Pharmacogenetics

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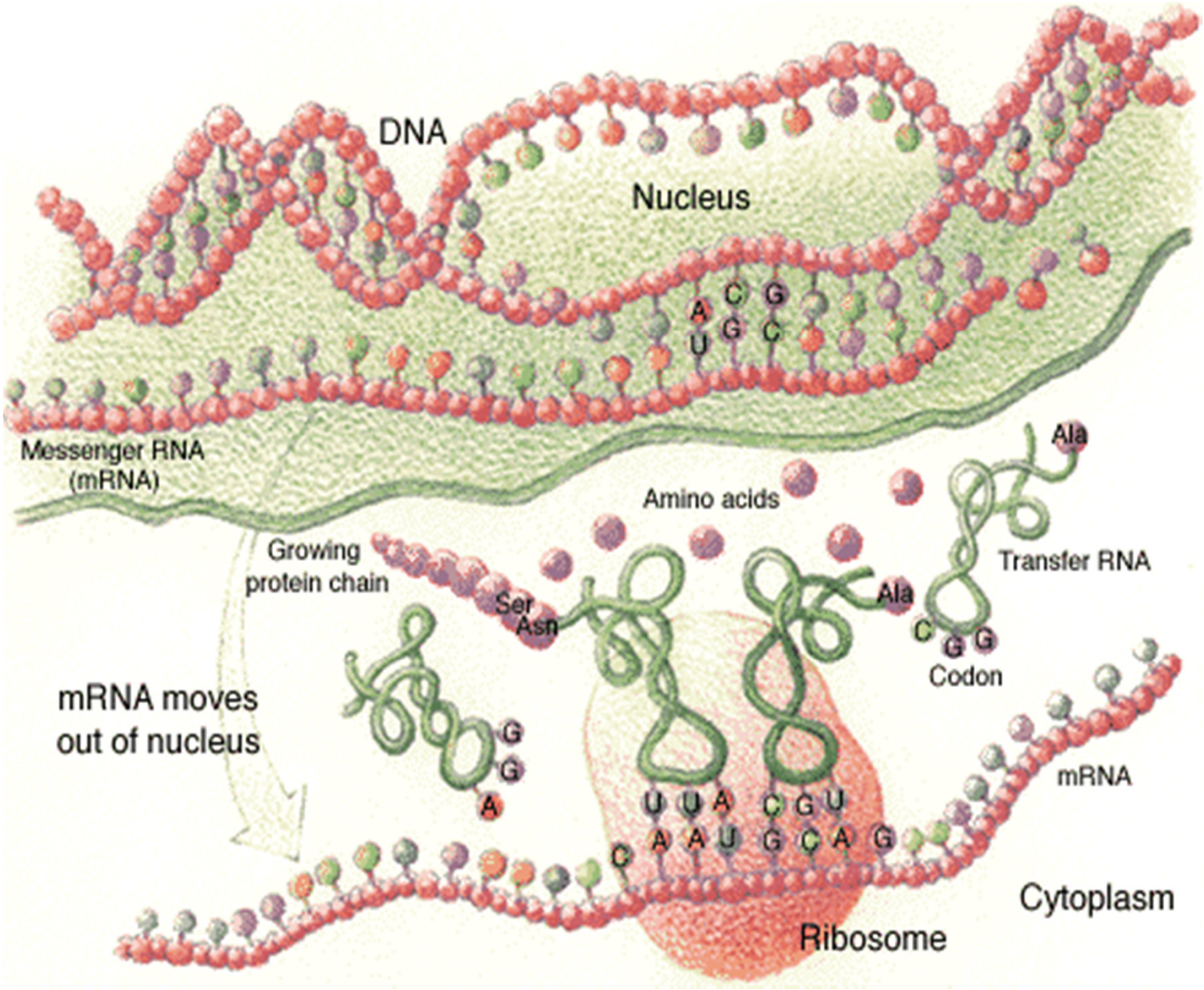
Human genome

- 46 chromosomes
  - 2 sex chromosomes - X, Y
  - 44 autosomal chromosomes (22 pairs)

- 3 billion DNA base pairs (haploid)
- 6 billion DNA base pairs (diploid)

- 21,000 genes

- ?? proteins


DNA
Nucleus
A
U
C
G
G
C
Messenger RNA
(mRNA)
Amino acids
Growing
protein chain
Ser
Asn
Ala
Ala
Transfer RNA
C
G
G
Codon
mRNA moves
out of nucleus
G
G
A
U
U
A
A
A
U
C
G
U
G
C
A
G
C
mRNA
Cytoplasm
Ribosome

Genotype

vs.

Phenotype

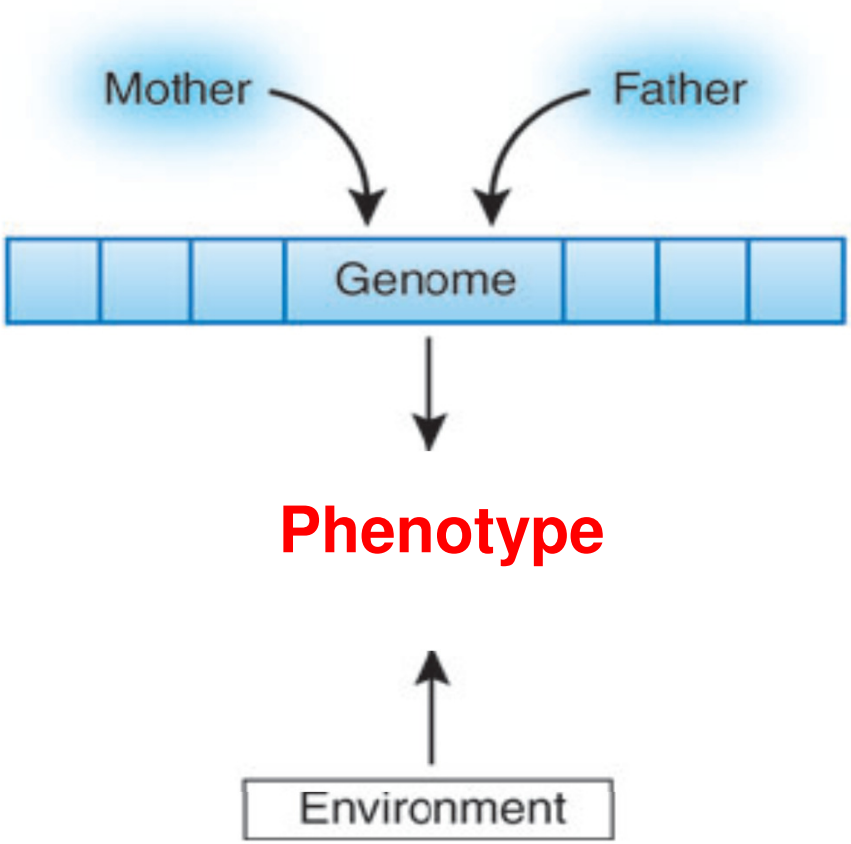
Genotype

An organism's genetic make-up that includes all alleles situated on homologous chromosomes

Phenotype

Any observable characteristic of an organism that results from an interaction of its genotype with the environment

Genotype versus phenotype



Mutations

We inherit hundreds of genetic mutations from our parents

The DNA in our own cells acquires many new mutations during a lifetime

Causes

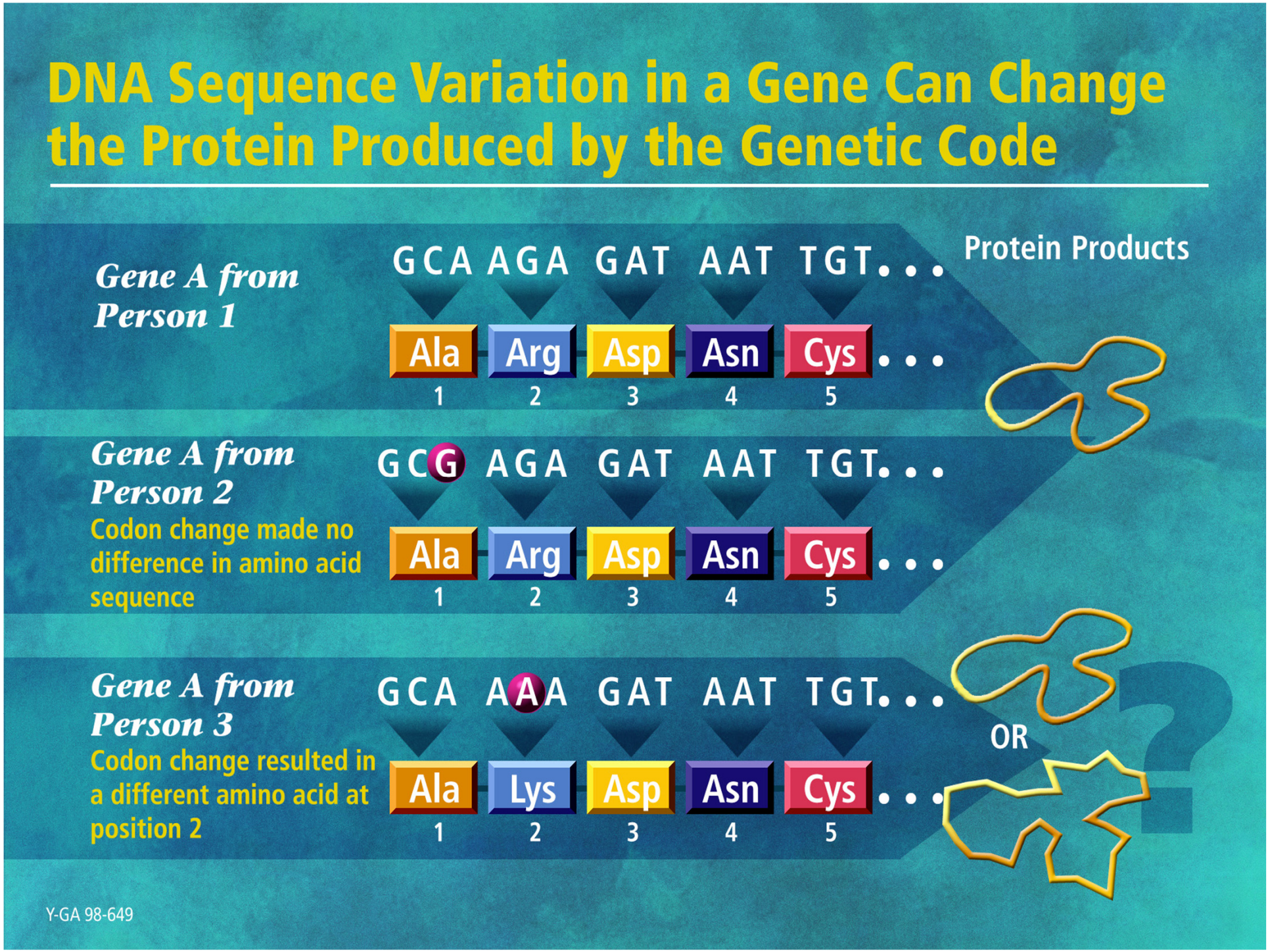
- environment (exogenous, endogenous) - majority
- cell division (DNA duplication)

  3 billion base pairs must be duplicated every time a cell divides

Most mutations occur outside coding and regulatory sequences

Problems occur when mutations affect protein synthesis or structure

DNA Sequence Variation in a Gene Can Change the Protein Produced by the Genetic Code



Y-GA 98-649

Pharmacogenetics

Definition

The study of variability in drug response due to heredity

Objective

To predict variations in drug efficacy and toxicity

$\rightarrow$ assist in determining drug choice and schedule

Scoline (succinylcholine) apnoea

- Butyrylcholinesterase (pseudocholinesterase)
  $\rightarrow$ scoline hydrolysis
- Activity reduced or absent in 1:3'500 Caucasians
- Unable to hydrolyze scoline
- Prolonged muscle paralysis $\rightarrow$ scoline apnoea

Glucose-6-phosphate dehydrogenase deficiency

- Drug-induced hemolytic anemia
- G6PD absent from 5-10% of individuals of African origin
- Risk of hemolysis from > 200 drugs

Pharmacogenetics

Clinical relevance

- Adverse drug reactions (compliance)
- Efficacy / response

Cost !

Adverse drug reactions

- 75% of ADRs are dose-dependent

USA (per annum)
- 100,000 deaths
- > 2,000,000 hospital patients experience a serious ADR

UK (per annum)
- 7% of all patients are affected by ADRs
- 1:10 NHS bed days used by patients with ADRs
- Cost: £ 380’000’000

- Pharmacogenetics: potential to be of benefit in 10-20% of ADRs

Inefficient therapy

Limited efficacy/response to drugs in current medical practice

	% non-responders
Cardiovascular	
ACE-inhibitors	10-30%
Beta-blockers	15-35%
Statins	10-60%
Anti-depressants	
SSRIs	10-25%
TCAs	20-50%

Pharmacokinetics

Characterized by four phases:

- Absorption
- Distribution
- Metabolism
- Elimination

Metabolism

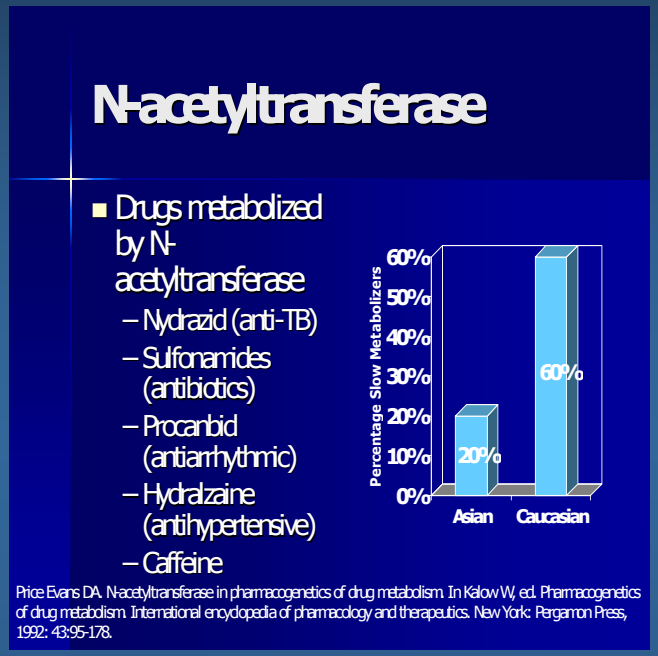
Enzymatic reactions which make a drug more water soluble to allow elimination from the body

Phase I - Biotransformation
- Oxidation
- Hydroxylation
- Reduction
- Hydrolysis

Phase II - Conjugation
- Addition of a new functional group

N-acetyltransferase

Metabolizes
- isoniazid / INH (anti-TB)
- hydralzine (anti-hypertensive)
- procainamide (anti-arrhythmic)



Polymorphisms in NAT2:
- slow and rapid acetylators
  slow → neurotoxicity and hepatotoxicity
  rapid → require higher doses
- genotyping – an alternative to therapetic drug monitoring

Metabolism

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Phase I - Biotransformation
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Phase II - Conjugation
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Cytochrome P450

>50 genes in human genome (>50 pseudogenes)

CYP450 gene has existed for more than 3.5 billion years

Liver (small intestine, lungs and other organs)
Cytoplasmic/endoplasmic reticulum (microsomal)

Oxidative metabolism (Fe^{3+}-dependent):
$NADPH + H^+ + O_2 + RH \rightarrow NADP^+ + H_2O + R\text{-}OH$

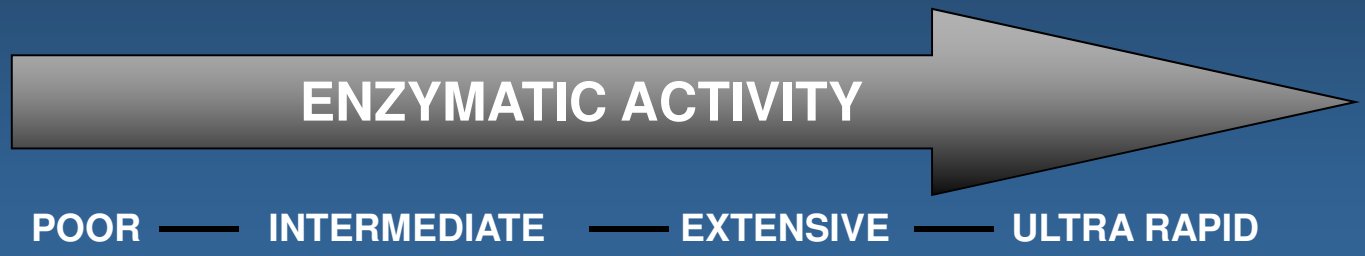
Cytochrome P450

Primary roles for the P450 system:

- formation of cholesterol, steroids and arachidonic acid metabolites
- metabolism and detoxification of many compounds after ingestion (xenobiotics)

Drug metabolism is a new and secondary role

Cytochrome P450



Poor metabolizers:

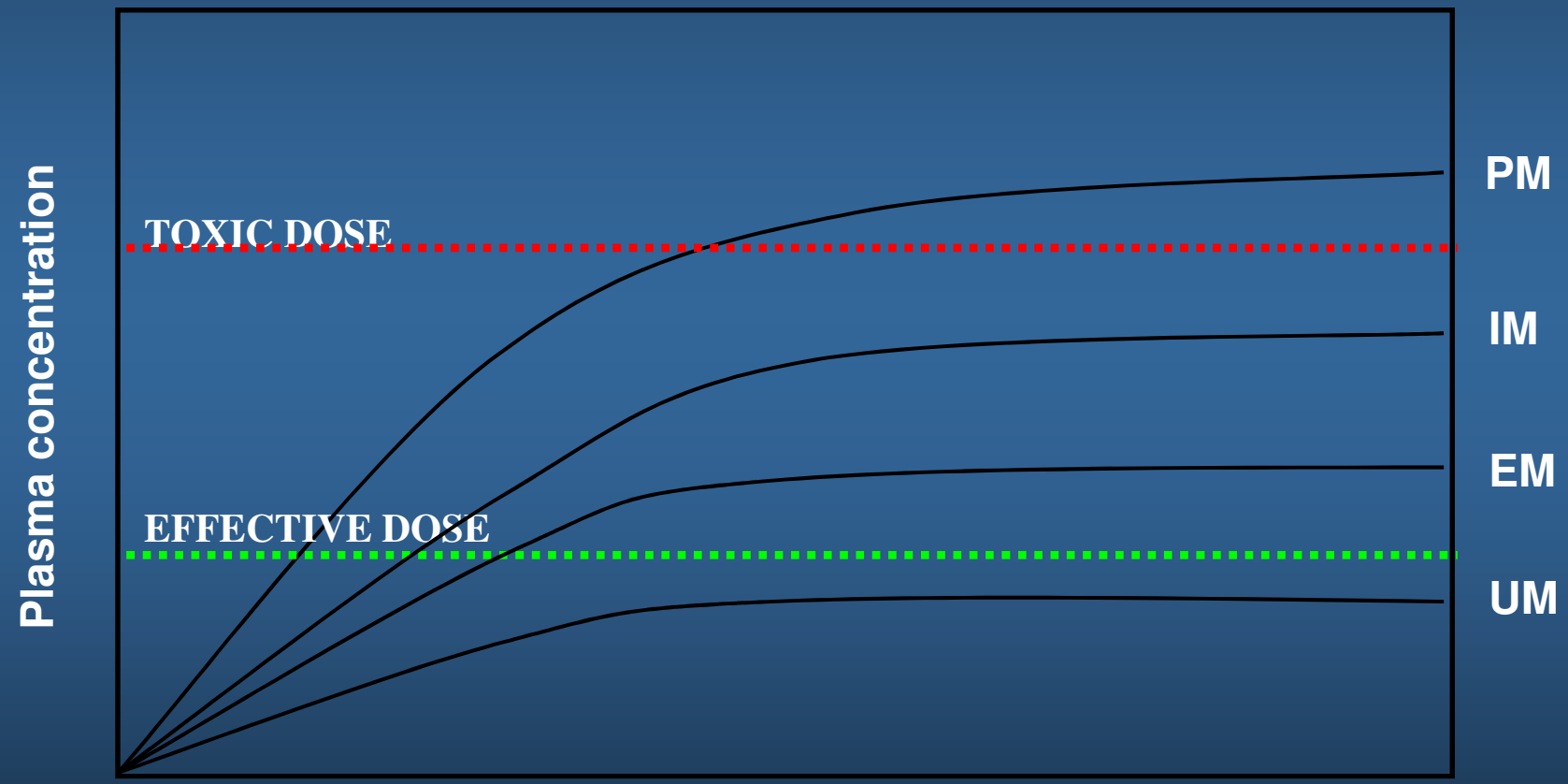
→ risk of toxicity

- Ultrarapid metabolizers:

→ therapeutic levels not achieved

Pro-drugs: opposite effect

Metabolizer status - rationale



Criteria for selection of a candidate gene for pharmacogenetic analysis

Genotype predictive of phenotype

→ minimal effect of environmental factors

Multiple genotypic variations leading to variations in phenotype (enzyme activity)

→ highly polymorphic

Drug metabolism by CYP450 genes

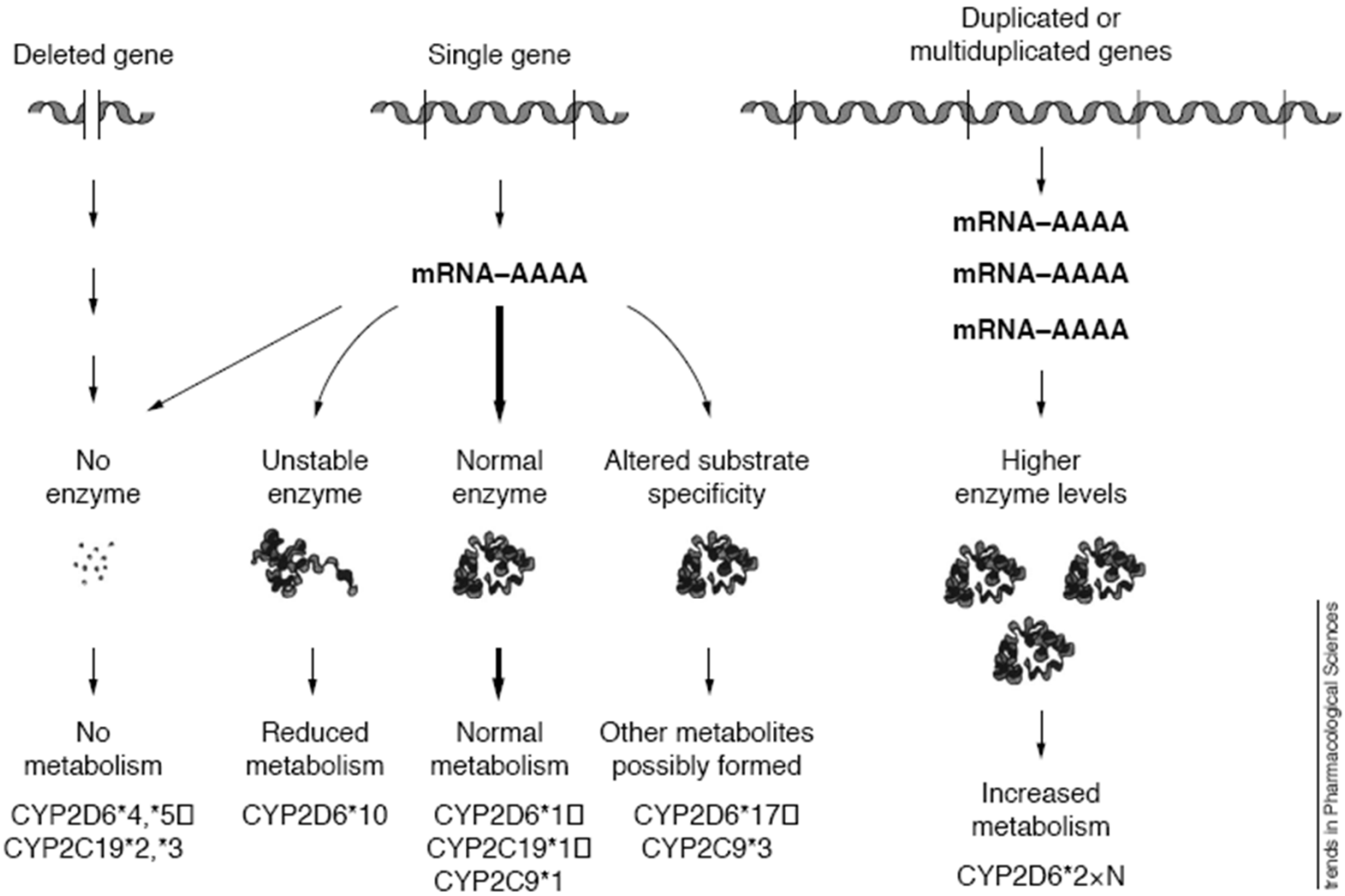
Gene	% drugs metabolized
CYP2A6	3%
CYP2B6	3%
CYP2E1	4%
CYP2C9	10%
CYP2C19	8%
CYP1A1/2	11%
CYP2C8/9	16%
CYP2D6	25%
CYP3A4/5	36%

CYP2D6

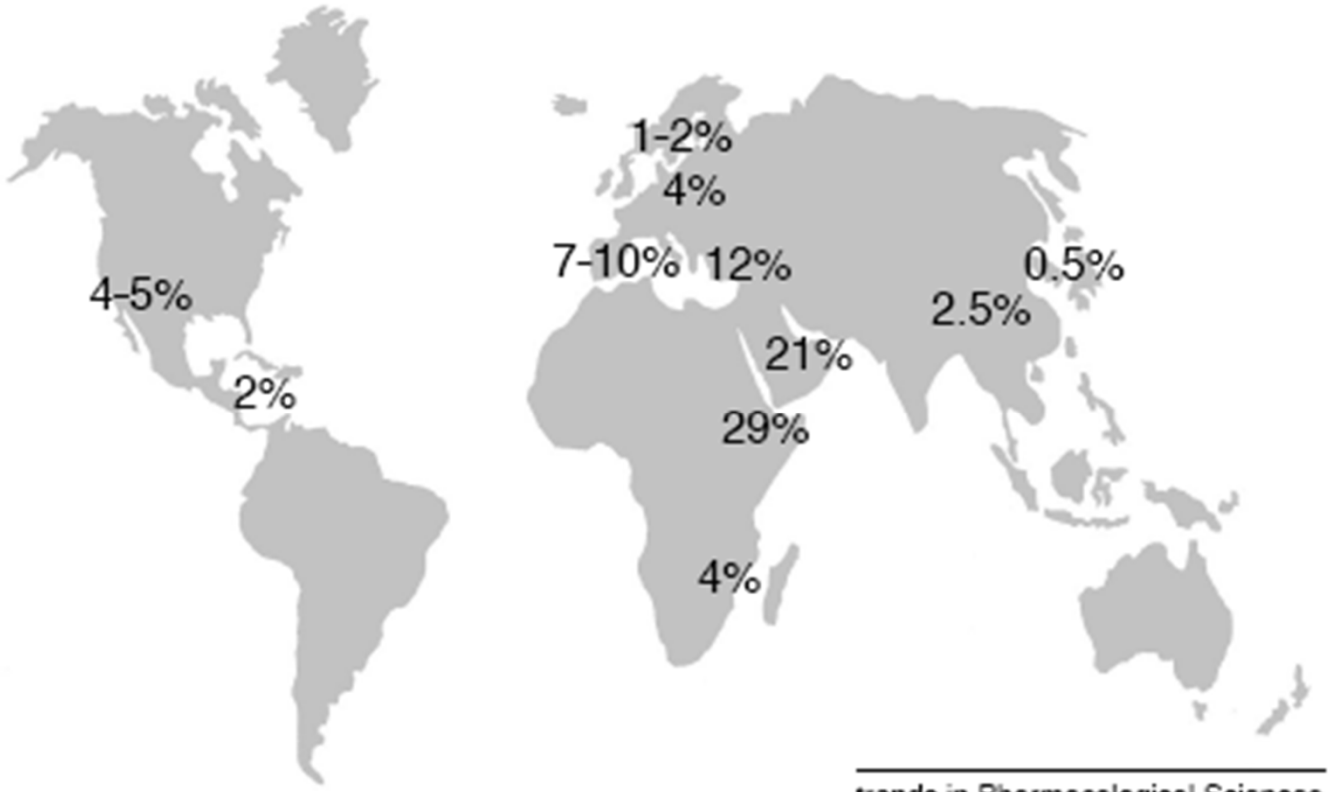
> 75 allelic variants known
→ affect expression and activity

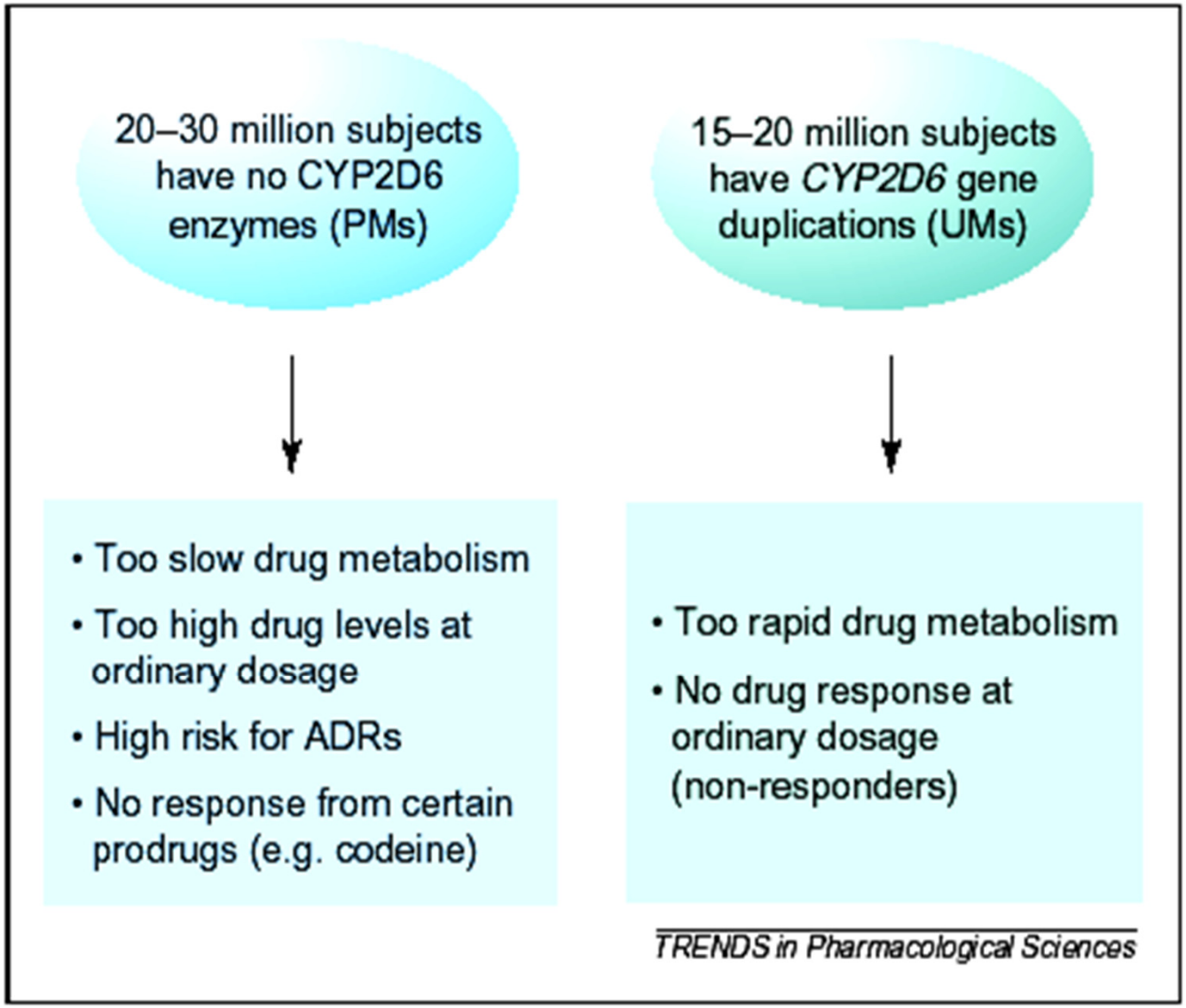
Large racial differences:
5-10% of Caucasians poor metabolizers
>25% East Africans ultrarapid metabolizers (multiple copies)

Metabolizes:
anti-depressants, anti-psychotics, analgesics, anti-arrhythmics, anti-emetics, beta-blockers…...


Deleted gene
Single gene
Duplicated or multiduplicated genes
mRNA–AAAA
mRNA–AAAA
mRNA–AAAA
mRNA–AAAA
No enzyme
Unstable enzyme
Normal enzyme
Altered substrate specificity
Higher enzyme levels
No metabolism
CYP2D6*4,*5□
CYP2C19*2,*3
Reduced metabolism
CYP2D6*10
Normal metabolism
CYP2D6*1□
CYP2C19*1□
CYP2C9*1
Other metabolites possibly formed
CYP2D6*17□
CYP2C9*3
Increased metabolism
CYP2D6*2×N
Trends in Pharmacological Sciences

Frequency of individuals carrying alleles with multiple CYP2D6 gene copies in different parts of the world





Ingelman-Sundberg, Trends Pharmacol Sci. 2004 Apr;25(4):193-200

CYP2D6 and psychiatry

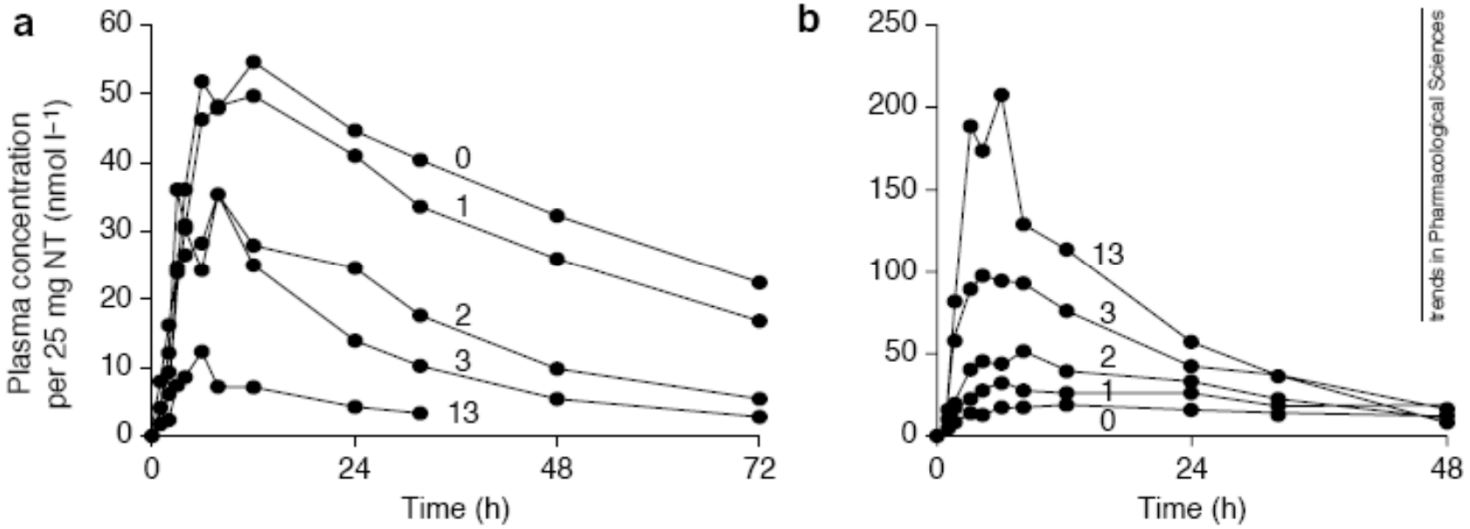
Psychotropic drugs

- drug selection and dosing are largely empirical
- 3-8 weeks to optimal effect
- many side effects → treatment failure

CYP2D6

- Tricyclic antidepressants
- SSRIs
- Antipsychotics (incl. risperidone)

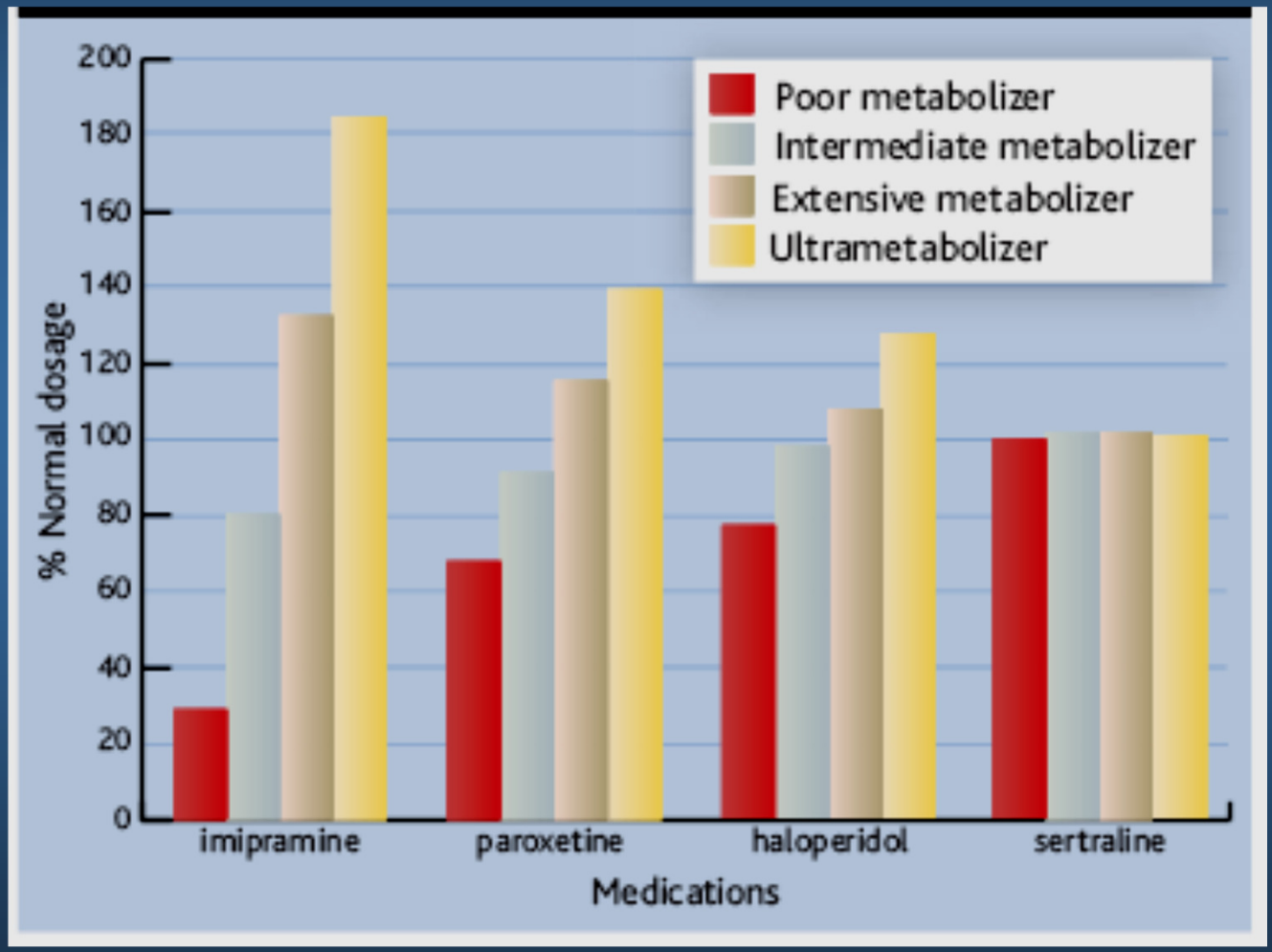
Effect of CYP2D6 gene copy number on metabolism of nortriptyline (TCA)



Mean plasma concentrations following a single oral dose of nortriptyline
a: nortriptyline
b: 10-hydroxynortriptyline

Number of CYP2D6 genes shown next to the curves

CYP2D6 and dose adjustment



CYP2D6 and analgesics

Codeine

pro-drug: metabolized to morphine by CYP2D6

PM: no or limited benefit from codeine
(also less likely to develop opioid dependence)

UM: dizziness, nausea, restlessness, hyperalertness

CYP2D6 and analgesics

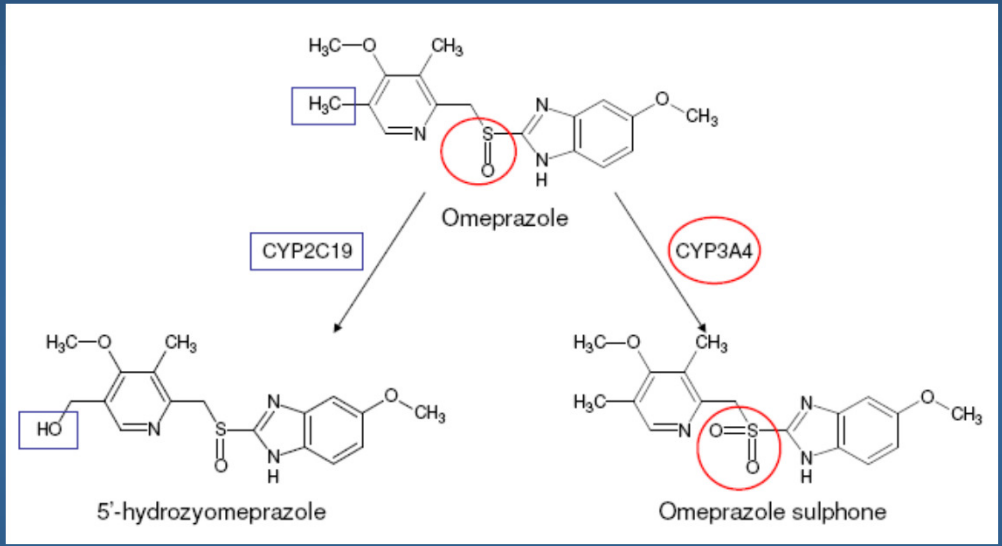
Tramadol

pro-drug: metabolised to 11 metabolites of which *O*-desmethyltramadol has > 200-fold higher affinity for the μ-opioid receptor than tramadol itself

PM: reduced analgesic efficacy

CYP2C19

Proton pump inhibitors



PMs - greater efficacy for gastritis and ulceration (duodenal and gastric)

EMs - reduced efficacy

CYP2C19

Proguanil

Malaria prophylactic

pro-drug: metabolized to active metabolite cycloguanil by CYP2C19

PMs - inadequate prophylactic cover

CYP2C19

Clopidogrel

Pro-drug: active metabolite inhibits platelet aggregation

Poor metabolizers:

- lower levels of the active metabolite
- less inhibition of platelets
- 3.58 times greater risk for major adverse cardiovascular events – heart attack, stroke and death

CYP2C9

Polymorphism leads to inactivity
→ 1-3% of caucasians are PMs

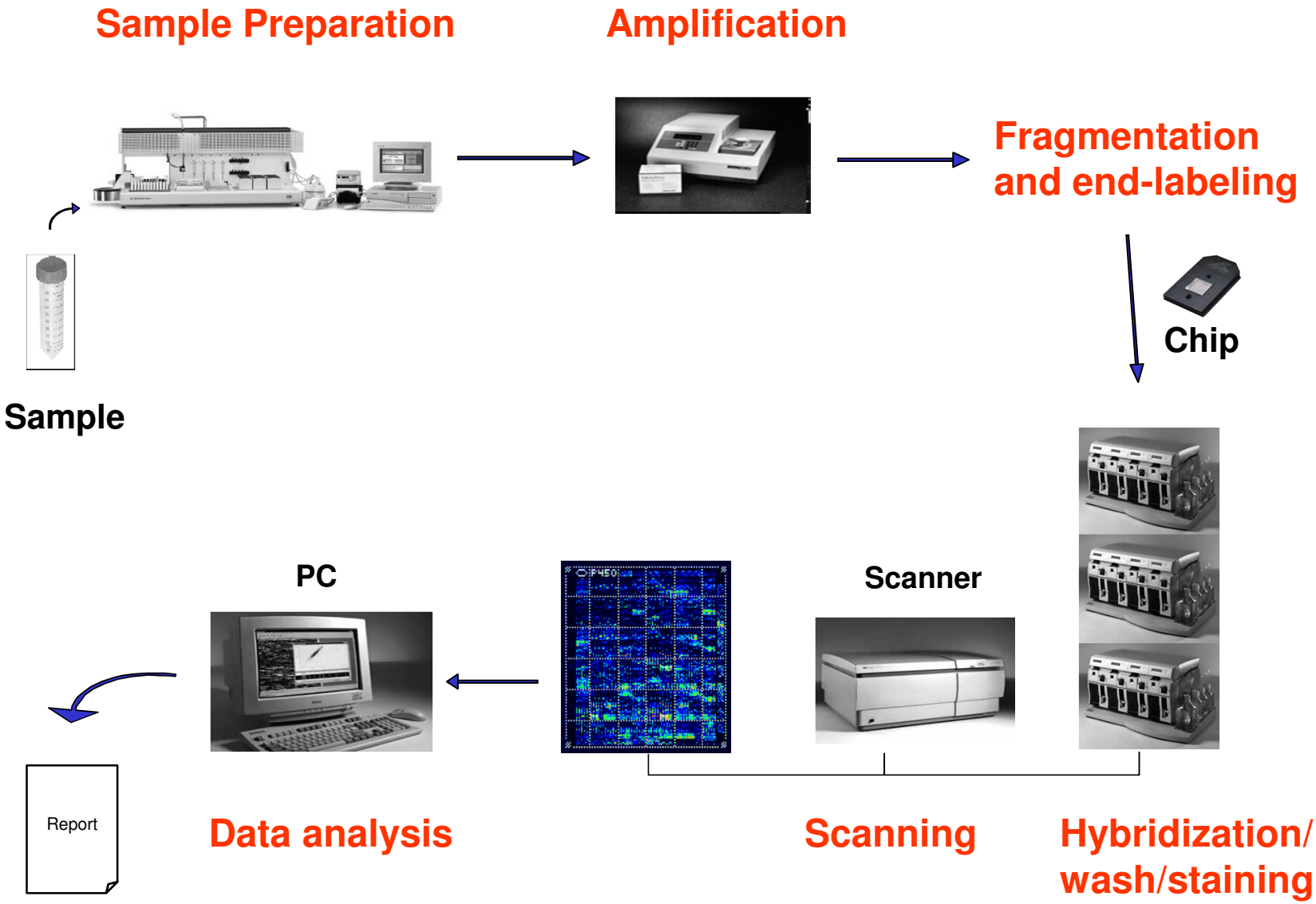
Inhibited by many drugs (INH)
→ potential for toxicity

Induced by many drugs (ritonavir and nelfinavir)
→ decreased efficacy

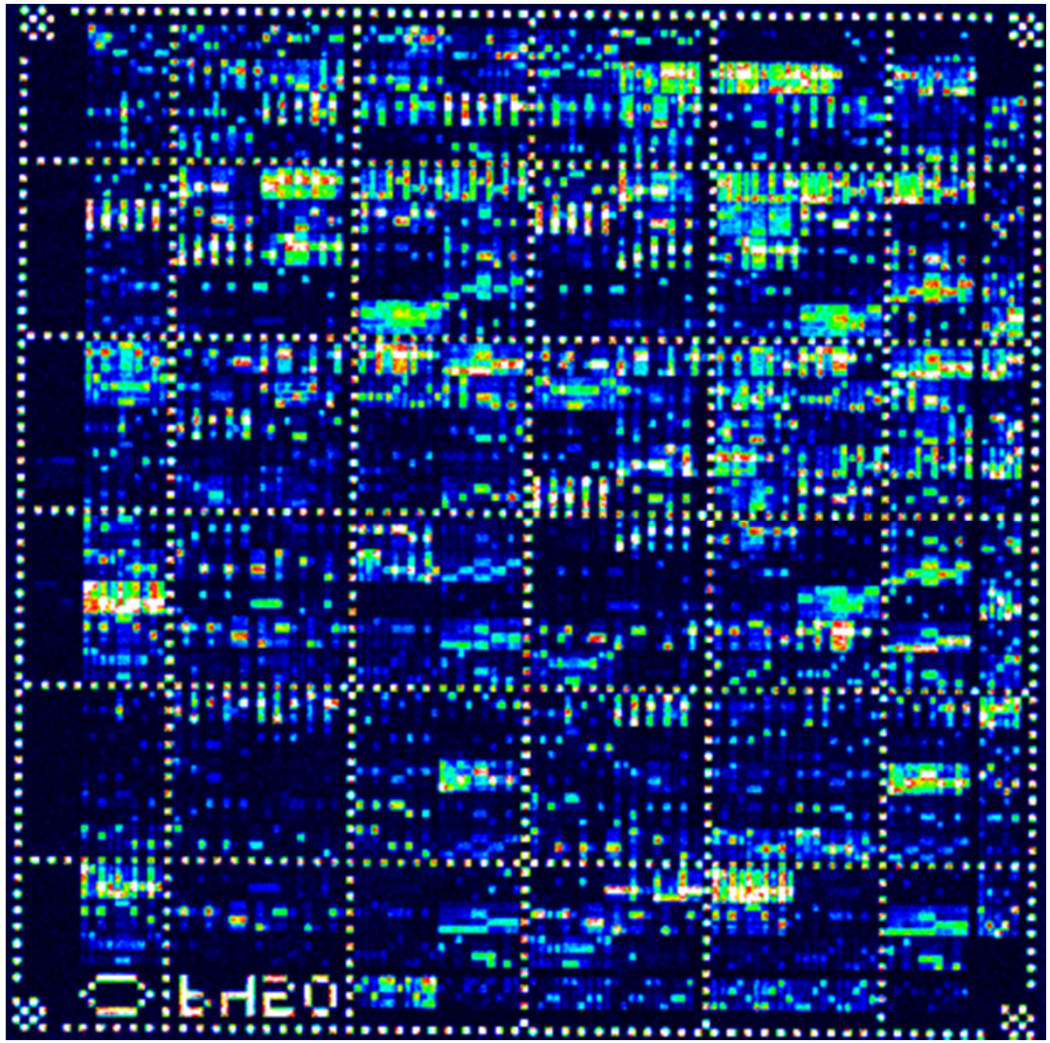
Metabolizes S-warfarin
absence or inhibition of CYP2C9
→ increased prothrombin time
→ risk of hemorrhage


Roche
AmpliChip™
CYP450 Array
LOT
A12345
2003/12
Powered by Affymetrix.

Microarray CYP450 genotyping assay flow chart



> 15,000 oligonucleotide probes



Cancer treatment

Sensitivity and toxity to chemotherapy
$\rightarrow$ narrow therapeutic window
$\rightarrow$ genetically determined

Chemotherapy ADRs
- increase overall hospital costs
- increase drug costs by 15%

Cancer treatment

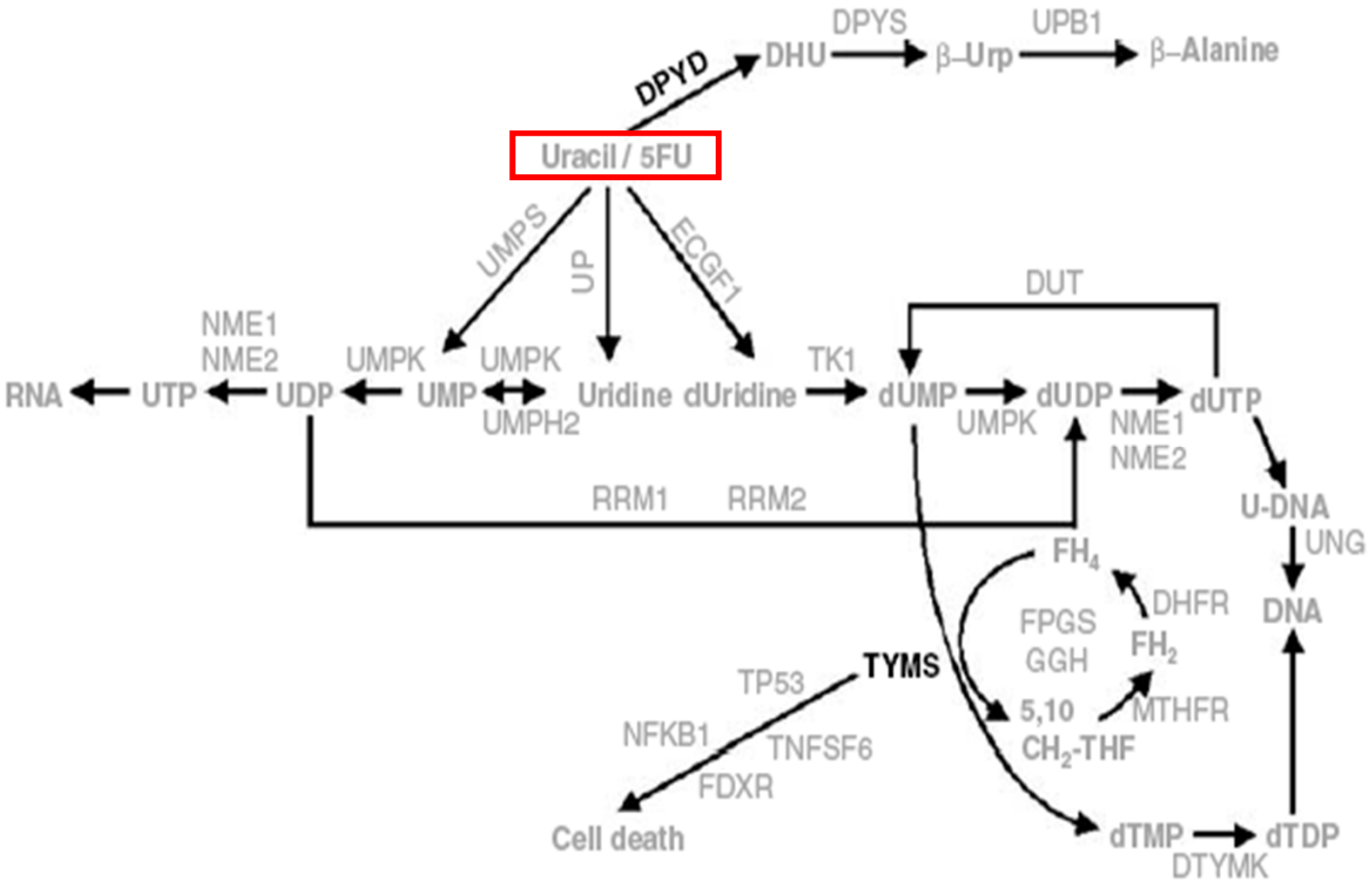
HER2

High expression in breast cancer
→ better response to herceptin therapy

Thiopurine S-methyltransferase

- Deficiency → hematopoietic toxicity with 6-mercaptopurine
- 10 low activity variants; 3 account for 95% of phenotypes
- Pretreatment genotyping realistic

5-Fluorouracil drug pathway



British Journal of Cancer (2004) **90**(1), 8–11

Cancer treatment

Dihydropyrimidine dehydrogenase (DPYD)

Deficiency $\rightarrow$ neurotoxicity with 5-fluorouracil
$\pm$ 20 polymorphisms described

Thymidylate synthase (TYMS)

Main target for 5-fluorouracil
Overexpression is linked to resistance

HIV / AIDS

Patient: side effects from drugs

- hepatotoxicity, hyperglycemia, hyperlipidemia, lactic acidosis, lipodystrophy, osteoporosis, skin rash
- abacavir: severe side effects in 50% of white males with specific HLA-B polymorphisms

Virus: resistance to therapy

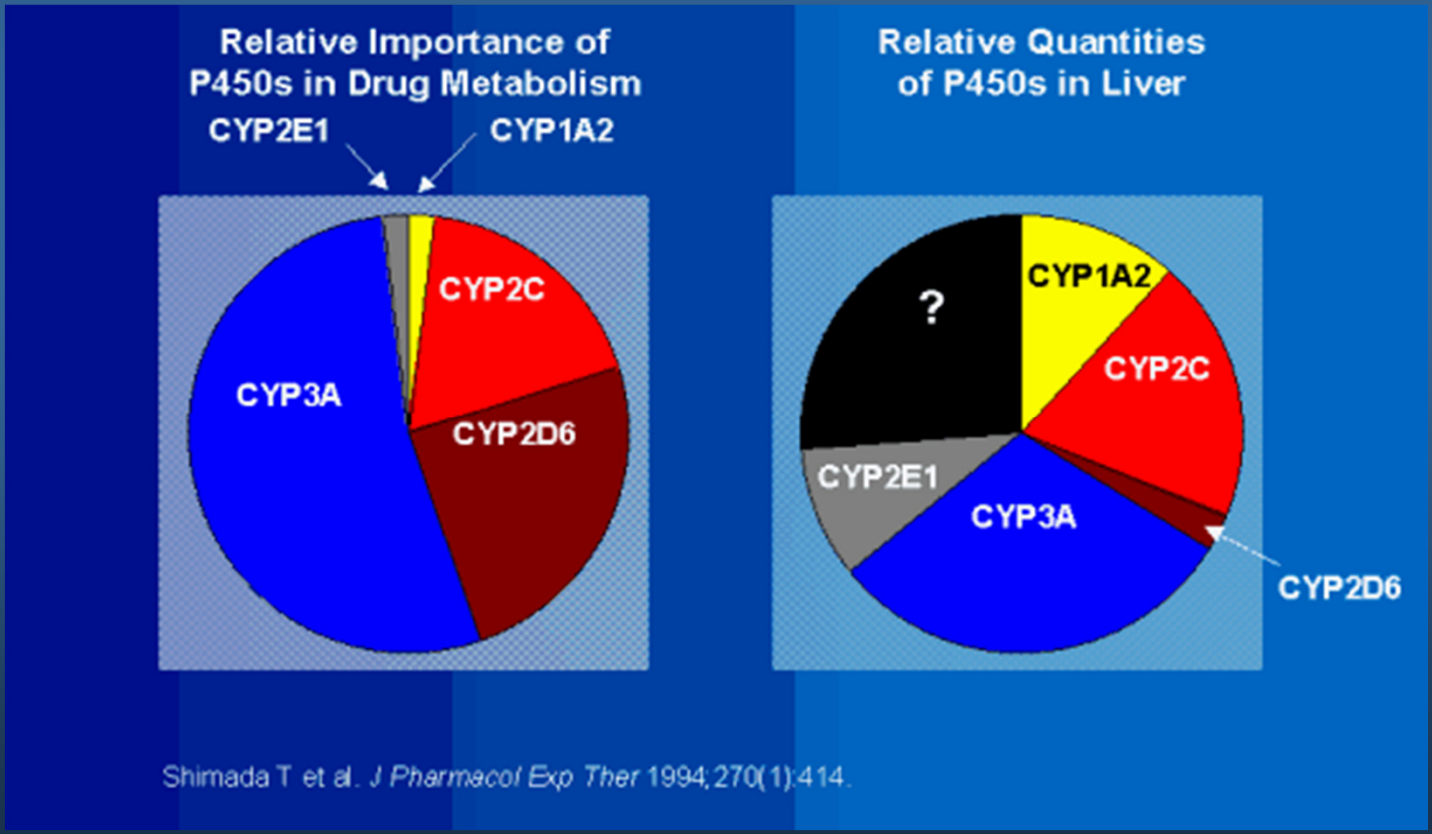
- > 120 known gene variants associated with resistance
- routine baseline resistance testing

Anti-retrovirals and CYP450

	Metabolized by	CYP inhibited	CYP induced
NNRTIs			
Delavirdine	3A4	3A4	None
Efavirenz	3A4 > 2B6	3A4, 2C9, 2C19	3A4
Nevirapine	3A4	None	3A4, glucuronyl transferase
PIs			
Amprenavir	3A4	3A4	Unknown
Indinavir	3A4	3A4	None
Lopinavir/ritonavir	3A4 > 2D6	3A4 > 2D6 > 2C19 >> 2A6 > 1A2 > 2E1	3A, 1A2, 2C9, glucuronyl transferase
Nelfinavir	3A4 >> 2C19, 2D6, 2C9, 2E1	3A4	glucuronyl transferase
Ritonavir	3A4 > 2D6	3A4 > 2D6 > 2C19 >> 2A6 > 1A2 > 2E1	3A, 1A2, 2C9, glucuronyl transferase
Saquinavir	3A4	3A4	None

CYP3A4

- most abundant CYP450 enzyme in humans
- metabolism of > 50% of all drugs



CYP3A4

Many polymorphisms
- implicated in hormone-dependent cancer (breast and prostate)

But…

CYP3A4 very sensitive to environmental factors
- other medications
- food e.g. inhibited by grapefruit

Therefore…
Poorly suited to pharmacogenetics

A new paradigm for therapy?

Current therapeutic approach: trial and error

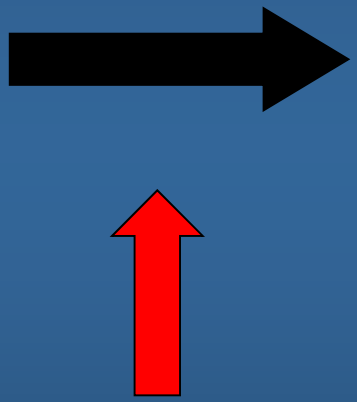
Interindividual differences in response to drugs

Future: pre-treatment genotyping
→ therapy maximally efficacious and well tolerated

Personalized medicine

Awareness/education

Rapidly evolving molecular technology including the human genome



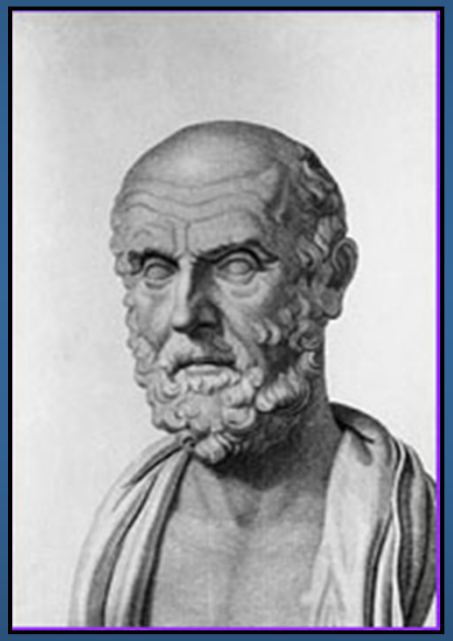
Incorporation into clinical practice

Education of health care professionals

The last word…..

Hippocratic oath (or equivalent)

I will neither give a deadly drug to anybody who asked for it, nor will I make a suggestion to this effect. Similarly I will not give to a woman an abortive remedy.



Hippocrates, the father of medicine

Failure to genotype before treatment ?

May one day be considered to be unethical !