MAYO CLINIC

CLINICAL VARIANT INTERPRETATION

USING ACMG/AMP CRITERIA TO CLASSIFY VARIANTS

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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

Review: Terminology …

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

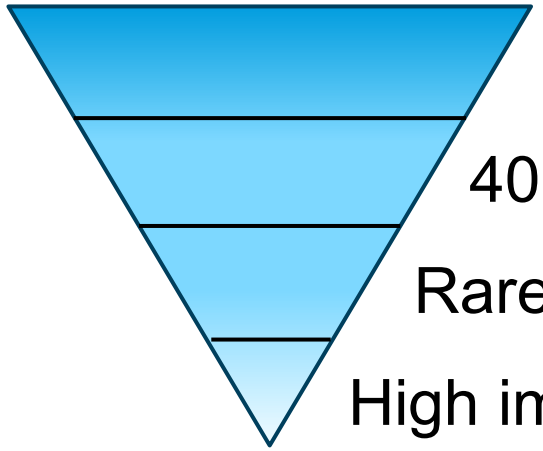
Variants

- **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

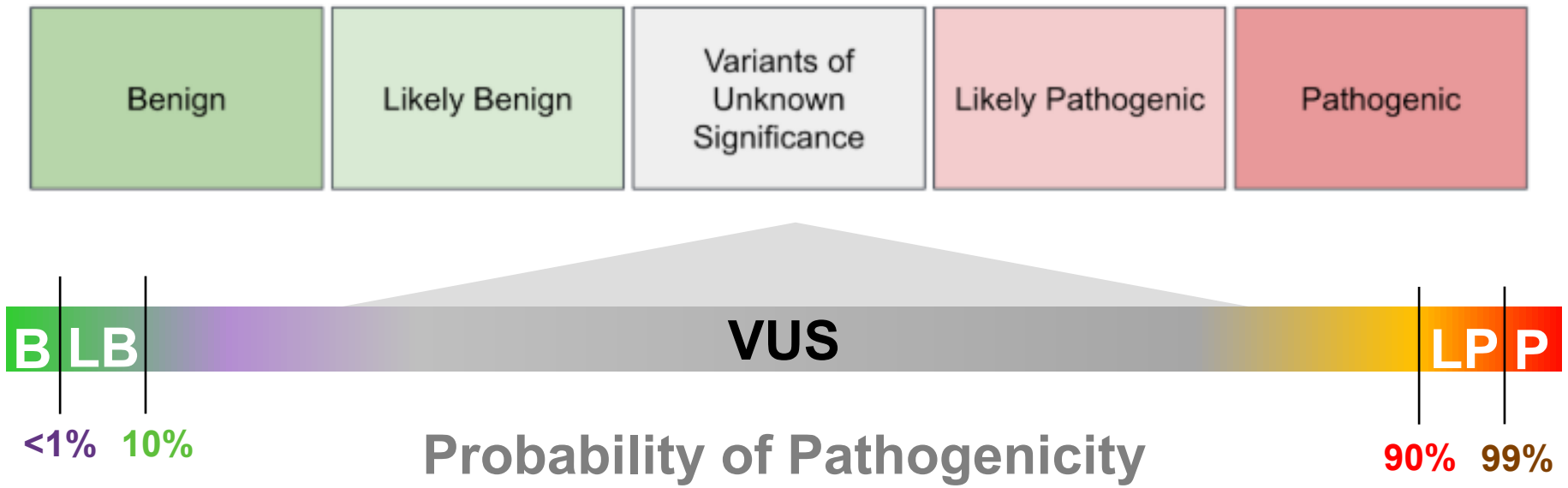
40,000 – 250,000 rare variants

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



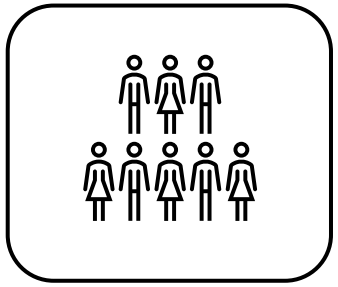
ACMG Criteria 2015

	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			

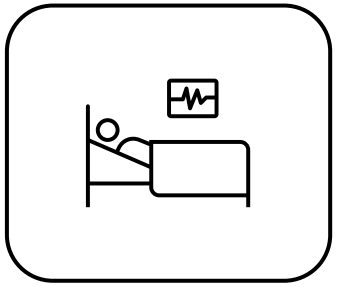
https://pubmed.ncbi.nlm.nih.gov/25741868/

- 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”

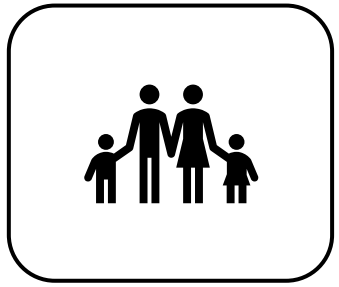
Criteria Evidence



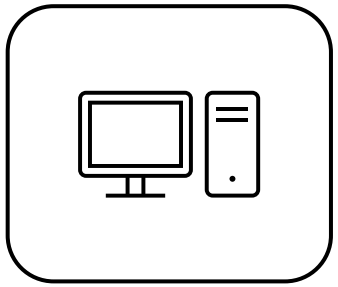
Population frequency
BA1, BS1, BS2, PM2



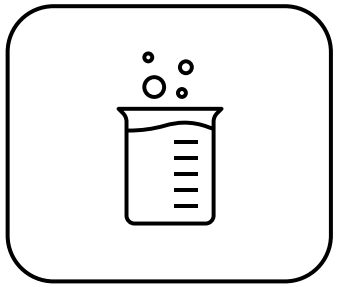
Case level evidence
PS1, PS4, PM3, PM5



Familial evidence
BS4, PS2, PM6, PP1



***In silico* predictions**
BP1, BP3, BP4, BP7, PVS1, PM4, PP2, PP3



Functional data
BS3, PS3, PM1

Specific phenotype
PP4

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

Criteria Naming Convention

PM1

type evidence; P = supports pathogenic, B = supports benign

strength of evidence

- P = supporting
- M = moderate
- S = strong
- VS = very strong

differentiating number

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

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 Today
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 Today
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

(Modified) Criteria Naming Convention

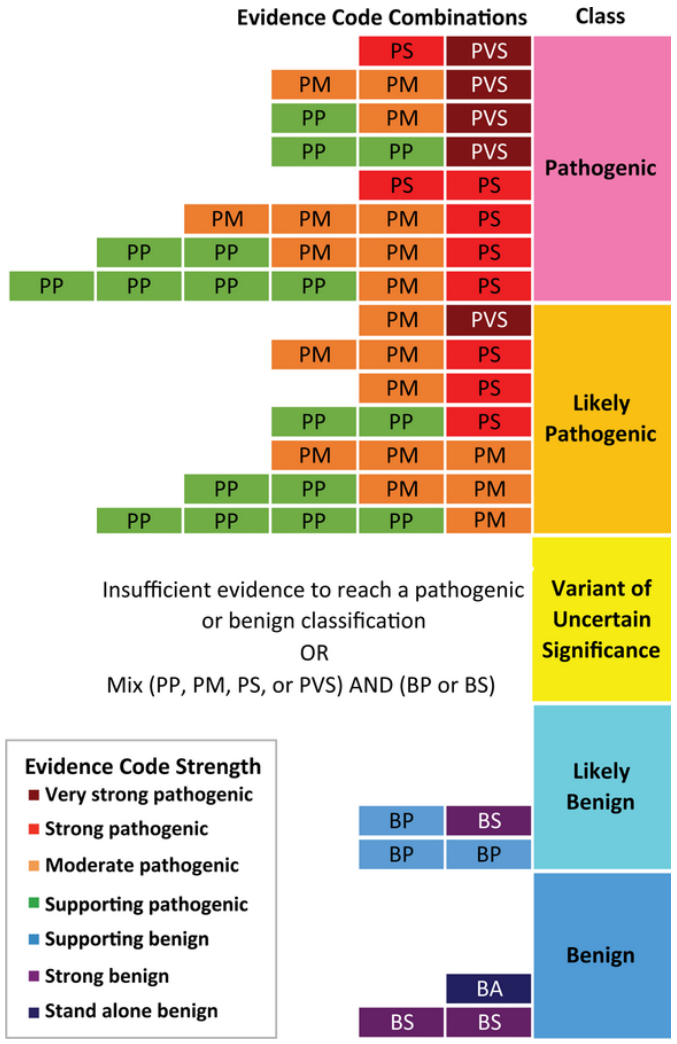
PM1_supp

modified strength

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

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.

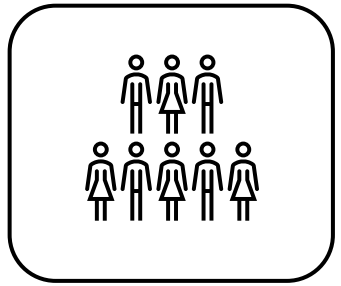
Summary of Overview

- 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

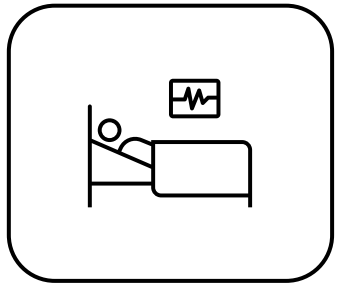
Specific Criteria

In this portion of the class, you will learn:

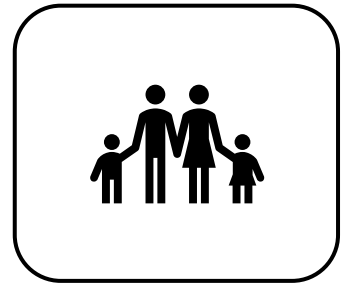
- How to apply the specific ACMG criteria.
- How strength of the criteria is determined.
- Tools / databases used to apply criteria.
- Specific examples of the criteria being applied to variants.



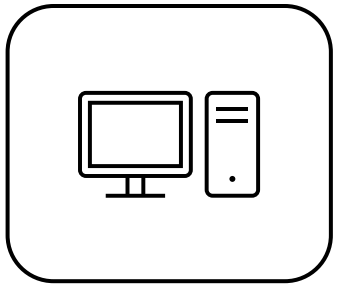
Population frequency
PM2, BA1, BS1, BS2



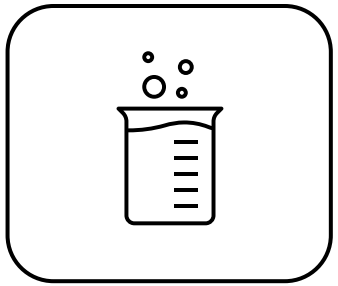
Case level evidence
PS1, PS4, PM3, PM5, BP2, BP5



Familial evidence
PS2, PM6, PP1, BS4

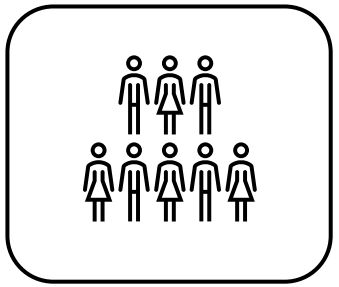


***In silico* predictions**
PVS1, PM4, PP3, BP1, BP3, BP4, BP7



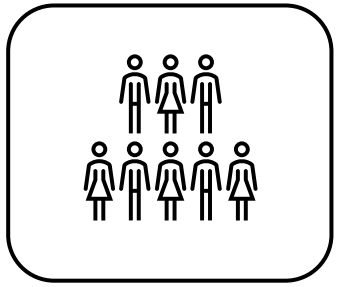
Functional data
PS3, PM1, PP2, BS3

Specific phenotype
PP4



Population frequency
PM2, BA1, BS1, BS2

Fundamental principle(s): disease-causing variants should not be more prevalent in healthy individuals than affected individuals; the rarer a condition, the rarer the variant should be.



Population frequency
BA1, BS1, BS2, PM2

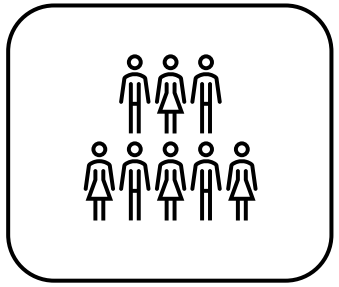
Criteria assignment:

- PM2: variant is absent/rare in control populations.
  - “Rarity” is specific for the gene, inheritance pattern, and penetrance of the disorder.
  - A good rule of thumb is ≤ frequent than the most frequent variant known to cause disease

- BA1: allele frequency is > 5% in control populations.

- BS1: allele frequency is greater than expected for the disorder (i.e., more common than the known incidence / carrier frequency of the disease).

- BS2: observed in homozygous state (for recessive disorders), heterozygous state (dominant disorders), or hemizygous state (X-linked disorders) in healthy adults for fully penetrant diseases with early onset



Population frequency
BA1, BS1, BS2, PM2

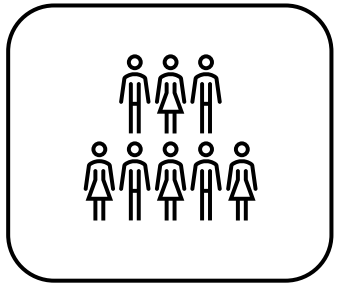
Strength determination:

- PM2 is applied only at supporting
  - Rationale: rare variants are rare at the population level, but it is very common for an individual to have several rare variants
  - See ClinGen PM2 recommendation 2020

- BA1: automatically makes a variant benign. These variants are usually filtered out before any formal classification occurs.

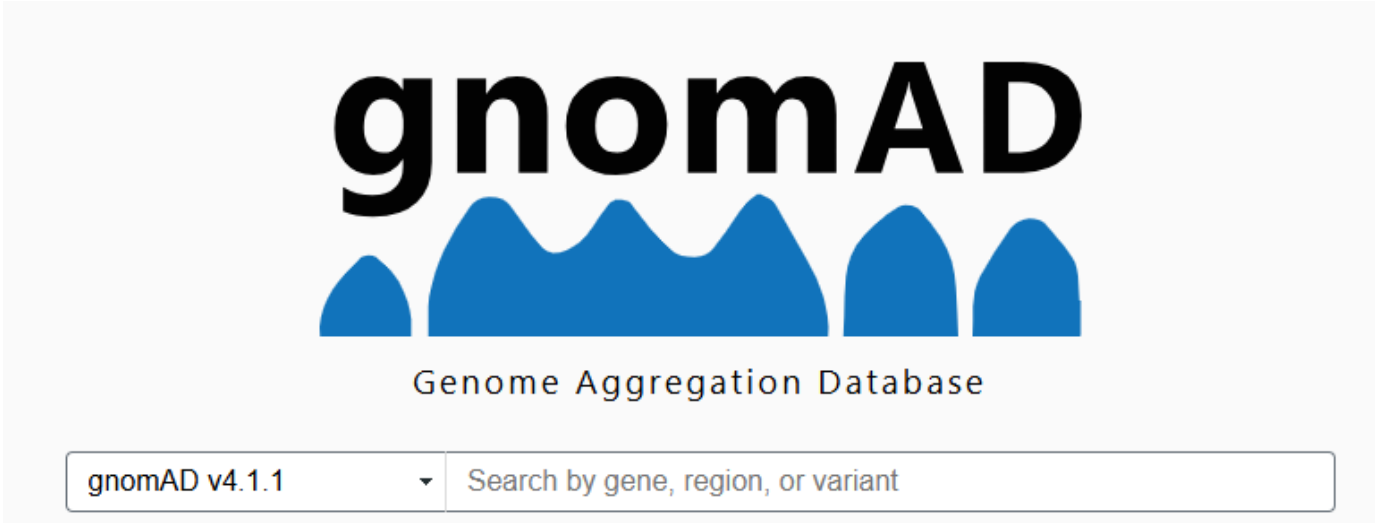
- BS1: usually applied at strong, but for certain genes, MAF cutoffs have been defined for applying at supporting. Difficult to apply because the disease incidence is not usually known for rare disorders.

- BS2: usually applied at strong, but for some genes, defined counts are allowed at supporting.



Population frequency
BA1, BS1, BS2, PM2

Tools / databases:



- 730,947 exomes and 76,215 genomes from individuals without severe pediatric disease
  - 5X larger than in 2023
- Allows for analysis of whether variants are rare or common in the generally healthy population
- Caveats:
  - Data has a sample bias towards individuals of European descent
  - Not as useful for adult-onset disorders

Population Data: gnomAD

Example: *ARID1B* variants:

Variant ID	Source	HGVS Consequence	VEP Annotation	LoF Curation	Clinical Significance	Flags	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
6-156779262-G-GCGC	E G	p.Pro533dup	inframe insertion		Benign		85948	1145648	7.50e-2	3584
6-156829362-A-G	E G	p.Ile643Val	missense		Benign		26369	1614148	1.63e-2	268
6-157186370-C-T	E G	p.Pro650Leu †	missense				23619	466958	5.06e-2	1266
6-157186564-G-C	E G	p.Gly715Arg †	missense				16741	470132	3.56e-2	397
6-156778871-CGGA-C	E G	p.Gly402del	inframe deletion		Benign/Likely benign		15204	1339750	1.13e-2	89
6-156778292-A-ACAGCAG	G	p.Gln213_Gln214dup	inframe insertion		Benign/Likely benign		11870	1517714	7.82e-3	32
6-156778665-G-A	E G	p.Gly329Ser	missense		Benign		8376	1488164	5.63e-3	32
6-156778268-C-CCAG	G	p.Gln214dup	inframe insertion		Benign/Likely benign		8240	1536626	5.36e-3	43
6-156777692-G-GGCA	E G	p.Ala14dup	inframe insertion		Benign		7474	148240	5.04e-2	237
6-156778889-CGGA-C	E G	p.Gly411del	inframe deletion		Benign		5933	1314634	4.51e-3	2
6-156777879-G-A	E G	p.Gly67Ser	missense		Benign		5667	1431612	3.96e-3	212
6-156778268-C-CCAGCAG	E G	p.Gln213_Gln214dup	inframe insertion		Benign/Likely benign		5377	1536714	3.50e-3	93
6-157206358-TGAC-T	E G	p.Asp1864del	inframe deletion		Benign		4769	1614046	2.95e-3	145
6-157201089-A-T	E G	p.Met1622Leu	missense		Benign		4233	1614190	2.62e-3	103
6-156778847-G-GGGC	E G	p.Gly402dup	inframe insertion		Benign/Likely benign		3034	1403452	2.16e-3	20
6-156778292-A-ACAG	E G	p.Gln214dup	inframe insertion		Benign/Likely benign		2918	1518244	1.92e-3	14
6-156778847-GGGCGGCG...	E G	p.Gly400_Gly402del	inframe deletion		Benign/Likely benign		2790	1403448	1.99e-3	10
6-156778889-CGGAGGA-C	E G	p.Gly410_Gly411del	inframe deletion		Benign/Likely benign		2746	1366178	2.01e-3	14
6-156778943-T-TGTGGCG	E G	p.Val422_Ala423dup	inframe insertion		Benign/Likely benign		2600	1272008	2.04e-3	3

Exceptions to BA1

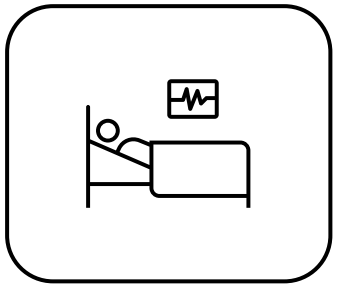
Gene	Variant	Classification	applied (not including BA1 or BS1)	ClinVar ID	ClinGen Allele Registry ID	Chr	Position	Ref	Alt	ExAC Source Pop	ExAC Source Pop MAF	ClinVar disease entry
ACAD9	NM_014049.4: c.-44_-41dupTAAG	VUS	PS3_Supporting; BS2	1018	CA114709	3	128,598,490	C	CTAAG	AFR	0.1261	Deficiency of Acyl-CoA dehydrogenase family, member 9
GJB2	NM_004004.5: c.109G>A (p.Val37Ile)	Pathogenic	PS4; PP1_Strong; PM3_VeryStrong; PS3_Moderate	17023	CA172210	13	20,763,612	C	T	EAS	0.07242	Deafness, autosomal recessive
HFE	NM_000410.3: c.187C>G (p.His63Asp)	Pathogenic*	PS4	10	CA113797	6	26,091,179	C	G	NFE	0.1368	Hereditary hemochromatosis
HFE	NM_000410.3: c.845G>A (p.Cys282Tyr)	Pathogenic*	PS4; PP3	9	CA113795	6	26,093,141	G	A	NFE	0.05135	Hereditary hemochromatosis
MEFV	NM_000243.2: c.1105C>T (p.Pro369Ser)	VUS	PM3; PM5	2551	CA280114	16	3,299,586	G	A	EAS	0.07156	Familial Mediterranean fever
MEFV	NM_000243.2: c.1223G>A (p.Arg408Gln)	VUS	PM3; PM5	2552	CA280116	16	3,299,468	C	T	EAS	0.05407	Familial Mediterranean fever
PIBF1	NM_006346.2: c.1214G>A (p.Arg405Gln)	VUS	PM3; BS2	217689	CA210261	13	73,409,497	G	A	AMR	0.09858	Joubert syndrome
ACADS	NM_000017.3: c.511C>T (p.Arg171Trp)	VUS	PS3_Moderate; PM3; PP3	3830	CA312214	12	121,175,678	C	T	FIN #	0.06589	Deficiency of butyryl-CoA dehydrogenase
BTD	NM_000060.4: c.1330G>C (p.Asp444His)	Pathogenic	PS3; PM3_Strong; PP3; PP4	1900	CA090886	3	15,686,693	G	C	FIN #	0.05398	Biotidinase deficiency

*ACMG/AMP criteria selected does not match the classification as these variants are common low-penetrant variants and the ACMG/AMP guidelines are not designed for this variant type

Detected at >5% MAF only in Finnish population (see text).

Genomic coordinates on build GRCh37

AFR: African/African American, EAS: East Asian, NFE: Non-Finnish European, AMR: Latino, FIN=Finnish



Case level data
PS1, PS4, PM3, PM5, BP2, BP5

Fundamental principle(s): the presence of the same or very similar variants in similarly affected individuals supports pathogenicity

Prevalence in Affected Population (PS4)

Criteria assignment & strength:

- Variant is associated with an odds ratio > 5.0 of causing disease with a lower bounded confidence interval > 1.0.
  - Odds ratio: $OR = \frac{(\frac{affected\ carriers}{affected\ non-carriers})}{(\frac{unaffected\ carriers}{unaffected\ non-carriers})}$
- The odds ratio calculation works best for more common variants that are associated with an incompletely penetrant phenotype (like cardiomyopathy or breast cancer).
- For most rare disease, the number of affected non-carriers is hard to estimate, and the number of unaffected carriers is often 0, making the equation mathematically meaningless.

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- For most rare disease, the number of affected non-carriers is hard to estimate, and the number of unaffected carriers is often 0, making the equation mathematically meaningless.

Population Data:
Prevalence in Affected Population (PS4)

Criteria assignment & strength:

- Most PS4 assessments are now “proband counting”:

Table 4 Overview of Case-Level Data Specifications: Point Value Thresholds per Strength Level for Proband Count Thresholds per Variant Curation Expert Panel for PS4

		Supporting	Moderate	Strong	Very strong
PS4	Cardiomyopathy	2 probands	6 probands	15 probands	N/A
	RASopathy	1 proband	3 probands	5 probands	N/A
	PTEN	1 point	2 points	4 points	16 points
	CDH1	1 proband	2 probands	4 probands	16 probands
	Hearing loss (AD)	2 probands	6 probands	15 probands	N/A

https://pubmed.ncbi.nlm.nih.gov/31479589/

Allelic Data (BP2, PM3)

Assignment:

- BP2 – *in cis* with a recessive pathogenic variant OR *in trans* with a dominant pathogenic variant. BP2 is used sparingly.
- PM3 – see table

Strength determination: see table.

Notes:

- Individual in whom the variants are present must be affected
- PM2 should be applicable for PM3 to be applied.
- Pay attention to the max point allowances for the homozygous occurrences and the *in trans* variant being of uncertain significance

Table 1. Points awarded per in *trans* proband

Classification/Zygosity of other variant [1]	Points per Proband	
	Confirmed in *trans*	Phase unknown
Pathogenic or Likely pathogenic variant	1.0	0.5 (P) 0.25 (LP)
Homozygous occurrence *(max point 1.0)*	0.5	N/A
Uncertain significance variant *(max point 0.5)*	0.25	0.0

[1]All variants should be sufficiently rare (meet PM2 specification); P - Pathogenic; LP - Likely pathogenic

Table 2. Recommendation for determining the appropriate evidence strength level for PM3

PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
0.5	1.0	2.0	4.0

https://clinicalgenome.org/site/assets/files/3717/svi_proposal_for_pm3_criterion_-_version_1.pdf

Other Data (BP5)

Assignment: Variant is found in a proband with an alternative cause of disease.

Strength determination: supporting.

Notes:

- Used VERY sparingly, as probands can have multiple variants that contribute to disease, the variant could be in a gene with incomplete penetrance, etc.

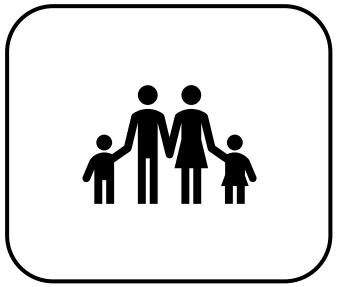
Highly similar variants known to cause disease (PM5, PS1)

Criteria assignment:

- **PM5** is assigned when a novel missense change is seen at the same position as a likely pathogenic or pathogenic variant.
    - LDLR: c.1721G>A (p.**Arg574His**)
      Likely pathogenic
    - LDLR: c.1721G>T (p.**Arg574Leu**)
      Variant under curation → **PM5**
- **PS1** is assigned when a novel nucleotide change leads to the same missense variant previously classified as likely pathogenic or pathogenic.
    - Major caveat: do NOT apply if either variant is predicted to have a different splice effect

Strength:

- Some ClinGen groups reduce PM5 strength for likely pathogenic variants or increase strength if multiple pathogenic variants are seen at the same residue.



Familial data
PS2, PM6, PP1, BS4

Fundamental principle(s):

- The presence of a *de novo* variant in a gene that matches the phenotype supports pathogenicity
- Family members that share a phenotype should share the variants being questioned

De Novo Data (PM6, PS2)

Assignment: Variant is either *de novo* with confirmed parentage (sequencing has determined parents are biological parents; PS2) or assumed parentage (only point testing was done on the parents; PM6).

Strength determination: see tables.

Notes:

- "Assumed de novo" does NOT mean *de novo* is assumed because of the severity of the condition. It means we assume the individuals presenting as mother and father are the proband's biological parents.
- For X-linked disorders that primarily affect males, you can apply *de novo* criteria if the variant is inherited from a mother in whom the variant was *de novo*.

Table 1. Points awarded per de novo occurrence

Phenotypic consistency	Points per Proband	
	Confirmed de novo	Assumed de novo
Phenotype highly specific for gene	2	1
Phenotype consistent with gene but not highly specific	1	0.5
Phenotype consistent with gene but not highly specific and high genetic heterogeneity*	0.5	0.25
Phenotype not consistent with gene	0	0

*Maximum allowable value of 1 may contribute to overall score

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for de novo occurrence(s)

Supporting (PS2_Supporting or PM6_Supporting)	Moderate (PS2_Moderate or PM6)	Strong (PS2 or PM6_Strong)	Very Strong (PS2_VeryStrong or PM6_VeryStrong)
0.5	1	2	4

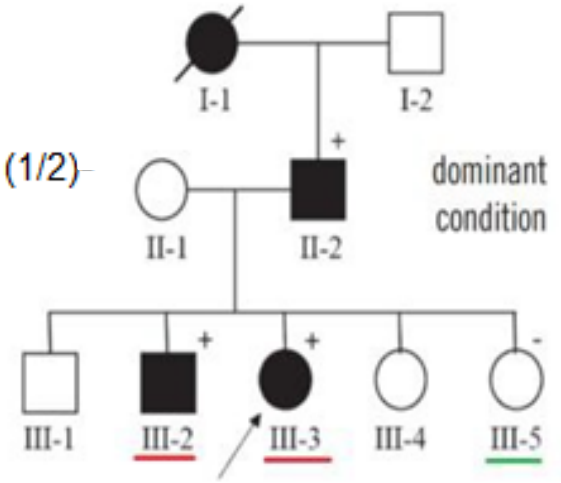
Segregation Data (BS4, PP1)

PP1 Strength	# Affected Individuals	Meiosis Method $(1/2)^m$
Weak (Supporting)	AD: ≥3 AR: ≥2	≤1/8 in 1 family ≤1/4 in >1 family
Moderate	AD: ≥6 AR: ≥3, ≥2 families	≤1/16 in 1 family ≤1/8 in >1 family
Strong	AD: ≥10 AR: ≥5, ≥2 families	≤1/32 in 1 family ≤1/16 in >1 family

Two additional meiosis
(II-2 and III-2) = $(1/2)^2$
An unaffected individual (III-5) = (1/2)
Total = 1/4 x 1/2 = 1/8

=Supporting evidence
(≤1/8 in single family)

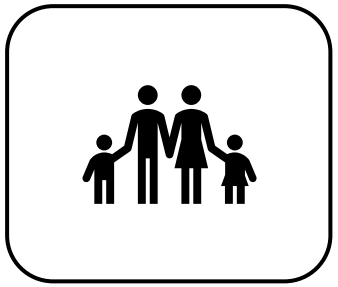
=Weak segregation
(≤ 3 affected individual)



Criteria application:

- PP1 can be applied based on the total number of probands within a family OR by the number of informative meioses
  - "Informative meioses" allows for the counting of unaffected non-carriers

- BS4 is applied when the variant does not segregate with disease within a family
  - Disease must be fully penetrant to apply BS4



Familial data
PS2, PM6, PP1, BS4

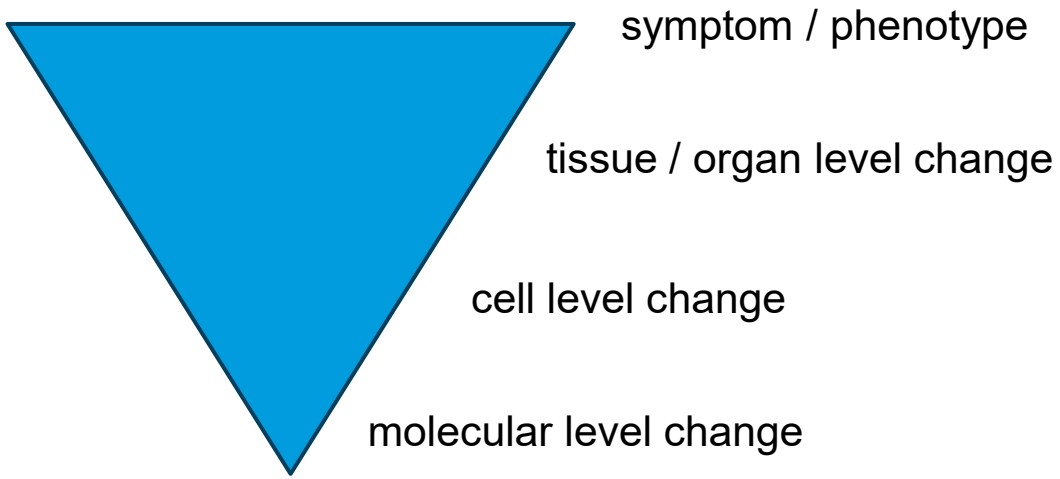
Fundamental principle(s):

- The presence of a *de novo* variant in a gene that matches the phenotype supports pathogenicity
- Family members that share a phenotype should share the variants being questioned

Phenotypic Data (PP4)

The patient's phenotype is **highly specific** for the affected gene:

- This means the phenotype points to a single (or a very limited number) gene. A variant in a gene that causes seizures in a proband with seizures cannot have PP4 applied on that basis alone because hundreds of genes cause seizures.



Phenotypic Data (PP4)

The patient's phenotype is **highly specific** for a gene:

- This means the phenotype points to a single (or a very limited number) gene. A variant in a gene that causes seizures in a proband with seizures cannot have PP4 applied on that basis alone because hundreds of genes cause seizures.

For example:

ClinGen PAH Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

Rule Set:	Rules For PAH
Disease(s)	phenylketonuria
Gene(s)	PAH
Genetype	nuclear

Criteria Code	Strength Specification
PP4 - Very Strong	NA
PP4 - Strong	NA
PP4 - Moderate	Plasma Phe >120 µmol/L and exclusion of a defect of BH4 cofactor metabolism.
PP4 - Supporting	Phenotype specific for disease with single genetic etiology.

ClinGen PAH Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

Showing 1 to 4 of 4 entries (filtered from 107 total entries)

Previous 1 Next

https://cspec.genome.network/cspec/ui/svi/summary

Phenotypic Data (PP4)

Important note:

- For some criteria, the patient's phenotype is considered as part of the strength determination (for example, in PS2). In these cases, **PP4 is not applied as a separate criteria**.

Table 1. Points awarded per de novo occurrence

Phenotypic consistency	Points per Proband	
	Confirmed de novo	Assumed de novo
Phenotype highly specific for gene	2	1
Phenotype consistent with gene but not highly specific	1	0.5
Phenotype consistent with gene but not highly specific and high genetic heterogeneity*	0.5	0.25
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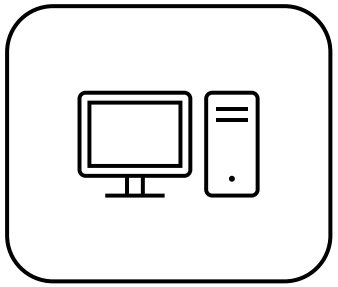
*Maximum allowable value of 1 may contribute to overall score

Table 2. Recommendation for determining the appropriate ACMG/AMP evidence strength level for de novo occurrence(s)

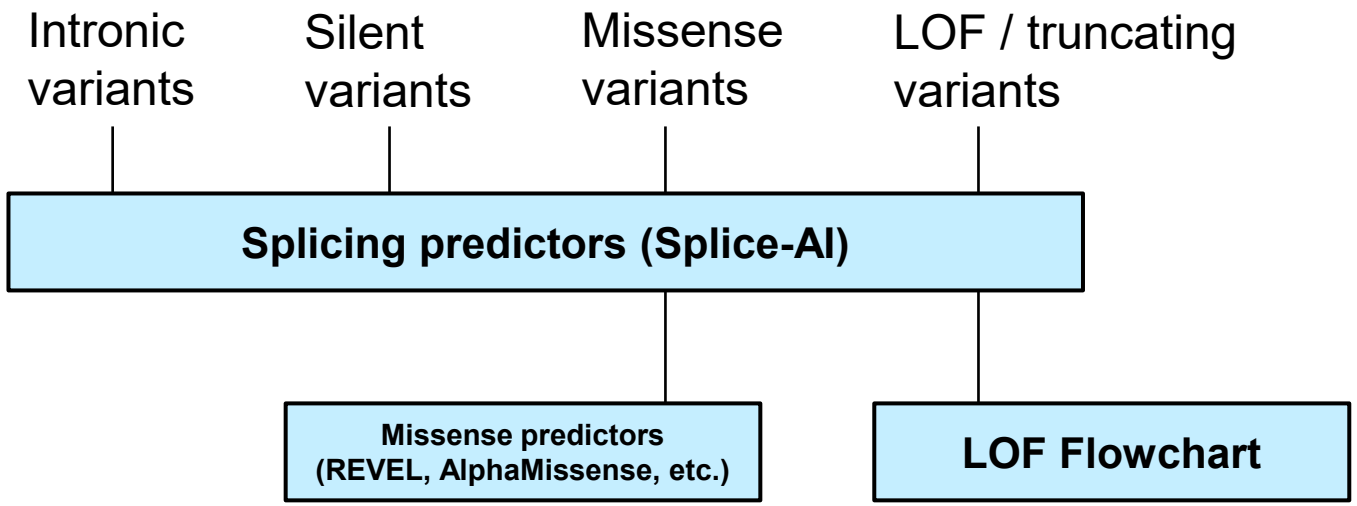
Supporting (PS2_Supporting or PM6_Supporting)	Moderate (PS2_Moderate or PM6)	Strong (PS2 or PM6_Strong)	Very Strong (PS2_VeryStrong or PM6_VeryStrong)
0.5	1	2	4



***In silico* predictions**
BP1, BP3, BP4, BP7, PVS1, PM4, PP3

Fundamental principle(s): pathogenic variants should significantly alter the function of the affected protein.

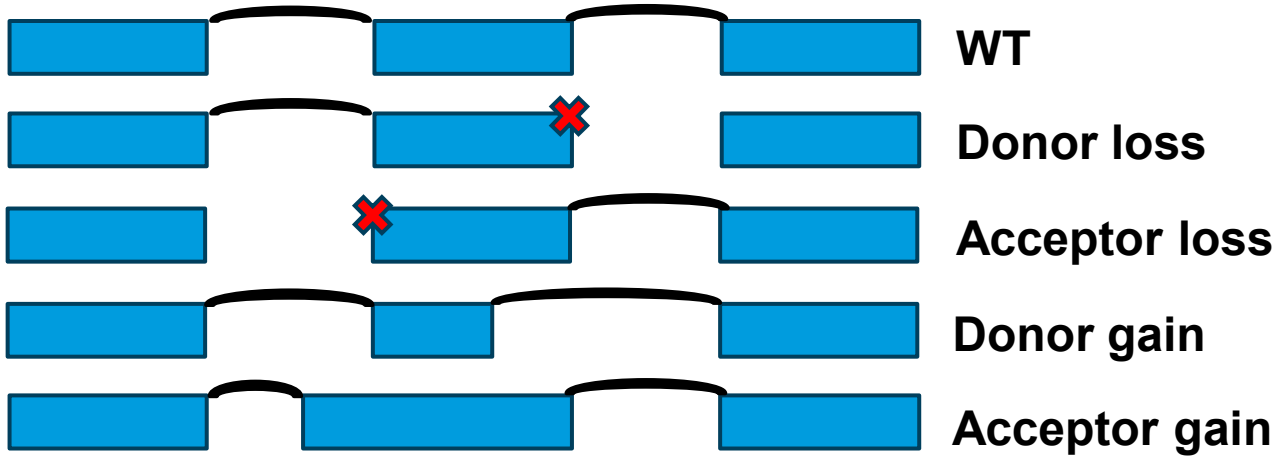
Quick Primer



ANY variant can cause errors in mRNA splicing and should be considered **first**

- mRNA splicing precedes protein translation!

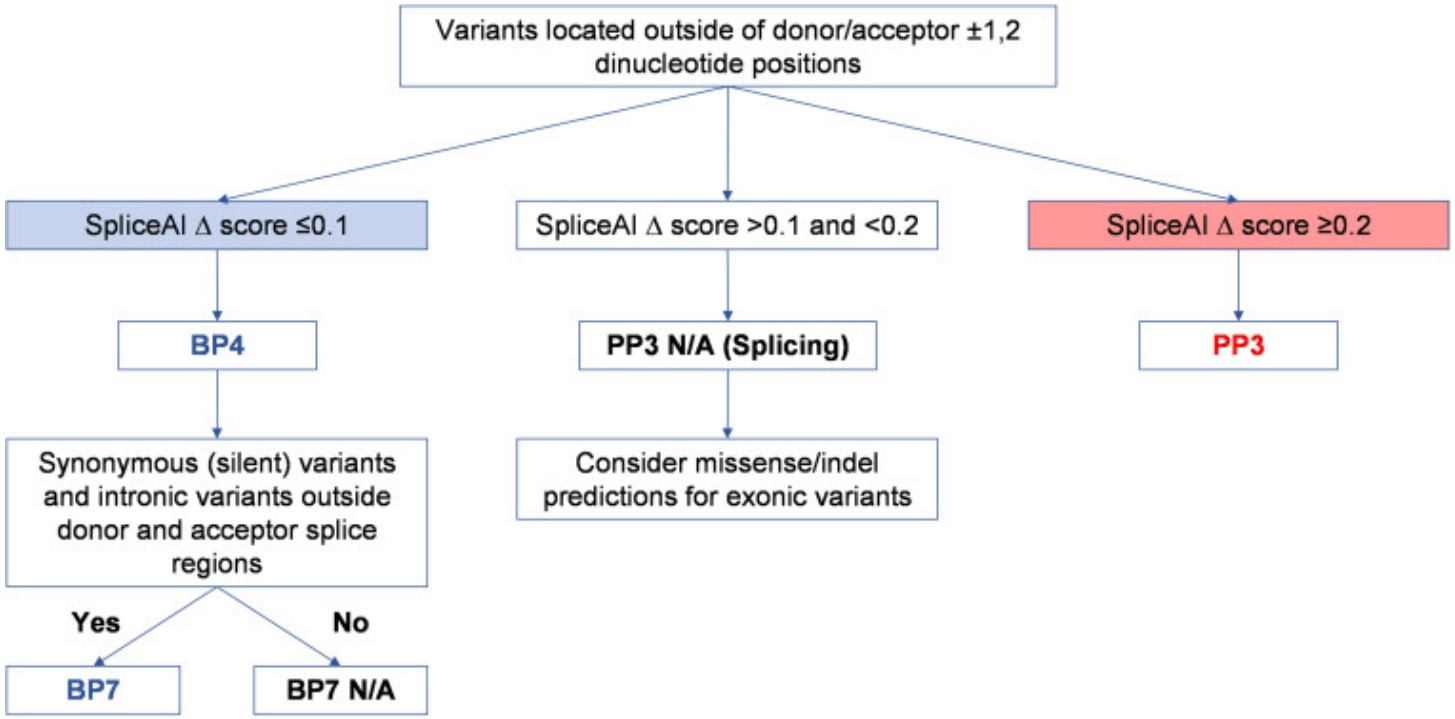
Computational and Predictive Data: Splicing *in silico* predictions (BP7, BP4, PP3)



- Splice AI is an *in-silico* tool that predicts:
  - Loss of canonical donors (DL)
  - Loss of canonical acceptors (AL)
  - Creation / strengthening of cryptic donors (DG)
  - Creation / strengthening of cryptic acceptors (AG)

- **ANY variant has a potential impact on splicing, not just variants within the intron.**

Computational and Predictive Data: Splicing *in silico* predictions (BP7, BP4, PP3)



https://www.cell.com/ajhg/fulltext/S0002-9297(23)00203-3

- Follow this decision tree to determine which criteria to apply
- All criteria applied on the basis of SpliceAI is applied at supporting strength.
- For missense variants that also have a predicted SpliceAI effect, apply whatever gives you the highest PP3 strength.

SpliceAI Example

SCN1A:c.3879+5G>A

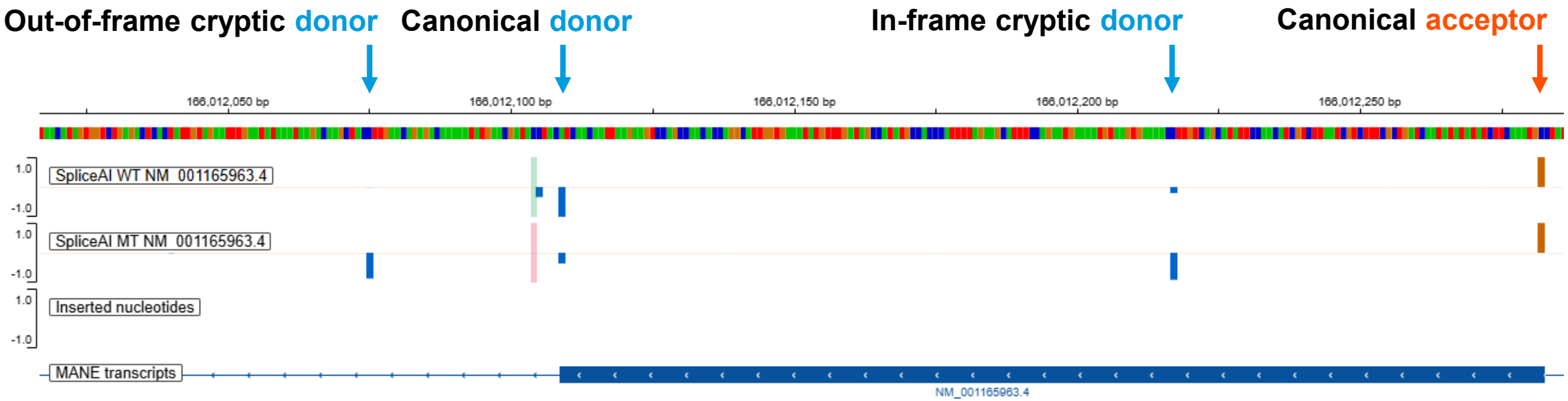
Variant	Gene	= MANE Select transcript	= non-coding transcript	Δ type	Δ score	position
chr2-166012104 C>T	SCN1A (ENSG00000144285.24 / ENST00000674923.1 / NM_001165963.4)			Acceptor Loss	0.00	178 bp
splice donor 5th base variant	protein coding MANE Select transcript (minus strand)			Donor Loss	0.64	5 bp
UCSC, gnomAD	OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards			Acceptor Gain	0.00	
				Donor Gain	0.86	-29 bp

- PP3 is applicable – BUT it is important to consider what is predicted to occur

SpliceAI Example

SCN1A:c.3879+5G>A

Mobi Details: https://mobidetails.iurc.montp.inserm.fr/MD/



- Splice-AI predicts weakening of the canonical donor, but not complete loss
- Additionally, 2 potential cryptic donors are predicted with 2 different consequences

Computational and Predictive Data: Missense *in silico* predictions (BP4, PP3)

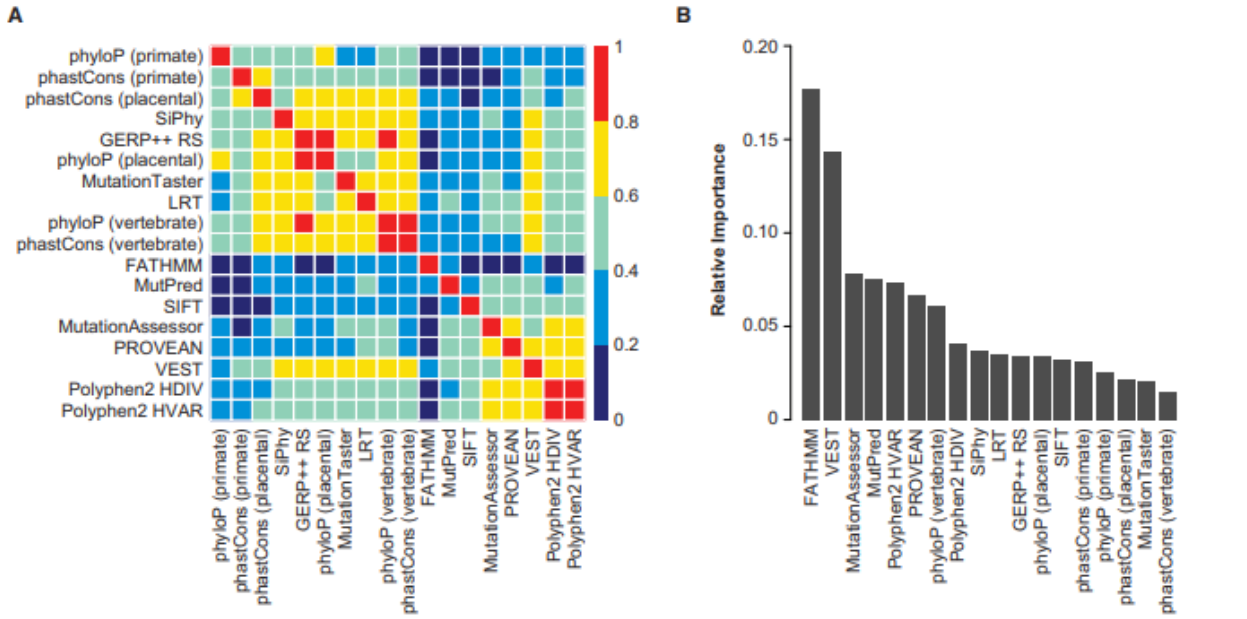
Criteria assignment:

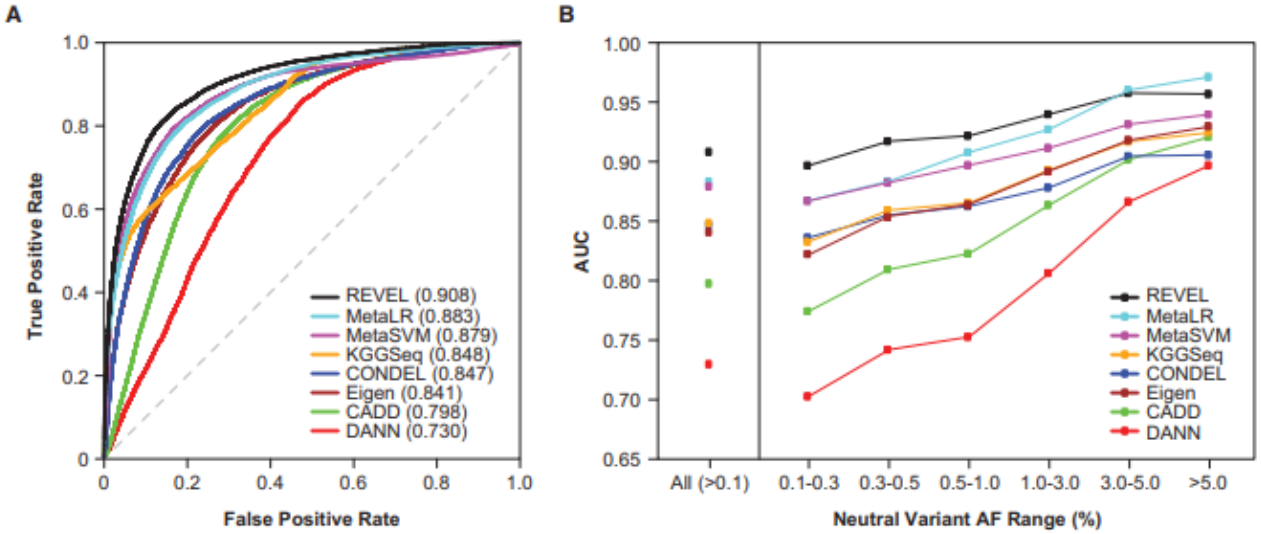
- Multiple *in silico* tools predict the variant is benign (BP4) or pathogenic (PP3). If conflicting, neither criteria is assigned.

- The most recent *in silico* tools like REVEL or AlphaMissense combine many forms of analysis into a single score. Thus, a REVEL score, for example, constitutes "multiple" *in silico* tools.

- It is recommended that groups pick a tool and **only use that tool** to prevent selection bias. ClinGen VCEPs primarily use REVEL currently.

Computational and Predictive Data: REVEL





- REVEL is a conglomeration of 18 *in silico* tools that assess how damaging a particular missense variant is
- REVEL gives you a 0 to 1 score of how "damaging" a variant is
- NOT as good at predicting gain of function variants:

REVEL Is Better at Predicting Pathogenicity of Loss-of-Function than Gain-of-Function Variants

Jasmin J. Hopkins, Matthew N. Wakeling, Matthew B. Johnson, Sarah E. Flanagan, Thomas W. Laver

Computational and Predictive Data: Missense *in silico* predictions (BP4, PP3)

Strength determination:

Table 2. Estimated threshold ranges for all tools in this study corresponding to the four pathogenic and four benign intervals

	Benign (BP4)				Pathogenic (PP3)			
Method	**Very Strong**	**Strong**	**Moderate**	**Supporting**	**Supporting**	**Moderate**	**Strong**	**Very Strong**
BayesDel	–	–	≤ −0.36	(−0.36, −0.18]	[0.13, 0.27)	[0.27, 0.50)	≥0.50	–
CADD	–	≤0.15	(0.15, 17.3]	(17.3, 22.7]	[25.3, 28.1)	≥28.1	–	–
EA	–	–	≤0.069	(0.069, 0.262]	[0.685, 0.821)	≥0.821	–	–
FATHMM	–	–	≥4.69	[3.32, 4.69)	(−5.04, −4.14]	≤ −5.04	–	–
GERP++	–	–	≤ −4.54	(−4.54, 2.70]	–	–	–	–
MPC	–	–	–	–	[1.360, 1.828)	≥1.828	–	–
MutPred2	–	≤0.010	(0.010, 0.197]	(0.197, 0.391]	[0.737, 0.829)	[0.829, 0.932)	≥0.932	–
PhyloP	–	–	≤0.021	(0.021, 1.879]	[7.367, 9.741)	≥9.741	–	–
PolyPhen2	–	–	≤0.009	(0.009, 0.113]	[0.978, 0.999)	≥0.999	–	–
PrimateAI	–	–	≤0.362	(0.362, 0.483]	[0.790, 0.867)	≥0.867	–	–
REVEL	≤0.003	(0.003, 0.016]	(0.016, 0.183]	(0.183, 0.290]	[0.644, 0.773)	[0.773, 0.932)	≥0.932	–
SIFT	–	–	≥0.327	[0.080, 0.327)	(0, 0.001]	0	–	–
VEST4	–	–	≤0.302	(0.302, 0.449]	[0.764, 0.861)	[0.861, 0.965)	≥0.965	–

A "–" implies that the given tool did not meet the posterior probability (likelihood ratio) threshold. See Table S1 for comprehensive results that include point estimates and one-sided confidence intervals. Intervals follow standard mathematical notation in which "(" and ")" indicate exclusion of the end value and "[" and "]" indicate inclusion of the end value

https://pubmed.ncbi.nlm.nih.gov/36413997/

- Most variant intepretation groups are not currently applying PP3 above moderate strength
- The combination of PP3 and PM1 cannot be > 4 points

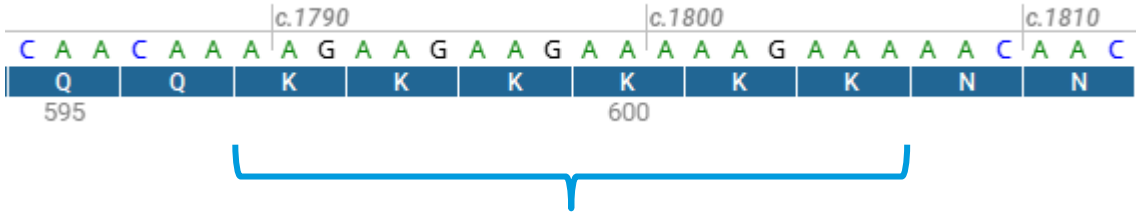
Computational and Predictive Data: In-frame indels (BP3, PM4)

Criteria assignment:

- PM4 is assigned for in-frame deletions or duplications in **non-repetitive regions**
- BP3 is assigned for in-frame deletions or duplications in **repetitive regions**

Strength:

- PM4 is often applied at supporting for single residue dels/dups. Otherwise, it is applied at moderate.
- BP3 is applied at supporting.



Example of a repetitive region in *CHD7*

Computational and Predictive Data: Missense variant in a LOF gene (BP1)

Criteria assignment:

- BP1 is assigned when a missense variant is seen in a gene where only truncating (LOF) variants are known to cause disease.
    - Be cognisant of confirmation bias when applying this criteria

Strength:

- BP1 is assigned at supporting.

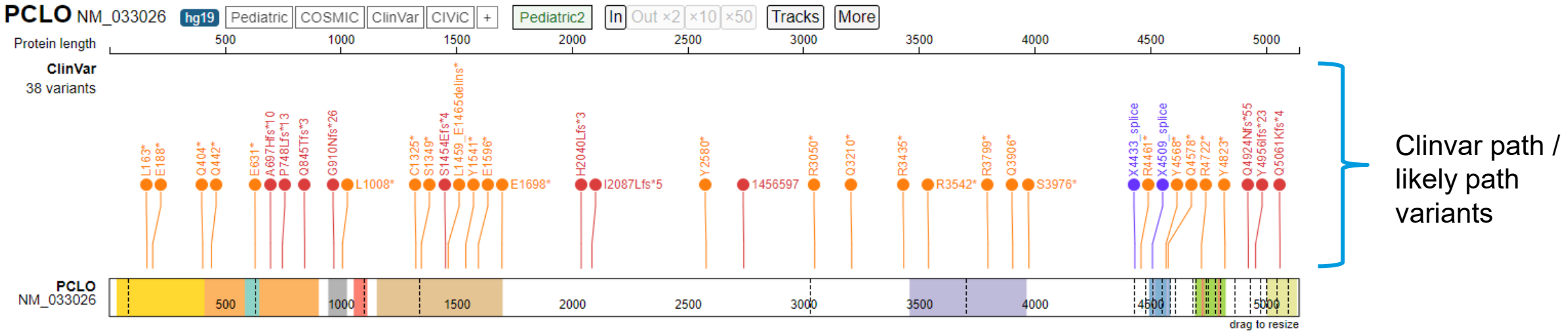


Image made in ProteinPaint (https://proteinpaint.stjude.org/)

Computational and Predictive Data (PVS1)

Criteria assignment:

- PVS1: LOF variant in a gene where LOF is an established disease mechanism
- How do you establish LOF as a disease mechanism?
  - Many LOF variants are associated with disease (best evidence)
  - LOF predictors (use caution)

Constraint | Variant co-occurrence

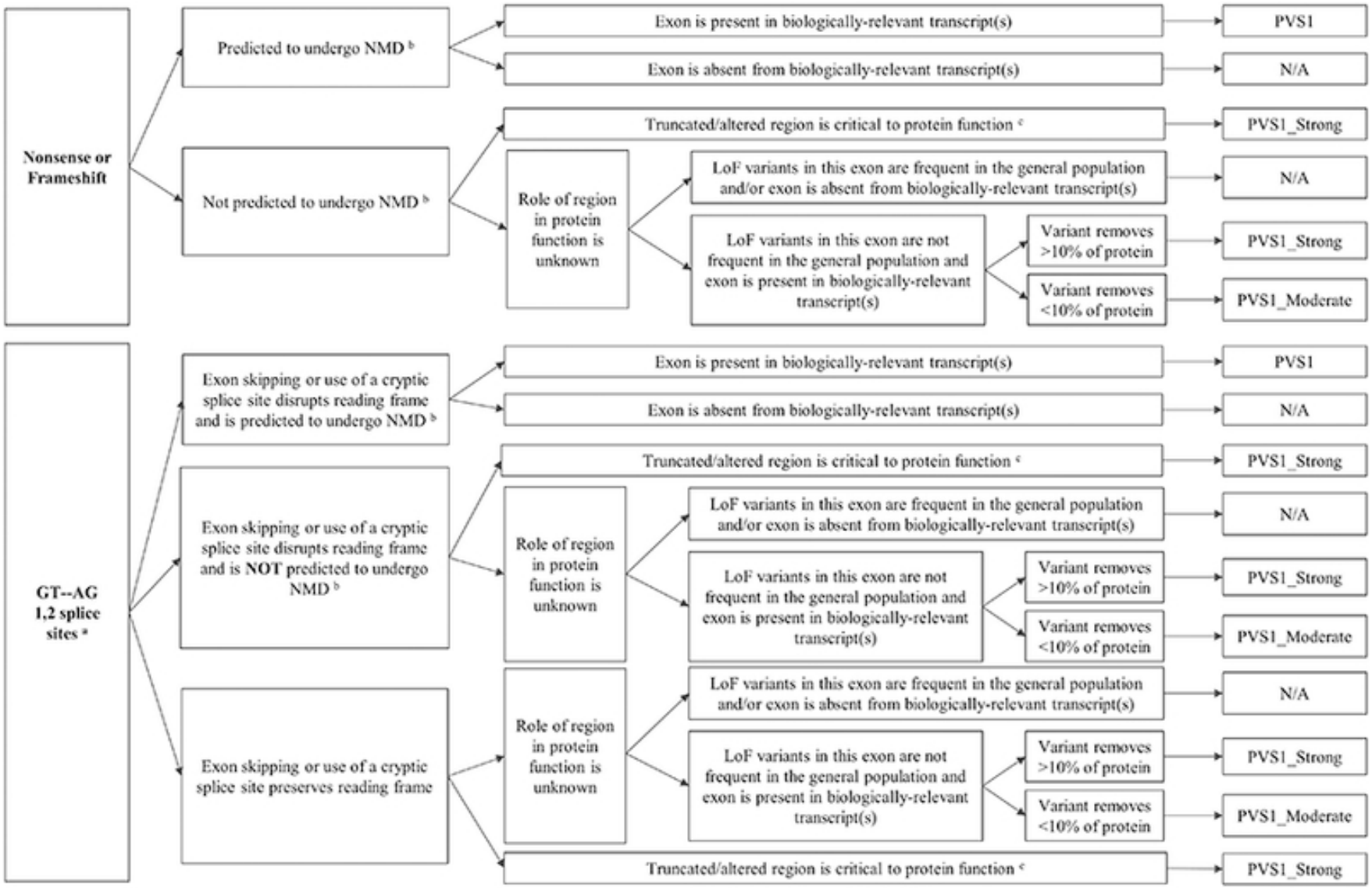
Category	Expected SNVs	Observed SNVs	Constraint metrics
Synonymous	1092.7	1013	Z = 1.31 o/e = 0.93 (0.88 - 0.98)
Missense	2707.8	2501	Z = 1.45 o/e = 0.92 (0.89 - 0.95)
pLoF	228.7	60	pLI = 1 o/e = 0.26 (0.21 - 0.33)

Strength determination:

Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion

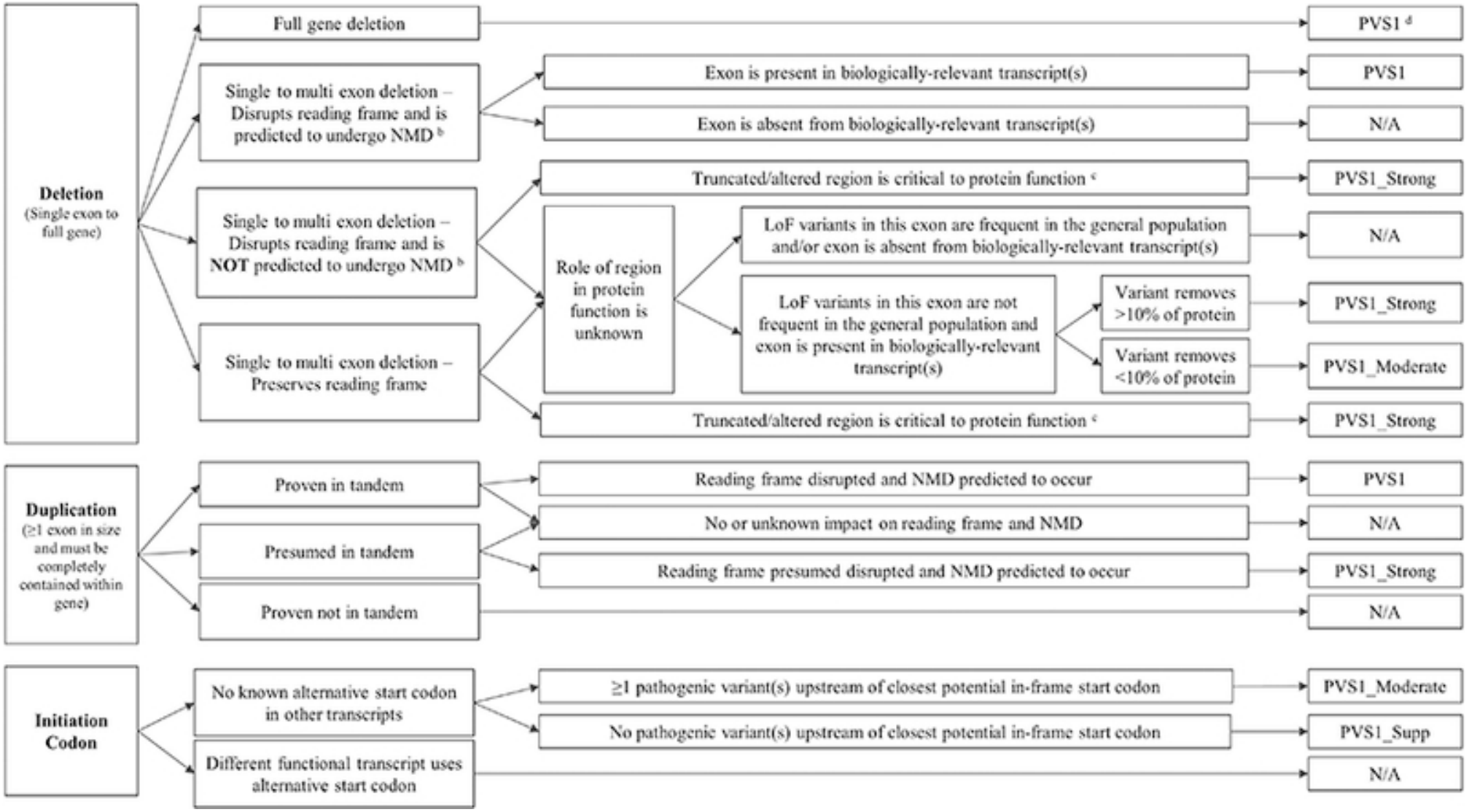
Ahmad N. Abou Tayoun, Tina Pesaran, Marina T. DiStefano, Andrea Oza, Heidi L. Rehm, Leslie G. Biesecker, Steven M. Harrison ... See all authors

Computational and Predictive Data (PVS1)



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6185798/

Computational and Predictive Data (PVS1)



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6185798/

Computational and Predictive Data (PVS1)

My quick and dirty guide to PVS1 strengths that doesn't involve blurry flowcharts …

PVS1 – any variant leading to NMD, deletion, or non-expression of the entire gene

PVS1_strong – any variant leading to *in-frame* removal of either a critical region of the gene (e.g. the active site) or > 10% of the protein

PVS1_moderate – any variant leading to *in-frame* removal of either < 10% of the protein or a region with unknown or non-critical function

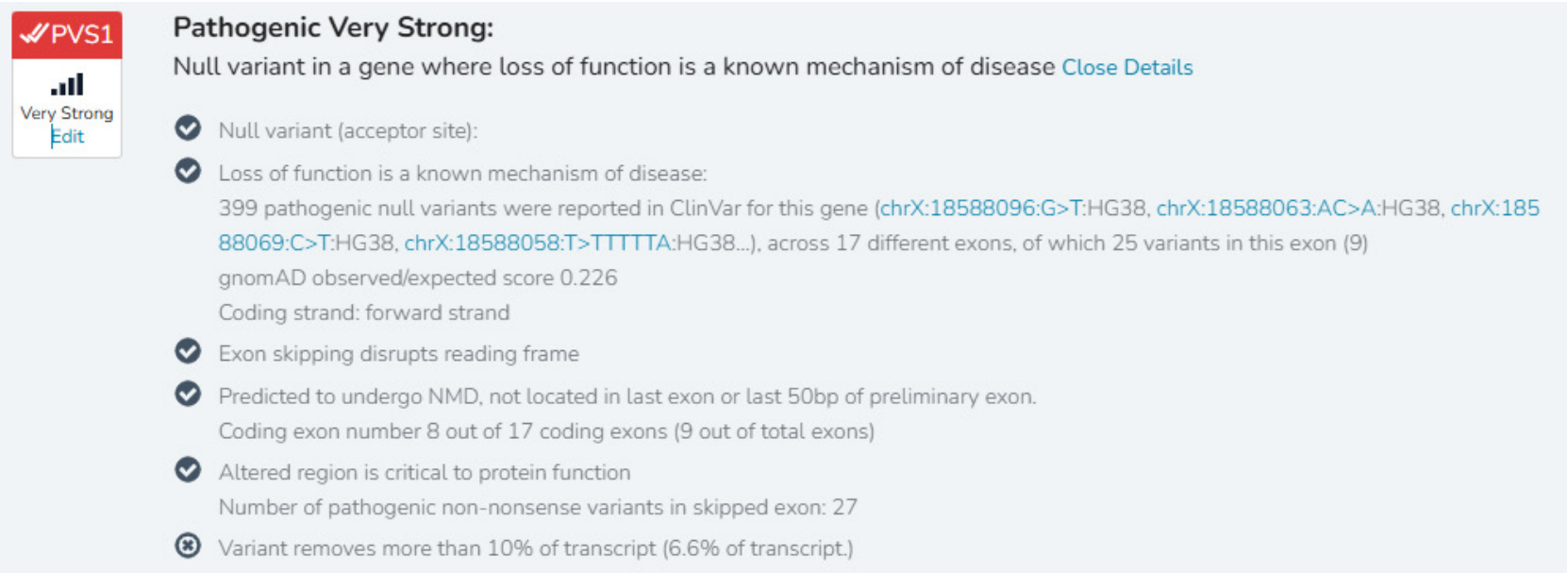
- This is essentially the same as **PM4**

Note: PVS1 is not applicable at any strength if LOF is not a disease mechanism, LOF variants are common in the affected exon, or if the affected exon is absent from disease-relevant transcripts

Computational and Predictive Data (PVS1): Selected examples

Example 1: *CDKL5*:c.555-1G>A, found in a proband with developmental encephalopathy

From Franklin:



Computational and Predictive Data (PVS1): Selected examples

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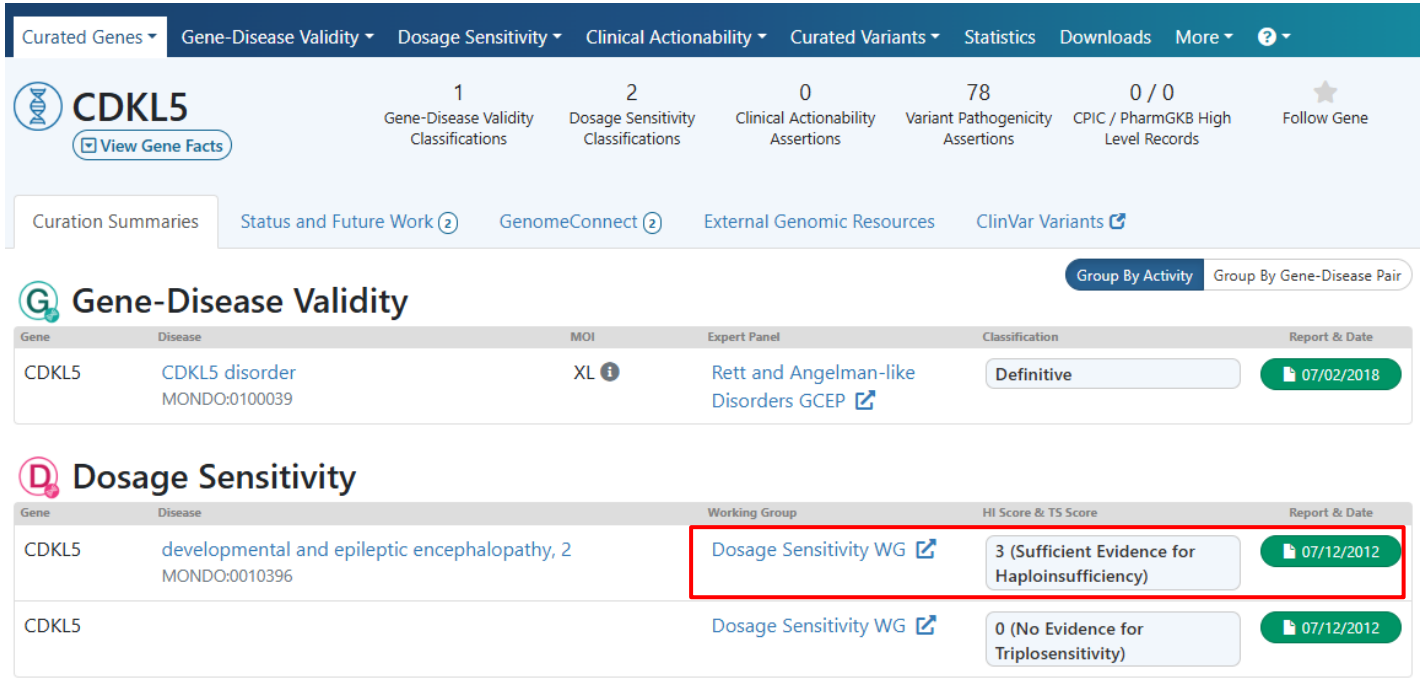
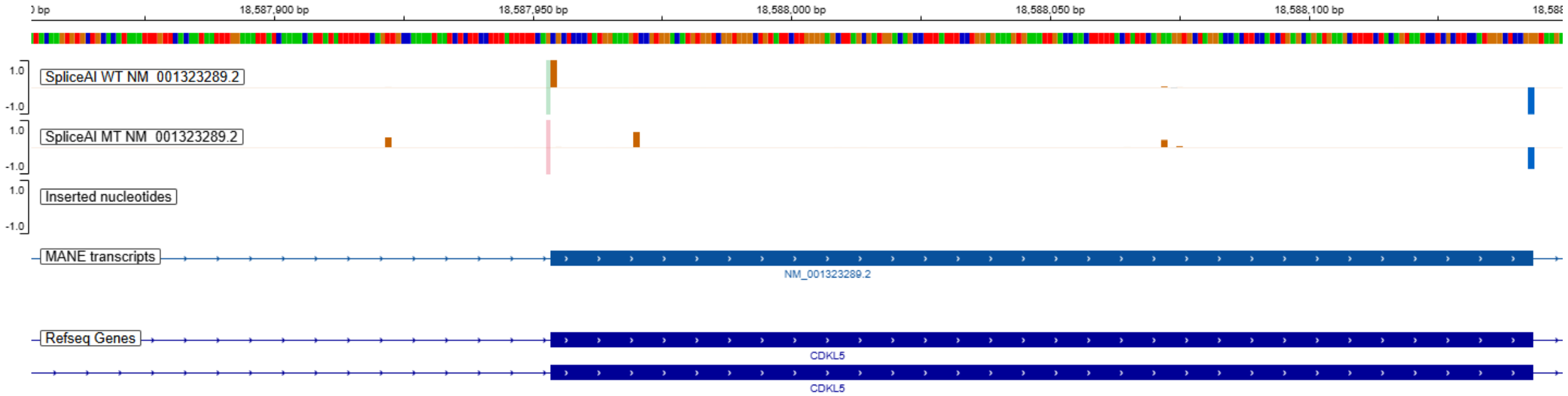


- ClinGen curates dosage sensitivity
- Pay attention to the report date – if it's old you may want to double check the current level of evidence

Computational and Predictive Data (PVS1): Selected examples

Example 1: *CDKL5*:c.555-1G>A, found in a proband with developmental encephalopathy



There ARE some cryptic acceptor sites, but all of them are out of frame.

Computational and Predictive Data (PVS1): Selected examples

Example 2: *SYN1*:c.1941_1947dup, p.A650Rfs*36 found in a proband with global delays and seizures

From Franklin:

Computational and Predictive Data (PVS1): Selected examples

Example 2: *SYN1*:c.1941_1947dup, p.A650Rfs*36 found in a proband with global delays and seizures

Nonsense mediated decay (NMD) is not predicted to occur if the premature stop codon is in the last exon or within the last 50 bp of the penultimate exon

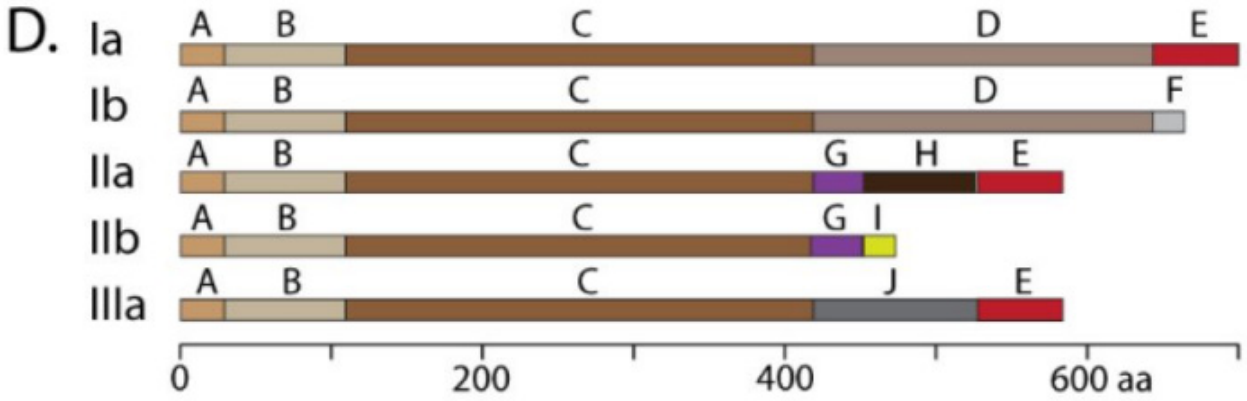
This frameshift occurs 35 bp from the final intron

PVS1 can be applied either at **strong** or **moderate**

- Strong if the truncated region is essential to disease-relevant protein function; moderate if not.

Computational and Predictive Data (PVS1): Selected examples

Example 2: *SYN1*:c.1941_1947dup, p.A650Rfs*36 found in a proband with global delays and seizures



SYN1 D-domain: residues 421-655
SYN1 E-domain: residues 656-705
Frameshift begins at residue 650

- Synapsin 1 encodes 2 of 5 of the major synapsin isoforms
- Synapsins regulate synaptic vesicle (SV) organization and neurotransmission by maintaining SV reserve pools
- The synapsins have 3 conserved N-terminal domains (A-C) whereas the C-terminal domains are more heterogenous (D-J)

Computational and Predictive Data (PVS1): Selected examples

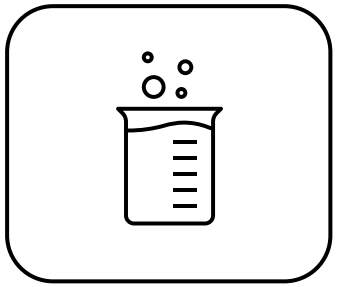
Example 2: *SYN1*:c.1941_1947dup, p.A650Rfs*36 found in a proband with global delays and seizures

Synapsin E-domain is essential for α-synuclein function

Alexandra Stavsky, Leonardo A. Parra-Rivas, Shani Tal, Jen Riba, Kayalvizhi Madhivanan, Subhojit Roy ✉, Daniel Gitler ✉

- In this paper, they “scramble” or randomize the E domain residues, essentially replicating the effect of a frameshift

- They found the loss of the E-domain:
  - Abolishes the attenuating effect of a-synuclein on synaptic responses
  - Completely abolishes the interaction between synapsin and a-synuclein
  - Re-introducing the E-domain (and only the E domain) restored the effect of a-synuclein



Functional data
PS3, PM1, PP2, BS4

Fundamental principle(s): experimental data demonstrating a variant's effect or the importance of a protein domain supports pathogenicity

Functional Data (BS3, PS3)

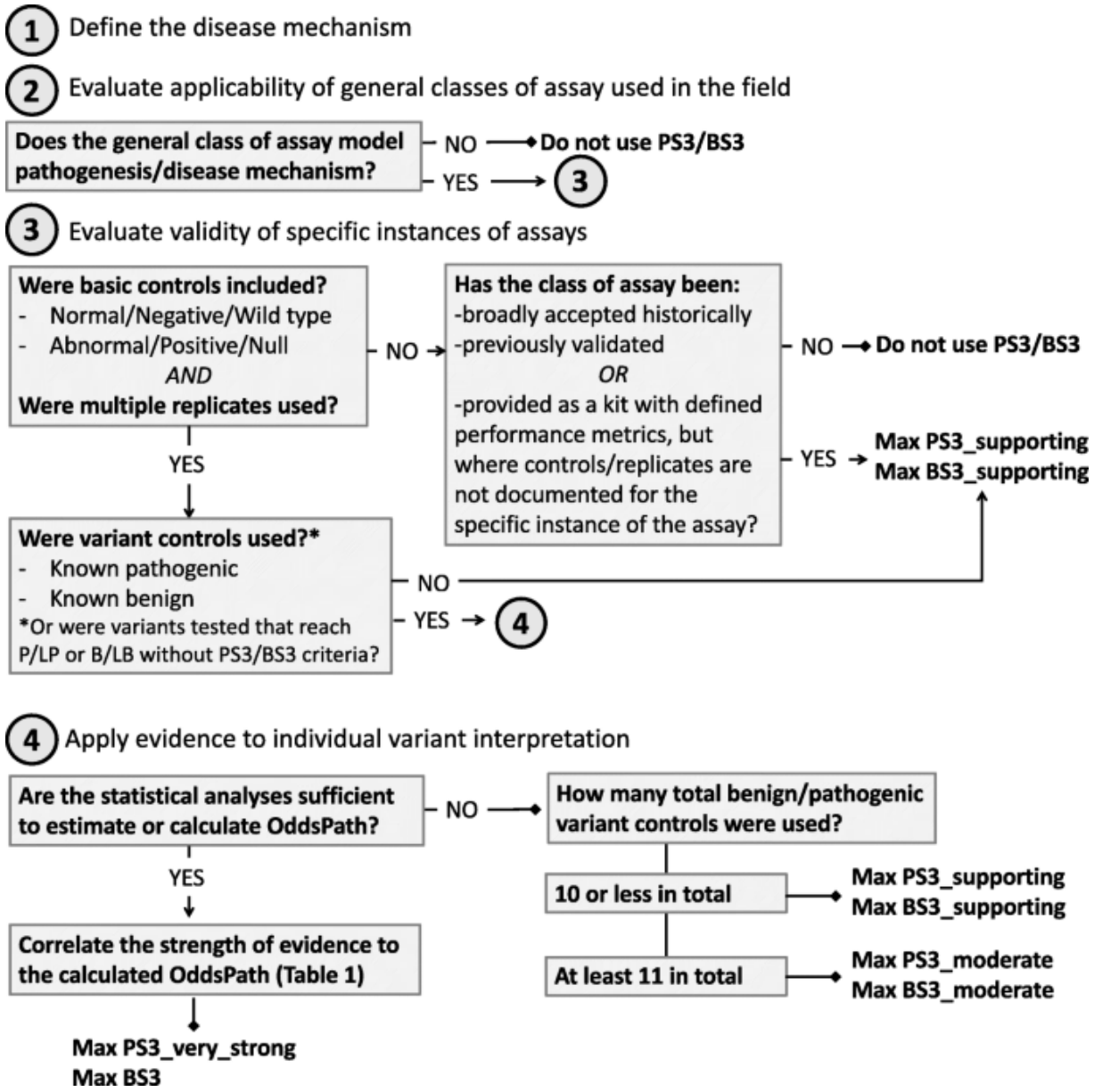
Criteria assignment:

- Very broadly: well-established functional evidence demonstrates your variant is deleterious (PS3) or has no effect (BS3)

- 2019 guidance (PMID:31892348) outlines 4 steps for applying PS3 and BS3, including what strengths are allowed:

  (1) define the disease mechanism
  (2) evaluate the applicability of general classes of assays used in the field
  (3) evaluate the validity of specific instances of assays, and
  (4) apply evidence to individual variant interpretation.

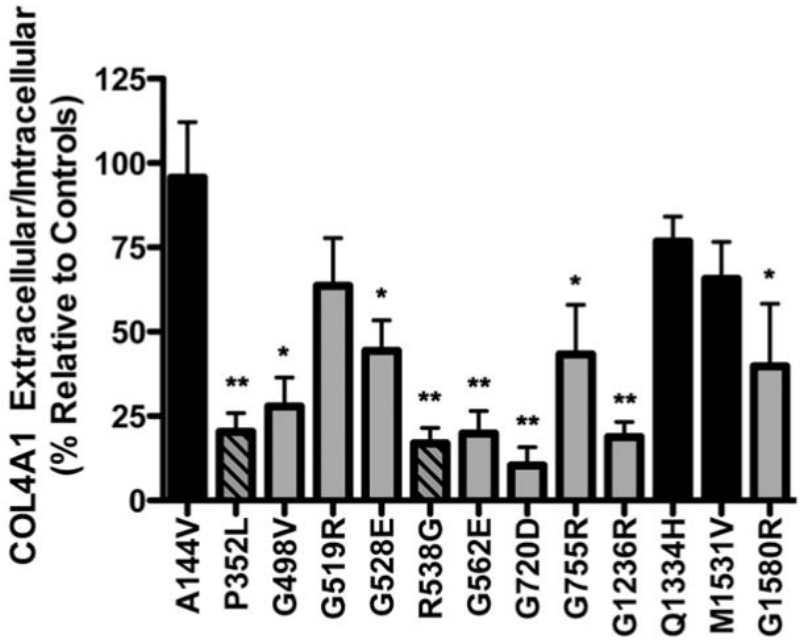
Functional Data (BS3, PS3)



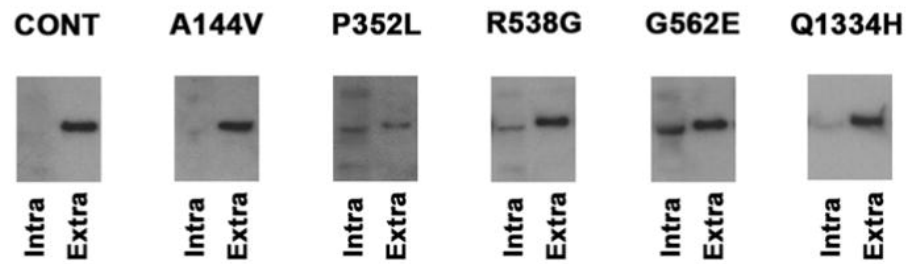
- PS3 is typically applied at supporting or moderate
- Statistical calculation of an odds of pathogenicity allows for PS3 at higher strength, but this is often only applicable in large-scale variant studies.

Functional Data (BS3, PS3): Example

A



B



A patient presents with sudden-onset hemiplegia. An MRI reveals a recent cerebral hemorrhage and evidence of several old microbleeds.

WGS reveals the variant *COL4A1*:c.2159G>A, G720D

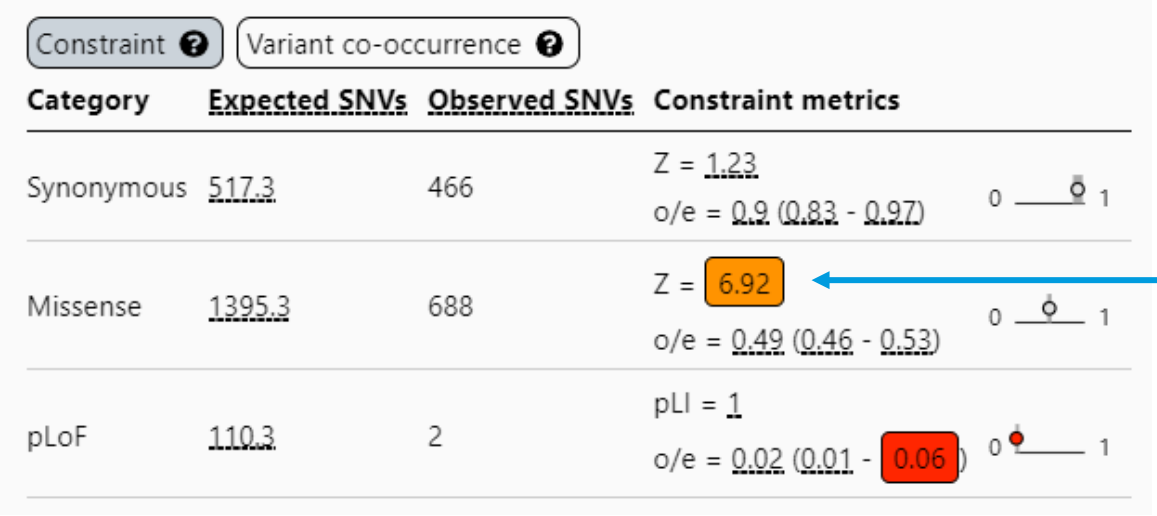
1. Define the disease mechanism:
- The disease mechanism is thought to result from a lack of stable collagen being exported, leading to decreased integrity of the extracellular matrix.

2. Evaluate the applicability of general assay classes used in the field.
- WB is very commonly used and applicable to the mechanism.

3. Evaluate the validity of specific instances of assays
- Done in triplicate with WT control

4. Apply evidence to individual variant interpretation.
- Not enough known pathogenic variants to reach PS3_moderate. Apply PS3_supporting.

Functional Data:
Gene intolerant to missense: (PP2)



Missense z-score

Criteria application: A gnomAD missense z-score of 3.09 corresponds to a p-value of 0.001 for the null hypothesis: the observed missense variants = expected missense variants. Genes with z-score > 3.09 can have PP2 assigned to missense variants.

Strength: PP2 is applied at supporting. Some groups / clinical labs do not apply this criteria.

Functional Data:
Functional Region: (PM1)

The variant lies in a mutational hotspot or well-studied functional domain without benign variation. Application & strength determination of this criteria is gene-specific; for example:

ClinGen Familial Hypercholesterolemia Expert Panel Specifications to the ACMG/AMP Variant Classification Guidelines Version 1.2

Rule Set:	Rules For LDLR
Disease(s)	hypercholesterolemia, familial, 1
Gene(s)	LDLR
Genetype	nuclear

Criteria Code	Strength Specification
PM1 - Very Strong	NA
PM1 - Strong	NA
PM1 - Moderate	Missense variant located in exon 4, or a missense change in one of 60 highly conserved cysteine residues (listed in Supp. Table 4). Caveat: variant must also meet PM2.
PM1 - Supporting	NA

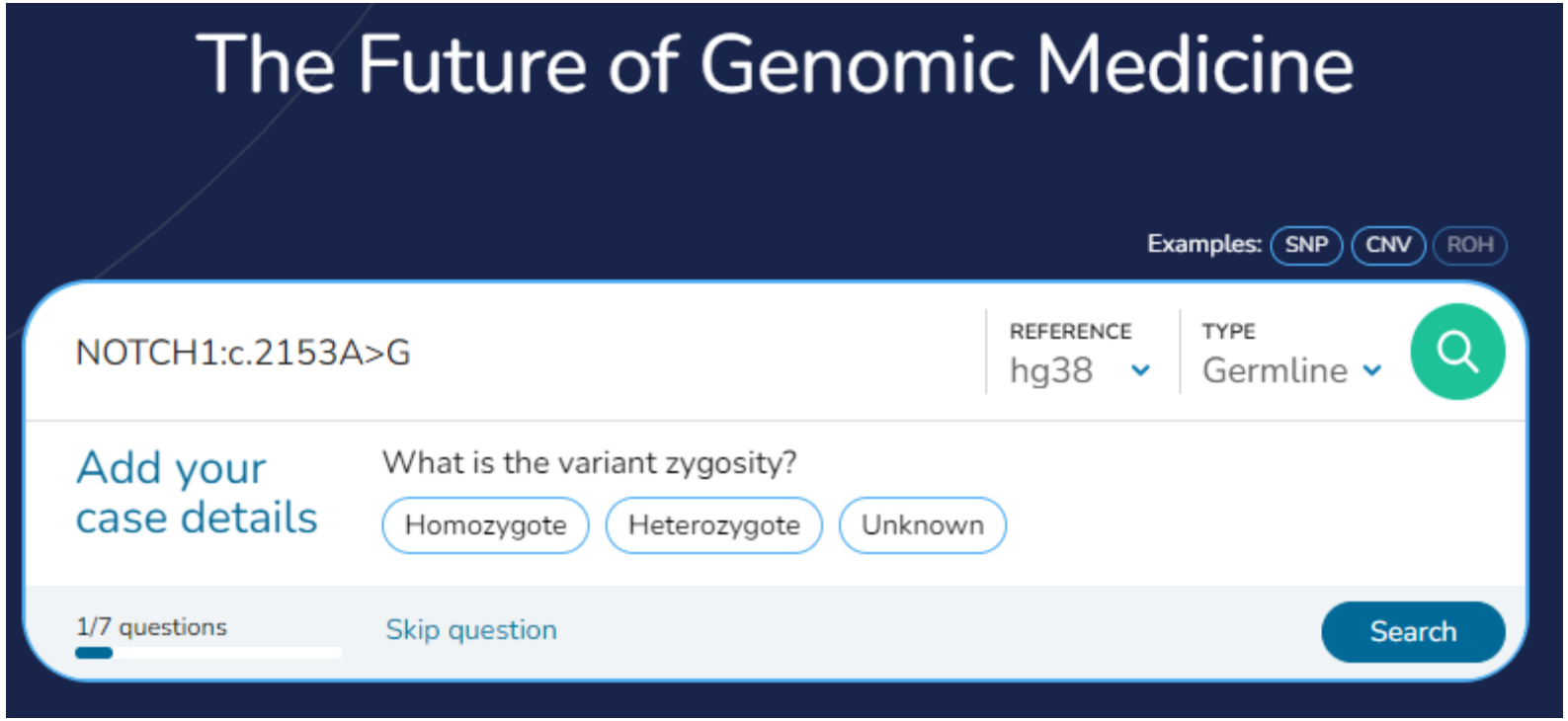
ClinGen Familial Hypercholesterolemia Expert Panel Specifications to the ACMG/AMP Variant Classification Guidelines Version 1.2

Showing 1 to 4 of 4 entries (filtered from 107 total entries)

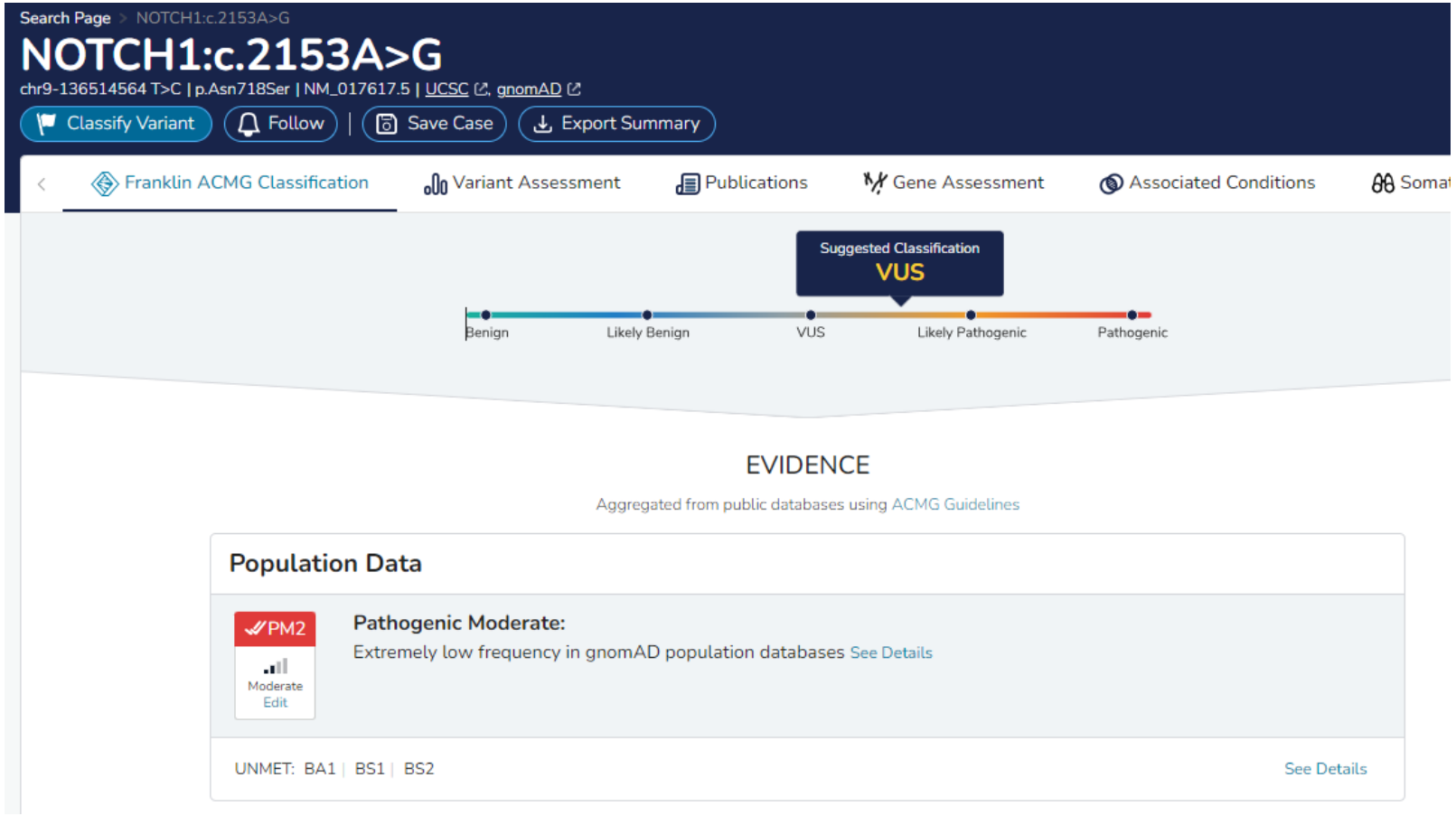
Previous 1 Next

https://cspec.genome.network/cspec/ui/svi/summary

General Tools: Franklin



General Tools: Franklin

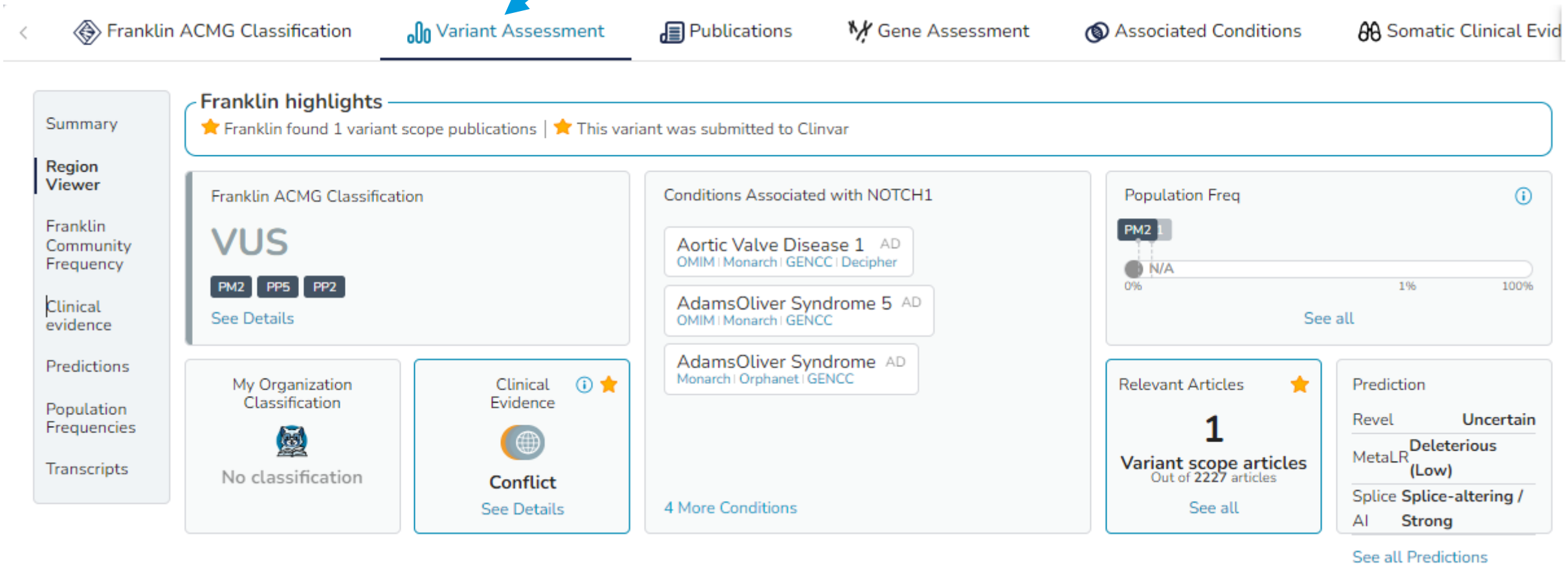


<u>Don't use the Franklin classification</u>

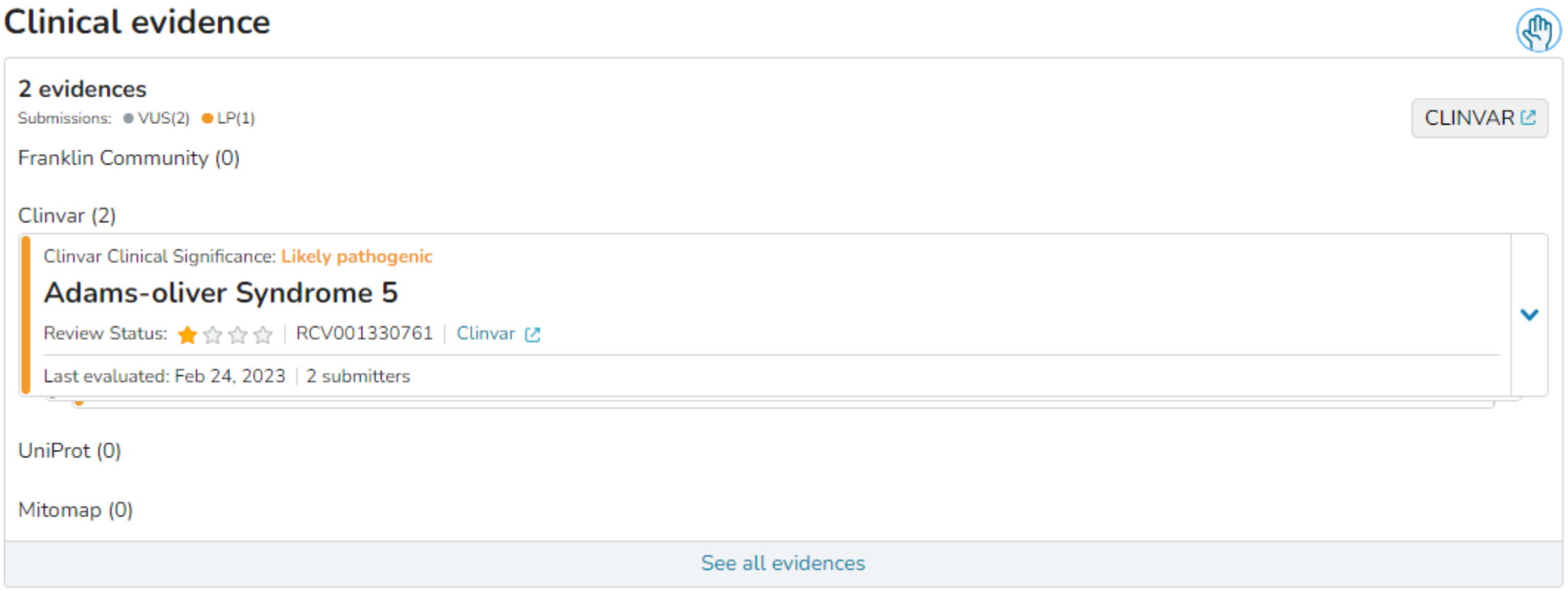
- They don't apply all ACMG criteria correctly

General Tools: Franklin

Most helpful tab

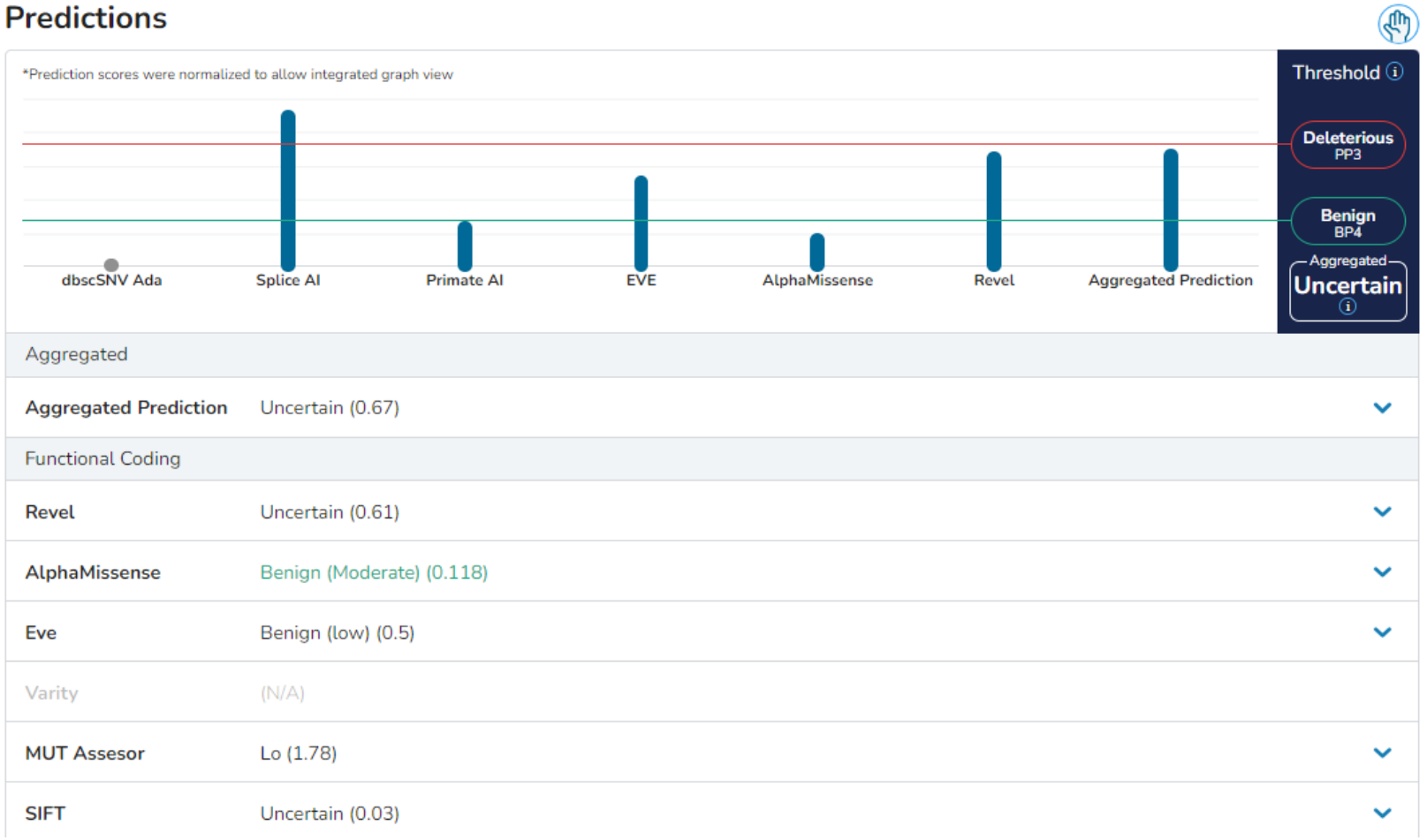


General Tools: Franklin



- They mine and link to ClinVar

General Tools: Franklin



- They give multiple *in silico* scores, including REVEL and Splice-AI

General Tools: Franklin

- They pull frequency data from gnomAD

