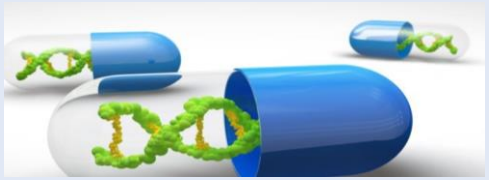
The integration of pharmacogenomics into clinical practice



Andriani Grigoratou,

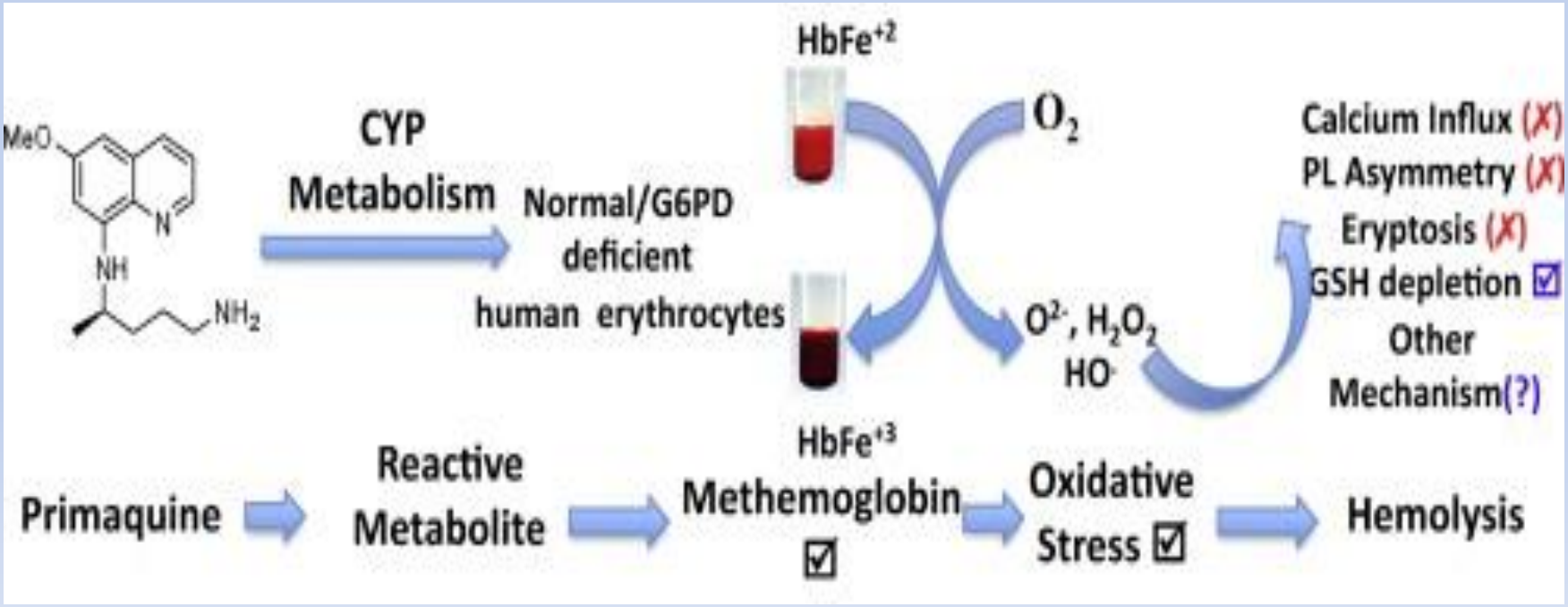
Evaggelismos Hospital,
Athens, Greece.

ΓΕΝΙΚΟ ΝΟΣΟΚΟΜΕΙΟ ΑΘΗΝΩΝ
Ο ΕΥΑΓΓΕΛΙΣΜΟΣ

- Therapeutic drugs save millions of lives a year.
- The Goal of Personalized Medicine demands the

the right dose of
the right drug for
the right indication for
the right patient at
the right time.

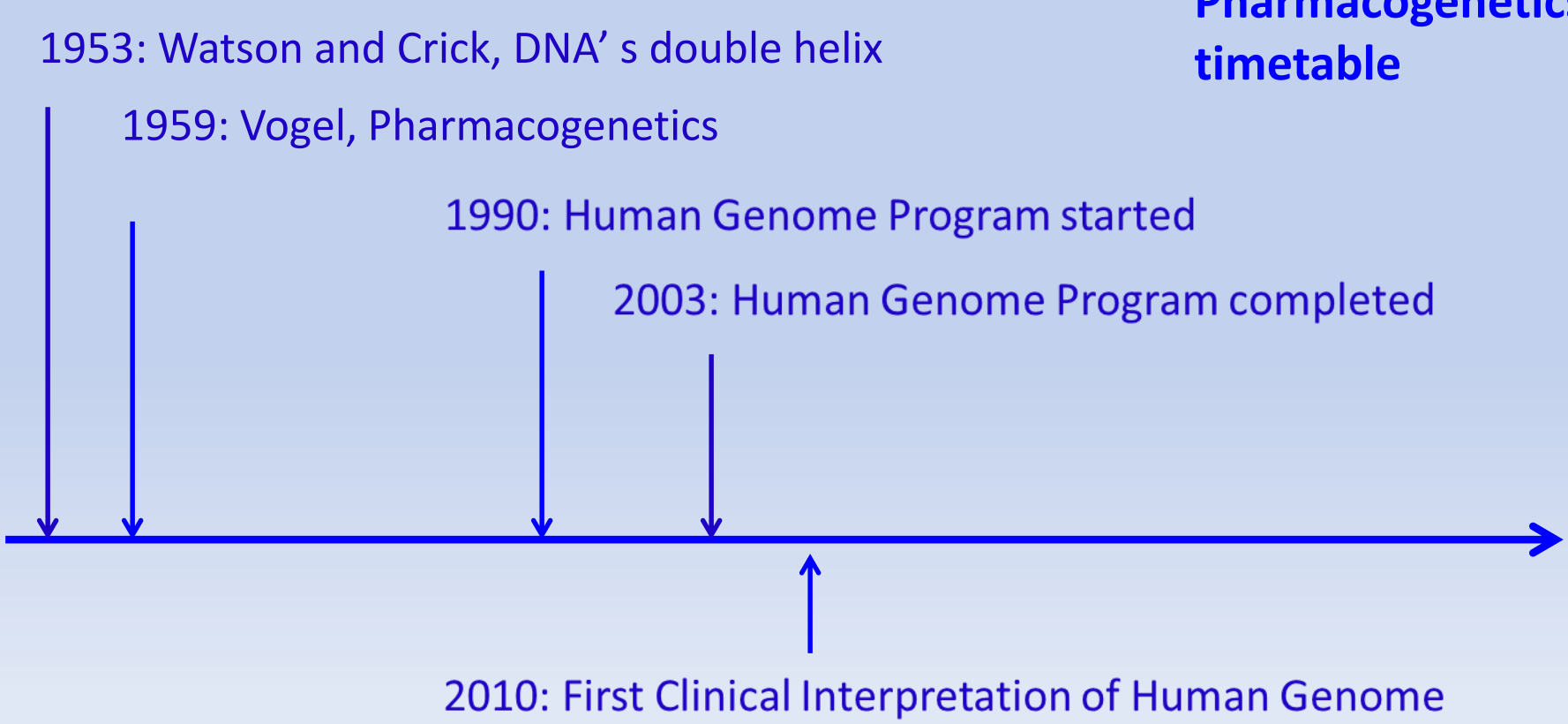
- The genetically determined differences in drug metabolism, drug distribution, drug target proteins and disease-associated proteins result in the observed **intrapatient** variability. Pharmacogenomics studies how a patient's genome influences the response to medications. In the recent years Pharmacogenomics has made important progress, but we are still in the early stages.
- The roots of Pharmacogenomics date back to Pythagoras (Pythagorean theorem), an ancient Greek philosopher, who noticed a connection between fava bean ingestion and illness or fatigue only for some individuals.



- Today is known that glucose-6-phosphate dehydrogenase G6PD is the most prevalent enzyme deficiency. Primaquine -an antimalarial drug- caused haemolytic anaemia to soldiers in World Wars I and II. Since 1956 haemolytic anaemia and jaundice have been associated not only with fava beans, but also with several medications.

Understanding the mechanisms for metabolism-linked hemolytic toxicity of primaquine against glucose 6-phosphate dehydrogenase deficient human erythrocytes: Evaluation of eryptotic pathway. Toxicology 294, (1), 2012, 54-60.

Pharmacogenetics timetable

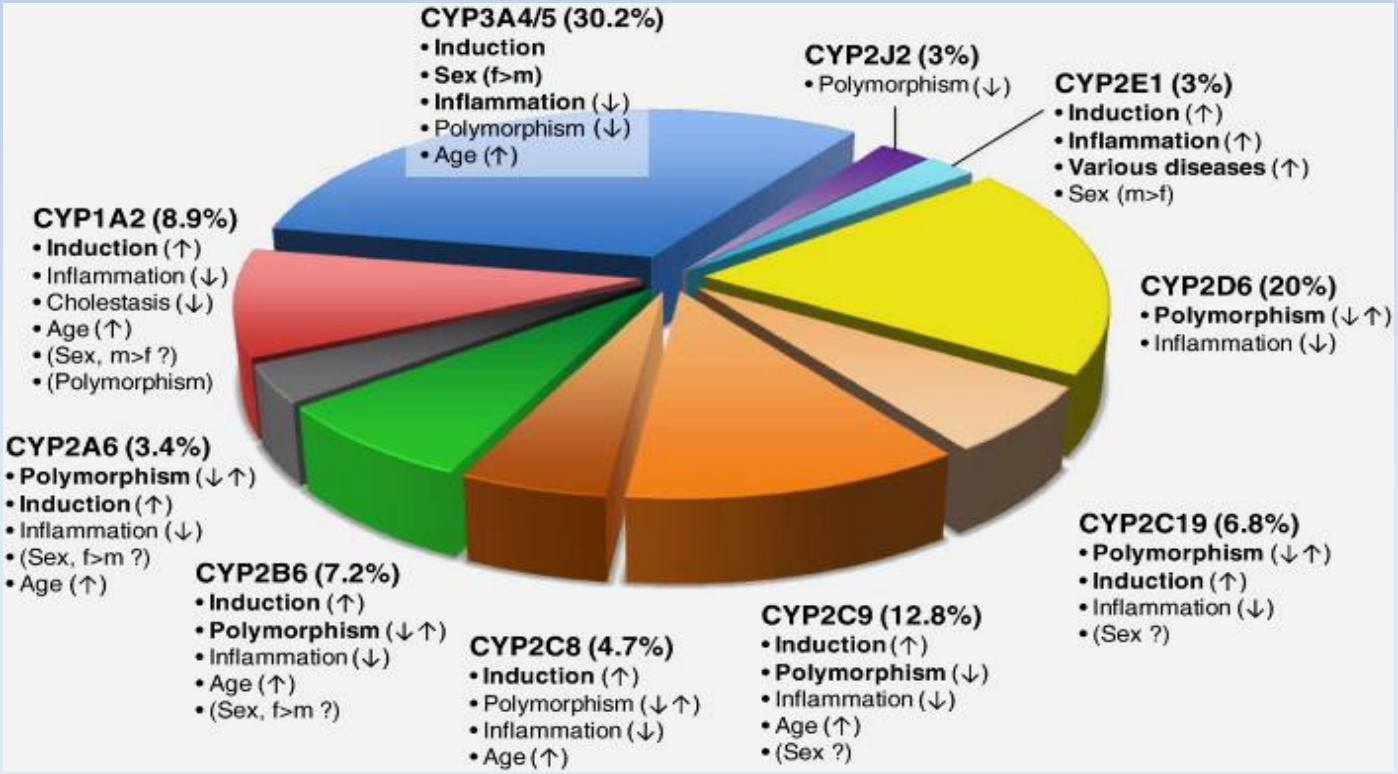


The term pharmacogenetics was coined by Friedrich Vogel (1959).

How is pharmacogenomic information helpful?

- Age, weight, sex, drug-drug interactions, drug-food interactions, disease state (hepatic/renal function), pregnancy, influence variability of patients' response. However genetic factors play a major role.
- Pharmacogenomics refers to the identification of genetic factors that influence ADME (absorption, distribution, metabolism, elimination) and action on receptor level.
- Pharmacogenomics studies the genetic sequence variants, the structural changes in chromosomes, the epigenetic phenotype changes (eg. methylation), changes in the expression profile (mRNA levels) and changes in microRNA. A genetic variation may be inherited or acquired. The most common type of these variations are SNPs (single nucleotide polymorphisms): nucleotide substitutions, insertions, deletions, copy number variations, short tandem repeats.
- The location of the variation is crucial for its functional consequences.
- A considerable part of studies refer to altered pharmacokinetics and concern the drug metabolizing enzymes DME.

- In humans there are 2 phases of xenobiotic metabolism.
- Phase I (modification: oxidation, reduction, hydrolysis) to improve water solubility. Cytochromes CYP450 are a superfamily of heam-containing monoxygenases. 58 genes encode the CYP isoenzymes that metabolize the therapeutic drugs (CYP3A4, CYP3A5, CYP2D6, CYP2C9 and CYP2C19).



CYP450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology & Therapeutics 138 (1), 2013, 103-141.

- Phase II (conjugation to non toxic metabolites).

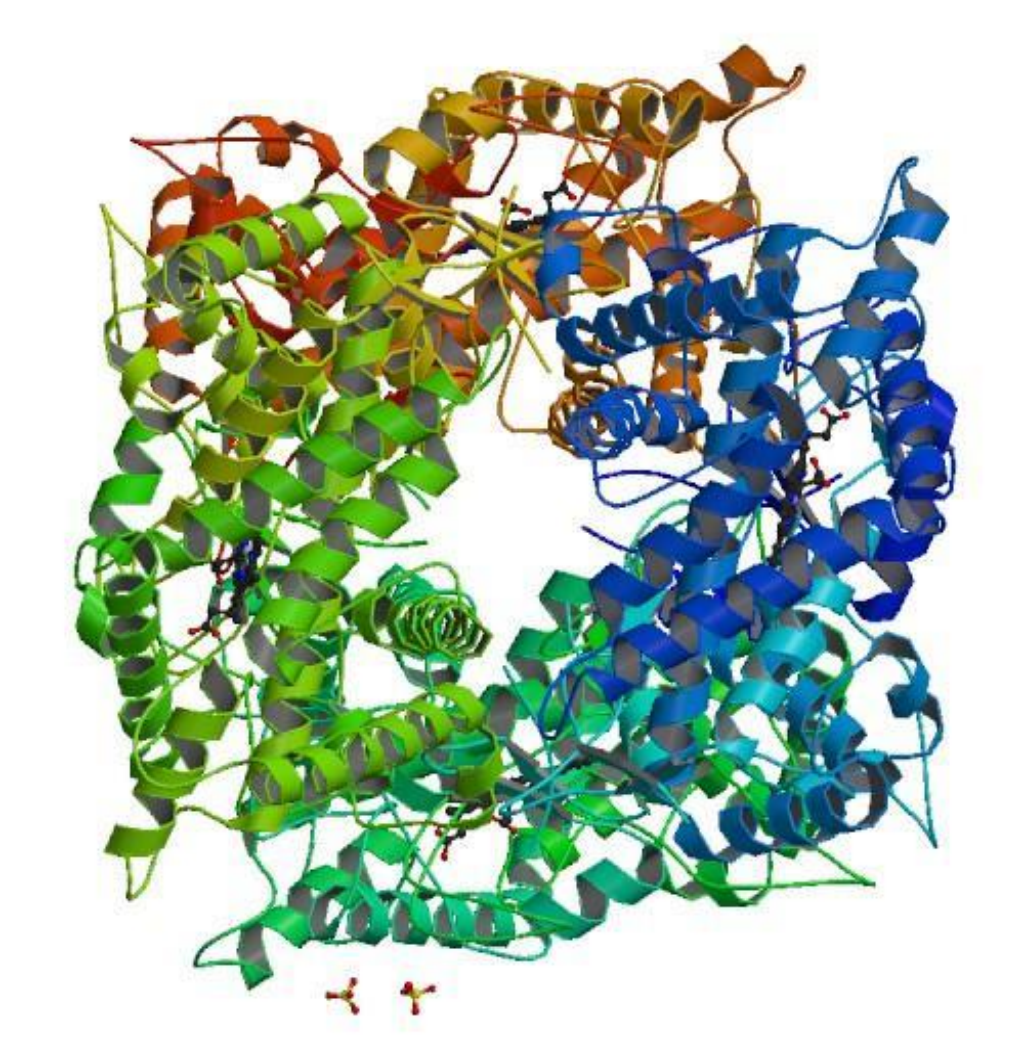
Most common CYP2D6 inhibitors according to FDA

Strong inhibitors[1]	Weak inhibitors[3]
Bupropion	Amiodarone
Fluoxetine	Celecoxib
Paroxetine	Cimetidine
Quinidine	Desvenlafaxine
	Diltiazem
	Diphenhydramine
	Echinacea
	Escitalopram
	Febuxostat
Moderate inhibitors[2]	Gefitinib
Cinacalcet	Hydralazine
Duloxetine	Hydroxychloroquine
Terbinafine	Imatinib
	Methadone
	Oral contraceptives
	Propafenone
	Ranitidine
	Ritonavir
	Sertraline
	Telithromycin
	Verapamil

Most common CYP2C9 inducers

Carbamazepine
Barbiturates
Rifampin
Phenytoin
Primidone
St. John's wort

https://www.uniprot.org/uniprot/P10635



CYP2D6

poor metabolizers

(none enzymatic activity):
6-10% Caucasians,

2-5% African Americans,
1% Asians.

CYP2D6

ultra rapid metabolizers:

4% North Americans,

10% Greeks,

10% Portuguese,

30% Ethiopians

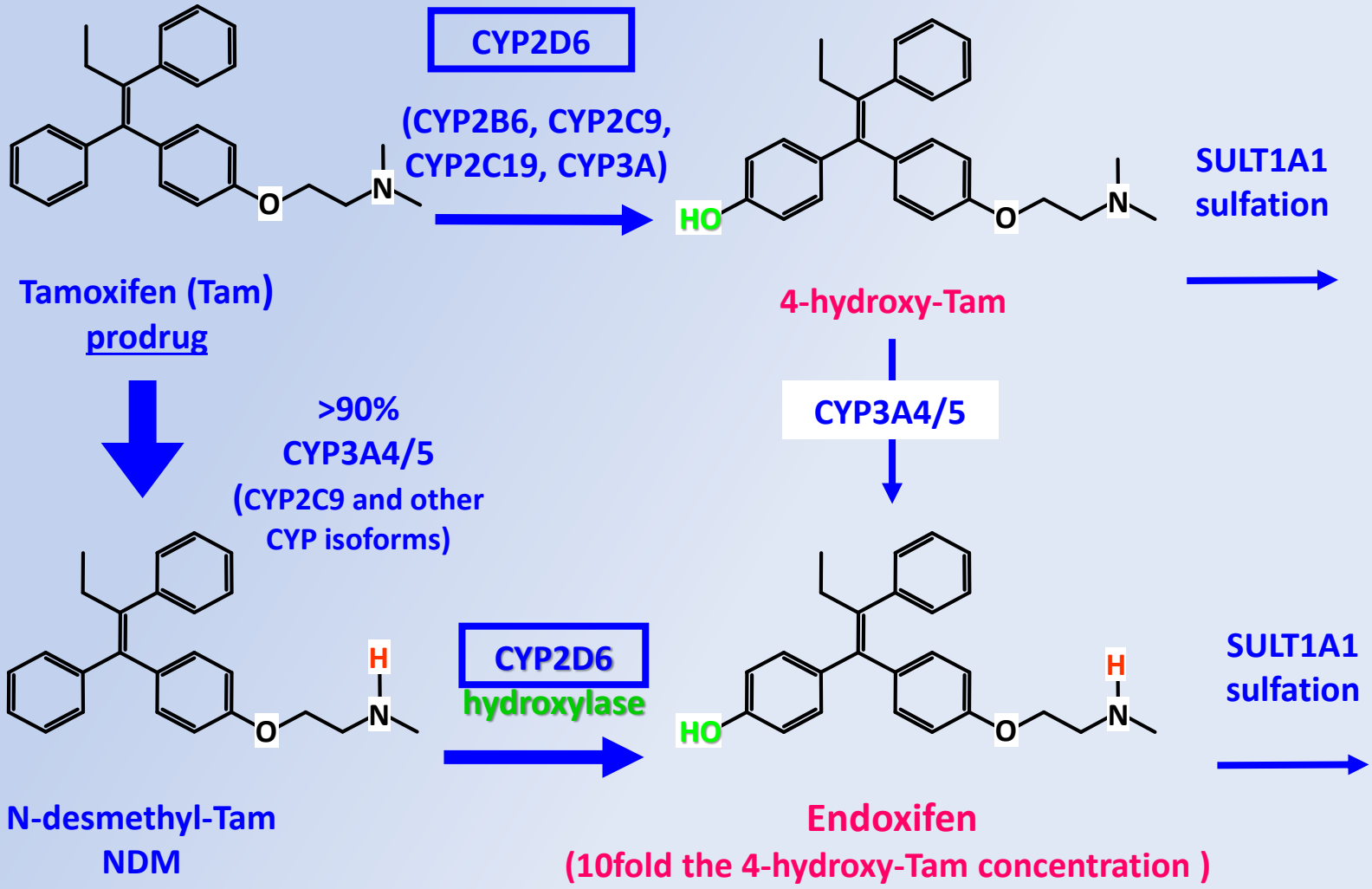
Human CYP2D6 3D structure, Oxidoreductase, EC 1.14.14.1
Molecular weight: 55,769 Da, Basal Isoelectric point: 6.77
Chromosomal Location of Human Ortholog: 22q13.2

- CYP2D6 is the most highly polymorphic and extensively characterized drug-metabolizing enzyme. There are 90 allelic variants of CYP2D6.

Table of CYP2D6 substrates

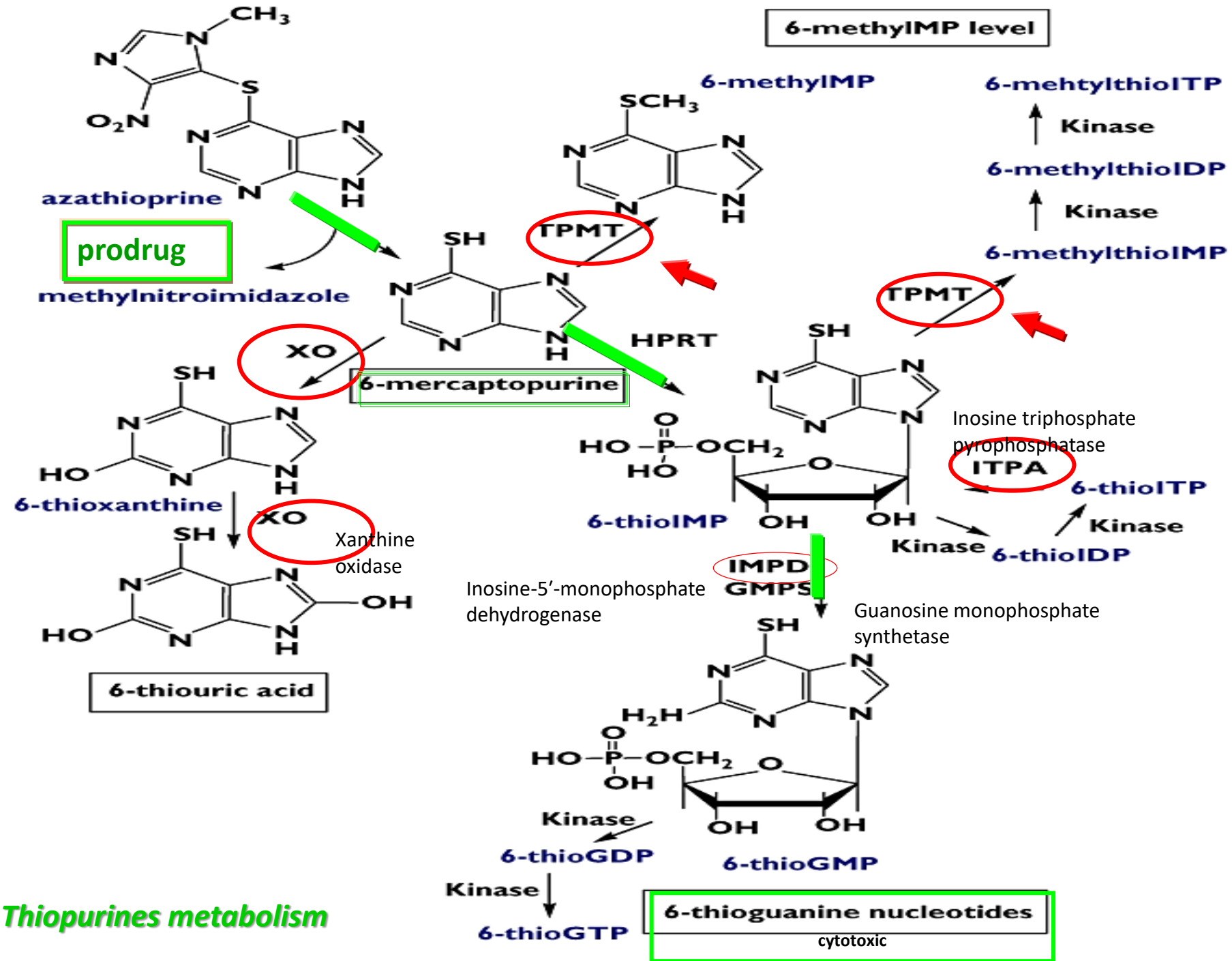
Cardioactive drugs	Amiodarone, encainide, flecainide, lidocaine, mexiletine, propafenone,
Antidepressants	Amitriptyline, clomipramine, desipramine, doxepin, fluoxetine, fluvoxamine, imipramine, nortriptyline, paroxetine, trazodone, venlafaxine
Antipsychotic	Chlorpromazine, haloperidol, perphenazine, quetiapine, risperidone, thioridazine,
Beta-blockers	Alprenolol, carvedilol, labetalol, metoprolol, penbutolol, pindolol, propafenone, propanolol, timolol
Antihypercholesterolemic	Simvastatin
Analgesics	Codeine, fentanyl, meperidine, oxycodone, propoxyphene, tramadol

Tamoxifen: anti-estrogen for the treatment of patients with hormone-dependent breast cancer



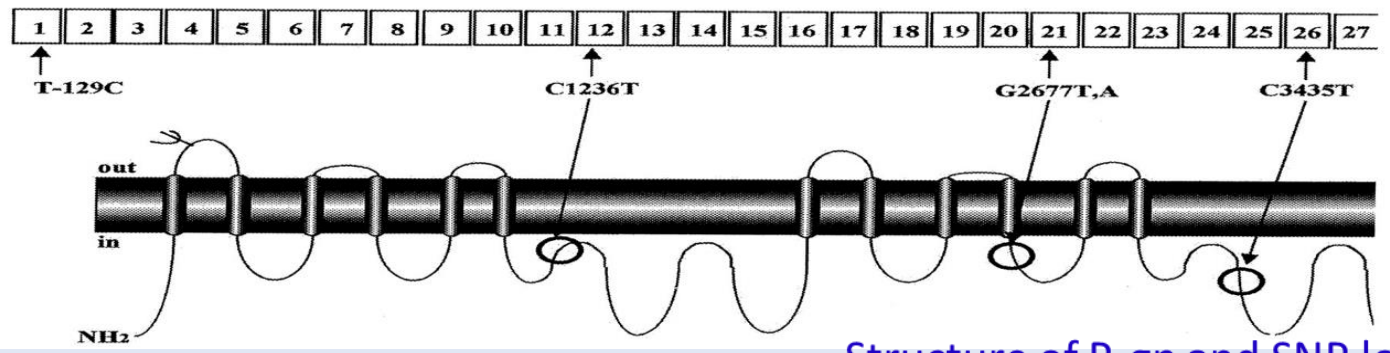
Jin Y. et al. *J Natl Cancer Inst.* 2005;97:30-39

- TPMT is a Phase II metabolic enzyme.
- TPMT (Thiopurine S-Methyltransferase) metabolizes azathioprine, 6-mercaptopurine and thioguanine, prescribed for the treatment of inflammatory, autoimmune and malignant disorders. The inactivation or decreased activity of TPMT increases the risk of severe treatment-related leucopenia.
- More than 24 low activity genetic variants have been detected and 2 of them account for more than 95% defective TPMT.
- 1/10 patients have reduced activity and 1/300 have zero enzymatic activity.
- Those patients need up to 90% dose reduction, in order to minimize the drug-induced toxicity. Specific dose recommendation (2013) is offered by CPIC (Clinical Pharmacogenetics Implementation Consortium).
- Routine TPMT genotyping has not been universally adopted and the available data on genotyping cost - effectiveness are conflicting.
- FDA doesn’t specifically recommend TPMT testing prior to treatment with thiopurines. Genotyping is ordered only for patients with prolonged myelosuppression.



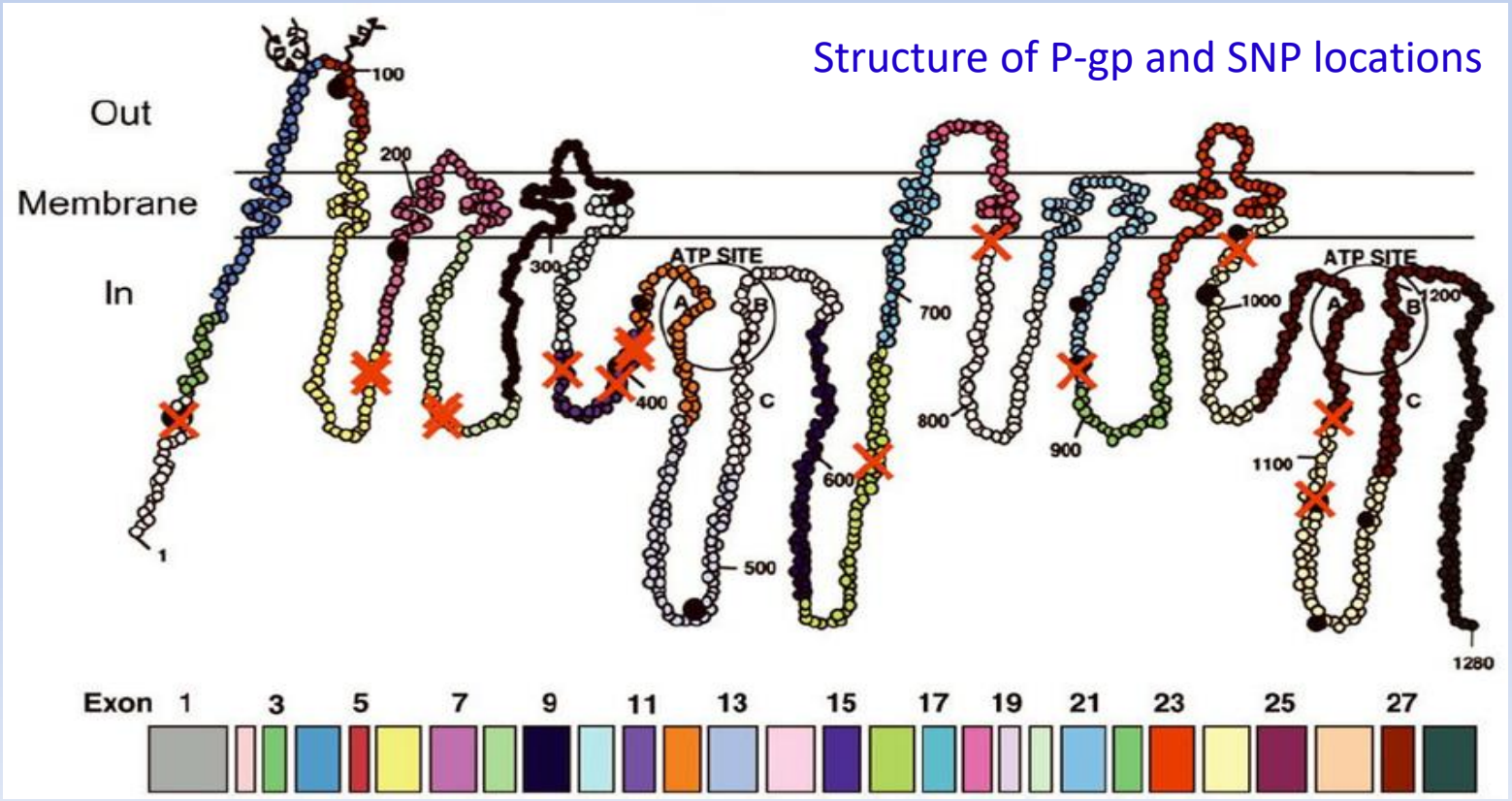
Thiopurines metabolism

- Drug transport. It is the number 2 major area of pharmacokinetics. The most extensively studied drug transport proteins are the membrane transporters ABC (ATP-Binding Cassette) family, that dispose and regulate drug action at cellular level.
- P-gp (Permeability glycoprotein), also called MDR1 (Multidrug Resistance Protein 1) encoded by *ABCB1* gene (ATP-Binding Cassette sub-family B, member 1), one of the most clinically important transmembrane transporters in humans, increases the energy dependent cellular efflux of many substances (bilirubin, antiretroviral drugs, chemotherapeutics).
- Certain P-gp SNPs predict drug plasma concentration or toxicity in cancer or HIV-infection.



Structure of P-gp and SNP locations

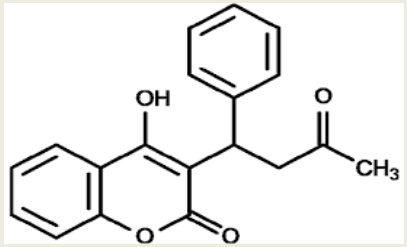
https://www.researchgate.net



The N-terminal half of P-gp contains 6 transmembrane domains, followed by a large cytoplasmic domain with an ATP-binding site, and then a second section with 6 transmembrane domains and an ATP-binding site. The two sections have 65% of amino acid homology. More than 50 SNPs have been identified, that have been extensively studied in cancer and been associated with altered mRNA levels, protein folding and drug pharmacokinetics (Cascorbi, 2006, Sauna et al. 2007, Longo et al. 2010).

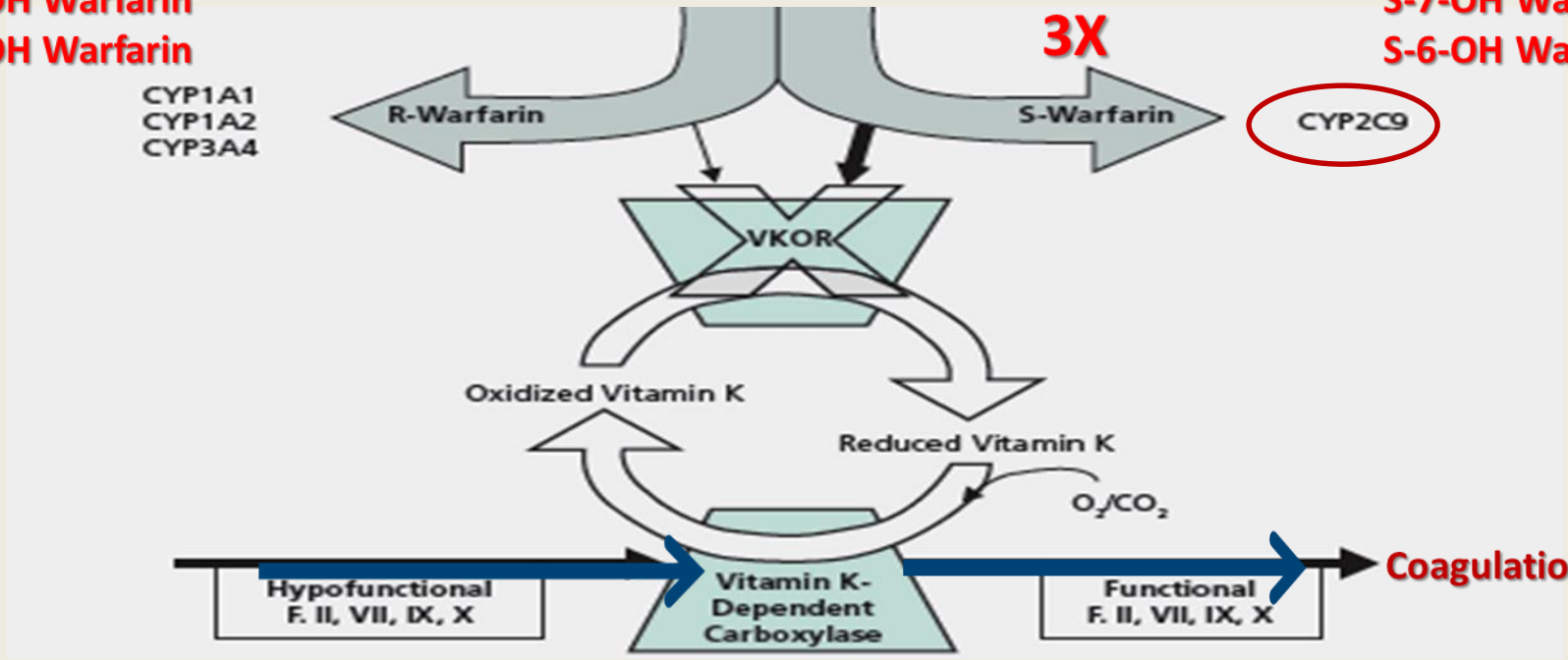
- **Pharmacodynamics** studies the effects of drug administration on the therapeutic target and other non target sites.
- Although the drug concentration at the target site may be appropriate, there are differences in the therapeutic results.
- Usually the physiology of the pathways to the drug target are complex, so it is difficult to find the differences due to genetic variation. In the case of drug metabolizing enzymes the differences are extremely high (sometimes 1000 fold).
- However it is difficult to find out in the differences in the drug binding at the target site, that are rather low (20 fold).
- In the case of anticoagulant **warfarin**, a drug with the narrow therapeutic index and wide variability in individual doses, the main issue is the gene *VKORC1* encoding the vitamin K epoxide reductase. This reductase is the target of warfarin and converts it to vitamin K. This is the rate limiting step of vitamin K recycling.
- There are also variants in CYP2C9 warfarin metabolizing enzyme.

R,S-Warfarin



R-4'-OH Warfarin
R-7-OH Warfarin
R-6-OH Warfarin

S-7-OH Warfarin
S-6-OH Warfarin



Warfarin is supplied as a racemic mixture of R- and S- (5 times more potent at inhibiting VKOR) enantiomers. It inhibits vitamin K reductase complex subunit 1 to interfere with the vitamin-K-dependent carboxylation and activate clotting factors prothrombin II, VII, IX, X) in the coagulation pathway. Genotyping *VKOR* and *CYP2C9* is proposed (delay of results).

- There are idiosyncratic, hypersensitivity or type B immune reactions, that occur rarely and unpredictably, are not related to any known pharmacological properties of a certain drug.
- Patients with one or two copies of the Human Leucocyte Antigen HLA-B*15:02:01 allele, treated with the antiepileptic carbamazepine, have an increased risk of induced Stevens-Johnson syndrome (CBZ-SJS) and toxic epidermal necrolysis. In Asian patients screening for HLA-B*15:02:01 has prevented CBZ-SJS. HLA-B*15:01 functions via binding the T-cell receptor protein using the antigen-presenting area and changing the shape and chemistry of the antigen-binding cleft. However, in a number of cases patients who had no HLA-B*15:02 developed CBZ-SJS.
- Abacavir is an antimetabolite, a reverse transcriptase inhibitor used in the treatment of HIV (Human Immunodeficiency Virus) infection. Conventional Major Histocompatibility Complex-class I (MHC-I) antigen presentation and activation of HLA-B*5701 induces the secretion of inflammatory mediators (TNF-α, γ-IFN) and leads to a delayed hypersensitivity reaction.

How we are familiar to access and use evidence-based pharmacogenomic test results and transform it into guided personalized therapy? There are 20000 citations on PubMed and clinician-friendly publicly available pharmacogenomics resources.

https://www.fda.gov/Drugs/default.htm

https://www.pharmgkb.org/

https://cpicpgx.org/

- 200 therapeutic drugs (10% of FDA approved drugs) have labels with pharmacogenomic and prescribing information.
- The Pharmacogenomics Knowledge Base, PharmGKB, is a public repository of genotype for researchers investigating how genetic variation affects drug response. PharmGKB (650 annotated drugs), provides information regarding 136 drug-centered pathways, pharmacogene summaries, 103 clinical annotations, pharmacogenomic – based drug-dosing guidelines, 513 drug-labeled annotations.
- Clinical Pharmacogenetics Implementation Consortium (CPIC) of the Pharmacogenomics Research Network established in 2009, is an international consortium with updated, evidence-based, freely accessible guidelines for gene/drug pairs, that help clinicians understand how available genetic test results should be used. These guidelines will facilitate the translation of pharmacogenomic knowledge from bench to bedside.
- Royal Dutch Association for the advancement of pharmacy (KNMP)
- Canadian pharmacogenomics network for drug safety (CPNDS)

- One size doesn't fit all. We must use pharmacogenomic information to tailor medication dosing.

- The difficulties to overcome in pharmacogenomic testing.

- The pharmacogenomics integration in the clinical practice is slow and the tests are not in routine use. There is need of education on the advantages of translation of the biological differences into clinical decisions.
- We need studies on clinical benefits of pharmacogenomics for the health system, the patients and the medical stuff. There is concern on the delay in therapy because of genotyping tests.
- There are few prospective randomized trials revealing better clinical results, when medication or its dose is selected according to pharmacogenomic data. It is difficult and expensive to study a large enough appropriate subpopulation that presents a strong phenotypic difference.
- Clinical trials are needed to identify links between genes and treatment outcomes but also to confirm initial findings, clarify the meaning of these associations and translate them into prescribing guidelines.

- Today the performed cost - effectiveness analyses, on the reimbursement of pharmacogenomic tests, are few. The cost - effectiveness of a pharmacogenomic test includes the high prevalence of the genetic variant.
- It is a very important issue under the present circumstances – a priori identification of patients in risk, fewer ADRs, reduction of the hospitalization days, lower morbidity and mortality.

- Ethical issues, regulatory concerns on the legislative framework.
- Privacy of testing. GINA (Genetic Information Non discrimination Act. 2008): Protection of patients rights for employment and health insurance.
- In the near future pharmacogenomics will be part of routine medical care — at least for some drugs and pave the way to personalized medicine.

Thank you for your attention