Intro to Variant Interpretation

Introduction

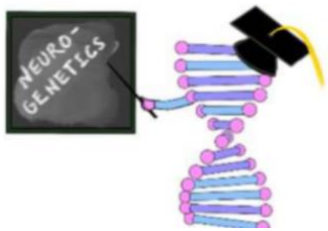
In this portion of the class, you will learn:

- The importance of ACMG criteria
- The 5 variant classifications
- The type of evidence considered by ACMG criteria
- An overview of how this evidence is weighted

Understanding the Report

Describe the type of genetic report and what the result is indicating:

1. arr[hg19]2q33.1(198,356,789-203,491,035)X1
2. c.346G>C (p.G116R)
3. c.1945dupT (p.S649FfsX40)
4. c.847C>T (p.R283X)
5. c.487+1G>T



Review: Terminology …

To make sure we're all on the same page, some terms:

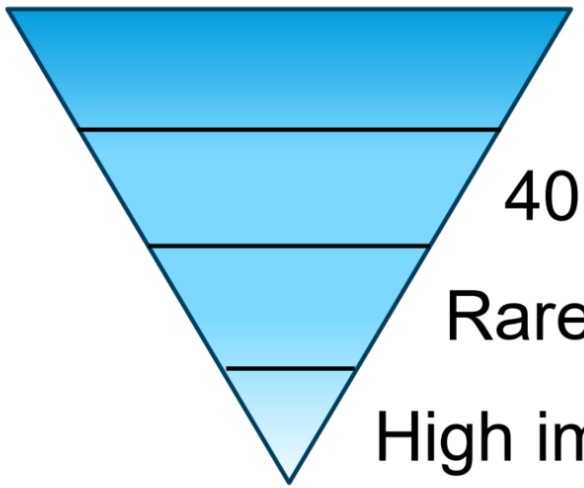
Variants

- **Missense variant:** variant leading to a protein change (ex. Arg515Ser)
- **Nonsense variant:** variant leading to the introduction of a premature stop codon (ex. Ser44Ter)
- **Silent variant:** variant leading to no protein change (but may have an effect on splicing)
- **Indel:** an (usually small) insertion and/or deletion
- **Loss of function (LOF) variant**: a variant leading to truncation of the gene / protein.

Other terms

- **Proband:** the individual presenting with disease
- **Penetrance:** the proportion of individuals with a pathogenic variant in a given gene who express the associated trait (disease).

Genome Sequencing Yields Many Variants



4.1 – 5.0 million total variants

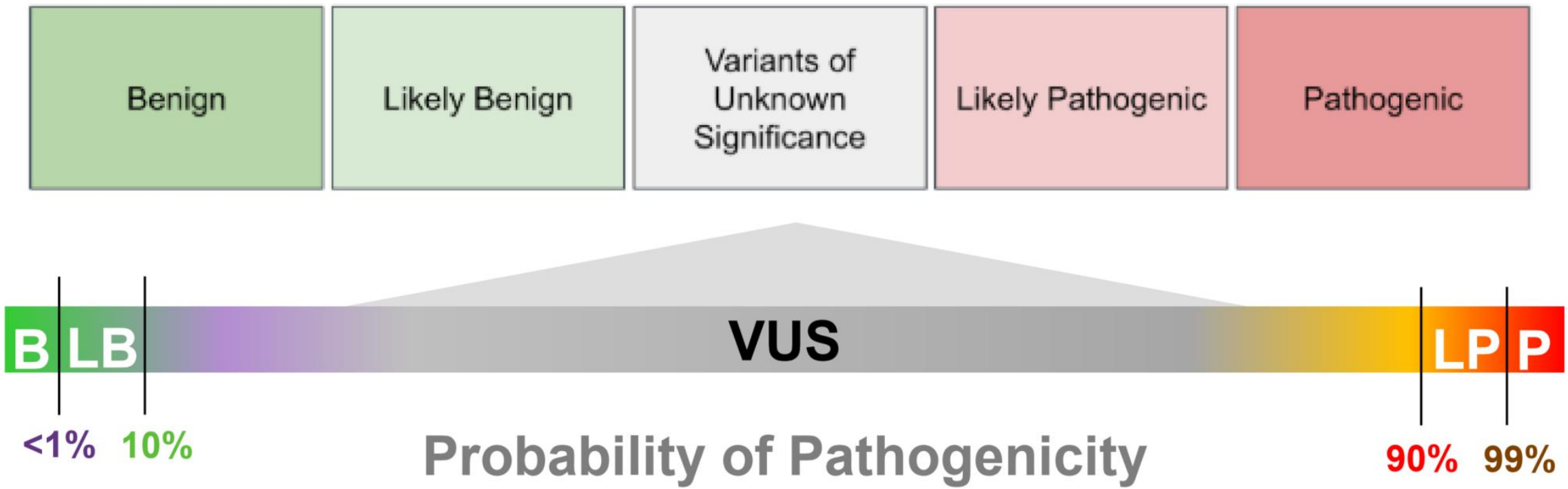
40,000 – 250,000 rare variants

Rare variants in disease relevant genes (n = ?)

High impact, rare variants in disease relevant genes (n = ?)

- How do we determine which variants are disease-causing in a consistent way?

ACMG Criteria 2015



ACMG Criteria 2015

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 **OR** observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

https://pubmed.ncbi.nlm.nih.gov/25741868/

- The American College of Medical Genetics (ACMG) criteria provide a common language for variant classification.
- 8 categories of evidence for either benignity or pathogenicity
- Evidence is ranked in different “strengths”

ACMG Criteria Beyond 2015 …

ACMG Publications

Major papers

- 2015 original paper - https://pubmed.ncbi.nlm.nih.gov/25741868/
- 2019 overview of updates - https://pmc.ncbi.nlm.nih.gov/articles/PMC6885382/
- 2020 recommendations for CNVs - https://pubmed.ncbi.nlm.nih.gov/31690835/

Specific criteria updates

- End of the “reputable source” criteria (BP6 / PP5): https://pubmed.ncbi.nlm.nih.gov/29543229/
- Updated recommendations for BA1: https://pubmed.ncbi.nlm.nih.gov/30311383/
- Updated recommendation for PM3: https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf
- Updated recommendation for PS2: https://clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_1.pdf
- Calibration of *in silico* tools for PP3 / BP4: https://pubmed.ncbi.nlm.nih.gov/36413997/
- Updated recommendation for PVS1: https://pubmed.ncbi.nlm.nih.gov/30192042/
- Applying PVS1 to splicing variants: https://pubmed.ncbi.nlm.nih.gov/37352859/
- PM2 transition to PM2_supp: https://clinicalgenome.org/site/assets/files/5182/pm2_-_svi_recommendation_-_approved_sept2020.pdf

Miscellaneous

- Transition to Bayesian (points) system - https://pubmed.ncbi.nlm.nih.gov/29300386/

Strengths of ACMG Criteria

The strength of most criteria is no longer static:

Criteria code	Brief Description	Strength 2015	Strength Range 2025
PVS1	Loss of function	very strong	moderate – very strong
PS1	Same AA change	strong	supporting – strong
PS2	*De novo*	strong	supporting – very strong
PS3	Functional evidence	strong	supporting – very strong
PS4	Prevalence in affected pop.	strong	supporting – strong
PM1	Functional domain	moderate	supporting – strong
PM2	Rare in pop. controls	moderate	supporting
PM3	*In trans*	moderate	supporting – very strong
PM4	Length changing	moderate	supporting – moderate
PM5	Same position, different AA	moderate	supporting – strong
PM6	Assumed *de novo*	moderate	supporting – very strong
PP1	Cosegregation	supporting – strong	supporting – strong
PP2	Intolerant to missense	supporting	supporting
PP3	*In silico*	supporting	supporting – moderate
PP4	Specific phenotype	supporting	supporting – moderate
PP5	Reputable source	supporting	discontinued

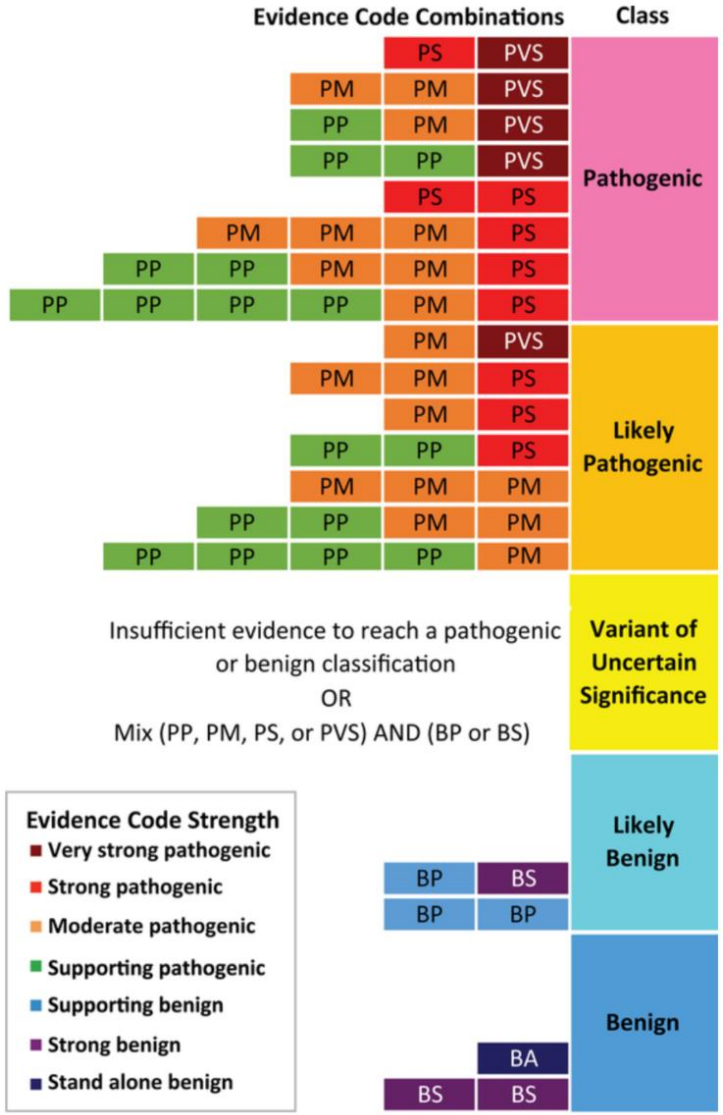
Strengths of ACMG Criteria

The strength of most criteria is no longer static:

Criteria code	Brief Description	Strength 2015	Strength Range 2025
BA1	Population prevalence	stand alone	stand alone
BS1	MAF is too high	strong	supporting – strong
BS2	Present in healthy adults	strong	supporting – strong
BS3	Functional evidence	strong	supporting – strong
BS4	Non-segregation	strong	supporting – strong
BP1	Missense in a LOF gene	supporting	supporting
BP2	In cis with recessive / in trans with dominant	supporting	supporting
BP3	Indel in a repeat region	supporting	supporting
BP4	*In silico*	supporting	supporting – moderate
BP5	Alternative cause found	supporting	supporting
BP6	Reputable source	supporting	discontinued
BP7	Splice variant with no prediction	supporting	supporting

ACMG Point System

Being Phased Out



Now

Type	Strength	Bayesian points
Pathogenic	very strong	+8
	strong	+4
	moderate	+2
	supporting	+1
Benign	strong	- 4
	moderate	- 2
	supporting	- 1

Score Range	Class
≤ -6	Benign
-5 to -1	Likely benign
0 to 5	VUS
6 to 9	Likely pathogenic
≥ 10	Pathogenic

- Rather than combinations of codes, classifications are now encouraged to be assigned with a Bayesian classification framework (Tavtigian 2018)
- By 2026, criteria code names will be changed, and the points system will be made “official”.
  - The underlying logic of the criteria will remain the same.

So, You Found a VUS…

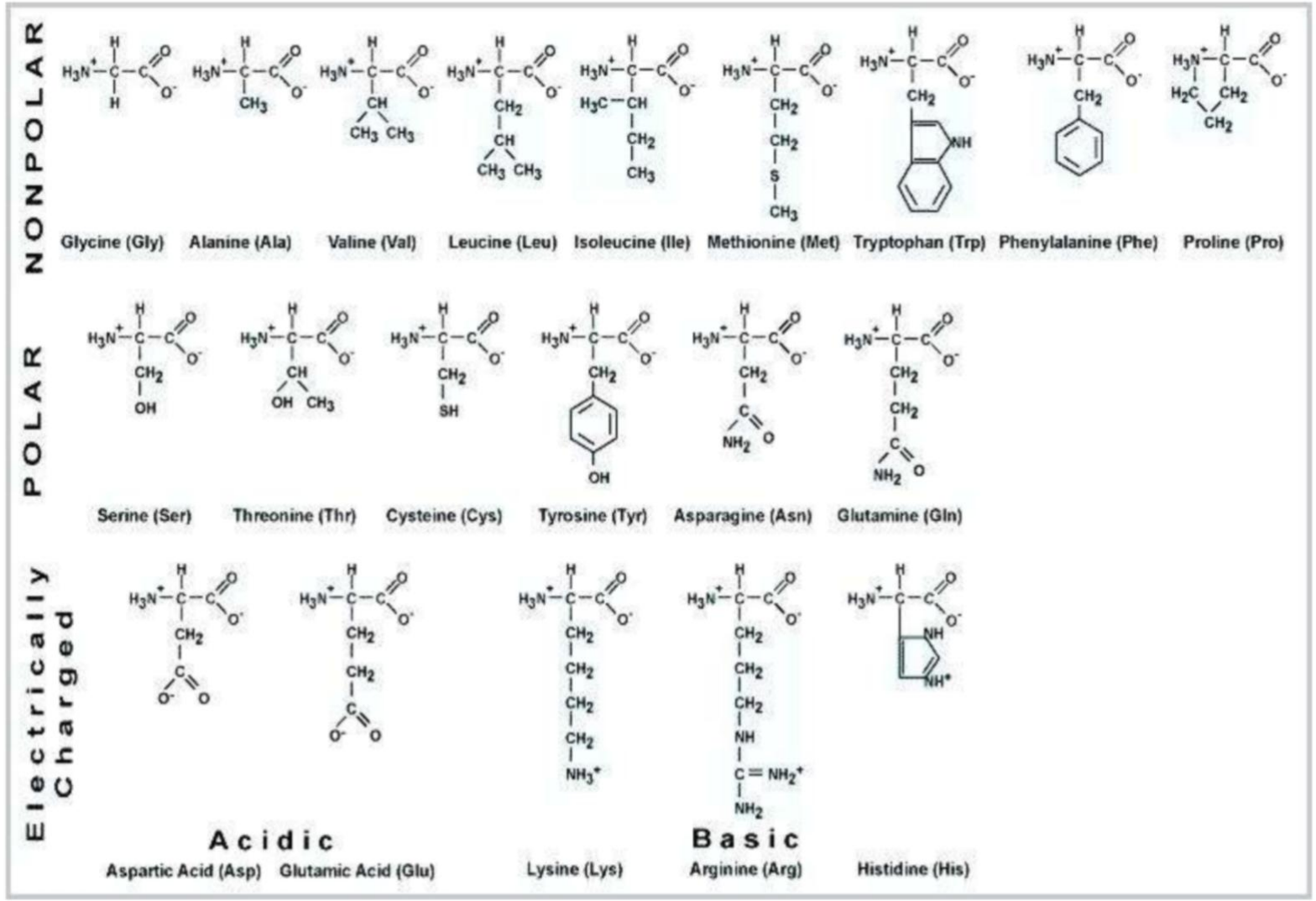
- For copy number variants:
  - What genes are included in the area?
  - How big is the area?
  - Is it inherited or de novo?
  - Is it a deletion or duplication?

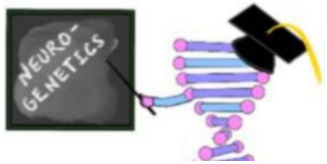
- For single nucleotide changes:
  - What type of change is it?
  - Is an amino acid changed?
  - How similar is the amino acid?
  - Is it conserved?
  - Does it occur in an important part of the protein?
  - Is it found in population databases? (ExAC, 1000 genomes)
  - Has it been shown to segregate with disease?
  - Have there been any functional studies?

Physiochemical Properties of Amino Acids

Relevant for missense variants:

Bigger physiochemical difference → more likely to alter protein conformation→ disrupted protein function





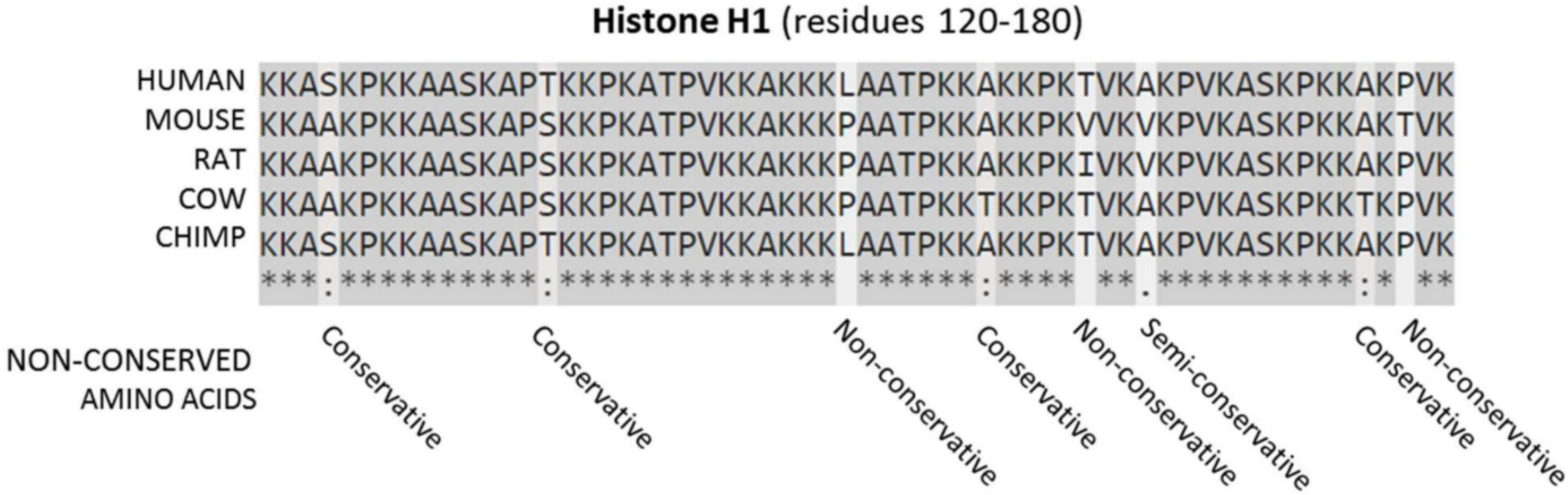
Conservation:

High = same base across species/evolution

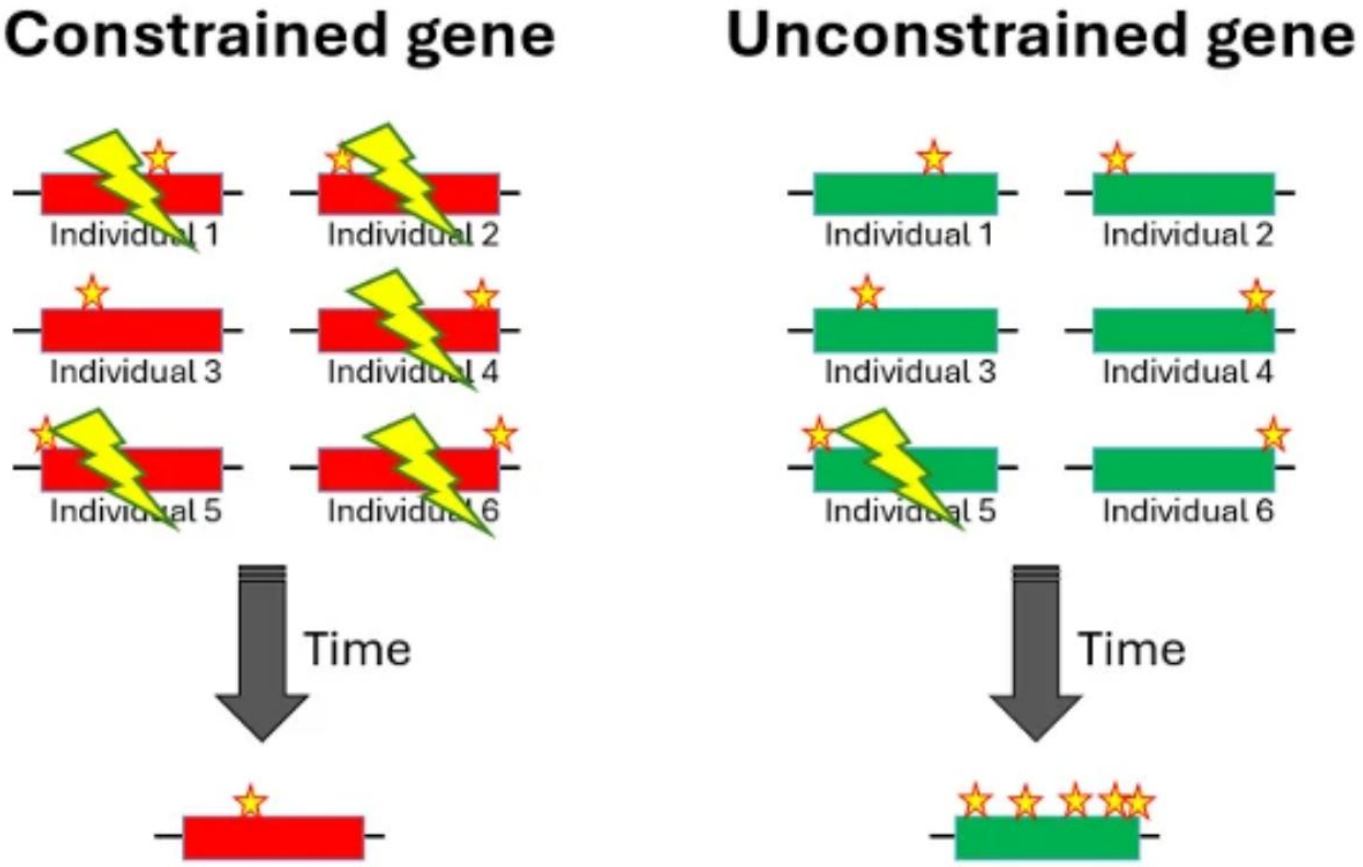
→ might be important

Low = varies across species/evolution

→ might not be important



Constraint: Intolerance to ‘mutation’ within species



Click to enlarge

Figure 1. The concept of constraint. Most mutations that arise in a constrained gene are not passed to subsequent generations as they are detrimental, therefore over time, there are few mutations within constrained genes. Mutations in an unconstrained gene are tolerated and, therefore accumulate over time. Stars represent mutations, lightning bolts represent that the mutation is removed by natural selection.

Summary of Overview

- ACMG criteria give us a common language with which we can characterize variants

- Variant interpretation involves both **assigning criteria** and **determining the strength** of the criteria assigned
    - Strength of criteria has evolved over time

- Summation of the assigned criteria's associated Bayesian points yields a final classification