433	11 760 (1.7)
Infectious	306	10 346 (1.5)†
Otologic	92	3209 (0.5)
Exposure without adverse effect at time of evaluation	2035	50 031 (7.1)
Unspecified overdose/toxicity	1091	32 065 (4.6)
Unspecified or generalized allergic reaction	657	16 096 (2.3)
Unspecified effect	242	6621 (0.9)

*Conditions were not mutually exclusive; therefore, percentages may total >100%.
†Estimates with coefficient of variation >30%: renal/genitourinary conditions, 33.4% and infectious, 32.6%.

Budnitz, D. S. et al. JAMA 2006;296:1858-1866.

La variabilità individuale nelle risposte ai farmaci ha *basi genetiche*

risiede in *differenze di sequenza*
esistenti a carico dei geni
codificanti per le proteine
coinvolte nella risposta
ad un determinato trattamento
farmacologico

Che cosa è la Farmacogenetica?

La FARMACOGENETICA
è la disciplina che studia le basi genetiche della risposta individuale ai farmaci

EMEA/CPMP 3070/01 Position paper on terminology in pharmacogenetics Londra, 21 novembre 2002

"La Farmacogenetica si occupa di studiare le variazioni inter-individuali nella sequenza del DNA in relazione alla risposta ai farmaci."

I geni che influenzano la risposta ad un determinato trattamento farmacologico possono essere distinti in due grandi classi

2

- **Geni codificanti per il *bersaglio terapeutico primario*, come per esempio recettori o enzimi**

- **Geni codificanti per proteine coinvolte *nell'assorbimento, metabolismo ed escrezione* del farmaco**

I geni che influenzano la risposta ai farmaci possono presentare *varianti alleliche* nella popolazione, cioè, in altri termini, essere *polimorfici*

Tali polimorfismi sono spesso a carico di singole basi e vengono pertanto definiti SNP

Single Nucleotide Polymorphisms

What is an SNP?

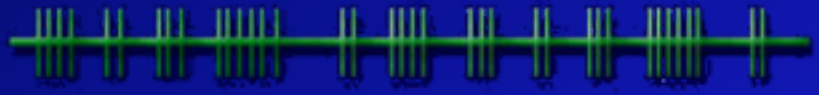
Different people can have a different nucleotide or base at a given location on a chromosome

. . . G G T A A C T G . . .

. . . G G C A A C T G . . .

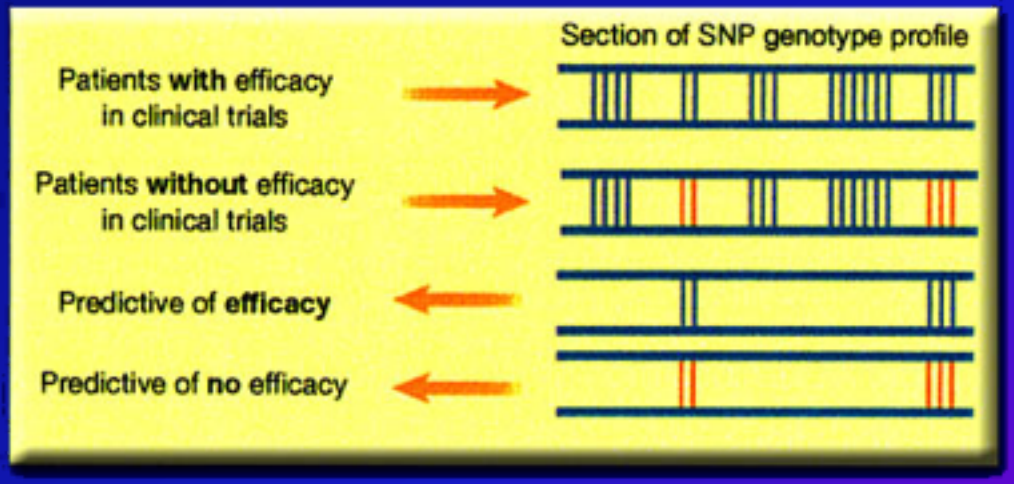
What is an SNP map?

Location of SNPs on human DNA

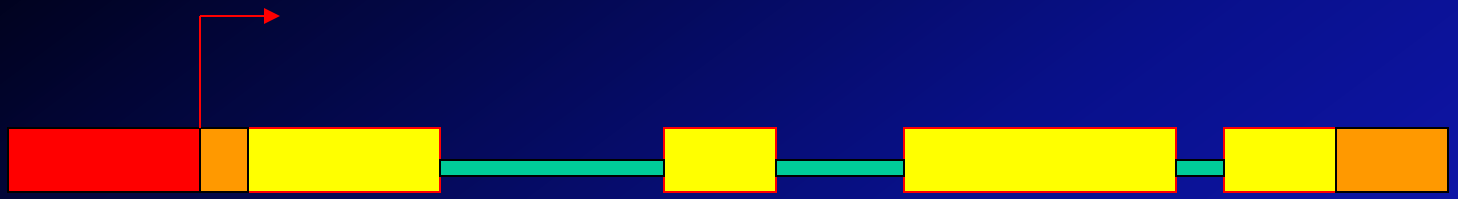


Human DNA

How can an SNP map be used to predict medicine response?



Si definiscono polimorfici tutti quegli alleli (o loci) che sono presenti almeno nell’1% della popolazione



start codon

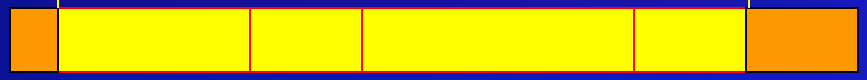
TRASCRIZIONE

stop codon

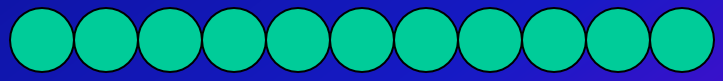
start codon

SPLICING

stop codon



TRADUZIONE



Gli *SNPs*

vengono classificati

in 3 distinte categorie

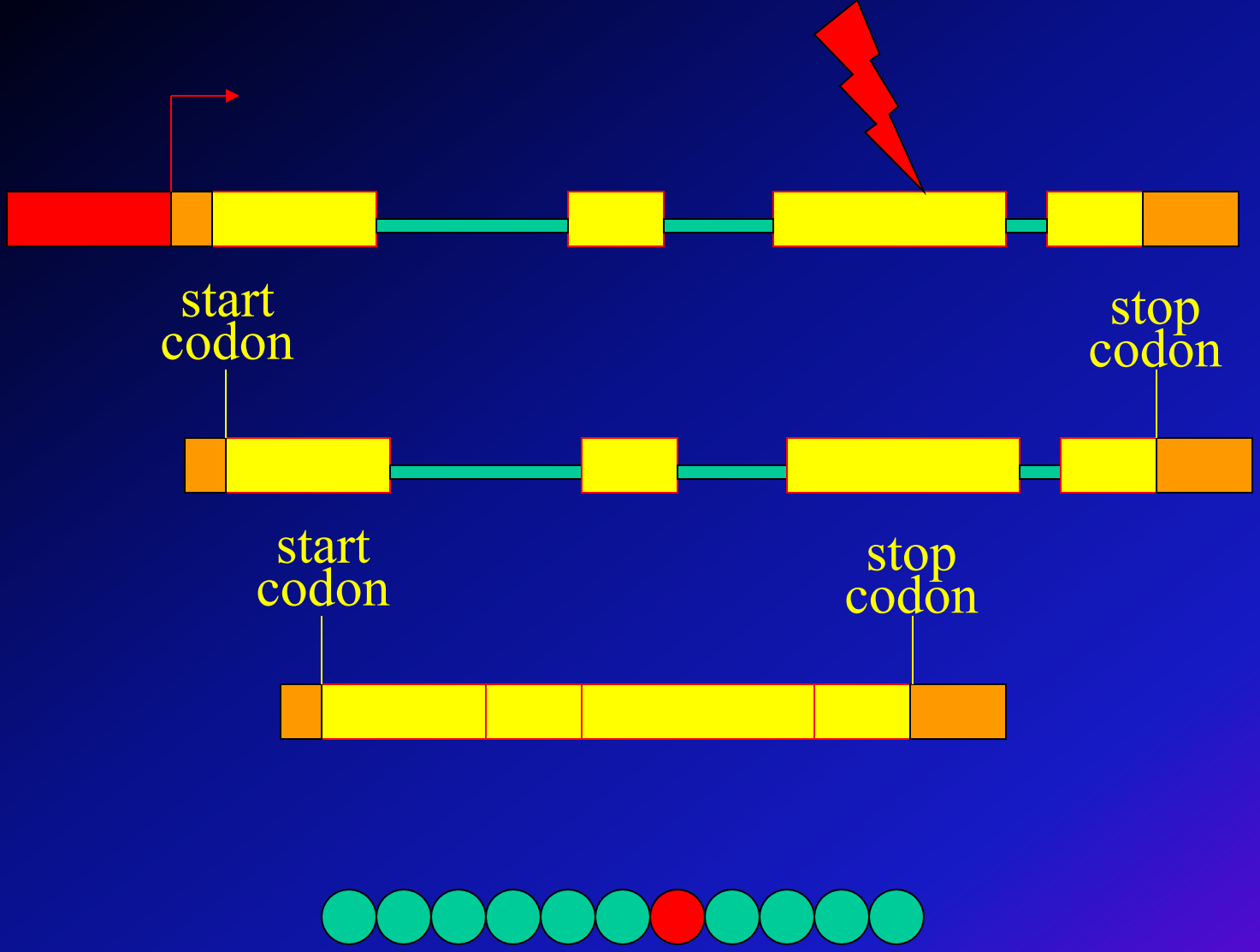
SNP delle regioni codificanti (cSNP).

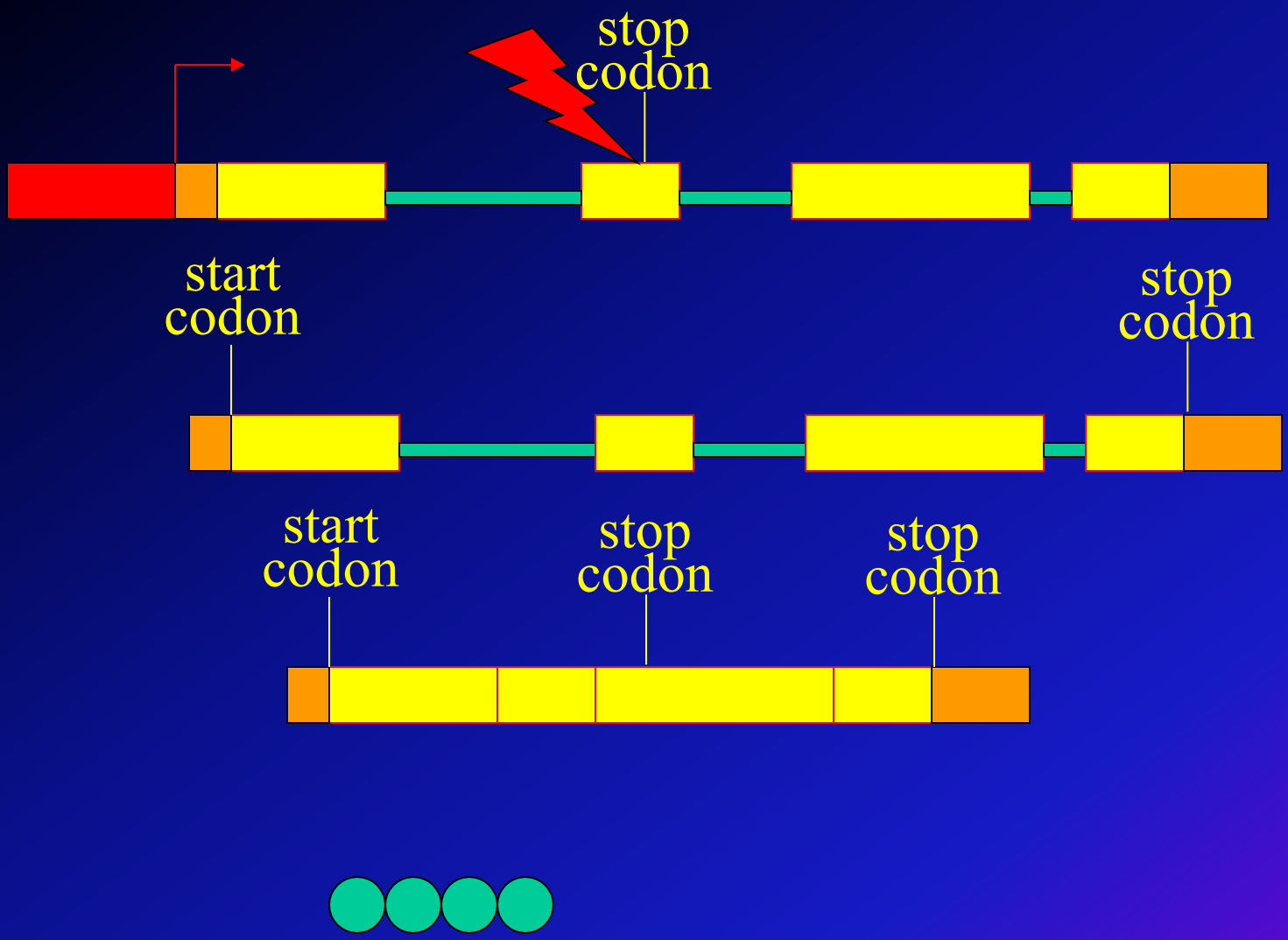
SNP perigenici (pSNP) che interessano le regioni regolatorie al 5', le regioni specificanti i 5' e 3' non tradotti del mRNA, gli introni.

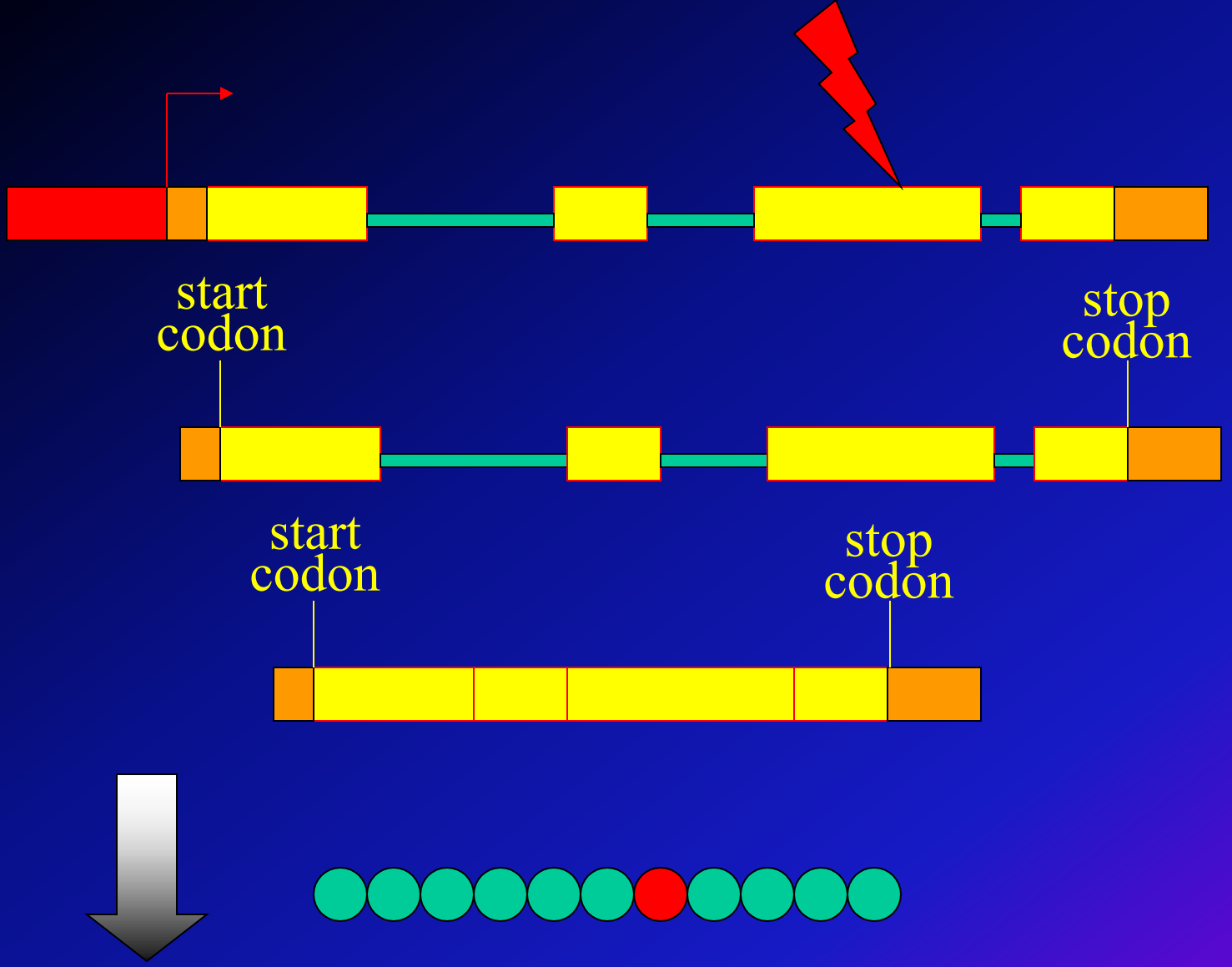
SNP che si trovano random (rSNP) nelle regioni intergeniche.

cSNPs

POLIMORFISMI A SINGOLO NUCLEOTIDE DELLE REGIONI CODIFICANTI

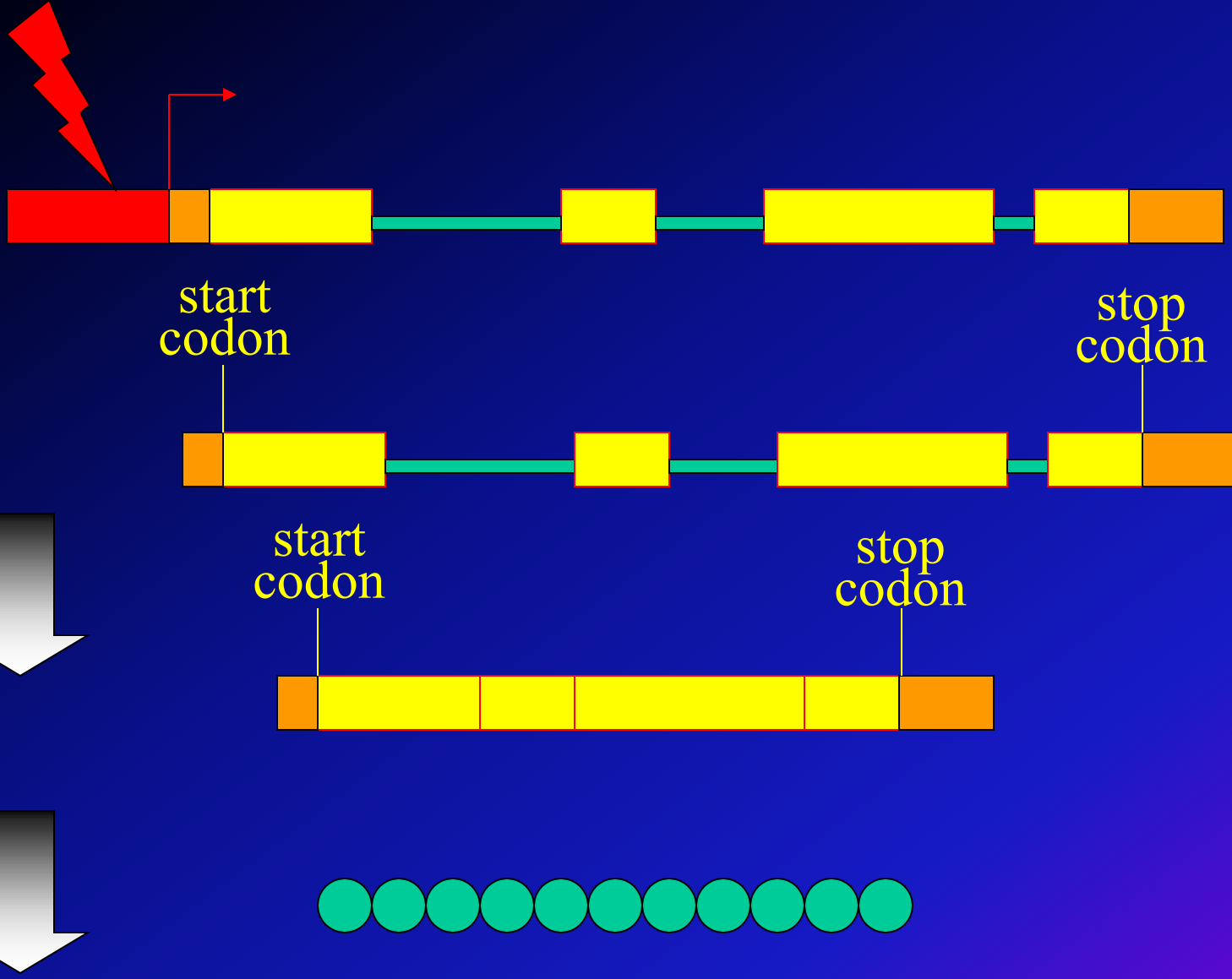

start
codon
stop
codon
start
codon
stop
codon


stop
codon
start
codon
stop
codon
start
codon
stop
codon
stop
codon


start
codon
stop
codon
start
codon
stop
codon

pSNPs

POLIMORFISMI A SINGOLO NUCLEOTIDE DELLE REGIONI PERIGENICHE


start
codon
stop
codon
start
codon
stop
codon

Si ritiene che in media vi siano 4 cSNP per gene; se si assume che nel genoma umano vi siano 30.000 geni, si stima che vi possano essere tra i 120.000 e i 200.000 cSNP che, con altri appropriati calcoli, si traducono nel fatto che un individuo-tipo è eterozigote per circa 30.000 aminoacidi

What is an SNP?

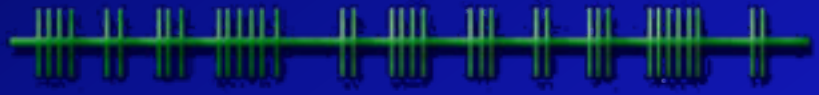
Different people can have a different nucleotide or base at a given location on a chromosome

. . . G G T A A C T G . . .

. . . G G C A A C T G . . .

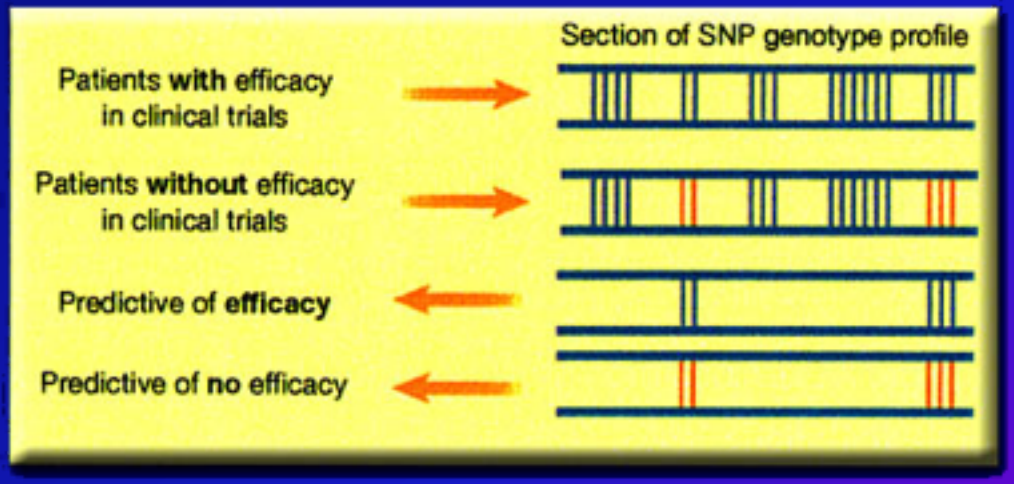
What is an SNP map?

Location of SNPs on human DNA



Human DNA

How can an SNP map be used to predict medicine response?



Polimorfismi in enzimi responsabili del metabolismo dei farmaci

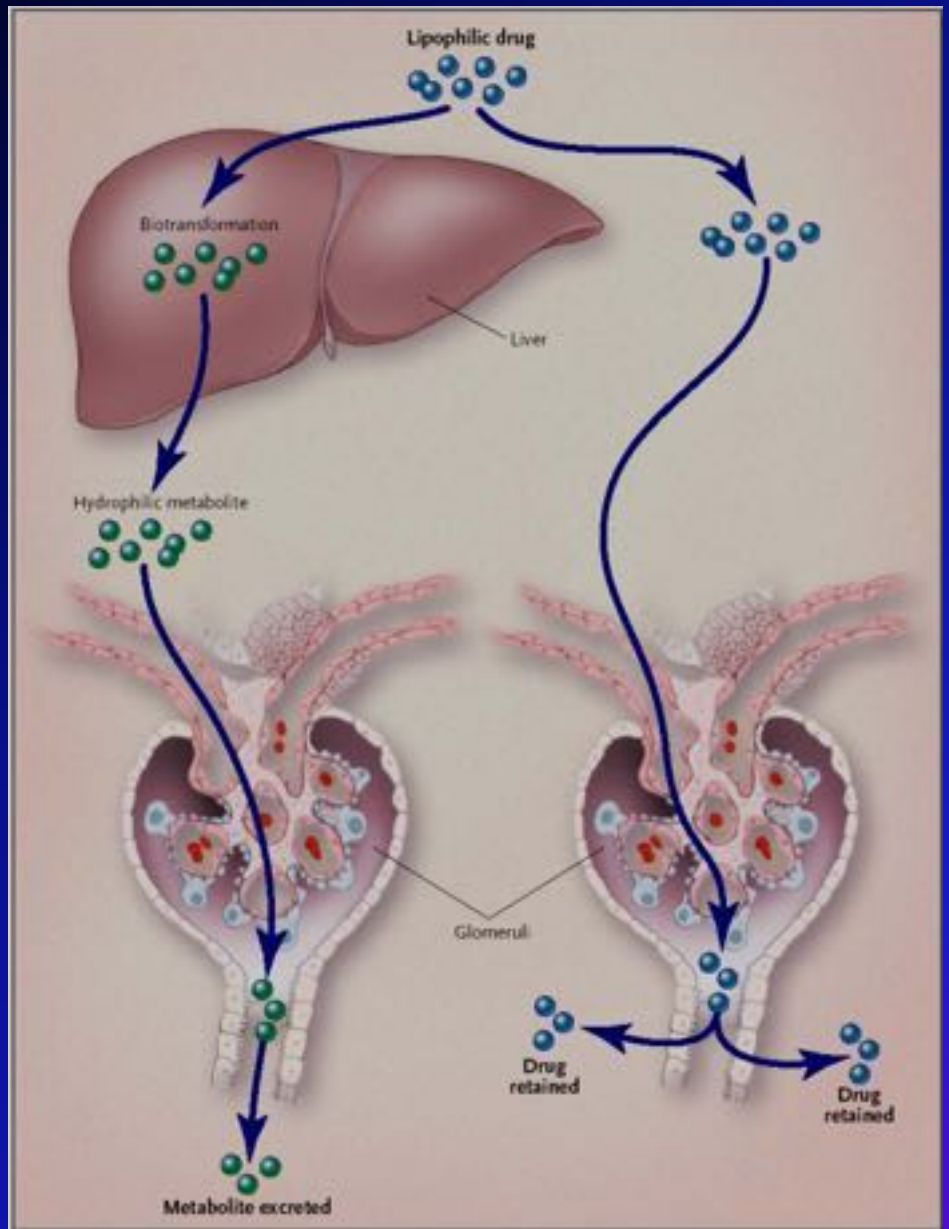
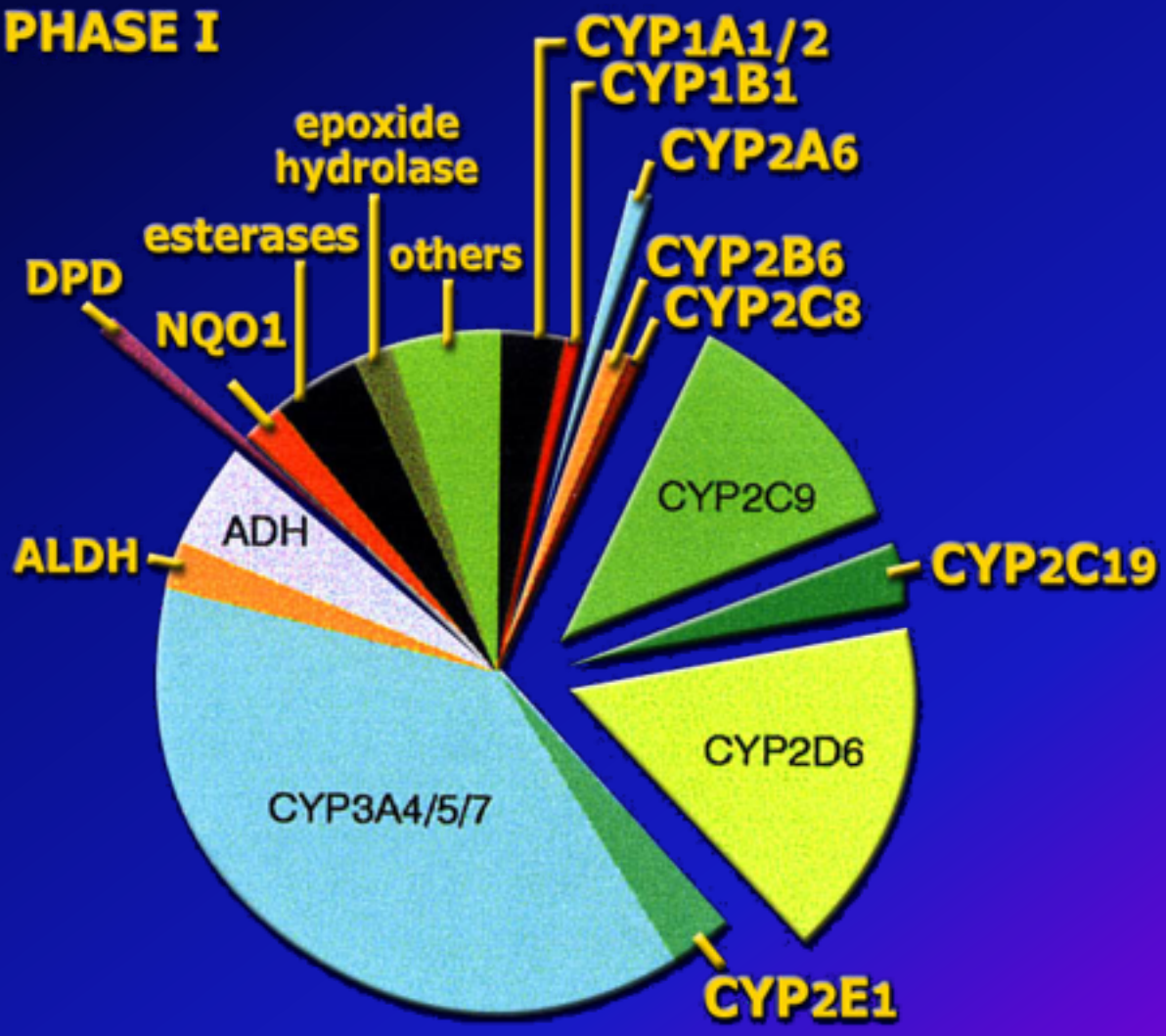
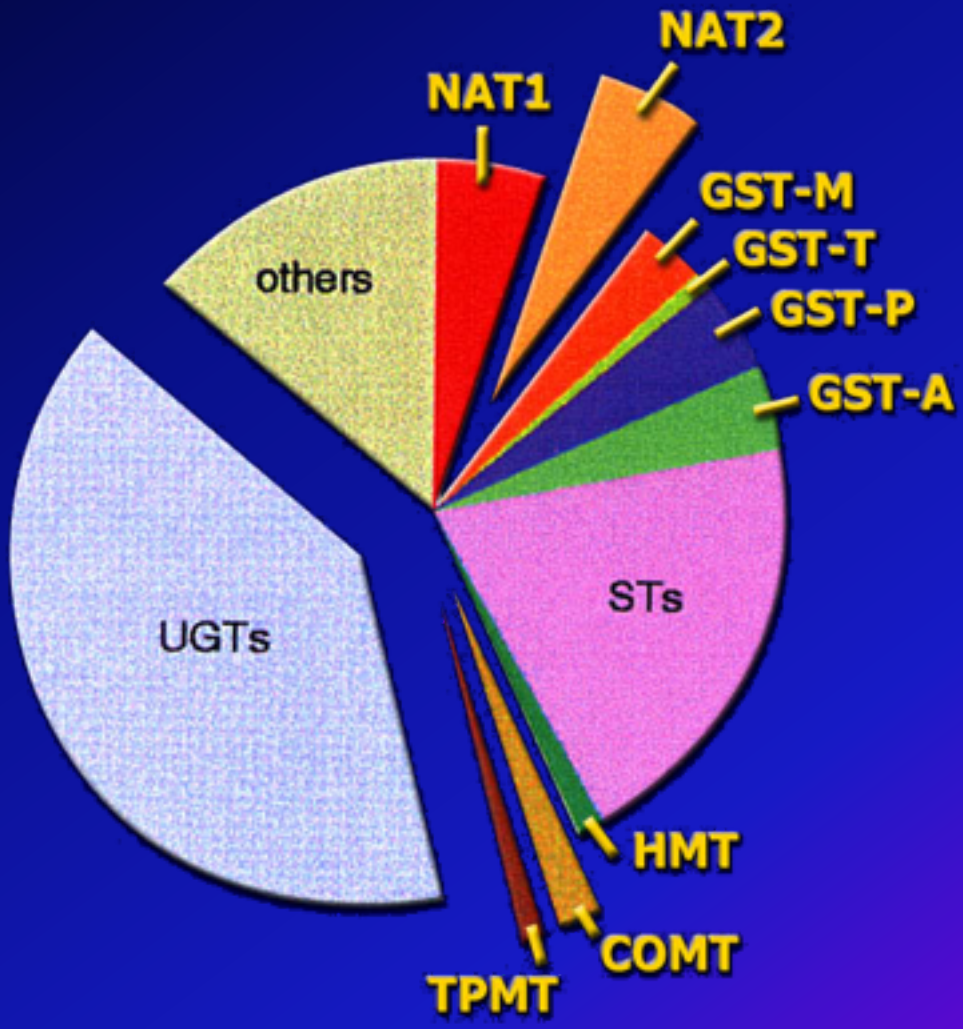


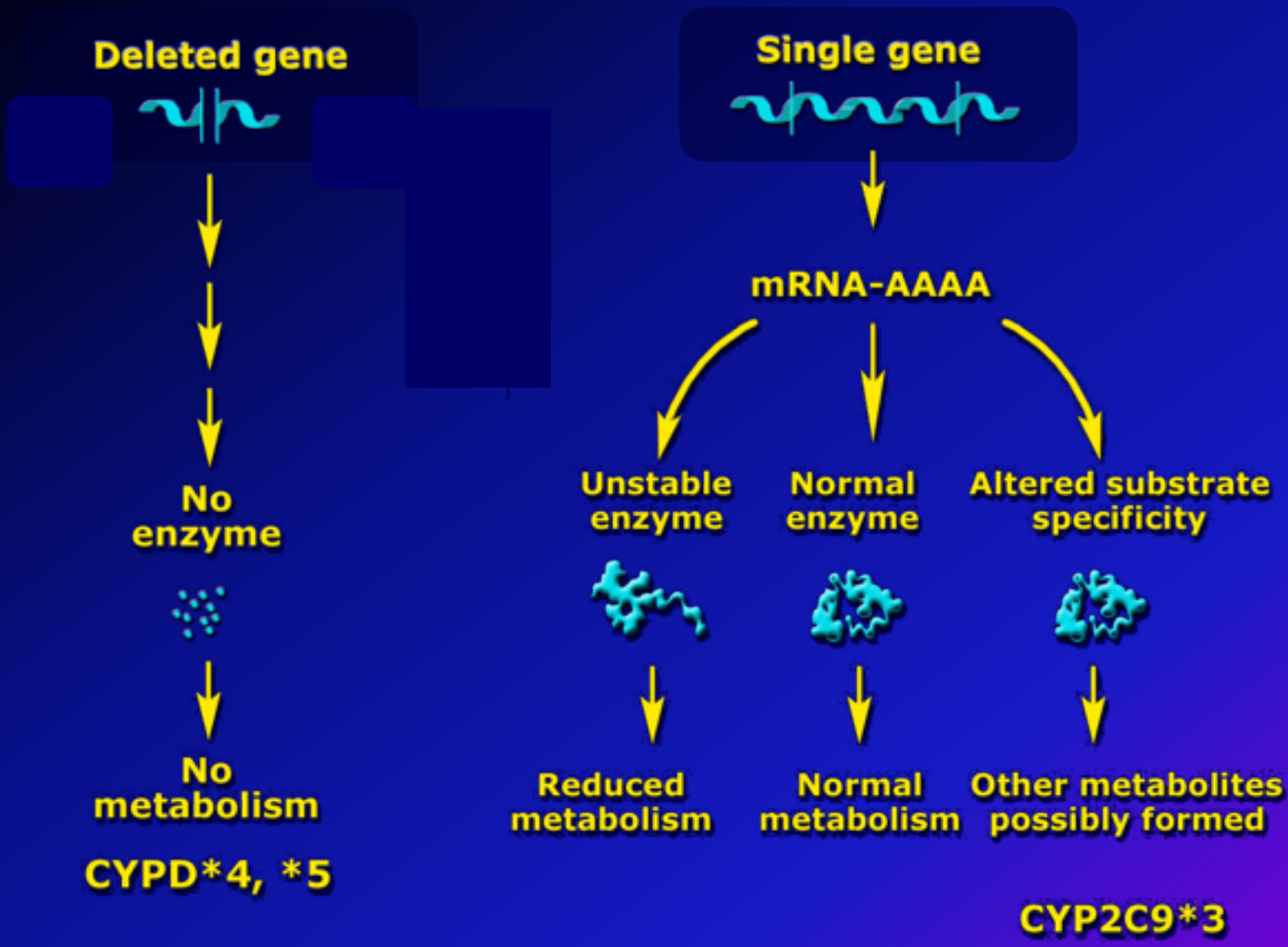
Figure 1. **The Effect of Drug Metabolism on Excretion.**
Lipophilic (or fat-soluble) drugs are metabolized to form relatively more hydrophilic (or water-soluble) metabolites than the parent drug, and these metabolites are thus more easily excreted.


PHASE I
CYP1A1/2
CYP1B1
CYP2A6
CYP2B6
CYP2C8
CYP2C9
CYP2C19
CYP2D6
CYP2E1
CYP3A4/5/7
ADH
ALDH
DPD
NQO1
esterases
epoxide hydrolase
others

PHASE II



Schema dei principali *meccanismi molecolari* che possono causare un alterato metabolismo dei farmaci nell'uomo


Deleted gene
No enzyme
No metabolism
CYPD*4, *5
Single gene
mRNA-AAAA
Unstable enzyme
Normal enzyme
Altered substrate specificity
Reduced metabolism
Normal metabolism
Other metabolites possibly formed
CYP2C9*3

Duplicated or multiduplicated genes

mRNA-AAAA
mRNA-AAAA
mRNA-AAAA

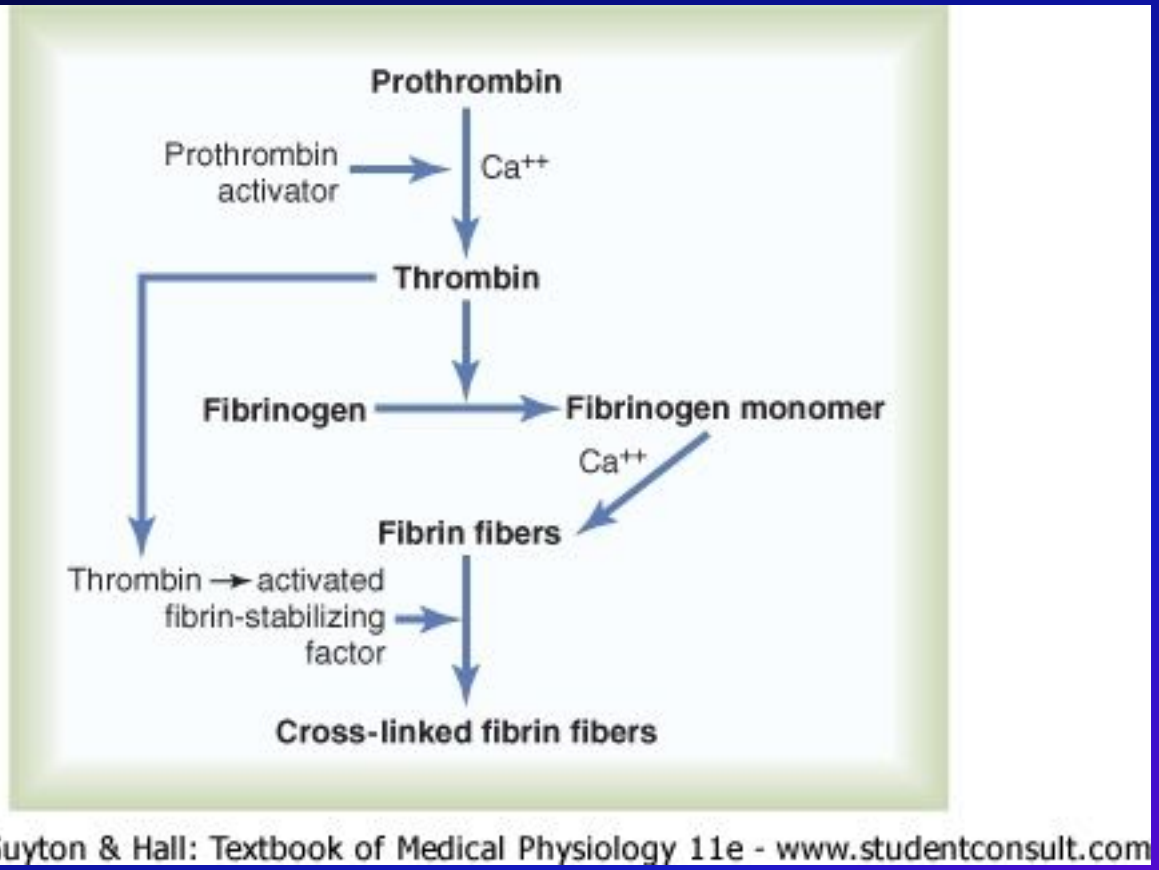
Higher enzyme levels

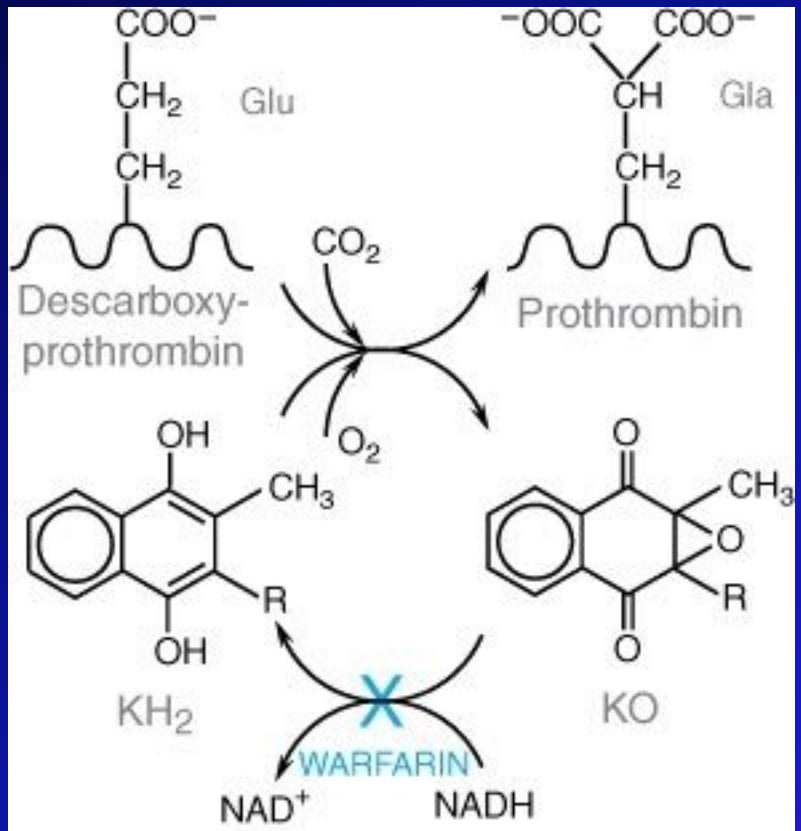
Increased metabolism

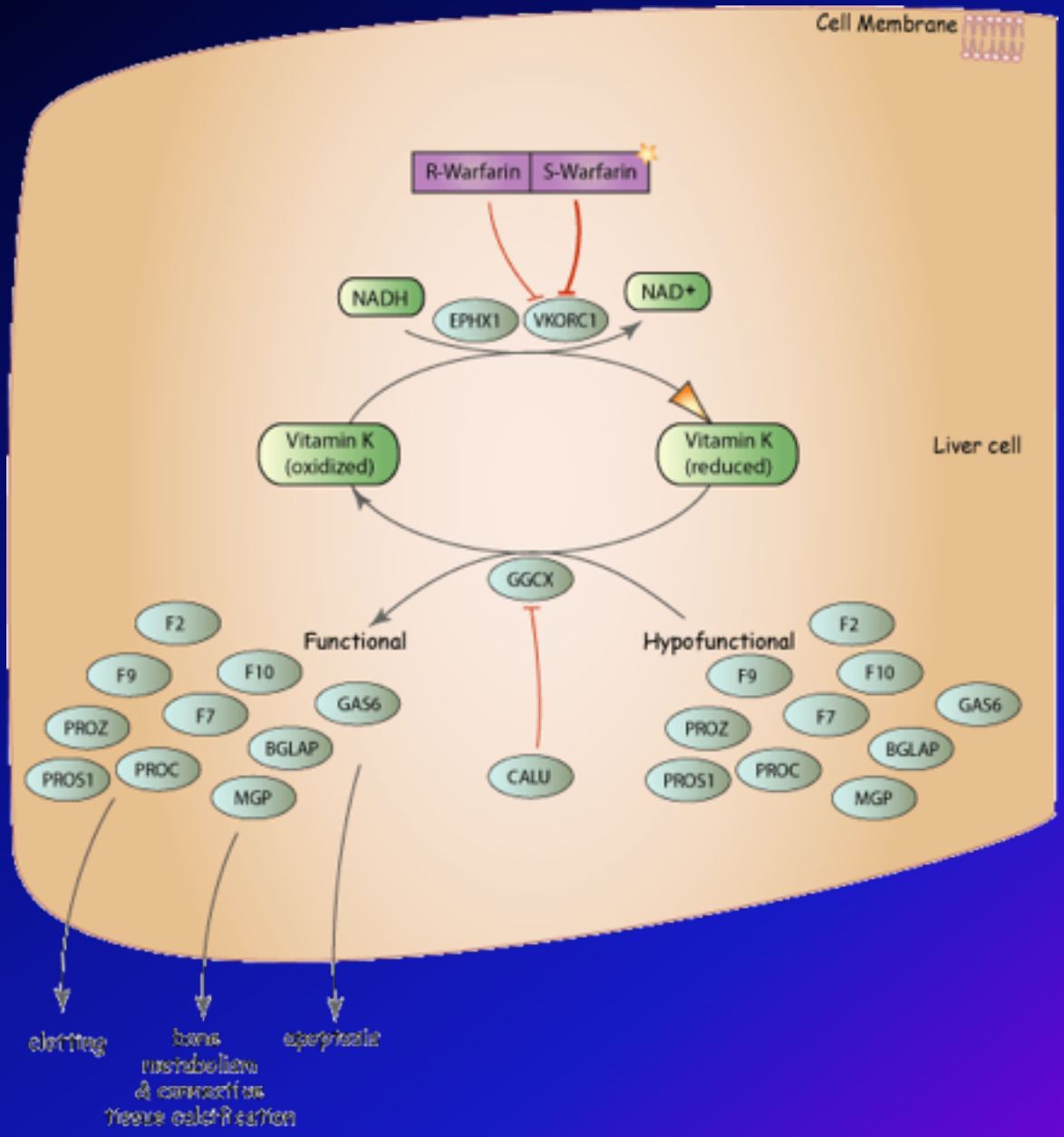
CYP2D6*2xN

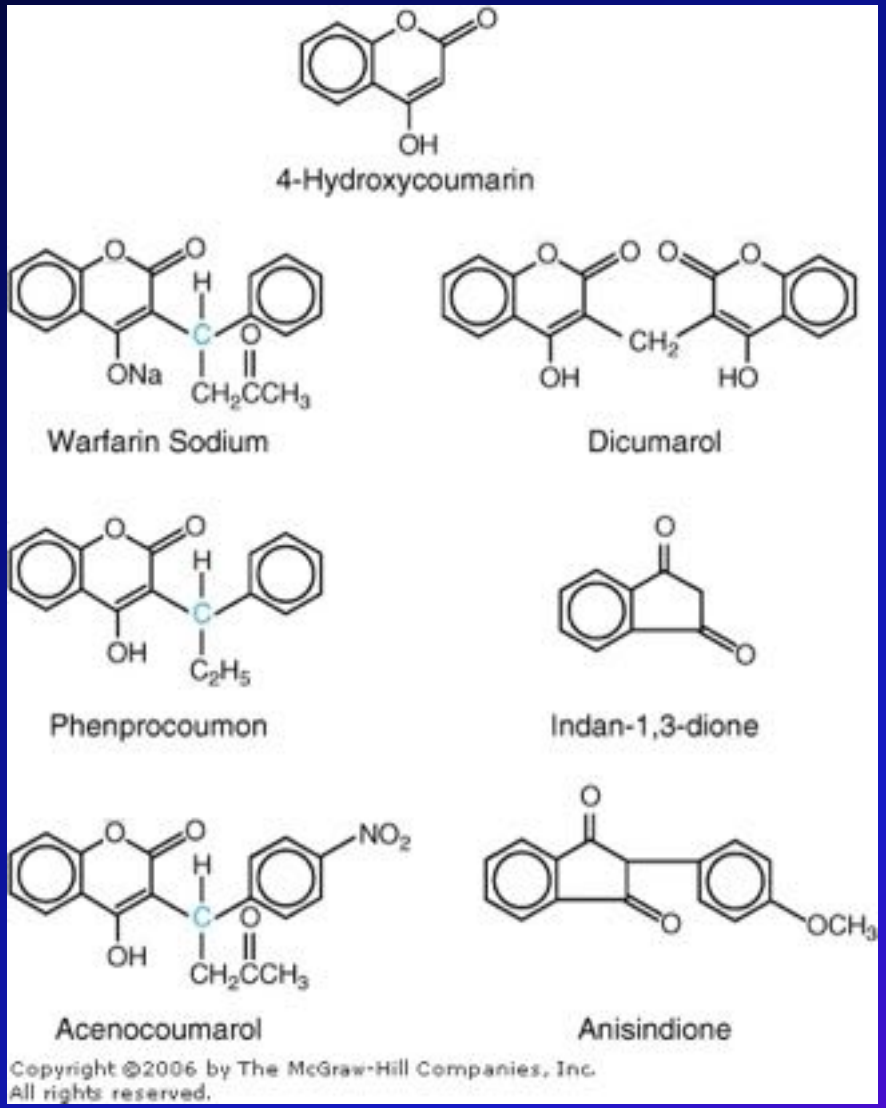
Effetti dei polimorfismi del citocromo P450 nel trattamento farmacologico di "poor metabolizers"

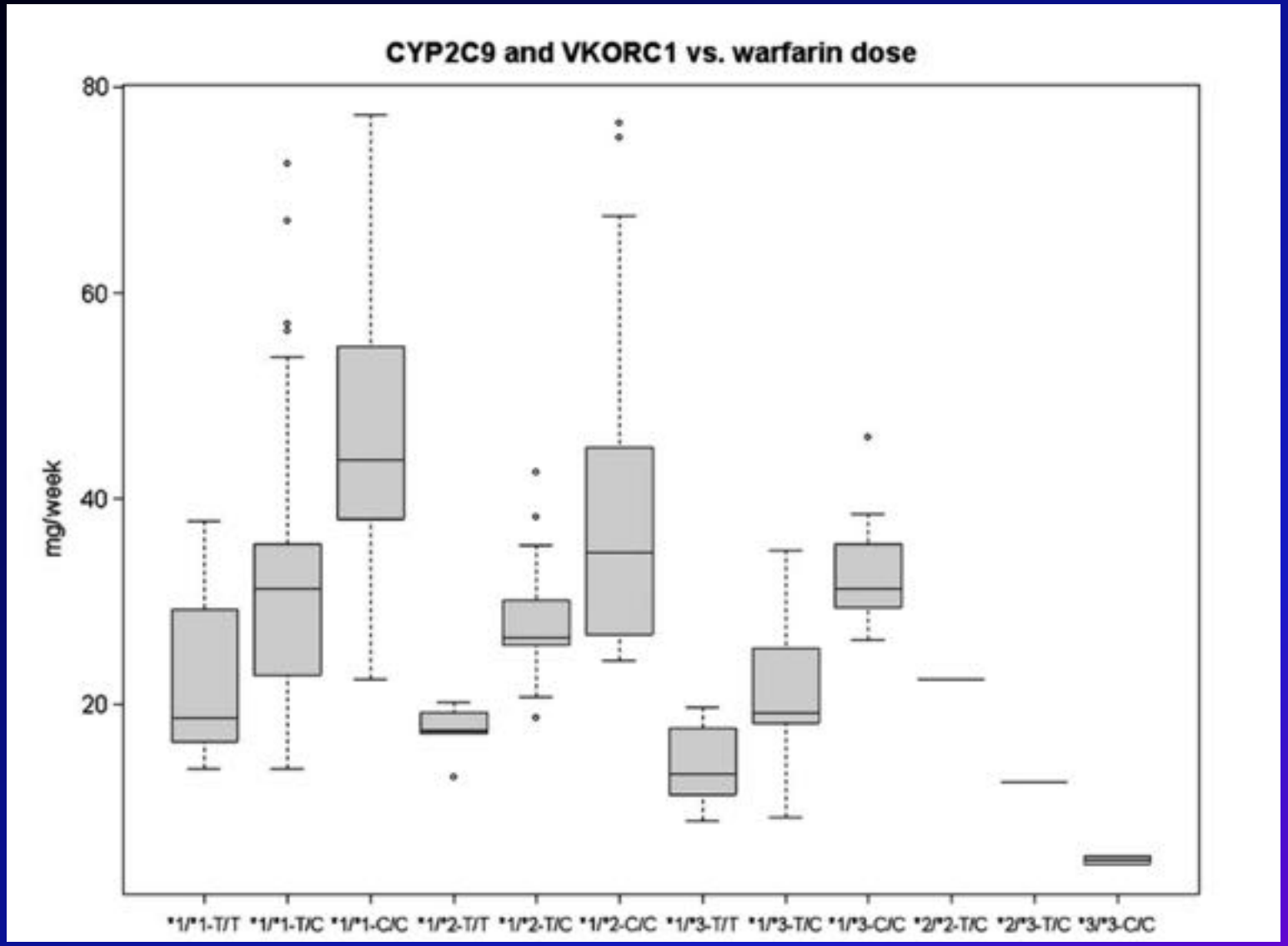
Polymorphic enzyme	Decreased clearance	Adverse effects	Reduced prodrug activation
CYP2C9	S-Warfarin	Bleeding	Losartan
	Phenytoin	Ataxia	
	Losartan		
	Tolbutamide	Hypoglycaemia	
	NSAIDs	GI-bleeding(?)	
CYP2C19	Omeprazole		Proguanil
	Diazepam	Sedation	






Cell Membrane
R-Warfarin
S-Warfarin
NADH
EPHX1
VKORC1
NAD+
Vitamin K (oxidized)
Vitamin K (reduced)
Liver cell
GGCX
Functional
Hypofunctional
F2
F9
F10
F7
GAS6
PROZ
BGLAP
PROS1
PROC
MGP
CALU
clotting
bone metabolism & connective tissue calcification
apoptosis





Wadelius et al., The Pharmacogenomics Journal; 2005

Number of Cases and Annual Estimate of Drugs Most Commonly Implicated in Adverse Events Treated in Emergency Departments--United States, 2004-2005*

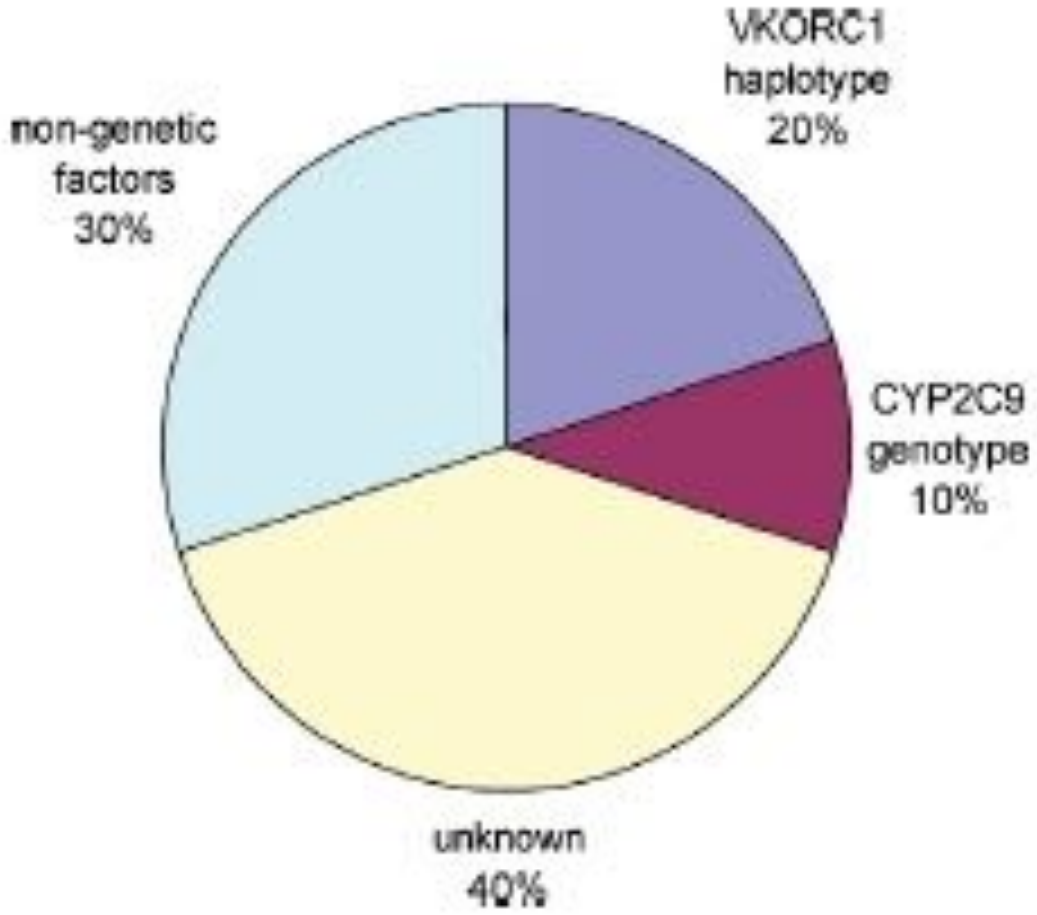
Table 5. Number of Cases and Annual Estimate of Drugs Most Commonly Implicated in Adverse Events Treated in Emergency Departments—United States, 2004-2005*

Drug	Adverse Drug Events	
	Cases, No.	Annual Estimate, No. (%)
Insulins	1577	55 819 (8.0)
Warfarin	1234	43 401 (6.2)†
Amoxicillin	1022	30 135 (4.3)
Aspirin	473	17 734 (2.5)
Trimethoprim-sulfamethoxazole	447	15 291 (2.2)
Hydrocodone-acetaminophen	420	15 512 (2.2)
Ibuprofen	526	14 852 (2.1)
Acetaminophen	497	12 832 (1.8)
Clopidogrel	241	10 931 (1.6)†
Cephalexin	293	10 628 (1.5)
Penicillin	270	9275 (1.3)
Amoxicillin-clavulanate	274	8959 (1.3)
Azithromycin	255	8794 (1.3)
Levofloxacin	230	8682 (1.2)
Naproxen	245	8634 (1.2)
Phenytoin	238	7937 (1.1)
Oxycodone-acetaminophen	227	7328 (1.0)
Metformin	179	6678 (1.0)

*Drugs implicated in ≥1% of adverse events. For 434 cases (annual estimate, 15 784 [2.2%]) 2 of these 18 drugs were implicated in the adverse event. Therefore, these 18 drugs accounted for adverse drug events in 8214 cases (annual estimate, 277 636 [39.6%]).
†Estimates with coefficient of variation >30%: warfarin, 32.5%; clopidogrel, 36.6%.

Budnitz, D. S. et al. JAMA 2006;296:1858-1866.

JAMA


VKORC1
haplotype
20%
CYP2C9
genotype
10%
unknown
40%
non-genetic
factors
30%

Polymorphic enzyme	Decreased clearance	Adverse effects	Reduced prodrug activation
CYP2D6	Tricyclic	Cardiotoxicity	Tramadol
	Antidepres.		Codeine
	Haloperidol	Parkinsonism	Ethylmor.
	Perphenazine		
	Perhexiline	Neuropathy	
	SSRIs	Nausea	
	Tolterodine		

Frequenza di individui portatori di alleli con copie multiple del citocromo P450 CYP2D6

Duplicated or multiduplicated genes

mRNA-AAAA
mRNA-AAAA
mRNA-AAAA

Higher enzyme levels

Increased metabolism

CYP2D6*2xN


1-2%
7-10%
12%
0.5%
4-5%
21%
2.5%
2%
29%
4%

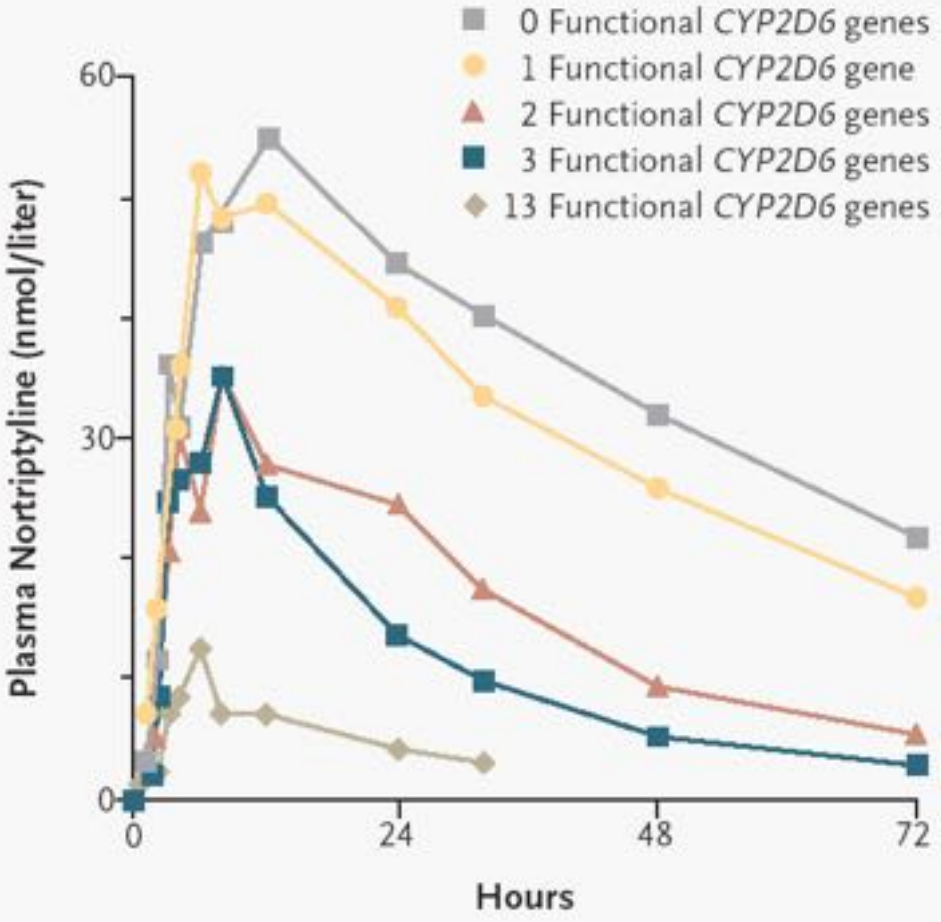
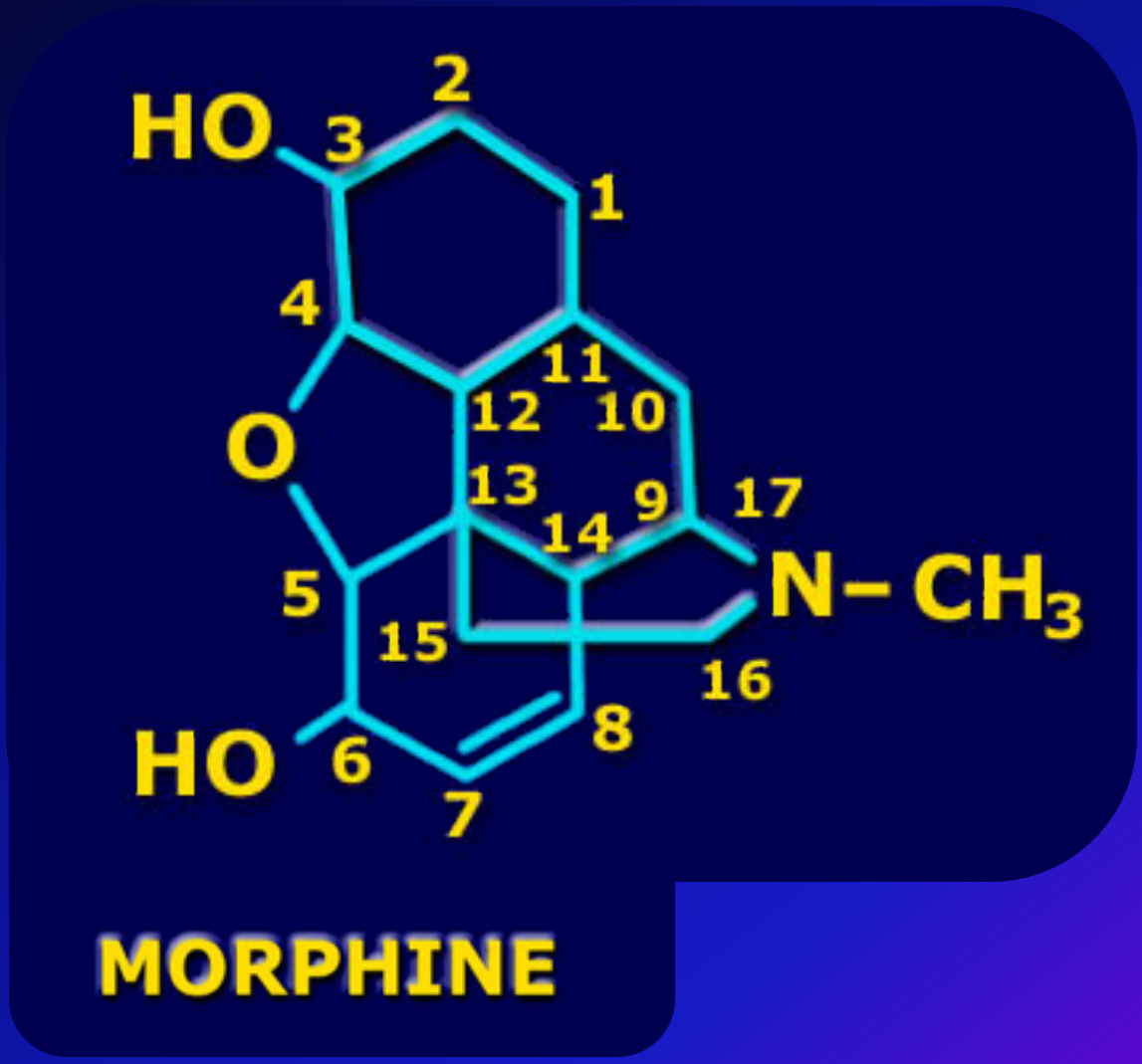


Figure 4. Pharmacogenetics of Nortriptyline.

Mean plasma concentrations of nortriptyline after a single 25-mg oral dose are shown in subjects with 0, 1, 2, 3, or 13 functional *CYP2D6* genes. Modified from Dalén et al.[23] with the permission of the publisher.

Struttura degli Oppioidi e degli antagonisti degli Oppioidi correlati chimicamente alla morfina


HO
2
3
1
4
11
12
10
O
13
9
17
14
5
N– CH3
15
16
8
HO
6
7
MORPHINE

NONPROPRIETARY NAME	CHEMICAL RADICALS AND POSITIONS		
	3	6	17
Morphine	-OH	-OH	-CH
Heroin	-OCOCH	$-OCOCH_3$	$-CH_3$
Hydromorphone	-OH	=O	$-CH_3$
Oxymorphone	-OH	=O	$-CH_3$
Levorphanol	-OH	-H	$-CH_3$
Levallorphan	-OH	-H	$-CH_2CH=CH_2$
Codeine	$-OCH_3$	-OH	$-CH_3$
Hydrocodone	$-OCH_3$	=O	$-CH_3$
Oxycodone	$-OCH_3$	=O	$-CH_3$

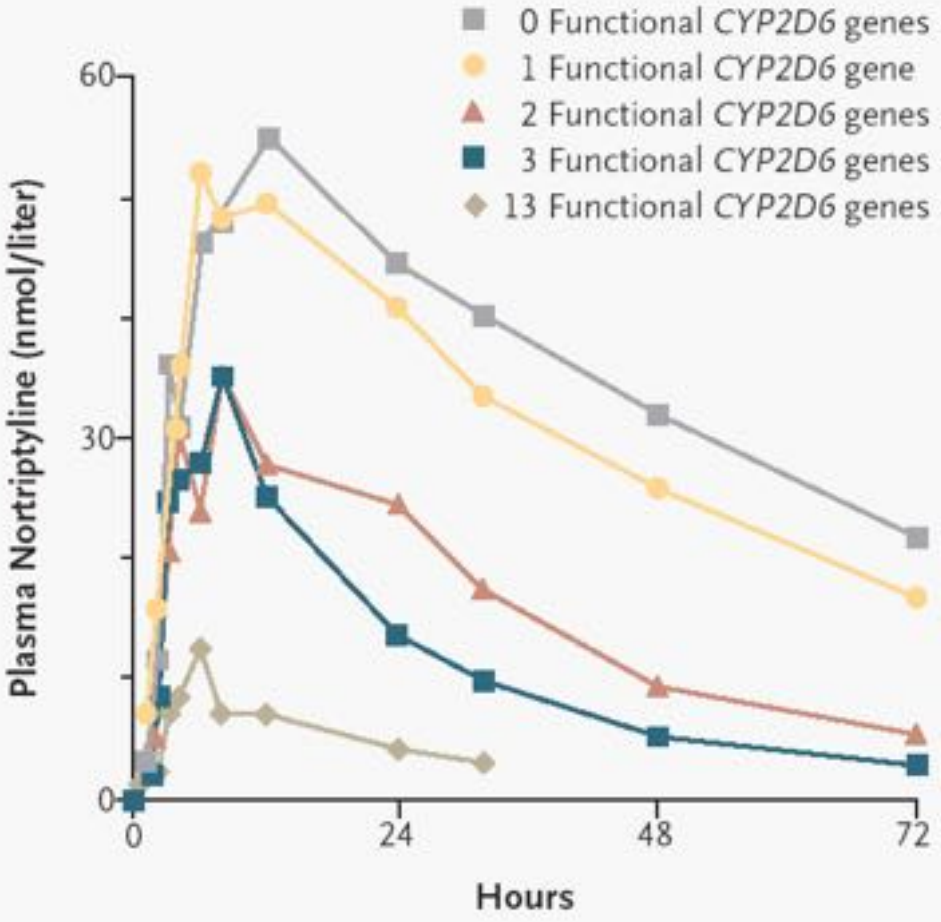


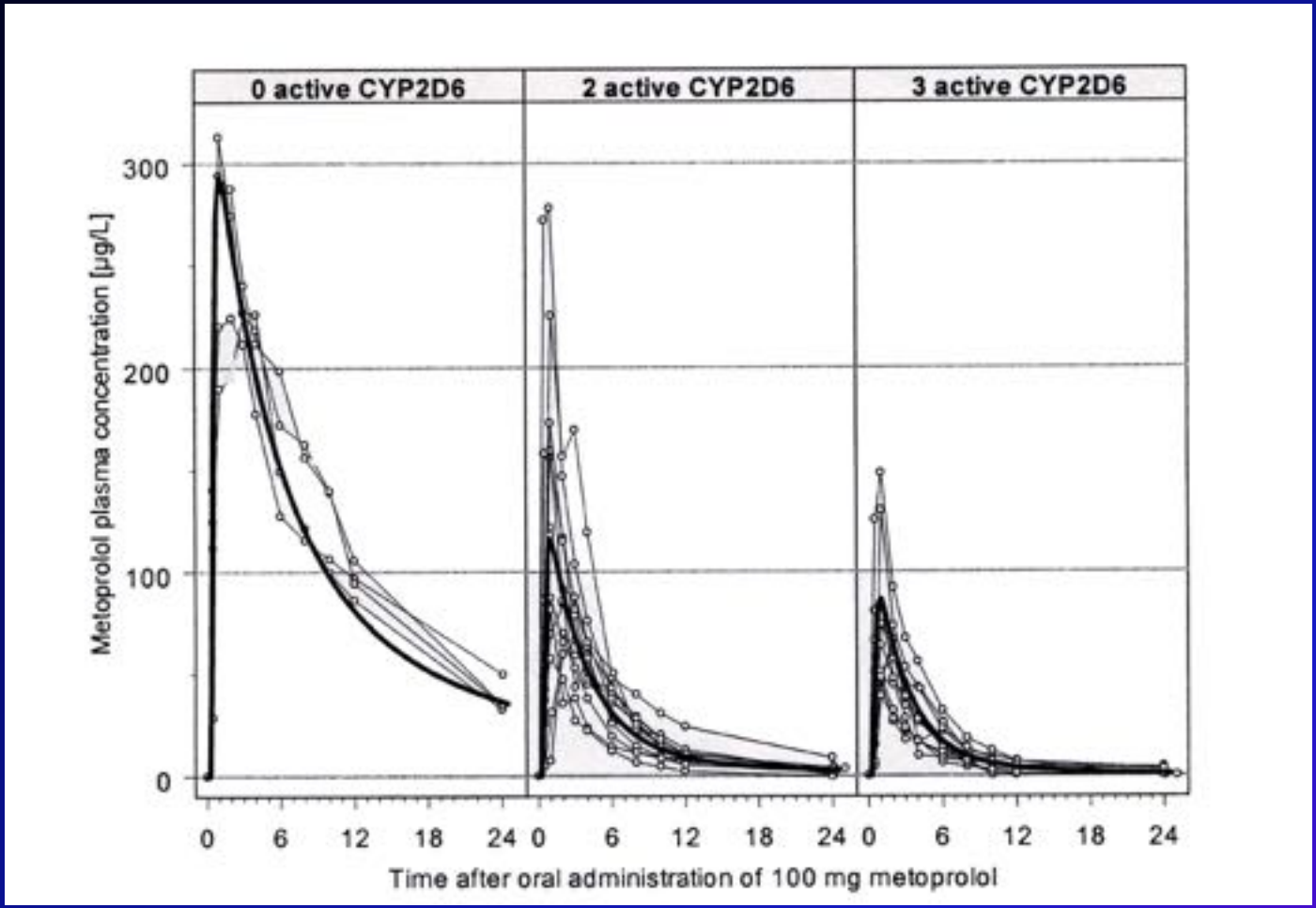
Figure 4. Pharmacogenetics of Nortriptyline.

Mean plasma concentrations of nortriptyline after a single 25-mg oral dose are shown in subjects with 0, 1, 2, 3, or 13 functional *CYP2D6* genes. Modified from Dalén et al.[23] with the permission of the publisher.

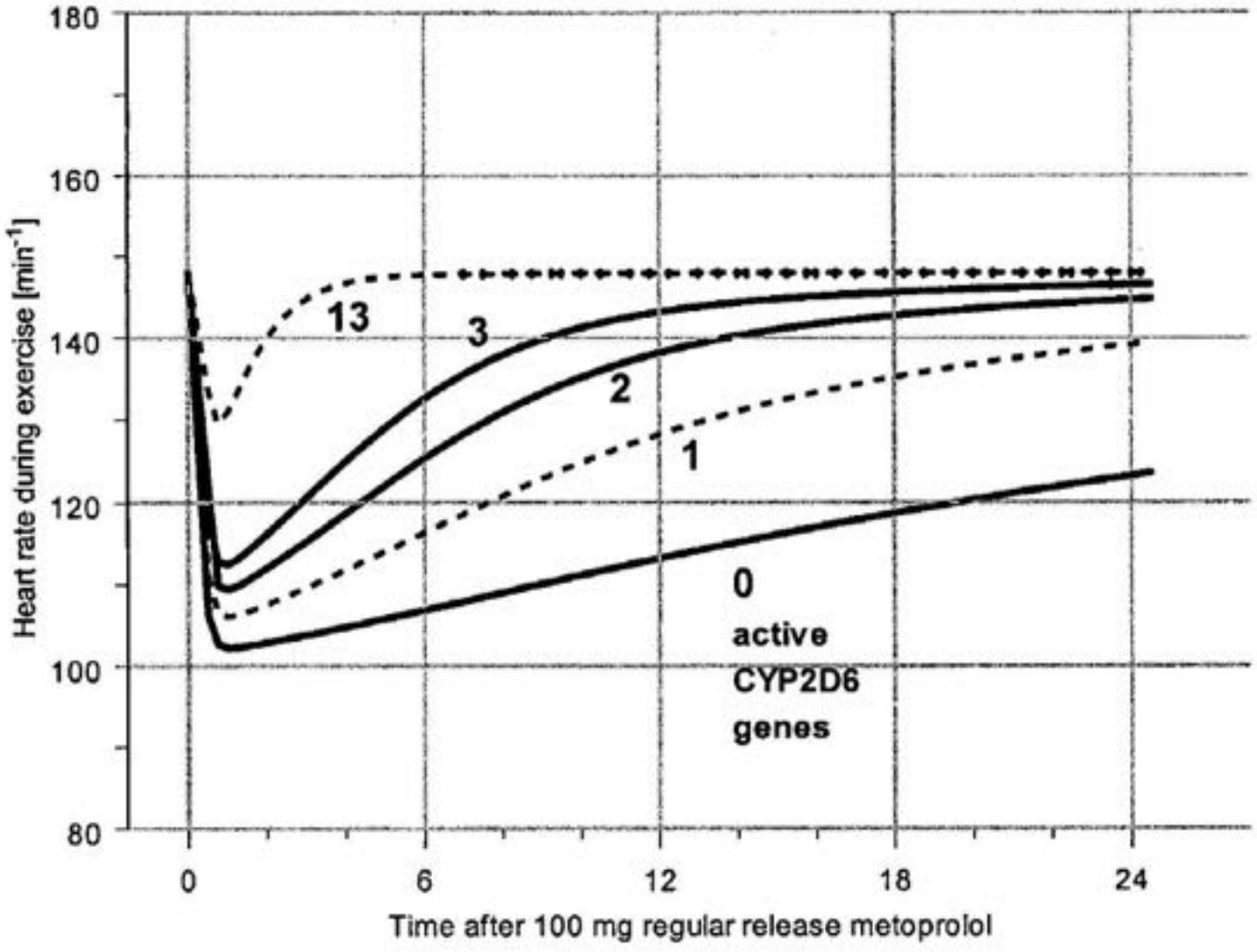
Table 3. Common Drug Substrates and Clinically Important Inhibitors of CYP2D6.

CYP2D6 Substrates	CYP2D6 Inhibitors
Beta-blockers	
Alprenolol	
Bufuralol	
Carvedilol	
Metoprolol	
Propranolol	
Timolol	
Tricyclic antidepressants	
Amitriptyline (in part)	Clomipramine
Clomipramine (in part)	
Desipramine	
Imipramine (in part)	
Nortriptyline	
Antiarrhythmic agents	
Flecainide	Quinidine
Mexiletine	
Propafenone	
Antipsychotic agents and SSRIs*	
Fluoxetine	Fluoxetine
Haloperidol	Haloperidol
Paroxetine	Paroxetine
Perphenazine	
Venlafaxine	
Opioids	
Codeine	
Dextromethorphan	

* SSRIs denotes selective serotonin-reuptake inhibitors.



Kirchheiner et al,Clin Pharmacol Ther; 2004



Kirchheiner et al,Clin Pharmacol Ther; 2004

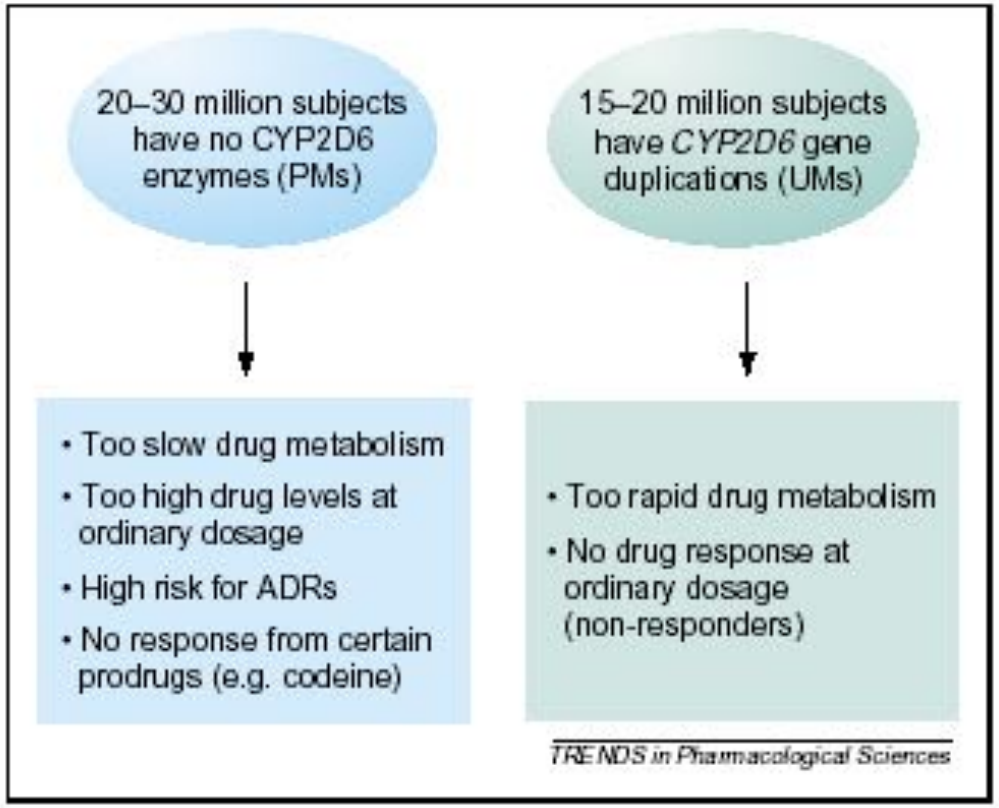
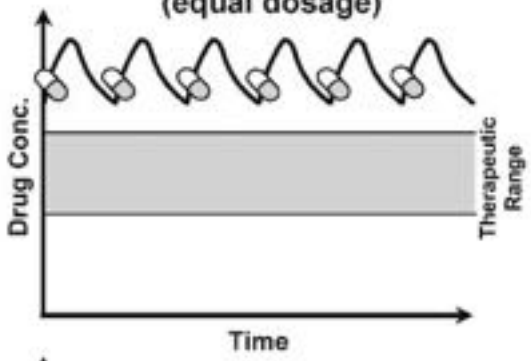
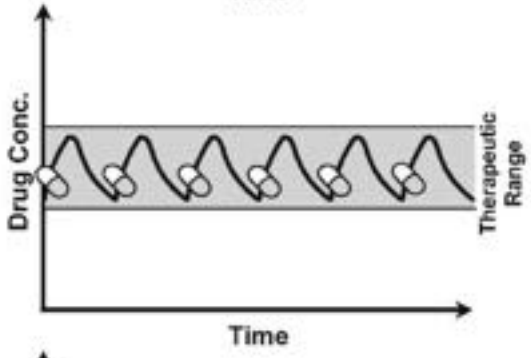
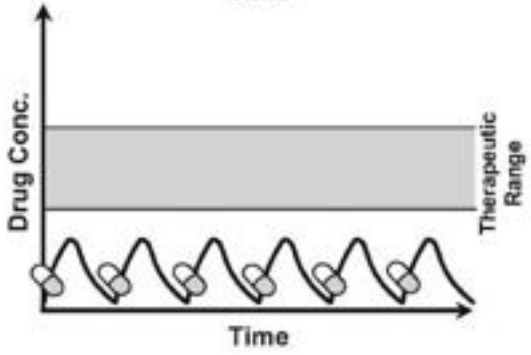


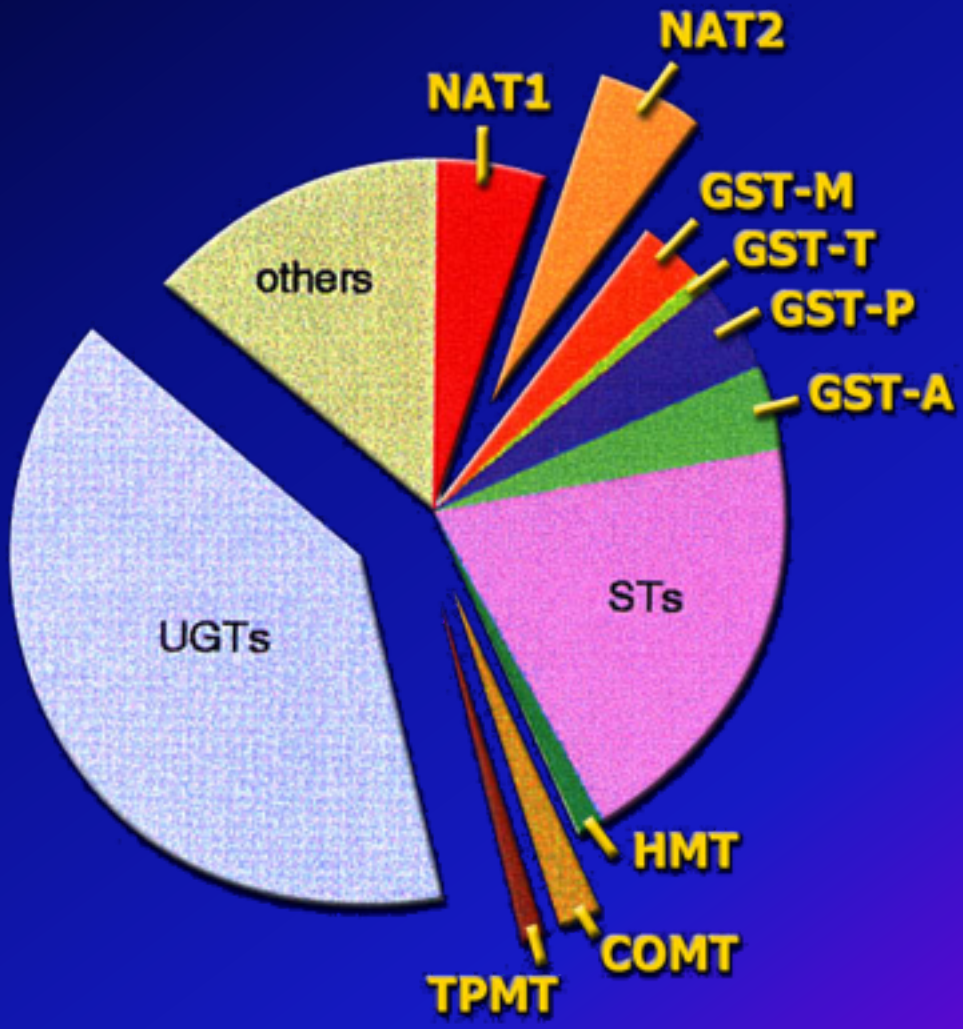
Figure 2. The consequences of outlier cytochrome P450 CYP2D6-dependent drug metabolism. 35–50 million Europeans are either poor metabolizers (PMs) (i.e. lack the functional enzyme) or ultrarapid metabolizers (UMs) (i.e. have multiple gene copies of *CYP2D6*, resulting in elevated enzyme levels), with respect to CYP2D6. As a result of the use of population-based dosing, drug treatment can result in many different effects in these subjects. Abbreviation: ADRs, adverse drug reactions.

Table 4. Number of Cases and Annual Estimate of Individuals With Adverse Drug Events Treated In Emergency Departments by Drug Class—United States, 2004-2005

Therapeutic Category (Drug Class)*	Adverse Drug Events†	
	Cases	Annual Estimate, No. (%)
Central nervous system agents	4698	150257 (21.4)
Opioid-containing analgesics	1167	41421 (5.9)
Non–opioid-containing analgesics	715	20887 (3.0)
Antidepressants and mood stabilizers	591	19817 (2.8)
Anticonvulsants	588	17887 (2.6)
Antipsychotics	443	13635 (1.9)
Benzodiazepines	288	9299 (1.3)
Non–benzodiazepine-derived sedatives	182	6375 (0.9)
Stimulants	177	4152 (0.6)
Anesthetics	92	3176 (0.5)
Other central nervous system agents or central nervous system agents from different classes	455	13608 (1.9)

Mutation	CYP2D6 Function	Effect on steady state concentration (equal dosage)	Clinical effect	Possible consequences
> 50 mutations with defective enzymes or complete deletions	reduced or completely absent → poor metabolizer (PM)		• toxicity • adverse drug effects	• reduce dose • change medication and avoid substrates of CYP2D6
homozygous or heterozygous wild type	normal → extensive metabolizer (EM)		• desired concentration range • efficient therapy	
duplication or multiplication of functional gene	enhanced metabolism → ultra rapid metabolizer (UM)		• ineffective therapy	• prescribe megadose or comedicate other CYP2D6 substrate and monitor concentration • avoid CYP2D6 substrates

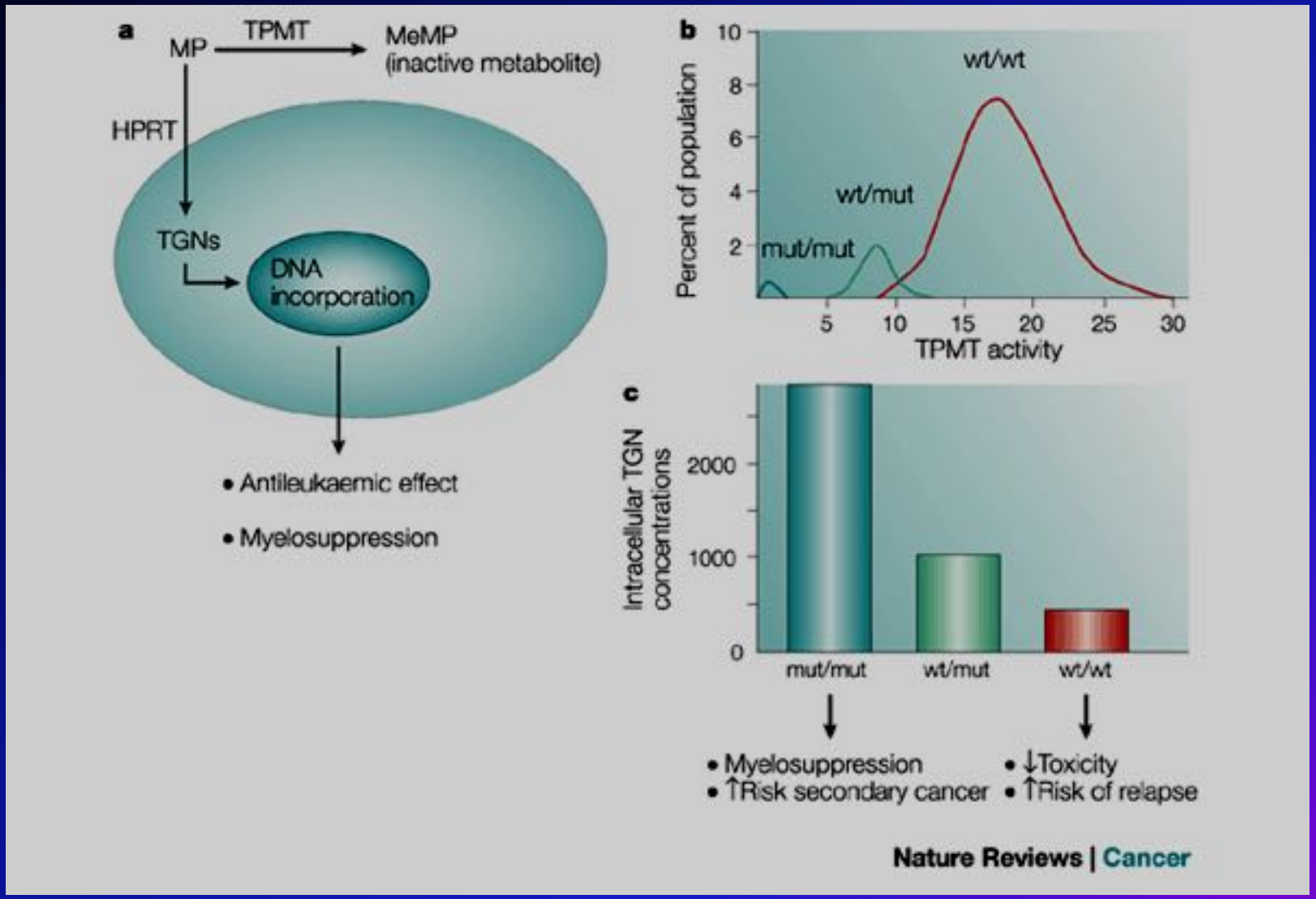
PHASE II



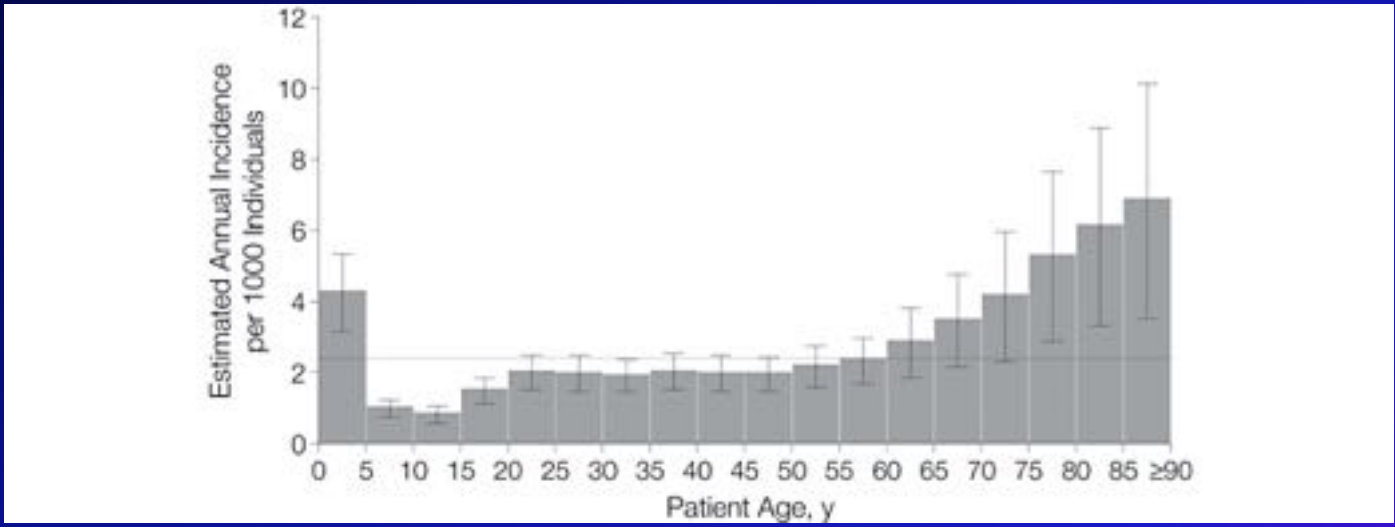
MERCAPTOPURINE

THIOGUANINE

AZATHIOPRINE


a
MP
TPMT
MeMP
(inactive metabolite)
HPRT
TGNs
DNA
incorporation
• Antileukaemic effect
• Myelosuppression
b
Percent of population
10
8
6
4
2
wt/wt
wt/mut
mut/mut
5
10
15
20
25
30
TPMT activity
c
Intracellular TGN
concentrations
2000
1000
0
mut/mut
wt/mut
wt/wt
• Myelosuppression
• ↑Risk secondary cancer
• ↓Toxicity
• ↑Risk of relapse
Nature Reviews | Cancer

Estimated Annual Incidence of Adverse Drug Events Treated in US Emergency Departments



Budnitz, D. S. et al. JAMA 2006;296:1858-1866.

JAMA

Systemic antimicrobial agents	3867	127 807 (18.2)
Amoxicillin-containing agents	1150	35 228 (5.0)
Quinolones	445	16 074 (2.3)
Sulfonamide-containing agents	446	15 593 (2.2)
Cephalosporins	454	15 369 (2.2)
Erythromycins and macrolides	329	11 833 (1.7)
Penicillin	233	7848 (1.1)
Antivirals, antiparasitics, and antifungals	141	4338 (0.6)
Tetracyclines	106	3662 (0.5)
Lincomycins	100	3332 (0.5)
Metronidazole	59	1815 (0.3)
Other antimicrobial agents, unspecified antimicrobials, or drugs from different classes of antimicrobial agents	404	12 715 (1.8)

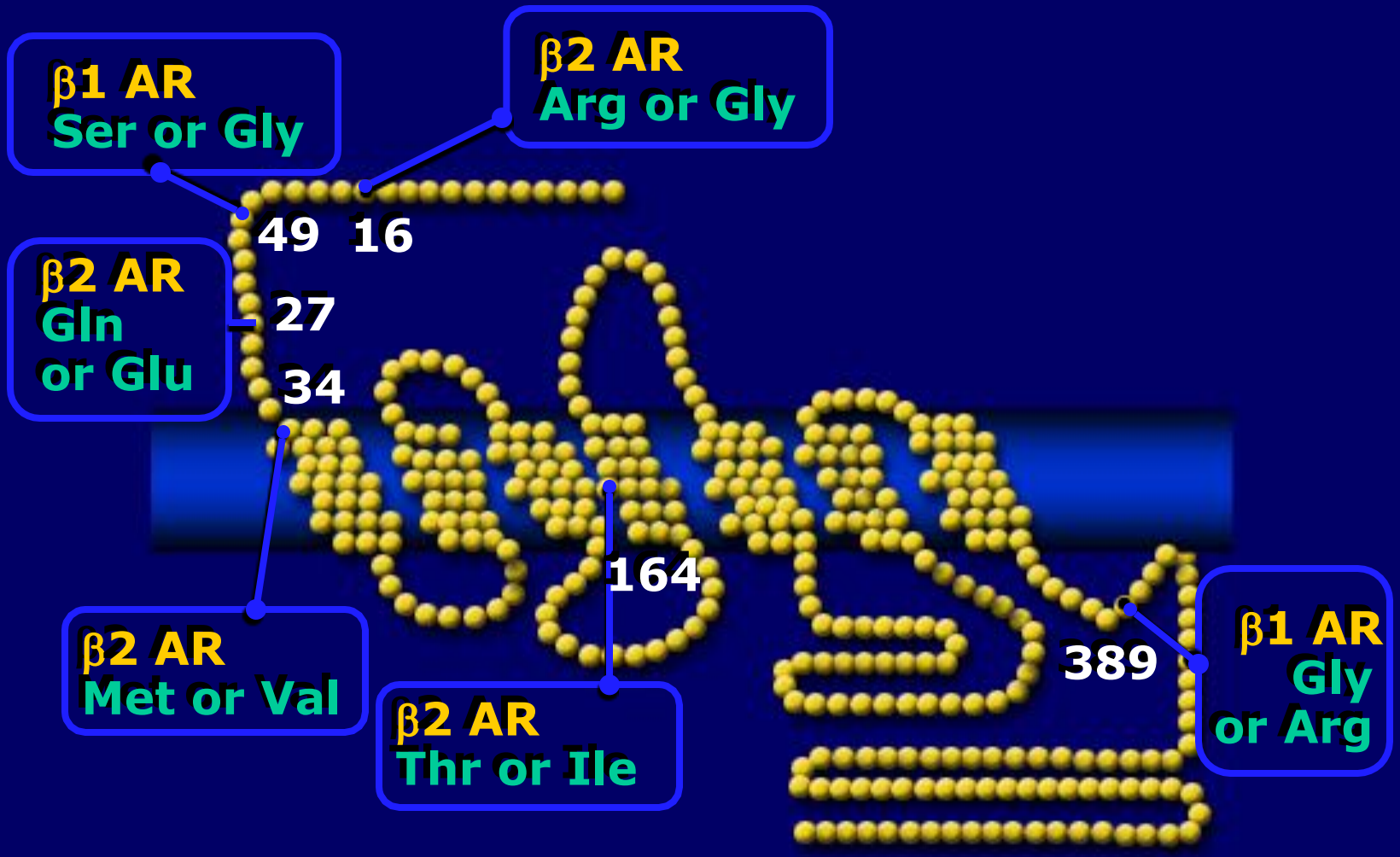
Polimorfismi genetici in bersagli primari di farmaci ed effetti sulla risposta farmacologica

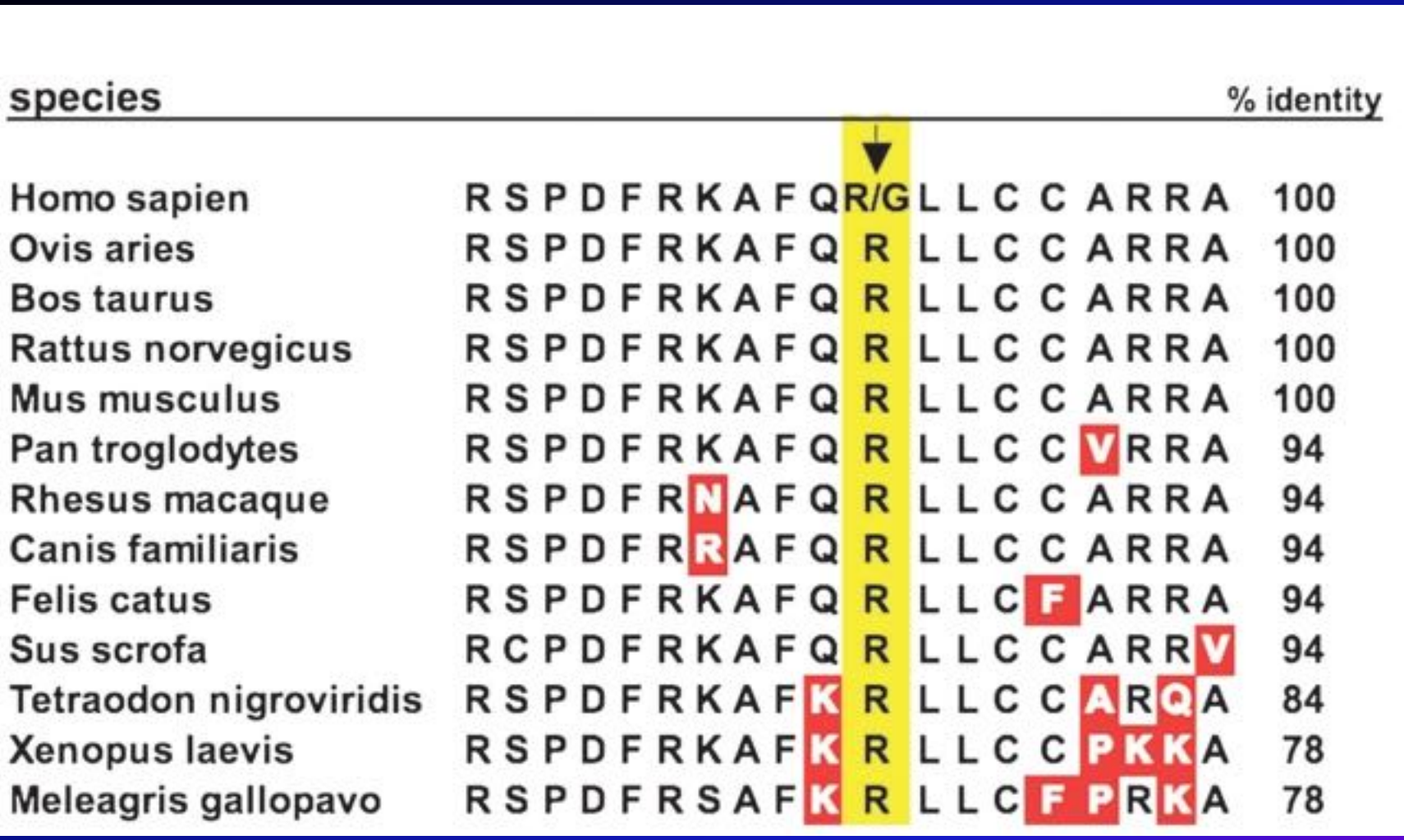
Receptor/target	Medication
b2-Adrenergic receptor	Albuterol
5-Lipoxygenase promoter	ABT-761 (zileuton)
Angiotensin-converting enzyme (ACE)	Enalapril, lisinopril, captopril
Cholesteryl ester transfer protein	Pravastatin
Stromelysin	Pravastatin
Angiotensin-II T receptor	Perindopril nitrendipine

Receptor/target	Medication
Sulfonylurea receptor	Tolbutamide
5-Hydroxytryptamine 2C receptor	Clozapine
5-Hydroxytryptamine 2A receptor	Clozapine and other neuroleptics
Serotonin transporter promoter	Fluvoxamine
Dopamine D2 and D3 receptors	Antipsychotics
Vitamin D receptor	1,25-Dihydroxy vitamin D3
Glucocorticoid receptor	Dexamethasone

Receptor/target	Medication
Nicotinic receptor	Acetylcholine (-) nicotine
Delta opioid receptor	Heroin
Potassium channels	
HERG	Quinidine
	Cisapride
KvLQT1	Terfenadine, disopyramide meflaquine
hHCNE2	Clarithromycin

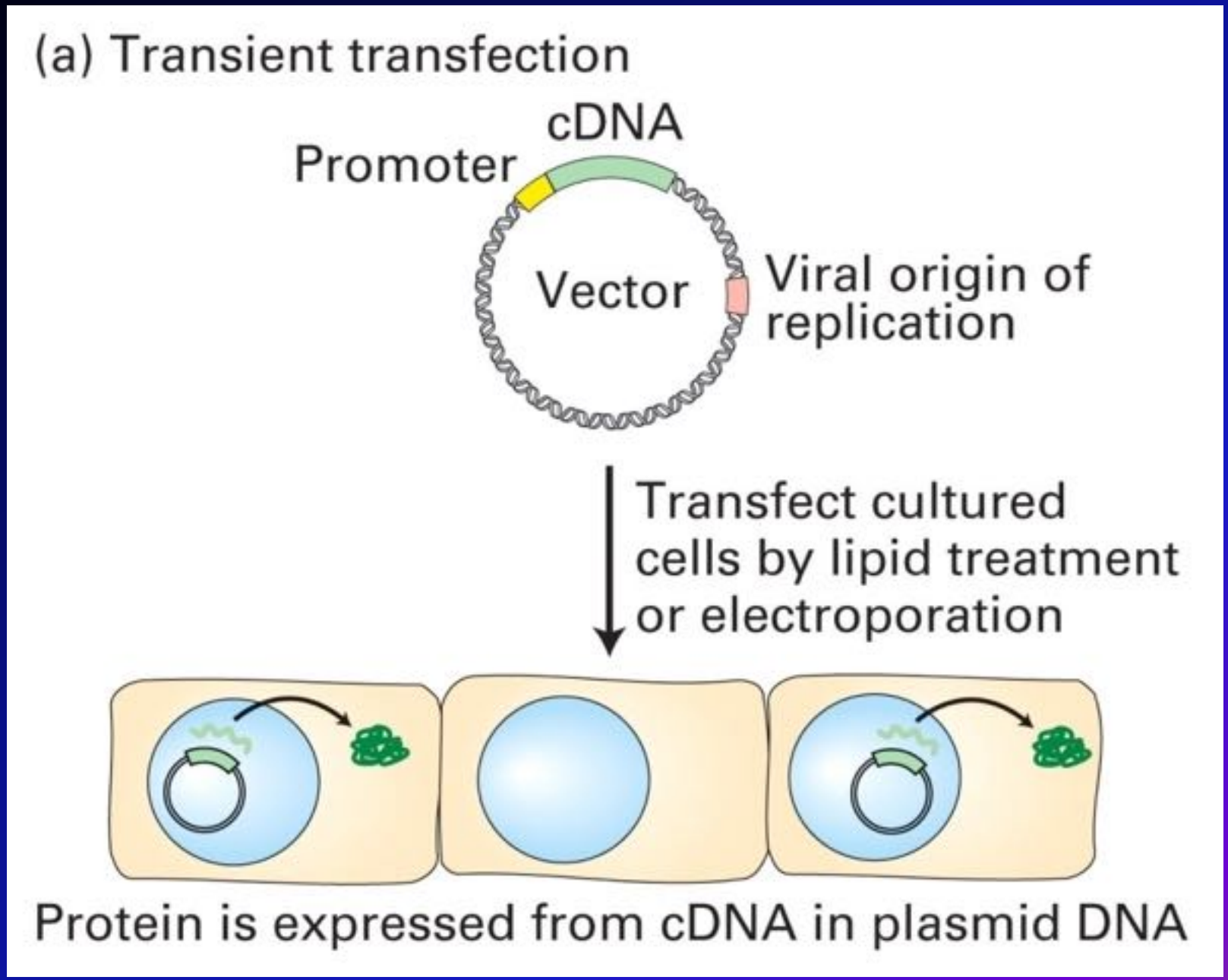
Receptor/target	Medication
Sodium channels	
SCN5A	Mexiletine
Inositol-p1p	Lithium
HLA-DRB1	Cyclosporin A
Apolipoprotein E4	Tacrine
Ryanodine receptor	Halothane or succinylcholine
Prothrombin	Oral contraceptives
Peroxisome proliferator-activated receptor	Insulin

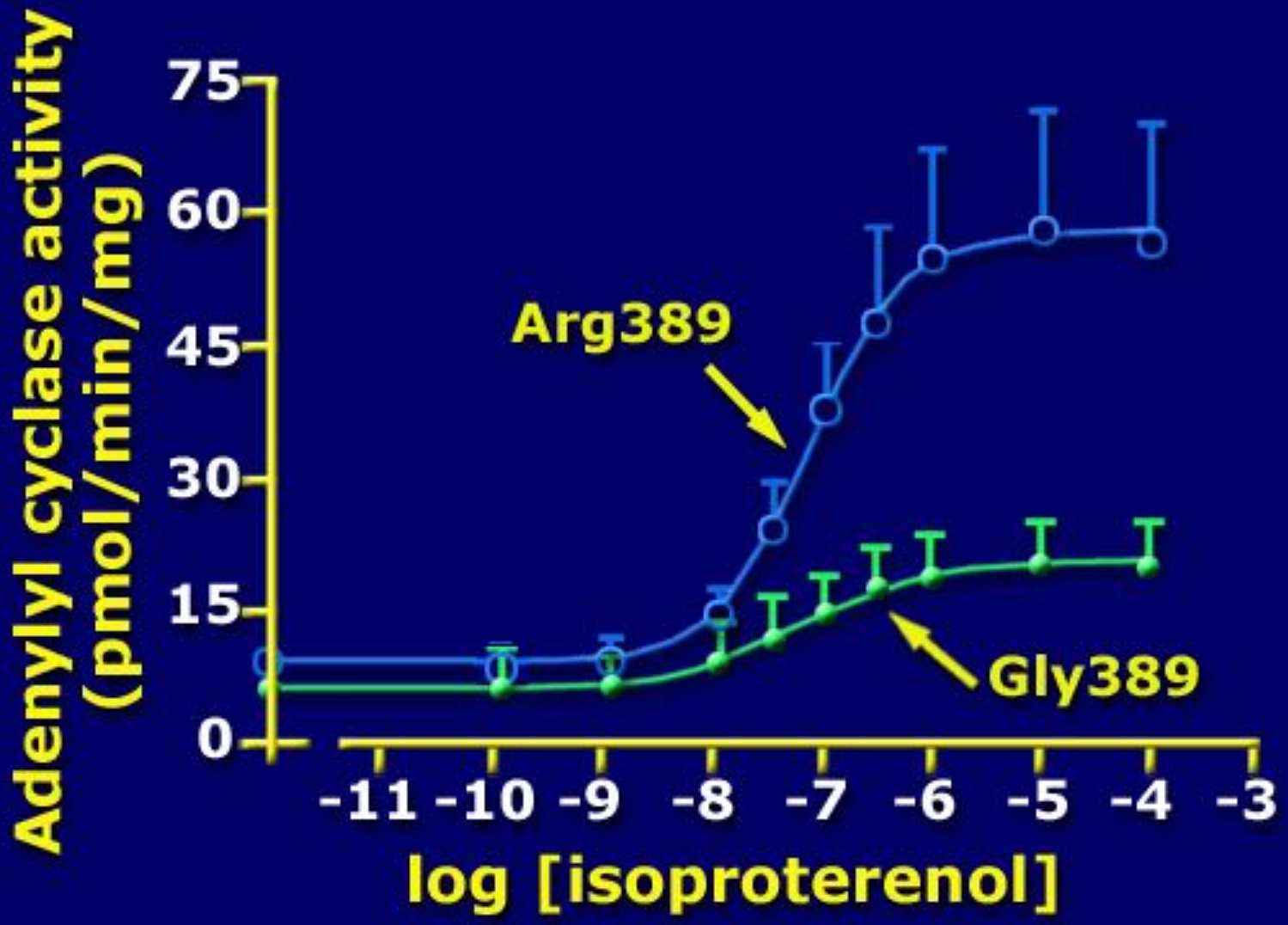

β1 AR
Ser or Gly
β2 AR
Arg or Gly
49
16
β2 AR
Gln
or Glu
27
34
164
β2 AR
Met or Val
β2 AR
Thr or Ile
389
β1 AR
Gly
or Arg

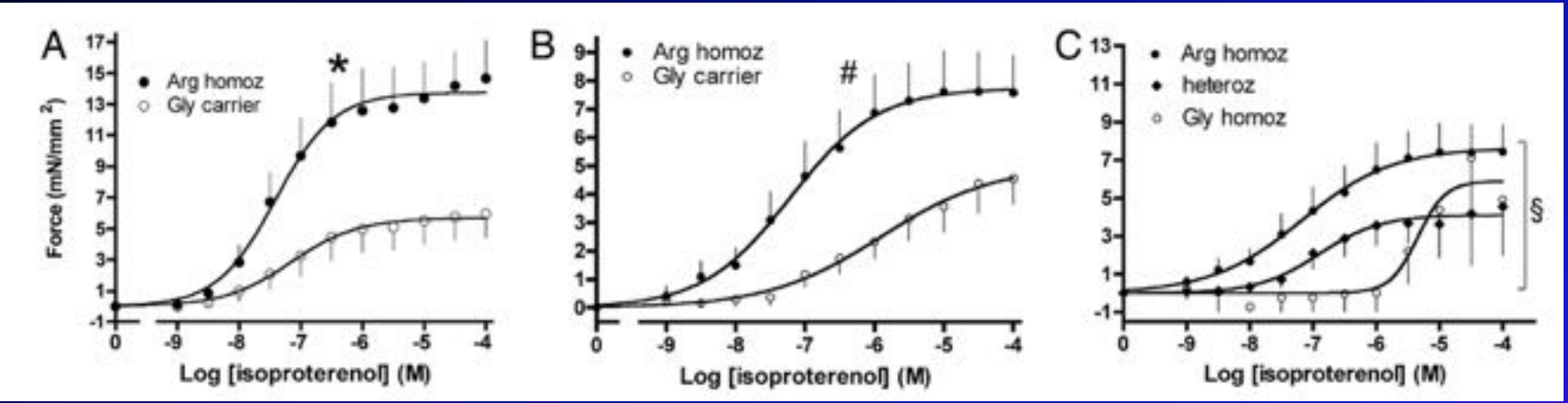


Liggett et al, PNAS; 2006

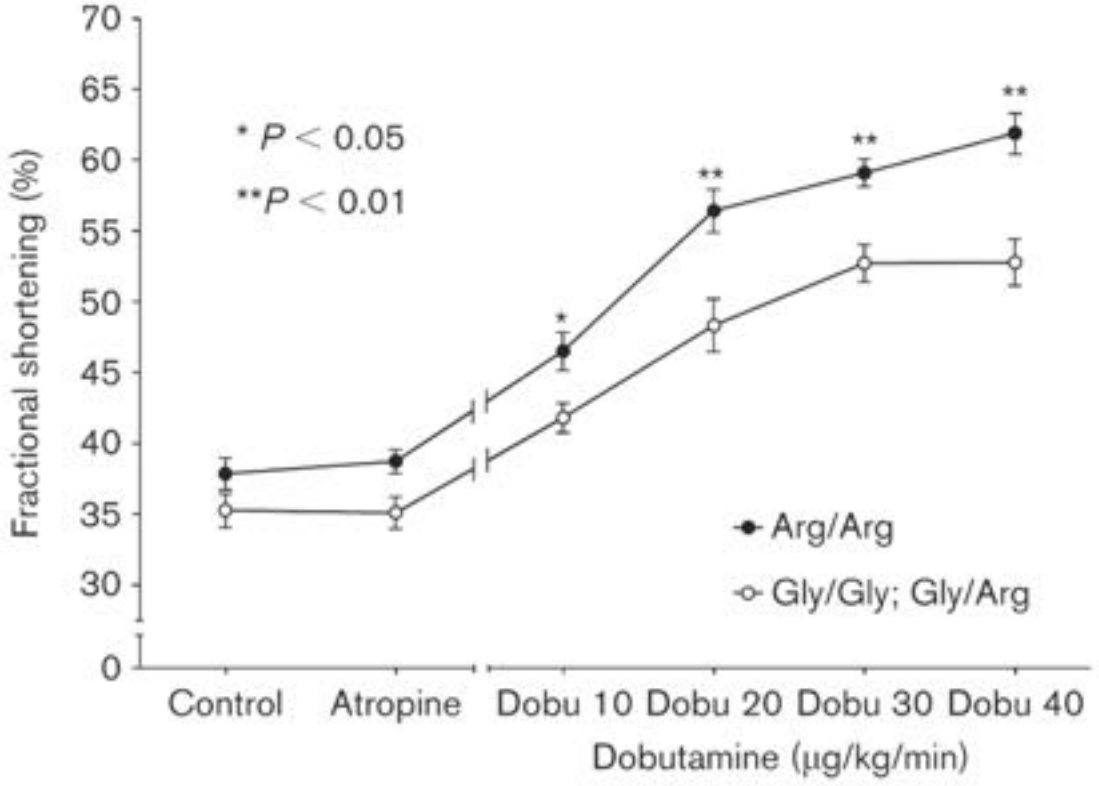
Effetti farmacologici del polimorfismo in posizione 389 del recettore Beta-1


(a) Transient transfection
cDNA
Promoter
Vector
Viral origin of replication
Transfect cultured cells by lipid treatment or electroporation
Protein is expressed from cDNA in plasmid DNA


Adenylyl cyclase activity
(pmol/min/mg)
75
60
45
30
15
0
Arg389
Gly389
-11 -10 -9 -8 -7 -6 -5 -4 -3
log [isoproterenol]

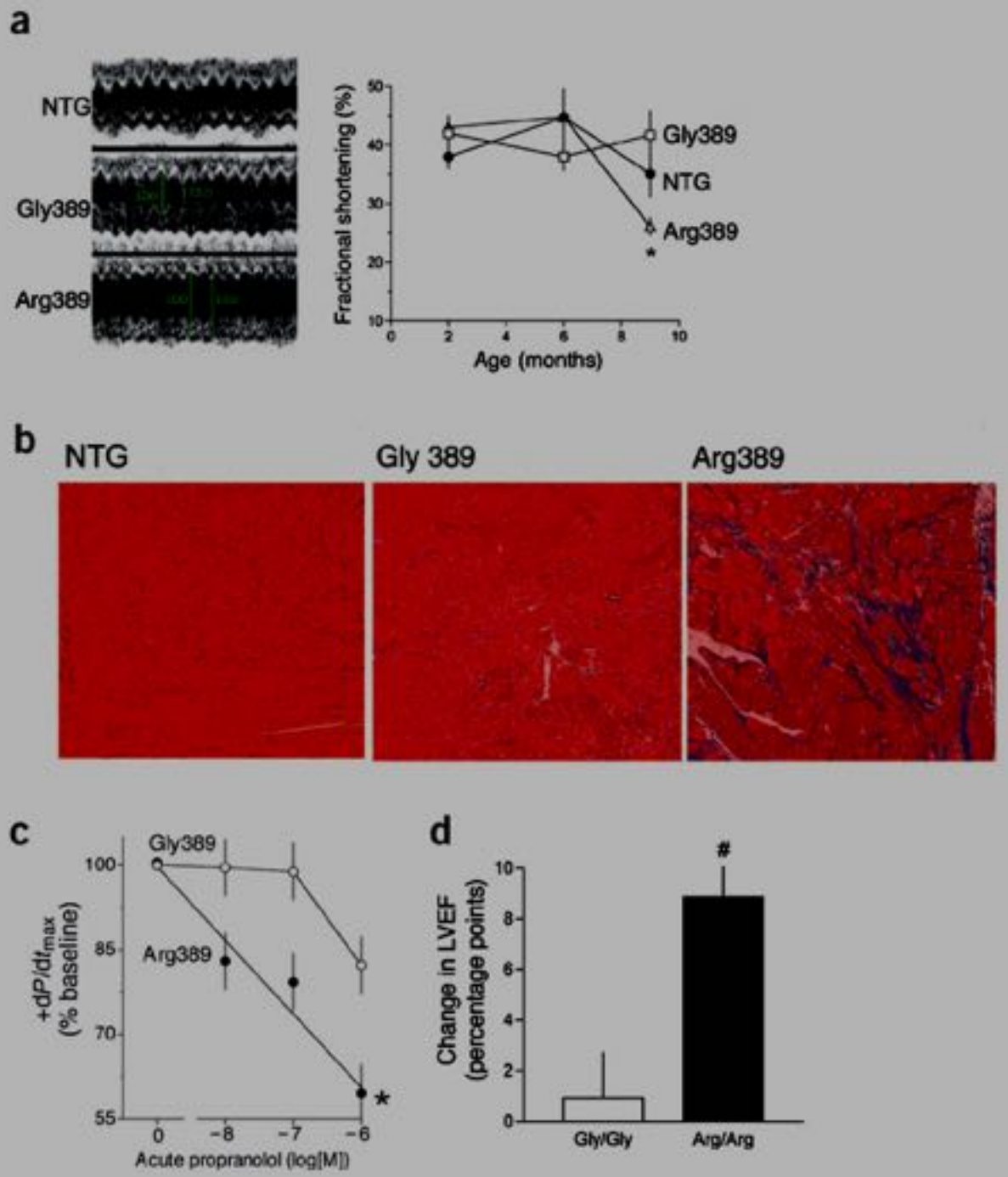


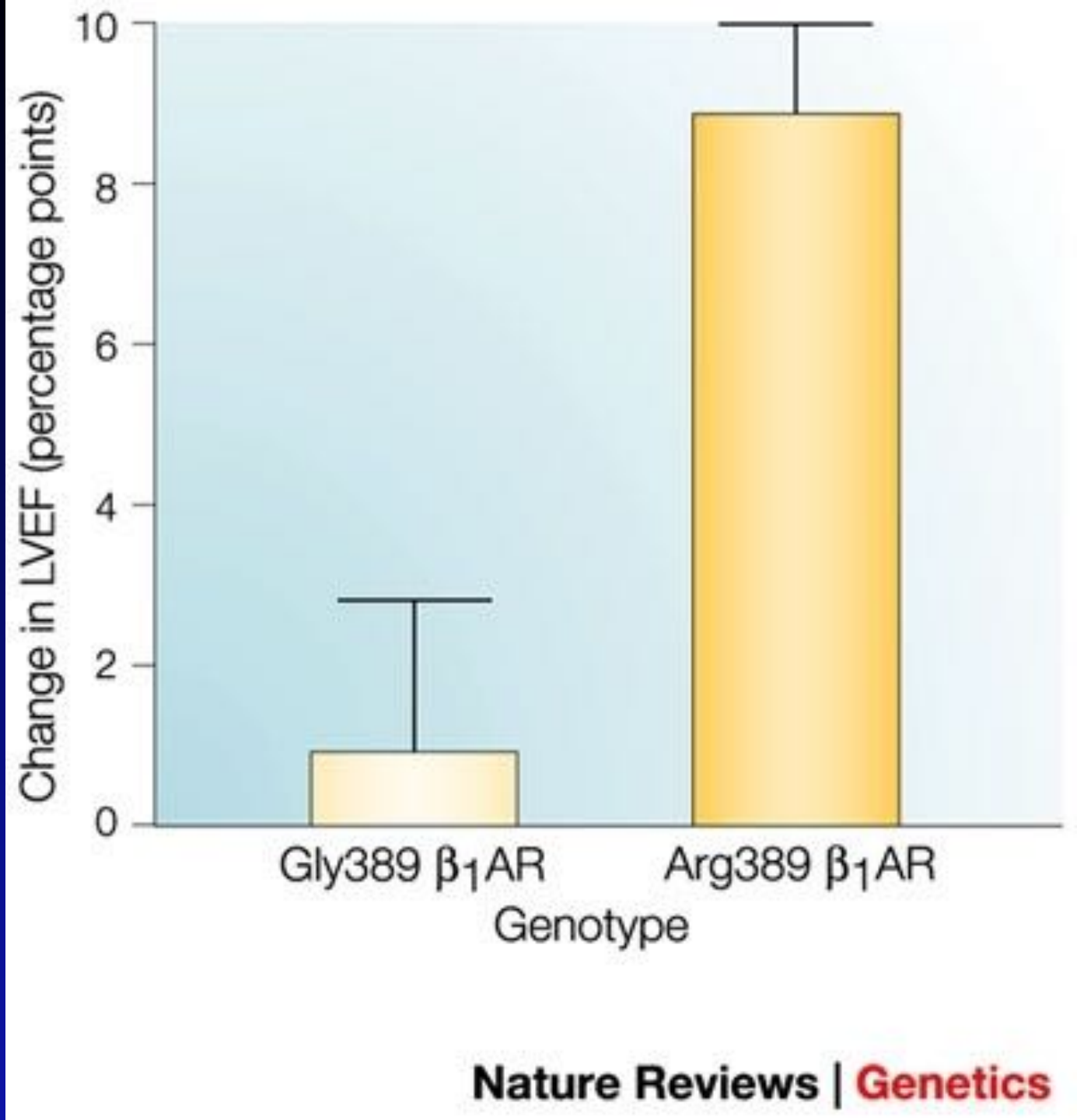
Liggett et al, PNAS; 2006

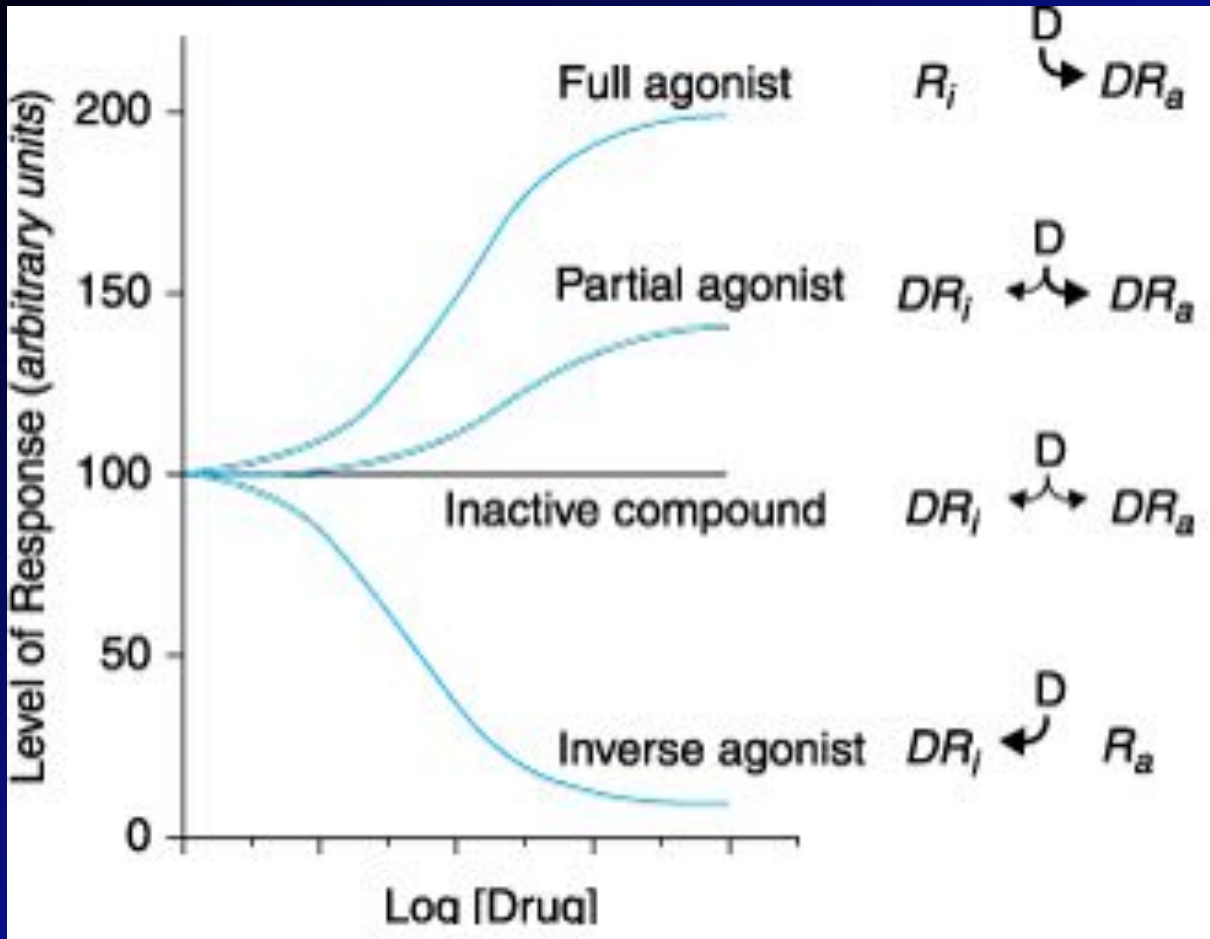


Dobutamine-induced increase in fractional shortening (FS) in healthy males measured by M-mode echocardiography. Continuous infusion of increasing doses of dobutamine (10, 20, 30, 40 µg/kg/min) was applied after bolus application of atropine (0.015 mg/kg) in healthy males homozygous for arginine (Arg/Arg) ($n = 20$) versus those homozygous ($n = 2$) or heterozygous ($n = 8$) for glycine (Gly/Gly; Gly/Arg).

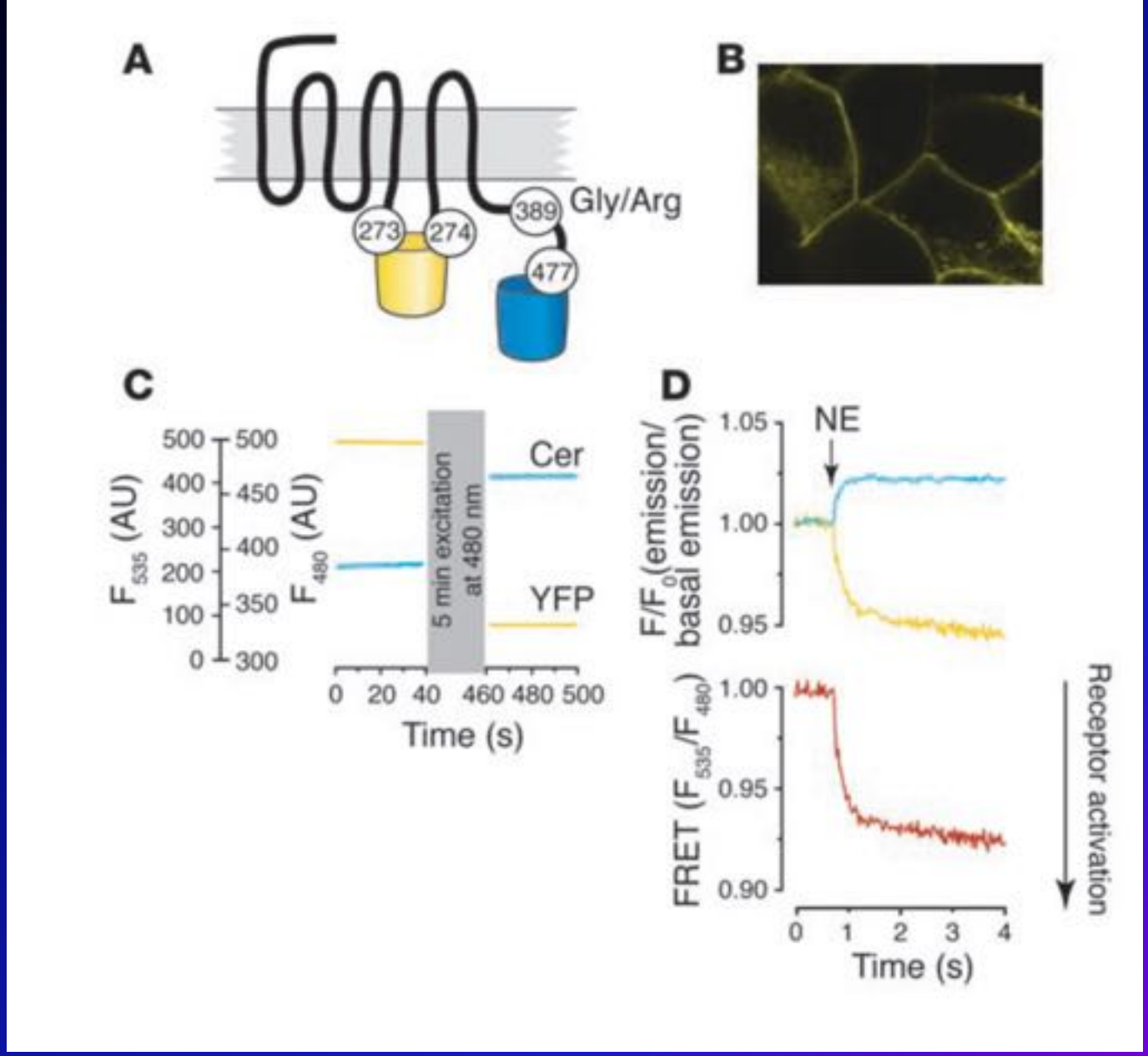
Rosee et al., Pharmacogenetics; 2004


a
NTG
Gly389
Arg389
Fractional shortening (%)
50
40
30
20
10
0
2
4
6
8
10
Age (months)
Gly389
NTG
Arg389
*
b
NTG
Gly 389
Arg389
c
Gly389
Arg389
100
85
70
55
+dP/dtmax (% baseline)
0
−8
−7
−6
Acute propranolol (log[M])
*
d
Change in LVEF (percentage points)
10
8
6
4
2
0
#
Gly/Gly
Arg/Arg

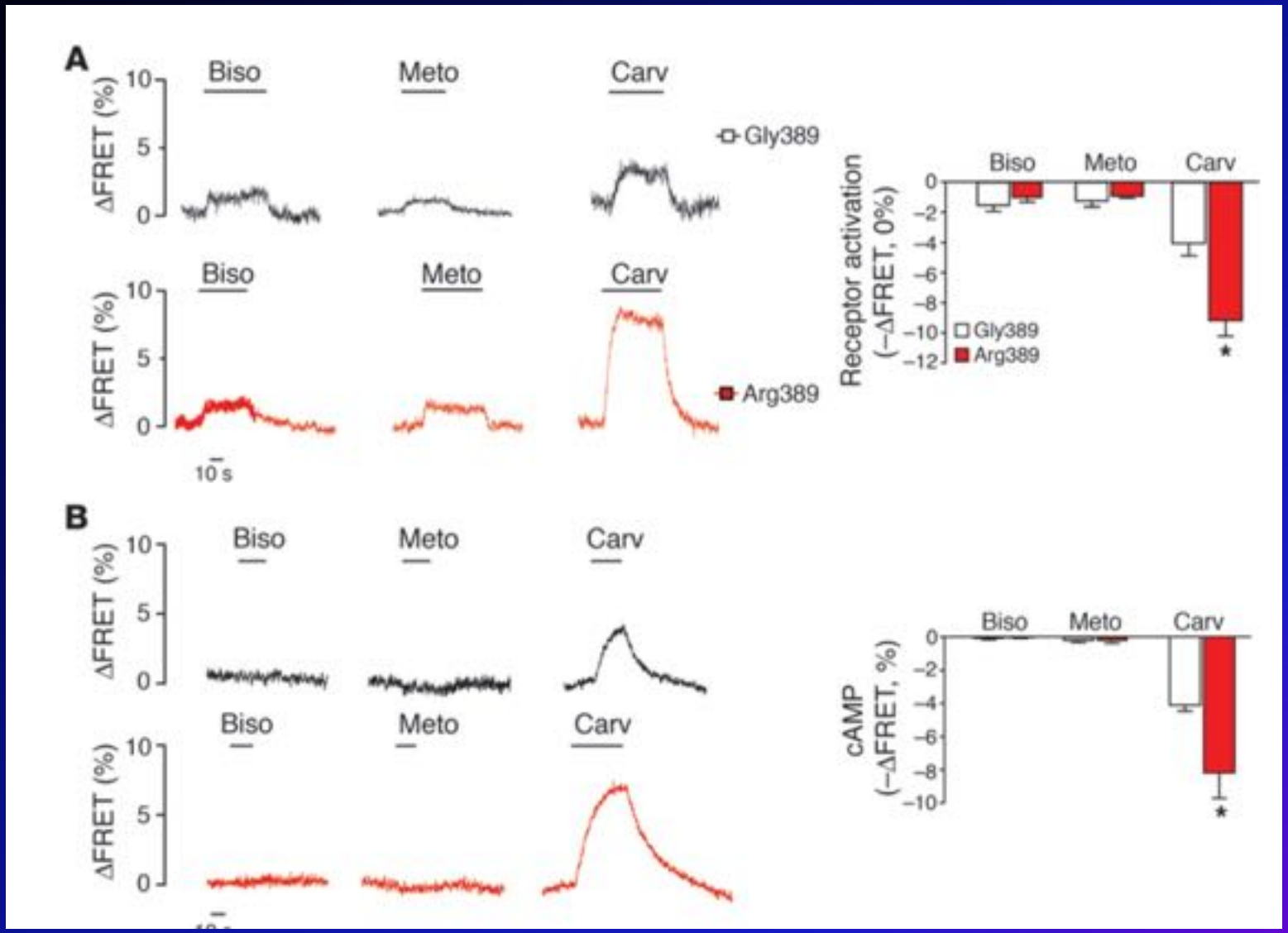

10
8
6
4
2
0
Change in LVEF (percentage points)
Gly389 β1AR
Arg389 β1AR
Genotype
Nature Reviews | Genetics



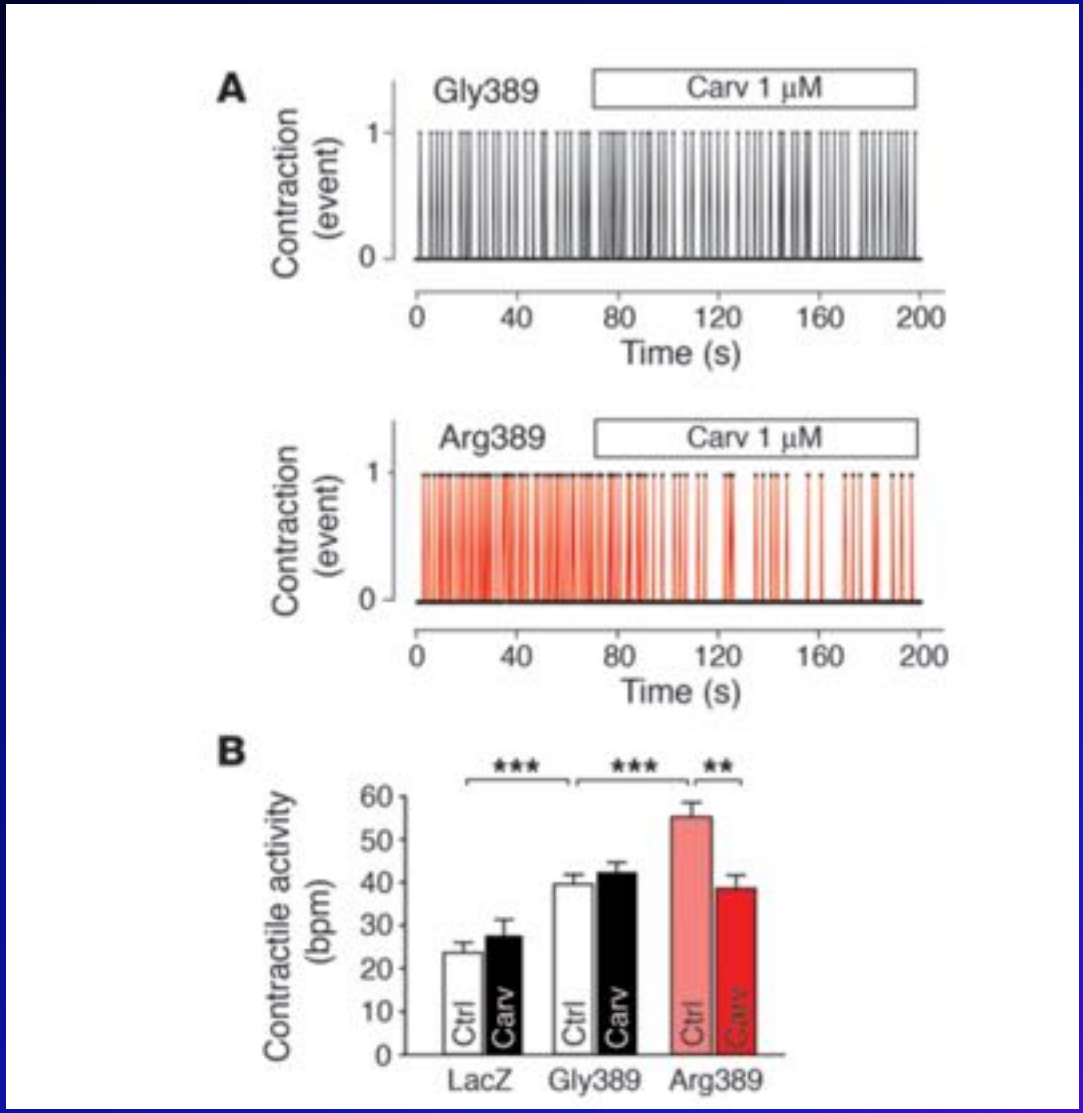
Source: Brunton LL, Lazo JS, Parker KL: *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 11th Edition: http://www.accessmedicine.com



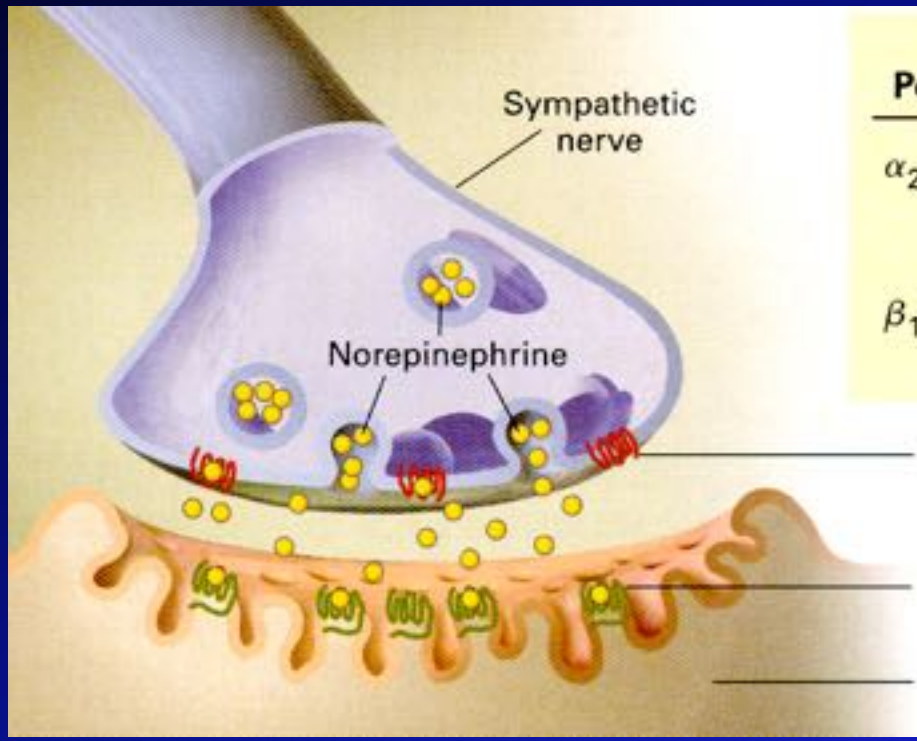
Rochais et al., The Journal of Clinical Investigation; 2007



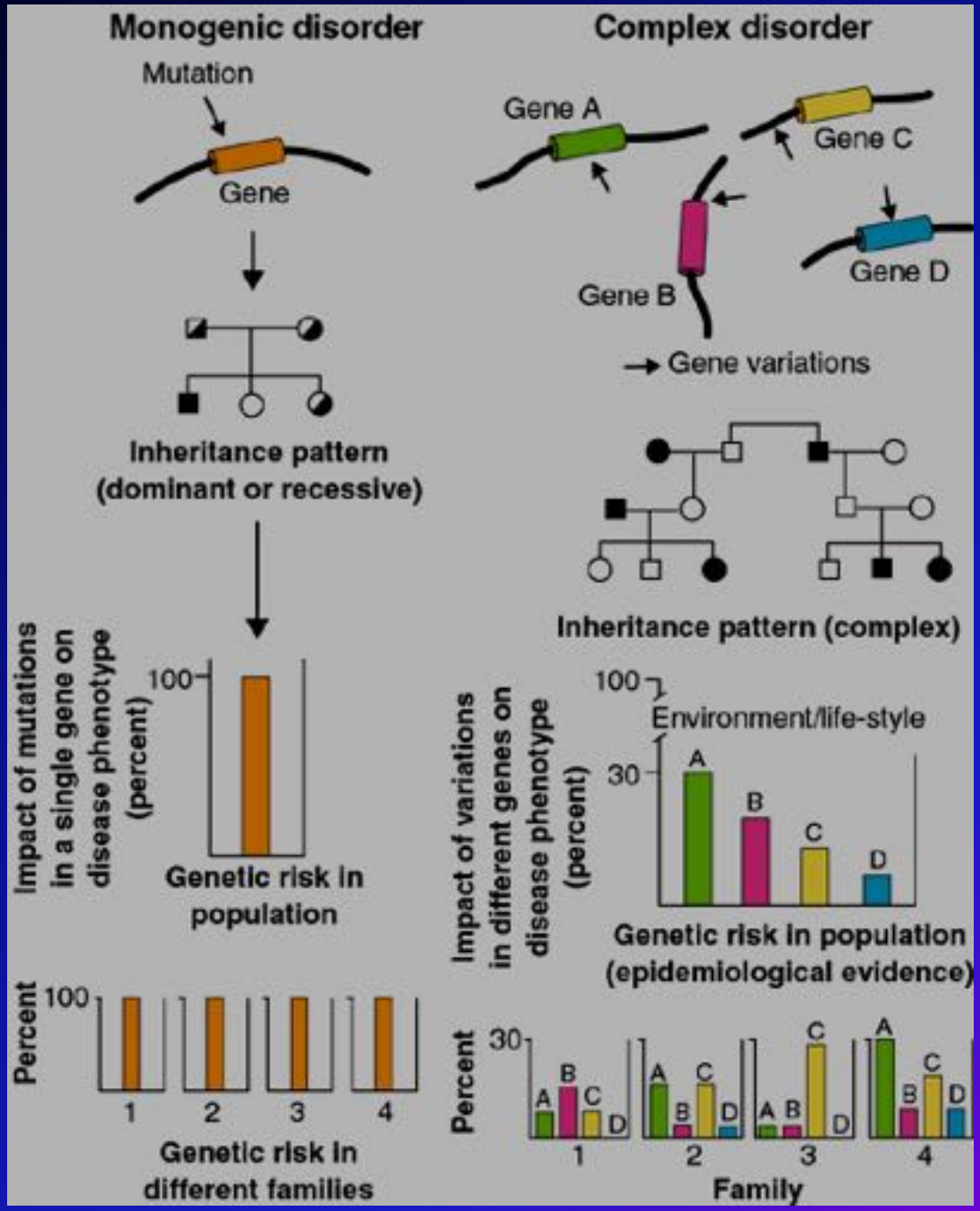
Rochais et al., The Journal of Clinical Investigation; 2007

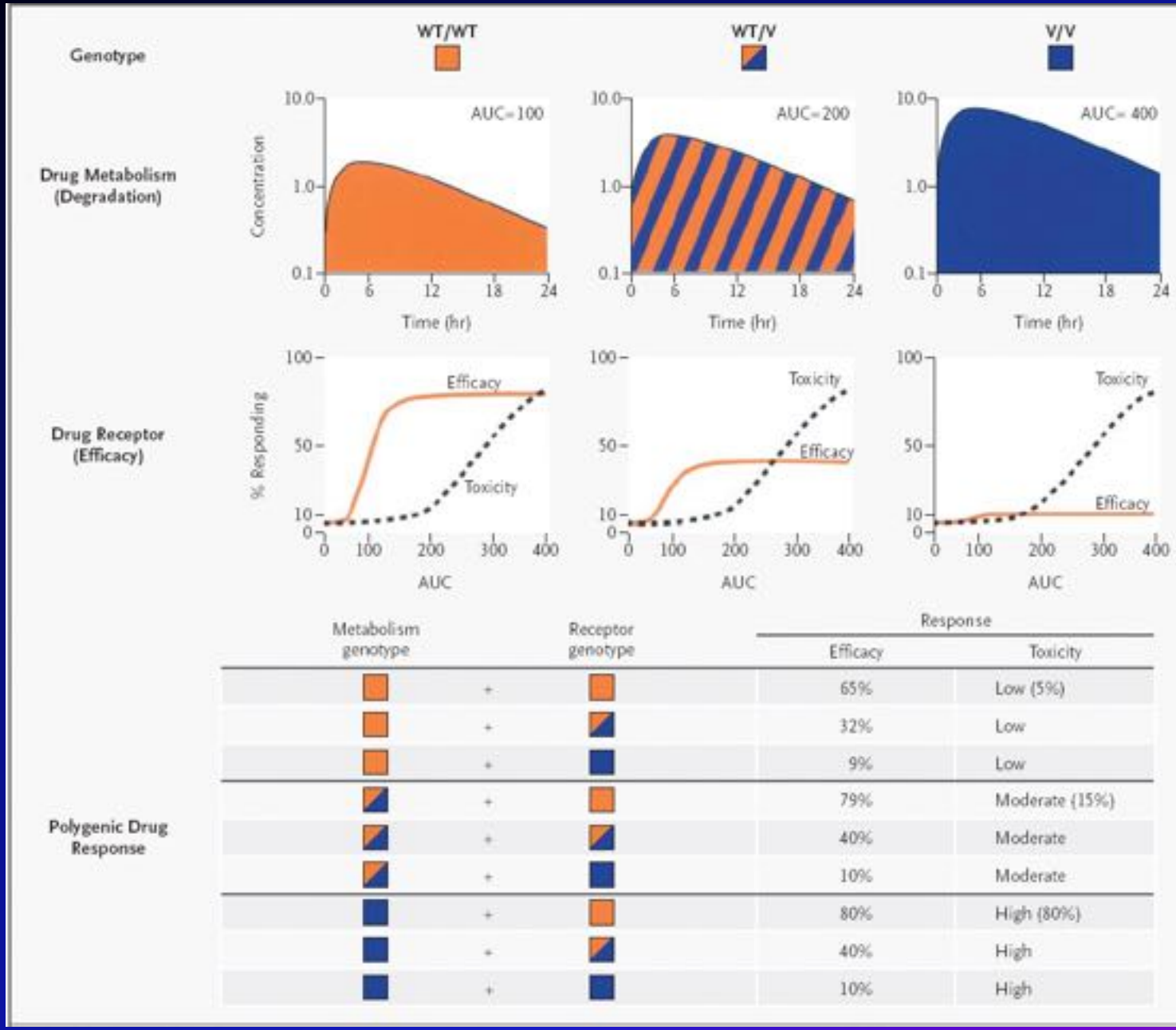


Rochais et al., The Journal of Clinical Investigation; 2007



Receptor Polymorphism	Effect in Vitro	Expected Effect in Vivo
α_{2C}Del322–325	Decreased function	Increased norepinephrine release at synapse
β_1Arg389	Increased function	Increased response at cardiomyocyte

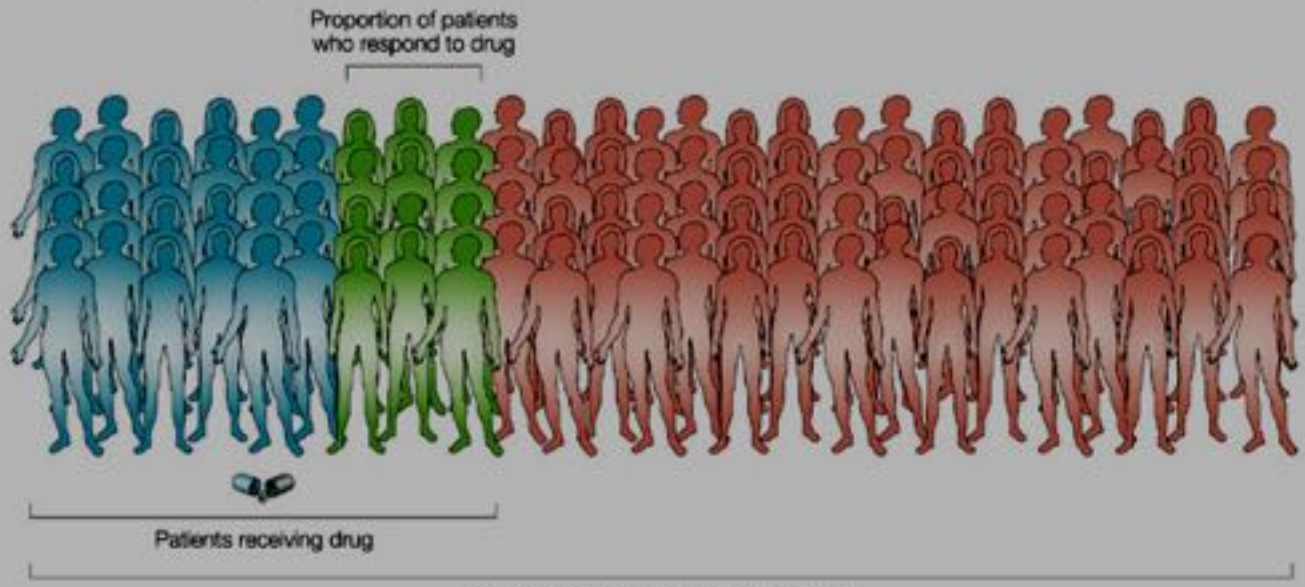

Monogenic disorder
Mutation
Gene
Inheritance pattern
(dominant or recessive)
Impact of mutations in a single gene on disease phenotype (percent)
100
Genetic risk in population
Percent
100
1
2
3
4
Genetic risk in different families
Complex disorder
Gene A
Gene C
Gene B
Gene D
Gene variations
Inheritance pattern (complex)
Impact of variations in different genes on disease phenotype (percent)
100
Environment/life-style
30
A
B
C
D
Genetic risk in population (epidemiological evidence)
Percent
30
A
B
C
D
1
2
3
4
Family


Genotype
WT/WT
WT/V
V/V
Drug Metabolism (Degradation)
Concentration
AUC=100
AUC=200
AUC=400
Time (hr)
Drug Receptor (Efficacy)
% Responding
Efficacy
Toxicity
AUC
Polygenic Drug Response
Metabolism genotype
Receptor genotype
Response
Efficacy
Toxicity
65% Low (5%)
32% Low
9% Low
79% Moderate (15%)
40% Moderate
10% Moderate
80% High (80%)
40% High
10% High

La Farmacogenetica è un processo che si sviluppa in 3 fasi distinte

- **La fase della scoperta e catalogazione degli SNPs**
- **La fase della ricerca di correlazioni tra la presenza di definiti SNPS (Aplotipi) e determinati profili farmacogenetici**
- **La fase diagnostica**

a Current state of drug development research



b Ideal future objective of drug development research