Basics of Pharmacogenomics

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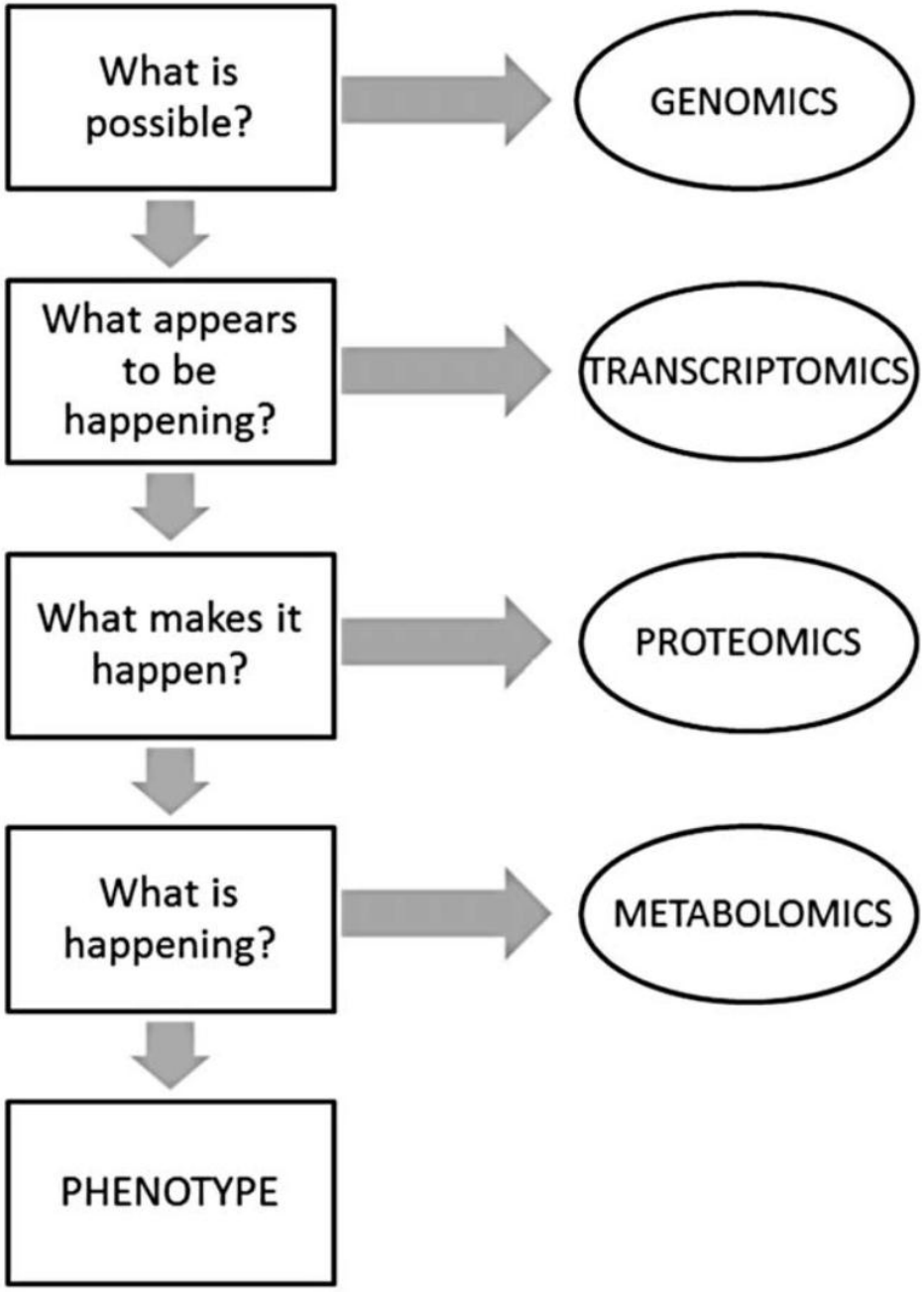
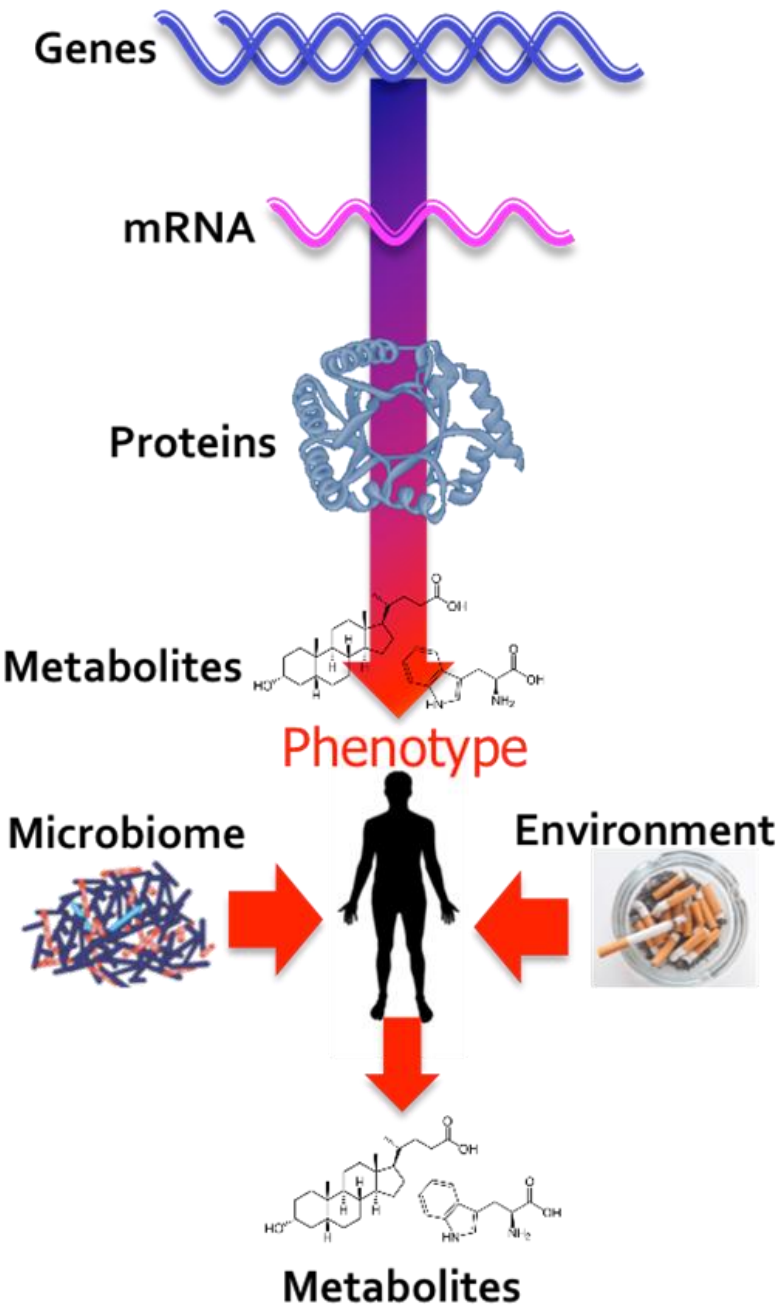
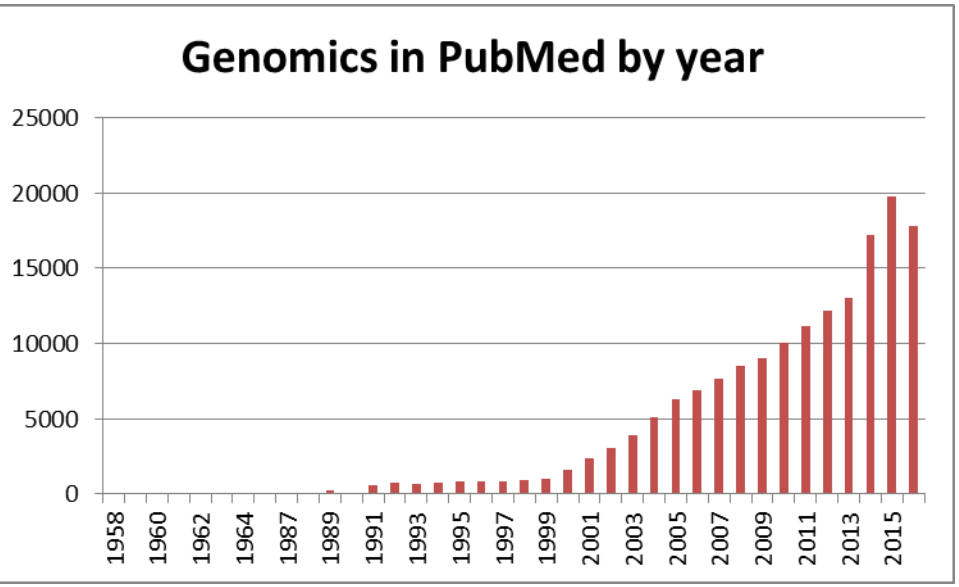
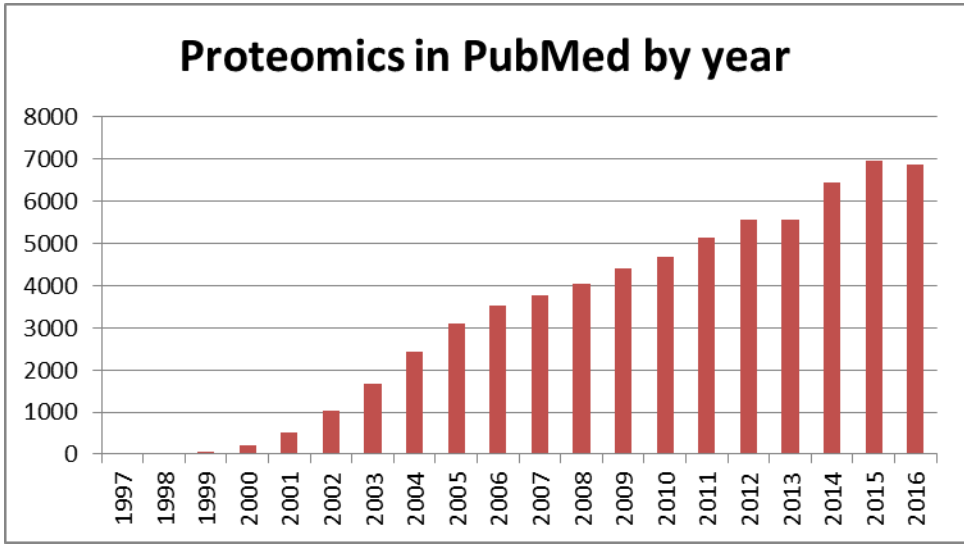
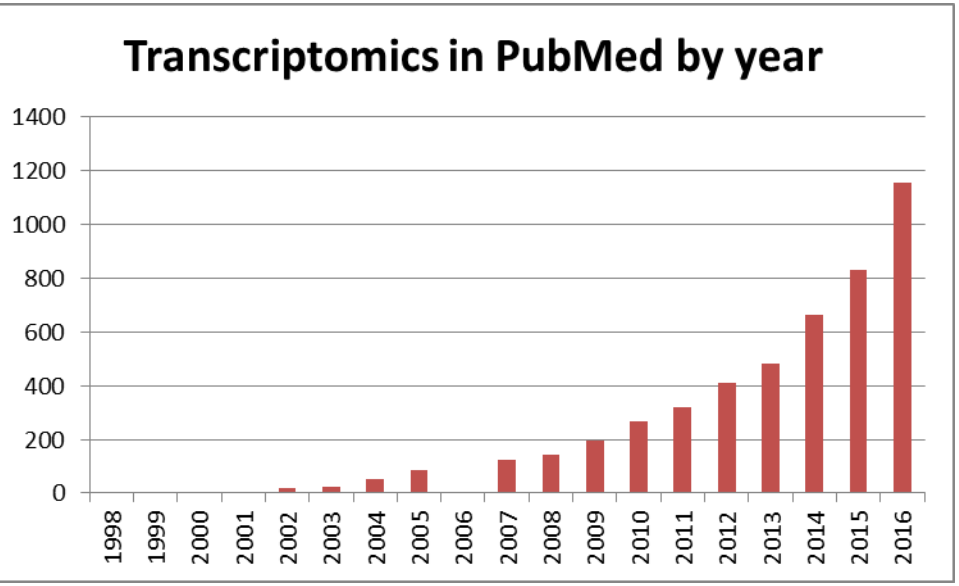

What is possible?
GENOMICS
What appears to be happening?
TRANSCRIPTOMICS
What makes it happen?
PROTEOMICS
What is happening?
METABOLOMICS
PHENOTYPE

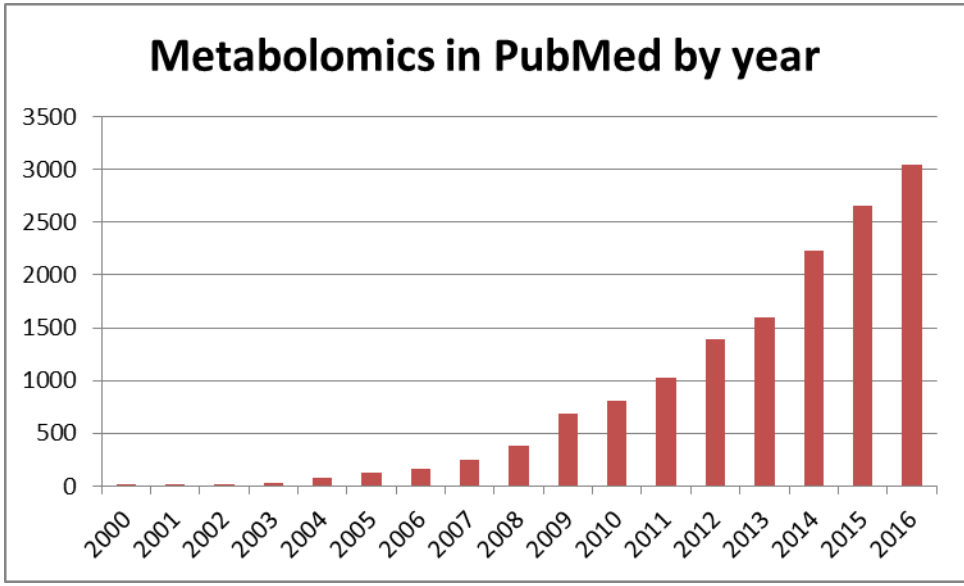

Genes
mRNA
Proteins
Metabolites
Phenotype
Microbiome
Environment
Metabolites

The era of OMICS

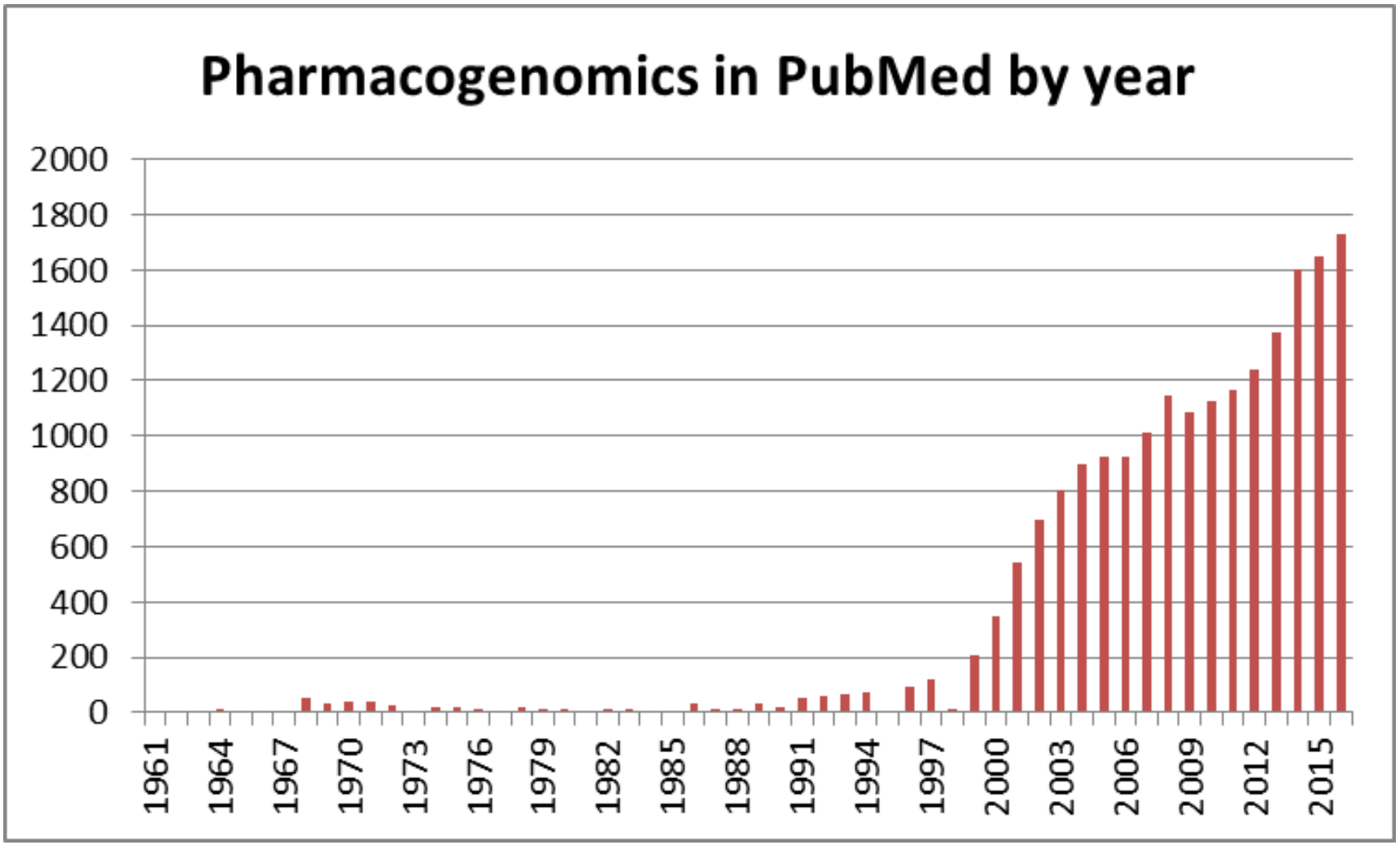




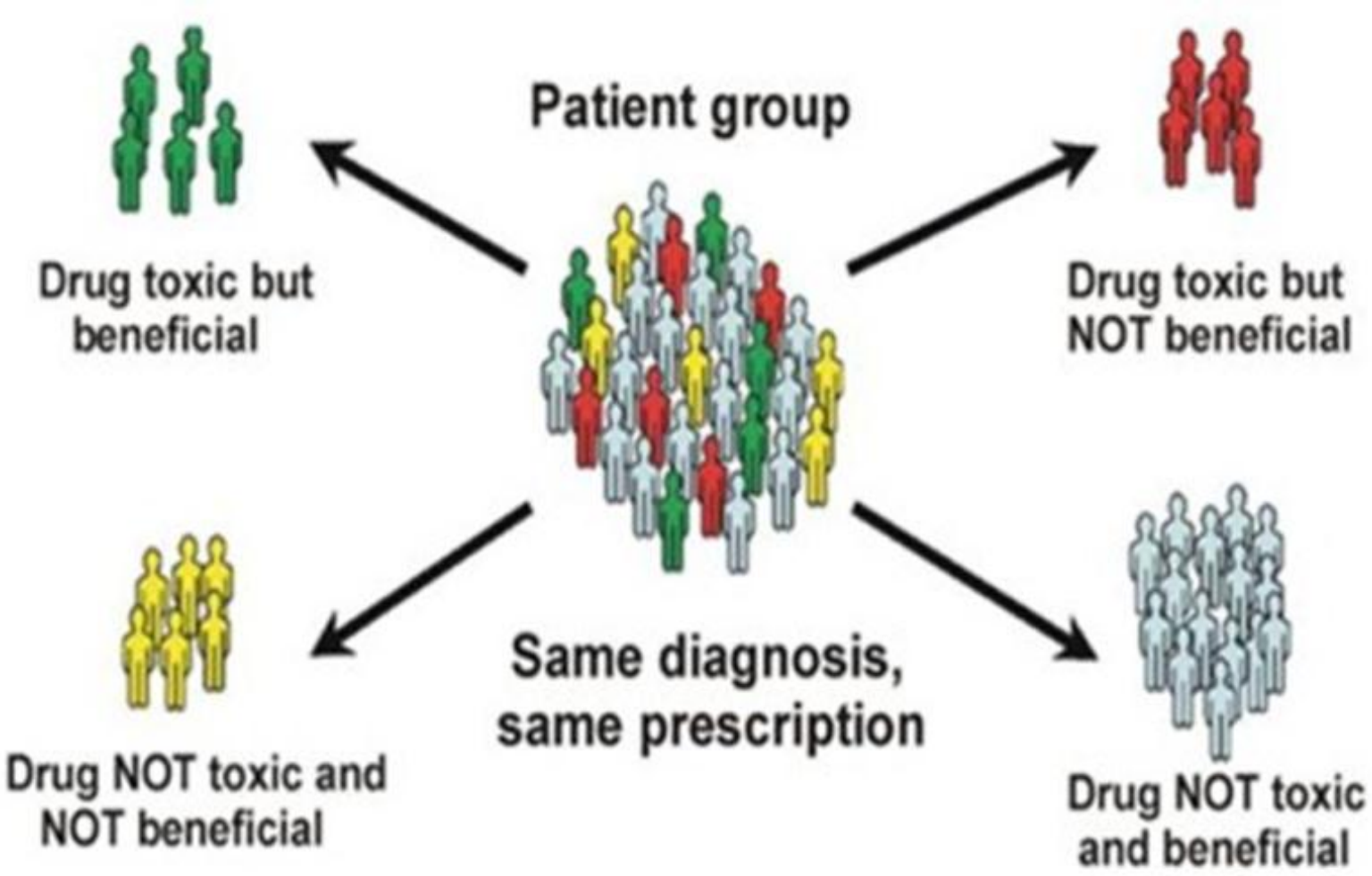




What about PharmacoGENOMICS?

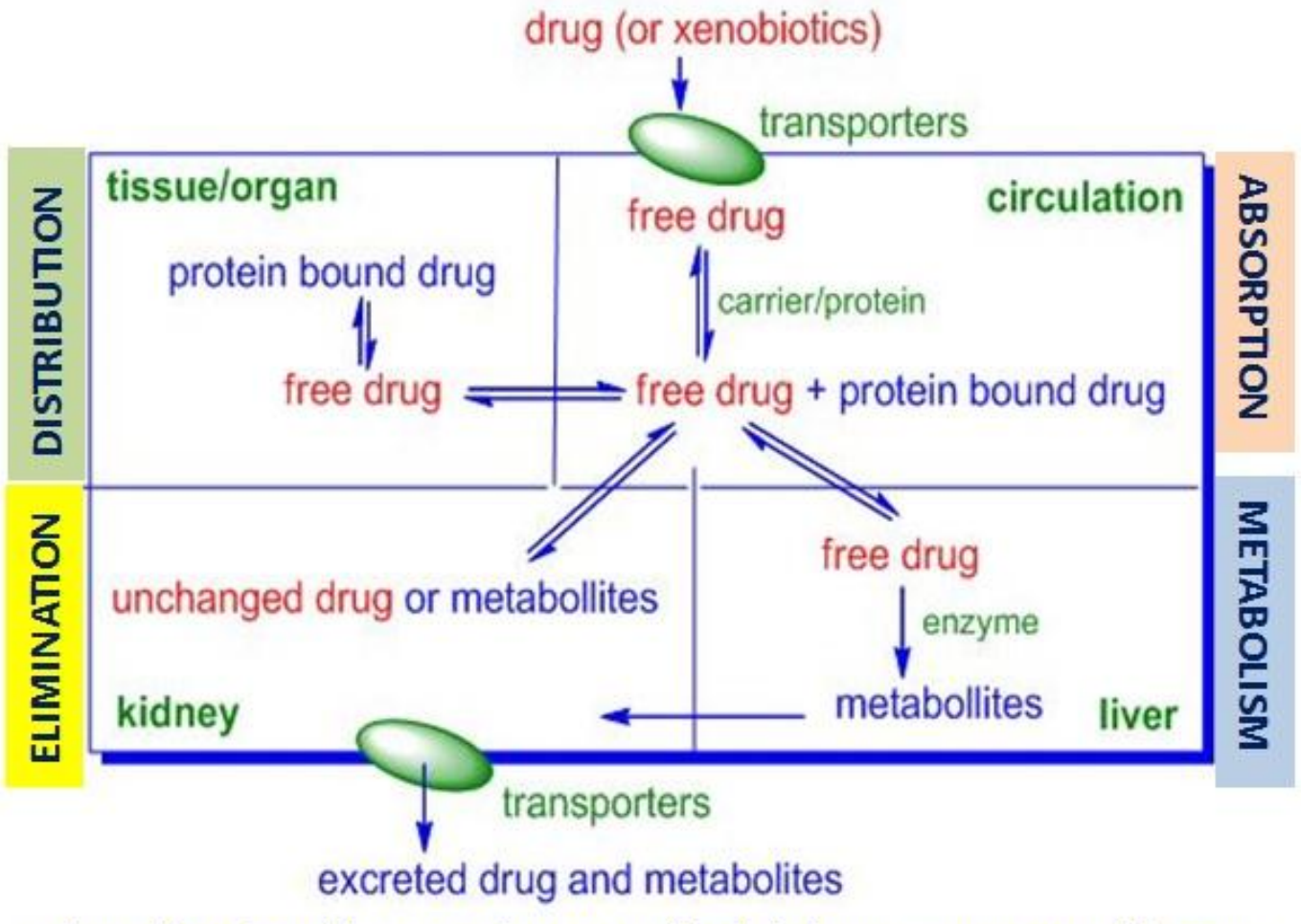


What is Pharmacogenomics?



Take home message: <u>One size does not fit all!</u>

What happens to the drug after administration?

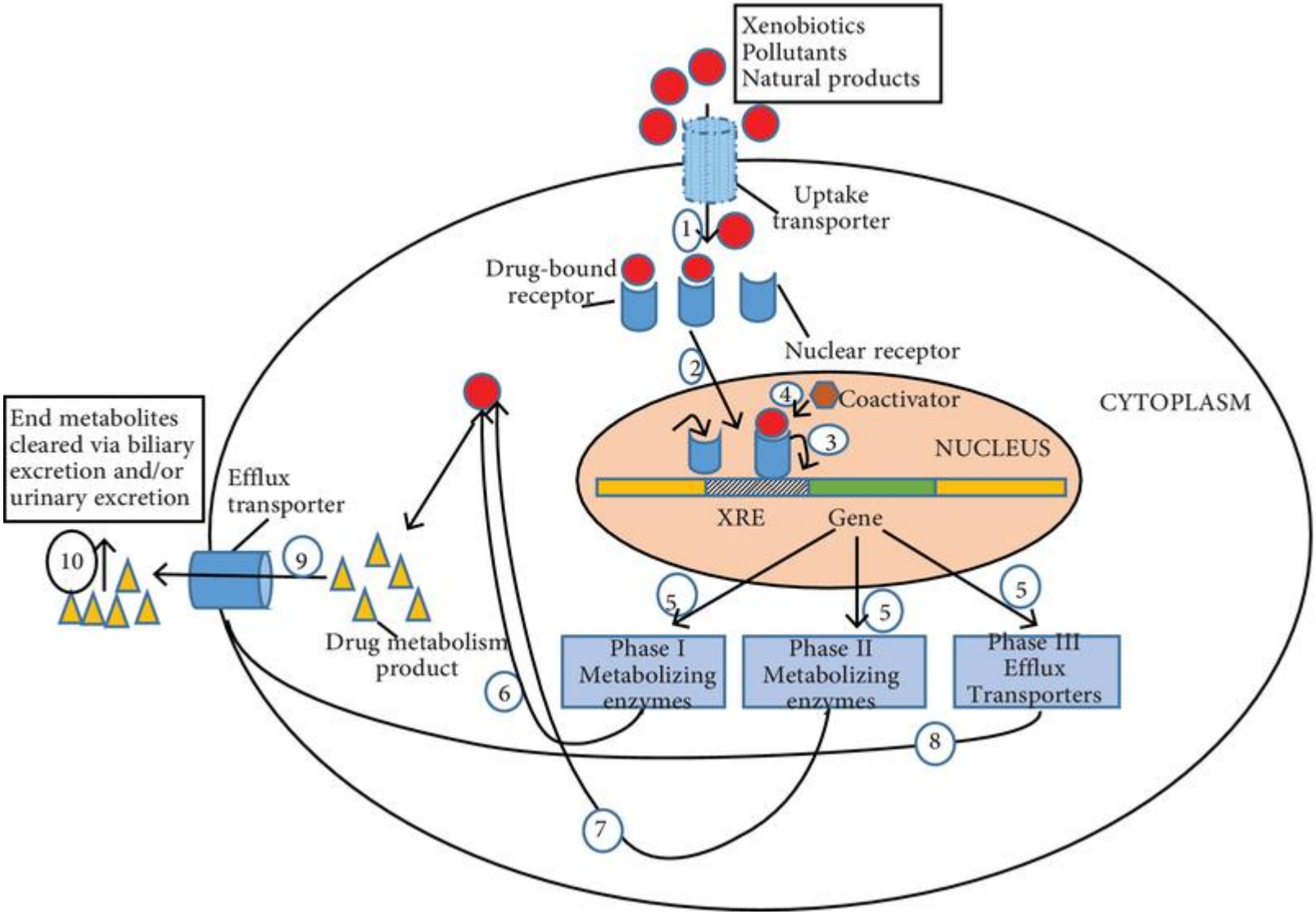


Absorption, Distribution, Metabolism, Elimination = ADME

What makes ADME possible?

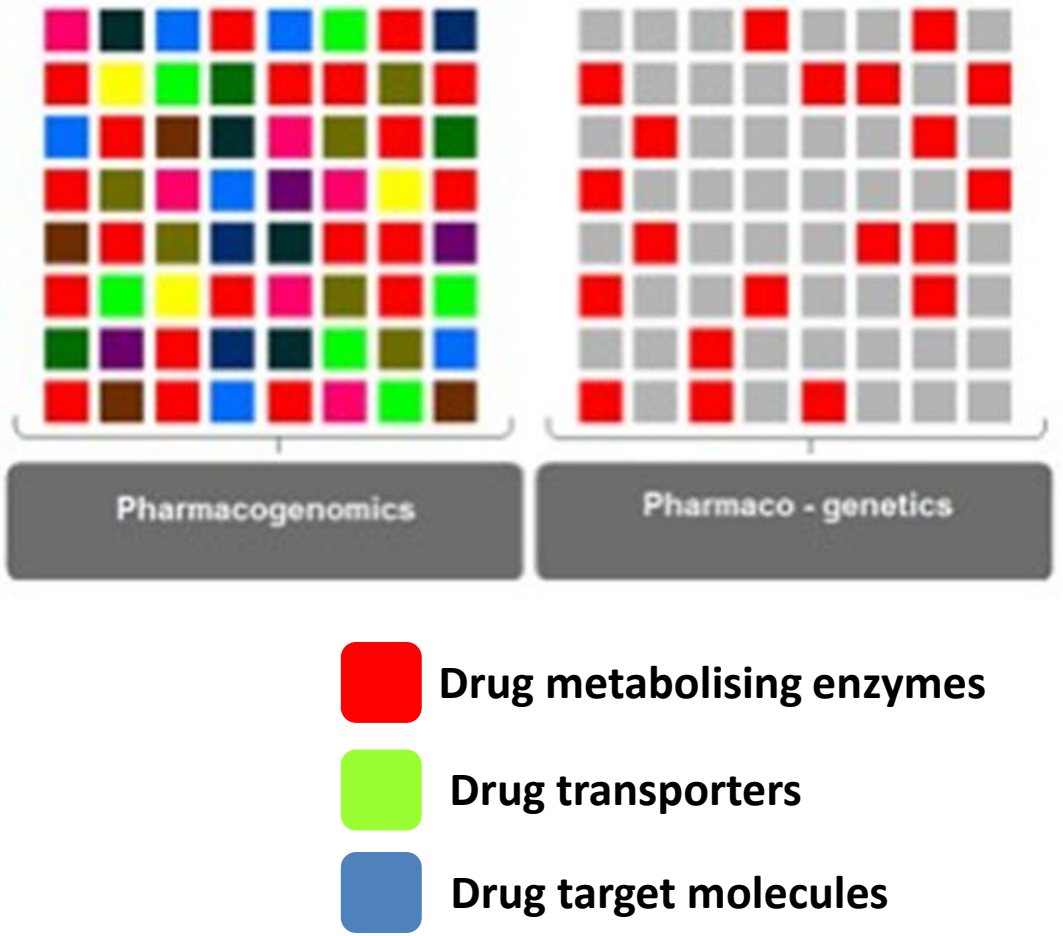
	Uptake transporters	Efflux transporters	Bidirectional transporters	CYP enzymes
Enterocytes	Intestine OATP2B1 OATP1A2 ASBT MCT1 Blood OCT1	Intestine MRP2 BCRP P-gp Blood MRP3	Blood OSTα-OSTβ	CYP3A4 CYP3A5 CYP2J2 CYP2C9 CYP2C19 CYP2D6
Hepatocytes	Blood OATP1B1 OATP1B3 OATP2B1 NTCP OCT1 OAT1 OAT7	Blood MRP3 MRP4 MRP6 Bile MRP2 BCRP P-gp BSEP MATE1	Blood OSTα-OSTβ	CYP1A2 CYP2A6 CYP2B6 CYP2C8 CYP2C9 CYP2C18 CYP2C19 CYP2D6 CYP2E1 CYP3A4 CYP3A5

Let`s go to the cellular level

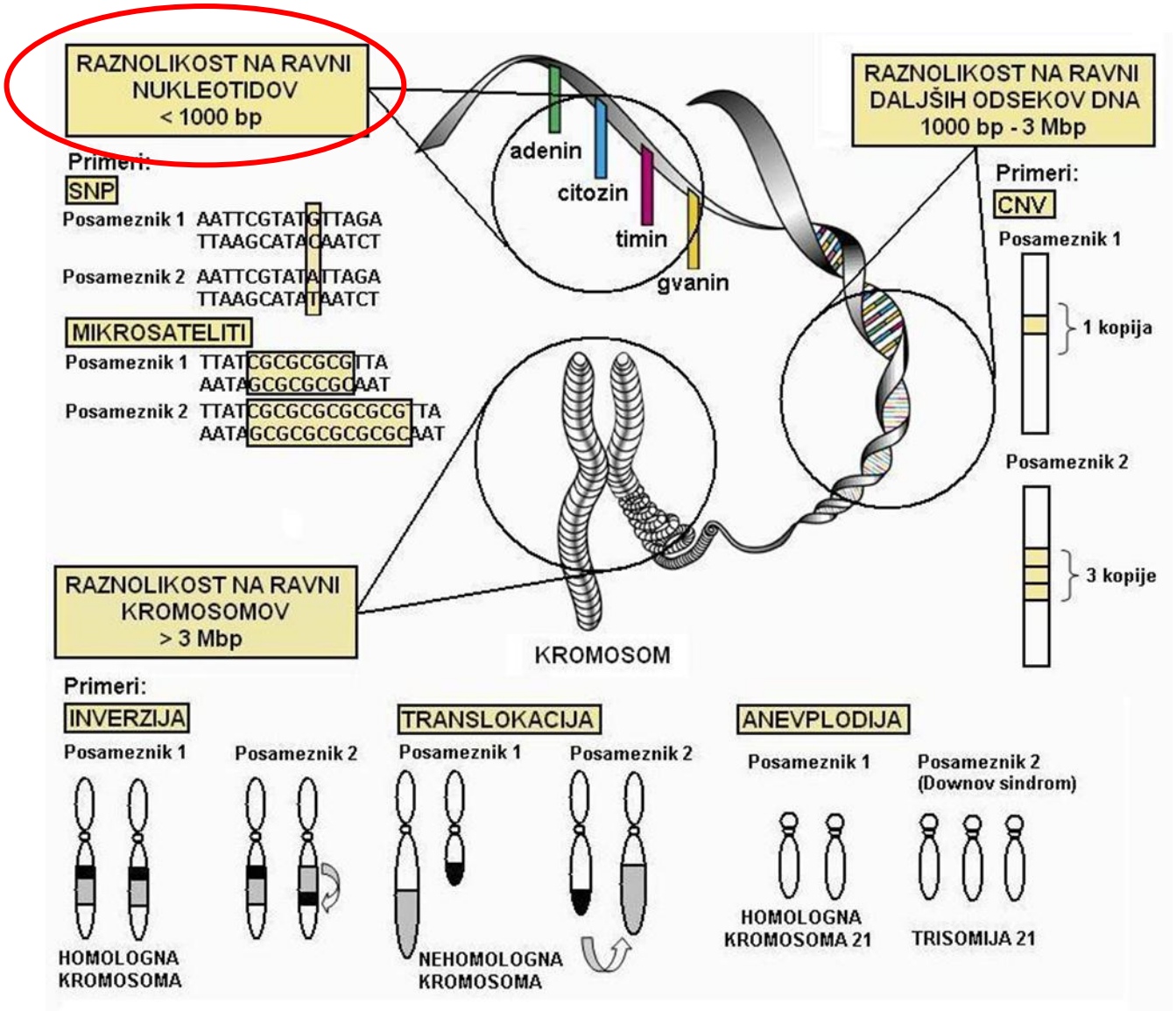


Pharmacogenomics vs. Pharmacogenetics

- The two terms are used interchangeably.
- Pharmacogenetics:
  - The study of variability in drug response due to heredity.
  - Focuses on **genes determining drug metabolism**
- Pharmacogenomics: encompasses **all genes in the genome that may determine drug response**

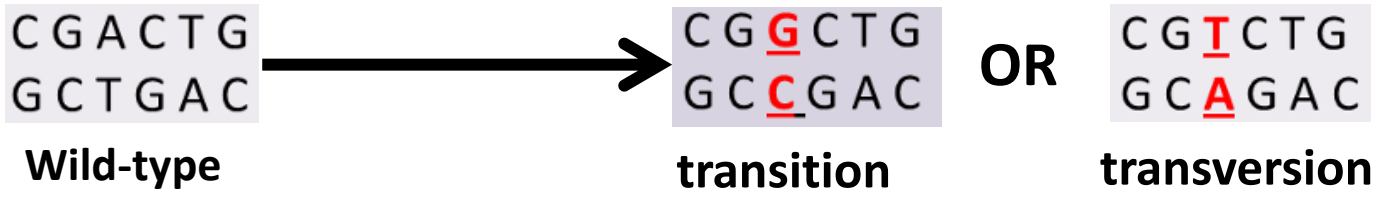


Types of genetic varitiability

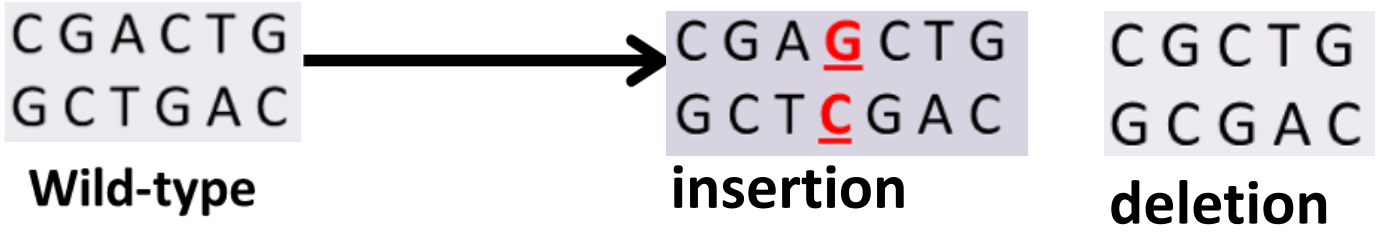


Short genetic variants (<1kbp)

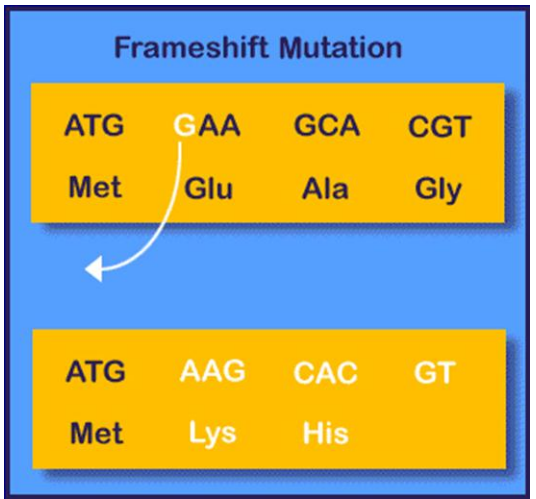
- **Single nucleotide polymorphisms (SNPs)**



- **Insertions/deletions (indels)**



Indels could result in the reading frame shift, if number of inserted/deleted bases is not divisible by 3!



Short genetic variants (<1kbp) cont`d

- **Microsatelites or short tandem repeats (STR):**
  - short nucleotide sequences ranging in length from 2 to 5 bp that are repeated 5-50 times (3% of human genome) → e.g. CACACACACA = $(CA)_5$
  - Number of tandem repeats has high inter-individual variability → $(CA)_3$, $(CA)_4$, $(CA)_6$, …
- **Multiple nucleotide polymorphisms (MNPs)**
  - Substitutions and indels involving more than 1 bp
  - Examples: CA/TG (substitution), -/ATC (insertion), TTTG/- (deletion)

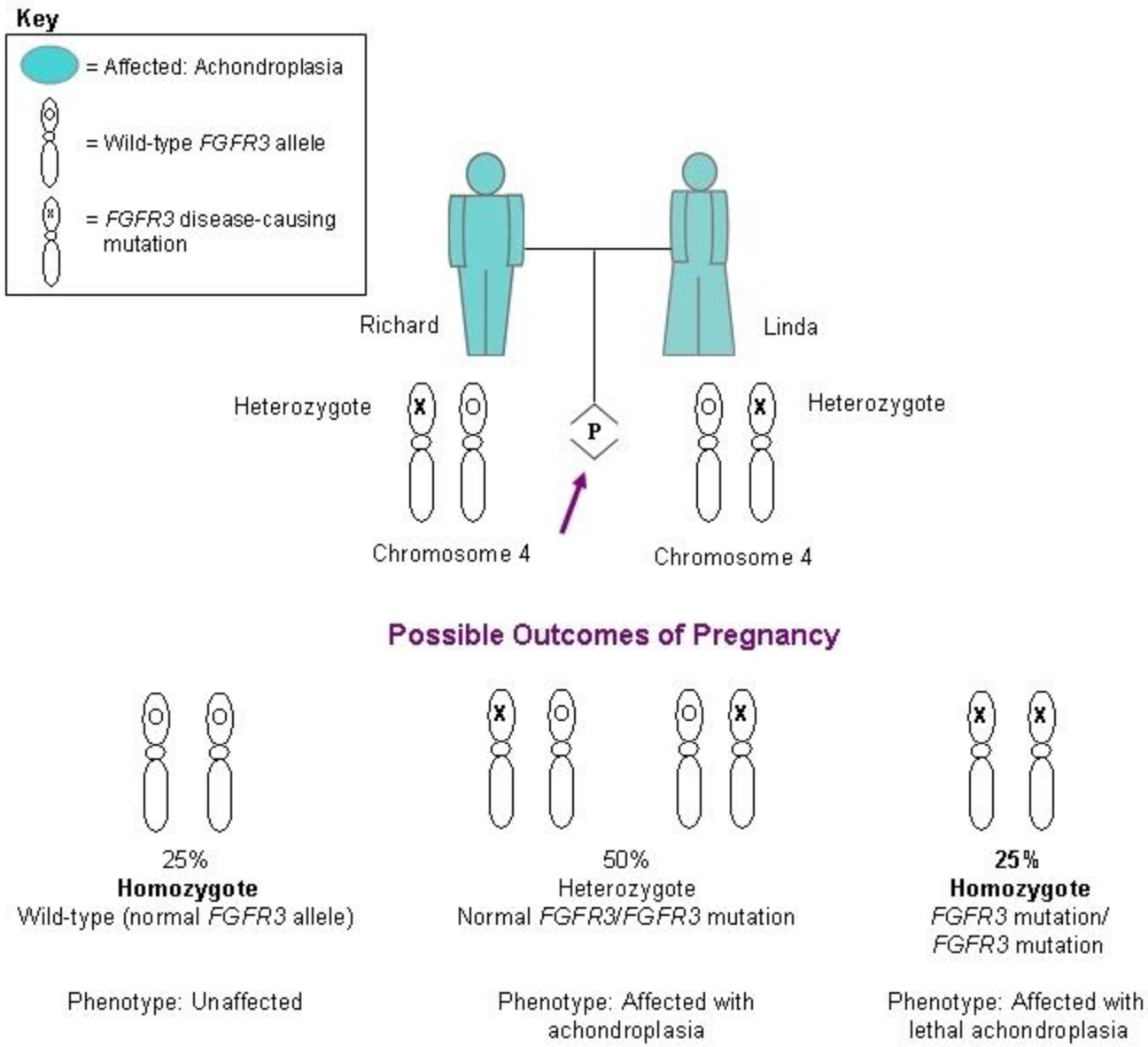
Influence of genetic variants on amino acid sequence and protein structure

- **Silent mutation**: coding for the same a.a. (or chemically similar a.a., that doesn`t affect the protein structure)
- **Missense mutation**: coding for another (chemically dissimilar) a.a.
- **Nonsense mutation**: creates premature STOP codon or abolishes it, resulting in shorter or longer protein
- **Frame shift mutation**: from the mutation site on, the a.a. sequence is completely changed

Allele zygosity

- **Homozygous:** both alleles of a diploid organism are the same
- **Heterozygous:** two alleles of a diploid organism are different

- **Hemizygous:** one of the alleles of a diploid organism is missing
- **Nullizygous:** both alleles of a diploid organism are missing



Influence of genetic variants on the protein function

- **Loss of function variants:**
  - Amorphic (Null) mutations → total loss of function
  - Hypomorphic (Leaky) mutation →partial loss of function

- **Gain of function variants:**
  - Hypermorphic mutations → increased function of a protein
  - Neomorphic mutations → lead to an atypically new function

- Dominant negative (antimorphic) mutations → changed protein product of the mutated gene antagonizes the activity of the wild-type gene product in a heterozygous individual

Determine the mutation type:

Types of Mutations

Normal gene

AS THE MAN SAW THE DOG HIT THE CAN END ITIS

AS THE MAN SAW THE DOT HIT THE CAN END ITIS

AS THE MAN SAW THE HIT THE CAN END ITIS

AS THE MAN SAW THE FAT DOG HIT THE CAN END ITIS

AS THE MAN SAW THE OGH ITT HEC ANE ND ITI S

Why we need Pharmacogenomics?

The annual incidence of adverse drug reactions (ADRs) in the USA:

- 5.000.000 ADRs
- 2.000.000 severe ADRs
- 100.000 deaths due to ADRs
- ADRs 4th leading cause of death

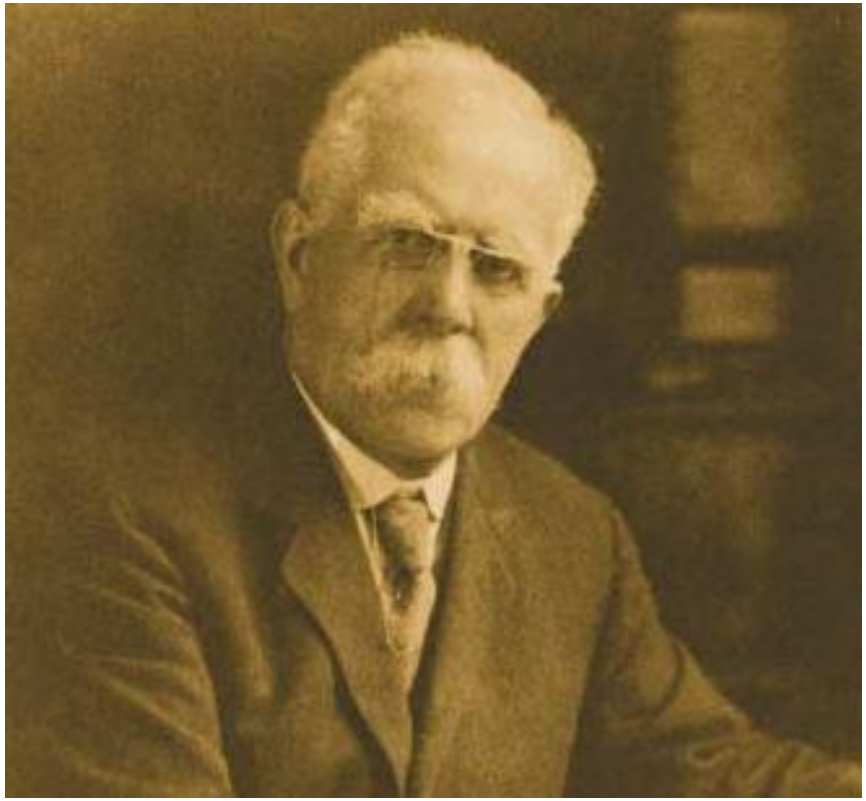
Armed with the knowledge of their patients' genetic status, physicians could predict their response to certain drugs, leading to **better efficacy**, **fewer adverse drug reactions**, and a **better cost-benefit ratio**.

Landmarks in Pharmacogenomics

Sir Archibald E. Garrod: concept of CHEMICAL INDIVIDUALITY:

„Every active drug is a poison, when taken in large enough doses; and **in some subjects a dose which is innocuous to the majority of people has toxic effects, whereas others show exceptional tolerance of the same drug.“**

(From the *The Inborn Errors of Metabolism, 1909)*



Landmarks in Pharmacogenomics

- **L.H.Snyder (1932):** the first example of a pharmacogenetic : study → Taste blindness:
  - 25% of individuals are unable to taste the bitterness of phenylthiocarbamide
  - Autosomal recessive inheritance
  - Frequency of non-tasters varied in populations of different ethnic origin
  - **The prototype for future studies of pharmacogenetic variation**

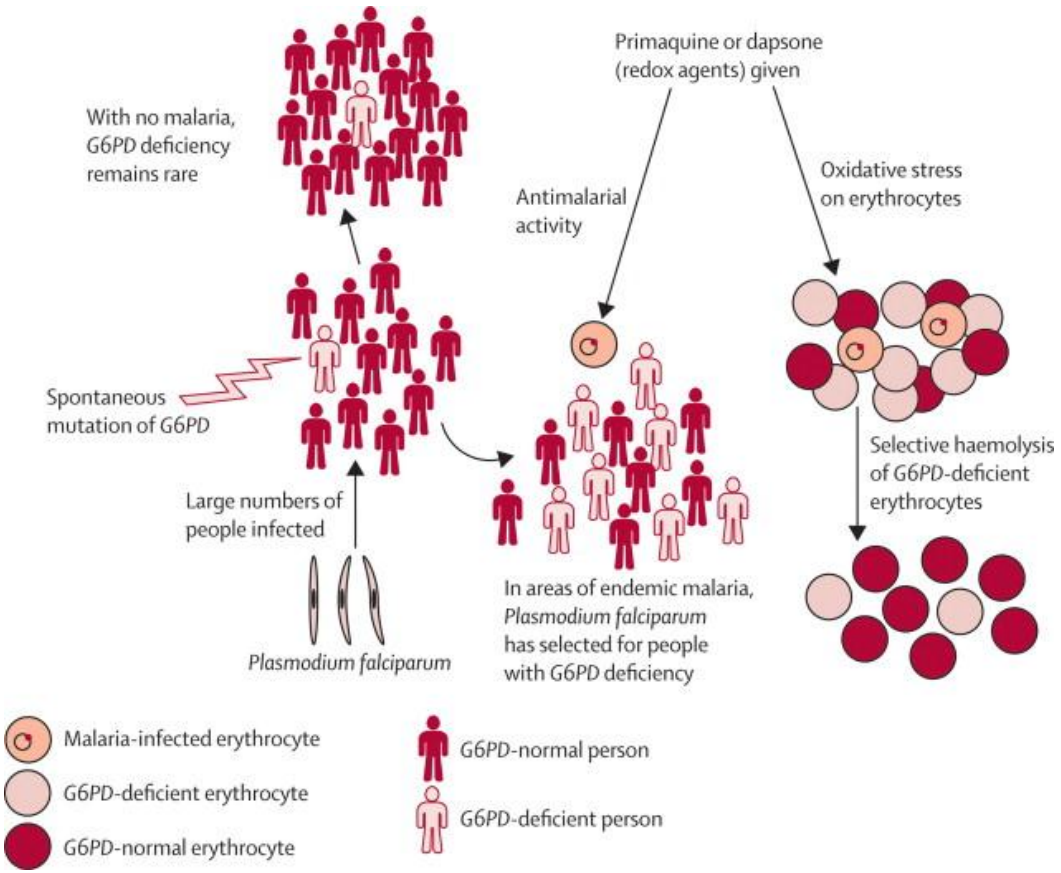
Landmarks in Pharmacogenomics

- Arno Motulsky (1957): *Drug reactions, enzymes and biochemical genetics* → This paper marked the true beginnings of PGx as a distinct discipline.
- Friedrich Vogel (1959): coined the term ‘pharmacogenetics’
- Kalow (1962): the first monograph on Pharmacogenetics published

Arno Motulsky

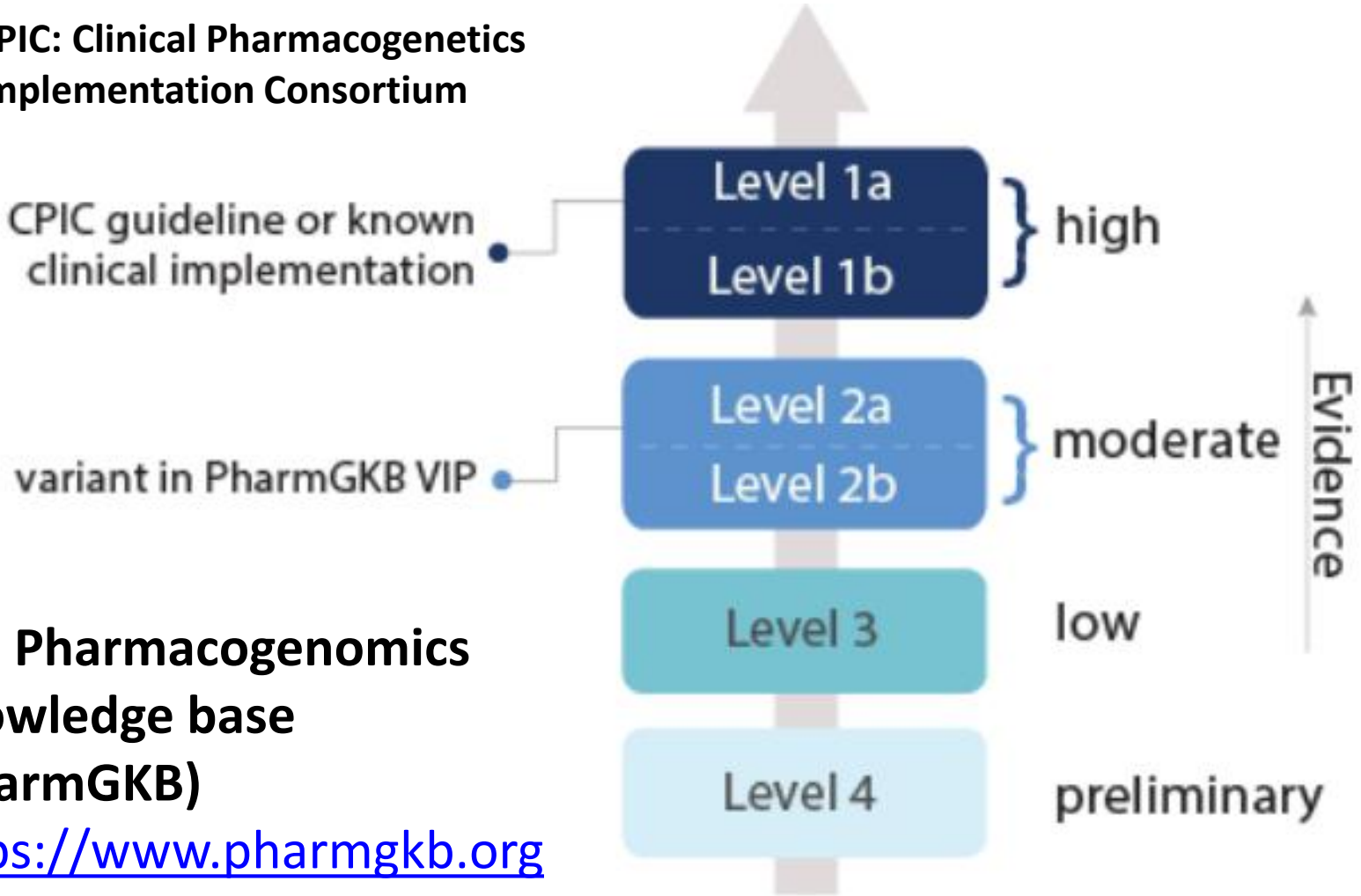
Some important early examples of pharmacogenetic traits

- **Antimalarics / glucose-6-phosphate DH**
  - ↓ G6PDH → hemolytic crisis
- **Succinylcholine / butyrylcholinesterase**
  - ↓ butyrylcholinesterase → prolonged apnea
- **Isoniazid / N-acetyltransferase-2**
  - ↓ NAT-2 (NAT2*5 and NAT2*6 slow acetylator alleles) → increased incidence of peripheral neuropathy



Clinical Annotation Levels of Evidence

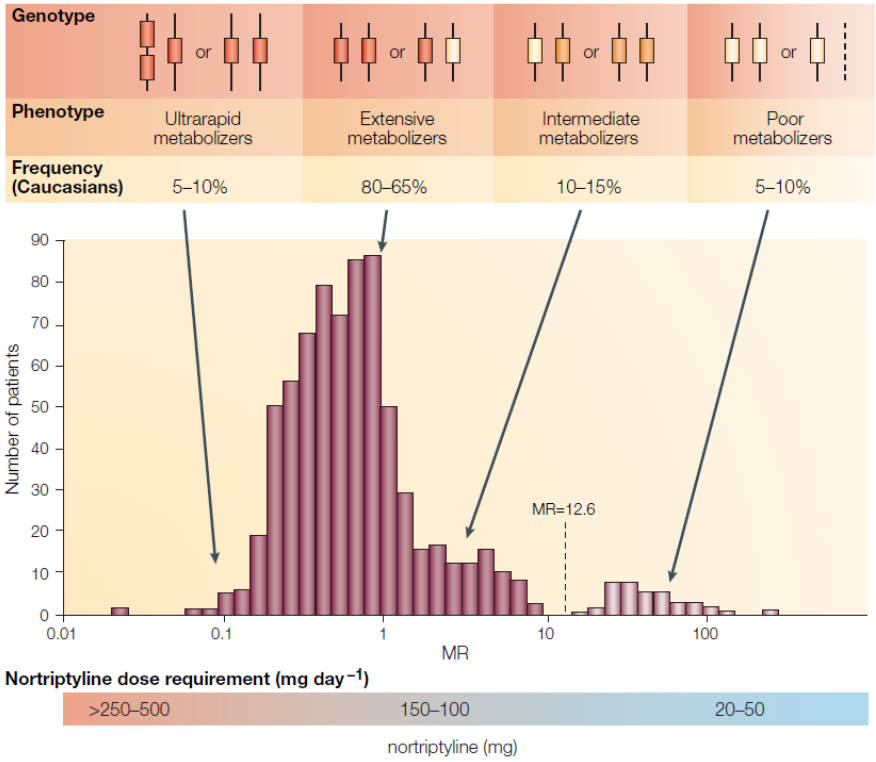
CPIC: Clinical Pharmacogenetics Implementation Consortium



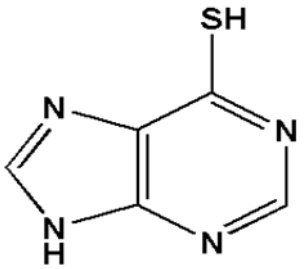
The Pharmacogenomics Knowledge base (PharmGKB)
https://www.pharmgkb.org/index.jsp

High level of evidence examples of pharmacogenetic traits

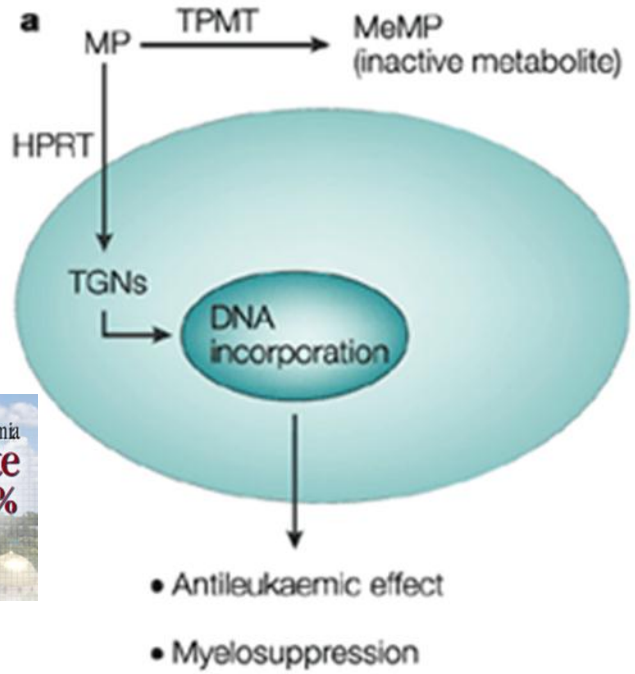
- Antidepressants, Anti-arrhythmics, Opioids / CYP2D6
- Antidepressants, Antithrombotic agents, Antimycotics / CYP2C19
- Warfarin, Phenytoin / CYP2C9
- Thioguanine drugs / thiopurine S-methyltransferase (TPMT)
- Irinotecan, Antiviral drugs / UDP-glucuronosyltransferase (UGT1A1)
- 5-fluorouracil / Dihydropyrimidine dehydrogenase (DPYD).
- Simvastatin / Solute carrier organic anion transporter SLCO1B1
- Gefitinib / epidermal growth factor receptor (EGFR, HER1)

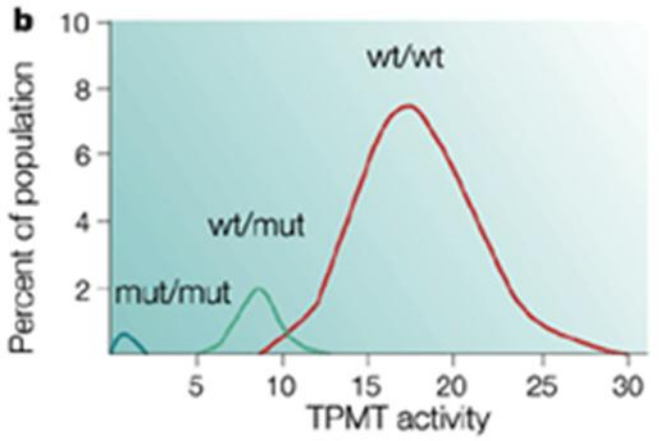


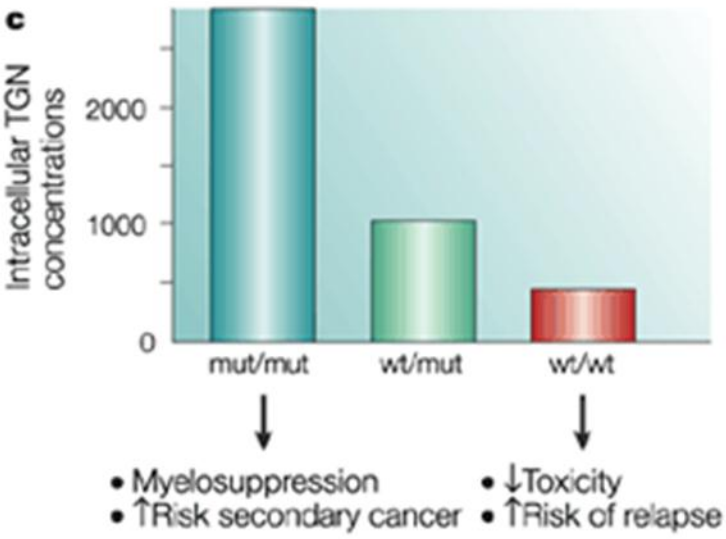
Thioguanine drugs / thiopurine S-methyltransferase (TPMT)



6-Mercaptopurine
(Purinethol ®)



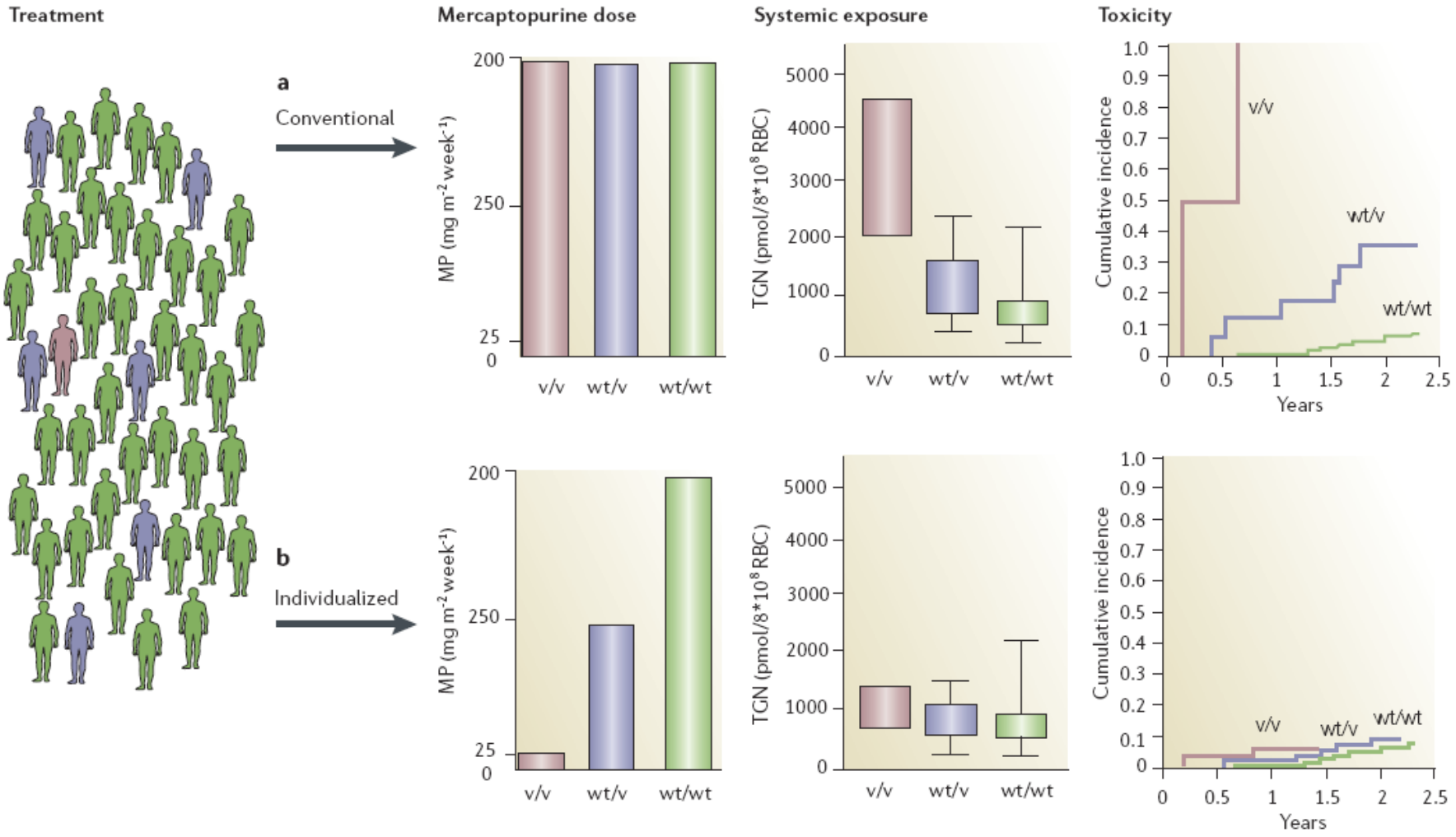




Based on the patient`s genotype her/his response to the drug might be predicted and optimal dose prescribed=
Pharmacogenetics/genomics, Personalized medicine

Individualization of thiopurine therapy

in perfect world

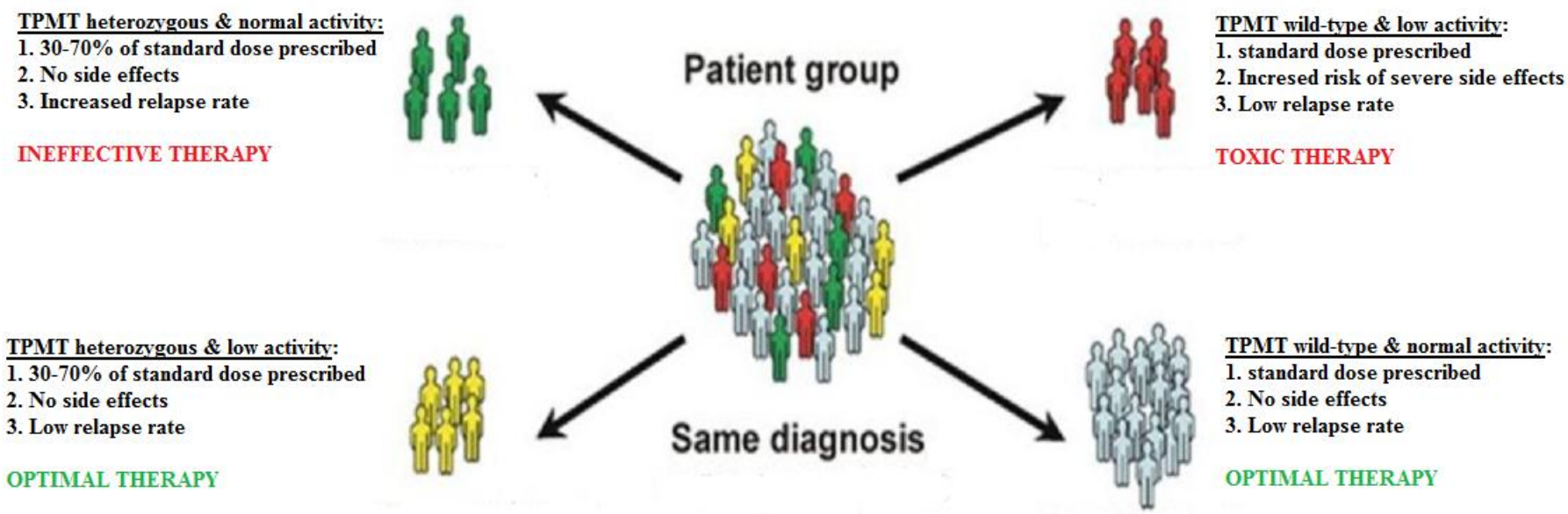


Meyling et al. Nature Rew Cancer 2006; 6: 117-129

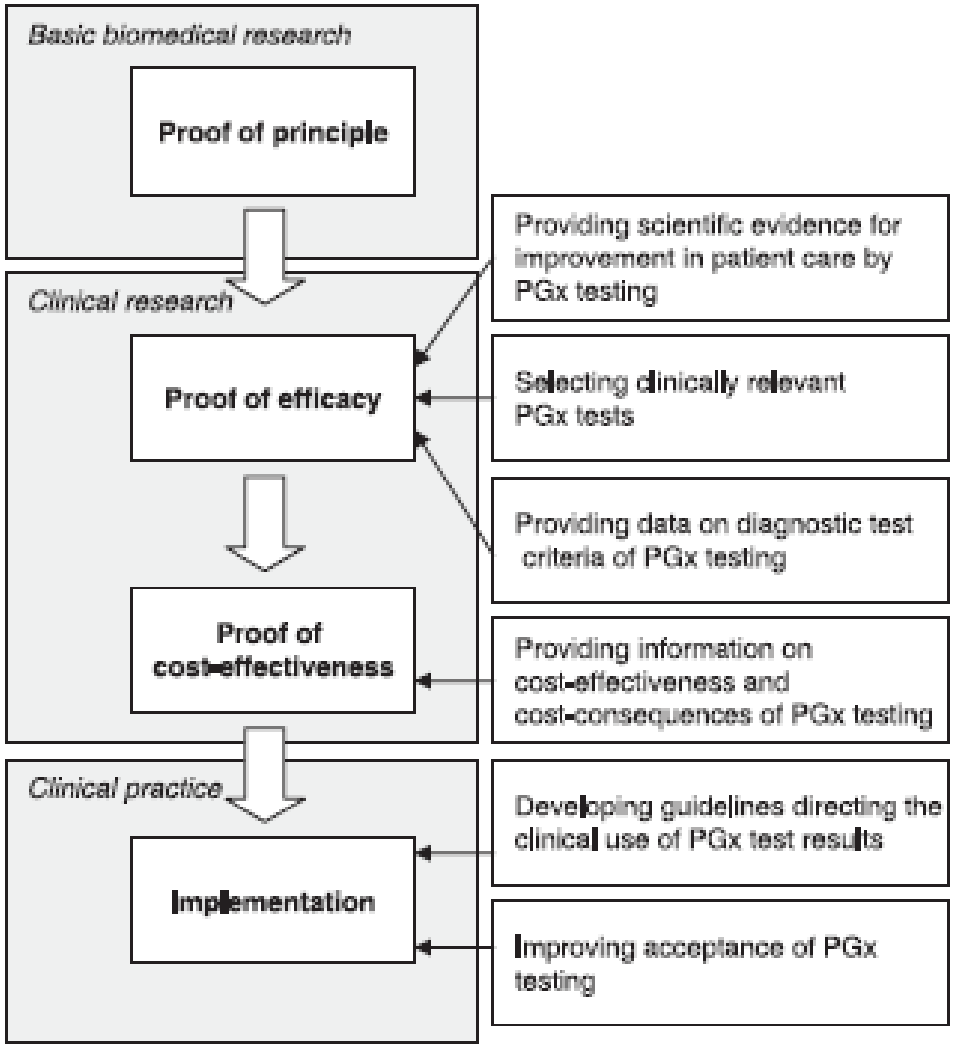
However, in the real world:

The things are not so straightforward

The unpleasant fact: If 6-MP dose is adjusted according to the patient`s TPMT genotype only, the small proportion of patients will be under-dosed or over-dosed:



Challenges to clinical implementation of Pharmacogenomics



doi:10.1371/journal.pmed.0040209.g001

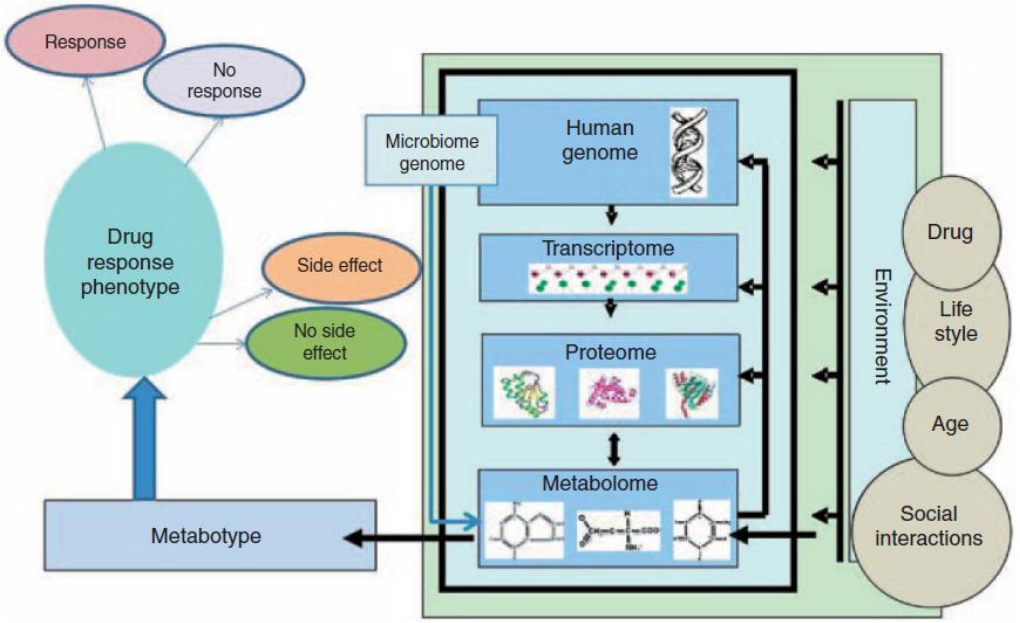
We need additional PGX markers: How to identify them?

Solution: using other OMICS to facilitate PGX discoveries → Precision medicine

- Despite some remarkable discoveries in the field of Pharmacogenomics, most drugs lack reliable PGX markers that could be used in a clinical setting.
- **Precision medicine initiative** (launched by pres. Obama in 2015): 215 million $ in the year 2016 awarded to NIH, NCI, FDA: www.whitehouse.gov/precision-medicine
- One of the goals of PMI is to **integrate the omics data such as proteomics, metabolomics, and genomics.**

Pharmacometabolomics

- Pharmacometabolomics investigates the integrated influence of the **genome, microbiome and the environment** on the drug efficacy and safety. It uses metabolic profiles (metabotypes) as markers of therapy response.
- Metabotypes can help:
  - **predict therapy response (good vs. poor responders)**
  - explain mechanisms of drug action (before vs. after drug exposure)
  - give insight into the influence of the microbiome on the drug`s pharmacokinetics (gut flora can modulate biological availability of the drug or increase production of toxic metabolites)
- **Translation of the results to the field of Pharmacogenomics → identification of new PGx markers, that would not be possible by using only Pharmacogenomic approach**



Example 1: Pharmacometabolomics of acetylsalicylic acid and discovery of new PGx marker

Drug exposure

- 165 healthy individuals, taking aspirin for 14 days (81 mg/day)
- For each individual measure 165 metabolites before & after exposure (GC, LC-MS)
- The greatest effects on **purines** levels (inosine ↑ 77%)

Therapy response

- 40 good & 36 poor responders, GC-MS
- After the aspirin exposure more pronounced increase of **inosine & adenosine** levels in poor vs. good responders

PGx study

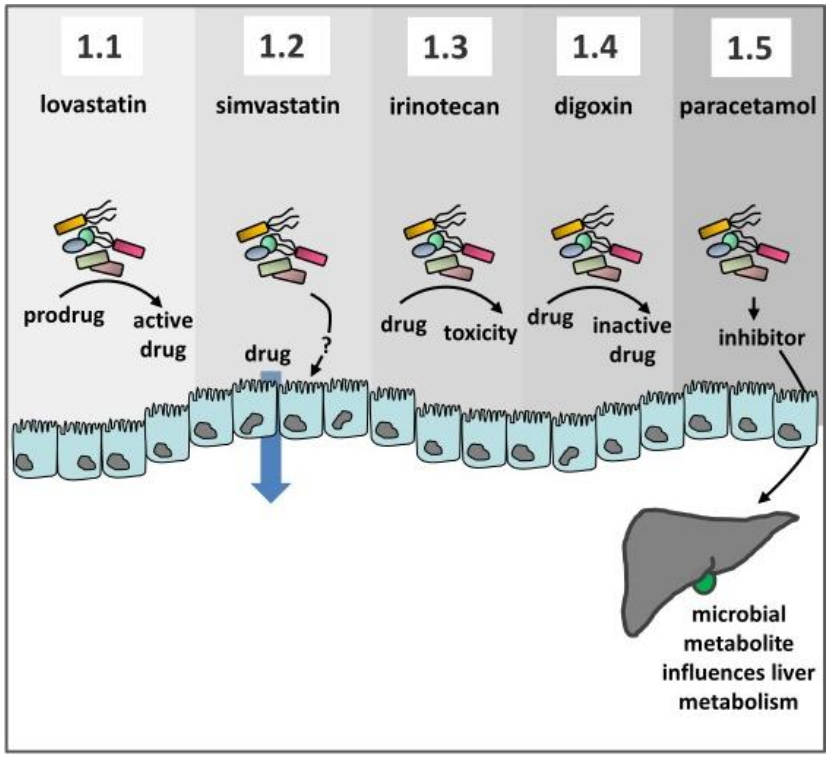
- 165 healthy individuals, taking aspirin for 14 days (81 mg/day)
- GWAS (poor vs. good responders – platelet aggregation)
- Best hit: **rs16931294 in the adenosine kinase (ADK) gene**

Replication study

- Independent cohort of 321 individuals
- **Rs16931294 A>G in ADK gene is associated with poor response to aspirin in terms of preventing platelet aggregation**

Example 2: Pharmacometabolomics of paracetamol (influence of microbiome)

- Pharmacometabolomic studies on mice and humans: higher urinary levels of **p-cresol** before the paracetamol (PA) administration are associated with higher risk of PA-induced hepatotoxicity.
- Mechanism: p-cresol is a product of microorganism *Clostridium difficile* (which is a normal part of gut flora). p-cresol competes with PA at the active site of **sulfotransferase SULT1A1** → at high levels of p-cresol, less PA is sulfonated to non-toxic products and consequently more of the hepatotoxic N-acetyl-p-benzoquinone imine is formed.



Useful links:

- **The Pharmacogenetics and Pharmacogenomics Knowledge Base (PharmGKB):** http://www.pharmgkb.org/

- Clinical Pharmacogenetics Implementation Consortium (CPIC): https://cpicpgx.org/
- On Line Mendelian Inherritance in Man (OMIM): https://www.omim.org/
- Pharmacogenomics Research network (PGRN): http://www.pgrn.org/