WASHINGTON STATE UNIVERSITY

Fundamentals of Pharmacogenomics (PGx), Genetic Variation in Drug Metabolism, and Interpretation of PGx Testing Results- Part 1

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Conflict of Interest Disclosure

- Nothing to disclose

LEARNING OBJECTIVES

- Describe fundamental principles of pharmacogenomics (PGx)
- Discuss how to navigate PGx database resources such as Clinical Pharmacogenetics Implementation Consortium (CPIC) and Pharmacogenomics Knowledge Database (PharmGKB)
- Illustrate general implications of genetic variants in drug metabolism in context of active parent drug versus inactive prodrug
- Outline function of major genetic variants associated with Phase I (CYP2C9, CYP2C19, CYP2D6) and Phase II (UGT1A1, TPMT) drug metabolism and describe potential impact on pharmacokinetics (PK) and drug response
- Define phenoconversion and predict CYP2D6 phenotypes by calculation of total activity scores
- Describe relevant FDA resources used for disseminating PGx-related drug-gene pair information

Pharmacogenomics in the clinic

Mary V. Relling[1] and **William E. Evans**[1]

[1]Department of Pharmaceutical Sciences, St. Jude Children's Research Hospital, Memphis, TN

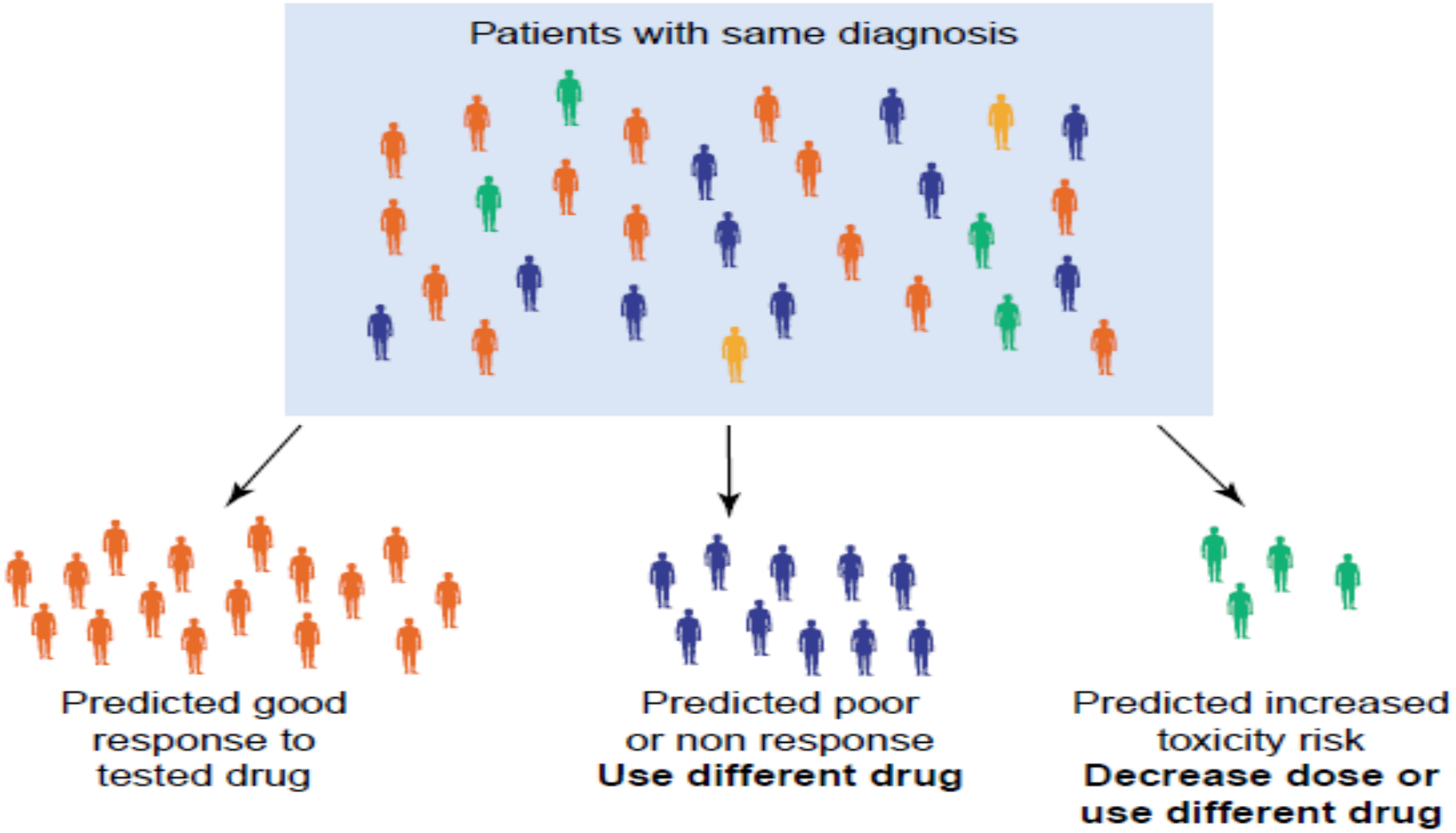
Preface

After decades of discovery, inherited variation in approximately 20 genes affecting about 80 medications has been identified as actionable in the clinic. Additional somatically acquired genomic variants direct the choice of "targeted" anticancer drugs for individual patients. Current efforts that focus on the processes required to appropriately act on pharmacogenomic variability in the clinic are systematically moving pharmacogenomics from discovery to implementation as an evidenced-based strategy for improving the use of medications, thereby providing an important cornerstone for precision medicine.

Introduction

Pharmacogenomics focuses on the identification of genome variants that influence drug effects, typically via alterations in a drug's pharmacokinetics (i.e., absorption, distribution, metabolism, elimination) or via modulation of a drug's pharmacodynamics (e.g., modifying a drug's target or perturbing biological pathways that alter sensitivity to the drug's pharmacological effects). For diseases other than cancer and infectious diseases, the genome

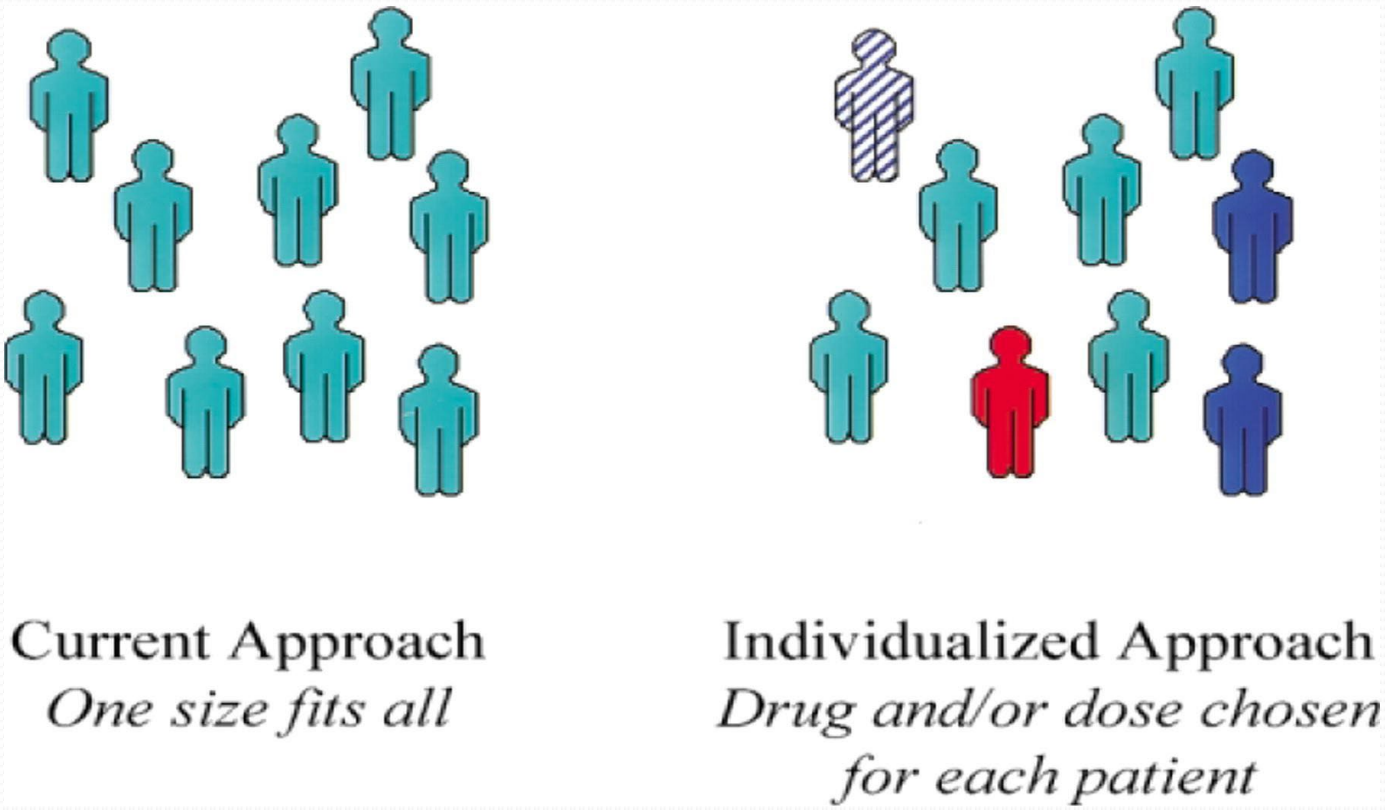
Mary V. Relling and William E. Evans. *Nature*. 2015; 526(7573): 343–350.



Tailored therapy can help to REDUCE adverse reactions and INCREASE efficacy rates

Johnson JA. *Trends Genet* 2003; 19(11): 660-666

Progressive movement toward using a personalized medicine approach

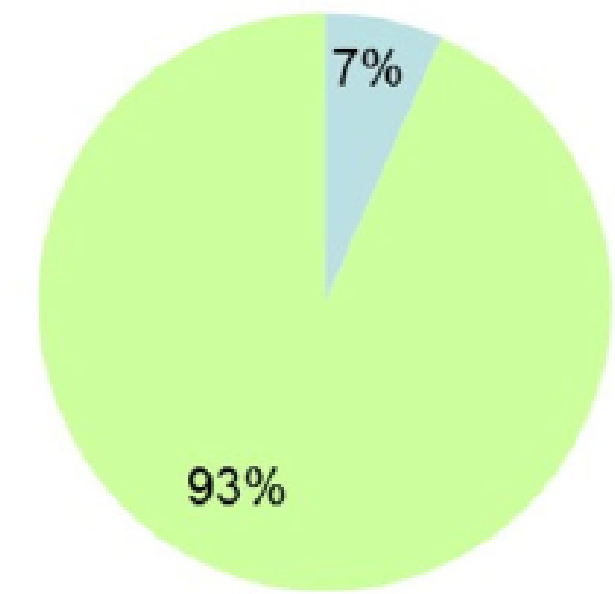


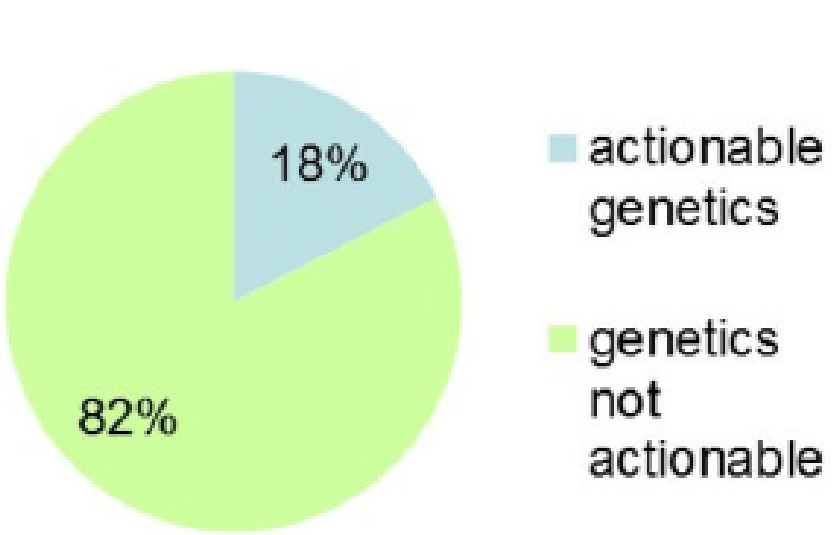
Current Approach
One size fits all

Individualized Approach
Drug and/or dose chosen for each patient

McLeod HL. *Clin Infect Dis* 2005; 41(7):S449-S452

Almost 1 in 5 prescriptions are affected by *actionable germline* PGx





Mary V. Relling and William E. Evans. *Nature*. 2015; 526(7573): 343–350.

Search Menu

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Table of Pharmacogenomic Biomarkers in Drug Labeling

Almost 500 therapeutic products recognized by the Food and Drug Administration (FDA) include PGx information in their drug labeling

https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling Accessed February 20, 2022

Competencies

Nurse	Physician Assistant	Pharmacist	Genetic Counselor	Physician
COMPETENCIES MAP	COMPETENCIES MAP	COMPETENCIES MAP	COMPETENCIES MAP	COMPETENCIES MAP
Print Version	Print Version	Print Version	Print Version	Print Version
Reference	**Reference**	**Reference**	**Reference**	**Reference**
Essentials of Genetic & Genomic Nursing: Competencies, Curricula Guidelines, & Outcome Indicators, 2nd Edition (2008)	Physician Assistant Genomic Competencies (2016)	Pharmacists Leading the Way to Precision Medicine: Updates to the Core Pharmacist Competencies in Genomics (2021)	Practice-Based Competencies for Genetic Counselors (2015)	Framework for Development of Physician Competencies in Genomic Medicine (2014)

https://www.genomicseducation.net/competency Accessed February 20, 2022

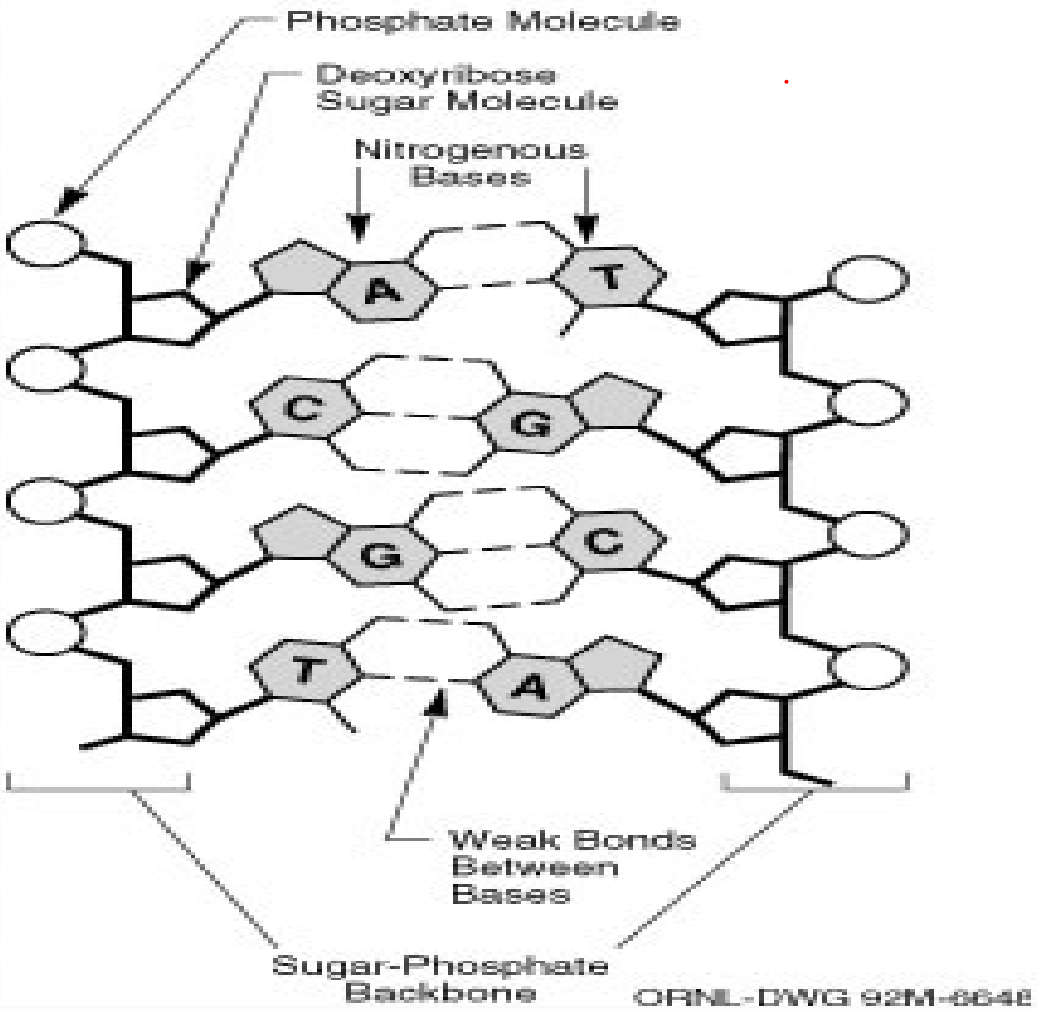
Describe the Fundamental Principles of Pharmacogenomics

Useful Resources for Genetic Terminology

- **Website References**
  - http://www.dnaftb.org/
  - https://medlineplus.gov/genetics/
  - https://www.genome.gov/about-genomics/teaching-tools/Genomics-Education-Websites
- **Genomics Glossary App**
  - https://www.genome.gov/genetics-glossary
- **Literature**
  - Feero WG, Guttmacher AE, and Collins FS. Genomic Medicine -An Updated Primer. *N Engl J Med.* 2010; 362(21): 2001-2011.

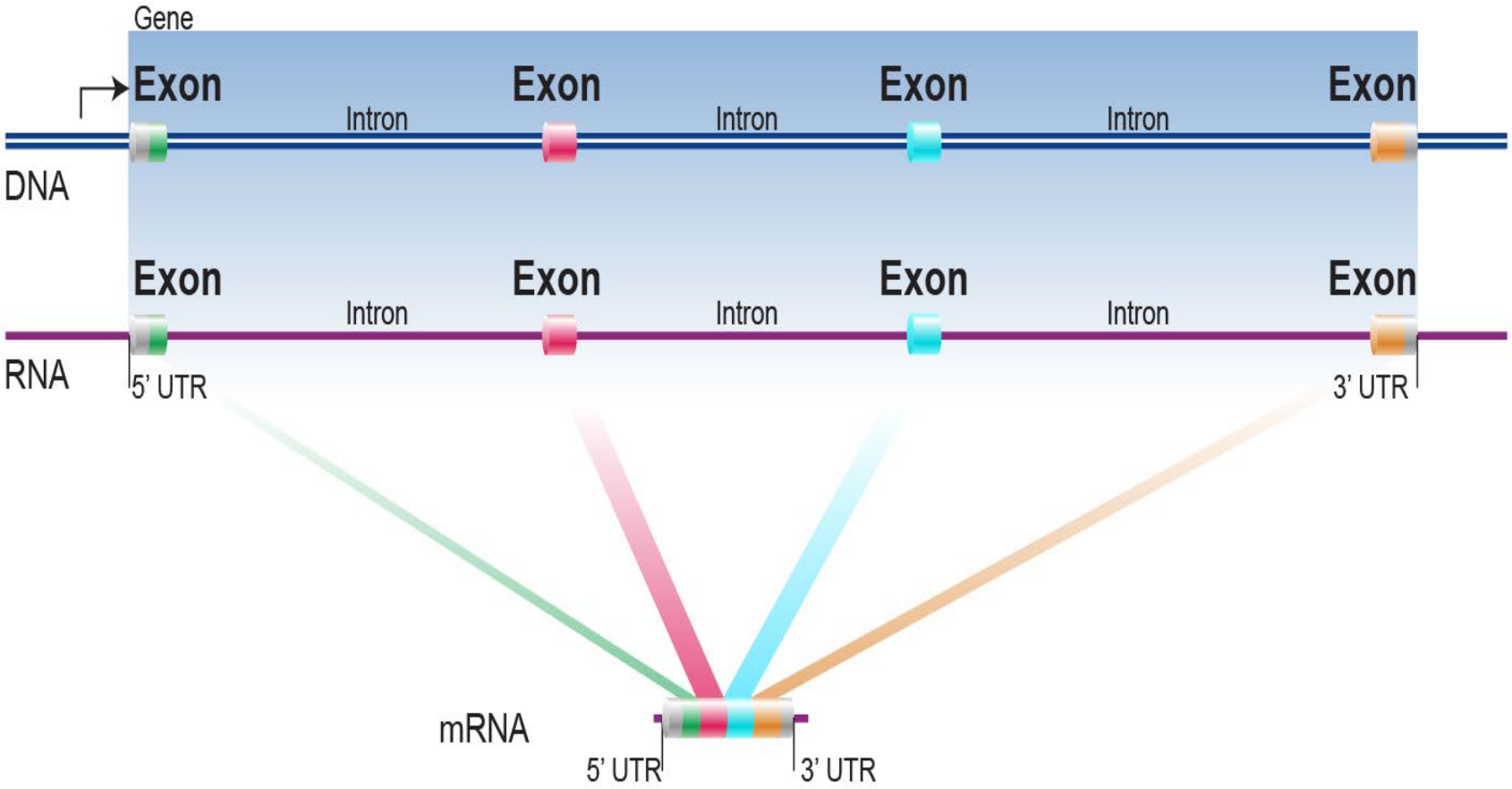
Building Blocks of DNA

- **Sugar-Phosphate backbone**
- **Four Nucleotides**
  - A: Adenine
  - T: Thymine
  - C: Cytosine
  - G: Guanine



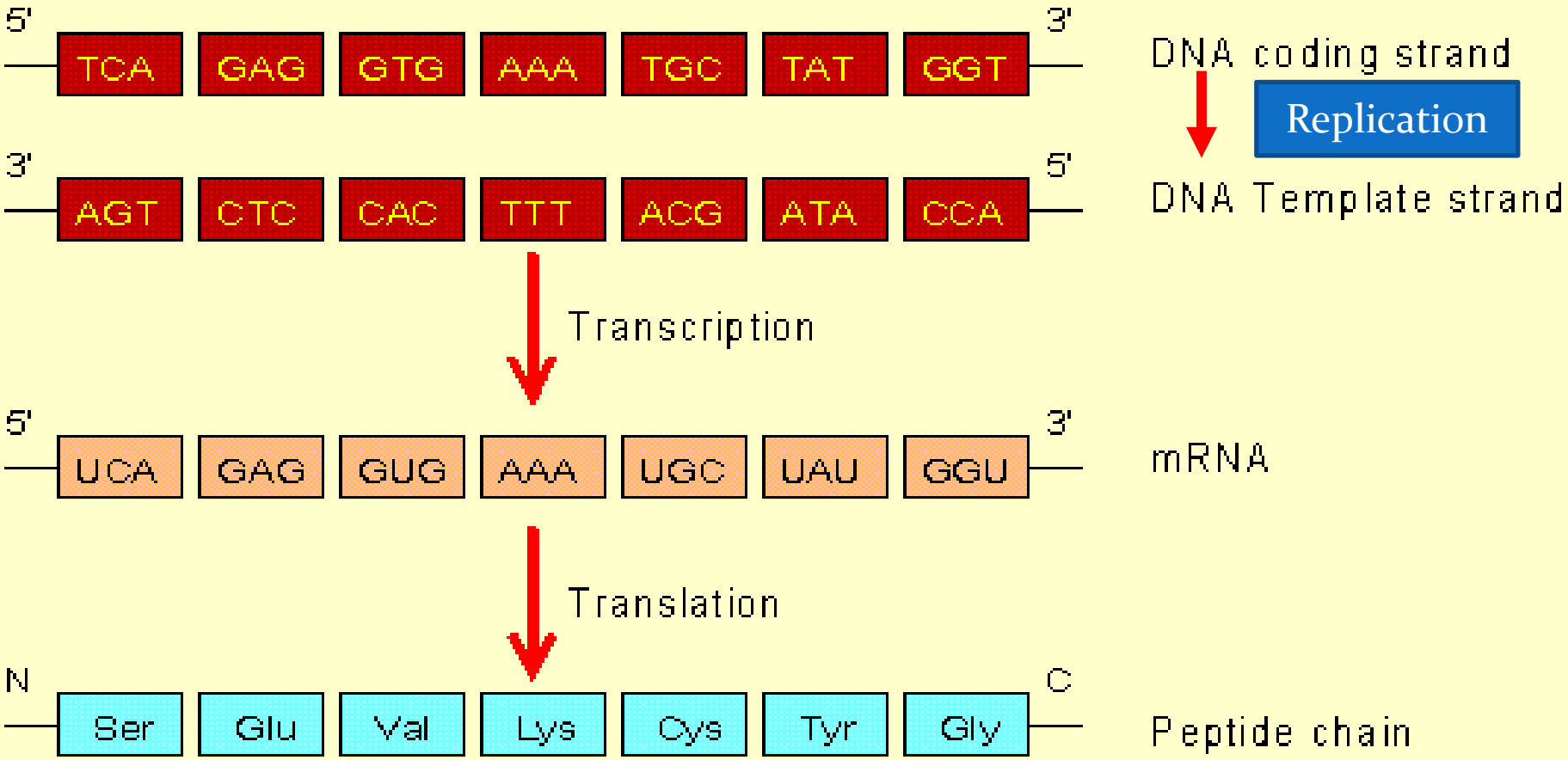
https://public.ornl.gov/site/gallery/detail.cfm?id=395&topic=&citation=&general=DNA&restsection=all
Accessed December 23, 2021

Gene Structure



https://www.genome.gov/genetics-glossary/Exon Accessed December 23, 2021

Central Dogma (Replication→ Transcription→ Translation)



http://www.web-books.com/MoBio/Free/Ch3E3.htm Accessed December 23, 2021

Transcription

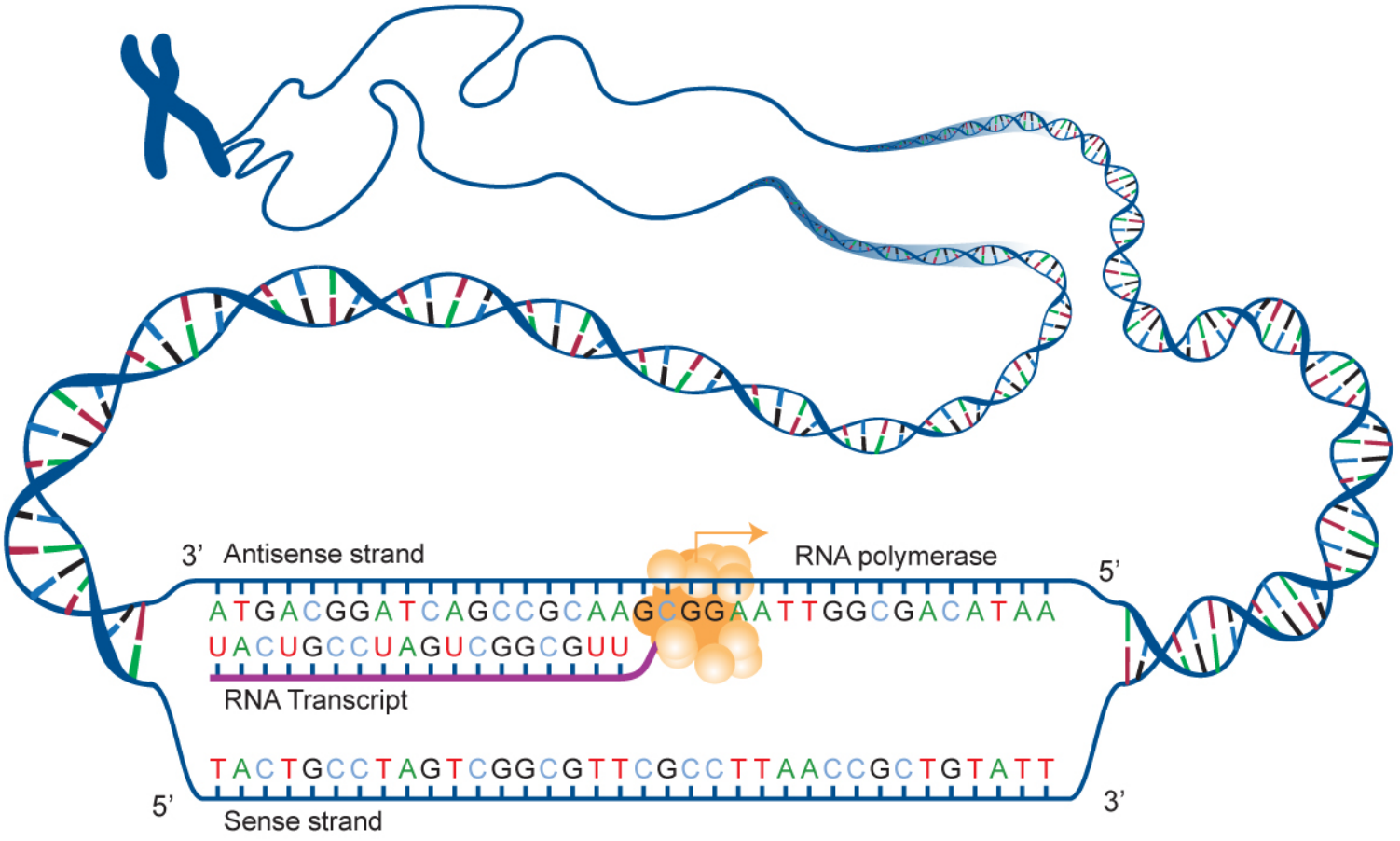
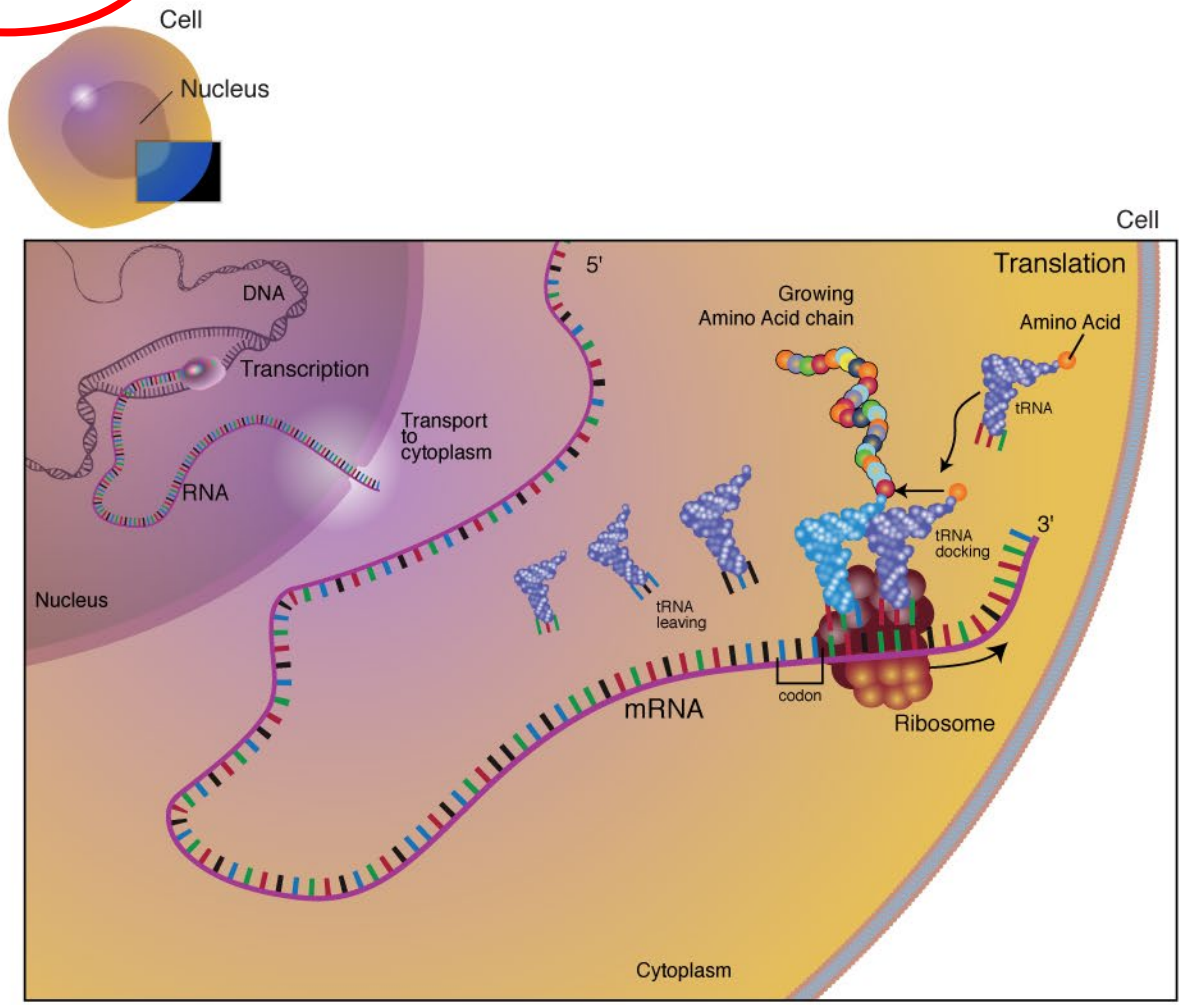
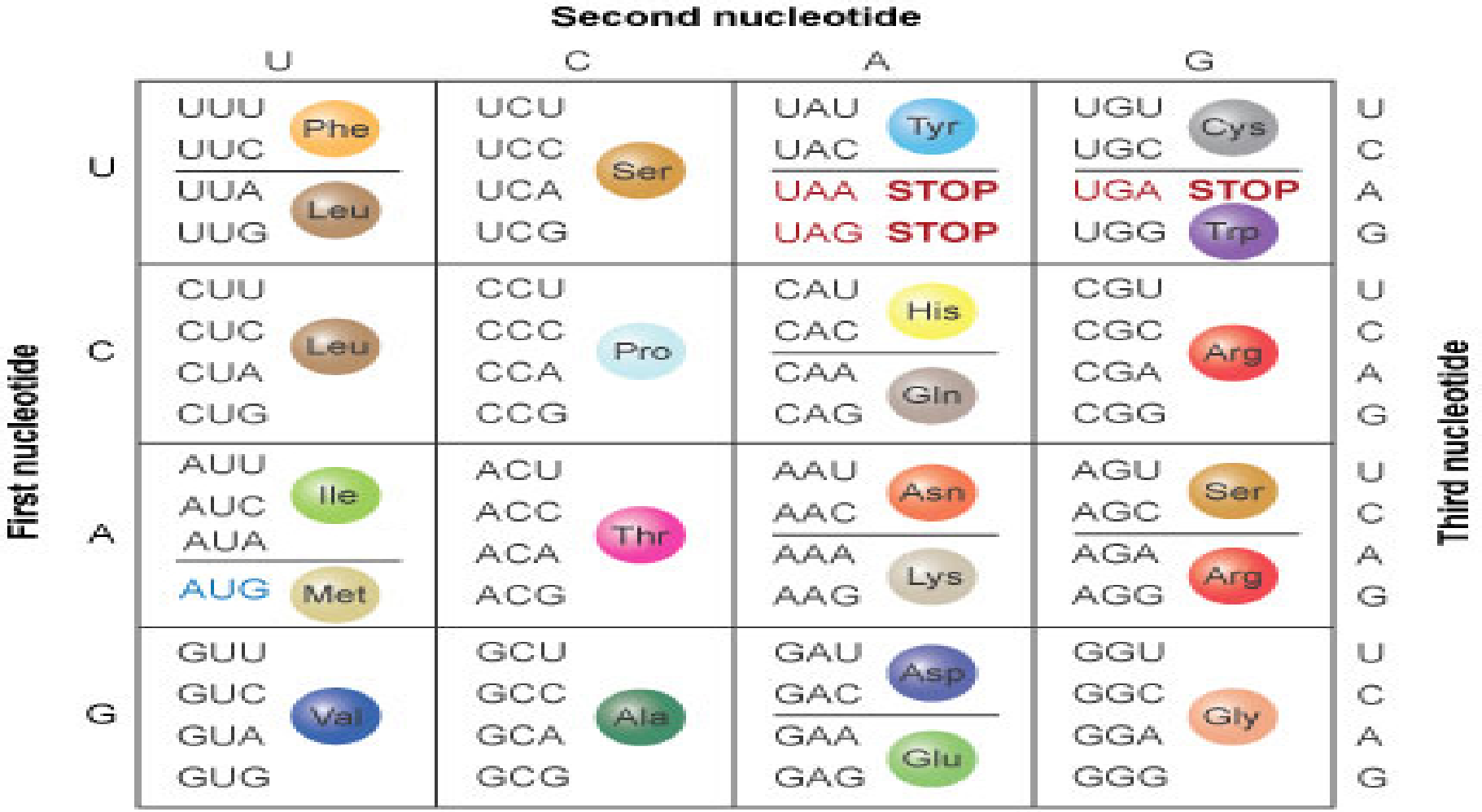


Illustration by Darryl Leja, NHGRI

Translation



Redundancy in genetic code…most amino acids are specified by more than one mRNA codon



https://www.nature.com/scitable/topicpage/the-information-in-dna-determines-cellular-function-6523228
Accessed February 20, 2022

Type of Polymorphisms

- **Polymorphism**: variation in a particular DNA sequence that is <u>different</u> from the reference/normal sequence at that location

- **4 primary types of polymorphisms**
  - Single nucleotide polymorphisms **(SNPs)**
  - Insertions/deletions **(indels)**
  - Variable number tandem repeats **(VNTRs)**
  - Copy number variants **(CNVs)**

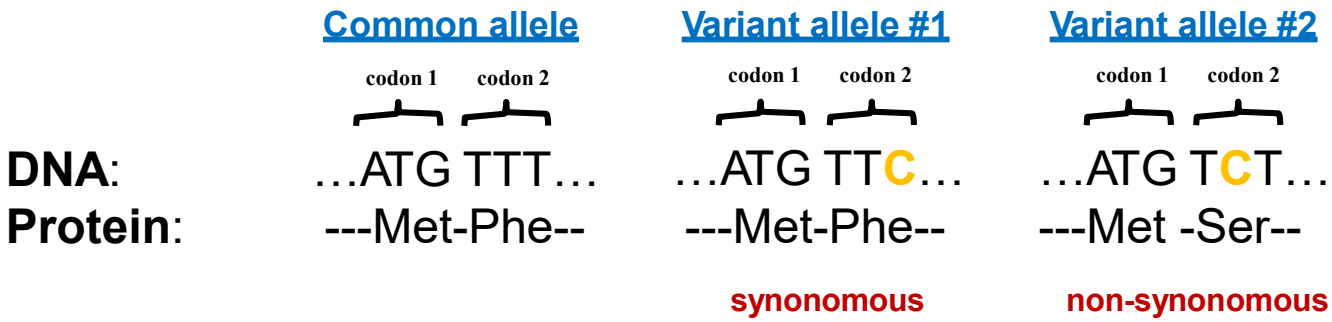
https://www.genome.gov/genetics-glossary/Polymorphism Accessed Dec 23,2021

Single Nucleotide Polymorphisms (SNPs)

- Most common sequence variation in human genome
- Change in single base pair or nucleotide in genomic sequence
  - For example: GAT**C**TGA (original sequence)
    GAT**T**TGA (change from original)
- On average, SNPs occur *every 100-300 base pairs*

Feero WG, Guttmacher AE, and Collins FS. Genomic Medicine -An Updated Primer. *N Engl J Med.* 2010; 362(21): 2001-2011.

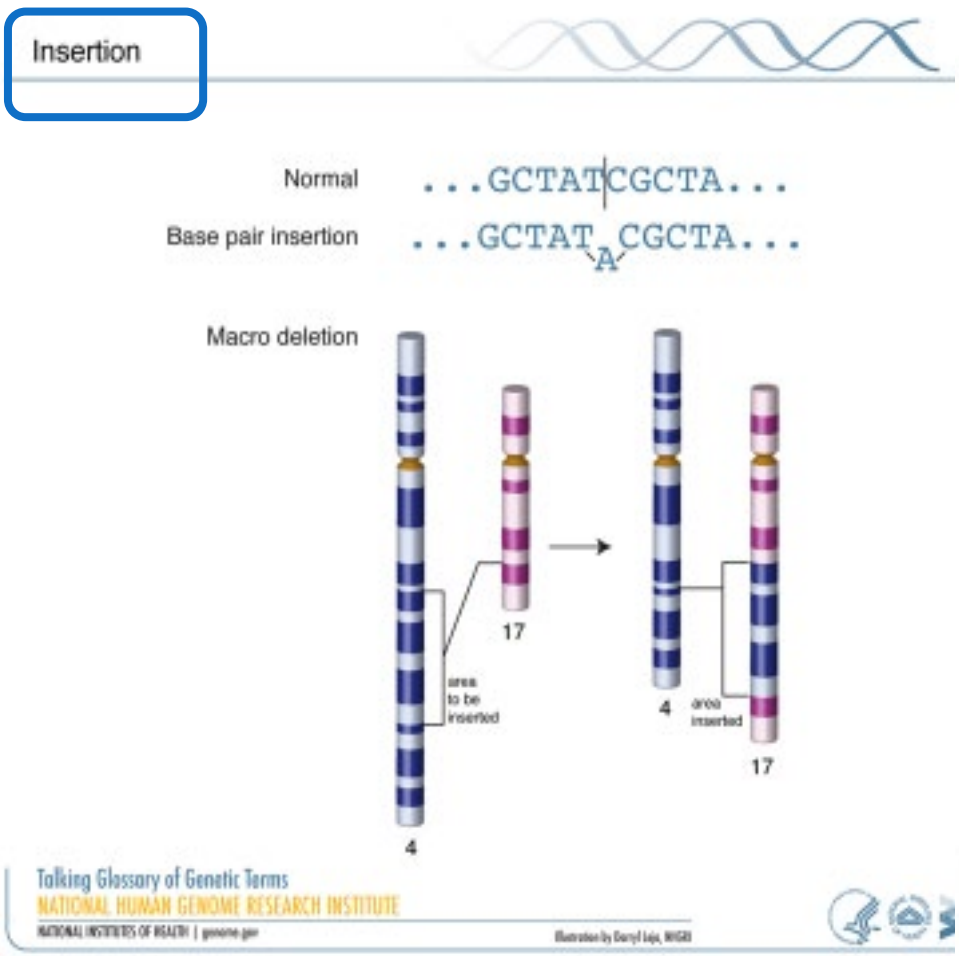
Synonymous SNP
versus
Non-synonymous SNP



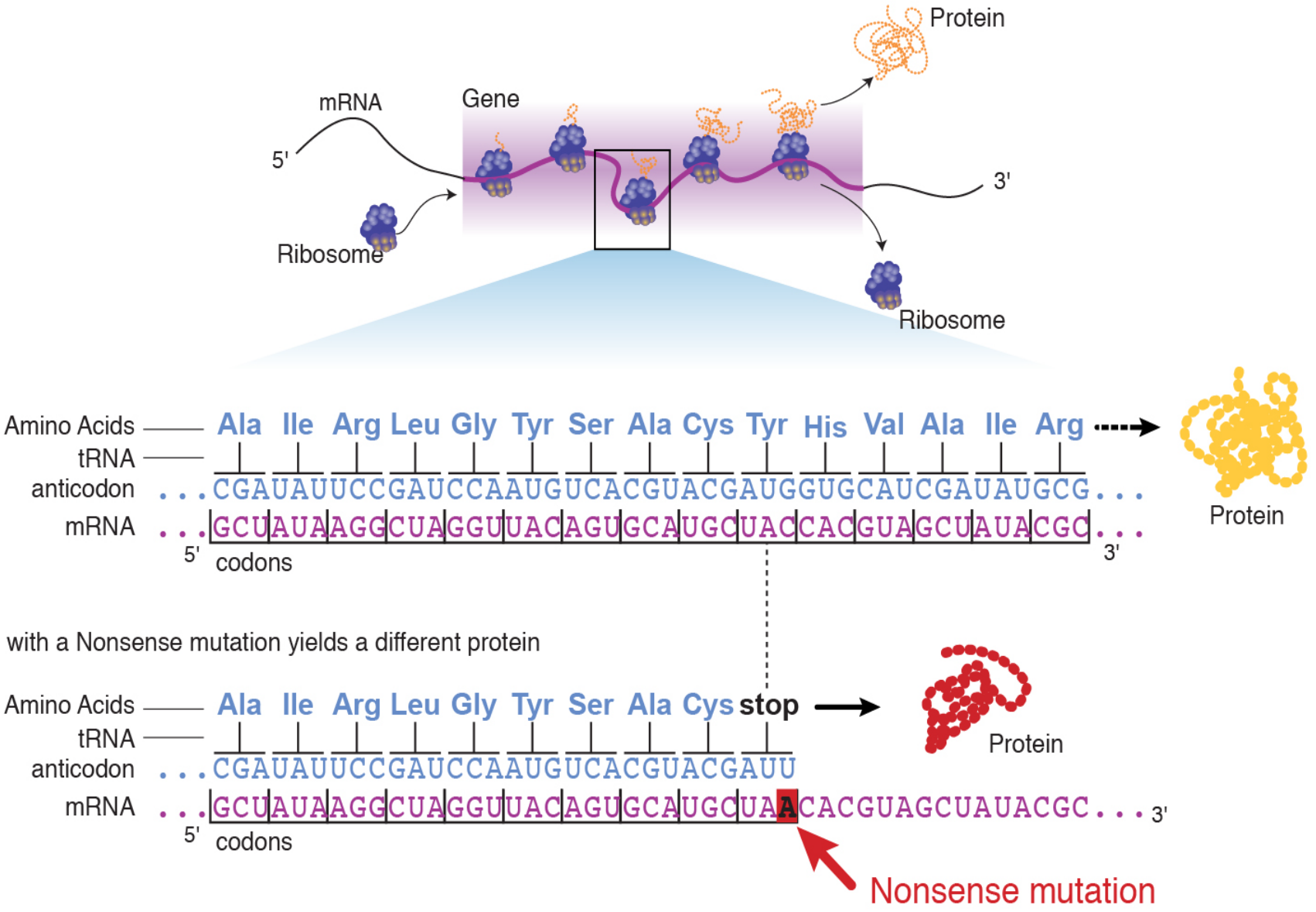
Synonymous SNP results in the SAME amino acid with no change in protein activity/function

Non-synonymous SNP results in a CHANGE in amino acid which may affect protein activity/function

Insertions/deletions (indels)



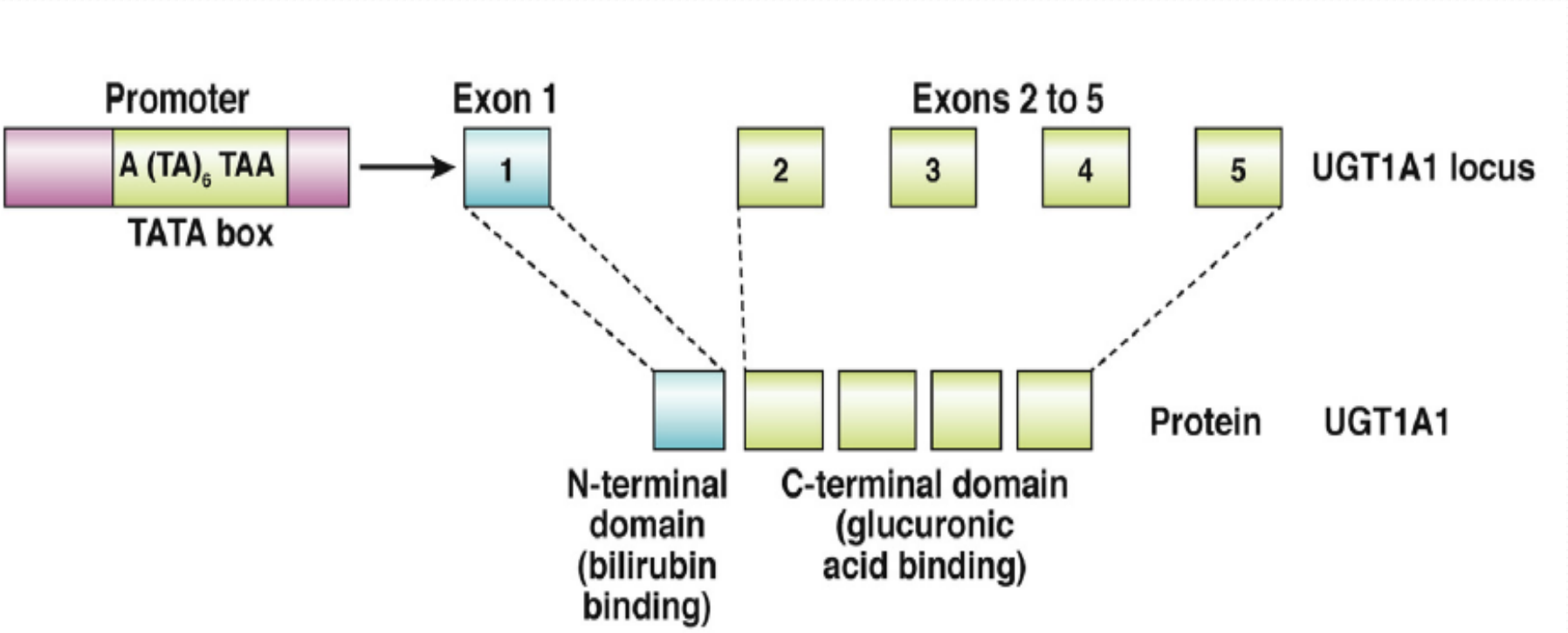
https://www.genome.gov/genetics-glossary/Insertion Accessed Dec 23, 2021
https://www.genome.gov/genetics-glossary/Deletion Accessed Dec 23, 2021



https://www.genome.gov/genetics-glossary/Nonsense-Mutation Accessed Dec 23, 2021

Variable Number Tandem Repeats (VNTRs)

"Consecutive base pair groups that are differentially *repetitive*"



https://www.genome.gov/genetics-glossary/Tandem-Repeat Accessed January 3,2022

Erlinger S, Arias IM, Dhumeaux D. *Gastroenterology* 2014;146:1625–1638

Copy Number Variants (CNVs)

- Variation from one person to the next in the number of copies of a particular gene or DNA sequence

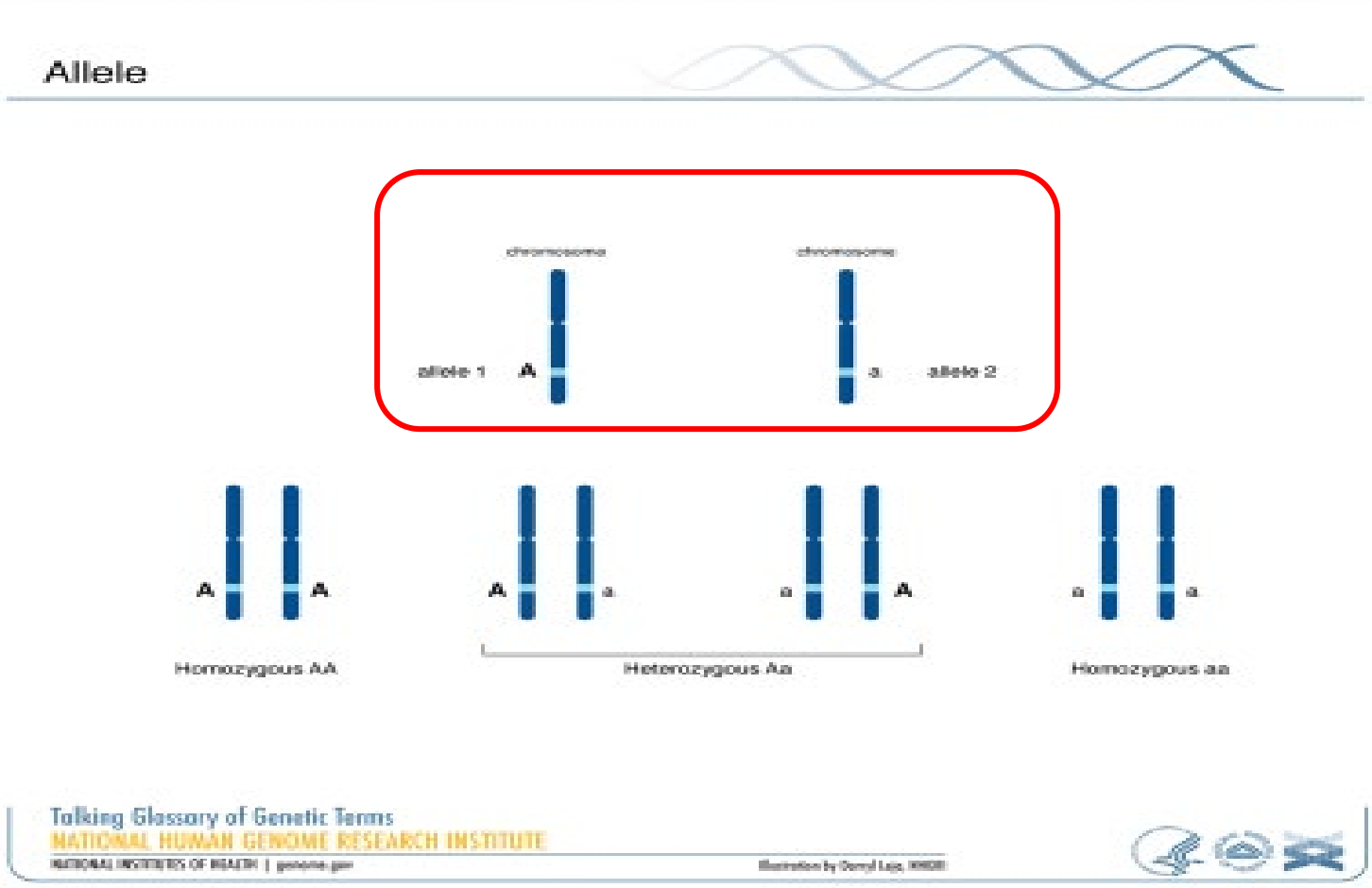
- Example: CYP2D6 ultra-rapid metabolizers (URMs)
  - *Functional alleles* (**CYP2D6*1xN and *2xN duplications**)

- Individuals who are URMs will metabolize drugs (CYP2D6 substrates) much *more quickly* relative to individuals who are normal metabolizers of CYP2D6 because they have additional copies of the CYP2D6 gene

Feero WG, Guttmacher AE, and Collins FS. Genomic Medicine -An Updated Primer. *N Engl J Med.* 2010; 362(21): 2001-2011.

Important Genetic Nomenclature

ALLELE is one of two or more versions of a gene



https://www.genome.gov/genetics-glossary/Allele Accessed Jan 3, 2022

Star Allele Nomenclature

- Specific allele is indicated by a *, followed by the allele number (for example: ***gene*allele number*** *or CYP2C9*2*)
- "Wild type" enzyme is designated as *1
  - *Reference* allele
  - Defined as "**normal" functional activity or metabolism**

- Each allele that is functionally distinct is given a new number after this reference allele
  - For example, variants are sequentially numbered, so the *4 allele in CYP2D6*4 is the 3rd variant identified
- Some alleles may have variations that are NOT functionally significant (i.e. silent SNPs or intronic SNPs)
  - Identified by a letter *after* the * number (for example-CYP2D6*4A)

Pharmacogenomics: Applications to Patient Care. Third Edition, 2015, ACCP publication

SNP Nomenclature

Basic SNP nomenclature

- *Gene*, *position*, *allele 1*>*2*
- For example: CYP2C19 681 G>A
  - This indicates a G to A SNP at nucleotide position 681 in the gene CYP2C19

Reference SNP (rs) nomenclature

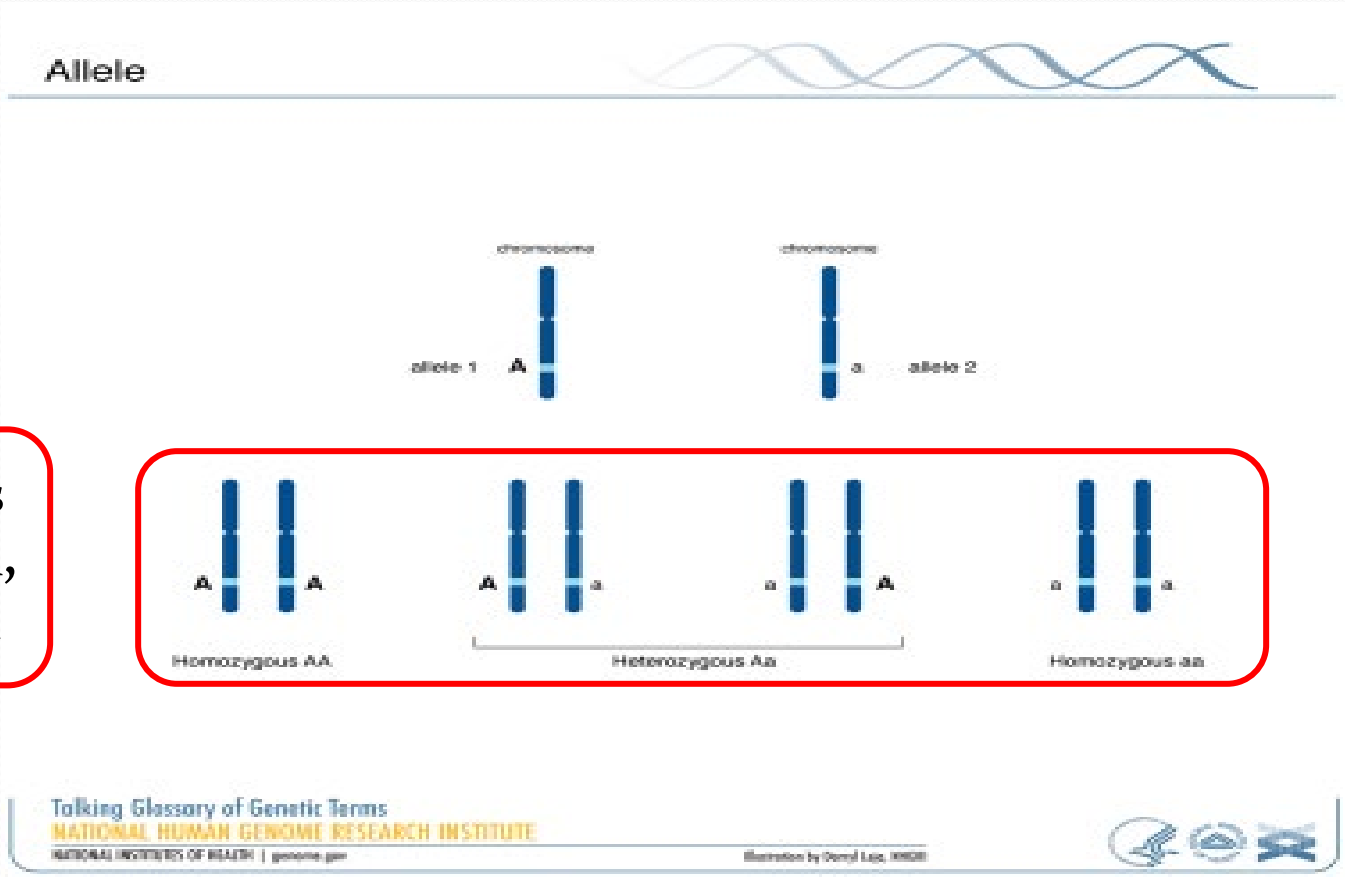
- Used by the NCBI (National Center for Biotechnology Information)
- An *rs number* is assigned to each SNP
- CYP2C19 681 G>A is also referred to as *rs4244285*
- Commonly used in research/publications

Pharmacogenomics: Applications to Patient Care. Third Edition, 2015, ACCP publication

Important Genetic Nomenclature

GENOTYPE is defined as two alleles inherited for a particular gene or variation at a particular location in the genome

Genotypes include **AA, Aa and aa**



https://www.genome.gov/genetics-glossary/genotype Accessed Jan 3,2022

Other Fundamental PGx Terms

- **Gene**--fundamental physical and functional unit of heredity
- **Haplotype**-- combination of alleles on the SAME chromosome (set of DNA variations, or polymorphisms, that tend to be inherited together)
- **Phenotype**- an individual's observable traits (can be determined by genotype and/or environmental factors)

Feero WG, Guttmacher AE, and Collins FS. Genomic Medicine -An Updated Primer. *N Engl J Med.* 2010; 362(21): 2001-2011.

Hardy-Weinberg Equilibrium (HWE) Principles

- "Describes the distribution of genotypes for a selected polymorphism within a population"

- **HWE assumptions**
  - Gene/genotype frequencies will remain *constant* from generation to generation
  - Infinitely large interbreeding population (mating is at random)
  - No selection, migration or mutation occurs

Pharmacogenomics: Applications to Patient Care. Third Edition, 2015, ACCP publication

Hardy-Weinberg Equilibrium (HWE) Principles

- **HWE Equation**
  - $p^2 + 2pq + q^2 = 1$
  - p refers to frequency of A (one allele)
  - q refers to frequency of a (other allele)
  - $p + q = 1$

  - *Genotype frequencies*
    - p^2 = A x A
    - 2pq = A x a
    - q^2 = a x a

	A	**a**
A	AA	Aa
a	Aa	aa

Pharmacogenomics: Applications to Patient Care. Third Edition, 2015, ACCP publication

Application of HWE Principles (practice example)

- Cytochrome P450 2C19 (CYP2C19*2) is a G to A change at nucleotide position 681 in CYP2C19 gene. Twelve percent of South/Central Asians are homozygous for the A allele at nucleotide position 681 in CYP2C19 gene.

- Using HWE equations, calculate both the allele AND genotype frequencies for G and A alleles in the South/Central Asian population.

Application of HWE Principles (practice example KEY)

- HWE equation
  - $p2 + 2pq + q2 = 1$
  - Assume p refers to frequency of G allele and q refers to frequency of A allele
  - $p + q = 1$
  - $q2$ (AA) = 0.12. Therefore, q (A) = $\sqrt{0.12}$ = 0.35.
  - Since $p + q = 1$, $p = 1 - q = 1 - 0.35 = 0.65$.
  - Thus, 35% and 65% of South/Central Asians carry A and G alleles, respectively.

  - GG genotype frequency is calculated as 0.65 x 0.65 x 100= 42.25%
  - GA genotype frequency is calculated as 2 x 0.65 x 0.35 x 100= 45.5%
  - AA genotype frequency is calculated as 0.35 x 0.35 x 100= 12.25%

- Since ~12% of South/Central Asians are poor metabolizers in CYP2C19, consider the clinical implications this may have for these individuals who take medication metabolized by this enzyme?

Discuss how to navigate PGx database resources such as Clinical Pharmacogenetics Implementation Consortium (CPIC) and Pharmacogenomics Knowledge Database (PharmGKB)

What is CPIC?

The Clinical Pharmacogenetics Implementation Consortium (CPIC®) is an international consortium of individual volunteers and a small dedicated staff who are interested in facilitating use of pharmacogenetic tests for patient care.

One barrier to implementation of pharmacogenetic testing in the clinic is the difficulty in translating genetic laboratory test results into actionable prescribing decisions for affected drugs.

CPIC's goal is to address this barrier to clinical implementation of pharmacogenetic tests by creating, curating, and posting freely available, peer-reviewed, evidence-based, updatable, and detailed gene/drug clinical practice guidelines (click here for all CPIC publications). CPIC guidelines follow standardized formats, include systematic grading of evidence and clinical recommendations, use standardized terminology, are peer-reviewed, and are published in a leading journal (in partnership with Clinical Pharmacology and Therapeutics) with simultaneous posting to cpicpgx.org, where they are regularly updated.

https://cpicpgx.org/ Accessed Jan 4,2022

CPIC provides guidelines that enable the translation of genetic laboratory test results into actionable prescribing decisions for specific drugs

Examples of Drug-Gene Pairs which have CPIC Guidelines

Cardiology
- Clopidogrel – ***CYP2C19***
- Simvastatin – *SLCO1B1*
- Warfarin – ***CYP2C9*** and *VKORC1*

Infectious disease
- Abacavir – *HLA-B*57:01*
- Atazanavir – *UGT1A1*
- PEG-interferon – *IL28B*

Neurology
- Carbamazepine – *HLA-B*15:02*
- Phenytion – ***CYP2C9***, *HLA-B*15:02*

Oncology
- Thiopurines – *TPMT*
- Capecitabine/5-FU – *DPYD*
- Rasburicase - *G6PD*

Pain management
- Codeine – ***CYP2D6***
- Tramadol – ***CYP2D6***
- Tricyclic antidepressants – ***CYP2C19, CYP2D6***

Psychiatry
- Tricyclic antidepressants – ***CYP2C19, CYP2D6***
- Selective serotonin reuptake inhibitors– ***CYP2C19, CYP2D6***

Rheumatology
- Thiopurines – *TPMT*
- Allopurinol – *HLA-B*58:01*

Solid organ transplant
- Tacrolimus – *CYP3A5*

Respiratory
- Ivacaftor - *CFTR*

Figure 1. Current drug-gene pairs with Clinical Pharmacogenetics Implementation Consortium guidelines grouped by disease state. The genes in bold (*CYP2C19*, *CYP2C9*, *CYP2D6*) are the backbone genes for a general pharmacogenomics implementation initiative.

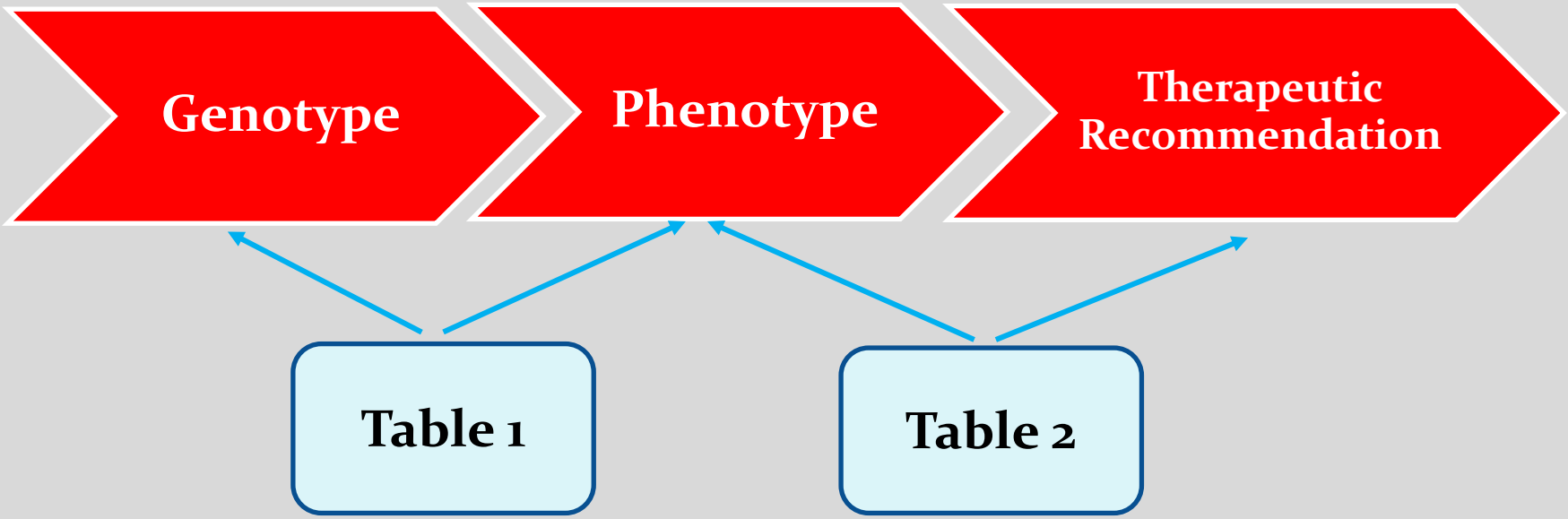
Vo TT et al. *Pharmacotherapy* 2017; 37:1014-1022
https://cpicpgx.org/

What standard information is included in a CPIC guideline?

Introduction
Literature Review Process
Gene(s)
Background
Genetic Test Interpretation
Available Genetic Test Options
Incidental Findings
Other Considerations
Drug(s)
Background
Linking genetic variability to variability in drug-related phenotypes
Dosage Recommendations/Therapeutic Recommendations
Recommendations for Incidental Findings
Other Considerations
Potential Benefits and Risks for the Patient
Caveats: Appropriate Use and/or Potential Misuse of Genetic Tests
Disclaimer
Assignment of likely [gene] phenotypes based on genotypes (Table 1[a] in CPIC guidelines)
Recommended Prescribing of [drug/s] by [gene] phenotype (Table 2[a] in CPIC guidelines)

Caudle KE et al. *Curr Drug Metab*. 2014; 15(2):209–217

Tables in each of the CPIC drug guidelines translate the genotype information TO phenotype information TO therapeutic recommendation for each drug



CPIC Level Evidence Definitions for Linking Genotypes to Phenotypes

- **High**: Evidence includes consistent results from well-designed, well-conducted studies.
- **Moderate**: Evidence is sufficient to determine effects, but the strength of the evidence is limited by the number, quality or consistency of the individual studies, generalizability to routine practice, or indirect nature of the evidence.
- **Weak**: Evidence is insufficient to assess the effects on health outcomes because of limited number or power of studies, important flaws in their design or conduct, gaps in the chain of evidence, or lack of information.

https://cpicpgx.org/levels-of-evidence/

CPIC Definitions for Assigning Strength to Each Prescribing Recommendation

- **Strong** recommendation for the statement**:** The evidence is high quality and the desirable effects clearly outweigh the undesirable effects.
- **Moderate** recommendation for the statement: There is a close or uncertain balance as to whether the evidence is high quality and the desirable clearly outweigh the undesirable effects.

- **Optional** recommendation for the statement: The desirable effects are closely balanced with undesirable effects, or the evidence is weak or based on extrapolations. There is room for differences in opinion as to the need for the recommended course of action.
- **No recommendation**: There is insufficient evidence, confidence, or agreement to provide a recommendation to guide clinical practice at this time.

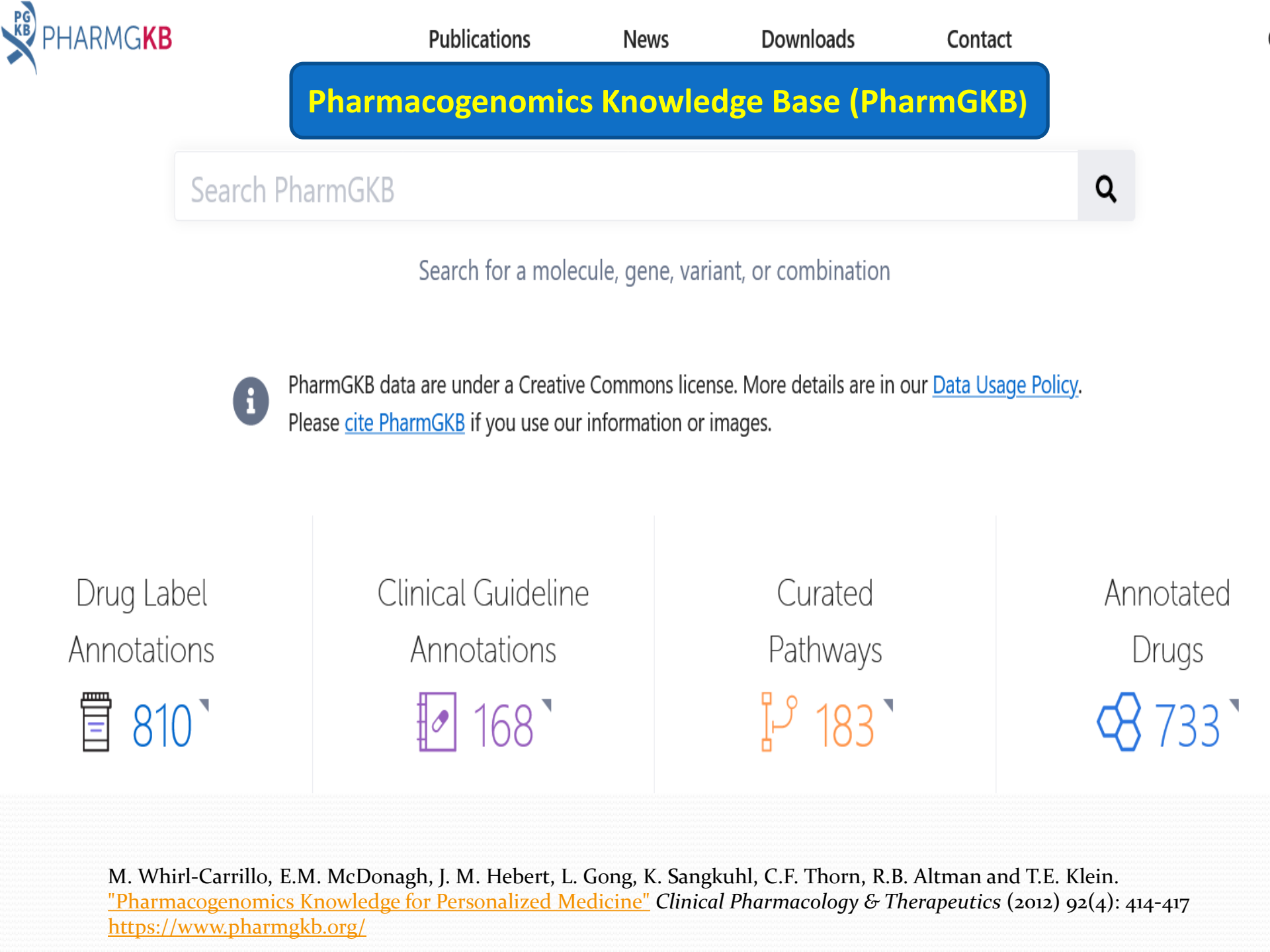
https://cpicpgx.org/strength-of-recommendations/

Level Definitions for CPIC Gene-Drug Pairs Updated

CPIC LEVEL	CLINICAL CONTEXT	LEVEL OF EVIDENCE	STRENGTH OF RECOMMENDATION
A	Genetic information should be used to change prescribing of affected drug.	Preponderance of evidence is high or moderate in favor of changing prescribing	At least one moderate or strong action (change in prescribing) recommended.
A/B	Preliminary review indicates it is likely that the definitive CPIC level will be either A or B.	Full evidence review needed to assess level of evidence, but prescribing actionability is likely	Full review by expert guideline group to assign strength of recommendation
B	Genetic information could be used to change prescribing of the affected drug because alternative therapies/dosing are extremely likely to be as effective and as safe as non-genetically based dosing.	Preponderance of evidence is weak with little conflicting data	At least one optional action (change in prescribing) is recommended.
B/C	Preliminary review indicates it is likely that the definitive CPIC level will be either B or C.	Prescribing actionability based on genetics is not clear without further evidence review	Full review by expert guideline group to assess strength of recommendation
C	There are published studies at varying levels of evidence, some with mechanistic rationale, but no prescribing actions are recommended because (a) dosing based on genetics makes no convincing difference or (b) alternatives are unclear, possibly less effective, more toxic, or otherwise impractical or (c) few published studies or mostly weak evidence and clinical actions are unclear. Most important for genes that are subject of other CPIC guidelines or genes that are commonly included in clinical or DTC tests.	Evidence levels can vary	No prescribing actions are recommended.
C/D	Preliminary review indicates it is likely that the definitive CPIC level will be either C or D.	Evidence levels can vary	No prescribing actions are recommended
D	There are few published studies, clinical actions are unclear, little mechanistic basis, mostly weak evidence, or substantial conflicting data. If the genes are not widely tested for clinically, evaluations are not needed.	Evidence levels can vary	No prescribing actions are recommended.

Navigating CPIC Resource: 7-step process for determining whether to use PGx data for patient care

1. Review the listed available PGx lab information for patient
2. If an abnormal phenotype is given (poor metabolizer, intermediate metabolizer, or rapid/URM), then review the *patient medication list* to determine whether any of these medications are listed under the gene-drug pairs tab on the CPIC website
3. If a gene-drug pair is listed for the medication of interest, determine the CPIC level of evidence (i.e. A or B; A has the strongest info)
4. If the gene-drug pair is associated with actionability (i.e. CPIC level evidence of A or B), then access the CPIC guideline if it is available
5. Once the CPIC guideline is accessed, review Table 2 for therapeutic recommendations for the patient's given phenotype for the gene of interest
6. Determine whether this therapeutic recommendation in Table 2 is associated with a moderate or strong recommendation
7. If a moderate or strong recommendation is listed, then this recommendation might be helpful to use in optimizing treatment for the disease state of interest for the patient



M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine" *Clinical Pharmacology & Therapeutics* (2012) 92(4): 414-417
https://www.pharmgkb.org/

Annotations

Clinical

CLINICAL GUIDELINE ANNOTATIONS	168
DRUG LABEL ANNOTATIONS	810
FDA DRUG LABEL ANNOTATIONS	382
CLINICAL ANNOTATIONS	4,898

Research

PATHWAYS	183
VIPs (Very Important Pharmacogenes)	68
VARIANT ANNOTATIONS	25,145
ANNOTATED DRUGS	733

Resources

- Cancer Pharmacogenetics
- Gene Information Tables
- Drugs with Prescribing Information
- Biogeographical Groups for PGx
- PGx Publication Tips
- Therapeutic Resource for COVID-19

M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine" *Clinical Pharmacology & Therapeutics* (2012) 92(4): 414-417
https://www.pharmgkb.org/whatIsPharmgkb

PGx Gene-specific Information Tables

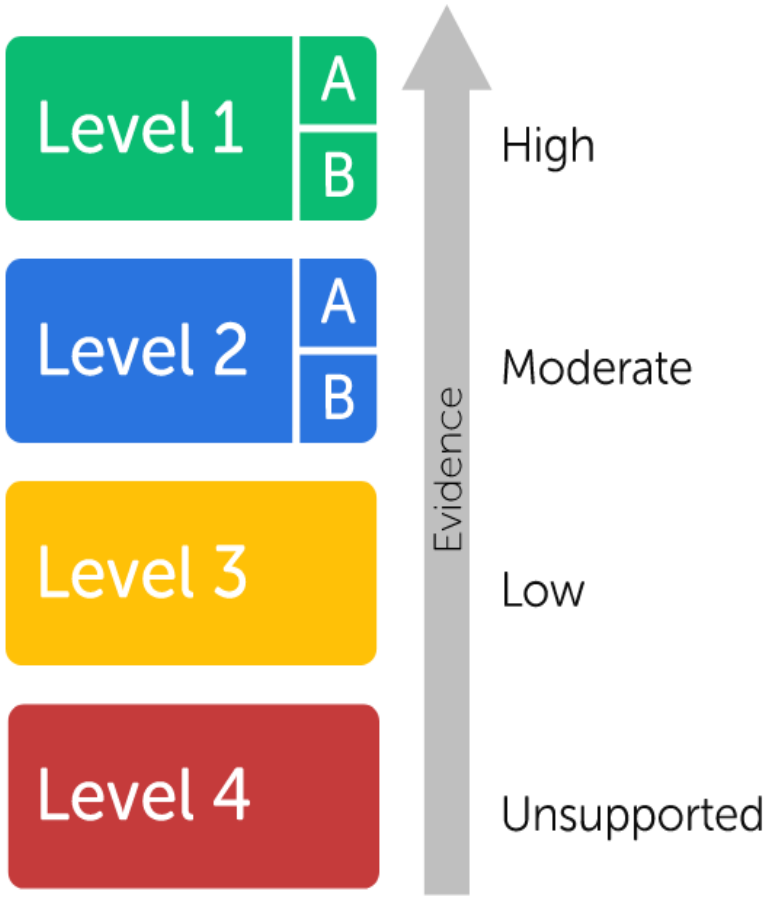
CACNA1S	CYP2B6	CYP2C19	CYP2C9	CYP2D6
CYP3A5	CYP4F2	CFTR	DPYD	G6PD
HLA-A/B	IFNL3	MT-RNR1	NUDT15	RYR1
SLCO1B1	TPMT	UGT1A1	VKORC1	

The above gene links lead to information tables created by PharmGKB and CPIC. The files support CPIC guidelines, but are also general resources for these PGx genes. The following types of files are provided by gene, as available:

- Allele Definition Table
  - Information about what variants define star (*) or named alleles
  - Mapping of variants to the human genome GRCh38, the RefSeq Gene sequence and protein sequence, and provides rsIDs, if available
- Allele Functionality Table
  - Allele function assignments using CPIC standardized terms
  - References for the allele function
- Frequency Table
  - Population-based allele frequency reported by references
  - Calculated allele frequency by PharmGKB biogeographical groups based on frequencies reported by references
    - Further details about the biogeographical grouping system can be found here or in [Article:30506572]
  - Calculated diplotype frequency (if applicable)
  - Calculated phenotype frequency (if applicable)

M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine" *Clinical Pharmacology & Therapeutics* (2012) 92(4): 414-417
https://www.pharmgkb.org/whatIsPharmgkb

Clinical Annotation Levels of Evidence



M. Whirl-Carrillo, E.M. McDonagh, J. M. Hebert, L. Gong, K. Sangkuhl, C.F. Thorn, R.B. Altman and T.E. Klein. "Pharmacogenomics Knowledge for Personalized Medicine" Clinical Pharmacology & Therapeutics (2012) 92(4): 414-417
https://www.pharmgkb.org/whatIsPharmgkb

Other Available Resources for Navigating PGx information

- Dutch Pharmacogenetics Working Group (DPWG)
  - https://www.pharmgkb.org/page/dpwg
- Professional society guidelines for gene-drug pairs
- Clinical Genome Resource (ClinGen)
  - https://clinicalgenome.org/
- **Pharm**acogene **Var**iation (**PharmVar**) Consortium
  - www.PharmVar.org
- **Pharm**acogenomics Global Research Network
  - www.pgrn.org/