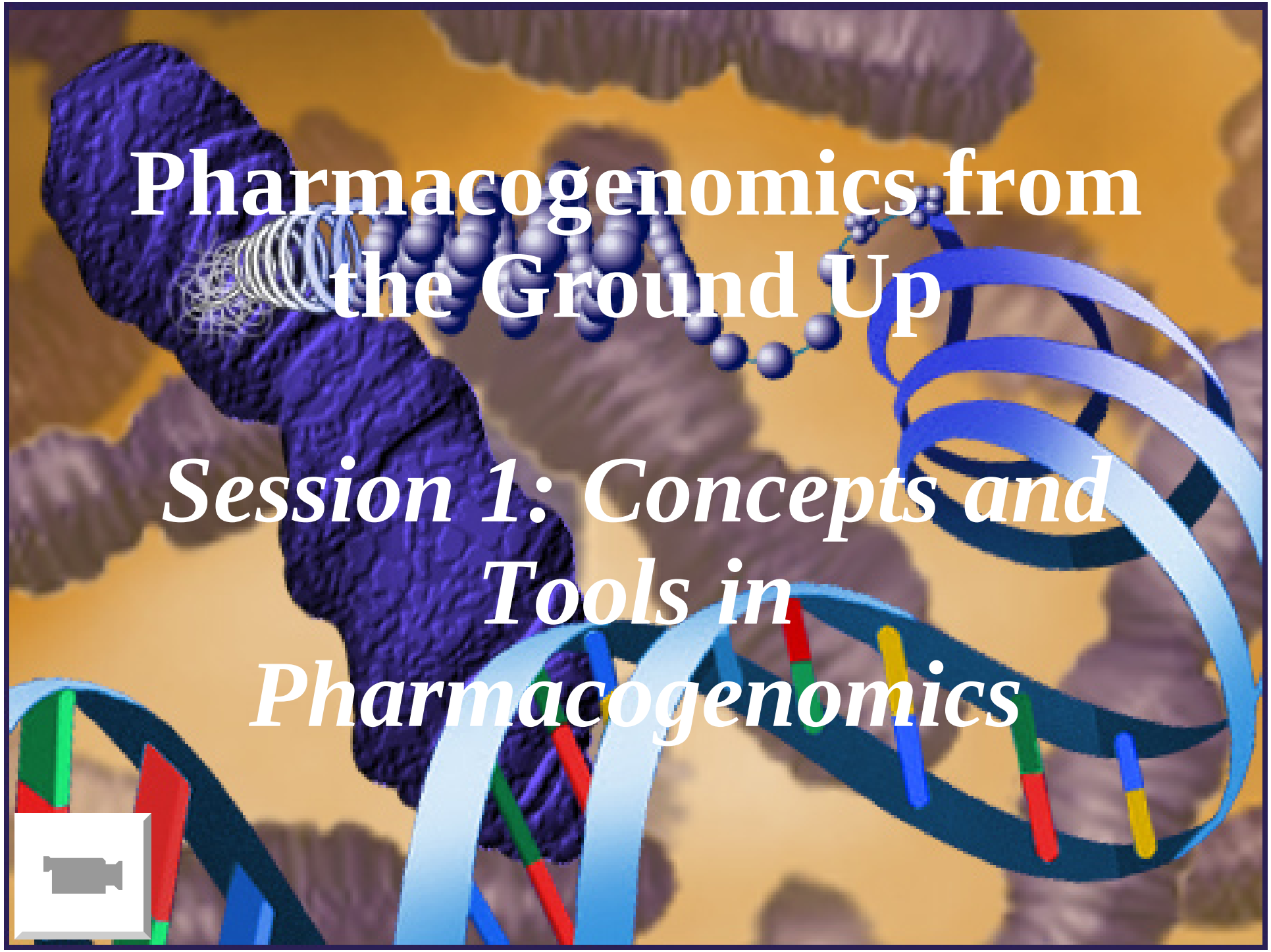

Pharmacogenomics from the Ground Up
Session 1: Concepts and Tools in Pharmacogenomics

The Human Genome

- The human genome contains 3164.7 million chemical nucleotide bases (A, C, T, and G).
- The average gene consists of 3000 bases, but sizes vary greatly, with the largest known human gene being dystrophin at 2.4 million bases.
- The total number of genes is estimated at 30,000.
- Almost all (99.9%) nucleotide bases are exactly the same in all people.
- The functions are unknown for over 50% of discovered genes.

The Human Genome

- Less than 2% of the genome codes for proteins.
- Repeated sequences that do not code for proteins ("junk DNA") make up at least 50% of the human genome.
- Repetitive sequences are thought to have no direct functions. Over time, these repeats reshape the genome by rearranging it, creating entirely new genes, and modifying and reshuffling existing genes.
- During the past 50 million years, a dramatic decrease seems to have occurred in the rate of accumulation of repeats in the human genome.

The Human Genome

- **The human genome's gene-dense "urban centers" are predominantly composed of the DNA building blocks G and C.**
- **In contrast, the gene-poor "deserts" are rich in the DNA building blocks A and T. GC- and AT-rich regions usually are the light and dark bands we see on chromosomes.**

The Human Genome

- Genes appear to be concentrated in random areas along the genome, with vast expanses of noncoding DNA between.

- Stretches of up to 30,000 C and G bases repeating over and over often occur adjacent to gene-rich areas, forming a barrier between the genes and the "junk DNA." These CpG islands are believed to help regulate gene activity.

- Chromosome 1 has the most genes (2968), and the Y chromosome has the fewest (231).

Session Goals

- **Concepts in Pharmacogenomics**
- **Metabolic Enzymes**
- **Pharmacogenomics of CYPs**
- **Clinical Examples**
- ***Pharmacogenomics Jeopardy!***

Concepts in Pharmacogenomics

Pharmacogenetics and Pharmacogenomics

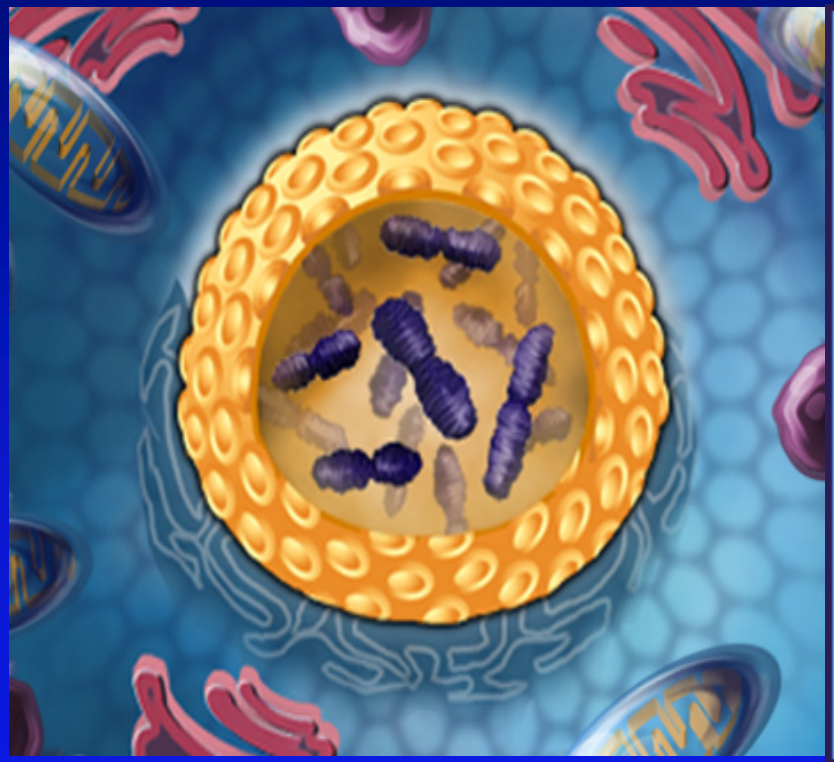
- ***Pharmacogenetics:*** **the effect of genetic variation on drug response, including disposition, safety and tolerability, and efficacy.**

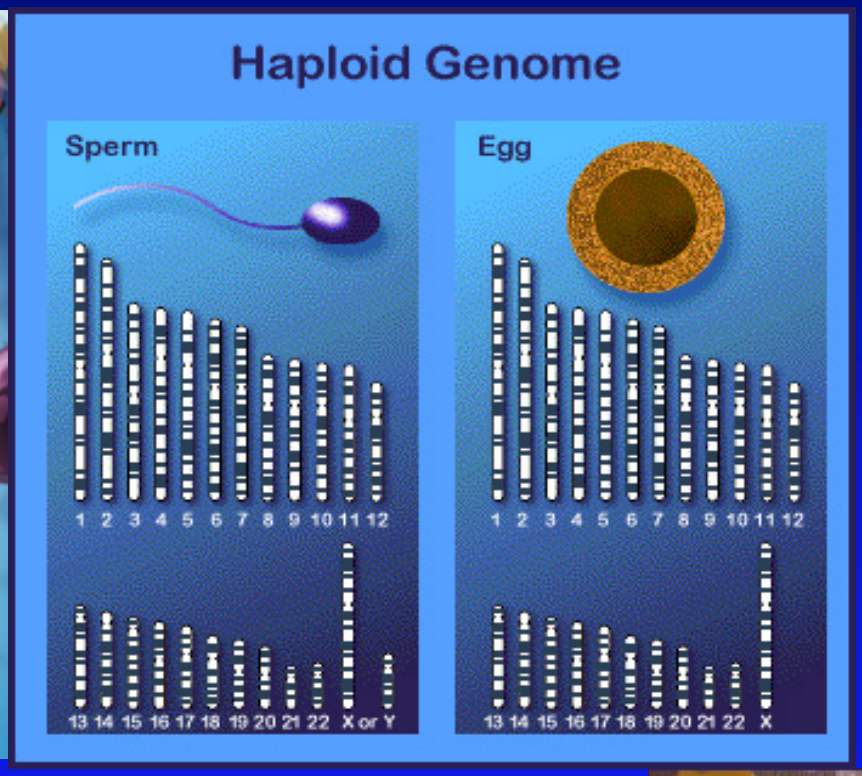
- ***Pharmacogenomics:*** **the application of genome science (genomics) to the study of human variability in drug response.**

- **We will study in the second session of this course *pharmacogenomics* applied to Epidermal Growth Factor Receptor (EGFR) drugs such as Tarceva, Iressa, and Erbitux.**

Genomic Organization: *Chromosomes*

- **Every human cell with the exception of the gametes contains 23 pairs of chromosomes.**

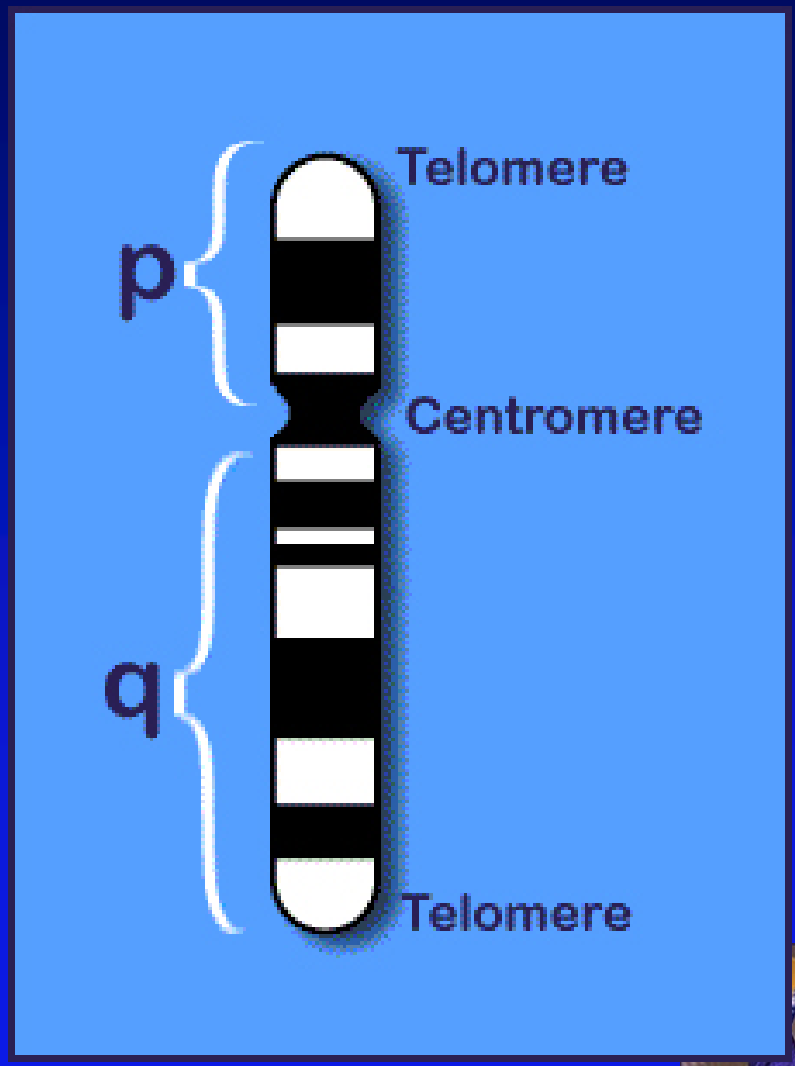




Genomic Organization: *Chromosome Structure*

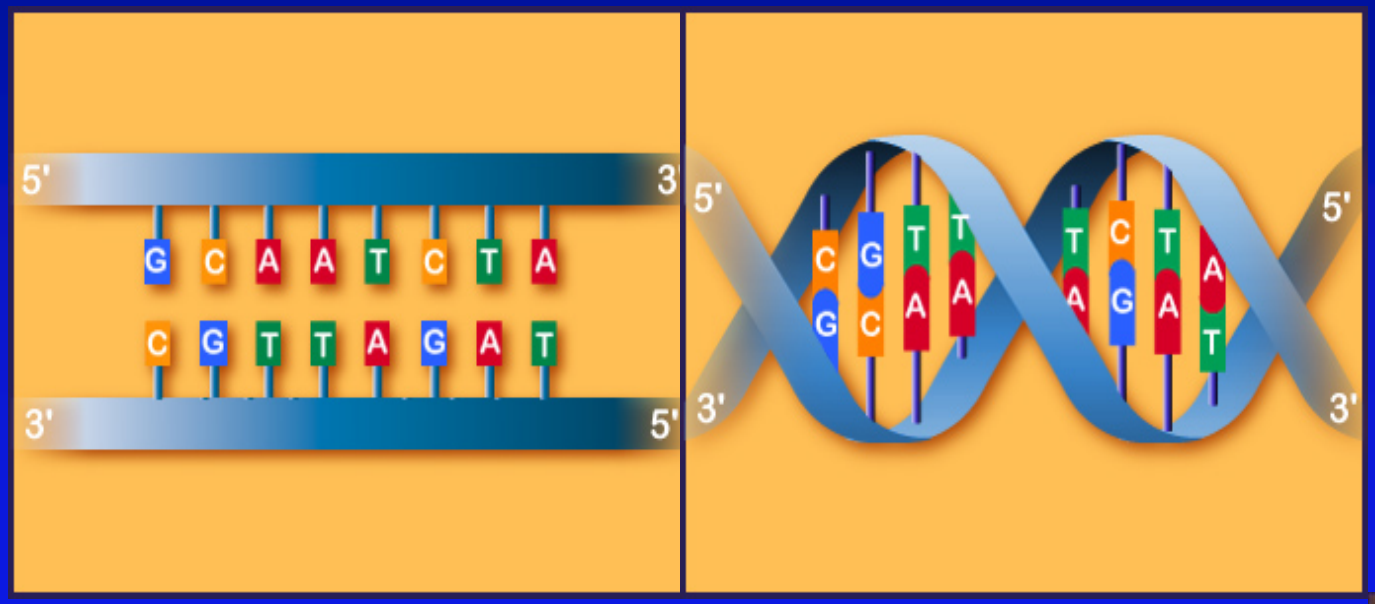
- **Chromosomes carry all of the genetic material coding for all the proteins in every cell.**

- **Chromosomes consist of DNA tightly wound around special protein structures called histones.**



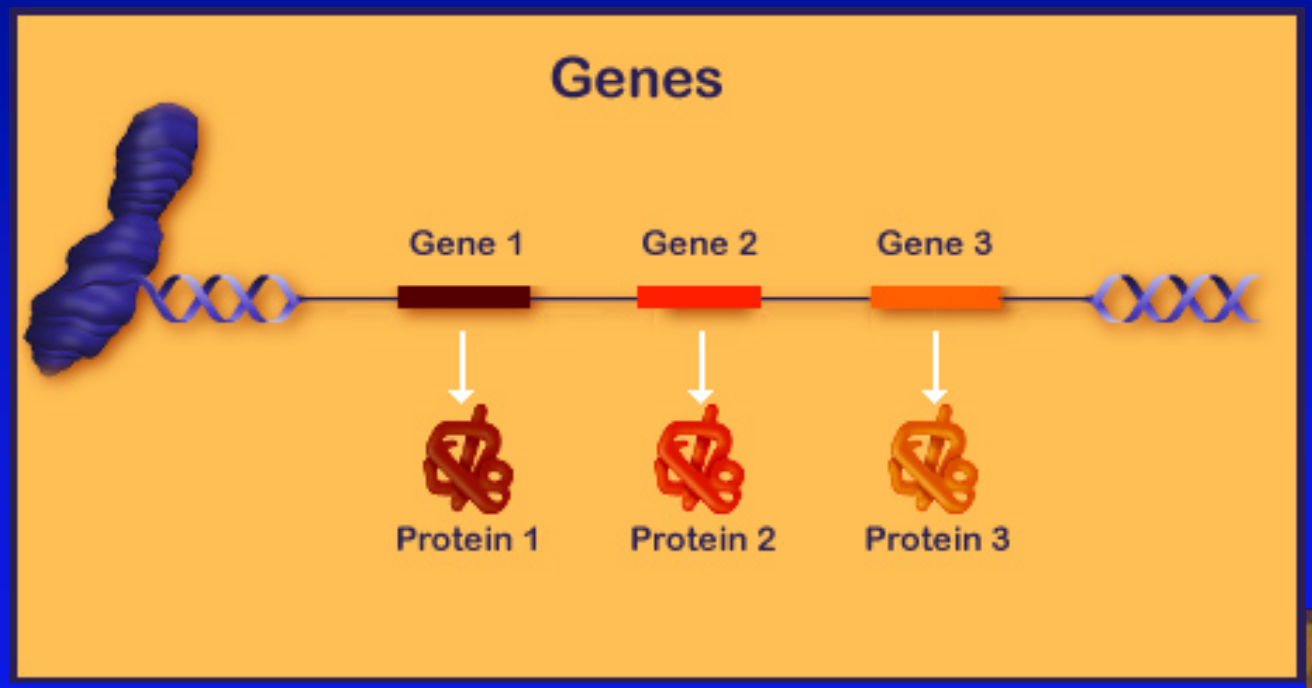
Structure of DNA

- **DNA is comprised of a string of 4 *nucleotide bases*, called A, G, T and C, that are linked together in a structure called the double helix. Bases on opposite strands are always matched A-T and C-G.**



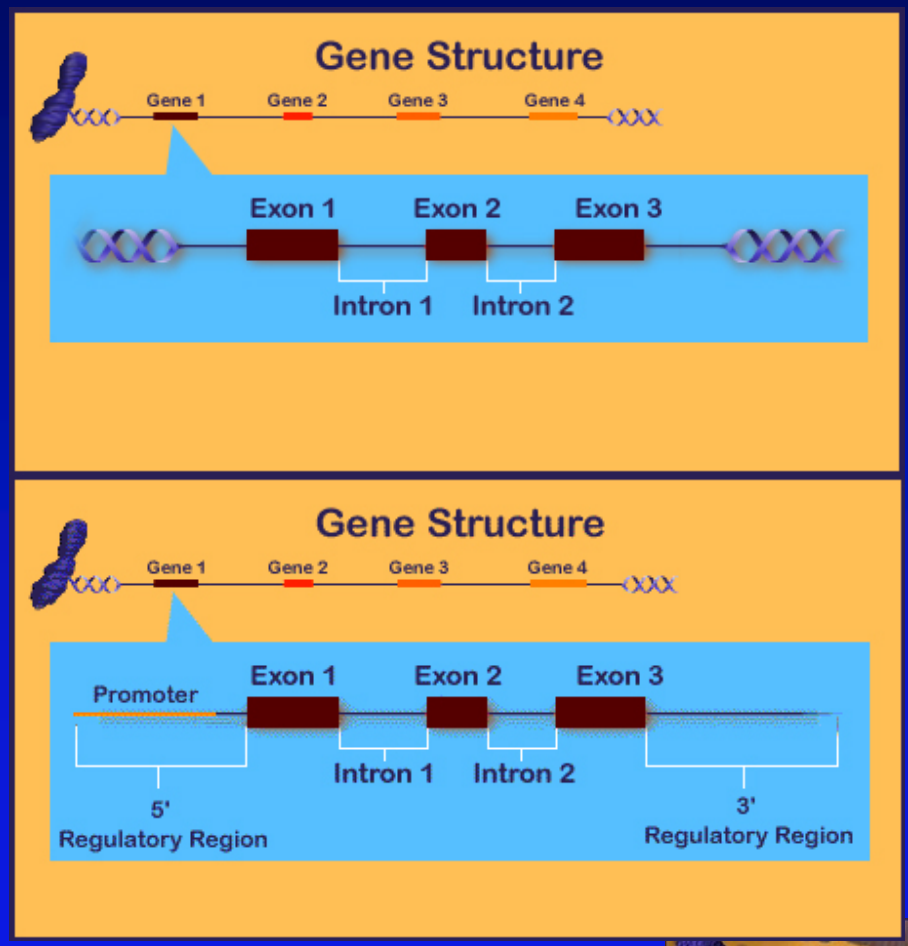
Structure of Genes

- **A segment of DNA containing all of the information needed to encode for one protein is called a *gene*. The order and sequence of the base pairs in a gene determine which protein is made.**



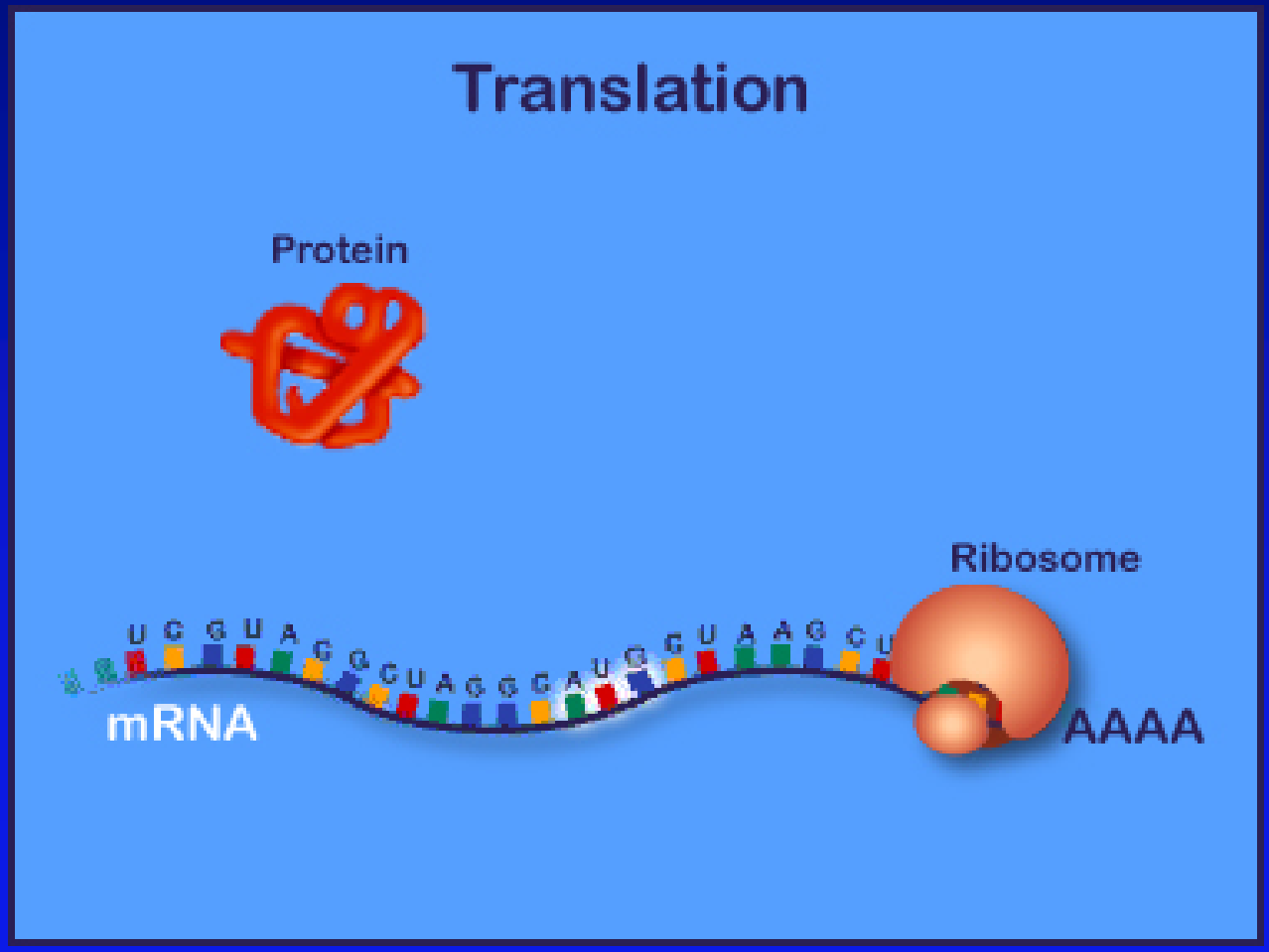
Transcription of Genes

- **The transcription of DNA into messenger RNA is often assembled from discontinuous sequences in the genome called *exons*, which are separated by sequences called *introns*.**
- **This process is referred to as *splicing*. A genomic sequence can yield more than one splicing product.**
- **Transcription can be controlled through regulatory sequences known as the *promoter sequences*.**

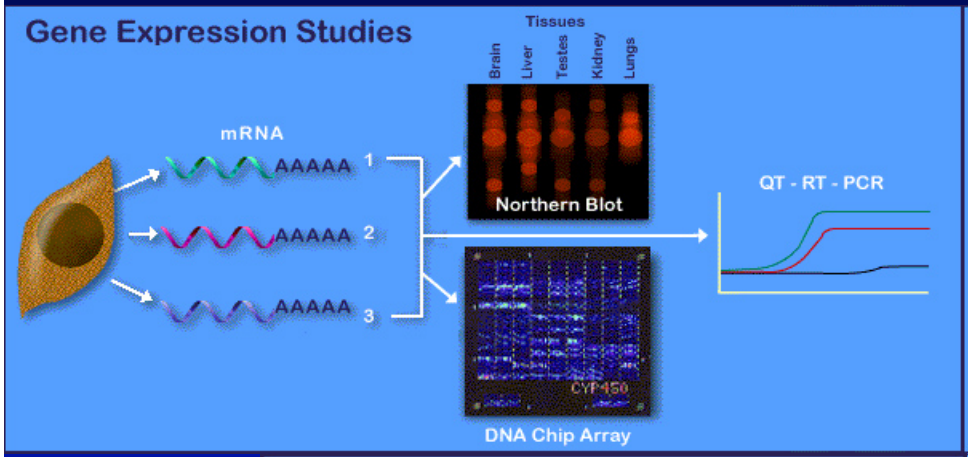


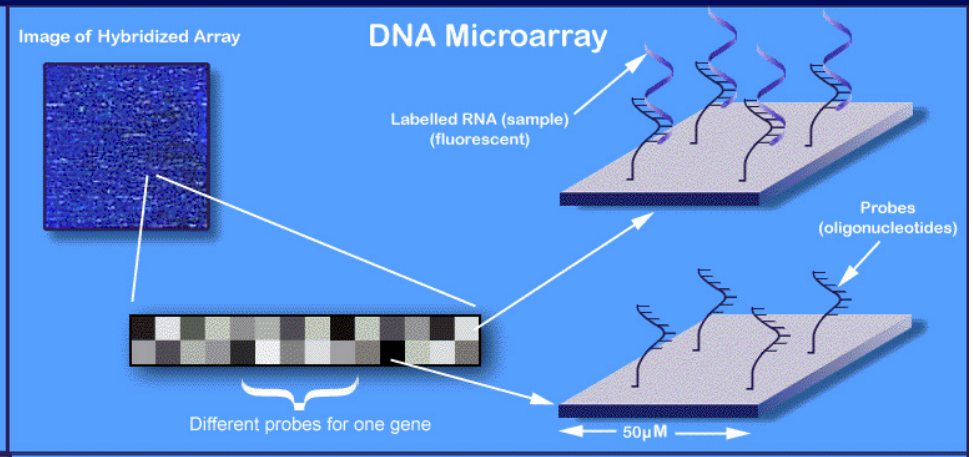
Putting It All Together: *Translation into Protein*

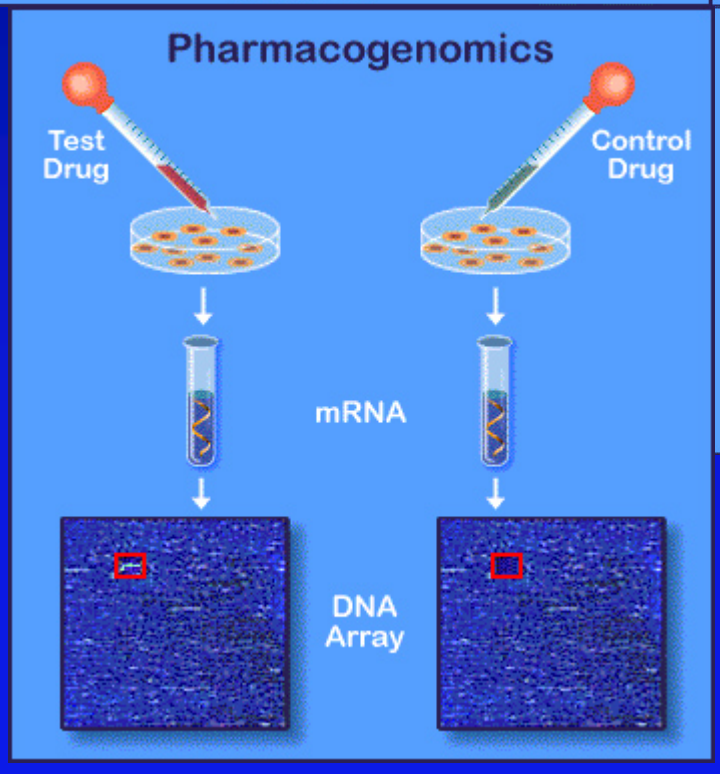
- **Translation into protein sequences is accomplished at the ribosome.**

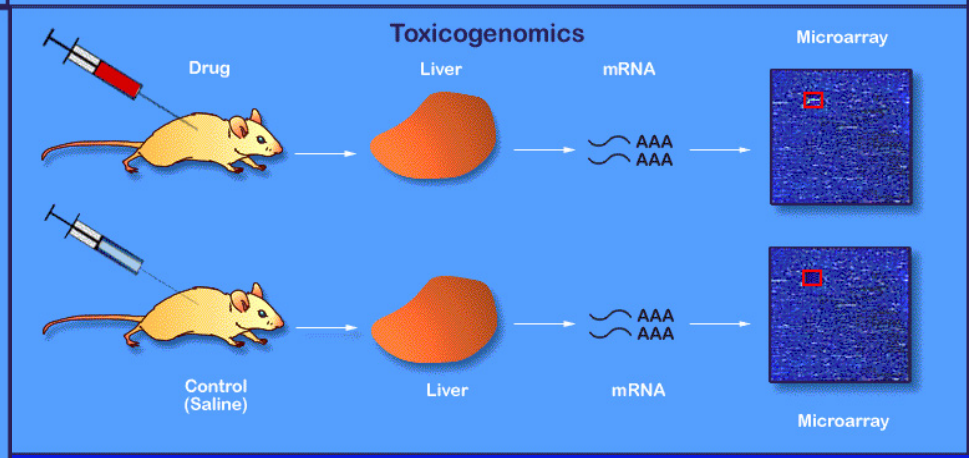


Gene Expression in Drug Development









The Genetic Code: *Translation into Protein Building Blocks*

- **The Genetic Code has some redundancy. But things can – and do – go wrong…**

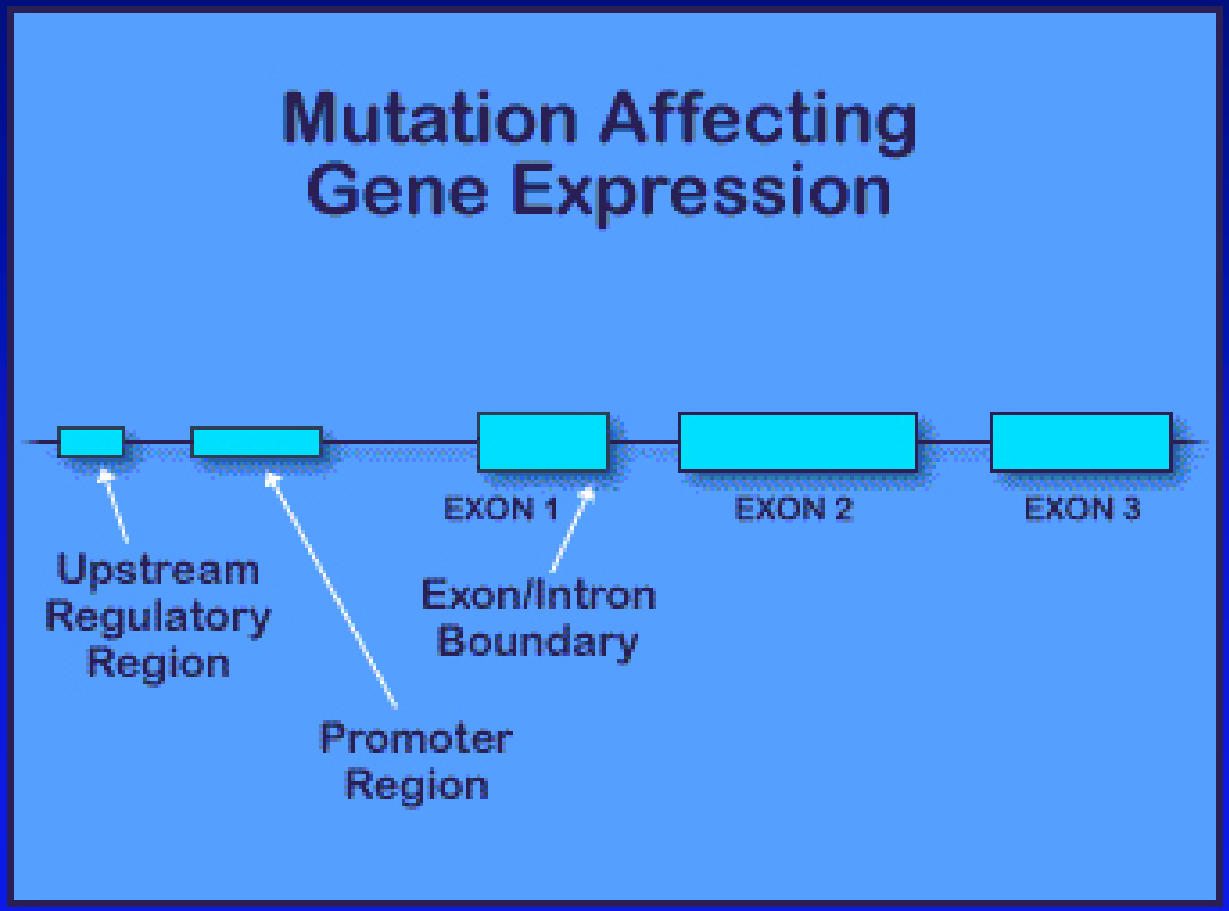
First Base	Second Base: U	Second Base: C	Second Base: A	Second Base: G	Third Base
U	UUU Phe	UCU Ser	UAU Tyr	UGU Cys	U
	UUC Phe	UCC Ser	UAC Tyr	UGC Cys	C
	UUA Leu	UCA Ser	UAA Stop	UGA Stop	A
	UUG Leu	UCG Ser	UAG Stop	UGG Trp	G
C	CUU Leu	CCU Pro	CAU His	CGU Arg	U
	CUC Leu	CCC Pro	CAC His	CGC Arg	C
	CUA Leu	CCA Pro	CAA Gln	CGA Arg	A
	CUG Leu	CCG Pro	CAG Gln	CGG Arg	G
A	AUU Ile	ACU Thr	AAU Asn	AGU Ser	U
	AUC Ile	ACC Thr	AAC Asn	AGC Ser	C
	AUA Ile	ACA Thr	AAA Lys	AGA Arg	A
	AUG Met / Start	ACG Thr	AAG Lys	AGG Arg	G
G	GUU Val	GCU Ala	CAU Asp	GGU Gly	U
	GUC Val	GCC Ala	GAC Asp	GGC Gly	C
	GUA Val	GCA Ala	GAA Glu	GGA Gly	A
	GUG Val	GCG Ala	GAG Glu	GGG Gly	G

Mutations in the Genome

- **One in every 1200 bases may be different in any two humans. This variation, which is called a *polymorphism*, is largely responsible for differences between how humans respond to drugs. Several types of mutations are associated with these variations:**

Mutations in the Genome

- **Mutations can also affect gene expression when they occur in regulatory or promoter sequences or in the exon/intron boundary.**



Clinically Important Polymorphisms

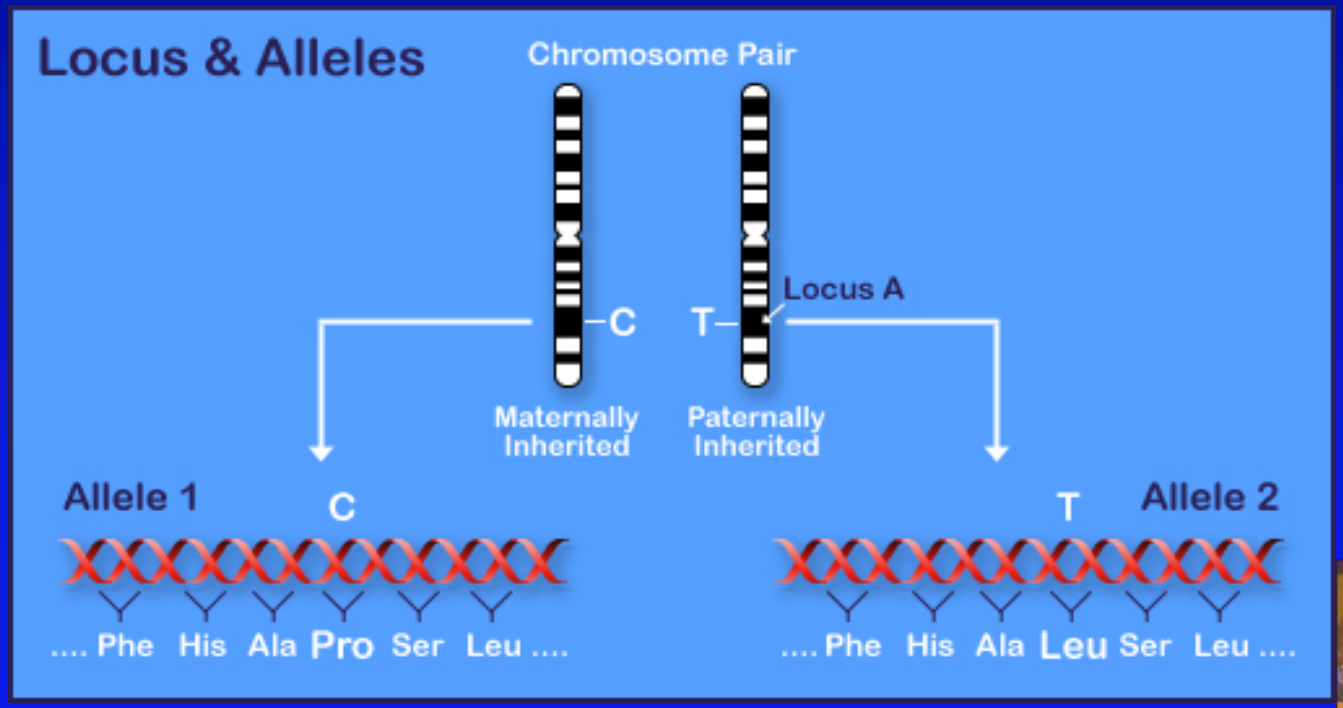
- Alter amino acid sequence of the corresponding protein (functional).
- Occur preferably within the exon or in the promoter region of the gene.
- Reasonable prevalence in the target population.

Alleles

- An allele is one of alternative forms at a genetic locus on a single chromosome. For loci in most of the genome, a human has two chromosomes, which may carry the same or two different alleles.

Genotype

- The specific genetic variants at one or more loci of an individual. Typically, the two chromosomal copies of a polymorphic site. For instance, A/A or A/G or G/G.



How to Calculate Allele and Genotype Frequencies: *The Hardy-Weinberg Equation*

$$(p^2 + 2pq + q^2) = 1, \text{ where } (p + q) = 1$$

- **Example: *cystic fibrosis***
  - **1/2000 = 0.0005 live births to caucasian parents have cystic fibrosis**
    - » **q^2 = 0.0005 (0.05% of the population are *homozygotes* for the recessive q allele)**
    - » **q = 0.02 (the *allele frequency* is 2%)**
  - **p = 1 – q = 0.98 (the *allele frequency* is 98%)**
    - » **p^2 = 0.96 (96% of the population are *homozygotes* for the dominant p allele)**
  - **2pq = 0.0392 (3.92% of the population are *heterozygotes*, or asymptomatic carriers of the cystic fibrosis gene)**

Metabolic Enzymes

Drug Metabolizing Enzymes

- **Drug Metabolism**
  - **Small number of metabolic pathways**
  - **Microsomal enzymes**
  - **Liver and small intestine**
- **Drugs**
  - **Lipophilic**
  - **Low water solubility**
  - **Metabolizing enzymes increase water solubility**
  - **Water-soluble metabolites are easier to excrete**

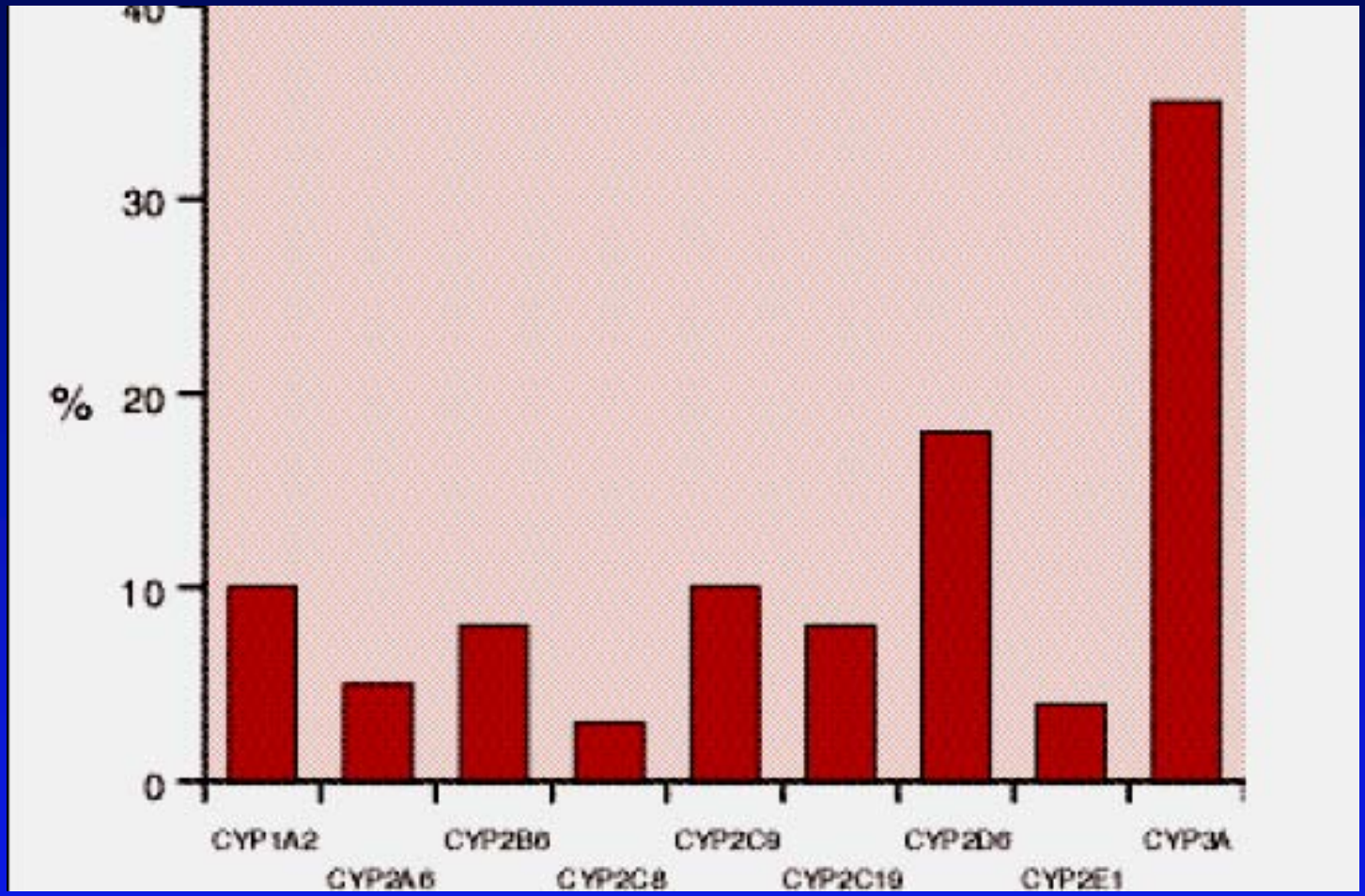
Phases of Drug Metabolism

Enzyme reaction	Metabolic reaction	Examples of enzymes
Phase I reactions		
Oxidation	Introduces hydroxyl, epoxide and ketone groups	Alcohol and aldehyde dehydrogenases
	Shortens alkyl side chains	Amine oxidases
	Converts alcohols to aldehydes and acids	Cytochromes P450
Reduction	Introduces hydrogen into ketones and nitro groups	Nitro- and azo-reductases
Hydrolysis	Breaks down esters to alcohols and acids	Esterases
Phase II reactions		
Acetylation	Adds acetate to polar sites	Acetyltransferases
Amino acid conjugation	Adds amino acids to polar sites	Glutathione transferases
Glucuronidation	Adds sugars to polar sites	Glucuronyl transferases
Methylation	Adds methyl groups to polar sites	Methyltransferases
Sulphation	Adds inorganic sulphate to polar sites	Sulphotransferases

P450 Enzymes

- 57 Different Active Genes
- 17 different families
- CYP1, CYP2 and CYP3 are primarily involved in drug metabolism.
- CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 are responsible for metabolizing most clinically important drugs
- Reactions
  - Aliphatic oxidation
  - Aromatic hydroxylation
  - Sulphoxide formation
  - N-oxidation and N-hydroxylation
  - N-/O-/S-dealkylation
  - Oxidative or reductive dehalogenation

P450s: *Who Work Most Often*



Major P450 Families and Their Isoforms

CYP family/subfamily	Isoform*	Organ localisation	Polymorphic substrates
1A	1A1	Primarily extrahepatic	Yes†
	1A2	Primarily hepatic	Yes†
	1B1	Primarily extrahepatic	Yes†
2A	2A6	Hepatic and extrahepatic	Coumarin, nicotine
	2A7	Hepatic and extrahepatic	
	2A13	Primarily extrahepatic	
2B	2B6	Primarily hepatic	Yes†
2C	2C8	Primarily hepatic	
	2C9	Primarily hepatic	Tolbutamide, warfarin
	2C18	Primarily hepatic	
	2C19	Primarily hepatic	Diazepam, propranolol
2D	2D6	Primarily hepatic	Codeine, nortriptyline, ecstasy
2E	2E1	Primarily hepatic	Yes†
2F	2F1	Primarily extrahepatic	
2G	2G1	Olfactory mucosa	
2J	2J2	Primarily extrahepatic	
3A	3A4	Primarily hepatic	Yes†
	3A5	Primarily hepatic	Yes†
	3A7	Foetal liver	
	3A43	Primarily hepatic	
4A	4A11	Primarily hepatic	
4B	4B1	Primarily extrahepatic	

*The role in drug metabolism of additional CYP isoforms in these families (eg, CYP4F2, CYP4F8) is not well described

† Multiple variants exist for these polymorphic isoforms but the consequences on the substrates they metabolise are not yet fully documented — see http://www.imm.ki.se/CYPalleles/default.htm

Nuclear Receptors in Enzyme Induction of Drug Metabolizing Enzymes

Receptor	CYPs induced	Inducers	Other enzymes induced
Aryl hydrocarbon (Ah/XRE*)	CYP1A1, CYP1A2, CYP1B1	Cigarette smoking, barbecued food and omeprazole	Glutathione transferases (GST) Glucuronlytransferases (UGT)
Constitutive androstane (CAR)	CYP2B6, CYP2A6, CYP3A†	Phenobarbital, amobarbital, secobarbital butobarbitone, heptobarbitone, glutethimide, promethazine	Epoxide hydrase, GST, UGT Cytochrome P450 reductase
Pregnane X (PXR)	CYP3A4, CYP3A5, CYP3A43†, CYPs2A†, CYPs2C†, CYP2E1†	Rifampicin, carbamazepine, dexamethasone, phenylbutazone, phenytoin, sulfadimidine, sulfinpyrazone; phenobarbital†, St John's Wort	Cytochrome P450 UGT†
Peroxisome proliferator-activated (PPAR)	CYP4A	Fibrate anti-hyperlipidaemics	Cytochrome P450 reductase
TR	CYP reductase	Thyroid hormone (T3)	
Unknown	2E1	Ethanol and chloral hydrate, isoniazid	

*XRE = xenobiotic responsive element (nuclear binding site) for the Ah receptor

†These items are suspected but not yet proven

Drug Pharmaceuticals	Associated CYP	Drug	Associated CYP	Drug	Associated CYP
Alfentanil	2B6, 3A4	Flecainide	1A2, 2D6	Nortriptyline	2D6
Alprazolam	2C19, 3A4	Fluconazole	2C9, 2C19, 3A4	Olanzapine	1A2
Amiodarone	1A2, 2C9, 3A4	Fluoxetine	2C9, 2C19, 2D6, 3A4	Omeprazole	1A2, 2C19, 3A4
Amitriptyline	2C9, 2D6, 3A4	Fluphenazine	2D6	Ondansetron	1A2, 2D6
Amprenavir	3A4	Flurbiprofen	2C9	Orphenadrine	2B6
Anastrozole	3A4	Flutamide	1A2, 3A4	Oxybutynin	1A2
Atorvastatin	2C9, 3A4	Fluvastatin	2C9	Paclitaxel	2C8, 3A4
Azatadine	3A4	Fluvoxamine	1A2, 2C19, 2D6, 3A4	Pantoprazole	2C19
Bromocriptine	3A4	Gestodene	3A4	Paracetamol	1A2, 2E1, 3A4
Budesonide	3A4	Glibenclamide	3A4	Paroxetine	1A2, 2D6
Bupropion	2B6	Granisetron	3A4	Pentazocine	2C19
Captopril	2D6	Griseofulvin	2A6	Perhexiline	2D6
Carbamazepine	1A2, 2B6, 2C8, 2C9, 3A4	Haloperidol	1A2, 2D6, 3A4	Perphenazine	2D6
Carisoprodol	2C19	Hexobarbital	1A2, 2C9, 2C19	Phenobarbital	1A2, 2A6, 2B6, 2C8, 2C9, 3A4
Carvedilol	2B6	Ibuprofen	2C9	Phenylbutazone	2C9
Chloramphenicol	2A6, 3A4	Ifosfamide	2A6, 2B6, 3A4	Phenytoin	1A2, 2C9, 2C19, 3A4
Chlordiazepoxide	1A2	Imipramine	1A2, 2C19, 2D6, 3A4	Pilocarpine	2A6
Chloroquine	2D6, 3A4	Indinavir	3A4	Piroxicam	2C9
Chlorpheniramine	2D6	Indomethin	2D6	Prednisolone	3A4
Cilansporin	3A4	Irinotecan	3A4	Prednisone	3A4
Cimetidine	2C9, 2C19, 3A4	Isoflurane	2E1	Primidone	3A4
Cinnarizine	2D6	Isoniazid	2C19, 2D6, 2E1, 2E1, 3A4	Procainamide	2B6
Ciprofloxacin	1A2, 2D6, 3A4	Isotretinoin	2C8, 3A4	Progesterone	3A4
Cisapride	3A4	Isradipine	3A4	Proguanil	2C19, 3A4
Citalopram	2C19, 2D6, 3A4	Itraconazole	3A4	Promethazine	2B6
Clarithromycin	1A2, 3A4	Ivermectin	3A4	Propafenone	2D6, 3A4
Clomipramine	2C19, 2D6	Ketoconazole	3A4	Propranolol	1A2, 2D6
Clopidogrel	2B6, 3A4	Lansoprazole	2C19, 3A4	Quetiapine	3A4
Clotrimazole	3A4, 3A4	Levonorgestrel	3A4	Quinidine	2D6, 3A4
Clozapine	1A2, 2D6, 3A4	Lignocaine	2B6, 3A4	Rabeprazole	2C19, 3A4
Cocaine	1A6, 3A4	Lindane	3A4	Retinol	2C8
Codeine	2D6, 3A4	Lopinavir	3A4	Rifabutin	2A6, 2C8, 2C9, 2C19, 3A4
Colchicine	3A4	Loratadine	3A4	Rifampicin	2A6, 2B6, 2C8, 2C9, 2C19, 3A4
Cortisol	3A4	Losartan	2C9, 3A4	Risperidone	2D6
Cyclophosphamide	2A6, 2B6, 3A4	Lovastatin	3A4	Ritonavir	1A2, 2D6, 3A4
Dapsone	3A4	Maprotiline	2D6	Ropivacaine	2B6
Debrisoquine	2D6	Mefenamic acid	2C9	Saquinavir	3A4
Desipramine	2D6	Meloxicam	3A4	Secobarbital	2A6, 2C9
Dexamethasone	2B6, 3A4	Methadone	2D6, 3A4	Sertindole	3A4
Dextromethorphan	2D6, 3A4	Methoxsalen	1A2, 2A6, 2E1	Sertraline	2D6, 3A4
Dextropropoxyphene	1A2	Methylprednisolone	3A4	Sevoflurane	2B6, 2E1
Diazepam	1A2, 2B6, 2C19, 2D6, 3A4	Metoprolol	2D6	Simvastatin	3A4
Diclofenac	2C9	Metronidazole	3A4	Sirolimus	3A4
Digitoxin	3A4	Mexiletine	1A2, 2D6	Sulfinpyrazone	3A4, 3A4
Digoxin	3A4	Mianserin	2D6	Sulfadiazine	2C9, 3A4
Diltiazem	3A4	Miconazole	2C9, 3A4	Sulfadimidine	3A4
Disulfiram	2E1	Midazolam	2B6, 3A4	Sulfamethoxazole	2C9, 3A4
Docetaxel	2C8, 3A4	Mifepristone	3A4	Tacrolimus (FK506)	3A4
Efavirenz	3A4, 3A4	Moclobemide	2C19	Tamoxifen	1A2, 2B6, 2D6, 3A4
Enalapril	3A4	Morphine	2D6	Temocicrin	2C9
Enflurane	2E1	Naproxen	1A2, 2C9	Terfenadine	2D6, 3A4
Ergotamine	3A4	Nelfinavir	3A4	Testosterone	2B6, 3A4
Erythromycin	1A2, 3A4	Nevirapine	2B6, 3A4	Theophylline	1A2, 2E1, 3A4
Estradiol	1A2, 3A4	Nicardipine	3A4	Thioridazine	2D6
Estrone	2B6	Nicotine	1A6, 2B6	Ticlopidine	2C19, 2D6
Ethinylestradiol	3A4	Nifedipine	3A4	Timolol	2D6
Etoposide	3A4	Nimodipine	3A4	Tolbutamide	2C8, 2C9
Felodipine	3A4	Nisoldipine	3A4	Torasemide	2C9
Fentanyl	3A4	Norfloxacin	1A2		

Drug	Associated CYP	Drug	Associated CYP	Drug	Associated CYP
Pharmaceuticals					
Toremifene	3A4	Valproic acid	2A6, 2B6, 2C9, 3A4	Warfarin (*R*)	1A2, 2A6, 2C19, 3A4
Tranylcypromine	2A6, 2C19	Venlafaxine	2D6	Warfarin (*S*)	2C9
Tretinoin	2C8, 3A4	Verapamil	1A2, 3A4	Zafirlukast	2C9
Trimethoprim	2C9	Vinblastine	3A4	Zuclopenthixol	2D6
Trimipramine	2D6	Vincristine	3A4		
Tropisetron	2D6	Vinorelbine	3A4		
Unlicensed products and environmental agents					
Aceclofenac 2C9	2C9	Flurythromycin	3A4	Phenacetin	1A2
Ajmalicine	2D6	Fumfylline	1A2	Phenformin	2D6
Alpidem	3A4	Germander	3A4	Ponsinomycin	3A4
Alprenol	2D6	Glutethimide	2B6	Propylajmaline	2D6
Amiflamine	2D6	Grapefruit juice	3A4	Quercetin	3A4
Aprindine	2D6	(naringenin)		Rauhimbine	2D6
Arachidonic acid	2B6	Guanoxan	2D6	Remoxipride	2D6
Benzphetamine	2C8, 3A4	Hydrocodone	2D6	Rokitamycin	3A4
Bufuralol	2D6	Isosafrole	1A2	Seratrodast	2B6, 2C9
Caffeine	1A2, 2E1	Josamycin	3A4	Sparteine	2D6
Cannabis (THC)	2C9, 3A4	Ketones 2E1	2E1	St John's Wort	3A4
Chlorproguanil	2C19	Lauric acid	2B6	Sulphamethizole	2C9
Chlorzoxazone	2E1	Lobeline	2D6	Sulphaphenazole	2C9
Coumarin 2A6	2A6	Lornoxicam	2C9	Suprofen	2C9
Cyclobenzaprine	3A4	Mephenytoin	2B6, 2C19	Tacrine	1A2
Delavirdine	2D6, 3A4	Metamphetamine	2D6	Teniposide	2C19, 3A4
Deprenyl	2D6	Methoxychlor	2B6	Terguride	3A4
Dichloralphenazone	2A6	Methoxyflurane	2B6, 2E1	Tienilic acid	2C9, 2D6
Dithiocarb	2A6, 2E1	Methoxyphenamine	2D6	Tomoxetine	2D6
Ebastine	3A4	Midecamycin	3A4	Triazolam	3A4
Ecstasy (MDMA)	2B6, 2D6	Minaprine	2D6	Trifluperidol	2D6
Encainide	2D6	Niludipine	3A4	Trimethadione	1A2, 2E1, 3A4
Ethanol	2C9, 2E1	Nitrendipine	3A4	Troglitazone	3A4
Felbamate	2C19	Norfluoxetine	2D6	Troleandomycin	3A4
Flunarizine	2D6	Oxidipine	3A4	Zonisamide	3A4

Readers are advised to check the extent of possible interactions shown here using additional sources eg, the British National Formulary (BNF)

Drugs are shown with their associated CYPs. Those CYPs in black are known to metabolise the drug, while those in blue are also inhibited and those in red also induced by the drug. Occasionally, the drug is not a substrate of the CYP it induces/inhibits eg, quinidine is *not* a substrate of CYP2D6.

Non-pharmaceutical agents may not be licensed in the UK but are shown because of their practical or theoretical importance.

Abbreviations: MDMA, methylenedioxymetamphetamine; THC, tetrahydrocannabinol.

Compiled from multiple sources including 7, 8, 11, 12, 14, 16, 17 and http://www.xenotechllc.com and http://medicine.iupui.edu/flockhart/

Pharmacogenomics of CYPs

Polymorphic P450s

- **Genetically Polymorphic P450s Associated with Changes in Drug Effects**
  - **CYP2C9**
  - **CYP2C19**
  - **CYP2D6**
  - **The correlation between pheno- and genotype for CYP3A is not fully understood.**

CYP Genotypes

- **Abnormal CYP alleles**
  - **Abolished enzyme activity**
    - » **Deleted gene or defect in allele**
  - **Reduced or altered activity**
    - » **Allele is not fully functional but some functionality remains**
      - **CYP2C9*2 and *3**
      - **CYP2D6*10 and *17**
  - **Increased activity**
- **CYP2D6: *genotype predicts phenotype***

CYP2D6 Potential Phenotypes

- ***Poor Metabolizers***
  - **lack functional enzyme.**
- ***Intermediate Metabolizers***
  - **heterozygous for one functional and one deficient allele**
  - **have two partially defective alleles that cause reduced metabolism**
- ***Extensive Metabolizers***
  - **two normal alleles**
  - **often majority of population**
  - **“normal metabolizers**
- ***Ultra-Rapid Metabolizers***
  - **duplicated or multiduplicated functional CYP2D6 genes with extremely high metabolic capacity.**

Phenotypic Effects of P450 Pharmacogenomics

Drug	Slow metabolizer phenotype	Fast metabolizer phenotype
Prodrug, needs metabolization to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Poor efficacy Possible accumulation of prodrug	Good efficacy, rapid effect
Active drug, inactivated by metabolization (example is omeprazole)	Good efficacy Accumulation of active drug can produce adverse reactions May need lower dose	Poor efficacy Need greater dose or slow release formulation

Phenotyping and Genotyping

- **Phenotyping**
  - **Dose subjects with a compound or compounds that are metabolized to a product exclusively by the enzyme systems in question.**
  - **Collect plasma or urine samples**
    - **Single time point**
    - **Over a period of time**
  - **Analyze for model compound and metabolite**
  - **Ratio of concentrations of compound and its metabolite is used to measure metabolic capacity for a specific P450.**

- **Genotyping**
  - **Collect blood (> 1 ml)**
  - **Isolate DNA from nucleated blood cells.**
  - **Amplify number of copies of DNA by the Polymerase Chain Reaction (PCR).**
  - **Genotype by sequencing or probing.**

Pharmacogenomics in Drug Development

- DNA samples taken for ADME genotyping in drug development
  - Routine if one enzyme is known as the predominant route of metabolism.
- Compounds with narrow safety margin
  - Reduce risk of developing concentration-dependent side effects when treated with standard doses
  - Exclude poor metabolizers (if the parent drug is predominantly biologically active)
  - Exclude ultra-rapid metabolizers (if metabolite is predominantly biologically active)
- Compounds with wide therapeutic window
  - Dose adjustments based on pharmacogenomic tests.
  - Increase opportunity for regulatory approval on subpopulation.
  - Less important if compound and metabolite have similar activity.
- Troubleshooting
  - Retrospective analysis in subjects with side effects or lack of therapeutic effect.
  - Prediction of ethnic variation explaining profiles in different populations.

Clinical Examples

Labeling Regulations

"If evidence is available to support the safety and effectiveness of the drug only in selected subgroups of the larger population with a disease, the labeling shall describe the evidence and identify specific tests needed for selection and monitoring of patients who need the drug."

- 21 CFR 201.57

How Does It Read?: Examples of Pharmacogenomic Information in the Drug Label

Brand Name (generic name)	Labeling section	Labeling Statement
HERCEPTIN® (trastuzumab) August 2002	INDICATIONS AND USAGE	HERCEPTIN should be used in patients whose tumors have been evaluated with an assay validated to predict HER2 protein overexpression (see PRECAUTIONS : HER2 Testing and CLINICAL STUDIES : HER2 Detection).
Purinethol (6-Mercapto-purine) July 2004	WARNINGS DOSAGE and ADMINISTRATIONS	Individuals who are homozygours for an inherited defect in the TPMT (thiopurint-S-methyltransferase) gene may be unusually sensitive to the myelosuppressive effects of mercaptopurine and prone to developing rapid bone marrow suppression following the initiation of treatment. …….. (see DOSAGE AND ADMINITRATION). Patients with inherited little or no thiopurine S-methyltransferase (TPMT) activity are at increased risk for severe PURINETHOL toxicity from conventional doses of mercaptopurine and generally require substantial dose reduction. The optimal starting dose for homozygous deficient patients has not been established (see CLINICAL PHARMACOLOGY, WARNINGS and PRECAUTIONS sections)
(thioridazine) July 2003	CONTRA-INDICATIONS	thioridazine is contraindicated ….. in patients, comprising about 7% of the normal population, who are known to have a genetic defect leading to reduced levels of activity of P450 2D6 (see WARNINGS and PRECAUTIONS).
STRATTERA (atomoxetine) March 2003	Drug-Drug Interactions Laboratory Tests	In EMs, inhibitors of CYP2D6 increase atomoxetine steady-state plasma concentrations to exposures similar to those observed in PMs. Dosage adjustment of STRATTERA in EMs may be necessary when coadministered with CYP2D6 inhibitors, e.g., paroxetine, fluoxetine, and quinidine (*see* Drug Interactions *under* PRECAUTIONS). In vitro studies suggest that coadministration of cytochrome P450 inhibitors to PMs will not increase the plasma concentrations of atomoxetine. CYP2D6 metabolism --Poor metabolizers (PMs) of CYP2D6 have a 10-fold higher AUC and a 5-fold higher peak concentration to a given dose of STRATTERA compared with extensive metabolizers (EMs). Approximately 7% of a Caucasian population are PMs. Laboratory tests are available to identify CYP2D6 PMs. The blood levels in PMs are similar to those attained by taking strong inhibitors of CYP2D6. The higher blood levels in PMs lead to a higher rate of some adverse effects of STRATTERA (*see* ADVERSE REACTIONS).

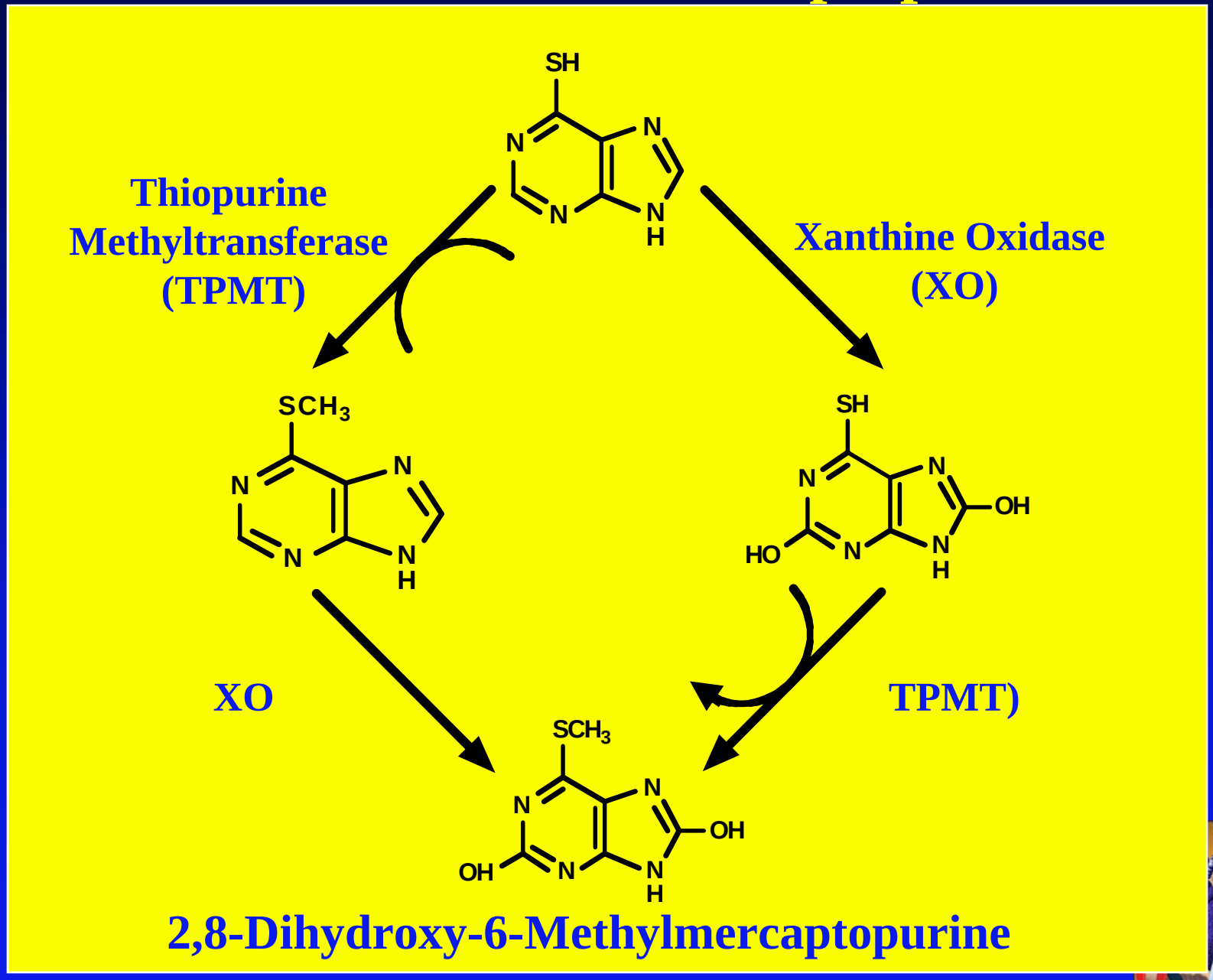
Mercaptopurines

- **Leukemia indication**
  - **Converted to nucleotides for incorporation into DNA by hypoxyxanthine phosphoribosyl transferase (HPRT).**
  - **Mercaptopurine-derived products block DNA replication and lead to tumor cell death.**
- **Mercaptopurine metabolism**
  - **Thiopurine methyltransferase (TPMT) converts mercaptopurine into an inactive metabolite called methylmercaptopurine.**
    - **90% homozygous for wild type allele and metabolize product normally**
      - **Toxicity is low, but relapse is high.**
    - **Some are poor metabolizers**
      - **Toxicity is high.**
    - **0.3% are homozygous for these variants**
    - **High risk of myelosuppression and secondary tumors.**
- ***Label:* "Recommendation to use pharmacogenetic testing to guide dosing".**

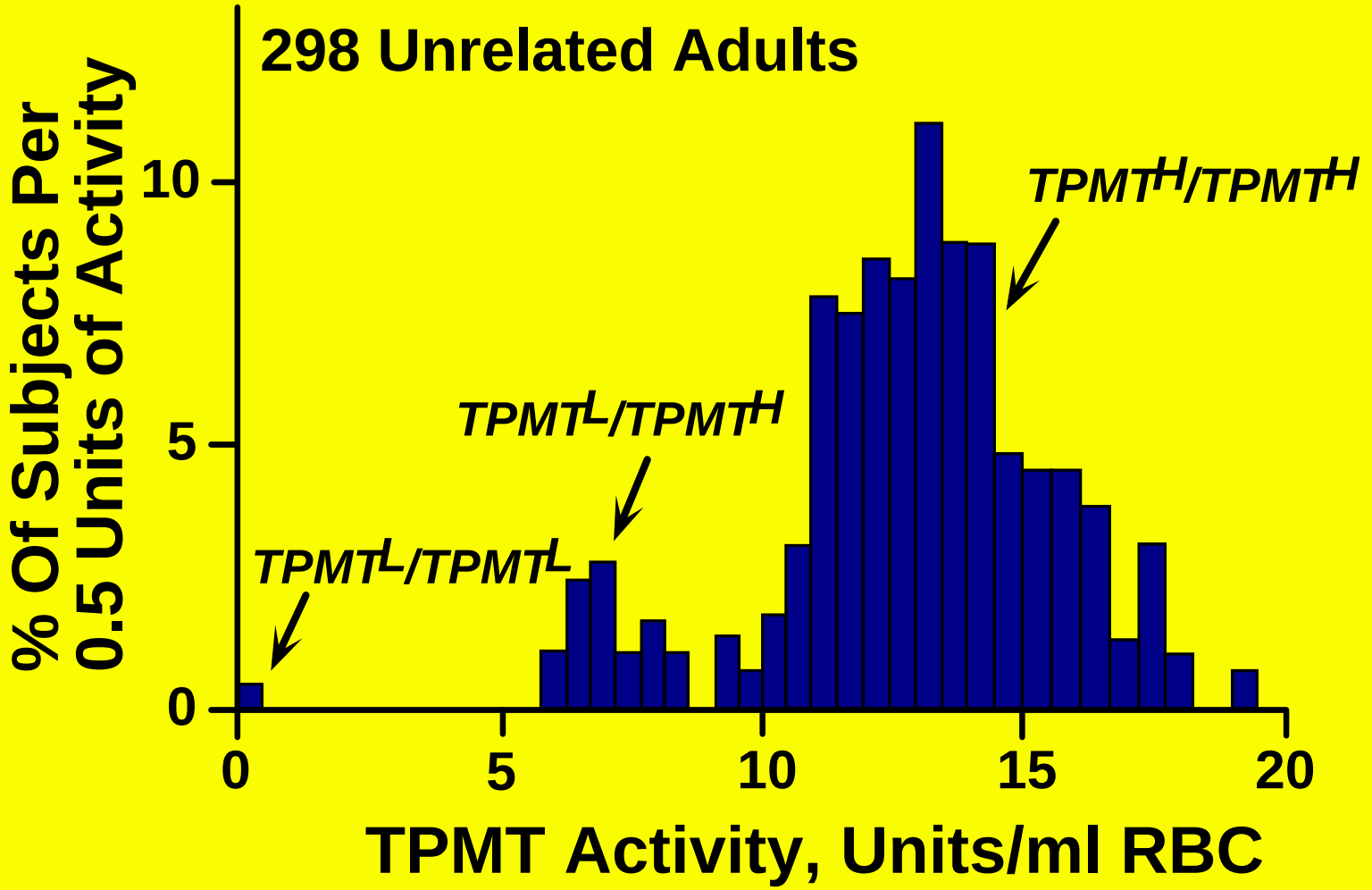
2005 FDA Pharmacogenomic Guidance *Valid Biomarkers*

- **Cytochrome P450 2D6 (CYP2D6)**
- **Thiopurine S-methyltransferase (TPMT)**

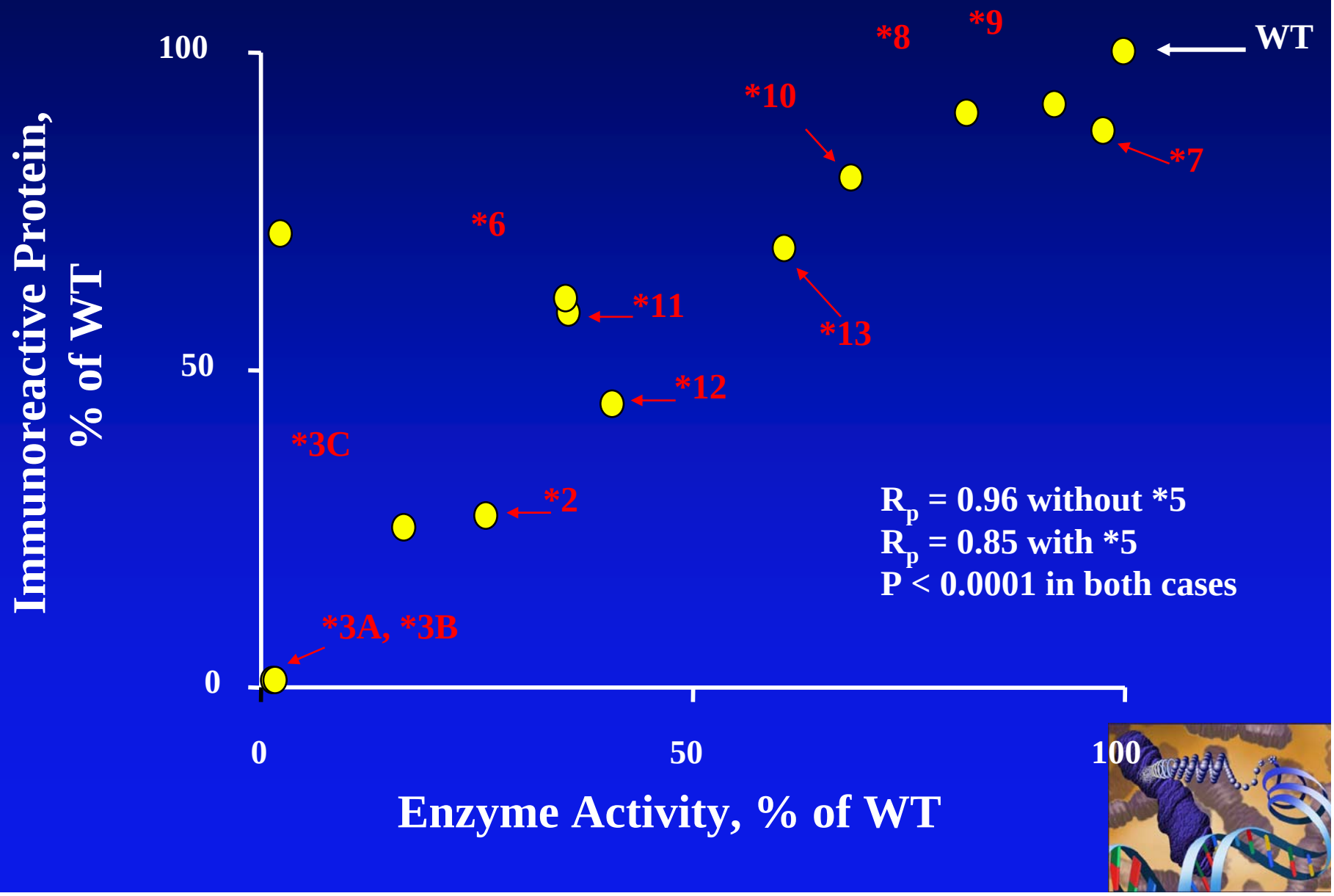
Metabolism of 6-Mercaptopurine



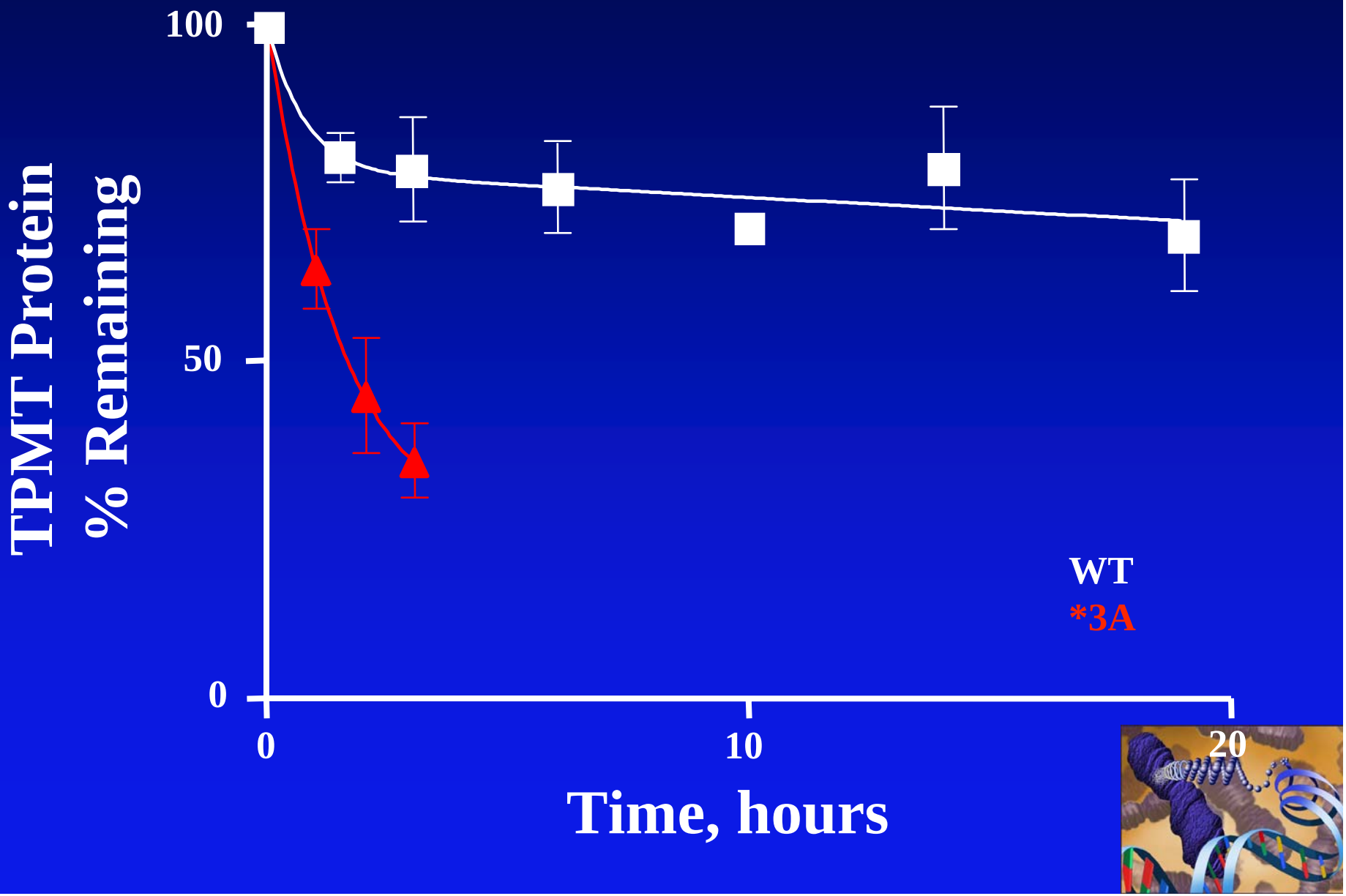
Human RBC TPMT



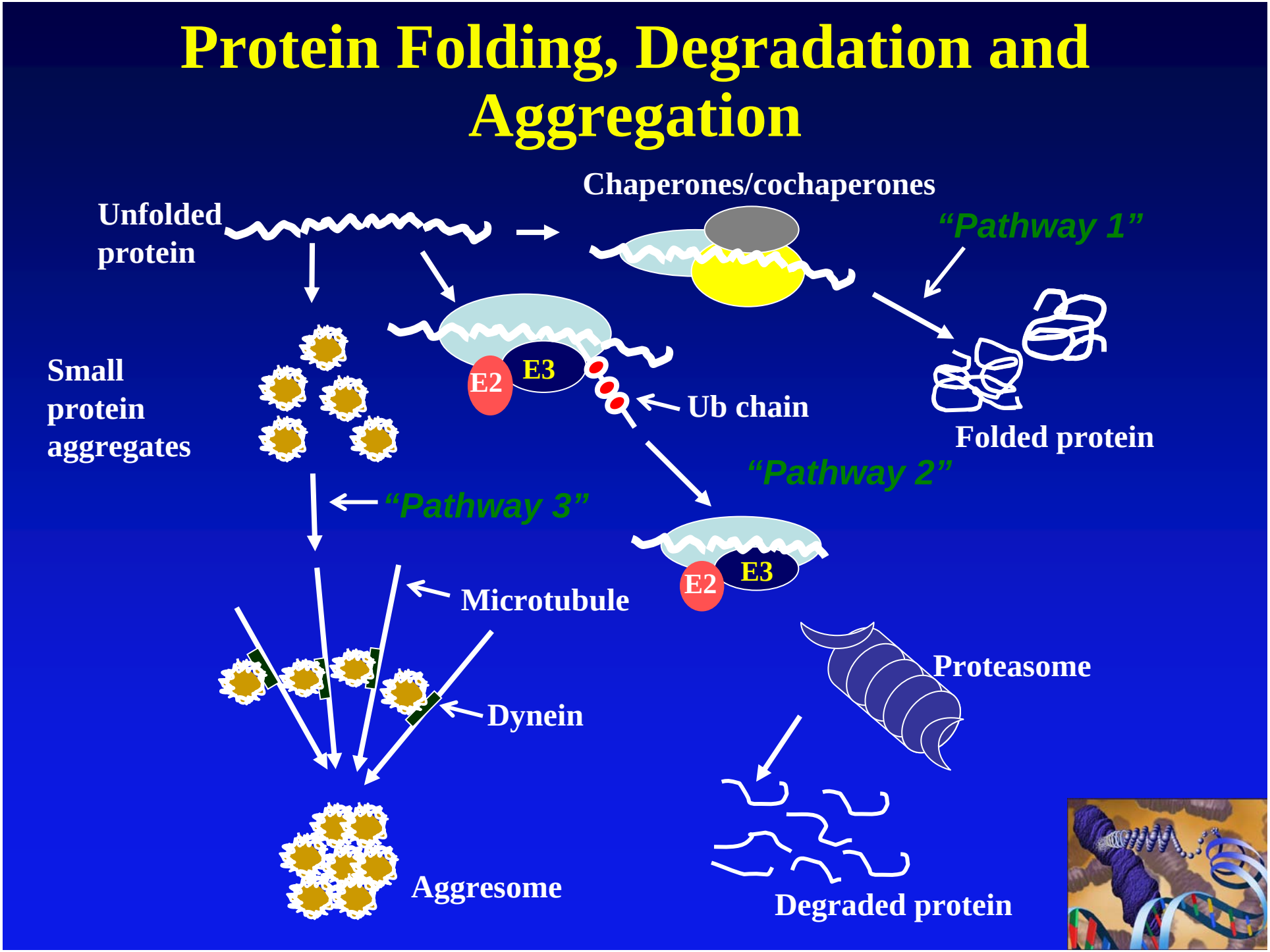
Human Recombinant TPMT Allozymes



Human TPMT Protein Degradation



Protein Folding, Degradation and Aggregation



TPMT Genetic Polymorphism Clinical Consequences

- Low TPMT
  - –Increased thiopurine toxicity
  - –Increased risk for secondary neoplasm
- High TPMT
  - –Decreased therapeutic effect

6-Mercaptopurine and TPMT Polymorphism

- ***Labeling*** **– absence of PGx information in label discussed at CPSC and Pediatric Oncology Subcommittee in 4/03 and 7/03**

- ***New labeling*** **- revised by sponsors in consultation with FDA- includes data on increased risk of severe myelosuppression for TPMT activity-deficient genotypes**

- **Informs clinicians about option of using TPMT testing to guide treatment with 6MP**

General Process for Updating Labels with PGx Information

- Develop the appropriate questions
- Capture the relevant evidence
- Abstract and summarize the evidence
- Evaluate the quality of studies
- Assess the overall strength of evidence
- Consider other factors in relabeling decision
- Determine specific language for label

Warfarin

- **Optimal use hampered by 10-fold interpatient variability in doses required for therapeutic response.**
- **CYP2C9 allelic variants are associated with impaired elimination of warfarin and exaggerated anticoagulatory responses to the drug.**
  - **CYP2C9*1 is the wild type**
  - **Other variants include CYP2C9*2, CYP2C9*3, CYP2C9*4 and CYP2C9*5**
    - » **Allelic frequencies depend on ethnicity**
- **185 patients genotyped for CYP2C9 and treated with warfarin**
  - **Variant groups required more time to achieve stable dosing with a median difference of 95 days.**
  - **Gene-dose effect relationship suggested when comparing the *1/*1, *1/*2, and *1/*3 genotypes, with corresponding mean maintenance doses of 5.63, 4.88, and 3.32 mg.**
  - ***Conclusion:* CYP2C9*2 and CYP2C9*3 polymorphisms are associated with increased risk of over coagulation and bleeding events.**
  - **In general, subjects who are heterozygous for these two alleles require a 60 to 75% lower dose of warfarin compared to homozygous wild-type patients.**

What would you do?