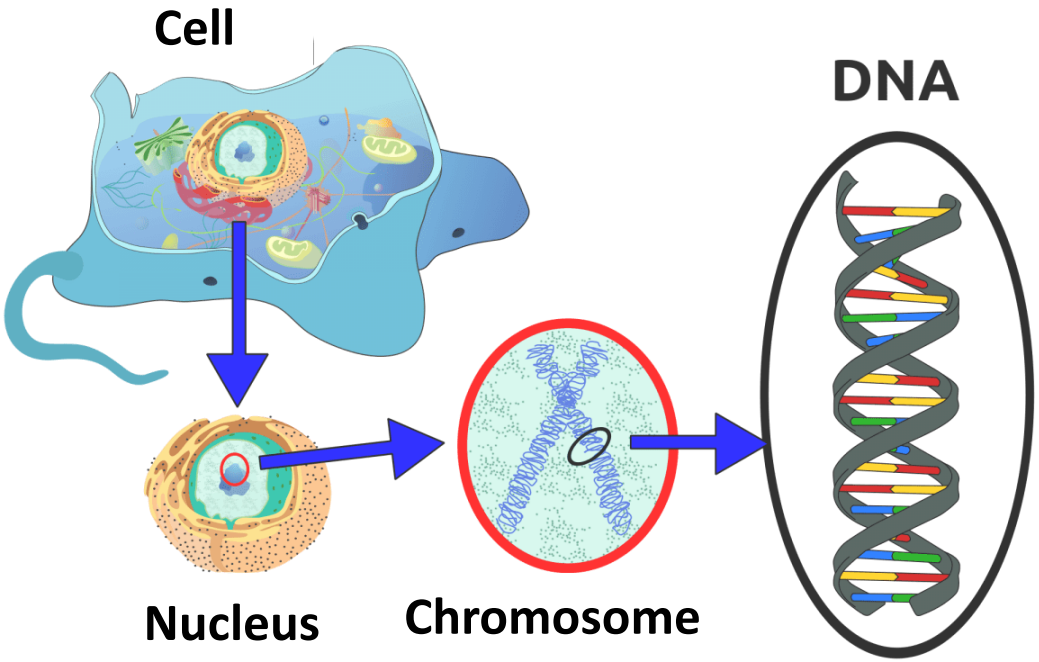
Chromosomes, genes and alleles

The human body is made up of millions of cells. Each cell has a nucleus containing DNA organized into **chromosomes**.

Every human cell (except gametes) contains **23 pairs of chromosomes**, 22 pairs of autosomes and one pair of sex chromosomes (homologous chromosomes). Each person has two versions of the same gene or **alleles** (one from each parent).



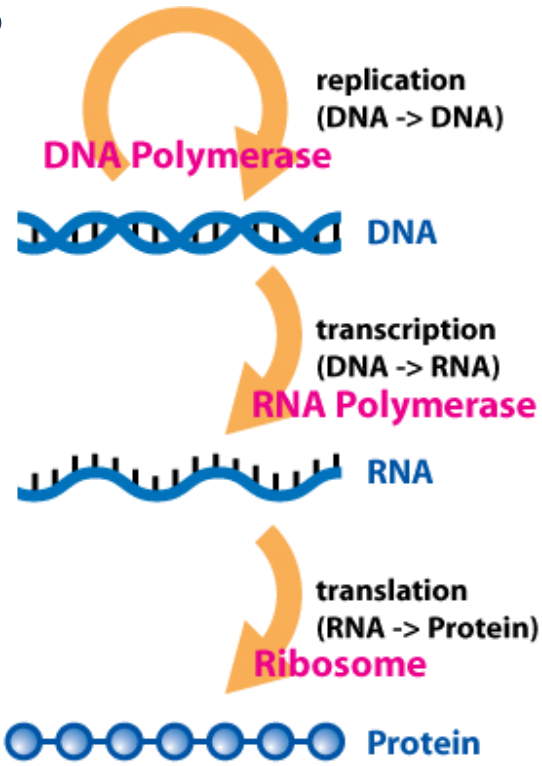
Modified from https://www.biopills.net/dna-cromosomi-e-cromatina/

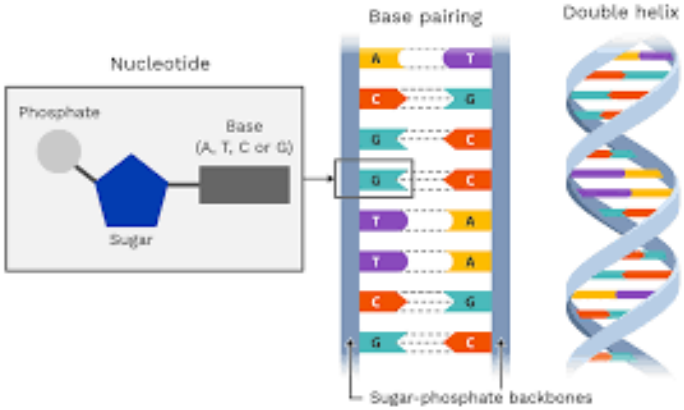
From DNA to proteins

Genes contain the code for synthesizing specific proteins.

Transcription is the process by which this information is trasferred from DNA to messenger RNA (mRNA), according to the rules of complementary base pairing.
It takes place in the nucleus

Translation is the stage of protein synthesis, in which the instructions carried by mRNA are translated into the correct sequence of amino acids to form a protein.
Each triplet of mRNA bases codes for a single amino acid.
It takes place in the cytoplasm at the level of ribosomes



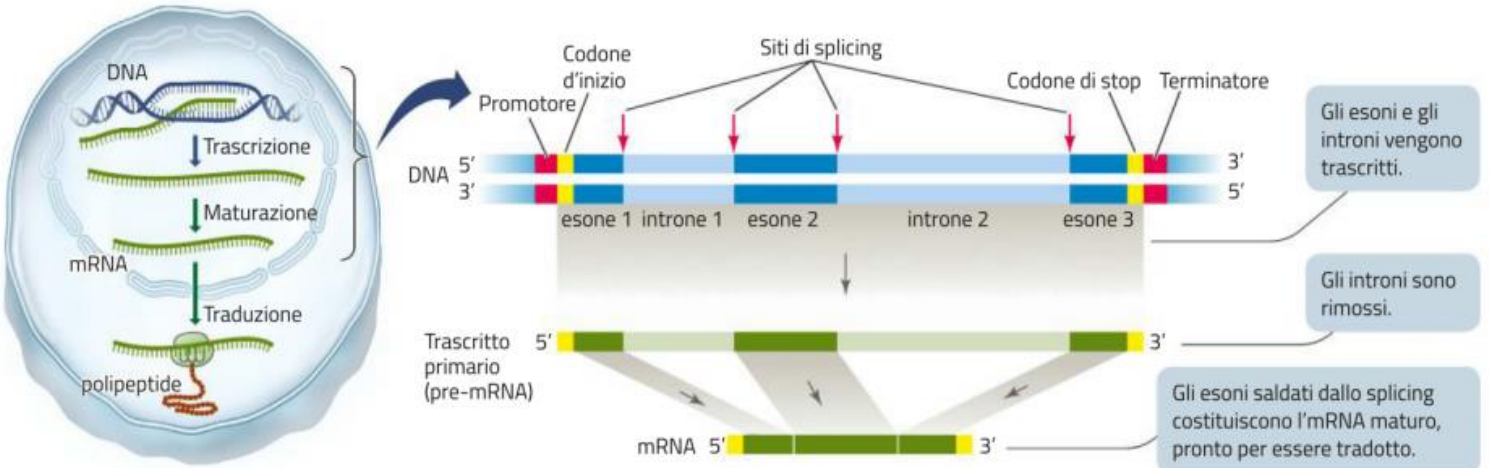


https://theory.labster.com/structure-dna/

Non-coding DNA

98.5% of the human genome consists of non-coding sequences not translated into proteins.

- **Introns:** non-coding sequences located in genes between coding sequences (exons). During transcription, the entire gene is copied into a pre-mRNA, which includes exons and introns. During the process of RNA splicing, introns are removed, resulting in mature mRNA.



Sadava et al. *La nuova biologia.blu* © Zanichelli 2016

Non-coding DNA

- **Gene expression regulatory sequences:** promoters or binding sites for transcription factors that can activate or inhibit transcription and protein synthesis (enhancers or silencers)

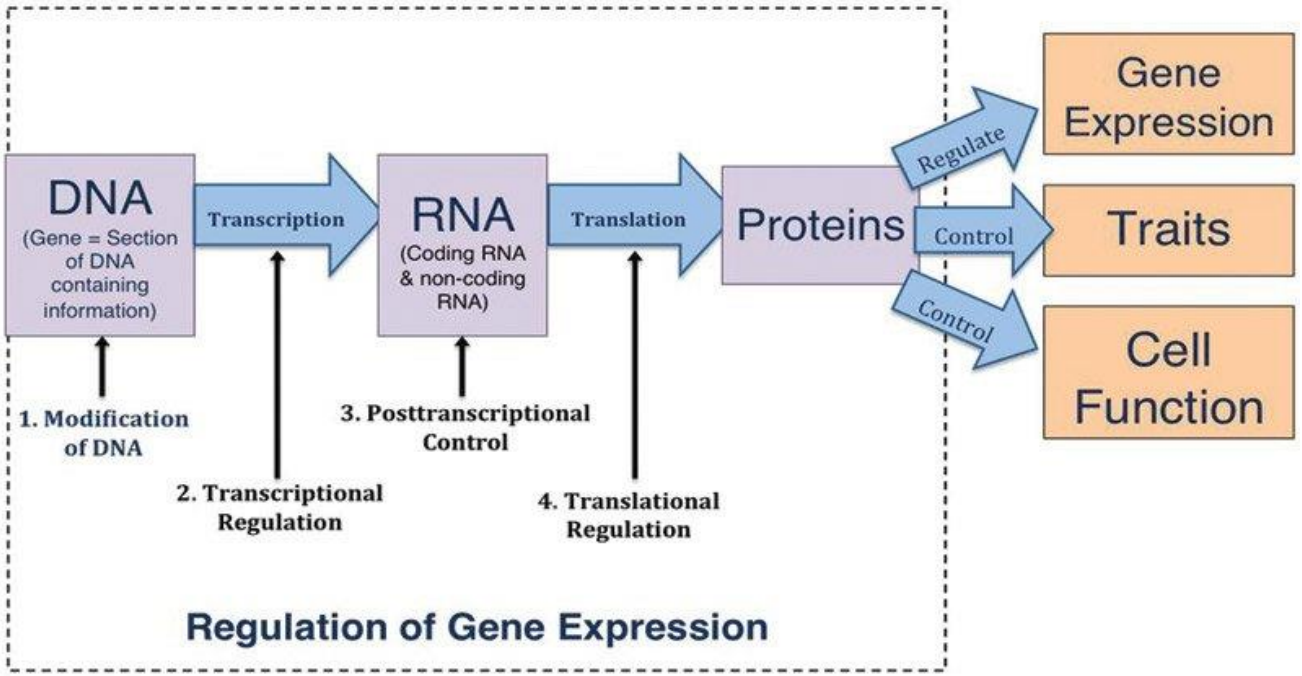
- **Telomers**: repeated non-coding nucleotide sequences at the end of chromosomes with a protective function

- **DNA sequences from which a non-coding RNA is transcribed:**

- transfer RNA (tRNA) and ribosomal RNA (rRNA), which are essential for protein synthesis
- microRNA (miRNA), which silences gene expression at the post-transcriptional level
- long non-coding RNAs, which play an important role in regulation of gene expression

Regulation of gene expression

All cells in the body have the same DNA, but different morphological and functional features. This is because they express different parts of their genome. Mechanisms controlling gene expression allow cells to differentiate and finely regulate all stages of protein synthesis.



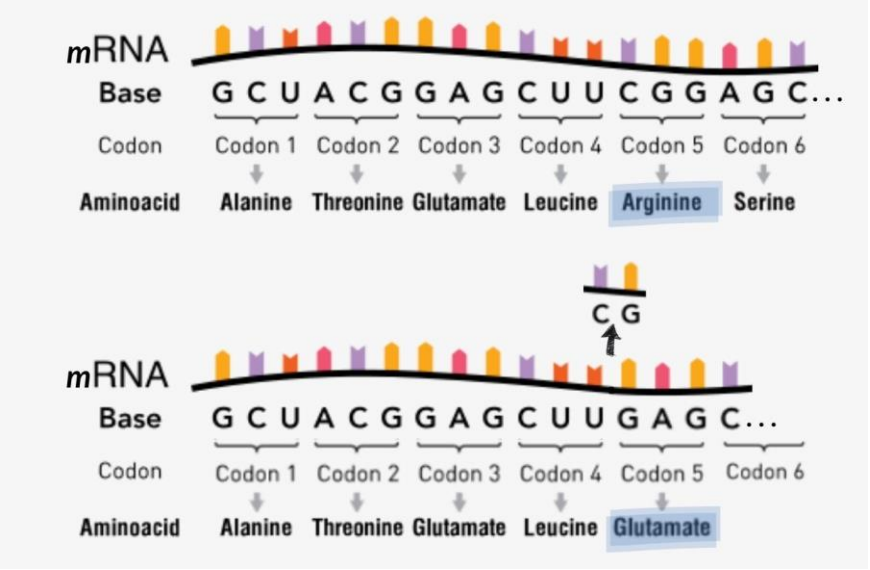
https://microbenotes.com/gene-expression/

Genetic polymorphisms

Genes can present sequence variations that lead to the existence of allelic variants of a given gene. ➡ **GENETIC VARIABILITY**

A **genetic polymorphism** can be defined as a variation in the DNA sequence that occurs in at least 1% of the population.

A **frameshift mutation** refers to the insertion or deletion of one or more nucleotides in the DNA sequence.

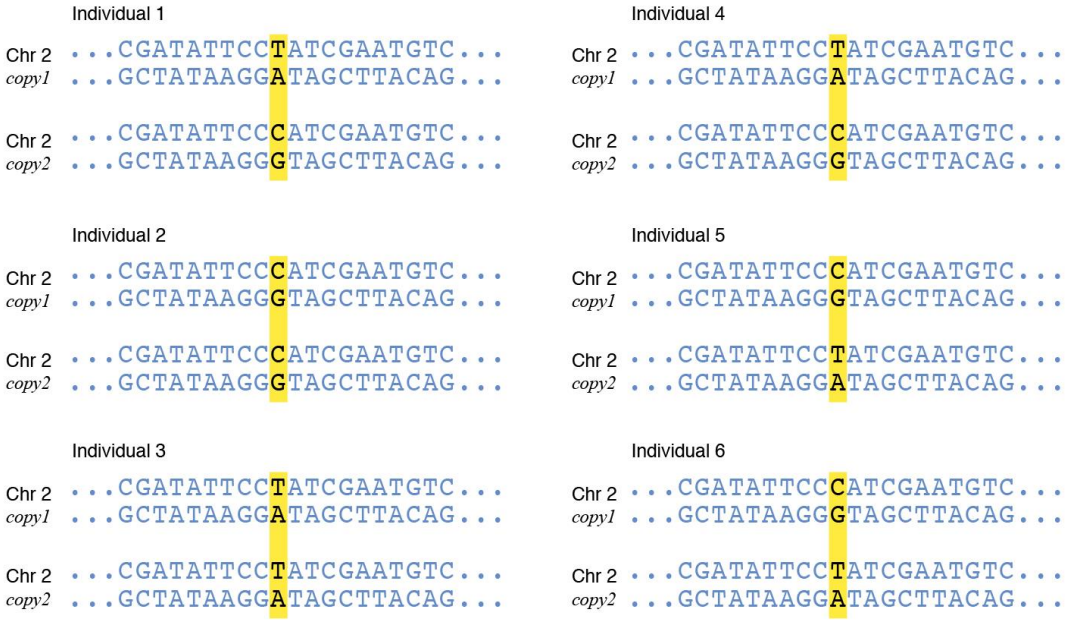


https://www.biologyonline.com/dictionary/frameshift-mutation

"Frame shift": shift in the way the sequence is read which leads to the synthesis of abnormal or nonfunctional proteins

Single nucleotide polymorphisms (SNPs)

SNPs are the are the most common type of genetic variation in the human genome and consist in the variation of a single base pair in the DNA sequence.



https://www.genome.gov/genetics-glossary/Single-Nucleotide-Polymorphisms

There are about 10 million SNPs in the human genome. The functional implications of these variations depends on their location within the DNA sequence.

Single nucleotide polymorphisms (SNPs)

1) Coding SNPs

They are located within the coding regions of a gene and they modify nucleotide triplets (codon) sequences, possibly changing the amino acid sequence of the gene's protein.

- ***Non-synonymous polymorphisms***: genetic variations that affect the encoded protein by an amino acid change, resulting in a change in protein primary structure and function. Proteins may not function, work less/more or be more quickly degraded.
- ***Synonymous polymorphisms:*** they change the nucleotide sequence, but do not alter the amino acid sequence. The genetic code is described as degenerate (or redundant), because a single amino acid can be coded by more than one codon (64 codons and 20 amino acids).

Single nucleotide polymorphisms (SNPs)

2) SNPs in perigenic regions

They can be located within:

- ***splice junctions*****,** leading to intron retention or exon loss in the mature transcript. The resulting protein is sequence aberrant and is usually degraded.
- ***regulatory regions,*** such as promoters or enhancers. A nucleotide substitution could change the binding site for a transcription factor. The effect on the protein is not qualitative but quantitative, i.e. the same protein is expressed more or less.

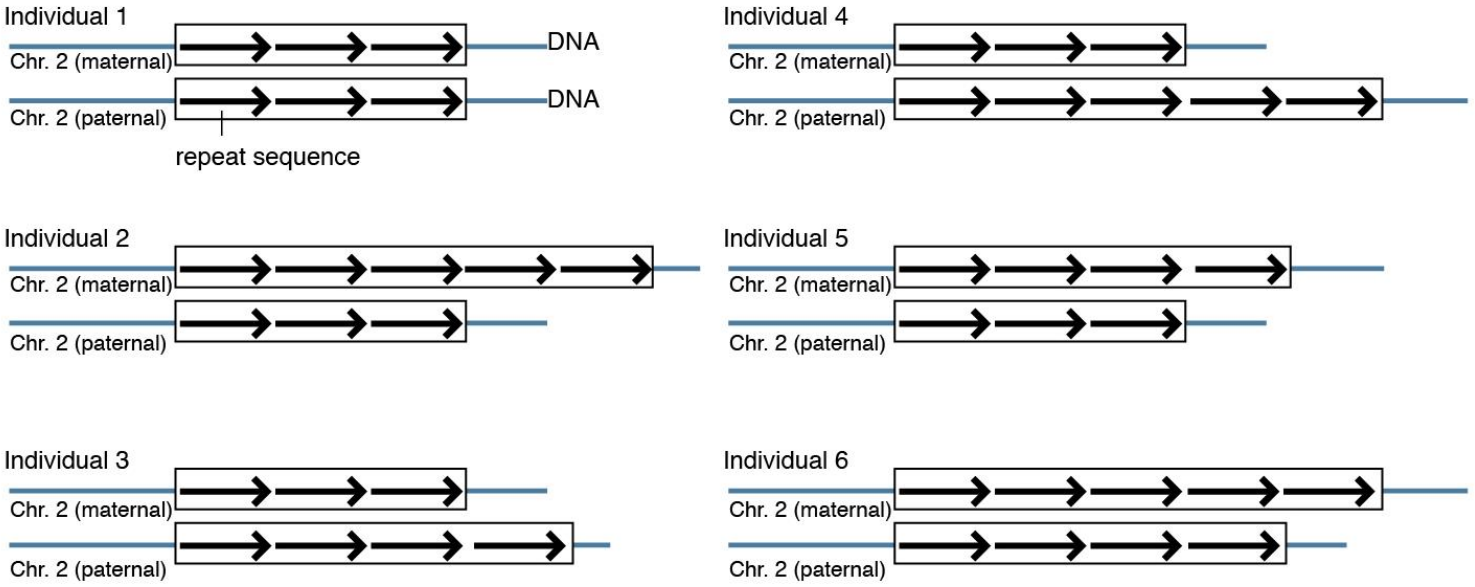
3) SNPs in intergenic regions

They are located in non-protein-coding DNA between genes. They generally do not have a major effect on protein function but they can indirectly affect gene expression by modifying the activity of miRNAs and long non-coding RNAs.

Copy number variation (CNV)

CNVs refers to variations in the number of copies of specific DNA sequences (between 50 bp and 3 Mb) among different individuals' genomes. These DNA segments may be absent, due to deletions, or present in numerous copies, due to duplications, in one or both chromosomes of an individual. If these sequences enclose a gene, a person may have no copies or many copies of that gene.

E.g.: CYP2D6, one of the most important CYP450 enzymes involved in drug metabolism, is encoded by a gene affected by CNV (0 to 13 copies).



https://www.genome.gov/genetics-glossary/Copy-Number-Variation

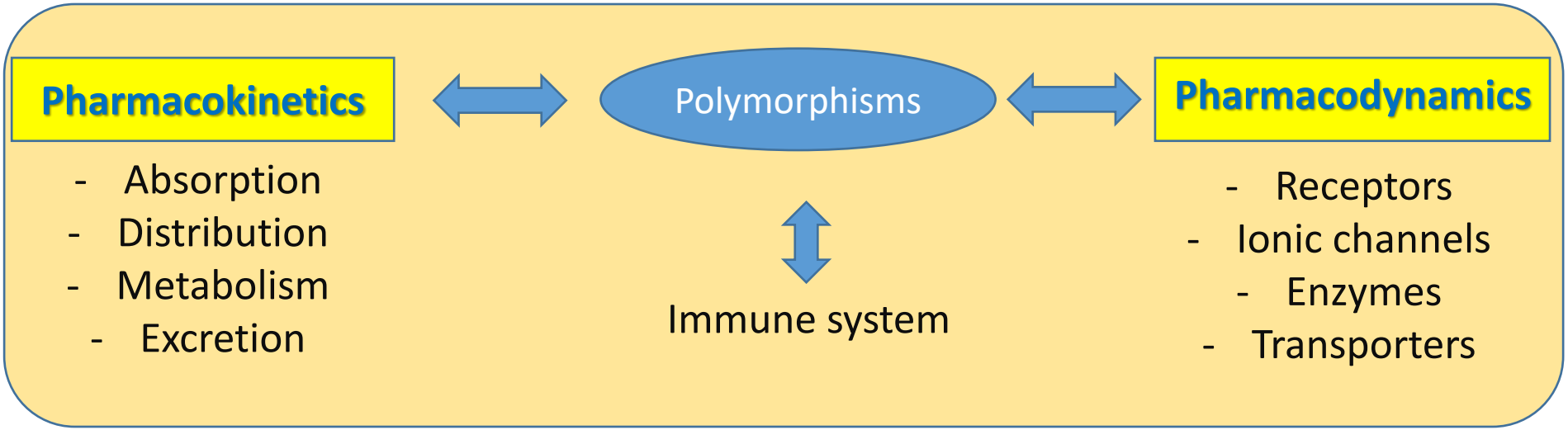
Pharmacogenetics

Pharmacogenetics is the study of how genes affect individual variability in drug response.

Many genes encoding proteins involved in drug response are polymorphic in the population.

These genes can be divided into two classes:

- genes encoding proteins involved in drug absorption, distribution, metabolism and excretion (ADME processes)
- genes encoding drug targets (receptors, enzymes, transporters and ion channels)



Polymorphisms in genes coding for proteins involved in drug metabolism

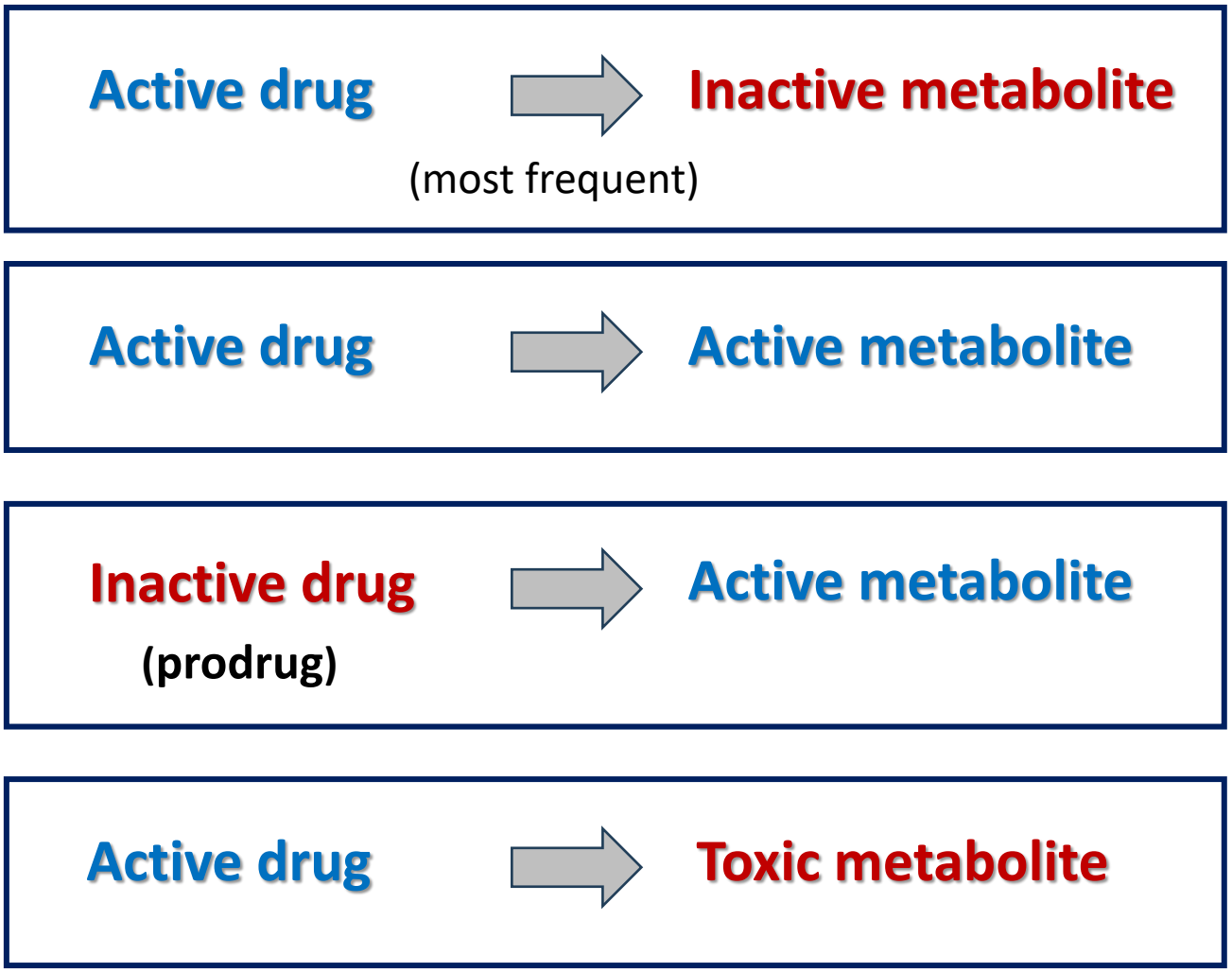
Drug metabolism

The reduction of the level of active drug in the body occurs through two processes: metabolism and excretion.
Metabolism refers to the the biotransformation reactions and chemical changes that a drug undergoes in the body.
These reactions are fundamental to reduce the biological activity of the drugs and favor their removal from the body.

Drug → **Polar and hydrophilic metabolites, which can be easily eliminated through urine or bile**

The main site of metabolic processes is the liver.
Other less important sites of metabolism are the intestine, the kidneys, and the lungs.

Possible outcomes of metabolic reactions



Drug metabolism

Phase I reactions (Functionalization)

Introduction or unmasking of polar functional groups on the chemical structure of the drug

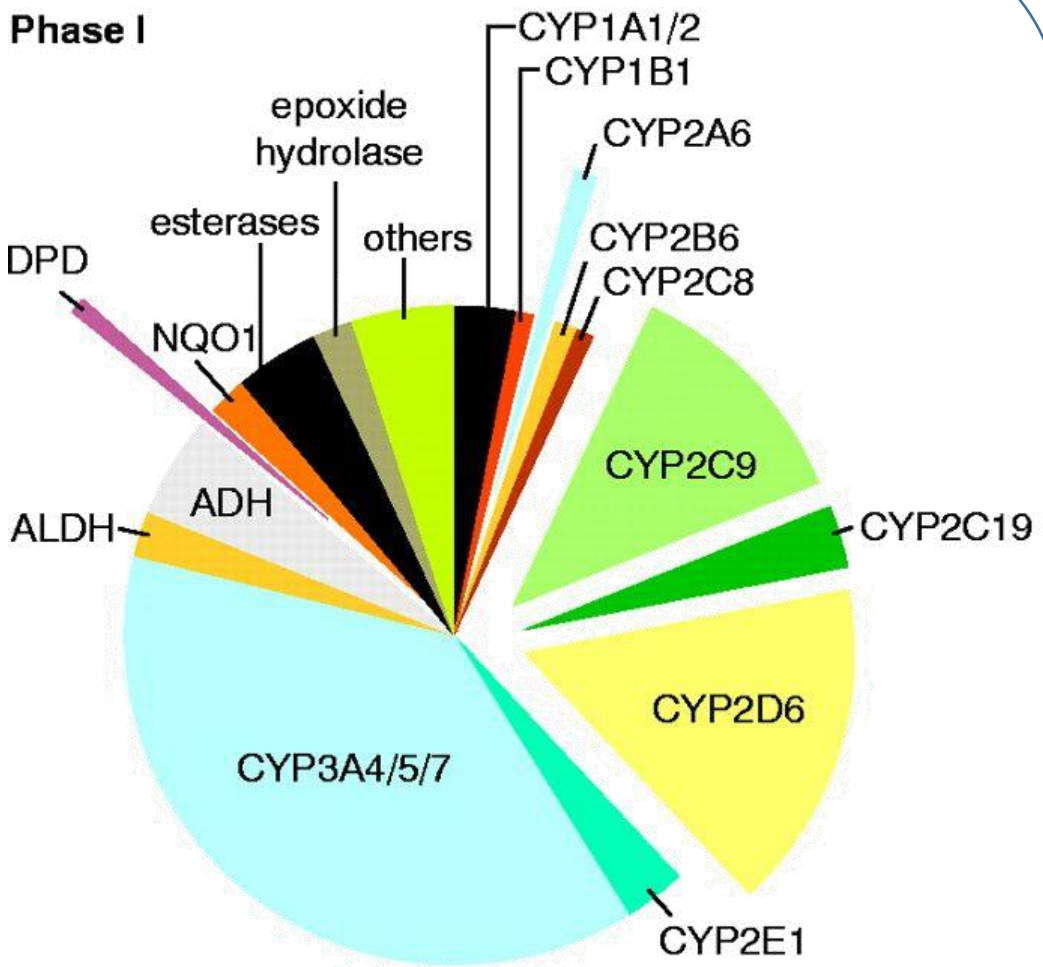
Oxidation
Reduction
Hydrolisis

Phase II reactions (Conjugation)

Binding of endogenous molecules to the functional groups that lead to highly water soluble and polar metabolites

Glucuronidation, sulfation, acetylation, methylation, conjugation with amino acid or glutathione.

Cytochrome P450 system (CYP450)



Evans and Relling, 1999. Science 286:487-91

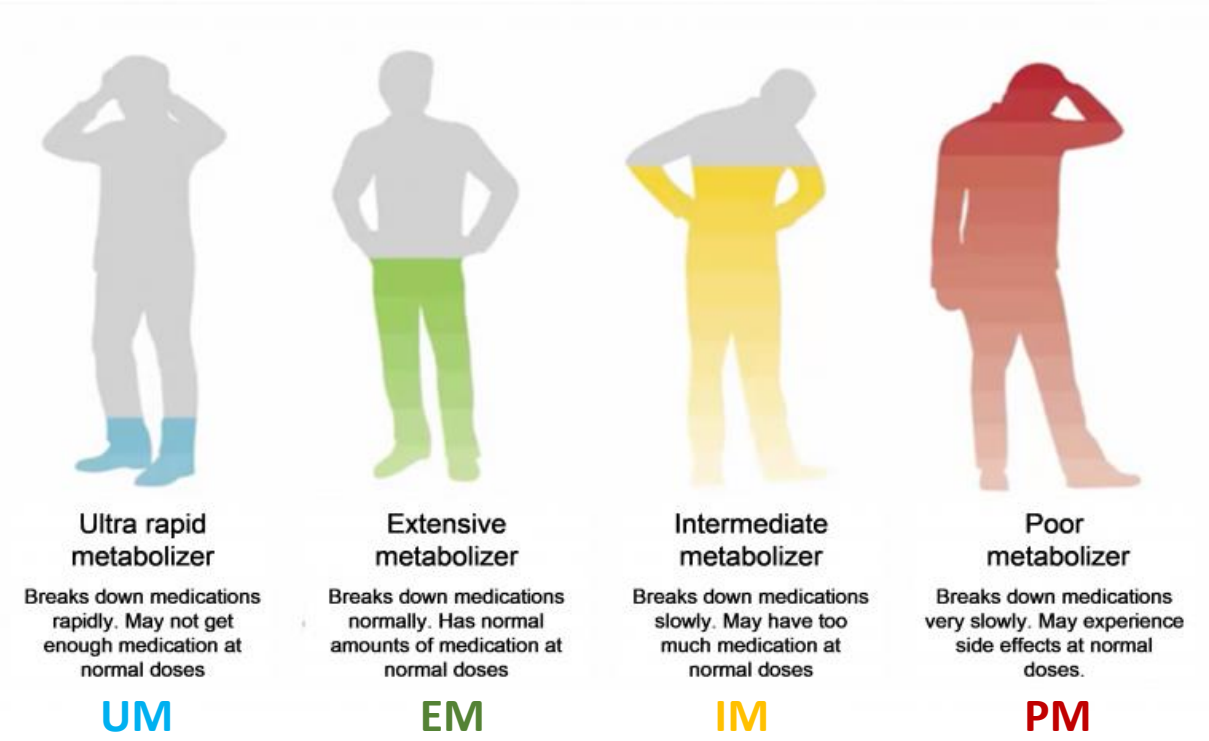
About 70% of drugs used in therapy are metabolized by CYP450 enzymes.

CYP450 genes are highly polymorphic contributing to the individual inter-variabilty in drug metabolism.

Therapeutic failure or adverse/toxic reactions

Polymorphisms can occur in one or both alleles of a selected CYP gene having different impact on individual's metabolic rate.

We can distinguish four main metabolic phenotypes:



PMs and UMs have respectively much lower or much higher metabolic activity compared to EMs and they are at increased risk of encountering adverse reaction/toxicity or therapeutic failure.

CYP2D6

This enzyme is involved in the metabolism of about 25% of drugs currently in use. These include antidepressants, antipsychotics, cardiovascular medicines (such as beta blockers) and analgesics.

CYP2D6 substrates

Antidepressants	Beta blockers	Anti-cancer	Antipsychotics		
Amitriptyline	Alprenolol	Tamoxifen	Haloperidol	Mexiletine	Methoxyamphetamine
Clomipramine	Carvedilol		Perphenazine	Minaprine	Bufuralol
Desipramine	Propafenone		Risperidone	Nebivolol	Chlorpheniramine
Imipramine	Bupranolol		Thioridazine	Nortriptyline	Chlorpromazine
Fluoxetine	Clonidine		Zuclopenthixol	Ondansetron	Clonidine
Paroxetine	Debrisoquine		Atomoxetine	Oxycodone	Codeine
Tamoxetine	Metoprolol		Alprenolol	Perhexiline	Debrisoquine
Trimipramine	Propranolol		Amphetamine	Phenacetin	Dexfenfluramine
Venlafaxine	Timolol		Aripiprazole	Phenformin	Dextromethorphan

Puangpetch et al. CYP2D6 polymorphisms and their influence on risperidone treatment. Pharmgenomics Pers Med.

CYP2D6

More than 90 allelic variants of CYP2D6 have been described. The gene is subjected to both SNPs and CNVs (deletion or duplication of the gene, up to 13x).

Polimorfismi del gene CYP2D6		
Allele	**Cambiamento Nucleotidico**	**Attività enzimatica**
*1	Nessuno	Normale
*2	2850C>T	Normale
*3	2549A>del	Nessuna
*4	1846G>A	Nessuna
*5	Delezione del gene	Nessuna
*6	1707T>del	Nessuna
*7	2935A>C	Nessuna
*8	1758G>T	Nessuna
*11	883G>C	Nessuna
*12	124G>A	Nessuna
*9	2613-2615 delAGA	Parziale
*10	100C>T	Parziale
*17	1023C>T	Parziale
*41	2988G>A	Parziale
Duplicazione genica	Duplicazione del gene	Aumentata

Association between polymorphims of CYP2D6 gene and enzyme activity

Torricelli e Giuliani. Farmacogenetica: aspetti diagnostici, applicazioni cliniche e prospettive future

Phenotype	Caucasian, %	Ethiopian/ Nigerian, %	Asian, %	Hispanic, %
Poor metabolizer	3–10	1.8–8.1	0–1.2	2.2–6.6
Intermediate metabolizer	1–2	N/A	51	N/A
Ultrarapid metabolizer	0.8–4.3	29	0.9	1.7

Abbreviation: N/A, not available.

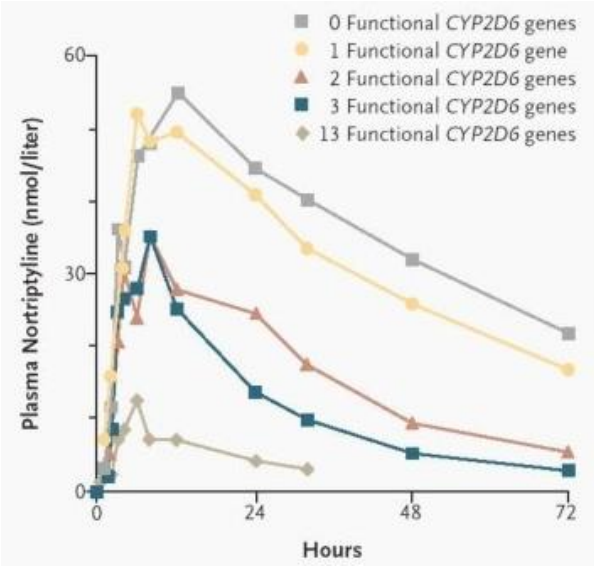
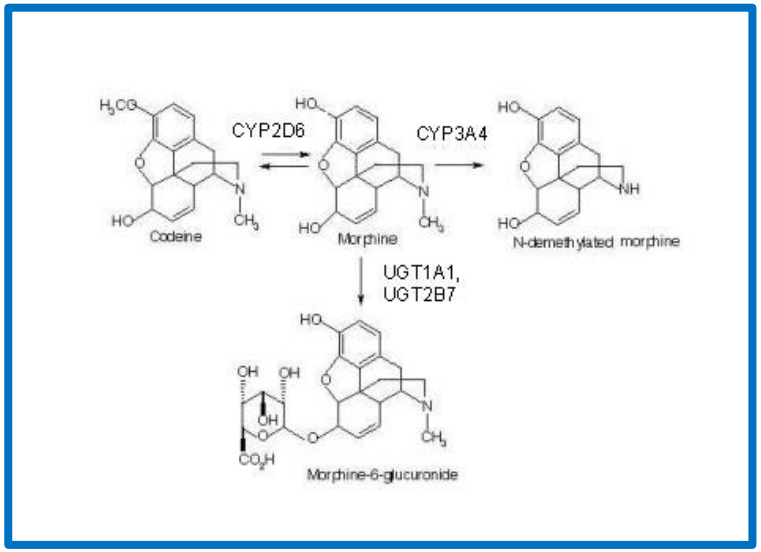


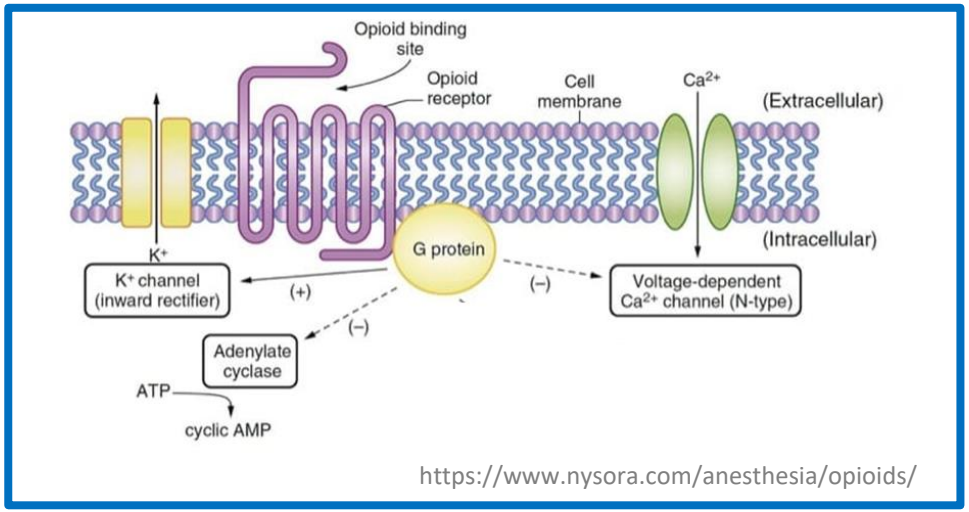
Table 1 | **Examples of specific polymorphisms reported to modulate drug action**

Gene product	Drug	Drug action linked to minor allele	References
Drug disposition			
CYP2C9	Warfarin	Reduced anticoagulant effect	22
	Phenytoin	Increased toxicity	69
	Tolbutamide	Lower dose requirement	70
CYP2C19	Omeprazole	Enhanced cure rate of *Helicobacter pylori*	23
	Mephenytoin	Increased adverse effects	71
CYP2D6	Codeine	Decreased analgesia (poor metabolizer)	17
		Euphoria, nausea (hyperextensive metabolizer)	72
	Propranolol, timolol, metoprolol, carvedilol	Augmented β-blockade (poor metabolizer)	20,73,74
	Desipramine and other tricyclics	Variable antidepressant effect	18
	Debrisoquine	Excessive hypotension	75
	Propafenone	Enhanced β-blockade	19
CYP3A5	Many	Variably expressed; function not yet established	36
P-glycoprotein	Digoxin	Altered blood level and effect	33
N-acetyl transferase	Procainamide, hydralazine	Slow acetylators; increased risk of the lupus syndrome	13
	Isoniazid	Slow acetylators; increased risk of hepatotoxicity	76
Thiopurine methyltransferase	6-mercaptopurine, azathioprine	Bone marrow aplasia (poor metabolizers)	57
		Suboptimal therapeutic response (rapid metabolizers)	24,58,77
Pseudocholinesterase	Succinylcholine	Prolonged apnoea	28
UDP-glucuronosyl-transferase	Irinotecan	Enhanced toxicity	25
Drug targets			
Choline acetyl-transferase	Tacrine	Decreased response in *APOE4* homozygotes	42
HERG/MiRP1	QT-prolonging drugs	Increased risk of arrhythmias in *MiRP1* mutation carriers	43
β_2-adrenoceptors	β_2-agonists	Variable response in asthma	78
5-lipoxygenase	5-lipoxygenase inhibitor (zileuton)	Diminished response among homozygotes for alleles reducing 5-lipoxygenase expression	44

Roden and George.The genetic basis of variability in drug responses.
Nature Reviews Drug Discovery volume 1, pages37–44 (2002)

CYP2D6: codeine metabolism





https://www.nysora.com/anesthesia/opioids/

Codeine is used to relieve mild-to-moderate pain, and it belongs to the drug class of opioid analgesics.

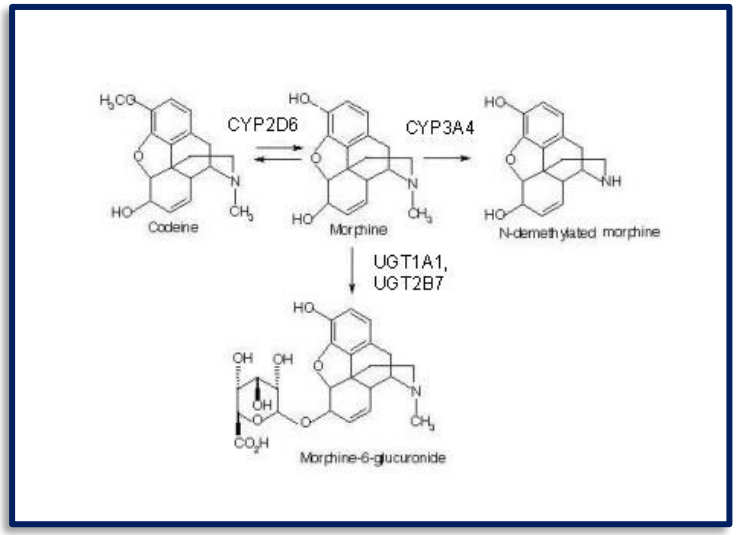
Codeine is a prodrug: its analgesic activity depends on its conversion to morphine that binds to the μ opioid receptors with 200-fold greater affinity than codeine.

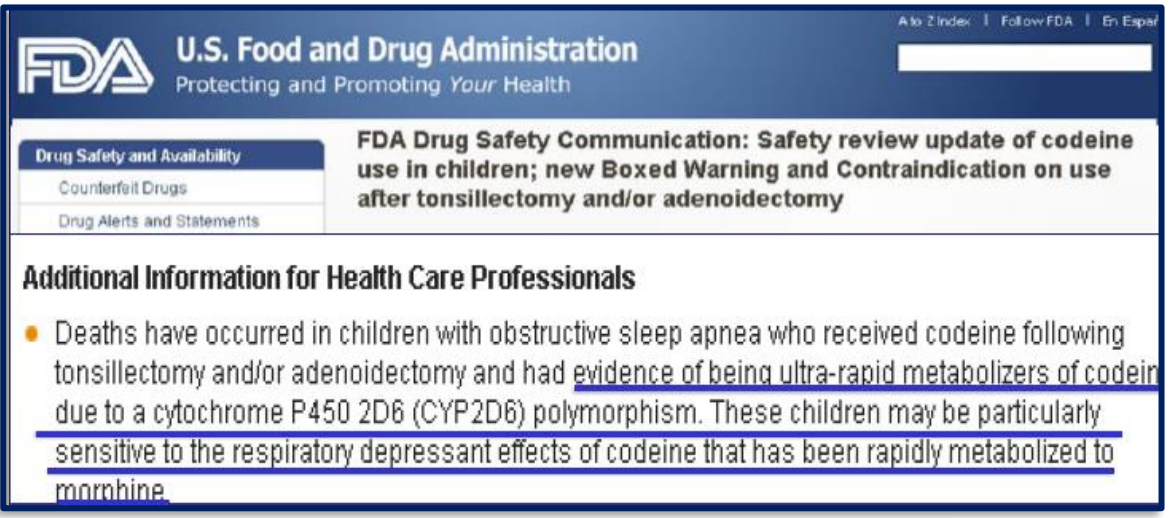
CYP2D6 metabolizes codeine to morphine.

Codeine is also used to treat cough at lower doses (depression of cough center in medulla)

CYP2D6: codeine metabolism

- Codeine therapeutic doses are set up on people with EM phenotype, who break down approximately 5-10% of codeine dose to morphine.
- PM patients are at risk of experiencing little or no therapeutic effect.
- UM patients are at risk of severe adverse reactions, such as central sedation and respiratory depression.
- Another drug should be used in PM or UM patients.





U.S. Food and Drug Administration
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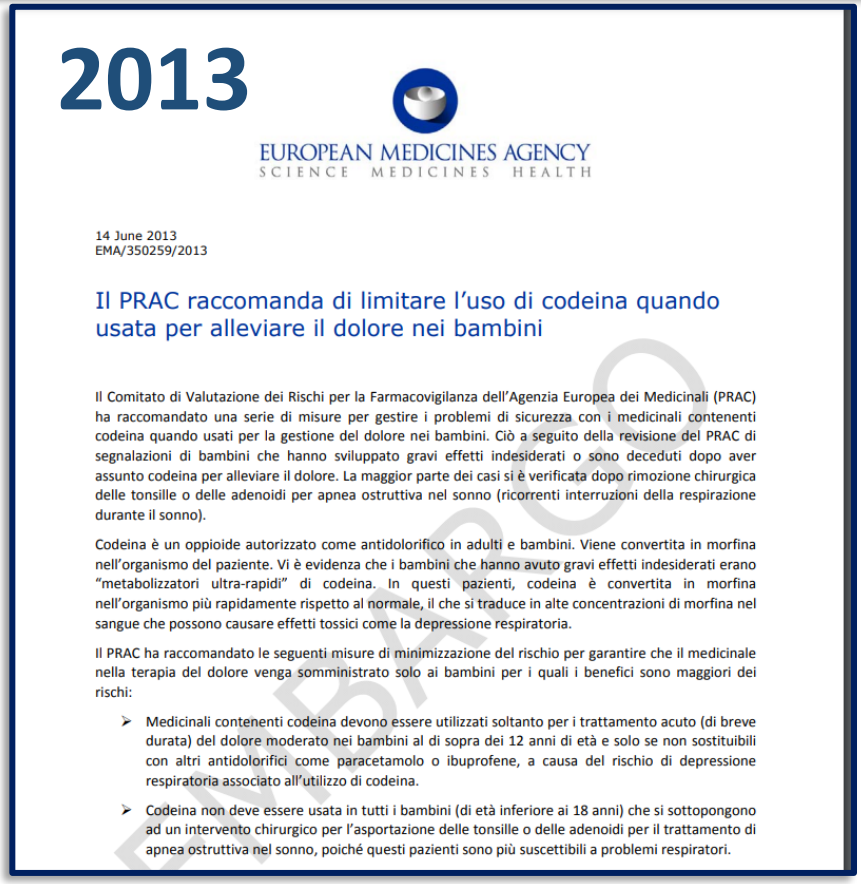
Drug Safety and Availability
Counterfeit Drugs
Drug Alerts and Statements

FDA Drug Safety Communication: Safety review update of codeine use in children; new Boxed Warning and Contraindication on use after tonsillectomy and/or adenoidectomy

Additional Information for Health Care Professionals

- Deaths have occurred in children with obstructive sleep apnea who received codeine following tonsillectomy and/or adenoidectomy and had evidence of being ultra-rapid metabolizers of codein due to a cytochrome P450 2D6 (CYP2D6) polymorphism. These children may be particularly sensitive to the respiratory depressant effects of codeine that has been rapidly metabolized to morphine.

In february 2013 a *boxed warning* was issued by FDA to contraindicate codeine use in children with specific CYP2D6 isoforms (UM profile) because of the increased risk of morphine-induced respiratory depressant effects.



2013

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

14 June 2013
EMA/350259/2013

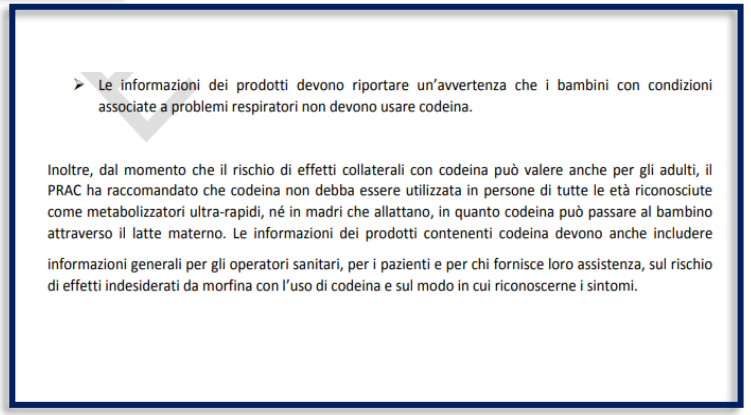
Il PRAC raccomanda di limitare l'uso di codeina quando usata per alleviare il dolore nei bambini

Il Comitato di Valutazione dei Rischi per la Farmacovigilanza dell'Agenzia Europea dei Medicinali (PRAC) ha raccomandato una serie di misure per gestire i problemi di sicurezza con i medicinali contenenti codeina quando usati per la gestione del dolore nei bambini. Ciò a seguito della revisione del PRAC di segnalazioni di bambini che hanno sviluppato gravi effetti indesiderati o sono deceduti dopo aver assunto codeina per alleviare il dolore. La maggior parte dei casi si è verificata dopo rimozione chirurgica delle tonsille o delle adenoidi per apnea ostruttiva nel sonno (ricorrenti interruzioni della respirazione durante il sonno).

Codeina è un oppioide autorizzato come antidolorifico in adulti e bambini. Viene convertita in morfina nell'organismo del paziente. Vi è evidenza che i bambini che hanno avuto gravi effetti indesiderati erano "metabolizzatori ultra-rapidi" di codeina. In questi pazienti, codeina è convertita in morfina nell'organismo più rapidamente rispetto al normale, il che si traduce in alte concentrazioni di morfina nel sangue che possono causare effetti tossici come la depressione respiratoria.

Il PRAC ha raccomandato le seguenti misure di minimizzazione del rischio per garantire che il medicinale nella terapia del dolore venga somministrato solo ai bambini per i quali i benefici sono maggiori dei rischi:

- Medicinali contenenti codeina devono essere utilizzati soltanto per i trattamento acuto (di breve durata) del dolore moderato nei bambini al di sopra dei 12 anni di età e solo se non sostituibili con altri antidolorifici come paracetamolo o ibuprofene, a causa del rischio di depressione respiratoria associato all'utilizzo di codeina.
- Codeina non deve essere usata in tutti i bambini (di età inferiore ai 18 anni) che si sottopongono ad un intervento chirurgico per l'asportazione delle tonsille o delle adenoidi per il trattamento di apnea ostruttiva nel sonno, poiché questi pazienti sono più suscettibili a problemi respiratori.



- Le informazioni dei prodotti devono riportare un'avvertenza che i bambini con condizioni associate a problemi respiratori non devono usare codeina.

Inoltre, dal momento che il rischio di effetti collaterali con codeina può valere anche per gli adulti, il PRAC ha raccomandato che codeina non debba essere utilizzata in persone di tutte le età riconosciute come metabolizzatori ultra-rapidi, né in madri che allattano, in quanto codeina può passare al bambino attraverso il latte materno. Le informazioni dei prodotti contenenti codeina devono anche includere informazioni generali per gli operatori sanitari, per i pazienti e per chi fornisce loro assistenza, sul rischio di effetti indesiderati da morfina con l'uso di codeina e sul modo in cui riconoscerne i sintomi.

Use of codeine for moderate pain is contraindicated in:

- children below 12 years
- children and adolescents below 18 years having respiratory issues
- CYP2D6 ultra-rapid metabolizers (children and adults)
- breastfeeding women

Codeine not to be used in children below 12 years for cough and cold

24 April 2015

CYP2C19

CYP2C19 accounts for approximately 4% of CYP450 enzymes in the liver. It is involved in the metabolism of several drugs, including antidepressants, omeprazole, diazepam, clopidogrel and mephenytoin.

More than 20 allelic variants of CYP2C19 gene have been described.

CYP2C19	Nucleotide change	Effect	Europeans	Blacks	Asians
*1		Wild type	85%	82%	65%
*2	681G→A	Truncated protein	13-19%	10-25%	20-30%
*3	636G→A	Truncated protein	<1%	0-2%	5-13%
*17	-806C→T -3402C→T	Increased translation	18%	18%	4%

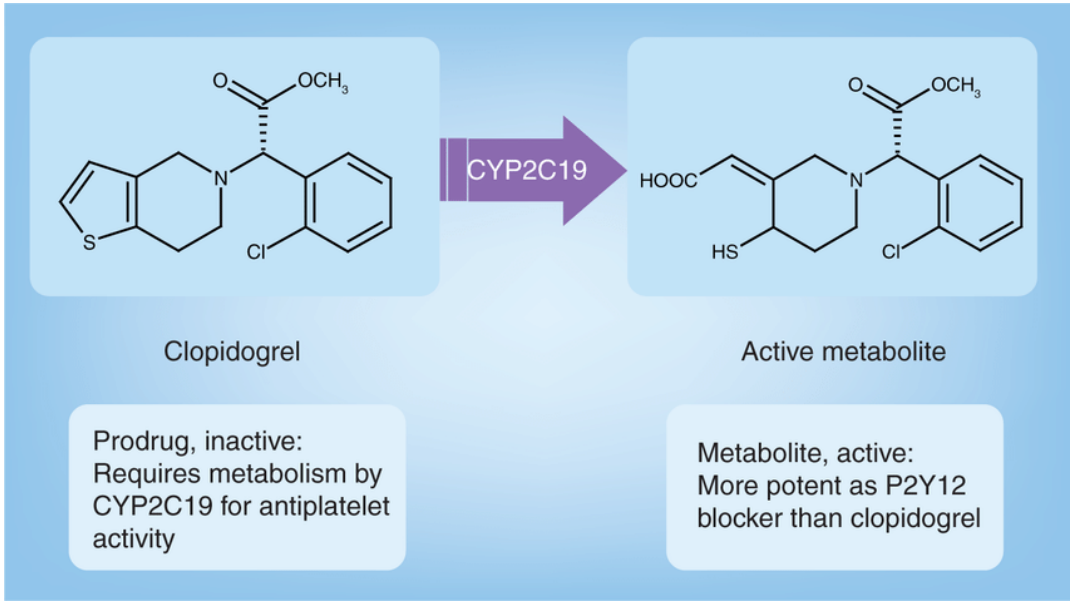
Variants generally lead to a decreased enzyme function due to the expression of inactive enzymes (PM phenotype). However, a SNP (CYP2C19*17) that leads to increased enzyme expression has also been identified (UM phenotype).

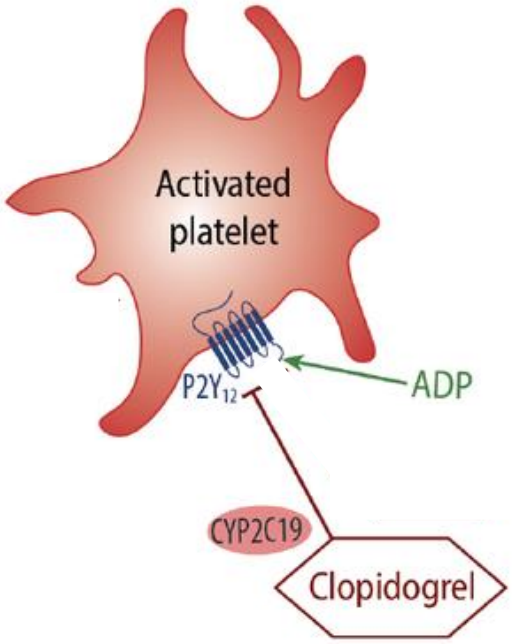
CYP2C19: clopidogrel metabolism

Clopidogrel is an antiplatelet agent used to prevent and treat cardiovascular diseases, such as stroke and myocardial infarction.

It's a prodrug and the active thiol metabolite is mainly produced by CYP2C19 metabolism.

Clopidogrel irreversibily blocks the $P2Y_{12}$ receptor on platelets, preventing the binding of ADP (adenosine diphosphate, which induces platelet activation and blood clotting) to the receptor.





Seip et al, 2010. Implementing genotype-guided antithrombotic therapy. Future cardiology. Indraswari et al., 2022. Antiplatelet Therapies After Ischemic Stroke. Practical Neurology

CYP2C19: clopidogrel metabolism

Likely phenotype	Genotypes	Examples of diplotypes	Implications for clopidogrel	Therapeutic recommendations
Ultra-rapid metabolizer: normal or increased activity (~5–30% of patients)	An individual carrying two increased-activity alleles (**17*), or one functional allele (**1*) plus one increased-activity allele (**17*)	**1/*17, *17/*17*	Increased platelet inhibition, decreased residual platelet aggregation	Clopidogrel label-recommended dosage and administration
Extensive metabolizer: homozygous wild-type or normal activity (~35–50% of patients)	An individual carrying two functional (**1*) alleles	**1/*1*	Normal platelet inhibition, normal residual platelet aggregation	Clopidogrel label-recommended dosage and administration
Intermediate metabolizer: heterozygote or intermediate activity (~18–45% of patients)	An individual carrying one functional allele (**1*) plus one loss-of-function allele (**2–*8*)	**1/*2, *1/*3*	Reduced platelet inhibition, increased residual platelet aggregation, increased risk for adverse cardiovascular events	Prasugrel or other alternative therapy (if no contraindication)
Poor metabolizer: homozygous variant, mutant, low, or deficient activity (~2–15% of patients)	An individual carrying two loss-of-function alleles (**2–*8*)	**2/*2, *2/*3, *3/*3*	Significantly reduced platelet inhibition, increased residual platelet aggregation, increased risk for adverse cardiovascular events	Prasugrel or other alternative therapy (if no contraindication)

Adapted from Scott SA, et al. Clin Pharmacol Ther 2013;94:317-23 [7] and Whirl-Carrillo M, et al. Clin Pharmacol Ther 2012;92:414-7 [92], with permission of the PharmGKB and Stanford University.
Abbreviation: ACS/PCI, acute coronary syndrome/percutaneous coronary intervention.

Kim et al., 2017. Clinical Pharmacogenetic Testing and Application: Laboratory Medicine Clinical Practice Guidelines. Ann Lab Med.

Individuals carrying the CYP2C19*2/*3 allelic variants have low circulating levels of the clopidogrel active metabolite. This can lead to low therapeutic action and to a higher risk of cardiovascular problems (stroke, heart attack, death). The variant has also been linked to stent thrombosis.

Patients with the CYP2C19*17 allelic variant are at increased risk of bleeding.

For PMs and IMs, other drugs are recommended, and careful clopidogrel dosage evaluation is recommended UMs.

CYP2C19: proton pump inhibitors (PPIs)

Polymorphisms in the CYP2C19 gene affect the pharmacokinetics and therapeutic efficacy of PPIs when used in combination with amoxicillin and clarithromycin or metronidazole for the eradication of Helicobacter pylori. PMs have a higher rate of eradication when compared to EMs.

CYP2C19: diazepam

Diazepam is demethylated by CYP2C19. The plasma half-life of diazepam is longer in individuals who are homozygous for the CYP2C19*2 allelic variant. Reduced metabolism may lead to diazepam accumulation with an increased risk of sedation and loss of consciousness.

CYP2C9

CYP2C9 is a CYP450 enzyme involved in the metabolism of approximately the 16% of drugs, including warfarin, phenytoin, NSAIDs, and sulfonylureas.

Table 1. Frequency of the *CYP2C9* alleles in different populations.

Allele	Nucleotide changes	Exon location	Protein variation	Activity compared with *CYP2C9*1/*1*	Allele frequency		
					Africans	Asians	Caucasians
*CYP2C9*2*	*430C→T*	Exon 3	Arg144Cys	Decrease	4%	0%	11%
*CYP2C9*3*	*1075A→C*	Exon 7	Ile359Leu	Decrease	2%	3%	7%
*CYP2C9*4*	*1076T→C*	Exon 7	Ile359Thr	-	0%	-	0%
*CYP2C9*5*	*1080C→G*	Exon 7	Asp360Glu	Decrease	1.8%	-	0%
*CYP2C9*6*	*818delA*	Exon 5	Null allele	No activity	0.6%	-	0%
*CYP2C9*7*	*55C→A*	Exon 1	Leu19Ile	-	-	-	-
*CYP2C9*8*	*449G→A*	Exon 3	Arg150His	Increase	6.7%	-	-
*CYP2C9*9*	*752A→G*	Exon 5	His251Arg	Decrease	-	-	-
*CYP2C9*10*	*815A→G*	Exon 5	Glu272Gly	-	-	-	-
*CYP2C9*11*	*1003C→D*	Exon 7	Arg335Trp	Decrease	2.7%	-	0.4%
*CYP2C9*12*	*1465C→D*	Exon 9	Pro489Ser	Decrease	-	2%	-

Summarized frequency data from [6,10,82,114-120]. A hyphen indicates lack of sufficient data. Note that such allele frequencies may even differ within the major ethnic groups.
CYP: Cytochrome P450.

Kirchheiner et al. The CYP2C9 polymorphism: from enzyme kinetics to clinical dose recommendations. Per Med. 2004 Dec;1(1):63-84.

CYP2C9

CYP2C9*2 and CYP2C9*3 alleles are the most widespread and they are linked to reduced or absent enzyme activity (IM or PM subjects). These patients require a reduced dose of the drug (20-60% less, depending on the genotype) to avoid serious adverse reactions (e.g. warfarin induced bleeding).

Common CYP2C9 Alleles With Known Activity Scores and Genotypes-to-Phenotypes Translation

Genotypes	Diplotypes	Activity Scores	Phenotypes
CYP2C9*1/*1	Homozygous for wild type	1+1 = 2	Normal (extensive) metabolizer
CYP2C9*1/*2	Heterozygous for reduced function	1+0.5 = 1.5	Intermediate metabolizer
CYP2C9*1/*3	Heterozygous for no activity	1+0 = 1	Intermediate metabolizer
CYP2C9*2/*2	Homozygous for reduced function	0.5+0.5 = 1	Intermediate metabolizer
CYP2C9*2/*3	Heterozygous for reduced and no function	0.5+0 = 0.5	Poor metabolizer
CYP2C9*3/*3	Homozygous for no function	0+0 = 0	Poor metabolizer

*CYP2C9*1: wild-type allele, activity score = 1; CYP2C9*2: reduced-activity allele, activity score = 0.5; CYP2C9*3: loss-of-function (no activity) allele, activity score = 0; Other alleles: undetermined, more data in future. Source: Reference 21.*

Theken et al. Clinical Pharmacogenetics Implementation Consortium guideline (CPIC) for CYP2C9 and nonsteroidal anti-inflammatory drugs. Clin Pharmacol Ther. 2020;108:191-200.

N-Acetyltransferase 2

N-acetyltransferase 2 (NAT2) is a key enzyme involved in the phase II metabolism of aromatic amines and heterocyclic aromatic amines.

NAT2 gene is highly polymorphic in the population.

Based on the genetic profile, we can distinguish:

- **rapid acetylators**
- **intermediate acetylators**
- **slow acetylators**

Slow acetylators have reduced detoxification capacity compared to rapid or intermediate acetylators and are at higher risk of adverse reactions.

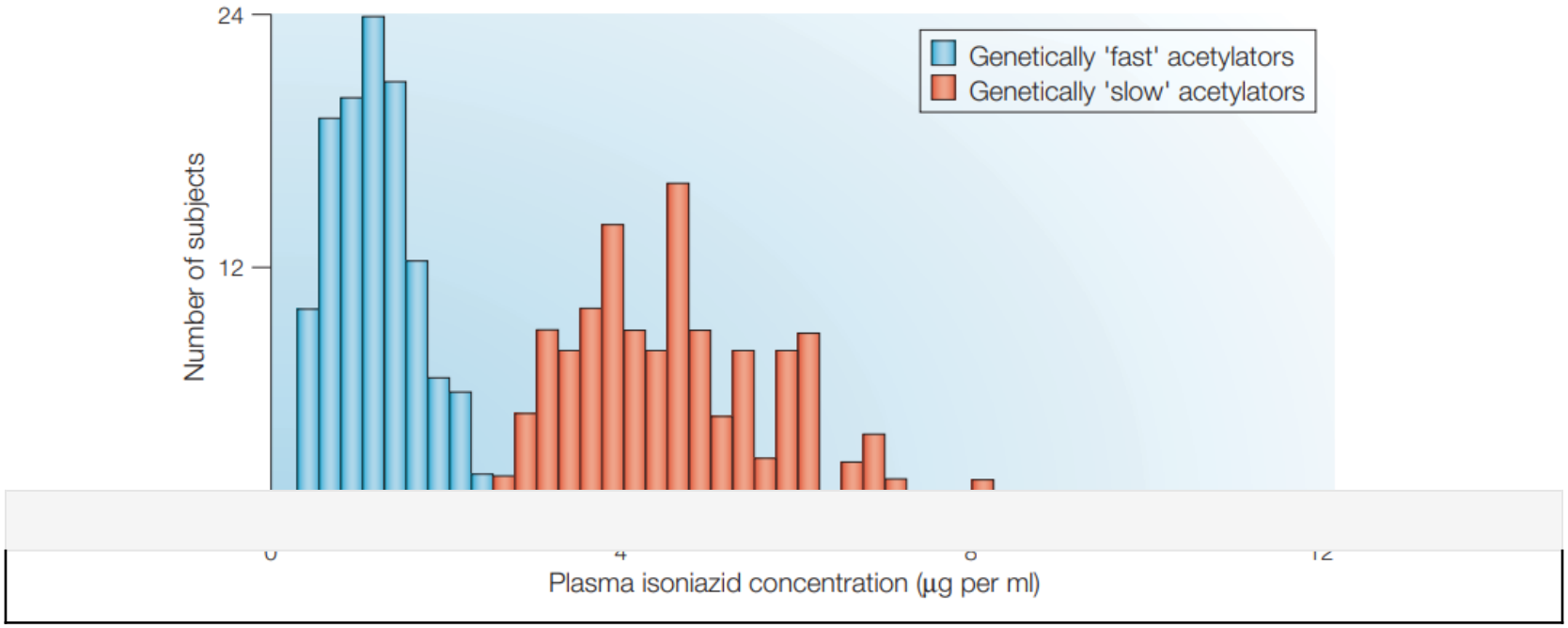
Slow acetylators frequency

80-90%	North-Africans
60%	Indians
50%	Europeans

Rapid acetylators frequency

80-90%	Asians
67%	Latin-Americans

NAT2: isoniazid metabolism



Plasma concentrations of the antituberculosis agent isoniazid in 267 subjects 6 hours after an oral dose. The bimodal distribution results from polymorphisms in the gene encoding N-acetyltransferase-2 (NAT2), which catalyses the metabolism of isoniazid.

Weinshilboum R, Wang L. Nature Rev Drug Discov 3, 739, 2004

Isoniazid is an antibiotic indicated in the first-line treatment of tuberculosis.

Slow acetylators have a higher risk of developing isoniazid-induced hepatotoxicity and peripheral neuropathy.

Gene-environment interaction

Many multifactorial diseases originate from a genetic predisposition, but are also influenced by environmental factors.

Gene-environment interaction (GxE) can be defined as:

1) different effects of an environmental exposure on disease risk in people with different genotypes;
2) a different effect of a genotype on disease risk in people with different environmental exposures.

GxE becomes a key factor in determining the risk of developing a disease.

NAT2 and bladder cancer

Review > Am J Epidemiol. 2007 Oct 1;166(7):741-51. doi: 10.1093/aje/kwm167. Epub 2007 Aug 4.

Joint effects of the N-acetyltransferase 1 and 2 (NAT1 and NAT2) genes and smoking on bladder carcinogenesis: a literature-based systematic HuGE review and evidence synthesis

Simon Sanderson [1], Georgia Salanti, Julian Higgins

GENE-ENVIRONMENT INTERACTION (GXE)

NAT2 is involved in the detoxification of aromatic amines, such as aniline and 2-naphthylamine, which are highly present in cigarette smoke, dyes, and paints.

The risk of developing bladder cancer link to smoking or to the occupational exposure to arylamines is increased in slow acetylators, although polymorphisms in the NAT2 gene causing a reduction of the enzyme activity are not a risk factor in themselves.

Polymorphisms in genes coding for drug transporters

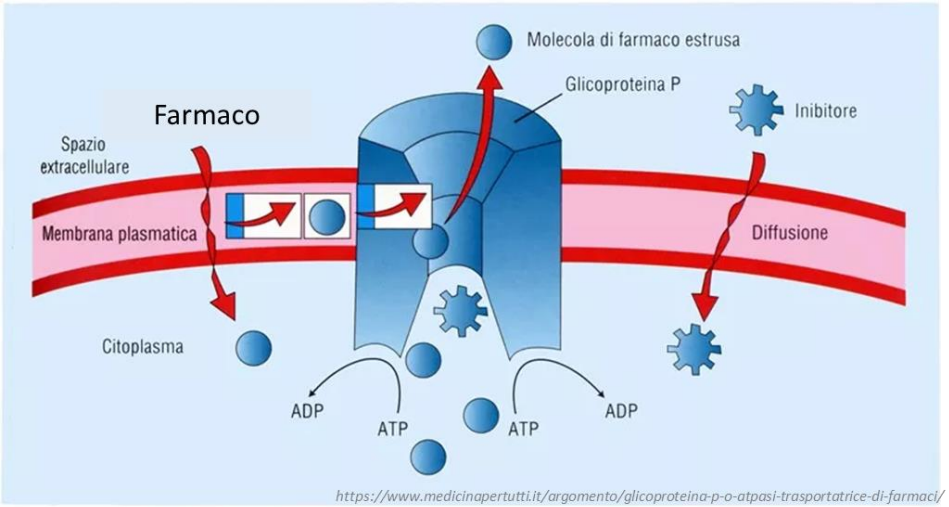
ABC transporters (ATP-binding cassette transporters)

They are the main drug transporters family.

They use the energy from the hydrolysis of ATP to transport drugs out of the cell (“*efflux pumps*”).

P-glycoprotein belongs to this transporter family. It is abundantly expressed in the apical membrane of enterocytes and renal tubular cells, in bile canaliculi of hepatocytes, in the blood–brain barrier, and in the placenta.

It plays a key protective role by controlling the absorption, distribution and excretion of drugs. It is also highly expressed in cancer cells, where it is involved in resistance to chemotherapeutic drugs.



https://www.medicinapertutti.it/argomento/glicoproteina-p-o-atpasi-trasportatrice-di-farmaci/

Anti-cancer drugs
- Anthracyclines; doxorubicin, daunorubicin
- Camptothecins; topotecan
- Epipodophyllotoxins; etoposide, teniposide
- Taxanes; paclitaxel, docetaxel
- *Vinca* alkaloids; vinblastine, vincristine

Tyrosine kinase inhibitors
- Imatinib mesylate
- Gefitinib

Natural products
- Flavonoids
- Curcuminoids
- Colchicine
- Actinomycin D

Fluorescent dyes
- Rhodamine 123
- Hoechst 33342
- Calcein-acetoxymethylester (AM)

Steroids
- Aldosterone
- Corticosterone
- Dexamethasone

Pesticides
- Methylparathion
- Endosulfan

Anti-epileptics
- Topiramate

Analgesics
- Morphine

Antibiotics
- Erythromycin

Cholesterol-lowering agents
- Lovastatin
- Simvastatin

Anti-helminthics
- Ivermectin

Anti-hypertensives
- Reserpine

Anti-histamines
- Terfenadine

Calcium channel blockers
- Nifedipine
- Diltiazem
- Verapamil

Calmodulin antagonists
- Trifluoperazine
- *trans*-Flupentixol

Cardiac glycosides
- Digoxin

HIV protease inhibitors
- Nelfinavir
- Ritonavir
- Saquinavir

Immunosuppressive agents
- Cyclosporin A
- Tacrolimus

Linear peptides
- ALLN
- Leupeptin
- Pepstatin A

Cyclic peptides
- PSC833
- Beauvericin

Ionophores
- Gramicidin A
- Valinomycin

Miscellaneous
- Reserpine
- Cimetidine
- Triton X-100

Examples of drugs that are substrates of P-glycoprotein

P-glycoprotein

It is encoded by the ABCB1 gene, also known as the MDR-1 (multidrug resistance 1) gene, which is located on chromosome 7.

This gene is highly polymorphic. Polymorphisms modify protein activity or expression, with a significant impact on the effect of drugs acting as its substrates (reduced bioavailability, risk of accumulation, altered passage through the blood-brain barrier and excessive elimination).

Table 3
Overview of the 15 most common *ABCB*1 (MDR1) genetic variants

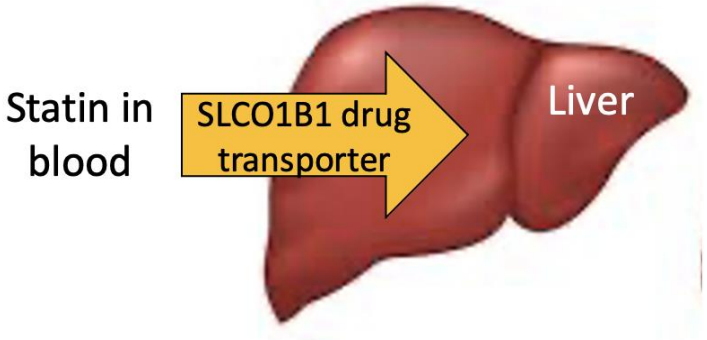
Position	Location	Effect	Allelic frequency (%)		
			CA	AS	AA
−129T>C	5′UTR	Non-coding	5	4	7
−1G>A	5′UTR	Non-coding	8	5	0
61A>G	Exon 2	Asn21Asp	8	2	2.5
Exon 5-25G>T	Intron 4		16	7	30
Exon 10-44A>G	Exon 10	Intron 9	45	69	26
1199G>A	Exon 11	Ser400Asn	2.5	0	<1
1236C>T	Exon 12	Synonymous	46	69	21
Exon 11+44C>T	Intron 12		5	0	17
Exon 12+24C>T	Intron 13		52	54	54
Exon 13+38A>G	Intron 14		50	68	54
Exon 19+24G>A	Intron 20		12	7	15
2677G>T/A	Exon 21	Ala893Ser/Thr	46/2	45/7	>1
3421T>A	Exon 26	Ser1141Thr	0	0	10
3435C>T	Exon 26	Synonymous	56	40	10
IVS+21T>C	Intron 28		0	8	0

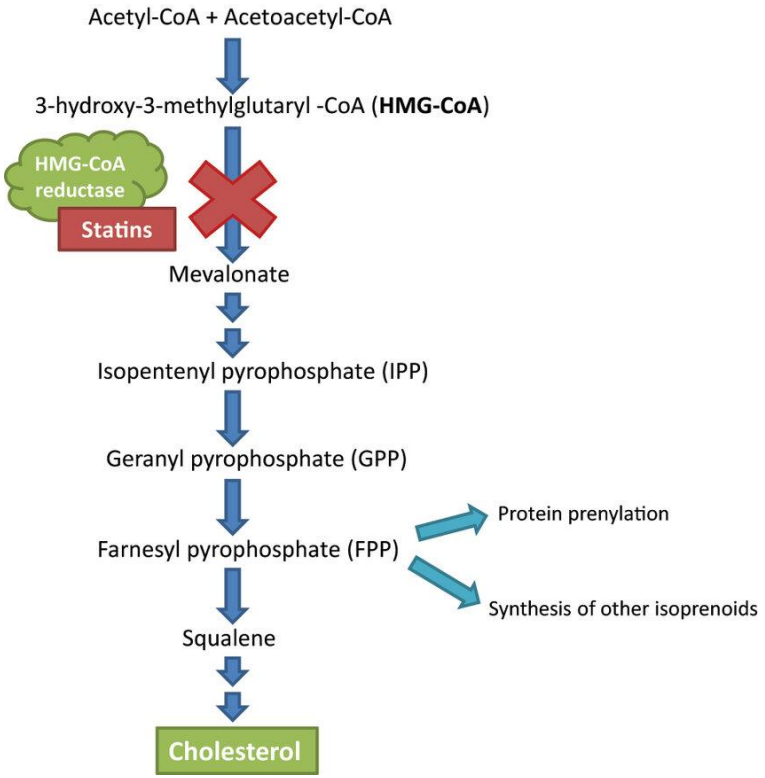
AA, African American; AS, Asian; CA, Caucasian.

OATP1B1 transporters

OATP1B1 transporter is encoded by SLC01B1 gene and belongs to the organic anion transporting polypeptides (OATPs) family, influx transporters which mediate the passage of drug across biological membrane.

OATP1B1 is considered the major transporter involved in the hepatic uptake of statins, cholesterol-lowering drugs.



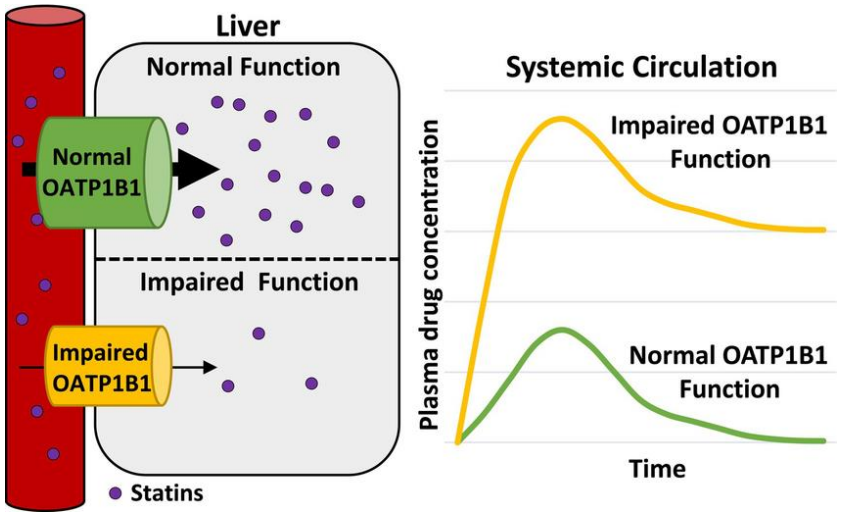


Sainz de Medrano Sainz et al. Influence of pharmacogenetics on the diversity of response to statins associated with adverse drug reactions. Adv Lab Med. 2023

Nucleotide Change (s)	rsID	Protein Variation (s)	Haplotype	Transporter Effect	OATP1B1 substrate serum conc.	Allele Frequency (%) [a]				Ref.
						AA	J	As	C	
None	N/A	N/A	*1A	Normal	Baseline					[60]
388A>G	2306283	Asn130Asp	*1B	Increased	Decreased	74–78	54	58–81	37–46	[60]
521T>C	4149056	Val174Ala	*5	Reduced	Increased	1–4	0.7	6–19	12–20	[60]
521T>C+ 388A>G	4149056+ 2306283	Val174Ala+ Asn130Asp	*15	Reduced	Increased		10			[60]

[a] AA = African American [61,62], J = Japanese [63], As = Asian [64,65], C = Caucasian [61,62,66,67].

The presence of polymorphisms which reduce the function of OATP1B1 can lead to reduced plasma clearance of statins, increasing the risk of accumulation in the blood, with reduced therapeutic effect and muscular side effects (e.g. myopathy)



Medwid S et al., 2024. SLCO1B1 variants in a patient of African ancestry presenting with rosuvastatin-induced rhabdomyolysis: A case report. Br J Clin Pharmacol. 91(1):232-235.

Canestaro et al.. Statin pharmacogenomics: opportunities to improve patient outcomes and healthcare costs with genetic testing. J Pers Med. 2012 2(4):158-74.