The ACMG/AMP Recommendations for Variant Classification – Resources and Challenges

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Agenda

- What is the ACMG/AMP Classification system and why is it essential for DNA Dx?
- Resources and Challenges for proper Variant Classification
- A glimps on ACMG/AMP V4

The ACMG/AMP Classification System (SNV)

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

- Determine if a variant (SNV) in a gene with a *definitive* role in a Mendelian disorder is pathogenic
- Reduce number of variants reported as „causative“ of disease without sufficient evidence
- Qualitative evaluation of different data types (**28 defined criteria with assigned code**), no „mutation“ but „**variant**“ …
  - **Pathogenic** (supporting, moderate, strong, very strong)
  - **Benign** (supporting, strong)

General Considerations:

- for variants in all Mendelian genes (single gene, gene panel, exome, genome or transcriptome)
- be carefull with candiate genes („genes of uncertain significance“; „GUS“; Jan 2026: OMIM 7,086 genes)
- intendend to be used by clinical labs who report DNA variants

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Evidence categories

	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			
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Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

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The ACMG/AMP system is based on a mathematical foundation

Tavtigian et al 2018

Odds Path: 1:18.7 | 1:2.08 | 2.08:1 | 4.33:1 | 18.7:1 | 350:1

.... and it can be fitted in a point-based system

Tavtigian et al 2018

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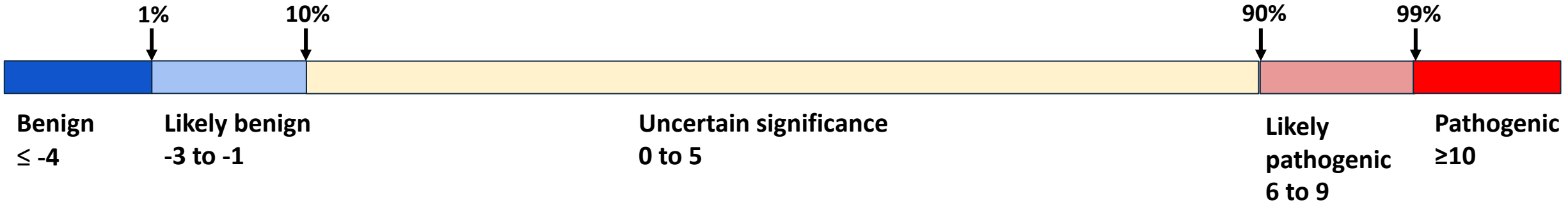
Tavtigian et al 2020

Points: -4 | -1 | 1 | 2 | 4 | 8

- Assigns a point value to each evidence strength level
- Classification can be determined by summing total points applied



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The classification of a variant forms the basis for clinical interpretation and clinical actionability

- Treatment
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This may sound a little dramatic, but:

- The correct classification of sequence variants (and clinical interpretation) literally determines life or death for many patients!

Table 1 InSiGHT variant classification scheme with accompanying recommendations for family management, adapted from the IARC five-tiered classification system

InSiGHT MMR gene variant class definition for Lynch syndrome[a]	Predictive testing of at-risk relatives	Surveillance for at-risk relatives	Research testing of relatives
5 (pathogenic)	Yes	Full high-risk guidelines	Not indicated
4 (likely pathogenic)	Yes[b]	Full high-risk guidelines	Yes
3 (uncertain)	No[b]	Family history and other risk factors	Yes
2 (likely not pathogenic)	No[b]	Family history and other risk factors; treated as having no mutation detected in this gene for this disorder	Yes
1 (not pathogenic)	No[b]	Family history and other risk factors; treated as having no mutation detected in this gene for this disorder	Not indicated

Adapted from Plon *et al.*[10]. Full high-risk surveillance guidelines for cancers in the Lynch syndrome spectrum are outlined in Vasen *et al.*[1]. Research testing entails cascade testing for the variant in affected and unaffected family members to facilitate segregation analysis and is indicated for variants in classes 2–4 to refine classification. Consent from subjects through a protocol approved by a human subjects committee should be obtained.

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Variant Classification ≠ Variant Interpretation

- Variant classification describes the process of aggregating all evidence to determine the probability that a specific variant in a gene is causally associated with a specific disease and a specific inheritance mechanism (‘probability of pathogenicity’).

- Clinical variant interpretation describes the process of assessing whether a particular variant is associated with a disease or disease risk in an individual patient (‘probability of the correctness of the diagnosis of the patient’).

It is important to distinguish between these two activities; the ACMG/AMP classification guidelines apply only to variant classification, not to clinical interpretation or diagnosis.

PMID: 31775856; **Leslie Biesecker**: Genomic screening and genomic diagnostic testing—two very different kettles of fish; Genome Med. 2019 Nov 27;11:75

The ACMG/AMP system is !

The ACMG/AMP system is not a “wishlist” !

	+**PS3** 108bp pseudoexon created in intron 54 detected by RNASeq
c.14566G>A (p.Ala4856Thr)	Likely pathogenic (PM1, PP2, PM2, PP3)
c.3215G>A (p.Arg1072Gln)	Likely pathogenic (PM2, PP3, PM3)
c.3215G>A (p.Arg1072Gln)	
c.93_113dup (p.Ala33_Gln39dup)	Likely pathogenic (PM2, PM4, PM6)
c.188T>A (p.Leu63Gln)	Likely pathogenic (PM2, PP3, PM3)
c.188T>A (p.Leu63Gln)	**Required functional studies** +**PS3** reduced dystroglycan in muscle biopsy
c.1522_1524del (p.Ser508del)	Likely pathogenic (PM2, PM4, PP5, PM3)
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c.909+7A>G	Likely pathogenic (PM2, PP3, PM3)
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c.579-1G>A	Pathogenic (PVS1, PM2, PP5)
c.579-1G>A	+PS3 exon 7 truncation detected by RNASeq
c.1741A>T (p.Met581Leu)	Pathogenic (PS4, PM1, PP2, PM2, PP3)
c.1660C>T (p.Pro554Ser)	Likely pathogenic (PM3, PM2, BP4)
Homozygous complex structural variant involving an inversion and a duplication in chr1:6524620-6529620	
c.114T>A (p.Tyr38Ter)	Likely pathogenic (PVS1, PM2)
c.26T>C (p.Leu9Pro)	Likely pathogenic (PM2, PM3, PP3, PP4)
c.4G>T (p.Glu2Ter)	Likely pathogenic (PVS1, PM2)
c.923T>G (p.Leu308Arg)	Likely pathogenic (PM2, PP3, PM3)
c.614T>C (p.Phe205Ser)	Likely pathogenic (PM2, PP3, PP2, PM6)
c.2004del (p.Glu668AspfsTer5)	Pathogenic (PM3, PVS1, PM2)
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PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of diseas

Not Applicable

Comments: RYR1 is not a gene that is constrained for missense variation. Hence PP2 is not applicable.

2 Mod + 1 Sup = VUS (5 pts)

2 Mod + 1 Sup = VUS (5 pts), PM2 not reduced to supporting (in all cases)

PP5 is not valid! PM3 cannot be used (for homoz max 1 point, supporting)

PP5 is not valid! Double counting: PVS1+PS3 can not come together,PVS1_RNA

1x in ClinVar and no clinical data! If choosen, max as supporting

PM3 + PM2 + BP4 = LP??? Crazy miscalculation… 2 pts = „cold VUS“ at best

1 Mod + 3 Sup = VUS (4 pts)

1 Mod + 2 Sup = VUS (3 pts)

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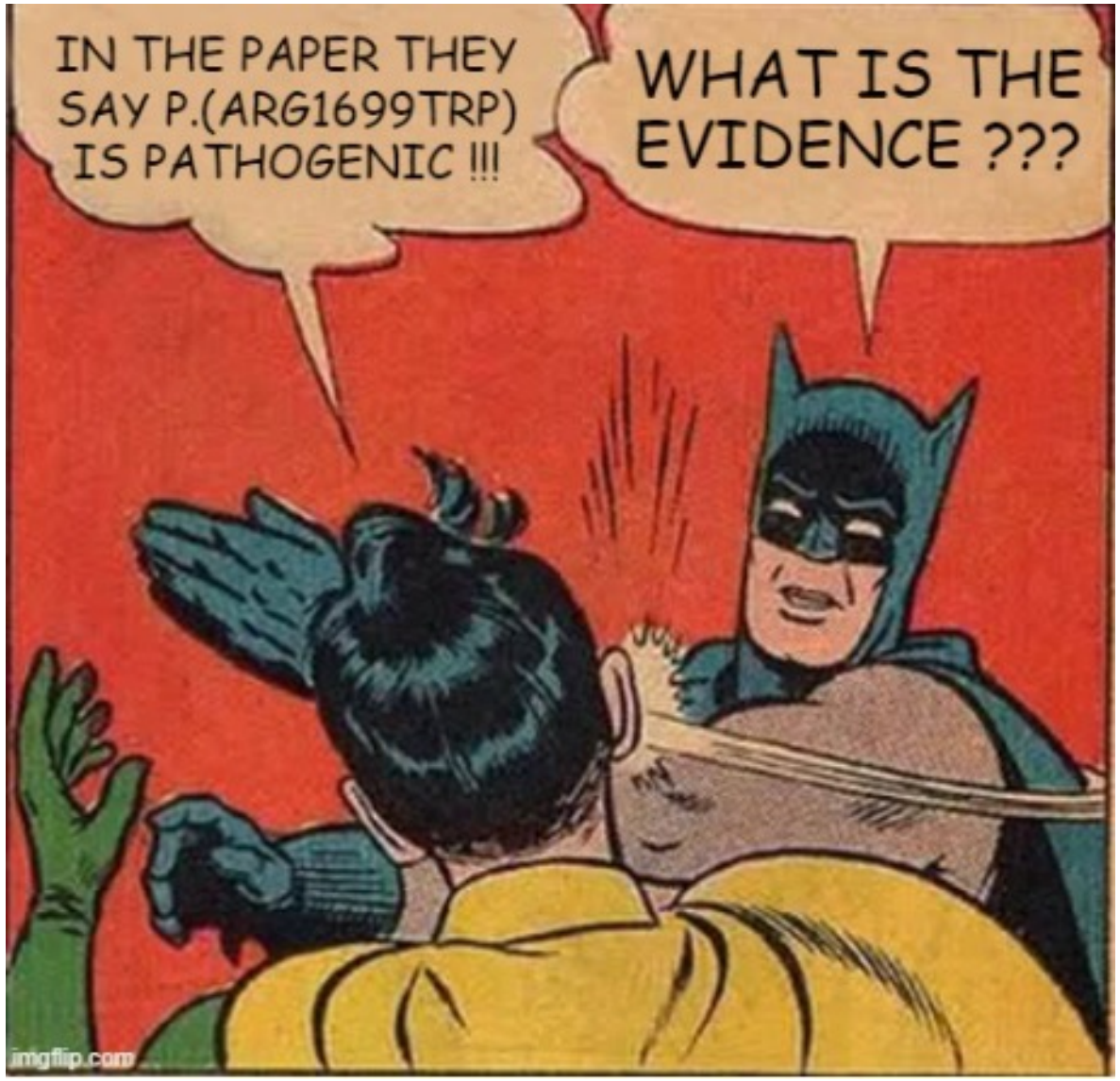
The “First Rule” in Variant Classification?



The “First Rule” in Variant Classification?

- Never uncritically adopt an opinion (=classification) e.g. from
  - ✓ Databases (*exception: *curated* data from LOVD, ClinVar-VCEP Expert Panel)
  - ✓ Literature (e.g. summary table: „known as pathogenic“, „found this pathogenic variant“)
  - ✓ (**AI**) Variant interpretation software tools (VarSome, QCI, Franklin, …)
  - ✓ Genetic reports (e.g. if no evidence given)
  - ✓ Meetings, Posters, Conferences (e.g. personal communication, yeah, especially me included)
- Always try to find the underlying evidence and, if necessary, interpret the variant according to all (new) available data
- Use the ACMG/AMP classification system (better: VCEP specifications)
- Always assume that the variant is not pathogenic and try to refute this claim (‘falsification’) instead of postulating that the variant is pathogenic and attempt to prove it (‘verification’).
- Be aware of our biased brain: We are programmed to want to ‘find’ something and tend to ignore evidence that does not fit our assumption/ expectation.

The challenge of using the ACMG/AMP System

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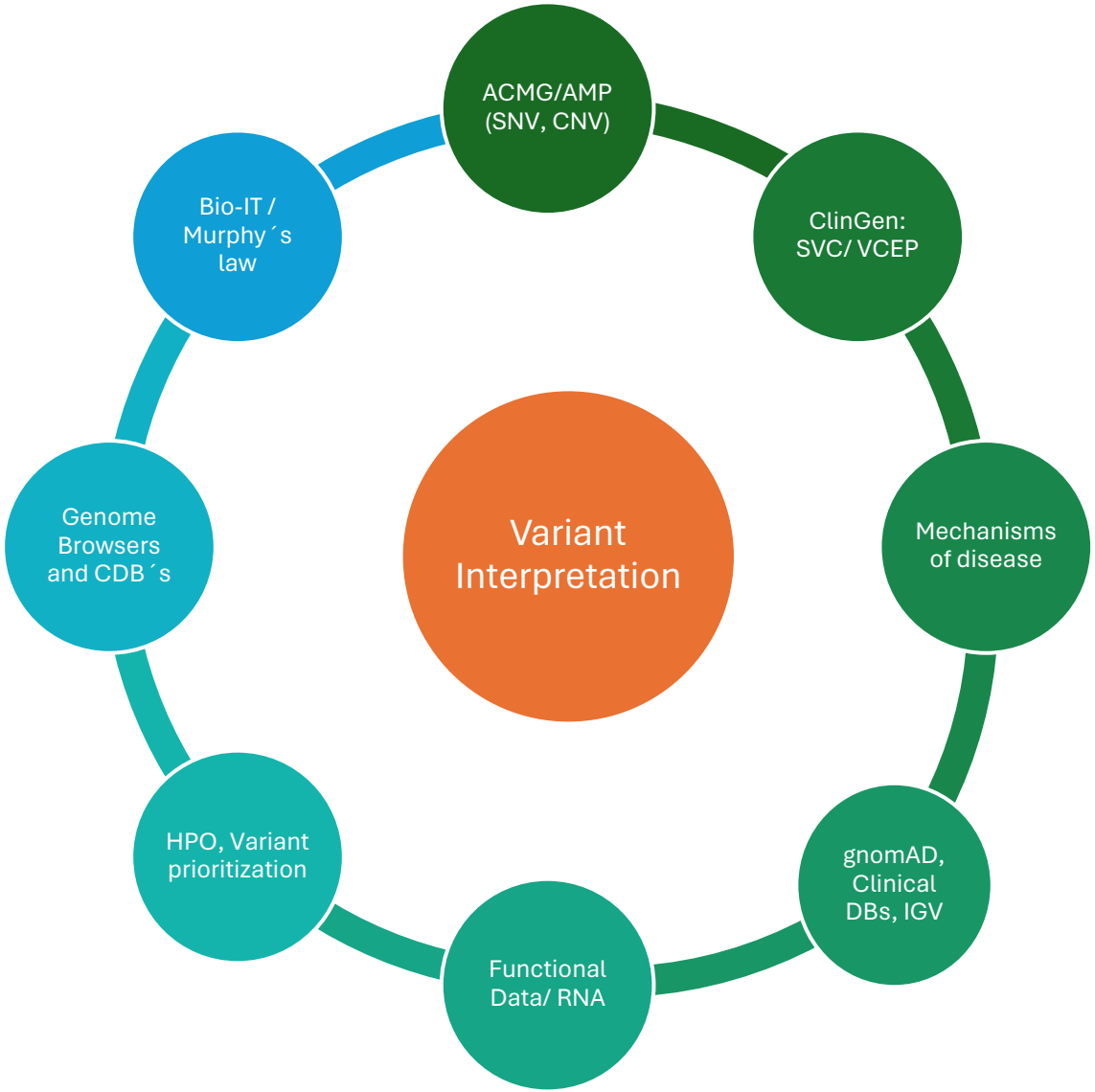

How can I learn all those things …?

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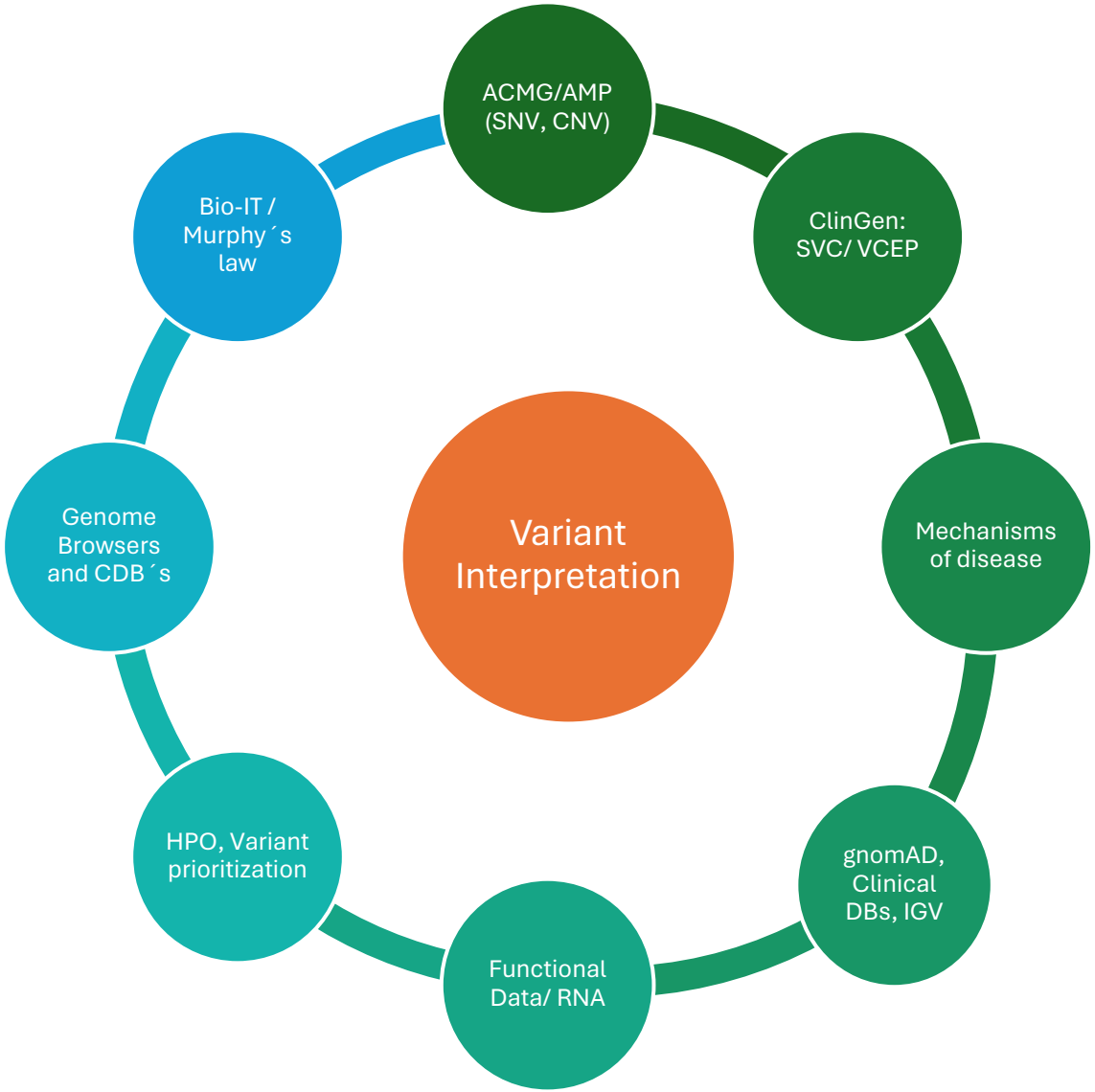
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			



…. HUGO´s veptc is the answer

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- Phenotype description - Human Phenotype Ontology

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- Ensembl - variant annotation with VEP
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- UCSC recommended track sets (SNV & CNV)
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RNA and other functional/prediction tests
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- Mechanisms of RNA disease
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- Online tools - MobiDetails (workshop)
- SNV variant classification basic (workshop)
- SNV variant classification advanced (workshop)
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Goals of ACMG V4

- Revise current standards to address:
  - appropriateness of criteria (PP5/BP6, PM2)
  - ambiguities in the application of criteria
  - strength assigned to criteria,

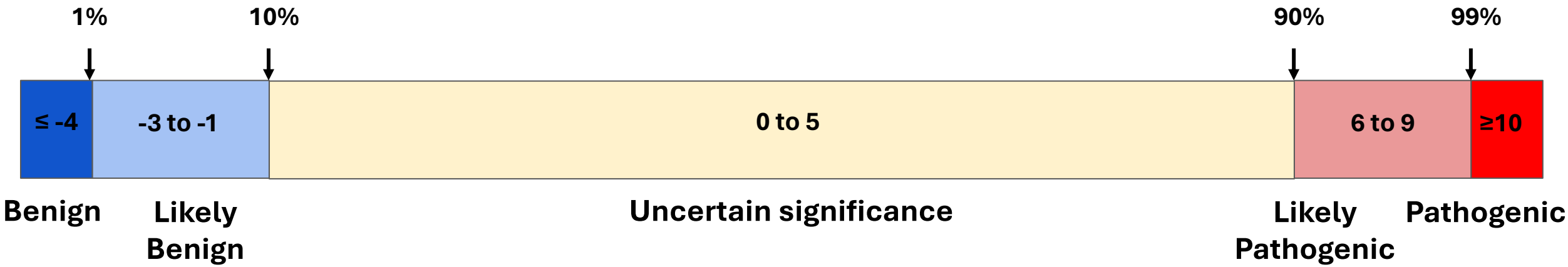
    individually and in combination
  - avoidance of double-counting
  - guidance for combining pathogenic and benign criteria
- Incorporate updates from ClinGen working groups, and develop mechanism for ongoing updates
- Where possible, establish more quantitative parameters

	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 *BP4* Missense when 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	Non-segregation with disease *BS4*		Co-segregation with disease in multiple affected family members *PP1*	Increased segregation data →		
De novo Data				*De novo* (without paternity & maternity confirmed) *PM6*	*De novo* (paternity & 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*			

Big Picture - v3 (Richards et al) versus v4

- Incorporate Gene Disease Validity
- Points based system
  - More granular, improving calibration
  - Allows combining of positive and negative evidence
- Evidence codes
- Decision trees
- Evidence types - some new, some eliminated, many altered weights

Bayesian ‘points’ adaptation of SVC v3 = v4 foundation



A few modifications on the LB/B side….

- Richards et al did not define boundaries for P and B
  - Bayesian framework assumed B = 0.1% probability of causing disease
- **For v4, we are using a 1% threshold for B**
- We maintaining the 10% threshold for LB, but lowering the relative weight needed to reach LB

Evidence Category	Evidence Concept	Evidence Code	Code Workflows	Evidence Code Cap	Evidence Concept Cap	Evidence Category Cap
Human Observational Data	Population Observations (POP)	**POP_FRQ** *Population Frequency*	Allele Frequency	-6 - 0	-10 - 0	N/A - N/A
		POP_HMZ *Population Homozygotes/Hemizygotes*	Hom/Hemi - Autosomal Dominant disorder	-4 - 0		
			Hom/Hemi - Autosomal Recessive / X-linked disorder			
	Clinical Observations (CLN)	**CLN_UAF** *Unaffected Observations*	Unaffecteds - Autosomal Dominant disorder	N/A - 0	N/A - N/A	
			Unaffecteds - Autosomal Recessive / X-linked disorder			
		CLN_ALT *Alternate Cause*	Alternate Cause (Variant) - Autosomal Dominant disorder	N/A - 0		
			Alternate Cause (Variant) - Autosomal Recessive / X-linked			
			Alternate Cause (Gene)			
		CLN_AFF *Affected Observations*	Affecteds - Autosomal Dominant disorder	0 - N/A		
			Affecteds - Autosomal Recessive / X-linked disorder			
		CLN_DNV *De Novo Observations*	De novo	0 - +12		
		CLN_CCS *Case-Control Studies*	Case - Control	-? - +?		
	Locus Specificity (LOC)	**LOC_PHE** *Specific Phenotype*	Specific Phenotype	0 - +4	0 - +4	
		LOC_SEG *Segregations with Disease*	Co-segregation - Autosomal Dominant disorder	0 - +4		
			Co-segregation - Autosomal Recessive disorder			
			Co-segregation - Autosomal Semi-Dominant disorder			
			Co-segregation - X-linked disorder			
Predictive and Functional Data	Variant Impact (XXX) *NB Applicable code determined based on evaluated variant impact*	**MIS_** *Single amino acid change*	**MIS_PRD** (*Predicted single amino acid change*)	-4 - +4	-8 - +6	-8 - +9
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Nonsense Variant in v3 framework

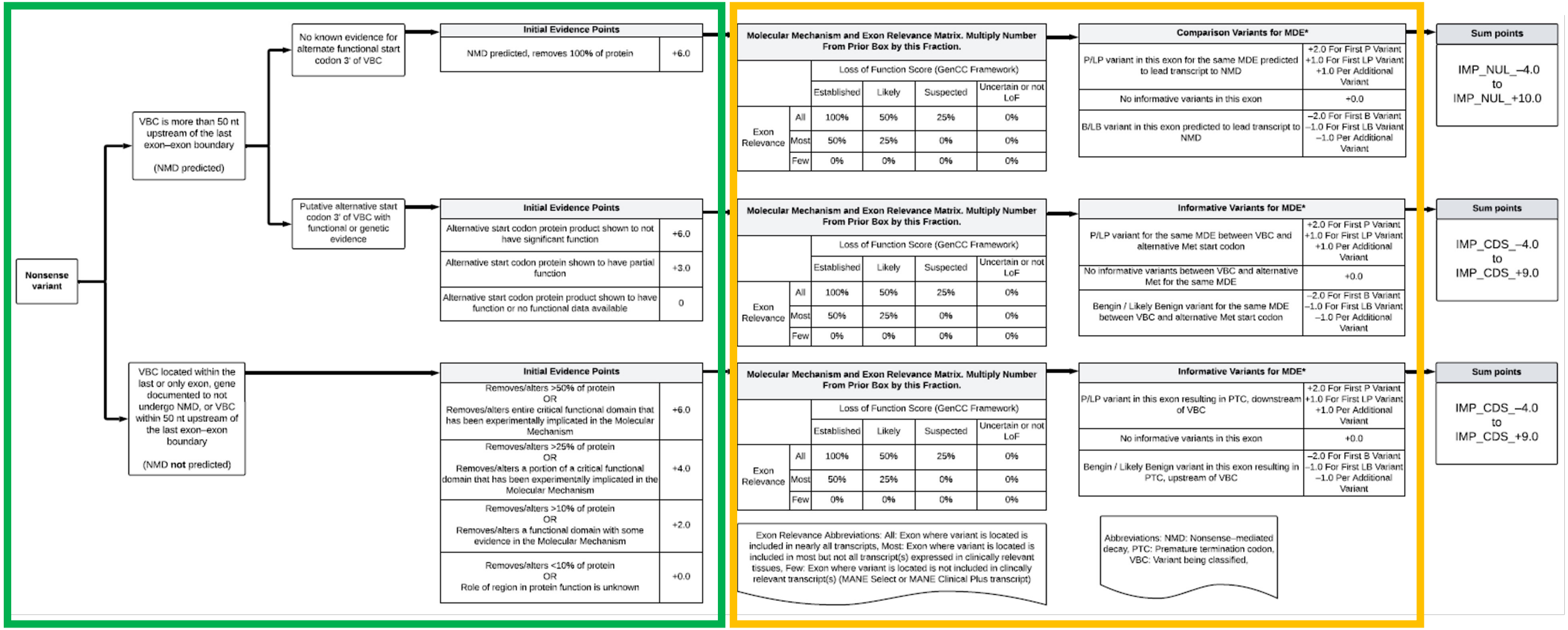
PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*)
- Use caution interpreting LOF variants at the extreme 3′ end of a gene
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts

Caveats and considerations are provided but no instructions on what to do with this information or how to assess it

Nonsense Variant in v4 framework



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