Principles of Genetic Medicine: Part II

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Disclosure

- I declare no conflicts of interest, real or apparent, and no financial interests in any company, product, or service mentioned in this program, including grants, employment, gifts, stock holdings, and honoraria.

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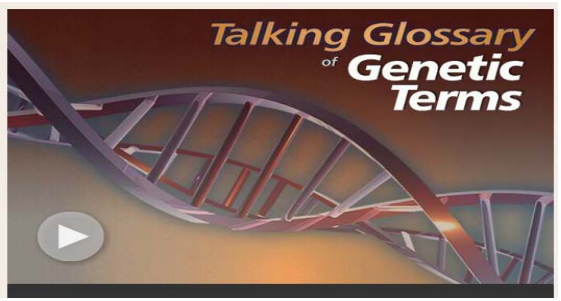
Learning Objectives

- Discuss the Types of Polymorphisms
- Define Allele, Genotype, Phenotype
- Define Linkage Disequilibrium, Haplotype, and Diplotype
- Introduce PharmGKB

Additional Resources

- Websites:
  - http://www.dnaftb.org/
  - http://genomics.energy.gov/
  - http://ghr.nlm.nih.gov/
  - https://www.genome.gov/10000464



- Genomics Glossary App:
  - http://www.genome.gov/glossary/index.cfm

- Literature:
  - Feero, Guttmacher, and Collins. Genomic Medicine – An Updated Primer. 2010. N Engl J Med. 362;21: 2001-2011.

The Human Genome

- 3.2 billion base pairs
- Diploid organisms (2 copies of each chromosome)
- Genes (DNA) → RNA → Protein
- What happens when there is a change in the DNA?

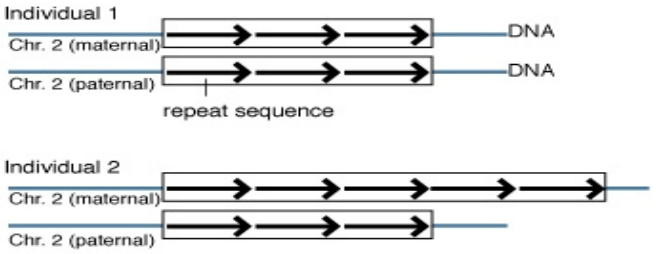
Polymorphism

- A variation in DNA sequence
- Types of polymorphisms:
  - Single Nucleotide Polymorphism (SNP)
    - pronounced ‘snip’
    - Change of a single base pair
  - Insertion/Deletion (I/D or In/Dels)
    - Presence or absence of a nucleotide(s)
  - Variable number of tandem repeats (VNTR)
    - Consecutive base pair groups that are differentially repetitive
  - Copy Number Variants (CNV)
    - When the number of copies of a particular gene vary from one individual to the next

http://www.genome.gov/Glossary/index.cfm?id=160

Examples of Genetic Polymorphisms

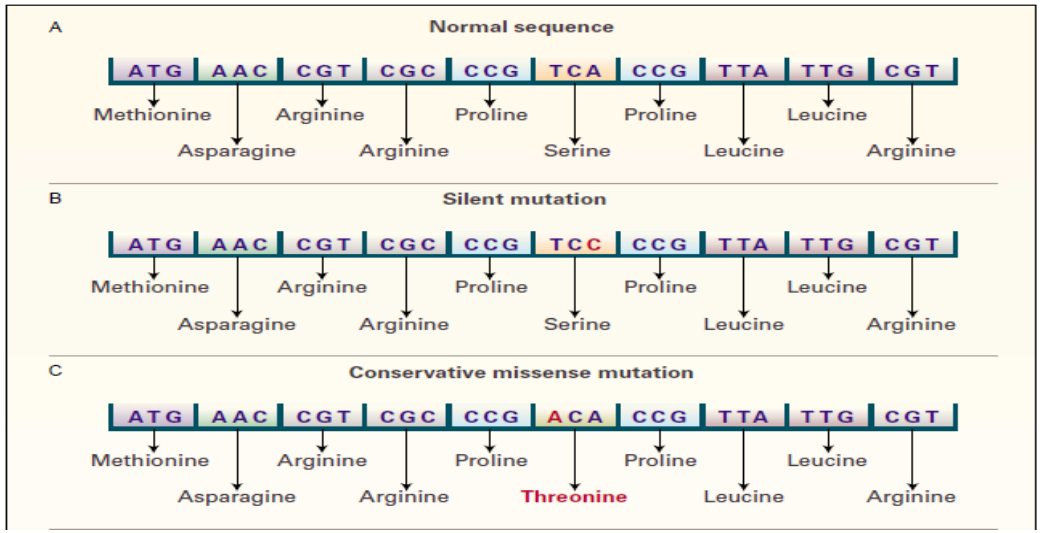
- SNP
  - Single base change
  - G A T C T G A
  - G A T T T G A
  - Occur approximately every 300 bp
- Insertion/Deletion (In/Del)
  - Insertion/Deletion of one or more base pairs
  - G A T C T G A
  - G A T C C T G A
- VNTR
  - Microsatellites
  - Minisatellites
  - Tandem repeats of short nucleotide sequences
- Copy Number Variation (CNV)



http://www.genome.gov/glossary/index.cfm?id=40

Consequences of Genetics Polymorphisms

- Polymorphisms can cause:
  - A change in the codon = A different amino acid
  - A premature stop codon
  - Different intron and exon splice junctions
  - An alteration in the stability of the mRNA
  - A change in the promoter activity
  - A change in enhancer activity
  - No consequence
  - Other consequences…



Guttmacher and Collins. N Engl J Med. 2002. 347(19): 1512-1520

Consequences of Genetic Polymorphisms in Different Regions

- Coding region:
  - Synonymous, Non-synonymous, Non-sense
    - Changes the protein

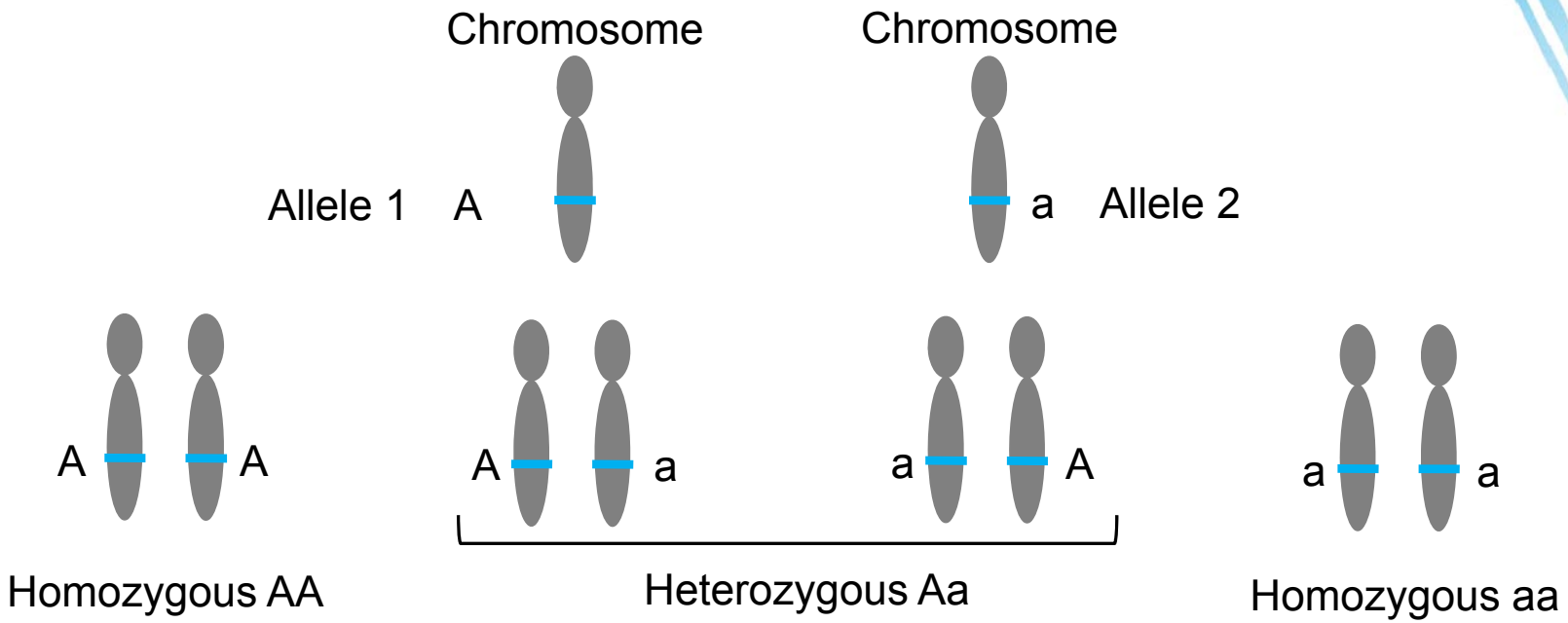
- Intronic or Untranslated (UTR) region:
  - Affect splicing, transcription factor binding
    - Changes the mRNA level and/or mRNA stability

- Promoter or Enhancer region:
  - Affect transcription factor binding, RNA polymerase binding
    - Changes the level of expression of the gene

- Intergenic region: ?

What is an Allele?

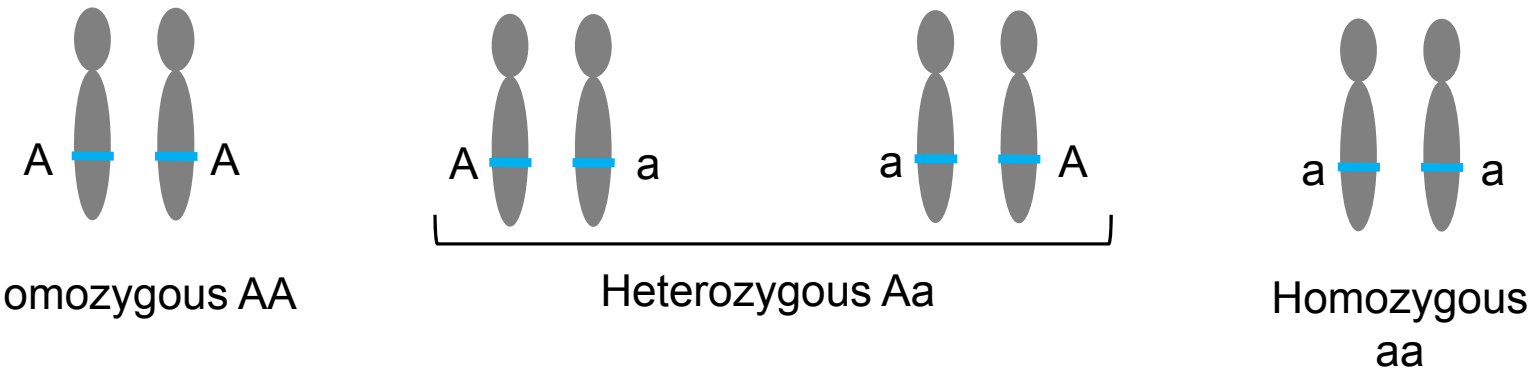
- An allele is one of two or more versions of a genetic sequence at a particular location in the genome



http://www.genome.gov/Glossary/index.cfm?id=4

What is a Genotype?

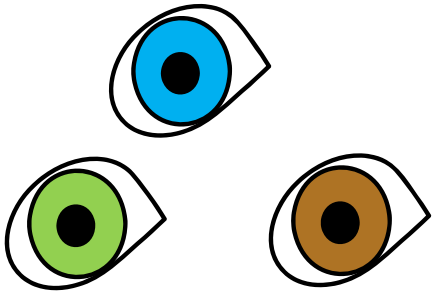
- A genotype is…
  - An individual's collection of genes
  - Two alleles inherited for a particular gene or variation



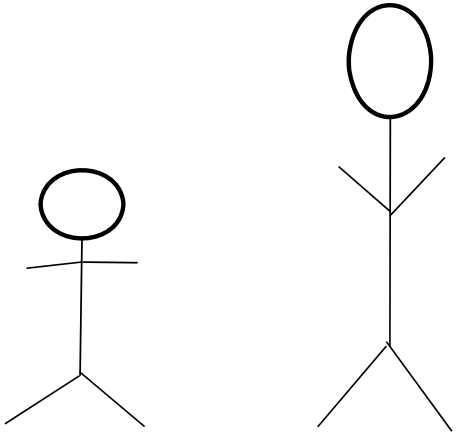
http://www.genome.gov/glossary/index.cfm?id=93

What is a Phenotype?

- A phenotype is an individual's observable traits



Eye Color



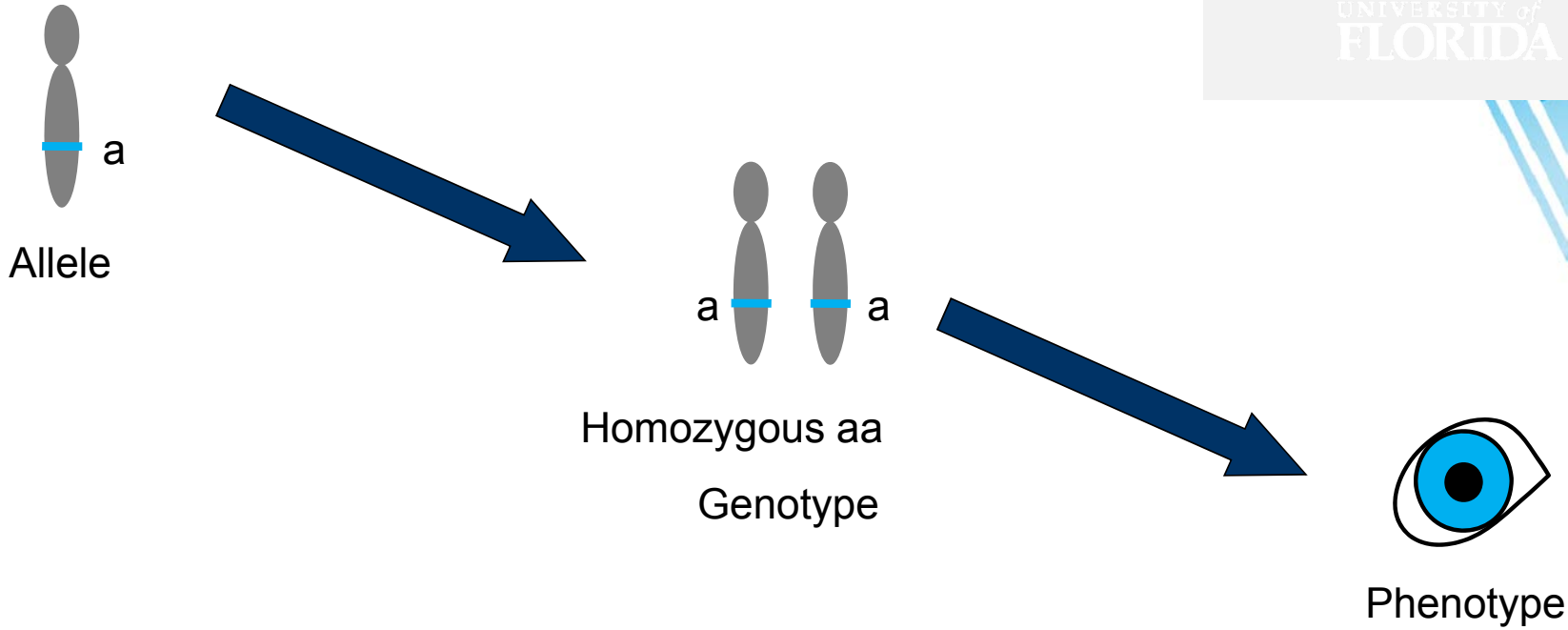
Height

Phenotype vs. Genotype

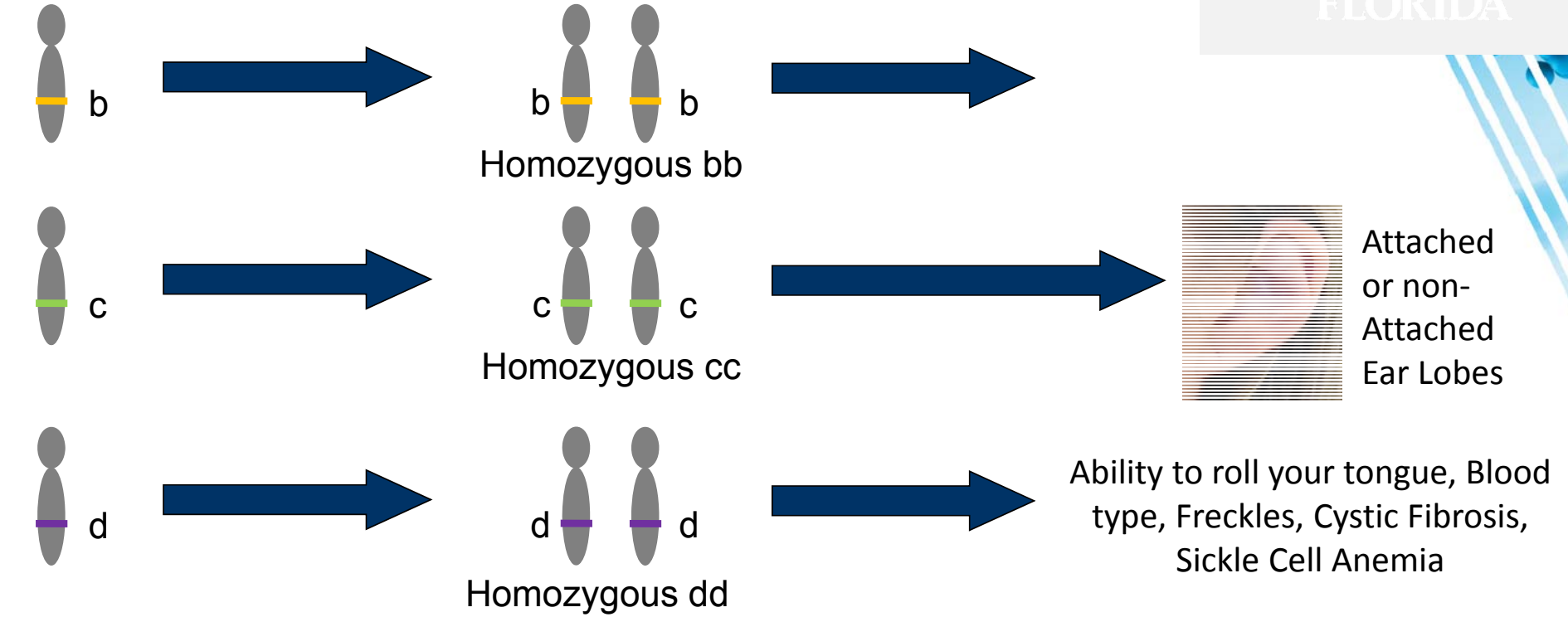
- Phenotype is the observable property of an organism; a trait such as height, weight, medical condition, etc.

- Genotype is the DNA sequence of an organism at a specific, defined location. The genotype is not necessarily observable. Genotype can be expressed in a variety of ways.

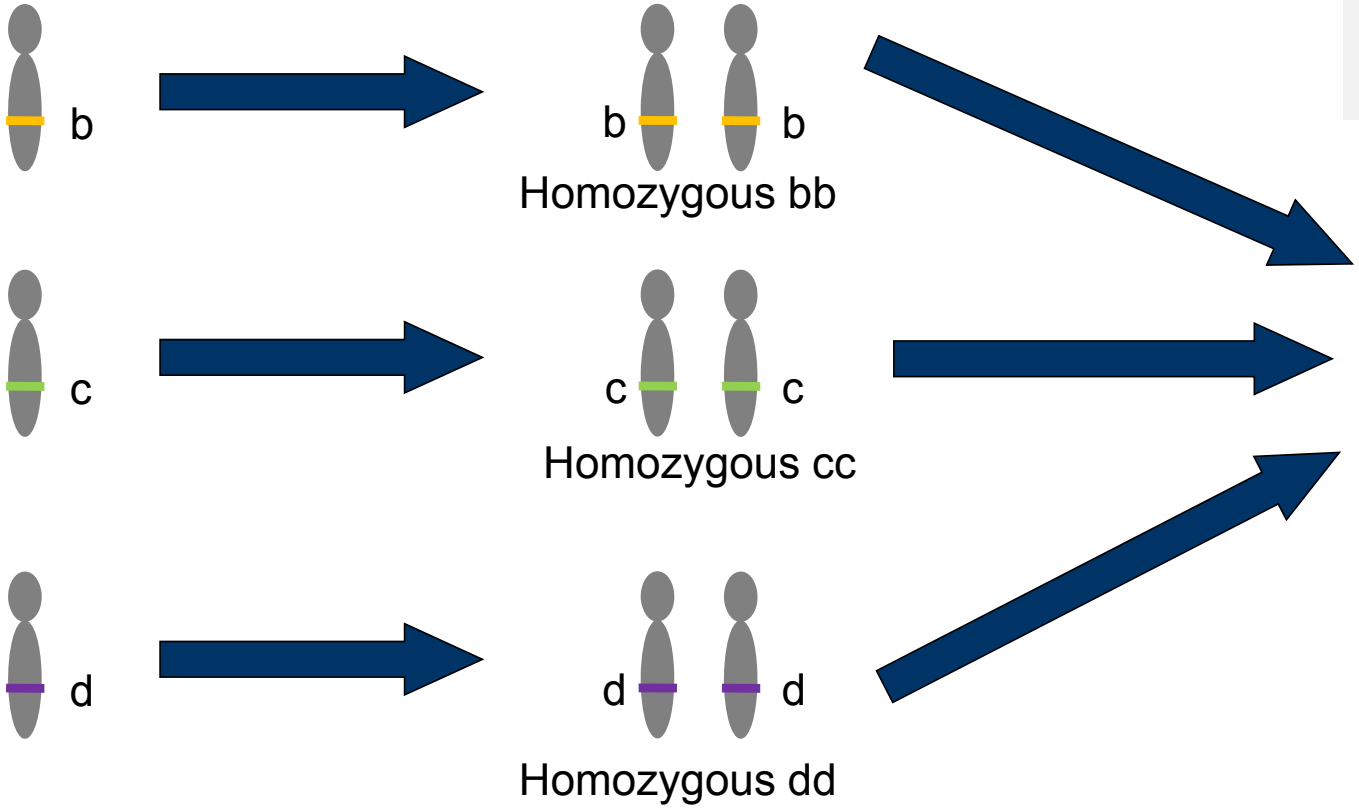
Allele to Genotype to Phenotype



Examples of Allele to Genotype to Phenotype



But it can be more complex



Complex traits: Hair Color, Facial Structure

Complex diseases: Diabetes, Cardiovascular Disease

Complex Drug responses:
Antihypertensives
Antidepressants
Pain management

SNP Nomenclature

- Basic Nomenclature
  - Gene Position Allele 1 > Allele 2
  - Example: CYP2C19 681 G>A

- Reference SNP (rs) Nomenclature
  - The ‘rs’ naming system is used by the National Center for Biotechnology Information (NCBI) SNP database (dbSNP)
  - Each SNP is assigned an rs number
  - CYP2C19 681 G>A → rs4244285

Allele Nomenclature

- Basic Alpha/Numeric Nomenclature
  - Gene Position Allele
  - CYP2C19 681 G OR CYP2C19 681 A
- Allele "Star" Nomenclature
  - Gene * Allele Number
  - CYP2C19 * 1 OR CYP2C19 * 2
- REMEMBER:
  - Allele nomenclature can look exactly the same but have different functional effects based on the specific protein
  - CYP2C19: *1 = normal BUT *2 = NO enzyme activity
  - CYP2C9 : *1 = normal BUT *2 = DECREASED enzyme activity

Genotype Notations and Nomenclature

- Genotype = two alleles inherited for a particular gene or variation
- Basic Genotype Nomenclature
  - CYP2C19 681 G/G = rs4244285 G/G
  - CYP2C19 681 G/A = rs4244285 G/A
  - CYP2C19 681 A/A = rs4244285 A/A
- "Star" Genotype Nomenclature
  - CYP2C19 *1/*1 = normal enzyme activity
  - CYP2C19 *1/*2 = reduced enzyme activity
  - CYP2C19 *2/*2 = NO enzyme activity

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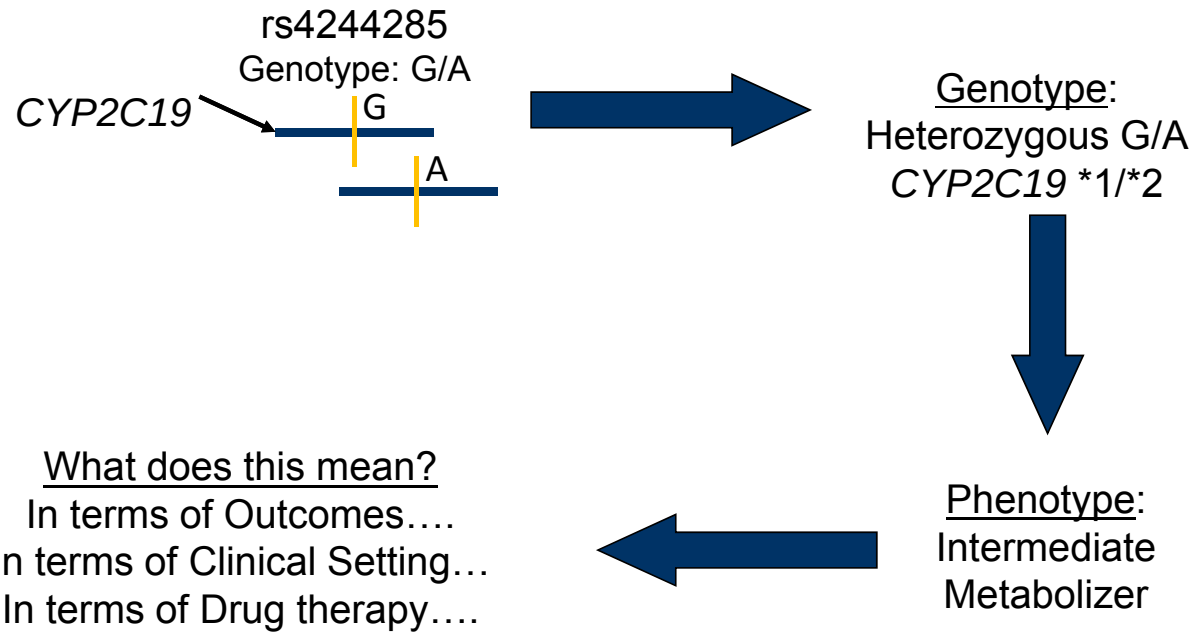
Genotype-Phenotype Pharmacogenomics Example

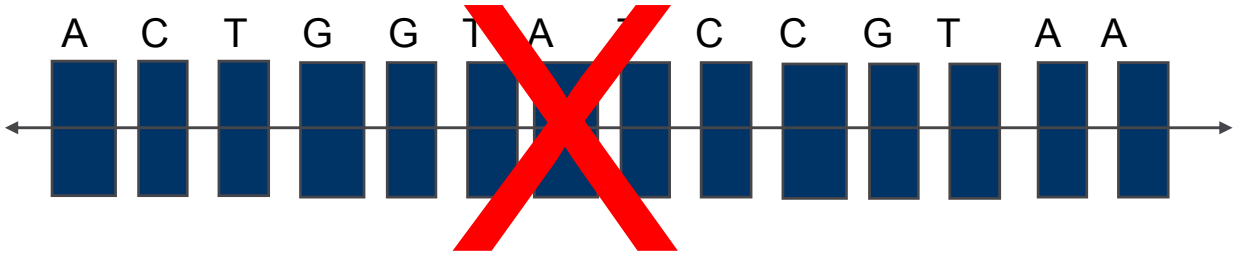
- *CYP2C19* and Clopidogrel



Scott SA. *Clin Pharmacol Ther*. 2011. 90:328-32.

BUT HOW ARE VARIANTS AND SNPS PASSED FROM ONE GENERATION TO THE NEXT?

Structure of the Human Genome



- Previously thought that recombination could occur at any point….
- WRONG!!

Structure of the Human Genome

– Human Genome is organized in a block structure

- Little or no recombination within the blocks
- Recombination occurs between the blocks

Linkage Disequilibrium

- Linkage Disequilibrium (LD)
  - The tendency for pairs of alleles at nearby loci to be associated with each other more often than expected by chance if the loci were segregating independently in the population
  - LD can be formed between two loci when a new mutation occurs at one locus on a chromosome that carries a certain allele at a near by locus

Locus: single gene, portion of a gene, or non-coding region of a chromosome

Reich et al. Nature 2001 May 10;411(6834):199-204 Ardlie et al. Nat Rev Genet 2002 Apr;3(4):299-309

Measuring LD

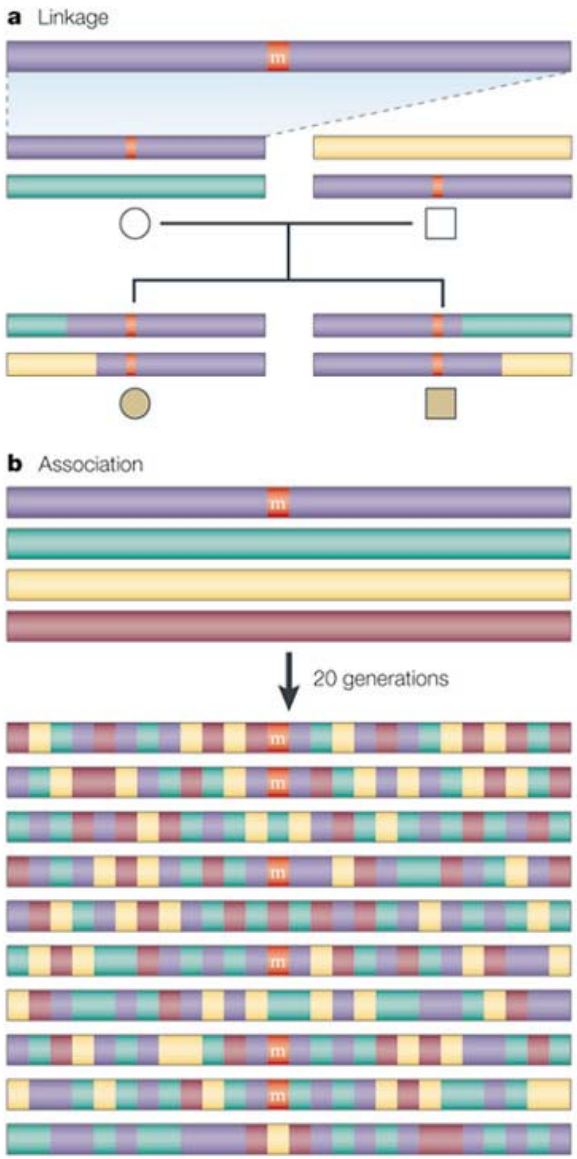
- LD is expressed by a measurement D’ or a measurement of r^2
- D’ can range from 1 (complete LD; no ancestral recombination) to 0 (no LD; independent assortment)
- r^2 can range from 1 (perfect LD, no ancestral recombination, and the two SNPs have the same allele frequency) to 0 (no LD; independent
- Values ≥0.70-0.80 is considered in “high” LD

Factors that Influence LD

- Recombination
  - Time – Evolution
    - The more time that passes, the more generations there are, and the more opportunities there are for recombination to occur between two loci
  - Distance
    - The more distance between two loci, the greater opportunity for recombination to occur between those two loci
- Mutation
  - Create LD with a previous mutation
  - High mutation rates can cause LD to decay in the region

- Admixture, Population Stratification, and Population Structure
- Population Growth, Natural Selection, Genetic Drift

Ardlie et al. Nat Rev Genet 2002 Apr;3(4):299-309



Linkage Disequilibrium and Human Disease

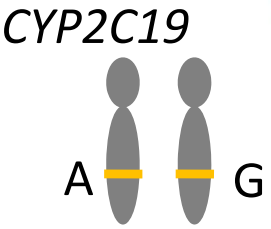
- Co-inheritance of adjacent DNA variants
- LD provides the genetic basis for most 'association' strategies
  - Use it as a tool to study PGx/genetics

Association study designs for complex diseases. Lon R. Cardon & John I. Bell. *Nature Reviews Genetics* 2, 91-99 (February 2001)

Haplotypes and Diplotypes

- What is a Haplotype?
  - A combination of alleles or SNPs along a chromosome that tend to be inherited together

- What is a Diplotype?
  - Two haplotypes that are inherited on a chromosome for a certain gene, or combination of alleles or SNPs



CYP2C19 *2 *CYP2C19*1

Diplotype: *CYP2C19* *1/*2

http://ghr.nlm.nih.gov/glossary=haplotype

Linkage Disequilibrium and Haplotypes

- Theory:
  - Number of possible haplotypes for a group of SNPs is 2^N
  - Where N= number of SNPs
  - If we have 13 SNPs, number of possible haplotypes would be 2^{13} = 8,192 combinations

- Reality:
  - There are much fewer haplotypes observed
  - Example – β_2 AR: While 13 SNPs in the gene, only 12 haplotypes and 5 haplotypes describe 90% of the population

PNAS. 2000; 97: 10483-88. and Science. July 20, 2001.

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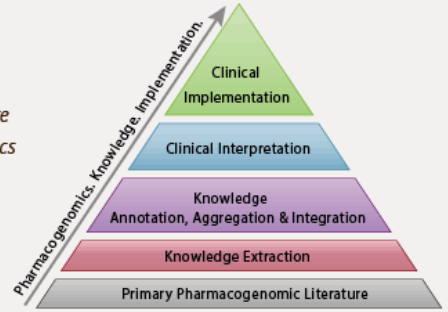

Latest News

- CPIC Meeting at ASCPT on March 15, 2017
- CPIC Seeks Feedback on Recommendation Strength and Gene/Drug Pair Level Definitions
- Pharmacogenomics discussed in the Summer 2016 issue of Genome

Clinically-Relevant PGx

- Selected Pharmacogenomic Associations
- Clinically relevant PGx summaries
- PGx drug dosing guidelines
- Drug labels with PGx info
- PGx gene haplotypes

PGx-Based Drug Dosing Guidelines

- See all CPIC guidelines
- Recent guidelines:
  - UGT1A1/atazanavir: article and supplement
  - SSRIs: citalopram/fluvoxamine/paroxetine article and supplement
- CPIC genes/drugs of interest
- TPP gene tables

CPIC: Implementing PGx
a PharmGKB & PGRN collaboration

PGx Research

- VIP: Very Important PGx gene summaries
- PharmGKB pathways
- Annotated SNPs by gene
- Drugs with genetic information
- Cancer PGx

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from search: REN

GENE:
REN
renin

Clinical PGx | PGx Research | Overview

Overview

Alternate Names:	None
Alternate Symbols:	None
PharmGKB Accession Id:	PA297

Details

Cytogenetic Location:	chr1 : q32.1 - q3
GP mRNA Boundary†:	chr1 : 20412394
GP Gene Boundary†:	chr1 : 20412094
Strand:	minus

† The mRNA boundaries are calculated using the gene's def
by no less than 10,000 bases upstream (5') and 3,000 bases

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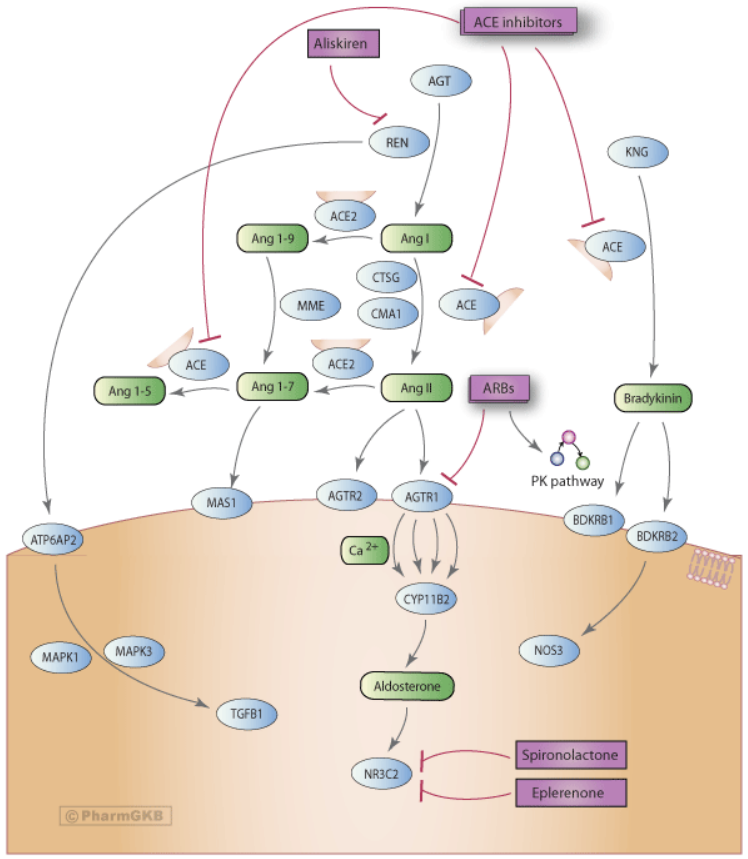
PATHWAY
Agents Acting on the Renin-Angiotensin System Pathway, Pharmacodynamics

Overview | Components | Related Pathways | Downloads/LinkOuts

Pharmacodynamics

Genes involved in the pharmacodynamics of the drugs that act on the renin-angiotensin-aldosterone system.



Summary

- Polymorphism
  - A variation in DNA sequence

- Alleles, Genotypes, and Phenotypes
  - is one of two or more versions of a genetic sequence
  - Two alleles inherited for a particular gene or variation
  - individual's observable traits

- Human Genome is organized in a block like structure
- Linkage Disequilibrium refers to the non-independence of alleles at different sites
  - Measured by D' and r2

- Haplotypes and Diplotypes

- PharmGKB