Principles of Pharmacogenetics

B.Sc. Pharmacology & Translational Medical Science, yr 2

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Learning objectives

- Context, definitions and history
- The molecular genetic basis
- Genetic Polymorphisms
- Quantitative genetic analysis
- Hardy-Weinberg Law
- Few examples

Context, definitions and history

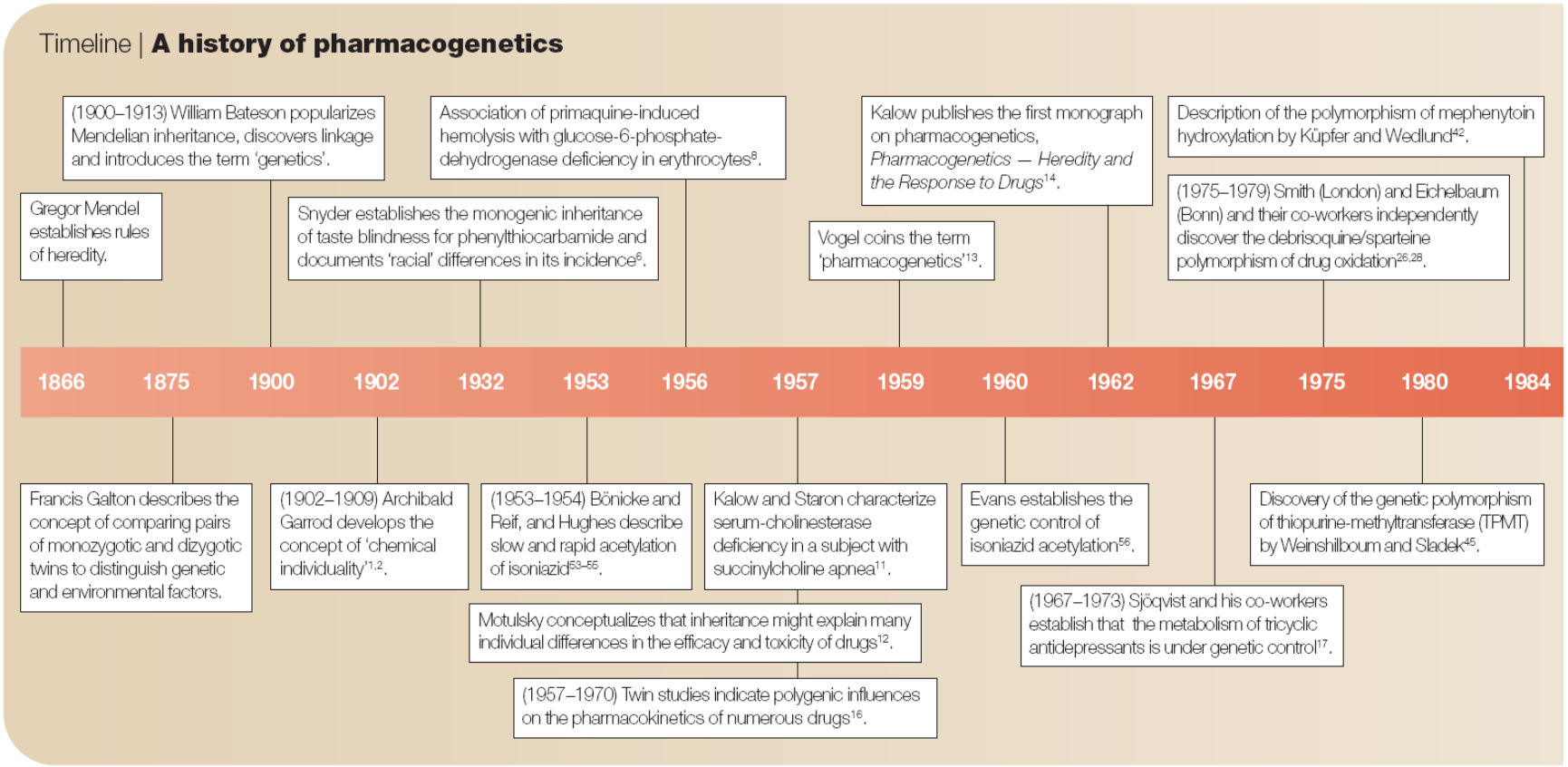
Pharmacogenetics

- Relatively new field of study within the realm of pharmacology
- Patients can respond differently to a given therapeutic agent even if they have the same illness
- The same dose of a given drug in some patients causes very different plasma levels and different therapeutic response that cannot be explained by weight, age or gender

Sir Archibald E. Garrod 1858-1936
Coined the term "Chemical Individuality"

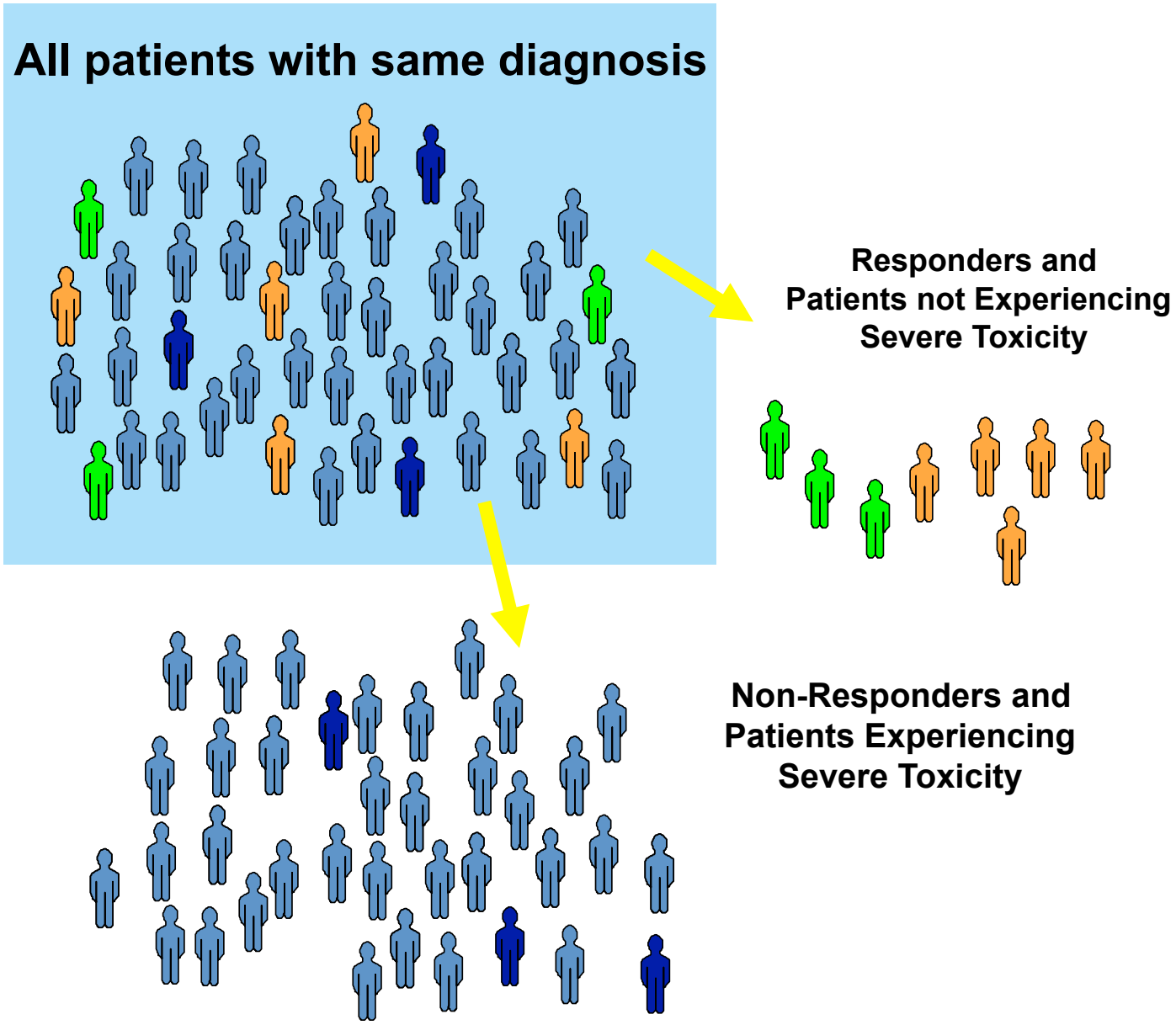
"Even against chemical poisons taken by mouth, or by other channels, there are some means of defense. Every active drug is a poison, when taken in large enough doses; and in some subjects a dose which is innocuous to the majority of people has toxic effects, whereas others show exceptional tolerance of the same drug. Some chemical poisons are destroyed in the tissues, provided that the dose given be not too large, and others are combined up with substances to hand, and so rendered innocuous and got rid of."

History: From Genetics to Pharmacogenetics



Meyer Nature Reviews Genetics 2004

What is Pharmacogenetics?



Definitions

- PHARMACOGENETICS: "The study of genetically determined inter-individual differences in therapeutic response to drugs and susceptibility to adverse effects"

⇒Restricted to one or few genes of interest

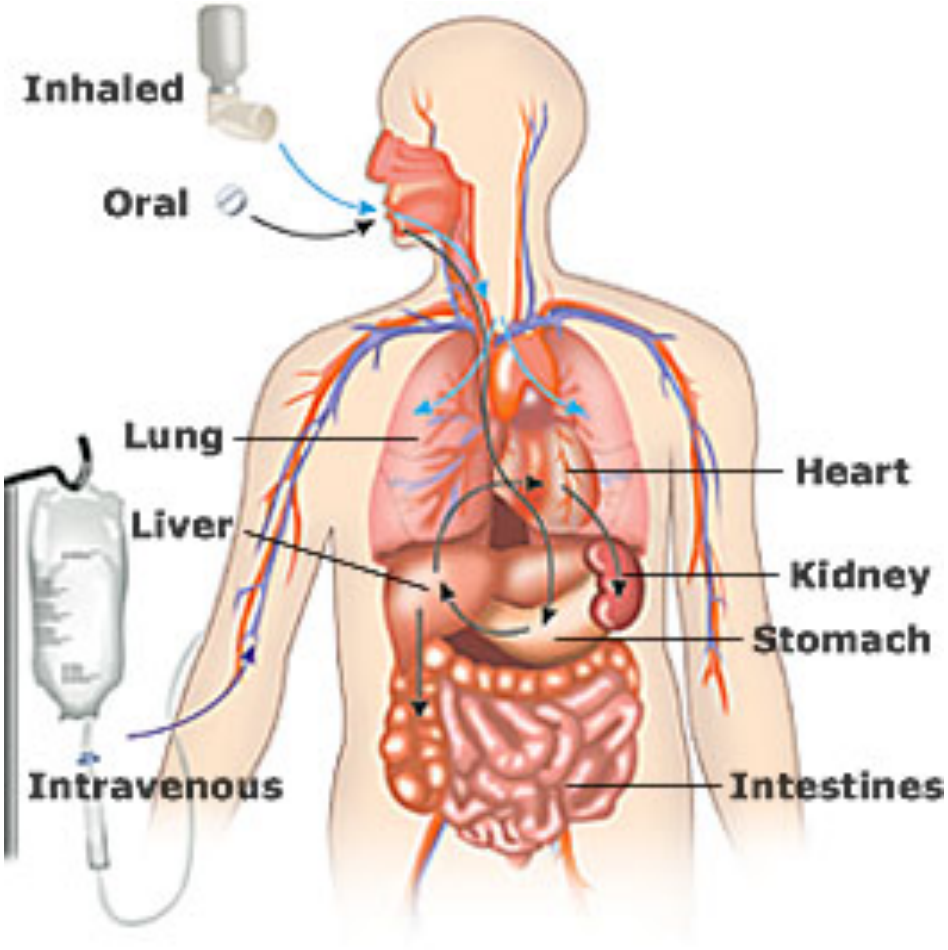
⇒Mendelian segregation

- PHARMACOGENOMICS: "Use of genome-based techniques in drug development"

⇒Not restricted to one or few genes

⇒Use of high-throughput technologies

Drug transport, targeting, and metabolism



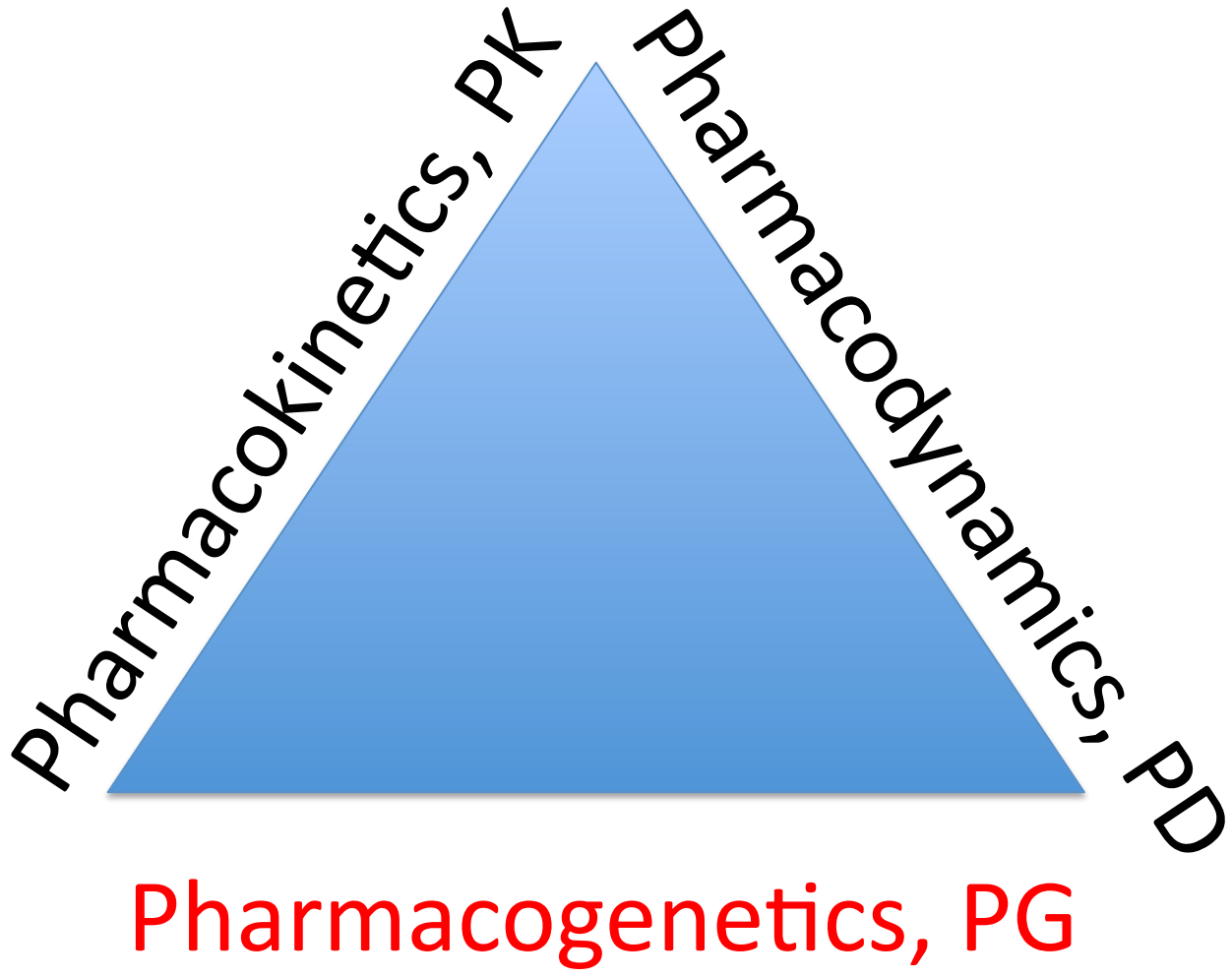
Genetic variation in drug response

The differences in the response to a given drug can be due to two major pharmacological factors that can vary with genetic influence:

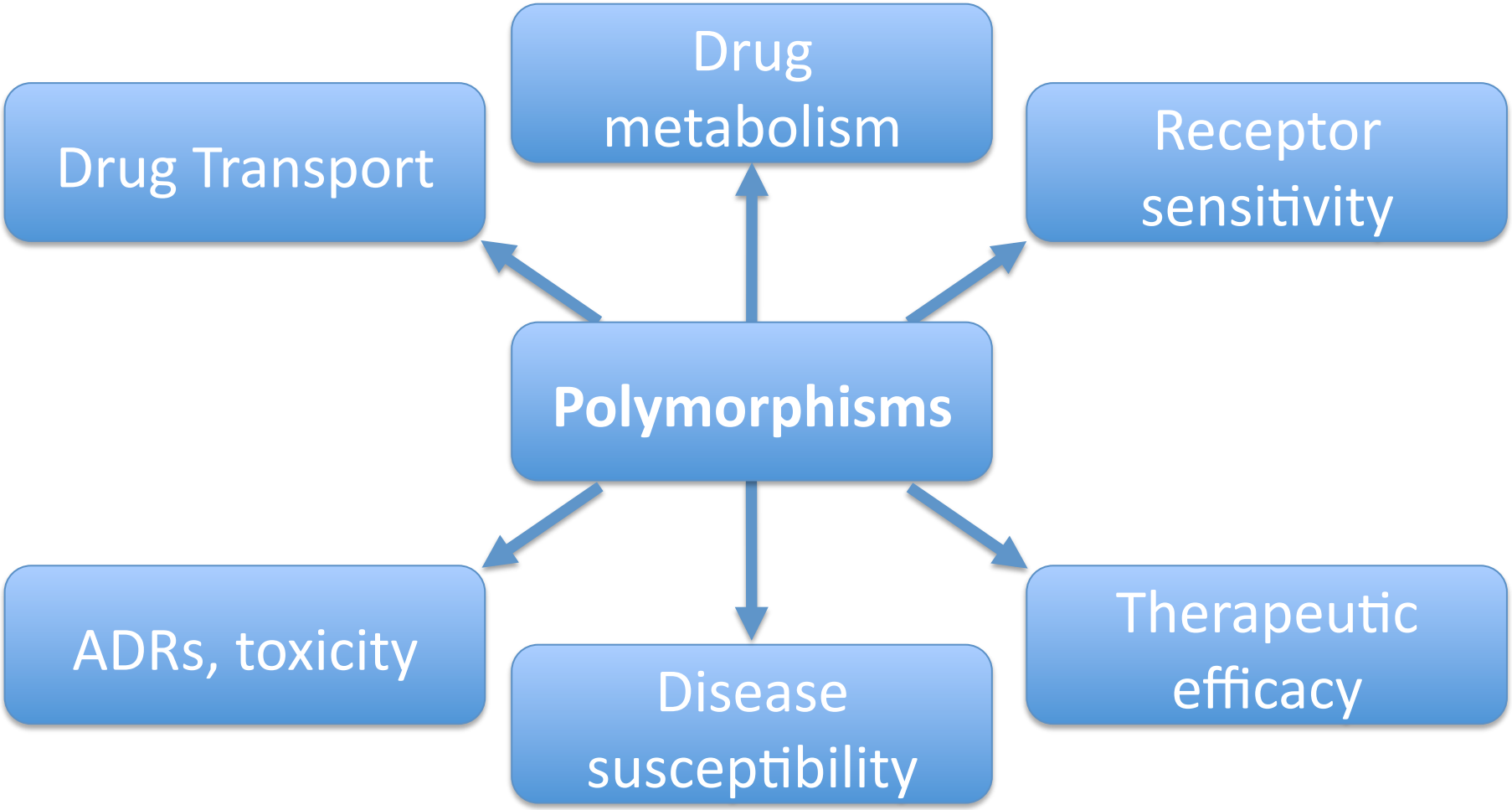
- **Pharmacokinetics - "the study of what the body does to a drug"**
  - The process by which a drug is absorbed, distributed, metabolized, and eliminated by the body
  - Genetically-based differences in the processes influencing bioavailability: absorption, distribution, metabolism, elimination

- **Pharmacodynamics: "the study of what a drug does to the body"**
  - How cells, organs or tissues respond to an equal stimulation
  - Genetically-based differences in the targets at which the drug acts
  - Receptors, enzymes, ion channels, etc

Pharmacology paradigm



Consequences of polymorphisms



Pharmacogenetics – one example

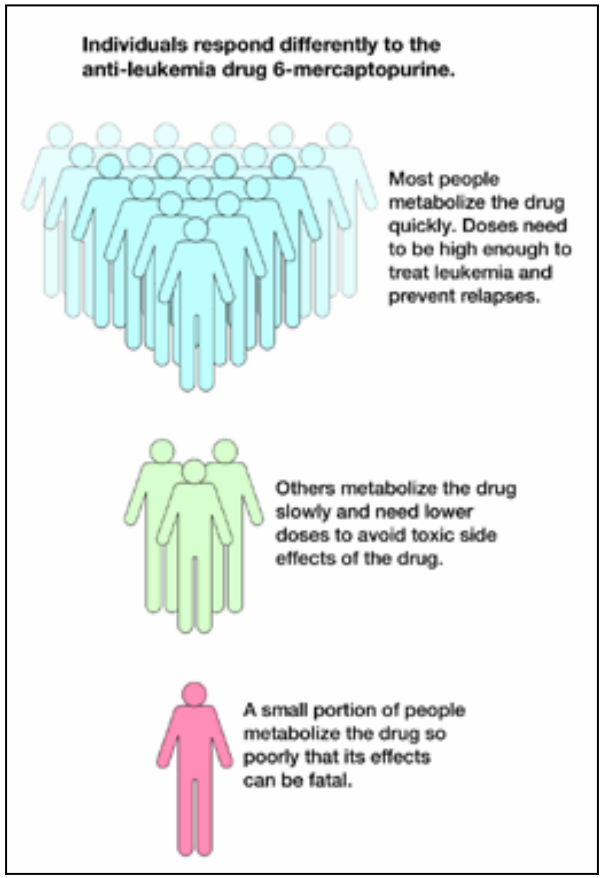
- Well known that the clinical response to a drug varies between individuals

1) In Europe ~10 % of population are deficient in cytochrome P450 enzymes responsible for metabolism of about 20 % of drugs on the market

⇒ Receive too high plasma concentrations at ordinary doses and increased susceptibility for adverse drug reactions

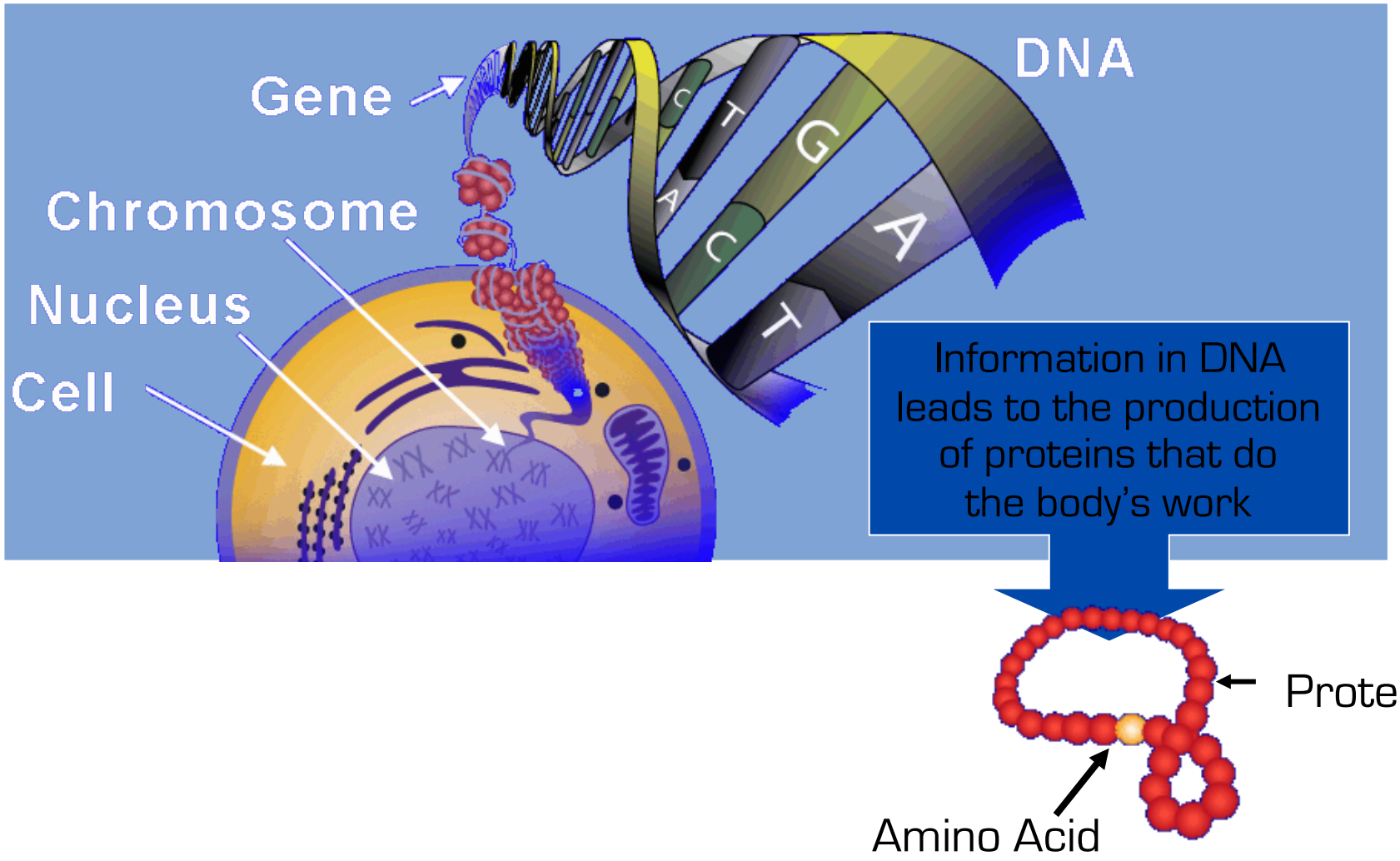
2) Ultra-rapid metabolisers (UMs) identified in 1992, where subjects have duplicated or multi-duplicated genes encoding drug metabolising enzymes

⇒ Too rapid drug metabolism occurs and the subjects get no response at ordinary dosage



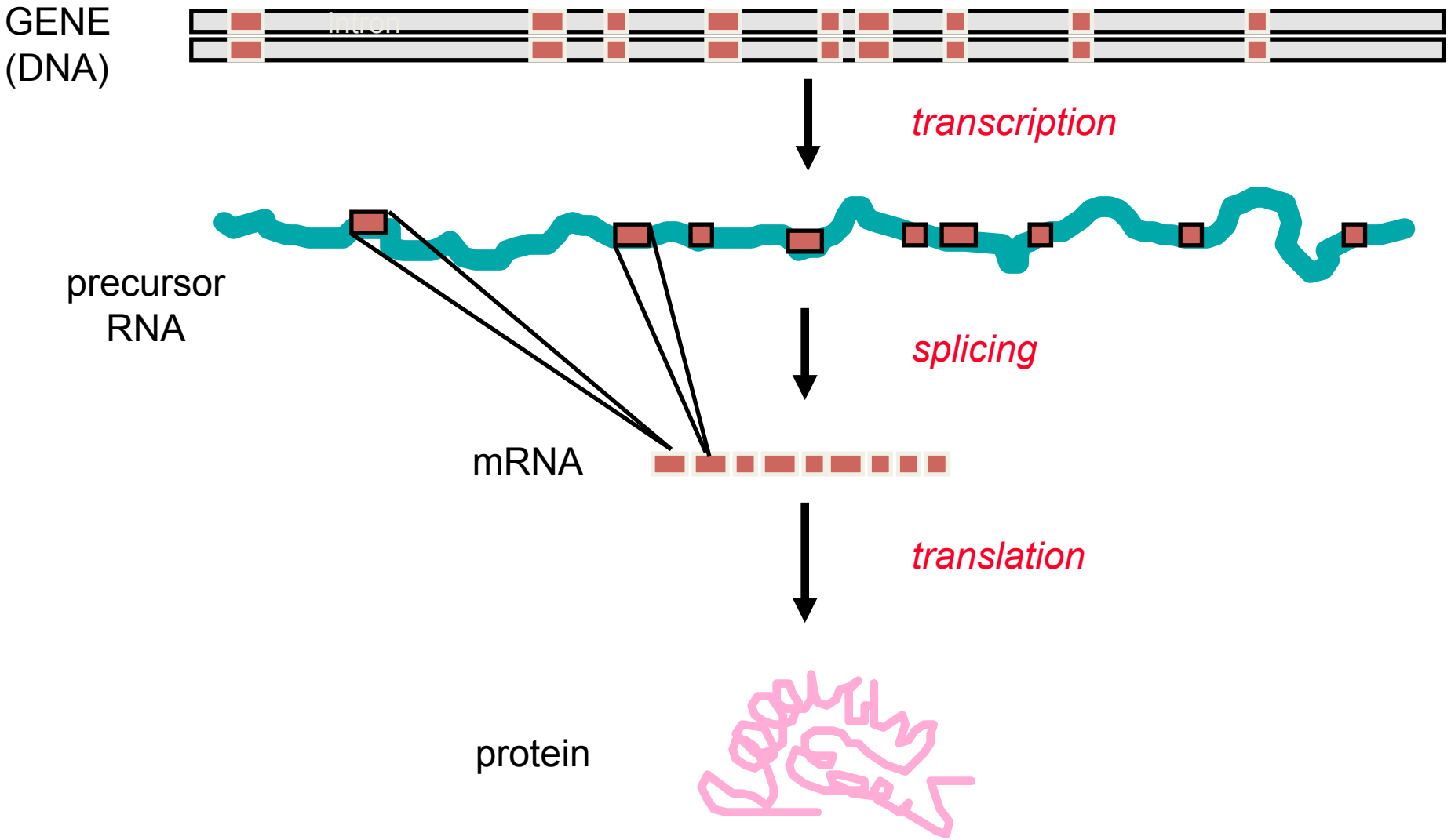
The molecular genetic basis

Basics: From DNA to proteins

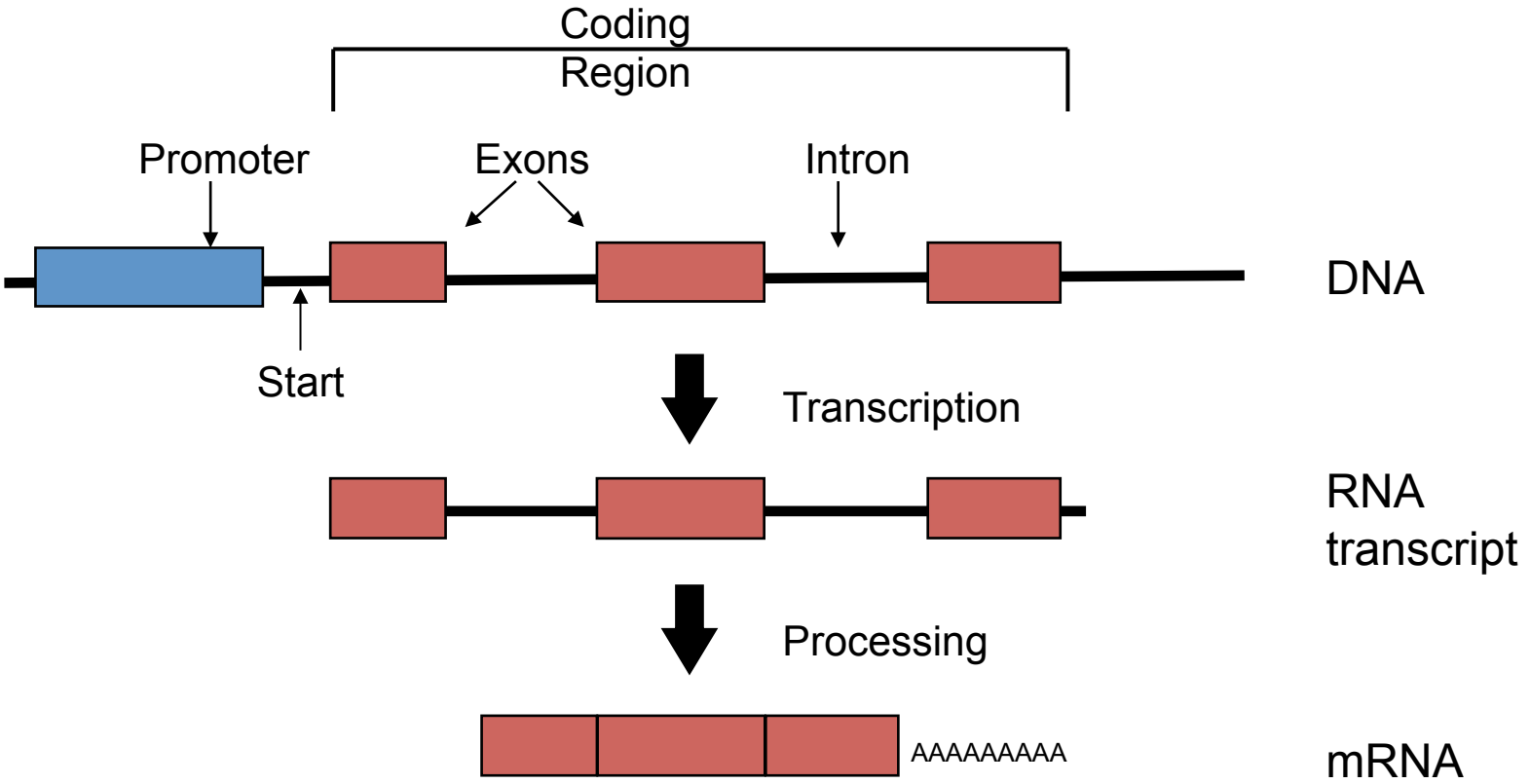


Basics: From DNA to proteins

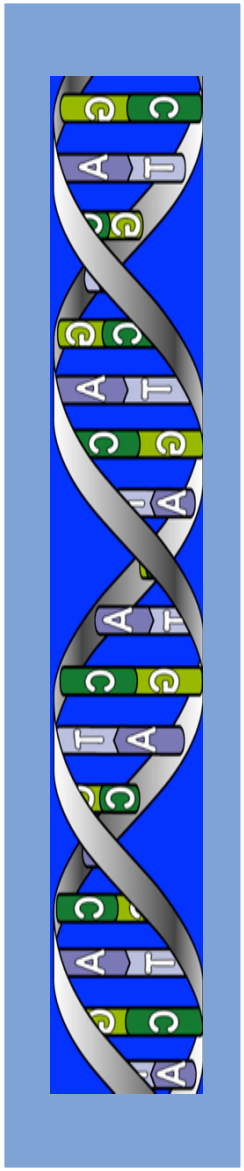
(35,000 genes in total)



Basics: Anatomy of a Gene



Basics: Genetic Variation

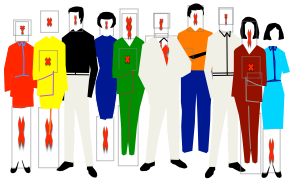


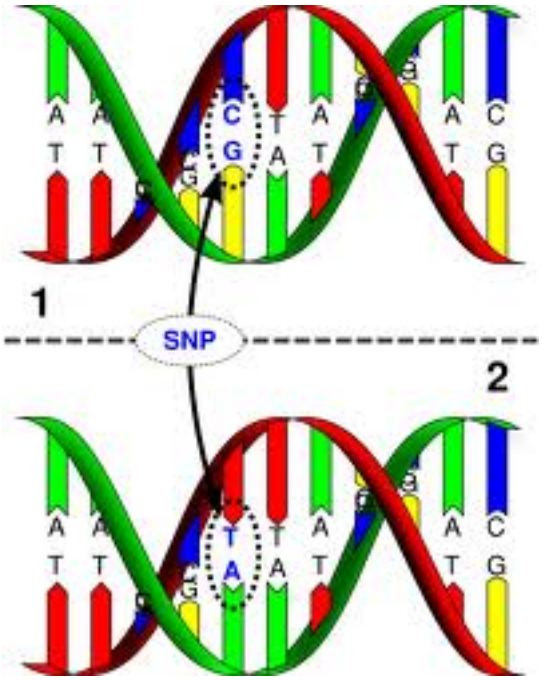
Human Genome has 3 billion DNA base-pairs

Polymorphic

...G G T A A C T G...

...G G C A A C T G...

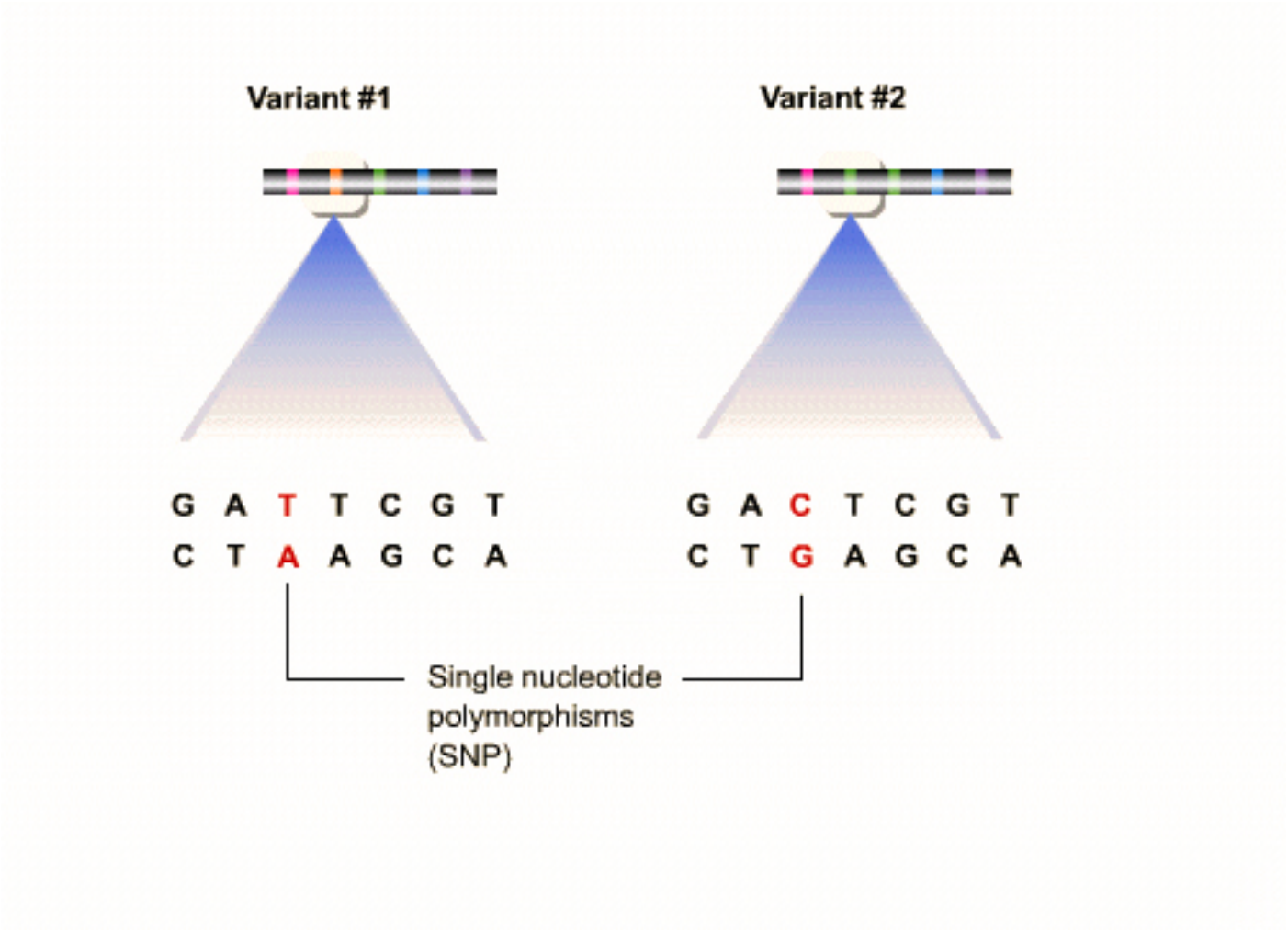




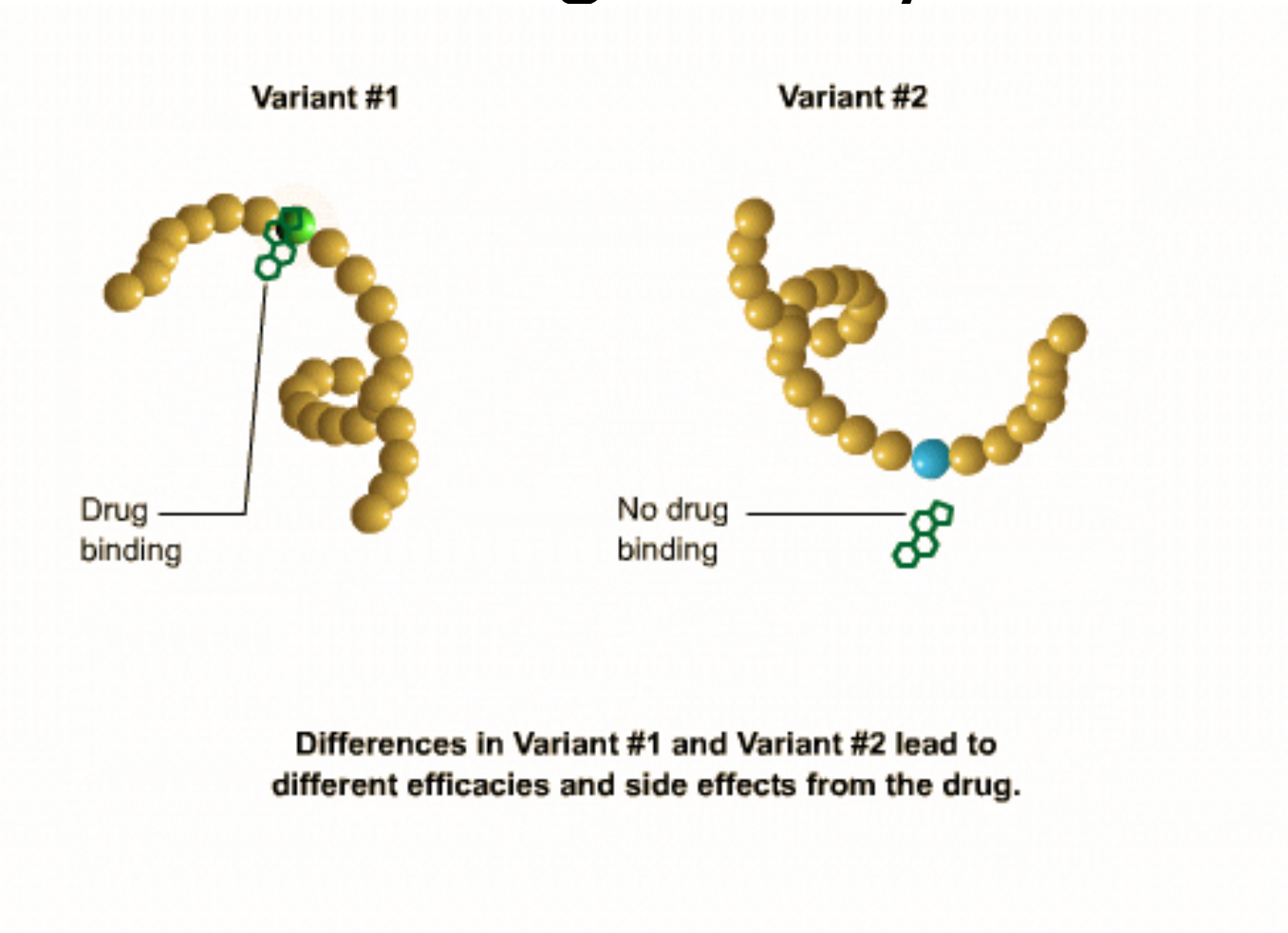
Some people have a different base at a given location:

This is a Single Nucleotide Polymorphism or SNP

Consequences of polymorphisms



Consequences of Polymorphisms on Drug Activity



Differences in Variant #1 and Variant #2 lead to different efficacies and side effects from the drug.

Genetic Polymorphisms

Polymorphisms (1): SNPs

- Genetic variation occuring with a frequency of 1% or more in the population (otherwise, mutation)

- SNP (single nucleotide polymorphism):
  - most frequent type
  - difference in a single base of the genomic sequence
  - usually 1/1000 base along the 3 billion base pairs of the human genome
  - The most common SNP is a change from cytosine to thymine (C→T) on one strand of DNA, with a change from guanine to adenine (G →A) on the other strand
  - most do not influence the structure or function of proteins
- SNP can occur
  - In exons (may alter the structure of proteins and may lead to functional consequences)
  - In introns (may influence splicing)
  - In the regulatory regions (may influence expression of the gene)

Polymorphism (2): other types

- Insertion/deletion polymorphism: insertion or deletion of a few nucleotides
- Variable number tandem repeats: variation in the number of times a sequence of several hundred base pairs is repeated
- Simple tandem repeats (microsatellites): 2-4 nucleotides repeated a variable number of times

The quantitative genetic basis

Key Concepts and Terms

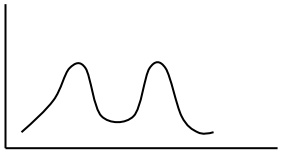
Genotype: gene structure encoding for the given characteristics

Phenotype: the manifestation of the genotype, which can be observed and can be influenced by other factors:
Other gene products
Environment
Acquired characteristics

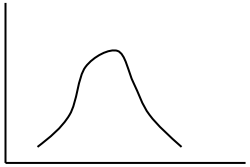
Determinism of a trait, or phenotype can be:
Monogenic: due to allelic variation at a single gene
Polygenic: due to variations at two or more genes
Polymorphic: frequently occurring monogenic variants at a frequency >1%

How is this determined? (1)

- Determination of genotype: PCR
- Determination of phenotype:
  - determination of metabolic rate (e.g. level of original drug/ metabolite in urine)
  - after administration of a given dose of the drug, pharmacokinetic parameters are measured (e.g. halflife, clearance, plasma levels)
- Distribution of phenotypes in the population:
  - Multimodal (usually bi- or trimodal) distribution indicates determination by a single gene having polymorphic variants



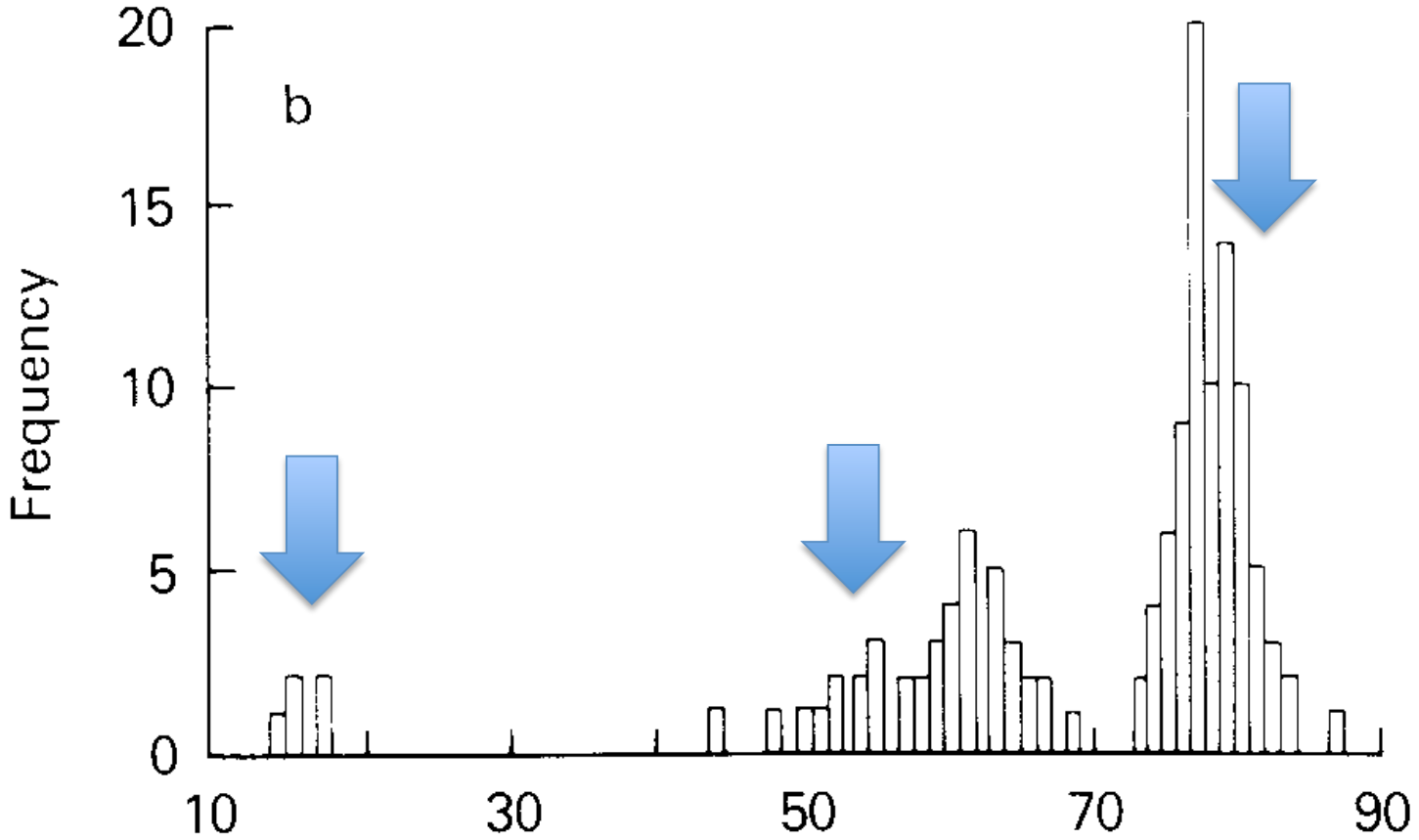
  - Unimodal distribution indicates polygenic multifactorial inharitance, or monogenic inheritance but no polymorphism



How is this determined? (2)

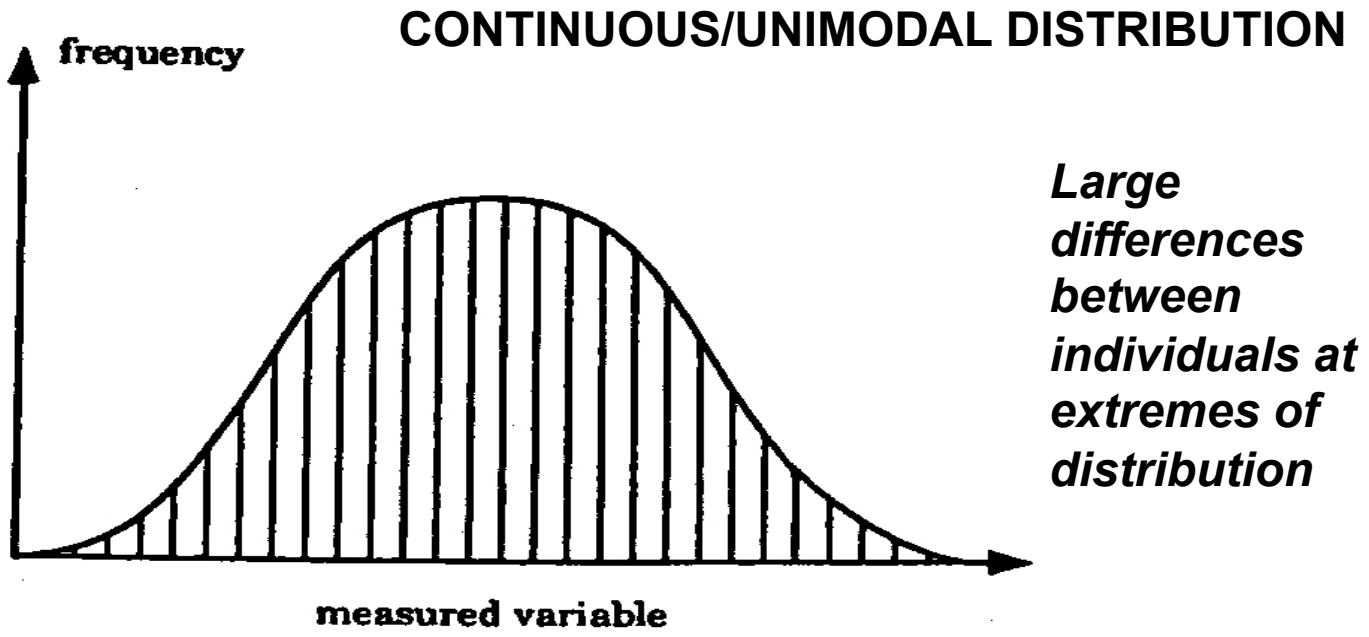
- The function is usually bi- or trimodal indicating two or three phenotypes
  - Enhanced/extensive metaboliser:
    - intensive metabolisation, resulting in low plasma concentration of the drug
    - usually heteozygote or homozygote dominant
  - Intermediate metaboliser
  - Poor metaboliser or non-metaboliser:
    - Slow or no metabolisation of the drug resulting in high plasma concentration for an extended time
    - Usually homozygote recessive

Polymorphic Distribution



Genetic control

Polygenic control



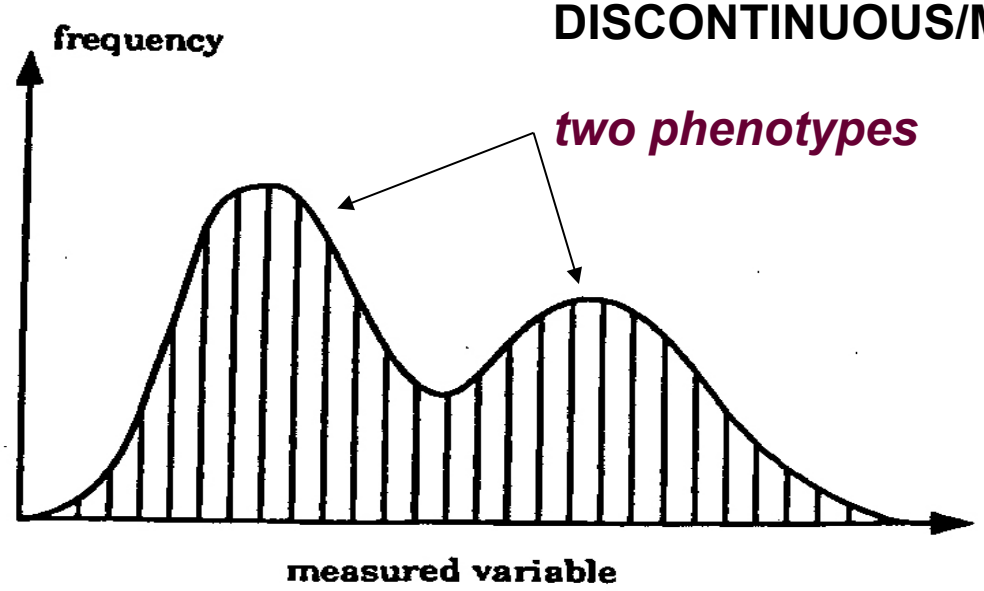
Several genes act together – not possible to recognise or discern the influences of single genes

⇒ Salicylate conjugation with glycine or glucuronic acid

Genetic control

Monogenic control

DISCONTINUOUS/MULTIMODAL DISTRIBUTION



Low incidence
Rare trait/inborn error

High incidence (>1%)
polymorphism

- Rare trait
  Succinylcholine – hydrolysis by plasma cholinesterase
- Polymorphism
  debrisoquine – oxidation by cytochrome P450 2D6

Hardy Weinberg Law

Hardy-Weinberg law (1)

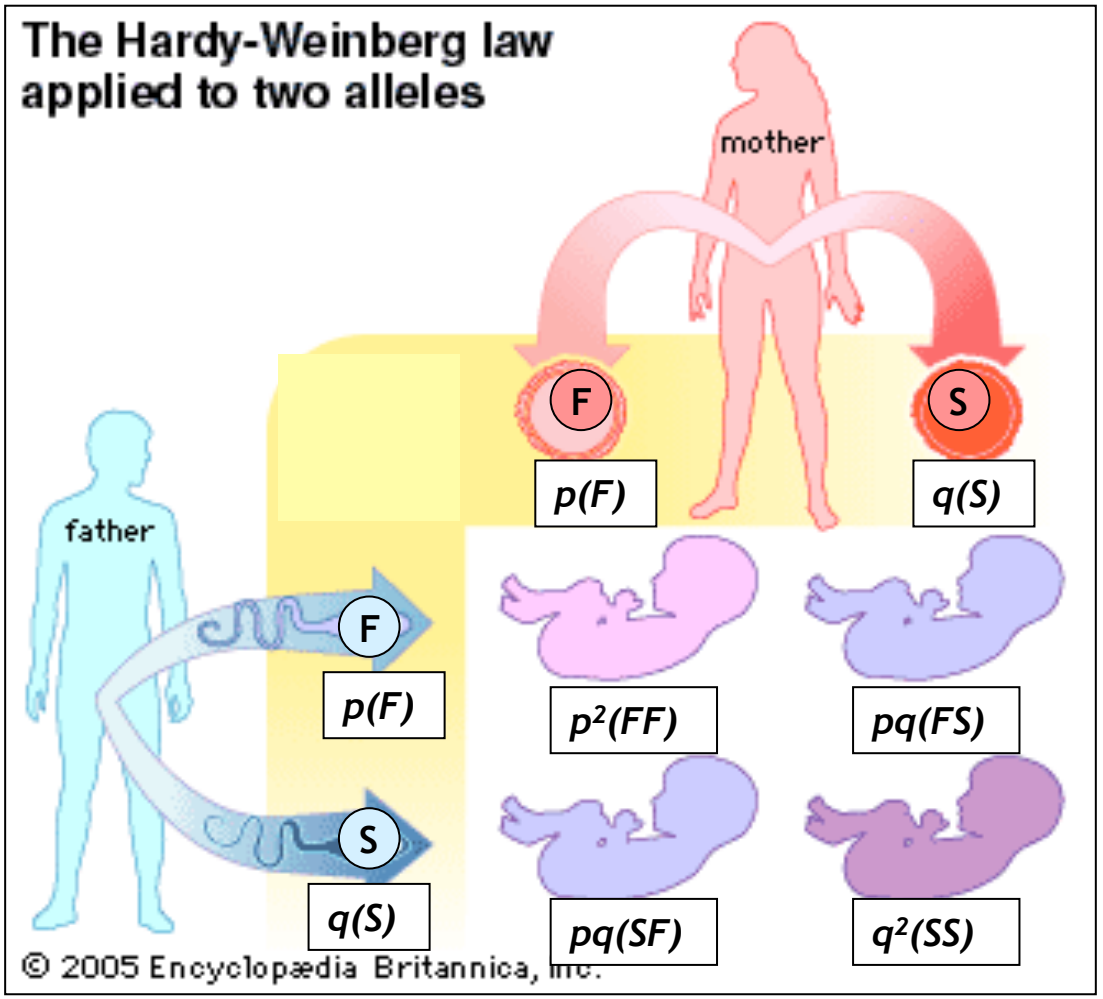
Hardy-Weinberg law (or principle) states that both **allele** and **genotype** frequencies in a population remain constant—that is, they are in equilibrium—from generation to generation unless specific disturbing influences are introduced.

Phenotype = observed FAST or SLOW metabolizer

Genotype = genetic make-up: FF, SS, FS or SF

Allele = individual copy of the gene F, or S

Hardy-Weinberg Law (2)



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

Hardy-Weinberg Law (3)

p(F) = p = frequency of wild-type allele(s)

p(S) = q = frequency of variant allele(s)

Only 2 alleles in this case, sum of frequencies = 100%

So that p + q = 1

p^2 = frequency of wild-type genotype

q^2 = frequency of variant genotype

2pq = frequency of heterozygote genotype

Hardy-Weinberg Law (4)

Generalization of binomial expansion

2-allele case :

$$(p + q)^2 = p^2 + 2pq + q^2 = 1$$

3-allele case :

$$(p + q + r)^2 = p^2 + q^2 + r^2 + 2pq + 2pr + 2qr = 1$$

Etc…

Consequences of Hardy-Weinberg Law

Based on observation of phenotypic frequencies

Allows computing the following frequencies:

-allelic frequencies: p, q

-genotypic frequencies: p^2, q^2, 2pq

Or based on allele frequencies (p and q),

Allows computing genotype frequencies

Few examples

More Examples

Gene or Gene Product	Medication	Drug Effect Associated with Polymorphism
ACE	ACE inhibitors (e.g., enalapril) Fluvastatin	Renoprotective effects, blood-pressure reduction, reduction in left ventricular mass, endothelial function[32-40] Lipid changes (e.g., reductions in low-density lipoprotein cholesterol and apolipoprotein B); progression or regression of coronary atherosclerosis[41]
Arachidonate 5-lipoxygenase	Leukotriene inhibitors	Improvement in FEV_1[42]
β_2-Adrenergic receptor	β_2-Agonists (e.g., albuterol)	Bronchodilatation, susceptibility to agonist-induced desensitization, cardiovascular effects[43-50]
Bradykinin B2 receptor	ACE inhibitors	ACE-inhibitor–induced cough[51]
Dopamine receptors (D2, D3, D4)	Antipsychotics (e.g. haloperidol, clozapine)	Antipsychotic response (D2, D3, D4), antipsychotic-induced tardive dyskinesia (D3), antipsychotic-induced acute akathisia (D3)[52-56]
Estrogen receptor-α	Conjugated estrogens Hormone-replacement therapy	Increase in bone mineral density[57] Increase in high-density lipoprotein cholesterol[58]
Glycoprotein IIIa subunit of glycoprotein IIb/IIIa	Aspirin or glycoprotein IIb/IIIa inhibitors	Antiplatelet effect[59]
Serotonin (5-hydroxytryptamine) transporter	Antidepressants (e.g., clomipramine, fluoxetine, paroxetine)	5-Hydroxytryptamine neurotransmission, antidepressant response[60-62]

Evans and Mcleod NEJM 2003

Even more Examples

Gene or Gene Product	Disease or Response Association	Medication	Influence of Polymorphism on Drug Effect or Toxicity
Adducin	Hypertension	Diuretics	Myocardial infarction or strokes[69]
Apolipoprotein E (APOE)	Progression of atherosclerosis, ischemic cardiovascular events	Statins (e.g., simvastatin)	Enhanced survival[70,71]
Apolipoprotein E (APOE)	Alzheimer's disease	Tacrine	Clinical improvement[72]
HLA	Toxicity	Abacavir	Hypersensitivity reaction[73,74]
Cholesterol ester transfer protein (CETP)	Progression of atherosclerosis	Statins (e.g., pravastatin)	Slowing of progression of atherosclerosis by pravastatin[75]
Ion channels (HERG, KvLQT1, Mink, MiRP1)	Congenital long-QT syndrome	Erythromycin, terfenadine, cisapride, clarithromycin, quinidine	Increased risk of drug-induced torsade de pointes[76-78]
Methylguanine methyltransferase (MGMT)	Glioma	Carmustine	Response of glioma to carmustine[63]
Parkin	Parkinson's disease	Levodopa	Clinical improvement and levodopa-induced dyskinesias[79]
Prothrombin and factor V	Deep-vein thrombosis and cerebral-vein thrombosis	Oral contraceptives	Increased risk of deep-vein and cerebral-vein thrombosis with oral contraceptives[80]
Stromelysin-1	Atherosclerosis progression	Statins (e.g., pravastatin)	Reduction in cardiovascular events by pravastatin (death, myocardial infarction, stroke, angina, and others); reduction in risk of repeated angioplasty[81]

Evans and Mcleod NEJM 2003

Conclusions: Pharmacogenetics

- Rational framework for evaluation of genetic variation of
  - Drug metabolising enzymes
  - Drug transporters
  - Receptors
  - Ion channels
- Which influences the risk of adverse drug reactions or therapeutic failure
- Reduction of trial-and-error choice of medication and dose
- Personalized treatment guidelines

Next pharmacogenetics lectures

- Variability in drug metabolism (but not only)
  - Phase 1 polymorphisms (+ drug absorption, distribution and elimination)
  - Phase 2 polymorphisms (+ drug activation, receptors)
- Modern methods
  - pharmacogenomics,
  - pharmacometabonomics
- Tutorial on pharmacogenetics