Pharmacogenomics and Clinical Correlation

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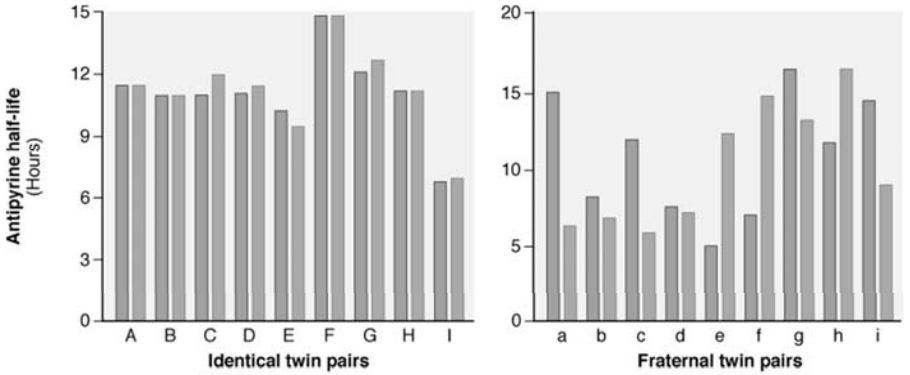
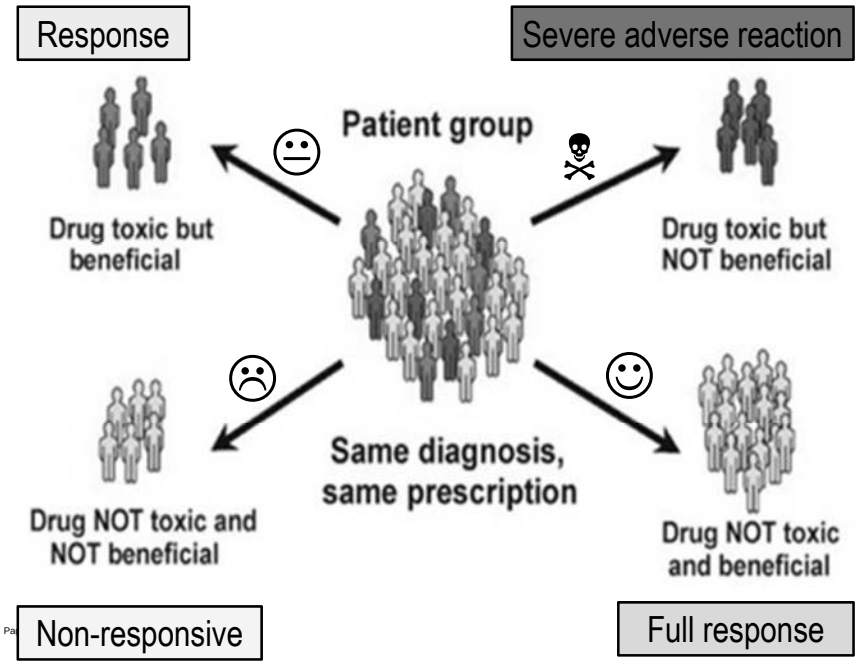
What do you think of this twin?



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition:* www.accessmedicine.com

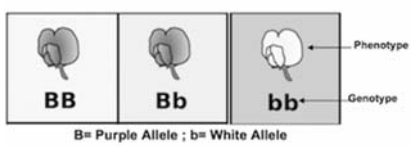


Objectives

- Define terms: pharmacogenetics, pharmacogenomics, drug efficacy, drugs safety, genotype, phenotype and drug response
- Understand an individual's genetic makeup is the key to creating personalized drugs with greater efficacy and safety
- Describe applications of pharmacogenomics in clinical setting
- Relate genetic variants of drug-metabolizing enzymes to variations in drug response

Genotype vs. Phenotype

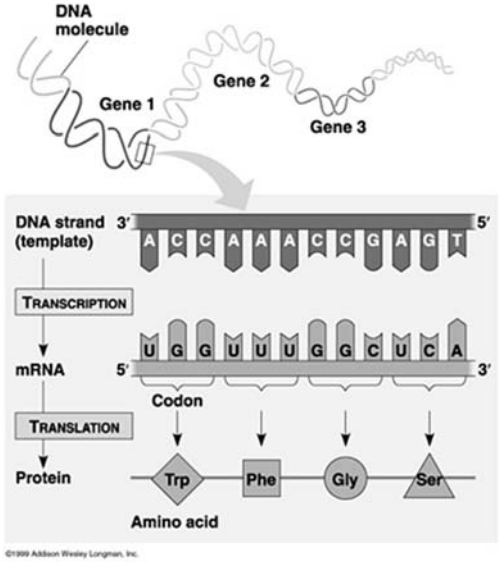
GENOTYPE: The genetic makeup of an individual, which may refer to the whole genome or to specific genes or regions of genes.



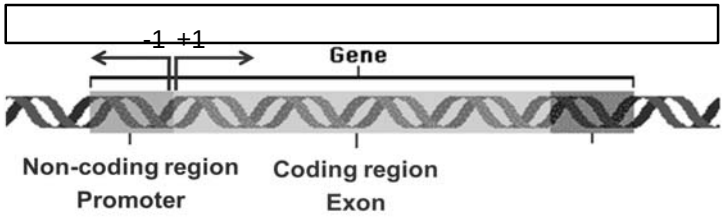
PHENOTYPE:

- The clinical presentation or characteristics
- Measurable characteristics of an organism (from genotype, environment, or combination)
- Organisms with the same phenotype can have different genotypes.
- Enzyme activity: responder and non-responder

From gene to protein



From gene to protein



Mutations on promoter region	→	Alteration of gene expression
Mutations on exon region	→	Structure & function of protein changes
Mutations on splice site	→	**Production of abnormal proteins**

Genomic variation

- The human genome contains approximately 3 billion nucleotides.
- The genome contains approximately 25,000 genes, through alternative splicing and post-translational modification, may encode 100,000 or more proteins.
- Any two people differ on average at about one nucleotide in every 1,000 in their genome, totaling an average interindividual difference of 3 million base pairs throughout the genome.
- The majority of these differences are called Single Nucleotide Polymorphisms (SNPs).

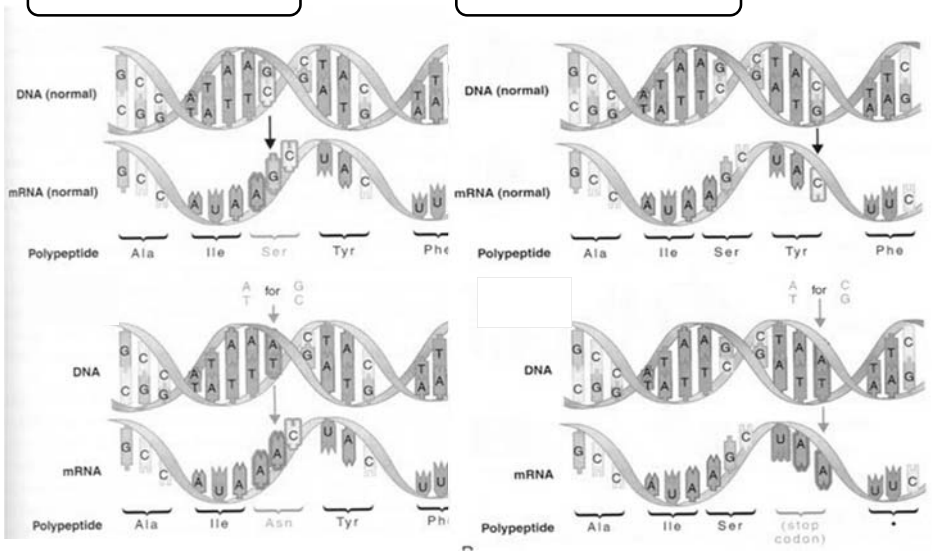
Mutation vs. Polymorphism

A **mutation** is defined as any change in a DNA sequence away from normal. This implies there is a normal allele that is prevalent in the population (wild-type) and that the mutation changes this to a rare and abnormal variant.

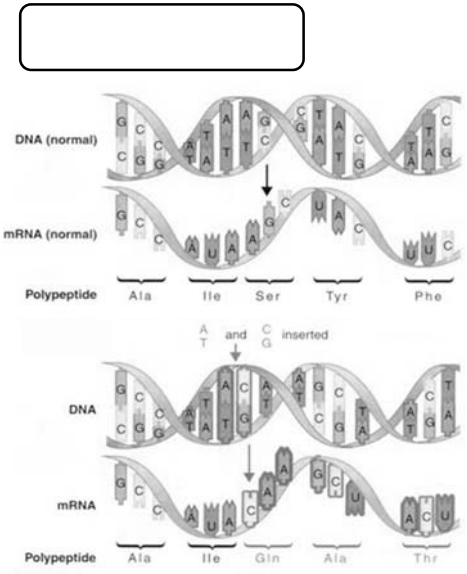
A **polymorphism** is a DNA sequence variation that is common in the population.

The arbitrary cut-off point between a mutation and a polymorphism is 1%.

Types of mutation



Types of mutation



TAT TGG CTA GTA CAT
Tyr Trp Leu Val His

TAC TGG CTA GTA CAT
Tyr Trp Leu Val His

wild-type sequence
ATCTTCAGCCATAAAAGATGAAGTT

3 bp deletion
ATCTTCAGCCAAAGATGAAGTT

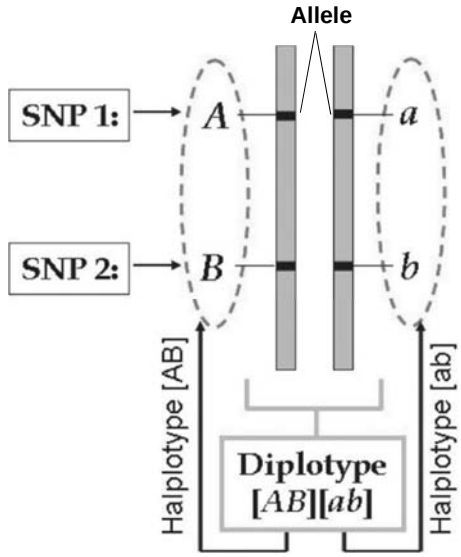
4 bp insertion (orange)
ATCTTCAGCCATATGTGAAAGATGAAGTT

Haplotype vs. Diplotype

Allele is one of a number of alternative forms of the same gene or same genetic locus.

Haplotype is a specific combination of SNPs all occurring on the same chromosome.

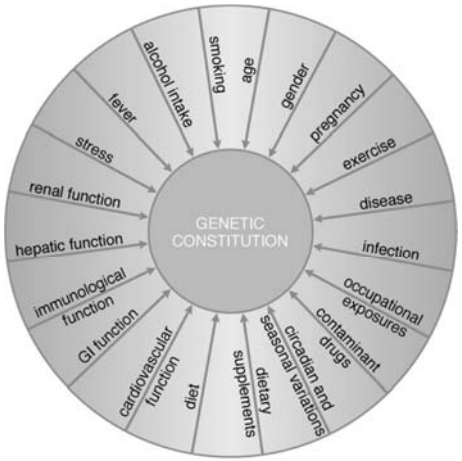
The pair of haplotypes is called a diplotype.



Factors contribute to variation in drug response

Importance of pharmacogenetics to variability in drug response

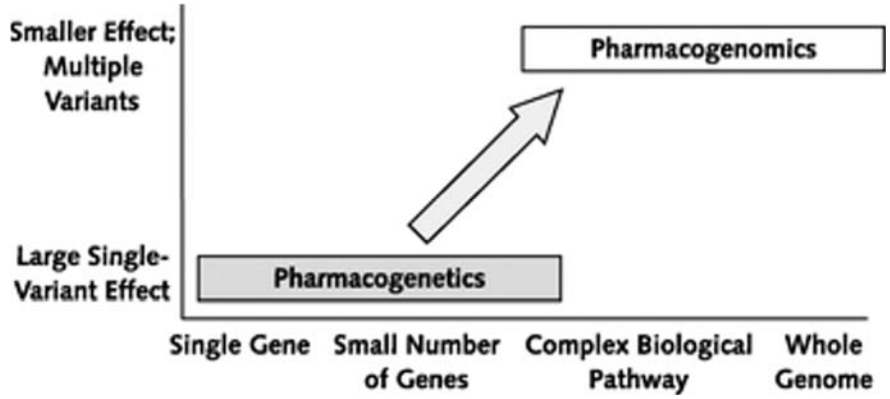
- Drug response is considered to be a gene-by-environment phenotype.
- Variation in drug response may be explained by variation in environmental and genetic factors, alone or in combination.



Source: Brunton LL, Chabner BA, Knollmann BC: *Goodman & Gilman's The Pharmacological Basis of Therapeutics, 12th Edition*: www.accessmedicine.com

Pharmacogenetics vs. Pharmacogenomics

What are pharmacogenetics (PGt) and pharmacogenomics (PGx)?



http://annals.org/article.aspx?articleid=730441

Pharmacogenetics vs Pharmacogenomics

Pharmacogenomics

- The science of how genes affect the way people respond to drugs
- How genes affect…
  - the way our body processes drugs (pharmacokinetics)
  - the interaction of drugs with receptors (pharmacodynamics)
  - the treatment efficacy and adverse side effects

Pharmacogenetics

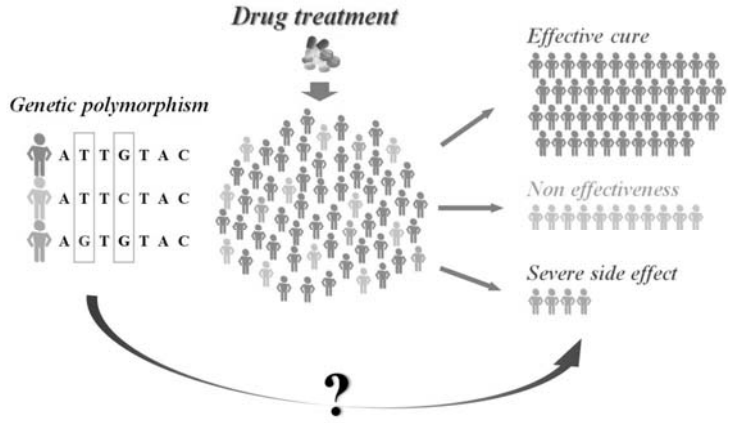
- A subset of 'pharmacogenomics'
- The study of how inherited variation affects drug response and metabolism

Table 1: Some important landmarks in the pharmacogenetics timeline

1953	James D Watson and Francis Crick published their paper on the double-helical structure of DNA.[2]
1957	Motulsky proposes that "inheritance might explain many individual differences in the efficacy of drugs and in the occurrence of adverse drug reactions."[3]
1959	The word "pharmacogenetics" appears for the first time in a paper published by Friedrich Vogel.[4]
1968	Vessel and Page show similar drug pharmacokinetics in identical twins who share 100% of their genes as contrasted to fraternal twins who only share 50%.[5]
1977	DNA sequencing technologies start to emerge.
1990	The human genome project (HGP) is initiated, and funded by the National Institutes of Health (USA) and other international partners. Projected project timeline is 15 years.[38]
2003	The HGP is completed, two years in advance of its original projected target date.[38]
2004	Roche AmpliChip Cytochrome P450 Genotyping test is given marketing clearance by FDA. This is the first pharmacogenetic test to be given FDA approval.[26]
2005	The European Medicines Agency (then known as EMEA, later known as EMA) establishes the Pharmacogenetics Working Party (PgWP). This later changed its name to the Pharmacogenomics Working Party, still maintaining the PgWP abbreviation.[32]
2005	The Food and Drug Administration (FDA) establishes the Interdisciplinary Pharmacogenomics Review Group (IPRG).[33]
2005	FDA gives marketing approval for The Invader UGT1A1 Molecular Assay. This is the first pharmacogenetic test to be approved by the FDA, following establishment of the IPRG.[28]
2009	Imperial College London announce their ongoing development of the SNP Dr pharmacogenotyping device.[37]
Today	Major pharmaceutical companies have incorporated pharmacogenomics into the drug discovery process,, published pharmacogenetic data is escalating exponentially, and translation from bench to bedside is well underway.

J Malta College Pharm Pract. 2011;17:25-33.

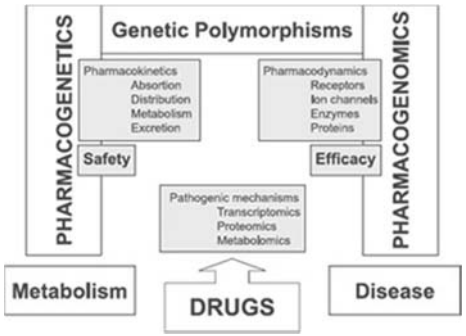
Personalized medicine

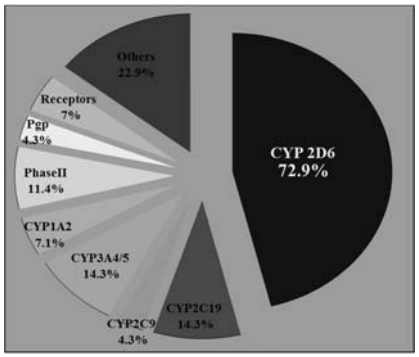


The goal is to reduce adverse effects to medications, lower the cost of therapies and improve efficacy.

Genotyping & phenotyping

Breakdown of genotyping and phenotyping in FDA survey:





- Genotyping and phenotyping performed in some submissions
- Phase II enzymes measured: NAT-2, UGT, GSTM1, etc.
- Receptors: Dopamine, 5-HT, β-adrenergic, α_1-adrenergic, potassium channels, etc.
- Others: HMC, CETP, ACE, α-reductase, AAG, CYP2B6, glyceraldehyde 3-phosphate dehydrogenase, ApoE etc.

Influence of PGt on the risk of adverse effects

Journal of the Malta College of Pharmacy Practice. 2011.

Drug	Gene/s involved	Potential adverse effect
Abacavir	HLA-B*1502	Increased risk of general hypersensitivity
Azathioprine	TMPT	Slower metabolism and greater risk of myelotoxicity
Carbamazepine	HLA-B*1502, CYP1A2	Increased risk of severe dermatological hypersensitivity reaction
Carvedilol	CYP2D6	Increased risk of adverse effects in slow metabolizers
Clopidogrel	CYP2C19	Reduced metabolism of the pro-drug clopidogrel, lower exposure to the active metabolite and lower therapeutic effect
Fluoxetine	CYP2D6	Increased risk of toxicity in slow metabolizers, especially if prescribed with other CYP2D6-metabolized drugs
Irinotecan	UGT1A1	Slower metabolism and increased risk of neutropenia
Isoniazid	NAT2	Increased risk of agranulocytosis, hepatotoxicity and seizures
Nilotinib	UGT1A1	Exacerbation of drug-induced jaundice
Rifampicin	NAT	Slower metabolism and greater risk of general adverse reactions
Warfarin	CYP2C9, VKORC1	Reduced metabolism and higher bleeding risk

HLA = Human Leukocyte Antigen; TMPT = Thiopurine methyltransferase; CYP = Cytochrome P450;
UGT = UDP-glucuronosyltransferase; NAT = *N*-acetyltransferase; VKORC = Vitamin K epoxide reductase complex

Variation in drug-metabolizing enzymes

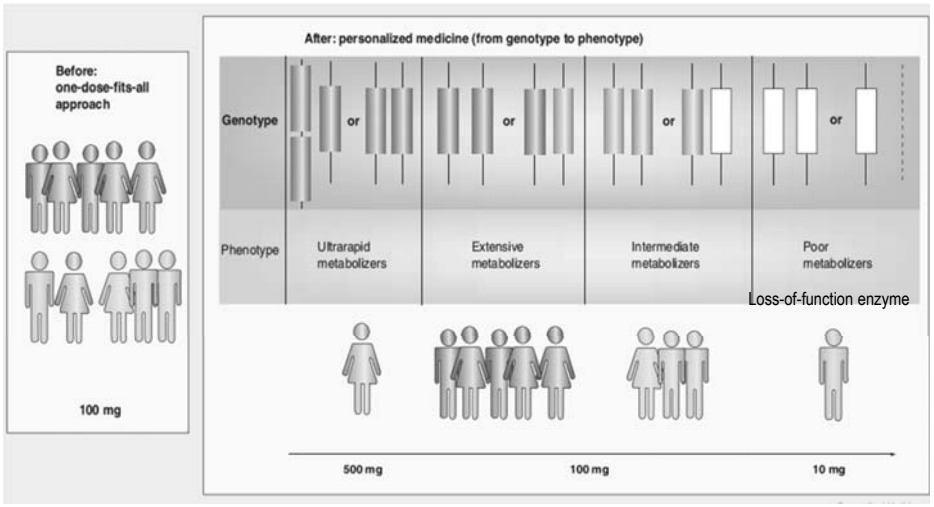
For drug biotransformation, there are 4 possible phenotype categories:

1) Poor metabolizer (slow)
2) Intermediate metabolizer
3) Extensive metabolizer (normal)
4) Ultra-rapid metabolizer (ultra-extensive)

Poor metabolizer

A person who metabolizes a probe drug–the rate of which is related to the metabolizing CYP450 enzyme–slower than others

Phenotype-driven terminology



Personalized Medicine. 2005;2(4):325-37.

CYP : root symbol for P450 genes

Family : >40% identity with other CYP2 proteins

Subfamily : >55% identity with other CYP2D or CYP2C proteins

CYP2D6*2 Allele

CYP2C19

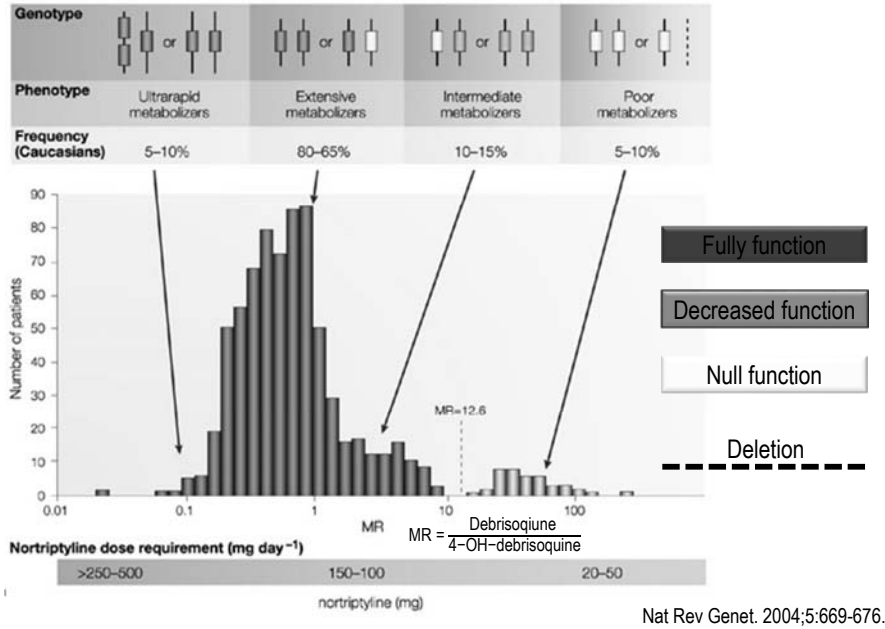
Isoform

Polymorphic CYP450s nomenclature

- The gene and the allele name is separated by an asterisk followed by Arabic numerals designating the specific allele (e.g., *CYP1A1*3*).
- The most popular gene in the CYP-allele database is *CYP2D6*, followed by *CYP2C9*, *CYP2C19* and *CYP3A4*.
- To be assigned a unique allele name, the sequence should contain at least one nucleotide change that has been shown to affect transcription, splicing, translation, posttranscriptional, or posttranslational modifications or result in at least one amino-acid change.
- For extra gene copies (N) placed in tandem on the same chromosome, the entire allelic arrangement should be referred to as, e.g., *CYP2D6*2xN.*

http://www.cypalleles.ki.se/criteria

Genotype-phenotype relationships of CYP2D6



Nat Rev Genet. 2004;5:669-676.

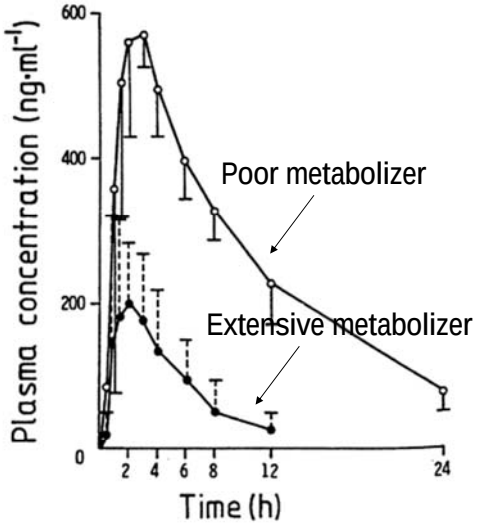


Fig. 3. Mean (±S.D.) plasma concentration–time curves after administration of a single oral dose of metoprolol (200 mg) to six extensive (●) and six poor metabolizers (○) of debrisoquine [reprinted with permission from [39], copyright (1982) Massachusetts Medical Society].

Genotype-phenotype relationships of *CYP2D6*

Functional Status	Activity Score	Alleles
Functional / normal activity/ wild-type[b]	1	*1[c], *2, *27, *33, *35, *45, *46, *39, *48, *53
Reduced-function / decreased activity	0.5	*9, *10, *17, *29, *41, *49, *50, *54, *55, *59, *69, *72
Non-functional, variant, or mutant / no activity	0	*3, *4, *5, *6, *7, *8, *11, *12, *13, *14, *15, *16, *18, *19, *20, *21, *31, *36, *38, *40, *42, *44, *47, *51, *56, *57, *62

CPIC Update. 2014;95(5):376-82.

Genotype-phenotype relationships of *CYP2D6*

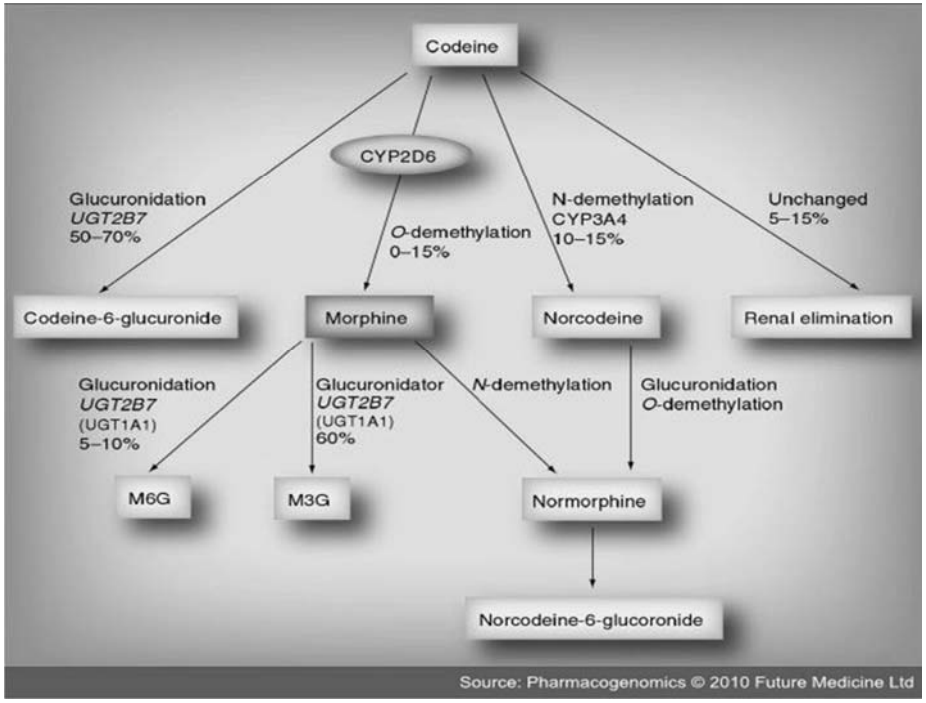
Likely phenotype[a]	Activity score	Genotypes	Examples of diplotypes
Ultrarapid metabolizer (~1–2% of patients)	>2.0	An individual carrying more than two copies of functional alleles	*1/*1xN, *1/*2xN
Extensive metabolizer (~77–92% of patients)	1.0–2.0[b]	An individual carrying two alleles encoding full or reduced function or one full function allele together with either one nonfunctional or one reduced-function allele	*1/*1, *1/*2, *2/*2, *1/*41, *1/*4, *2/*5, *10/*10
Intermediate metabolizer (~2–11% of patients)	0.5[b]	An individual carrying one reduced and one nonfunctional allele	*4/*10, *5/*41
Poor metabolizer (~5–10% of patients)	0	An individual carrying no functional alleles	*4/*4, *4/*5, *5/*5, *4/*6

CYP2D6 phenotypes among different ethnic populations

Population	PM phenotype (%)	Diminished activity of IMs (%)	UM phenotype (%)
White		1–2	
American	7.7		4.3
British	8.9		
Polish	8.3		
Swiss	10		
Danish			0.8
German	7.7		0.8
Swedish			1
Spanish			10
Turkish	1.5		8.7
Croatian	3.0		4.0
African			
African-American	1.9–7.3		4.9
Nigerian	0–8.1		
Ghanaian	6.0		
Ethiopian	1.8		29
South African	19		
Asian		51	
Japanese	0		
Chinese	<1.0		0.9
Thai	1.2		
Indian	1.8–4.8		
Saudi Arabian	1–2	3–9	21.0
Hispanic			
Colombian	6.6		1.7
Mexican	3.2		
Panamanian (Amerindian)	2.2–4.4		
Nicaraguan	3.6		

Abbreviations: IM, intermediate metabolizer; PM, poor metabolizer; UM, ultrarapid metabolizer.

The Oncologist. 2006;11(2):126-35.



CYP2D6 - Recommendation for codeine use

Diplotype - **1/*1xN, *1/*2xN*
Phenotype - UM (>2.0)

- Increase formation of morphine following codeine administration leads to higher risk of toxicity
- Alternative analgesic, e.g. morphine or non-opioid

Diplotype - **1/*1, *1/*2, *2/*2, *1/*41, *2/*5*
Phenotype - EM (1.0–2.0)

- Normal morphine formation
- Standard starting dose

CYP2D6 - Recommendation for codeine use

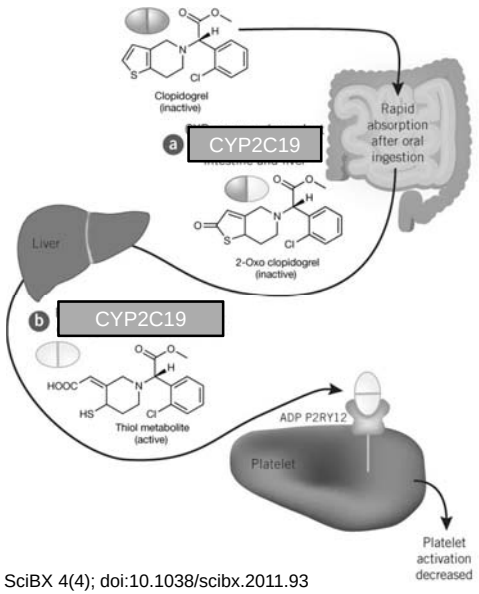
Diplotype - **4/*10, *5/*41*
Phenotype - IM (0.5)

- Standard starting dose; monitor closely for lack of analgesic response due to reduced morphine formation
- Consider alternate analgesic, e.g. morphine or non-opioid

Diplotype - **3/*4, *4/*4, *4/*5, *5/*5, *4/*6*
Phenotype - PM (0.0)

- Greatly reduced morphine formation, leading to insufficient pain relief
- Alternative agent, e.g. morphine or non-opioid analgesic
- Avoid higher doses, as central side effects do not differ in PMs

Clopidogrel therapy



SciBX 4(4); doi:10.1038/scibx.2011.93

- Clopidogrel is used to prevent heart attacks and strokes in persons with heart disease (recent heart attack), recent stroke, or blood circulation disease (peripheral vascular disease).
- FDA labeling warns of reduced effectiveness in those with impaired CYP2C19 function.

Clopidogrel therapy and *CYP2C19* genotype

Likely Phenotype	Genotype	Implications for Clopidogrel	Therapeutic Recommendations	Classification of recommendations
Ultrarapid Metabolizer (5-30%)	CYP2C19: *1/*17 CYP2C19: *17/*17	Increased platelet inhibition	Standard Clopidogrel dosing	Strong
Extensive Metabolizer (35-50%)	CYP2C19: *1/*1	Normal platelet inhibition	Standard Clopidogrel dosing	Strong
Intermediate Metabolizer (18-45%)	CYP2C19: *1/*2 CYP2C19: *1/*3 CYP2C19: *2/*17	Reduced platelet inhibition = increased risk of CV events	Alternative antiplatelet: Prasugrel or Ticagrelor*	Moderate
Poor Metabolizer (2-15%)	CYP2C19: *2/*2 CYP2C19: *2/*3	Significantly reduced platelet inhibition = increased risk of CV events	Alternative antiplatelet: Prasugrel or Ticagrelor*	Strong

* only if no contraindications to these alternatives

Clin Pharmacol Ther. 2011;90(2):328-32.

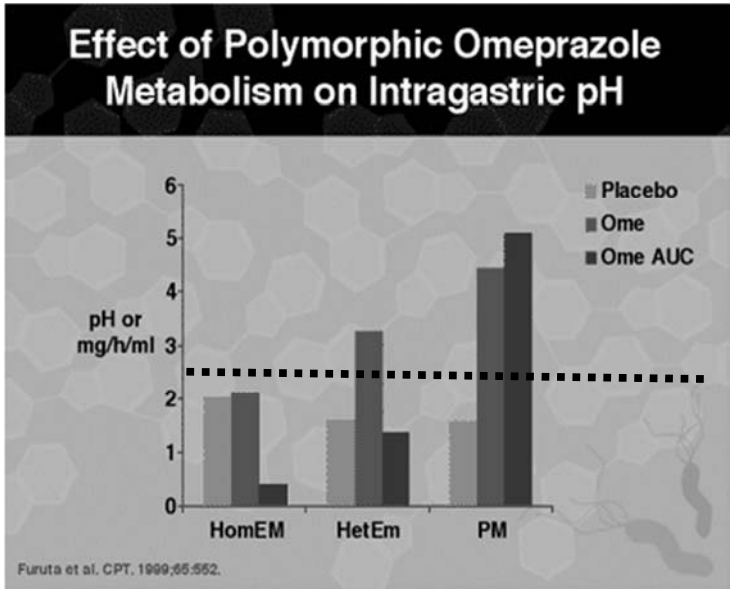
CYP2C19 and proton pump inhibitors

- Proton pump inhibitors are used to treat acid reflux and stomach ulcers.
- Ulcer cure rates using omeprazole and amoxicillin by CYP2C19 phenotype:

	Cure Rate
– Rapid metabolizers (RM)	29%
– Intermediate metabolizers (IM)	60%
– Poor metabolizers (PM)	100%

Ann Intern Med. 1998;129:1027-1030.

CYP2C19 and proton pump inhibitors



Metabolizer Phenotype	Drug Type	
	Standard drugs	Prodrugs*
Poor Metabolizer (PM)	Reduced elimination Increased toxicity risk	Decreased effectiveness Decreased activation
Intermediate Metabolizer (IM)	Increased drug-to-gene and drug-to-drug interaction risk Possible increased toxicity risk	Increased drug-to-gene and drug-to-drug interaction risk Possible reduced effectiveness
Normal Metabolizer (NM) (EM)	Performs according to FDA label specifications	
Rapid or Ultra Rapid Metabolizer (RM, URM)	Reduced effectiveness Increased elimination	Increased activation Increased toxicity risk

Pharmacogenomic biomarkers in drug labeling

Pharmacogenomics can play an important role in identifying responders and non-responders to medications, avoiding adverse events and optimizing drug dose. Drug labeling may contain information on genomic biomarkers and can be described:

- Drug exposure and clinical response variability
- Risk for adverse events
- Genotype-specific dosing
- Mechanisms of drug action
- Polymorphic drug target and disposition genes

FDA-approved drugs with PGx-related label information

Biomarker	Therapeutic Area	Drugs
CYP2C9	Analgesics Psychiatry Hematology	Celecoxib Fluvoxamine Warfarin
CYP2C19	Musculoskeletal Psychiatry Neurology Cardiovascular Gastroenterology	Carisoprodol Citalopram, Diazepam, Fluvoxamine Clobazam Clopidogrel, Prasugrel Esomeprazole, Omeprazole, Pantoprazole
CYP2D6	Psychiatry Cardiovascular Dental Analgesics	Citalopram, Fluoxetine, Paroxetine, Venlafaxine Carvedilol, Metoprolol, Propranolol Cevimeline Codeine

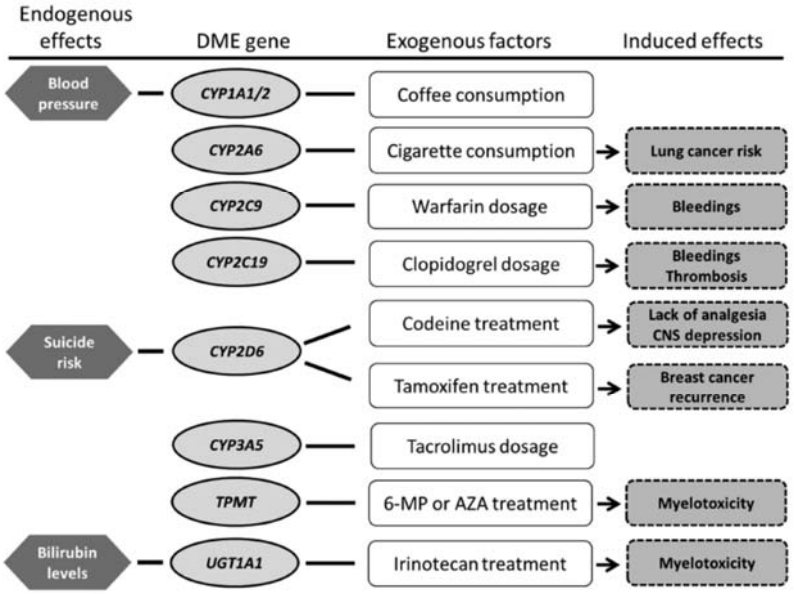
http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm

FDA-approved drugs with PGx-related label information

Biomarker	Therapeutic Area	Drugs
UGT1A1	Oncology Pulmonary	Irinotecan, Nilotinib Indacaterol, Arformoterol
TPMT	Oncology Rheumatology	Mercaptopurine, Cisplatin Azathioprine
NAT1-2	Cardiology Infectious diseases	Isosorbide, Hydralazine Rifampin, Isoniazid, Pyrazinamide

TPMT = Thiopurine methyltransferase; NAT = N-acetyltransferase

http://www.fda.gov/drugs/scienceresearch/researchareas/pharmacogenetics/ucm083378.htm



The Pharmacogenomics Journal. 2013;13:1–11.

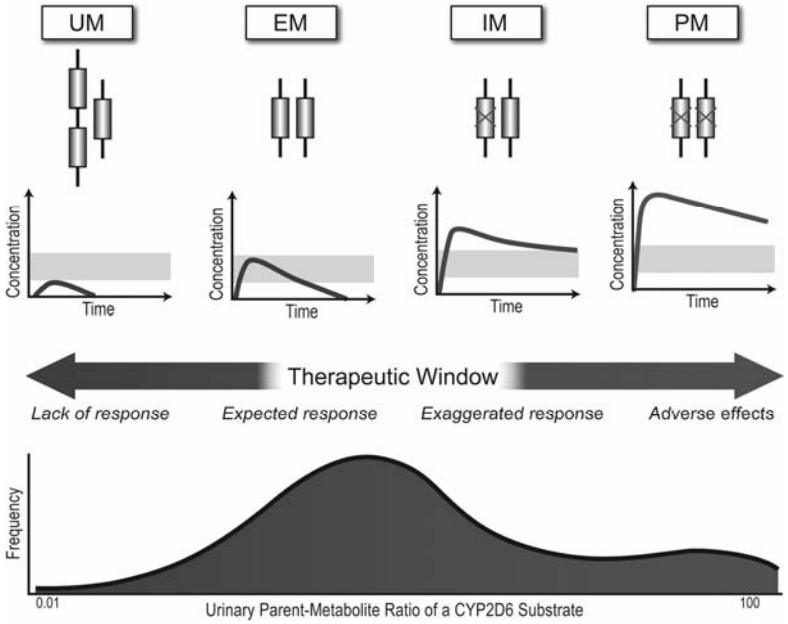
CYP450 Test - AmpliChip

- AmpliChip CYP450 Test (Roche) - Comprehensive detection of gene variations for the *CYP2D6* and *CYP2C19* genes.
- The first chip-based *in vitro* diagnostic with CE Mark certification and FDA clearance.
- Detects up to 33 *CYP2D6* alleles and 3 *CYP2C19* alleles.
- Detects *CYP2D6* gene duplication and deletions.
- The AmpliChip CYP450 Test performs genotyping of *CYP2D6* and *CYP2C19* and provides the predictive phenotype of the associated enzymatic activities, using DNA purified from human blood.

http://molecular.roche.com/assays/

Summary

- DNA variations may be responsible for inter-patient differences in drug efficacy and toxicity.
- One specific DNA variant may influence the response of several drugs.
- Variations in genes which code for drug receptor, drug transporters, drug metabolizing enzymes and proteins involved in signaling pathways may be especially relevant.
- Pharmacogenomics aims to predict drug response from patient genotypes and therefore provide a tool for a personalized optimization of drug and dose selection.
- A specific pharmacogenetics test is usually only applicable to the population for which it was developed and not to other ethnically diverse groups.



Anesthesiology. 2011;115:1316-1327.