

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:

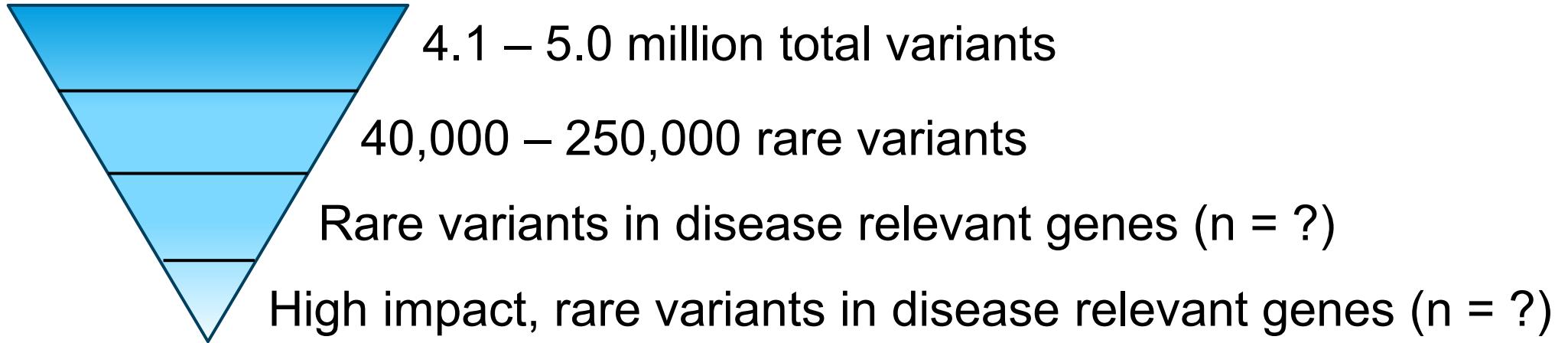
Variants

- **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



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

ACMG Criteria 2015

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, PhD¹, Nazneen Aziz, PhD^{2,16}, Sherri Bale, PhD³, David Bick, MD⁴, Soma Das, PhD⁵, Julie Gastier-Foster, PhD^{6,7,8}, Wayne W. Grody, MD, PhD^{9,10,11}, Madhuri Hegde, PhD¹², Elaine Lyon, PhD¹³, Elaine Spector, PhD¹⁴, Karl Voelkerding, MD¹³ and Heidi L. Rehm, PhD¹⁵; on behalf of the ACMG Laboratory Quality Assurance Committee

These recommendations primarily apply to genetic tests used in clinical laboratories including genotyping, single genes, panels, exomes and genomes.

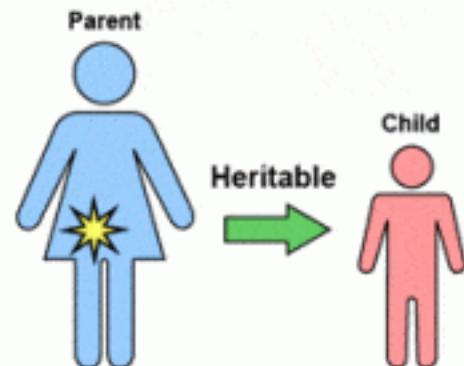
It is not intended for the interpretation of somatic variation, pharmacogenomic variants, or variants in genes associated with multigenic non-Mendelian complex disorders.

Somatic mutations
• Occur in *nongermline* tissues
• Cannot be inherited



Mutation in tumor only
(for example, breast)

Germline mutations
• Present in egg or sperm
• Can be inherited
• Cause cancer family syndrome

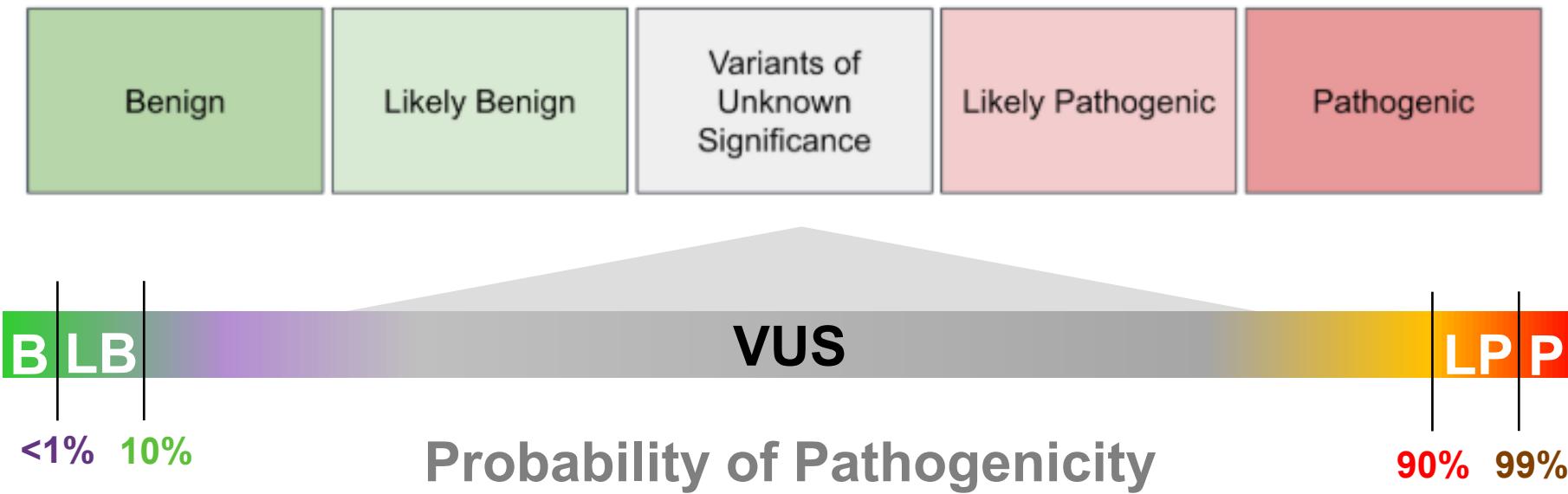


Mutation in
egg or sperm

| Class |
|-----------------------------------|
| Pathogenic |
| Likely Pathogenic |
| Variant of Uncertain Significance |
| Likely Benign |
| Benign |

Adapted from the National Cancer Institute and the American Society of Clinical Oncology

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 <i>trans</i> with a dominant variant BP2 Observed in <i>cis</i> with a pathogenic variant BP2 | | For recessive disorders, detected in <i>trans</i> 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 | | | |

- 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://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 | <i>De novo</i> | 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 | <i>In trans</i> | moderate | supporting – very strong |
| PM4 | Length changing | moderate | supporting – moderate |
| PM5 | Same position, different AA | moderate | supporting – strong |
| PM6 | Assumed <i>de novo</i> | moderate | supporting – very strong |
| PP1 | Cosegregation | supporting – strong | supporting – strong |
| PP2 | Intolerant to missense | supporting | supporting |
| PP3 | <i>In silico</i> | supporting | supporting – moderate |
| PP4 | Specific phenotype | supporting | supporting – moderate |
| PP5 | Reputable source | supporting | discontinued |

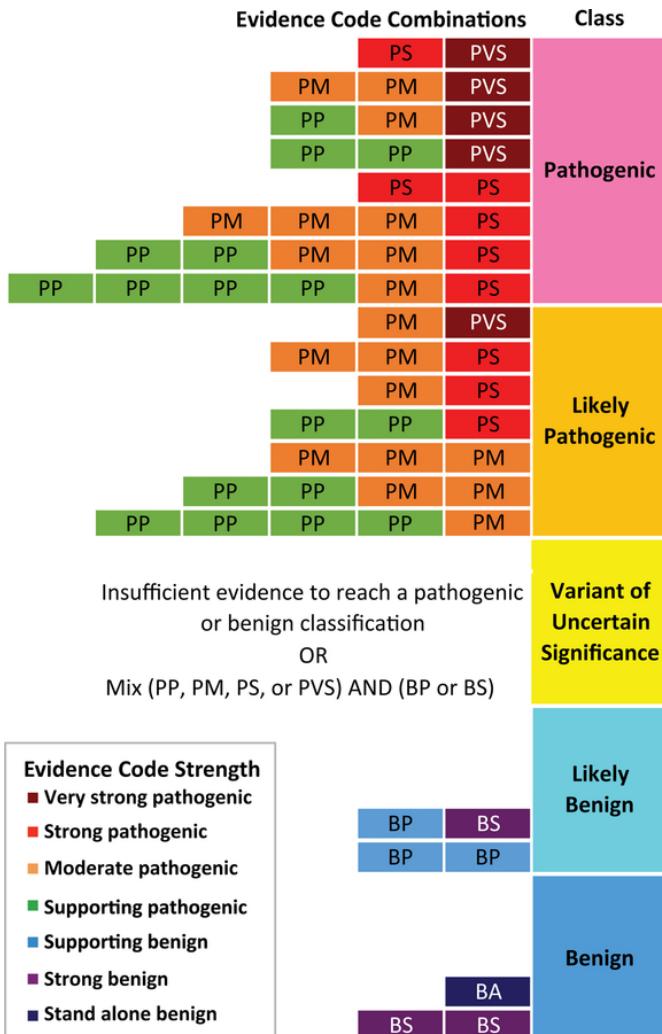
Strengths of ACMG Criteria

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 | <i>In silico</i> | 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.

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:

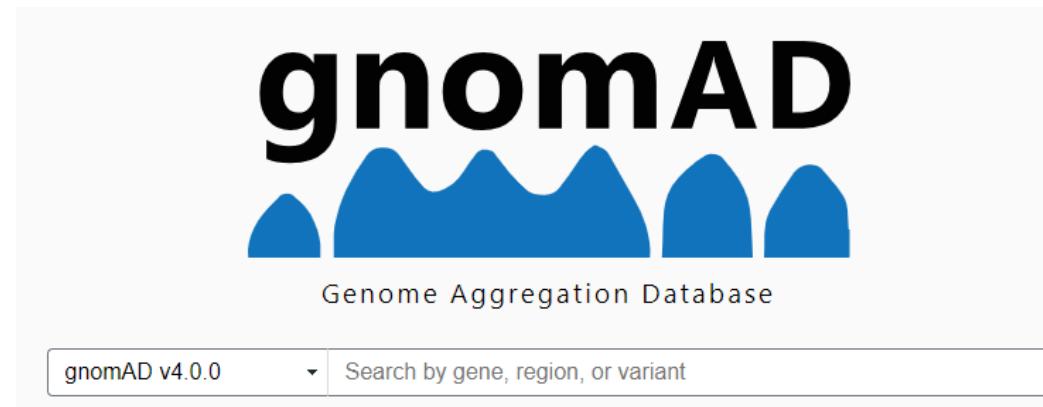
- How to apply the specific ACMG criteria.
- How strength of the criteria is determined.
- Tools / databases used to apply criteria.
- Specific examples of the criteria being applied to variants.

Population Data: Control Populations (BA1, BS1, BS2, PM2)

Criteria assignment:

- The reference control database is gnomAD, but other databases are available
- PM2: variant is absent/rare in control populations.
 - “Rarity” is specific for the gene, inheritance pattern, and penetrance of the disorder.
 - Max MAF < 0.0005 is often used as a “general” cutoff
- 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 Data: gnomAD



- 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-GGGCGGC... 6-156778889-CGGAGGAA-C | E G | p.Gly400_Gly402del p.Gly410_Gly411del | ● inframe deletion | | Benign/Likely benign | | 2790 | 1403448 | 1.99e-3 | 10 |
| 6-156778943-T-TGTGGCG | E G | p.Val422_AlA423dup | ● inframe insertion | | Benign/Likely benign | | 2600 | 1272008 | 2.04e-3 | 3 |

Population Data: Control Populations (BA1, BS1, BS2, PM2)

Strength determination:

- PM2 is recommended to be applied only at supporting
 - Rare variants are common
 - See [ClinGen PM2 recommendation 2020](#)
- BA1: automatically makes a variant benign. These variants are often filtered out before any classification.
- 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 Data: 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 | Biotinidase 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

Population Data: Prevalence in Affected Population (PS4)

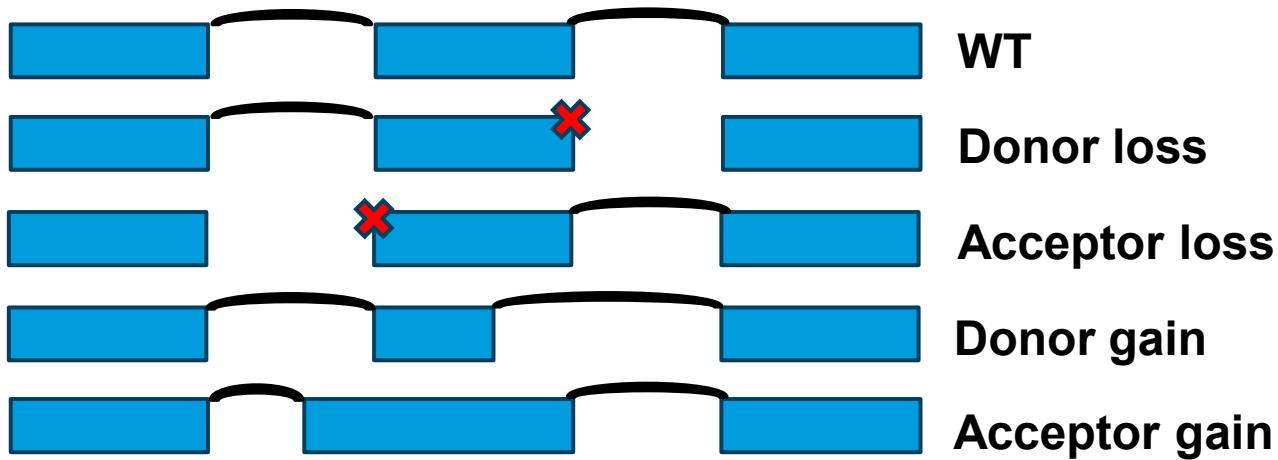
Criteria assignment & strength:

- Variant is associated with an odds ratio > 5.0 of causing disease with a lower bounded confidence interval > 1.0 .
- For many disease-causing variants (which are very rare causing very rare diseases) you don't get enough data to calculate a significant odds ratio. In this case, ACMG allows "proband counting" thresholds determined for a particular gene / disorder:

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

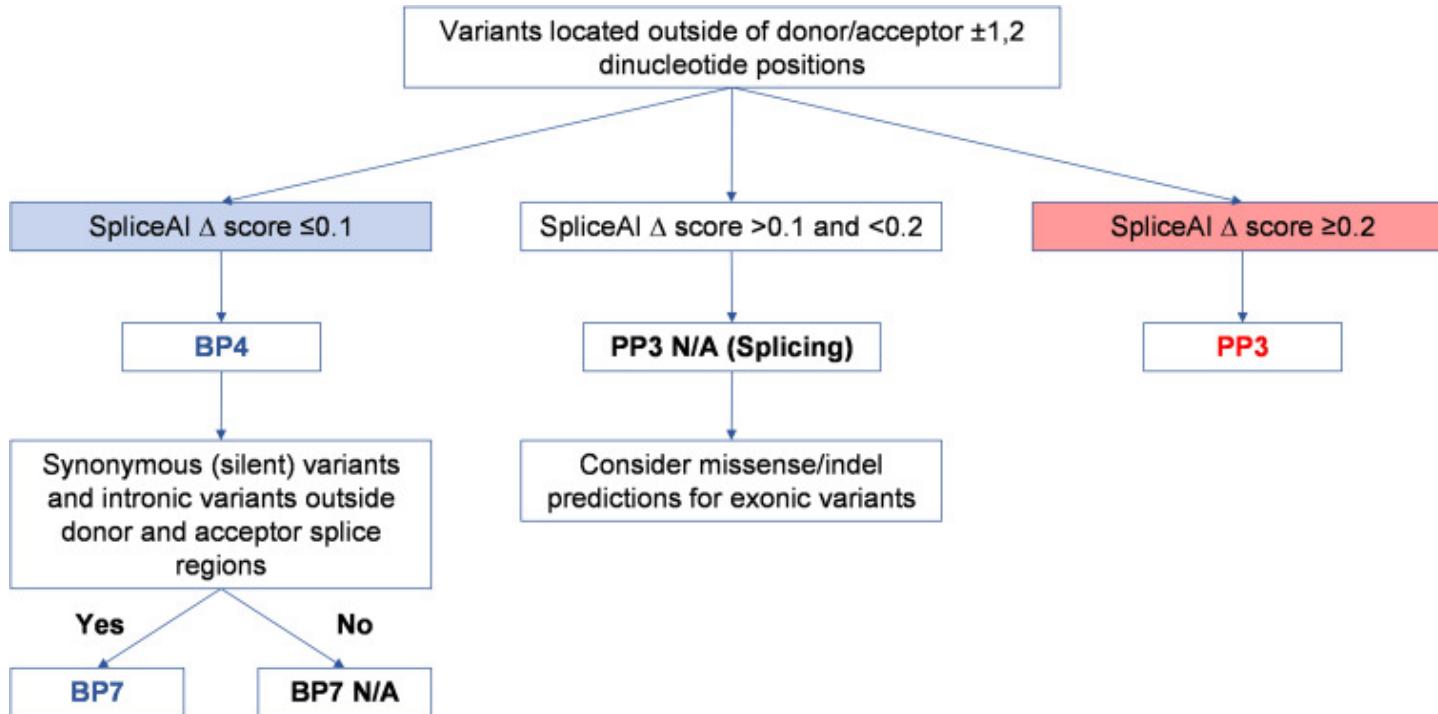
| | | Supporting | Moderate | Strong | Very strong |
|-----|-------------------|------------|------------|-------------|-------------|
| PS4 | Cardiomyopathy | 2 probands | 6 probands | 15 probands | N/A |
| | RASopathy | 1 proband | 3 probands | 5 probands | N/A |
| | PTEN | 1 point | 2 points | 4 points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

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)



- 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

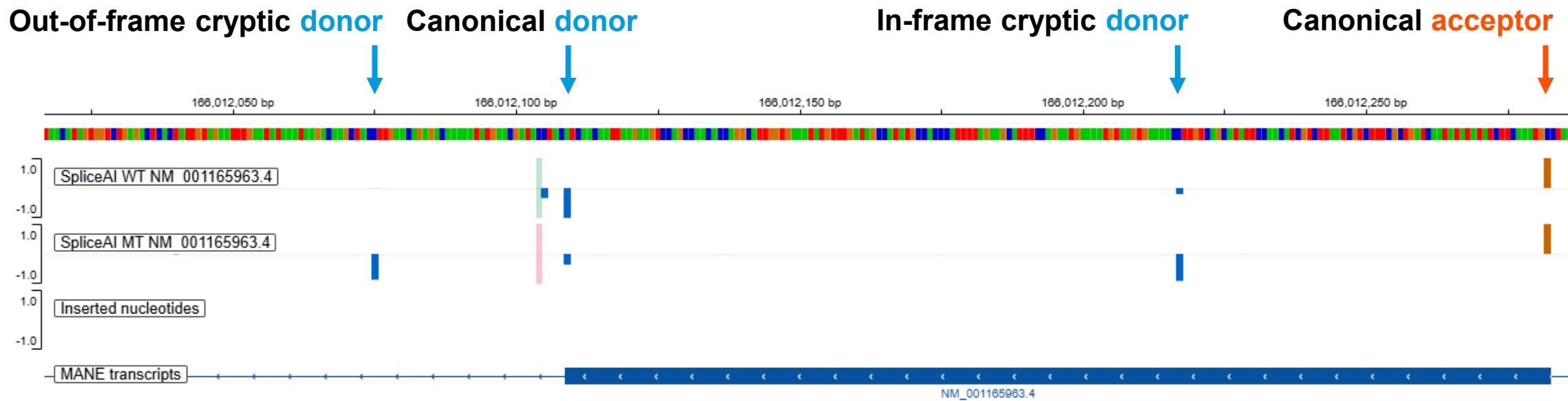
| Variant | Gene | MANE Select transcript non-coding transcript | Δ type | Δ score | position |
|-------------------------------|---|---|---------------|---------|----------|
| chr2-166012104 C>T | SCN1A (ENSG00000144285.24 / ENST00000674923.1 / NM_001165963.4) | | Acceptor Loss | 0.00 | 178 bp |
| splice 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

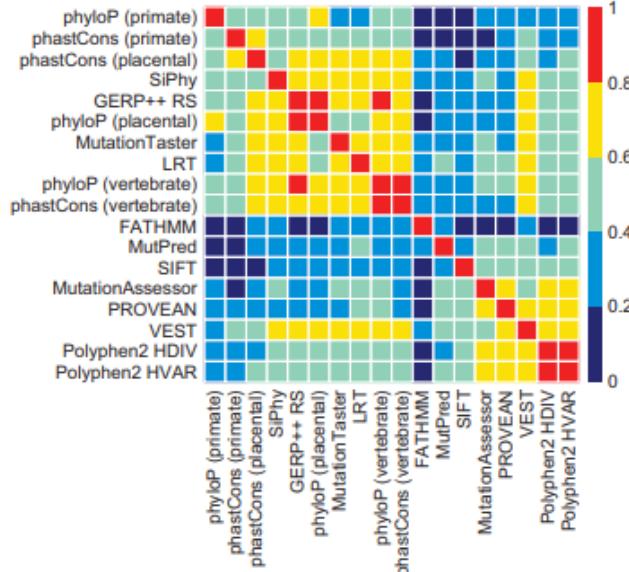
Computational and Predictive Data: Missense *in silico* predictions (BP4, PP3)

Criteria assignment:

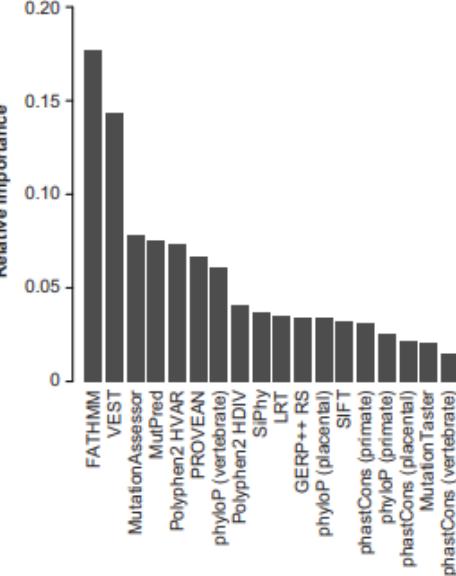
- Multiple *in silico* tools predict the variant is benign (BP4) or pathogenic (PP3). If conflicting, neither criteria is assigned.
- The most recent *in silico* tools like REVEL or AlphaMissense combine many forms of analysis into a single score. Thus, a REVEL score, for example, constitutes “multiple” *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

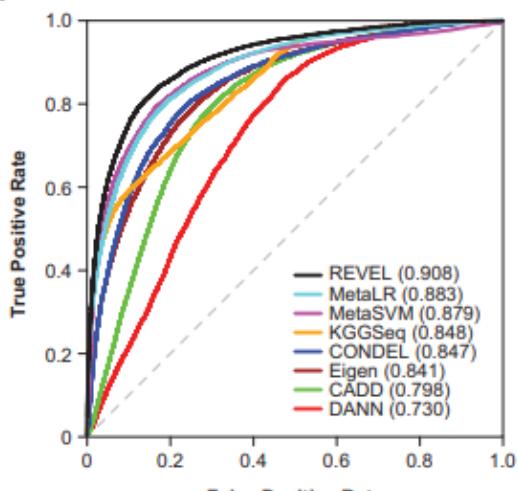
A



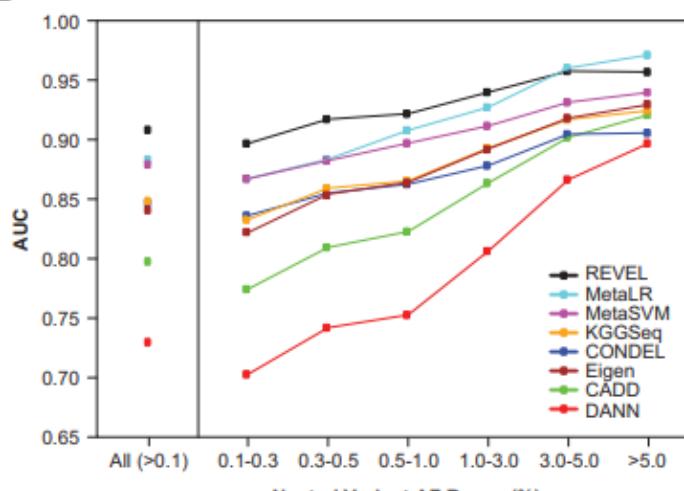
B



A



B



- 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

| Method | Benign (BP4) | | | | Pathogenic (PP3) | | | |
|-----------|--------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------|-------------|
| | 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

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

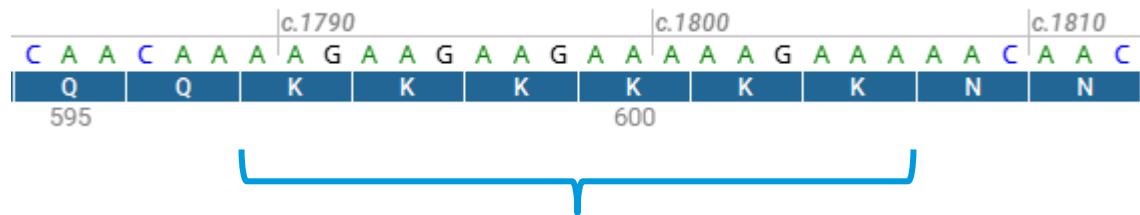
Computational and Predictive Data: In-frame indels (BP3, PM4)

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.

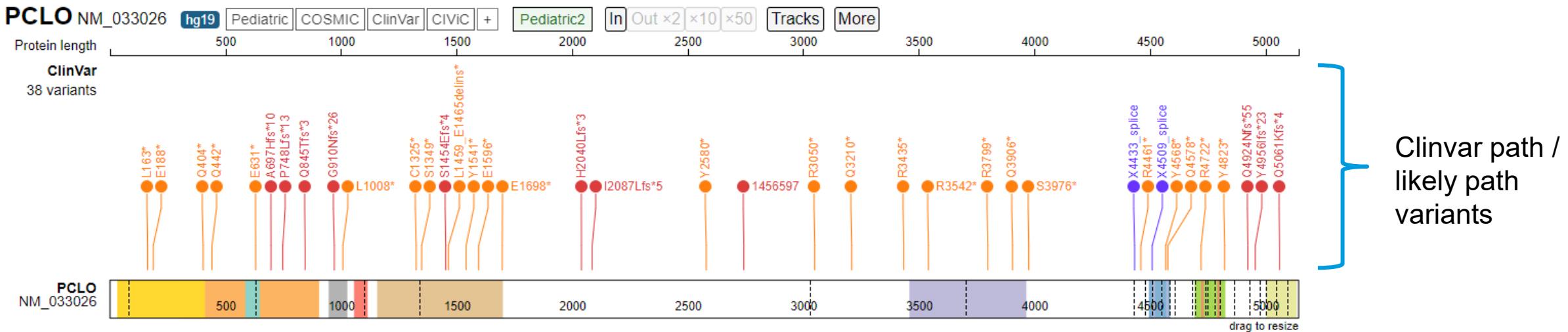


Image made in ProteinPaint (<https://proteinpaint.stjude.org/>)

Computational and Predictive Data (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.

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)

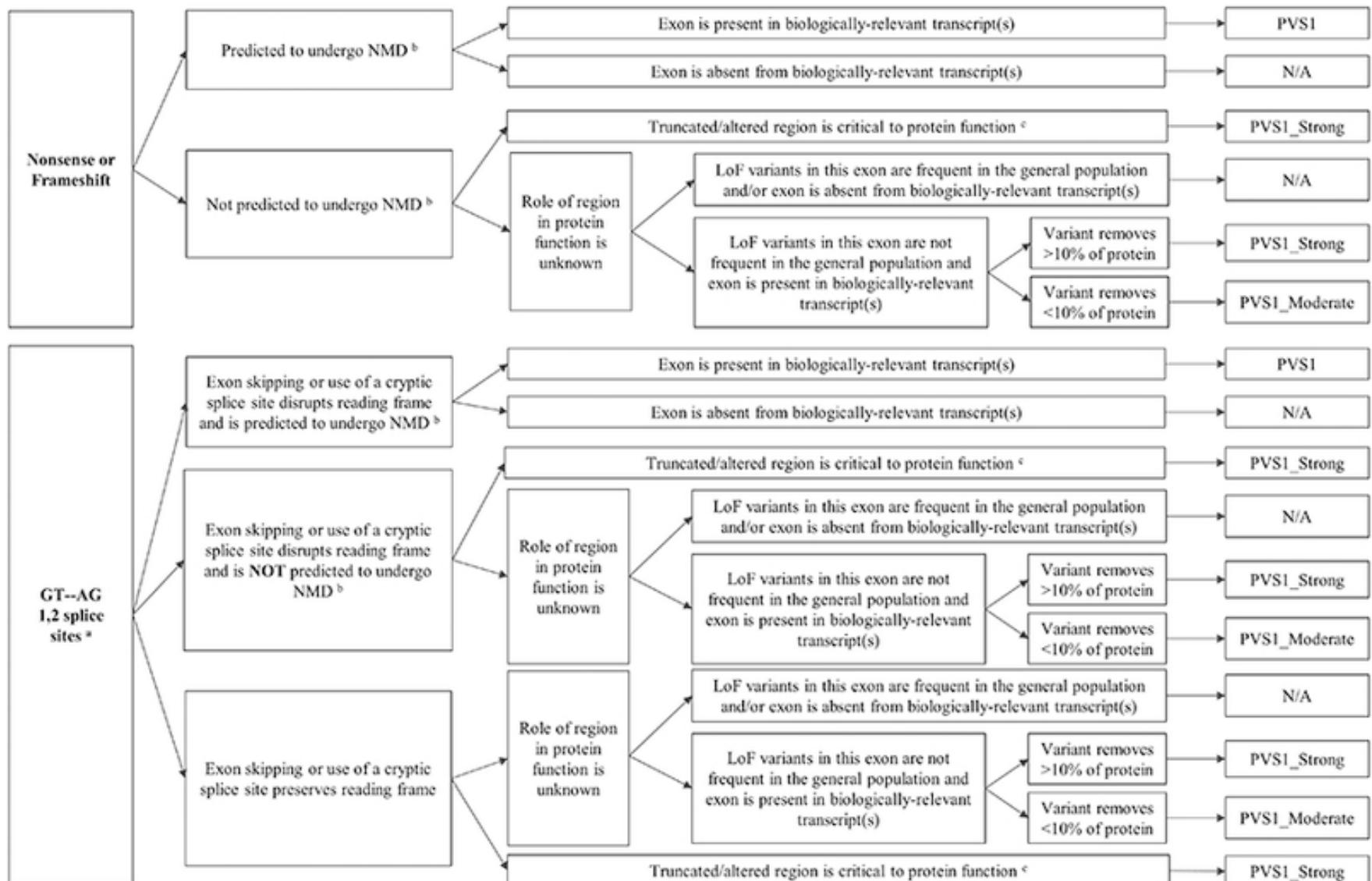
| Category | Expected SNVs | Observed SNVs | Constraint metrics |
|------------|---------------|---------------|--|
| Synonymous | 1092.7 | 1013 | Z = <u>1.31</u> o/e = <u>0.93</u> (0.88 - 0.98) 0 — 1 |
| Missense | 2707.8 | 2501 | Z = <u>1.45</u> o/e = <u>0.92</u> (0.89 - 0.95) 0 — 1 |
| pLoF | 228.7 | 60 | pLI = <u>1</u> o/e = <u>0.26</u> (0.21 - 0.33) 0 — 1 |

Strength determination:

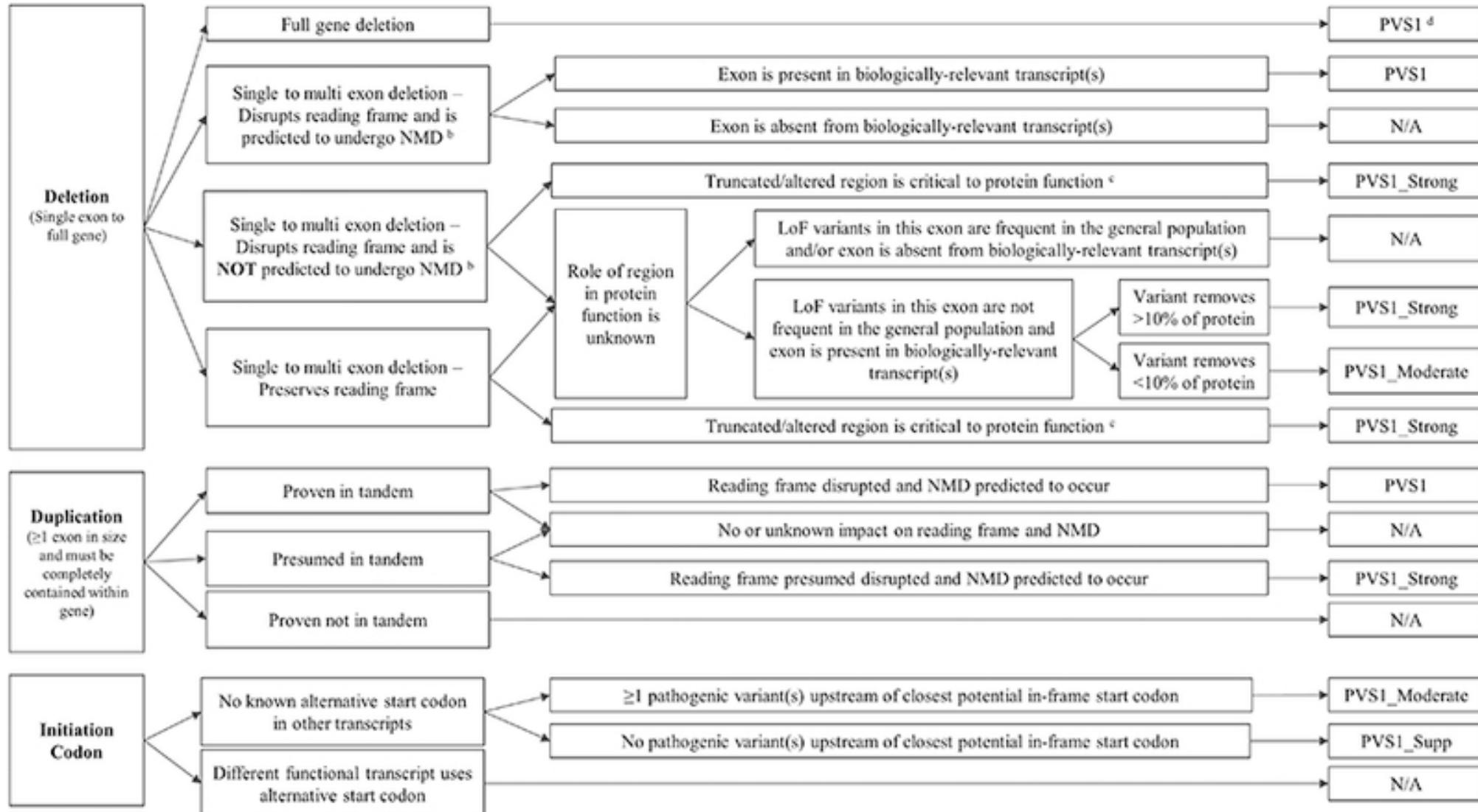
**Recommendations for interpreting the loss of function PVS1
ACMG/AMP variant criterion**

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)



Computational and Predictive Data (PVS1)



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

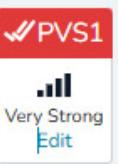
- This is essentially the same as **PM4**

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:

 PVS1
 Very Strong
[Edit](#)

Pathogenic Very Strong:
Null variant in a gene where loss of function is a known mechanism of disease [Close Details](#)

- ✓ Null variant (acceptor site):
- ✓ Loss of function is a known mechanism of disease:
399 pathogenic null variants were reported in ClinVar for this gene ([chrX:18588096:G>T:HG38](#), [chrX:18588063:AC>A:HG38](#), [chrX:18588069:C>T:HG38](#), [chrX:18588058:T>TTTTA:HG38...](#)), across 17 different exons, of which 25 variants in this exon (9)
gnomAD observed/expected score 0.226
Coding strand: forward strand
- ✓ Exon skipping disrupts reading frame
- ✓ Predicted to undergo NMD, not located in last exon or last 50bp of preliminary exon.
Coding exon number 8 out of 17 coding exons (9 out of total exons)
- ✓ Altered region is critical to protein function
Number of pathogenic non-nonsense variants in skipped exon: 27
- ✗ Variant removes more than 10% of transcript (6.6% of transcript.)

Computational and Predictive Data (PVS1): Selected examples

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

The screenshot shows the ClinGen Curated Gene Summary page for the gene *CDKL5*. The top navigation bar includes links for Curated Genes, Gene-Disease Validity, Dosage Sensitivity, Clinical Actionability, Curated Variants, Statistics, Downloads, More, and a help icon. Below the header, the gene name *CDKL5* is displayed with a DNA icon, and a "View Gene Facts" button. Key metrics shown are: 1 Gene-Disease Validity Classifications, 2 Dosage Sensitivity Classifications, 0 Clinical Actionability Assertions, 78 Variant Pathogenicity Assertions, and 0/0 CPIC / PharmGKB High Level Records. A "Follow Gene" button is also present.

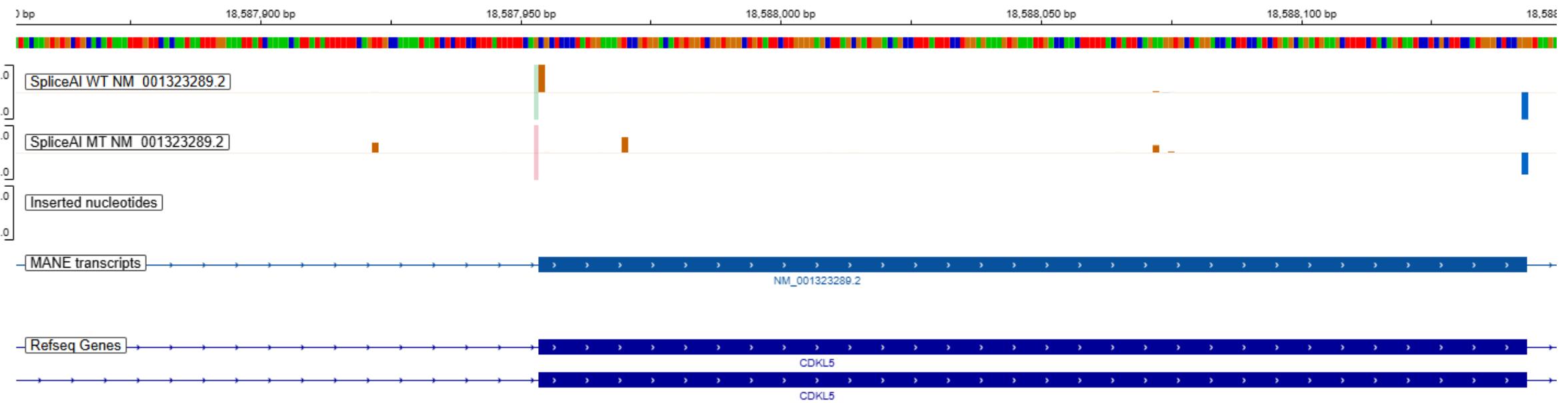
Below the metrics, tabs for Curation Summaries, Status and Future Work (2), GenomeConnect (2), External Genomic Resources, and ClinVar Variants are visible. The "Gene-Disease Validity" section is currently active, showing a table with columns for Gene, Disease, MOI, Expert Panel, Classification, and Report & Date. One entry for *CDKL5* is listed: Disease is *CDKL5 disorder* (MONDO:0100039), MOI is XL (1), Expert Panel is Rett and Angelman-like Disorders GCEP, Classification is Definitive, and Report & Date is 07/02/2018.

The "Dosage Sensitivity" section shows a table with columns for Gene, Disease, Working Group, HI Score & TS Score, and Report & Date. Two entries for *CDKL5* are listed: one under "Dosage Sensitivity WG" with a red box highlighting the classification "3 (Sufficient Evidence for Haploinsufficiency)" and report date 07/12/2012, and another under "Dosage Sensitivity WG" with classification "0 (No Evidence for Triplosensitivity)" and report date 07/12/2012.

- 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:

 PVS1
 Strong


Pathogenic Strong:
Null variant in a gene where loss of function is a known mechanism of disease [Close Details](#)

- Null variant (frameshift indel):
- Loss of function is a known mechanism of disease:
60 pathogenic null variants were reported in ClinVar for this gene ([chrX:47574328:CG>C:HG38](#), [chrX:47574077:AGCTGTGGTTGGGA](#)
[CGTCCAGCGGGGCCGCGCTGGGCCGAGGCTGCGTGGTGGGTGGCCCAGTGCAGGGACCCGCCTGGCTGGCCTGGCG](#)
[TGTGGGGCCG>A:HG38](#), [chrX:47574021:GT>G:HG38](#), [chrX:47574390:G>A:HG38...](#)), across 12 different exons, of which 18 variants in this exon (12)
gnomAD observed/expected score 0.251
Coding strand: reverse strand
- Predicted to undergo NMD, not located in last exon or last 50bp of preliminary exon.
Coding exon number 12 out of 13 coding exons (12 out of total exons)
- Truncated region is critical to protein function
Last pathogenic position [chrX:47572955:GC>G](#) last pathogenic variants [chrX:47572955:GC>G:HG38](#), [chrX:47574077:AGCTGTGGTT](#)
[GGGACGTCAGCGGGGCCGCGCTGGGCCGAGGCTGCGTGGTGGGTGGCCCAGTGCAGGGACCCGCCTGGCTGGCC](#)
[TGGCGTGTGGGGCCG>A:HG38](#), [chrX:47574193:T>TGGGCCTGGGGTTCTGGGGCG:HG38](#)
Number pathogenic variants in non-NMD region: 17 variants ([chrX:47574512:TG>T:HG38](#), [chrX:47574390:G>A:HG38](#), [chrX:47574077:AGCTGTGGTTGGGACGTCCAGCGGGGCCGCGCTGGGCCGAGGCTGCGTGGTGGGTGGCCCAGTGCAGGGACCCGC](#)
[CTGGCTGGCCTGGCGTGTGGGCCG>A:HG38](#), [chrX:47574328:CG>C:HG38...](#))

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



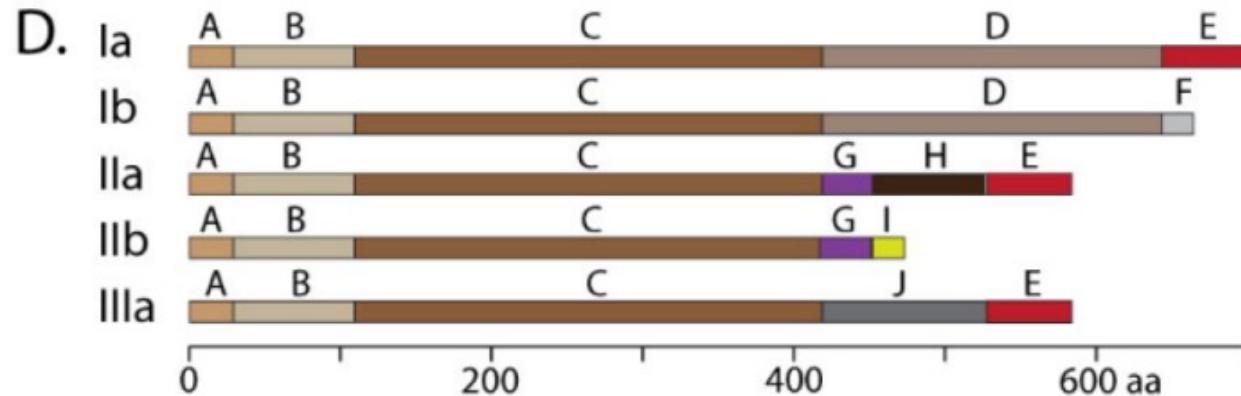
This frameshift occurs 35 bp from the final intron

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 α -synuclein on synaptic responses
 - Completely abolishes the interaction between synapsin and α -synuclein
 - Re-introducing the E-domain (and only the E domain) restored the effect of α -synuclein

Functional Data (BS3, PS3)

Criteria assignment:

- Very very broadly: well-established functional evidence demonstrates your variant is deleterious (PS3) or has no effect (BS3)
- New guidance (<https://pubmed.ncbi.nlm.nih.gov/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)

1 Define the disease mechanism

2 Evaluate applicability of general classes of assay used in the field

Does the general class of assay model pathogenesis/disease mechanism?

— NO → Do not use PS3/BS3

— YES → 3

3 Evaluate validity of specific instances of assays

Were basic controls included?

- Normal/Negative/Wild type
 - Abnormal/Positive/Null
- AND

Were multiple replicates used?

— NO →

YES

Were variant controls used?*

- Known pathogenic
- Known benign

*Or were variants tested that reach P/LP or B/LB without PS3/BS3 criteria?

4 Apply evidence to individual variant interpretation

Are the statistical analyses sufficient to estimate or calculate OddsPath?

— YES

Correlate the strength of evidence to the calculated OddsPath (Table 1)

Max PS3_very_strong

— NO → How many total benign/pathogenic variant controls were used?

10 or less in total

Max PS3_supporting
Max BS3_supporting

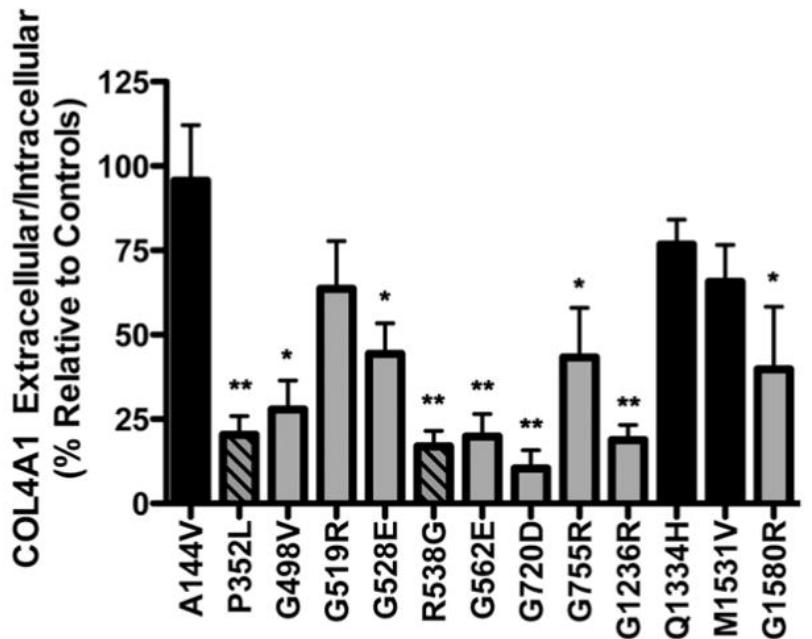
At least 11 in total

Max PS3_moderate
Max BS3_moderate

- 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



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.

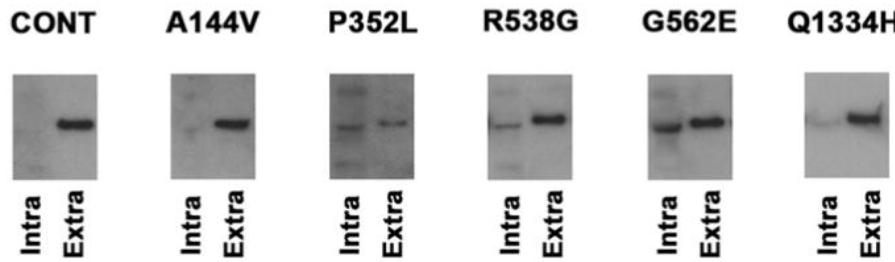
3. Evaluate the validity of specific instances of assays

- Done in triplicate with WT control

4. Apply evidence to individual variant interpretation.

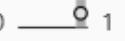
- Not enough known pathogenic variants to reach PS3_moderate. Apply PS3_supporting.

B



Functional Data: Gene intolerant to missense: (PP2)

Constraint  Variant co-occurrence 

| Category | Expected SNVs | Observed SNVs | Constraint metrics |
|------------|---------------|---------------|--|
| Synonymous | 517.3 | 466 | Z = <u>1.23</u> o/e = <u>0.9</u> (<u>0.83</u> - <u>0.97</u>)  |
| Missense | 1395.3 | 688 | Z = 6.92  o/e = <u>0.49</u> (<u>0.46</u> - <u>0.53</u>) |
| pLoF | 110.3 | 2 | pLI = 1 o/e = <u>0.02</u> (<u>0.01</u> - 0.06)  |

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 |
| Criteria Code | Strength Specification |
| Disease(s) | hypercholesterolemia, familial, 1 |
| Gene(s) | LDLR |
| Genotype | nuclear |
| 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>

Segregation Data (BS4, PP1)

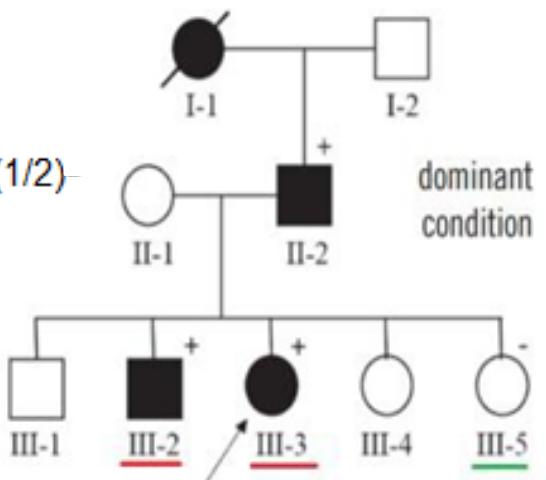
| PP1 Strength | # Affected Individuals | Meiosis Method (1/2) ^m |
|-------------------|--|---|
| Weak (Supporting) | AD: ≥ 3 AR: ≥ 2 | $\leq 1/8$ in 1 family $\leq 1/4$ in >1 family |
| Moderate | AD: ≥ 6 AR: $\geq 3, \geq 2$ families | $\leq 1/16$ in 1 family $\leq 1/8$ in >1 family |
| Strong | AD: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

Two additional meiosis
(II-2 and III-2) = $(1/2)^2$

An unaffected individual (III-5) = $(1/2)$
Total = $1/4 \times 1/2 = 1/8$

= Supporting evidence
($\leq 1/8$ 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

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 |

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 <i>trans</i> | 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 |

¹All 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.

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 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 |
| Genotype | 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

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 |
| 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 |

General Tools: Franklin

The Future of Genomic Medicine

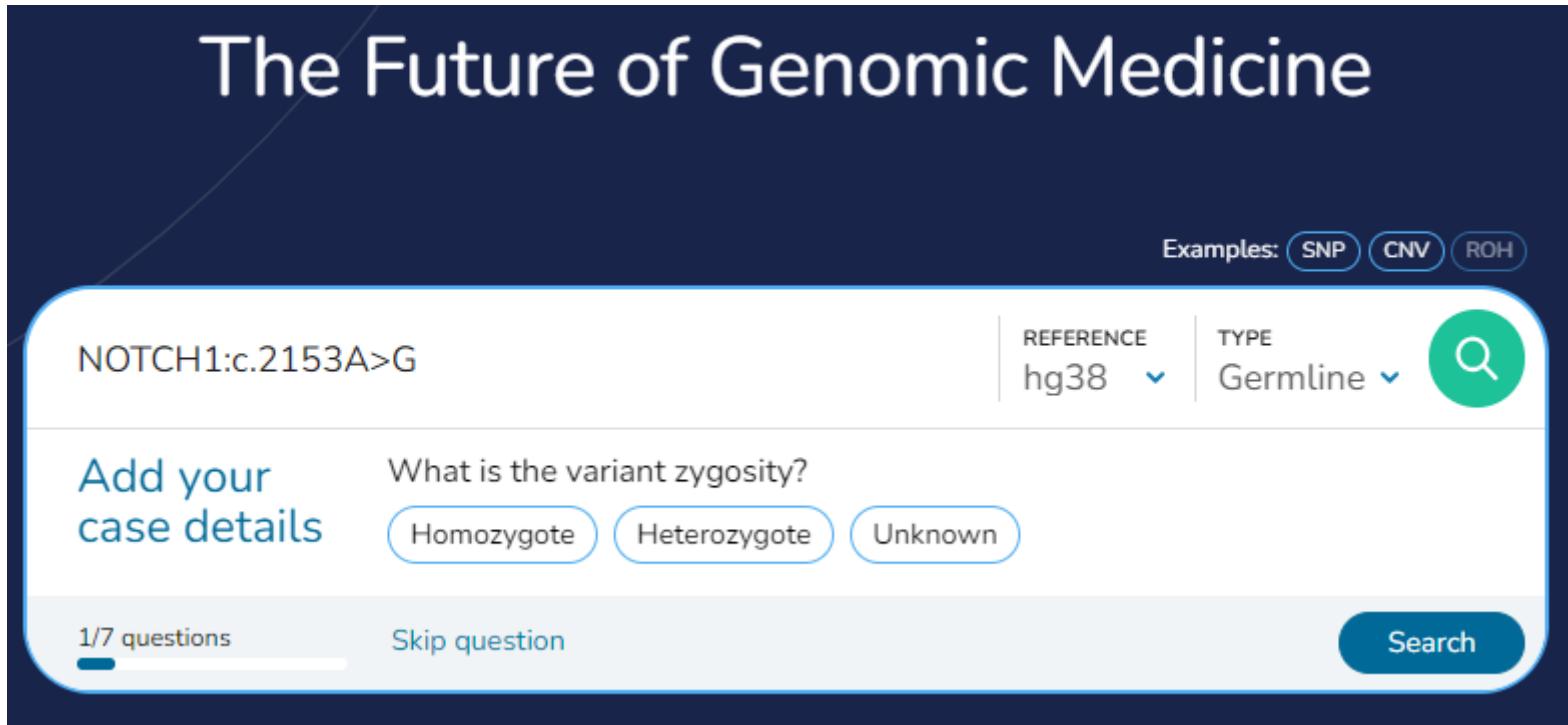
Examples: SNP CNV ROH

NOTCH1:c.2153A>G

REFERENCE hg38 TYPE Germline 

Add your case details What is the variant zygosity?
Homozygote Heterozygote Unknown

1/7 questions Skip question Search



General Tools: Franklin

Search Page > NOTCH1:c.2153A>G

NOTCH1:c.2153A>G

chr9-136514564 T>C | p.Asn718Ser | NM_017617.5 | UCSC, gnomAD

Class Variant | Follow | Save Case | Export Summary

Franklin ACMG Classification | Variant Assessment | Publications | Gene Assessment | Associated Conditions | Somatic

Suggested Classification
VUS

Benign Likely Benign VUS Likely Pathogenic Pathogenic

EVIDENCE

Aggregated from public databases using ACMG Guidelines

Population Data

PM2 Pathogenic Moderate:
Extremely low frequency in gnomAD population databases [See Details](#)

UNMET: BA1 | BS1 | BS2 [See Details](#)

Don't use the Franklin classification

- They don't apply all ACMG criteria correctly

General Tools: Franklin

Most helpful tab

Variant Assessment

Franklin ACMG Classification Publications Gene Assessment Associated Conditions Somatic Clinical Evid

Franklin highlights

★ Franklin found 1 variant scope publications | ★ This variant was submitted to Clinvar

Franklin ACMG Classification
VUS
PM2 PPS PP2
See Details

Conditions Associated with NOTCH1
Aortic Valve Disease 1 AD
OMIM | Monarch | GENCC | Decipher

AdamsOliver Syndrome 5 AD
OMIM | Monarch | GENCC

AdamsOliver Syndrome AD
Monarch | Orphanet | GENCC

Population Freq
PM2 1
N/A
0% 1% 100%
See all

Relevant Articles 1 Variant scope articles Out of 2227 articles
See all

Predictions

Population Frequencies

Transcripts

My Organization Classification
No classification

Clinical Evidence ★
Conflict
See Details

4 More Conditions

Population Freq
PM2 1
N/A
0% 1% 100%
See all

Relevant Articles 1 Variant scope articles Out of 2227 articles
See all

Prediction
Revel Uncertain
Deleterious MetaLR (Low)
Splice Splice-altering / AI Strong
See all Predictions

General Tools: Franklin

Clinical evidence



2 evidences

Submissions: ● VUS(2) ● LP(1)

[CLINVAR](#)

Franklin Community (0)

Clinvar (2)

Clinvar Clinical Significance: **Likely pathogenic**

Adams-oliver Syndrome 5

Review Status: ★★★★ | RCV001330761 | [Clinvar](#)



Last evaluated: Feb 24, 2023 | 2 submitters

UniProt (0)

Mitomap (0)

[See all evidences](#)

- They mine and link to ClinVar

General Tools: Franklin

Predictions



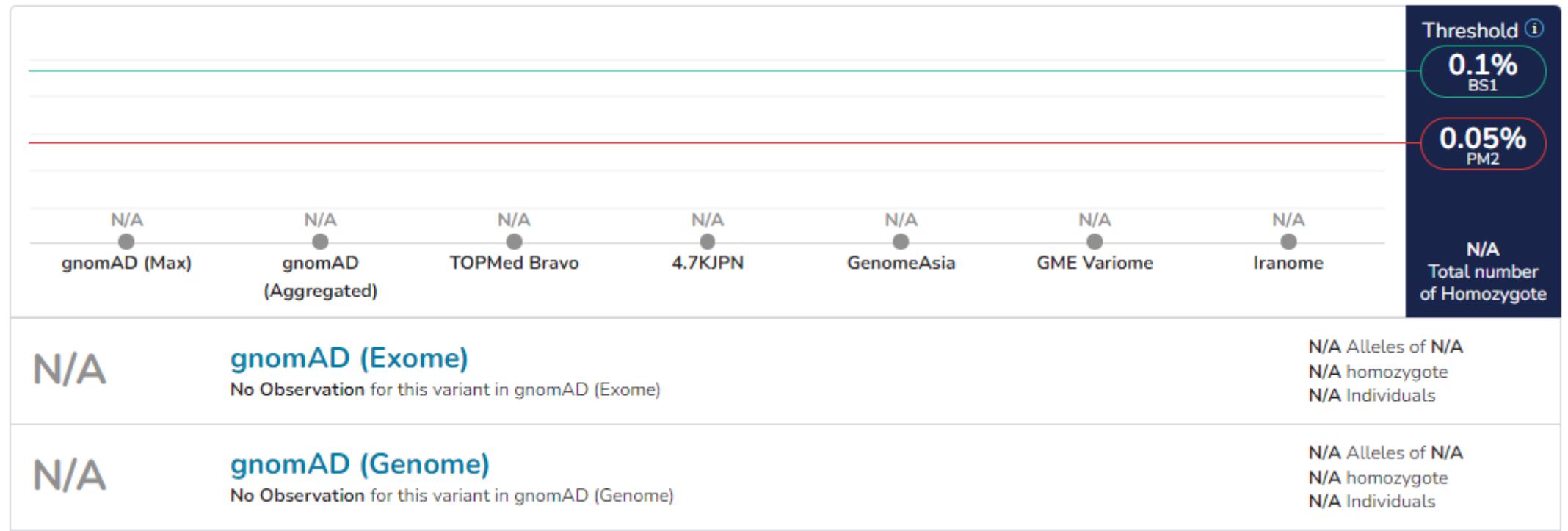
- They give multiple *in silico* scores, including REVEL and Splice-AI

General Tools: Franklin

Population Frequencies



Threshold ⓘ
0.1% BS1
0.05% PM2
N/A Total number of Homozygote



- They pull frequency data from gnomAD

Example

In this portion of the class, you will:

- See an example of a full variant classification! YAY!

Classification Example

Patient phenotype:

- Abnormality of male external genitalia, high palate, retrognathia, low-set ears, patent ductus arteriosus, hypoglycemia, abnormal pattern of respiration, ascending tubular aorta aneurysm, abnormality of the external nose, abnormal digit morphology, fetal choroid plexus cysts, short fetal femur length, heart murmur, abnormal atrioventricular valve physiology

Variant identified via trio genome:

- CCDC22:c.1634A>G, Lys545Arg
- Inheritance: maternal (X-linked gene). Follow-up sequencing found the variant was not inherited from the mother's parents.

| Criteria being considered | Strength being applied | Evidence | Points |
|---------------------------|------------------------|----------|--------|
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| Variant classification: | | | Sum: |

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 insertion 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 changing variant  | 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 w/out paternity/maternity confirmation BP6 | De novo (paternal) and maternity confirmed PS2  | |
| Allelic data | | Observed in trans with a dominant allele BP2 Observed with a pathogenic variant BP2 |   | For recessive disorders observed in trans pathogenic at PM3 | | |
| Other database | | Reputable database w/out shared de novo benign BP6 | Reputable database = pathogenic PP5 | | | |
| Other data | | Found in association with an alternative disease BP5 |  | Patient's phenotype or FH highly specific for gene PP4 | | |

PM2

Population Frequencies



Threshold

1.5%
BS1

1%
PM2

N/A
Total number
of Homozygote

N/A

gnomAD (Max)

N/A

gnomAD
(Aggregated)

N/A

TOPMed Bravo

N/A

4.7KJPN

N/A

GenomeAsia

N/A

GME Variome

N/A

Iranome

N/A

gnomAD (Exome)

No Observation for this variant in gnomAD (Exome)

N/A Alleles of N/A
N/A homozygote
N/A Individuals

N/A

gnomAD (Genome)

No Observation for this variant in gnomAD (Genome)

N/A Alleles of N/A
N/A homozygote
N/A Individuals

N/A

gnomAD (Aggregated)

No Observation for this variant in gnomAD (Aggregated)

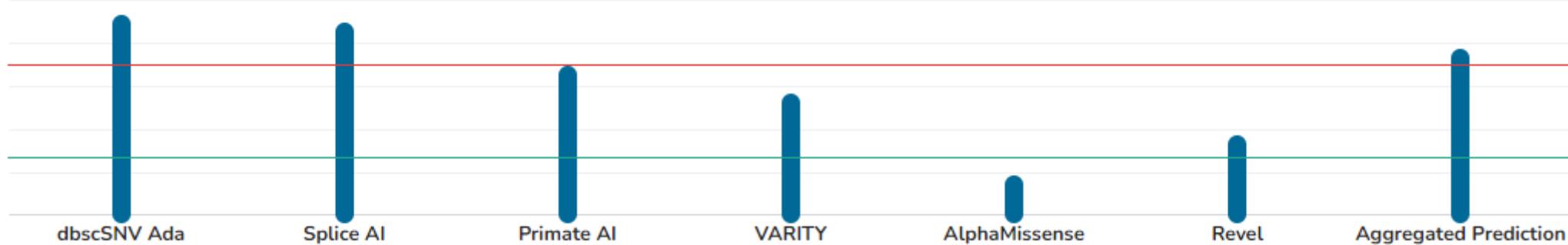
N/A Alleles of N/A
N/A homozygote
N/A Individuals

ACMG Criteria 2015

| | Benign | | | Pathogenic | | |
|-----------------------------------|--|---|---|--|---|---|
| | Strong | Supporting | Supporting | Moderate | Strong | Very strong |
| Population data | MAF is so high for disorder OR observed in controls incongruent with disease gene evidence 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 insertion 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 changing variant | 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 w/out paternity/maternity confirmation BP6 | De novo (paternal) and maternity confirmed PS2 | |
| Allelic data | | Observed in trans with a dominant allele BP2 Observed with a pathogenic variant BP2 | | For recessive disorders observed in trans pathogenic variant PM3 | | |
| Other database | | Reputable database w/out shared database = benign BP6 | Reputable database = pathogenic PP5 | | | |
| Other data | | Found in association with an alternative disease BP5 | Patient's phenotype or FH highly specific for gene PP4 | | | |

PP3

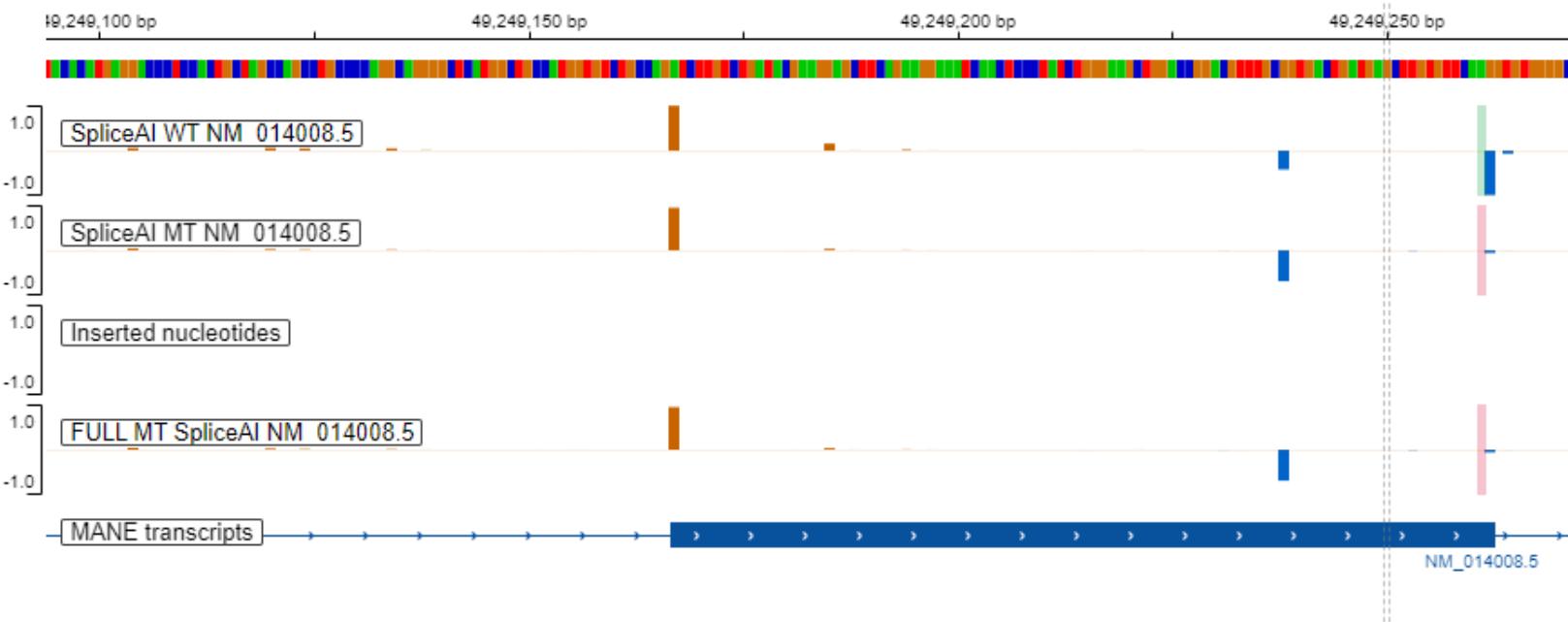
*Prediction scores were normalized to allow integrated graph view



| Functional Coding | |
|-------------------|-----------------------------|
| Revel | Uncertain (0.38) |
| AlphaMissense | Benign (Moderate) (0.085) |
| Eve | (N/A) |
| Variety | Deleterious (low) (0.42) |
| MUT Assesor | Med (2.04) |
| SIFT | Benign (Supporting) (0.128) |

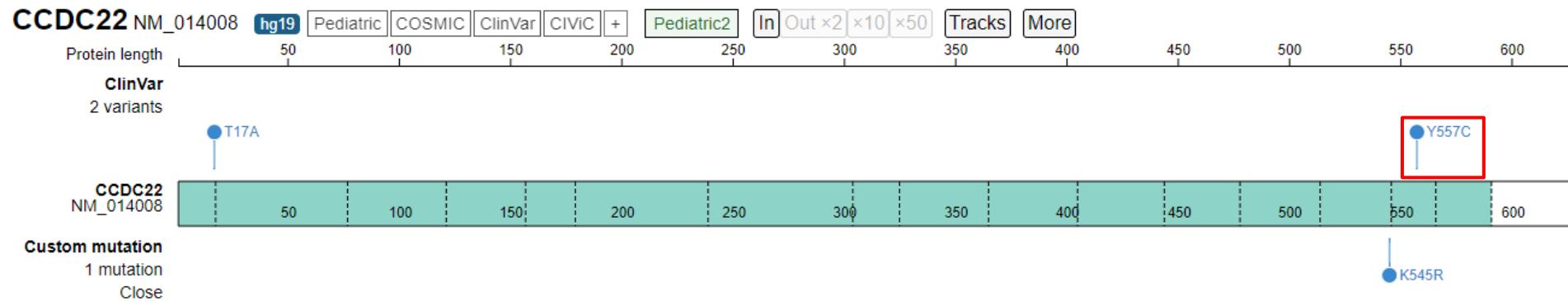
| | |
|--------------|------------|
| spliceAI AG: | 0.01 (1) |
| spliceAI AL: | 0.00 (-40) |
| spliceAI DG: | 0.26 (-23) |
| spliceAI DL: | 0.92 (1) |

PP3



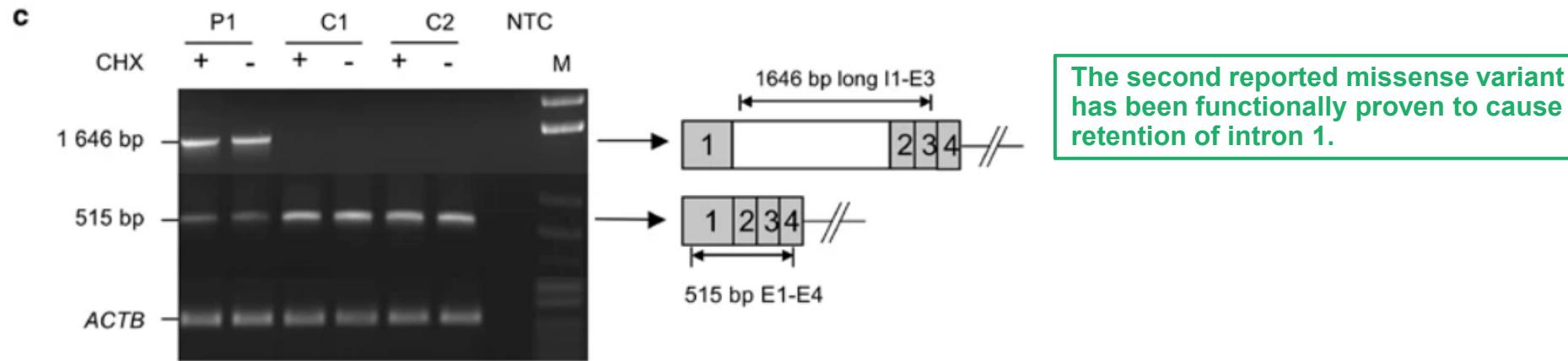
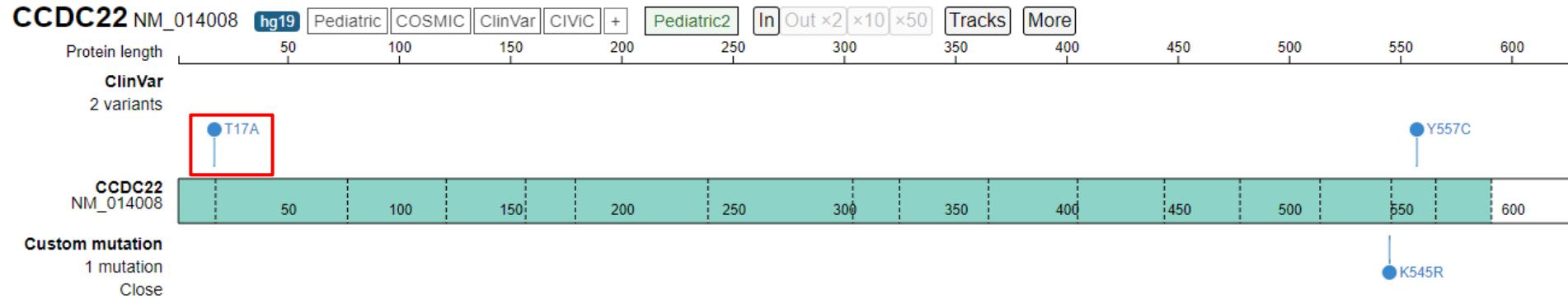
| | |
|--------------|------------|
| spliceAI AG: | 0.01 (1) |
| spliceAI AL: | 0.00 (-40) |
| spliceAI DG: | 0.26 (-23) |
| spliceAI DL: | 0.92 (1) |

PP3



| variant | gene | ☐ = canonical transcript | ☐ = non-coding transcript | Δ type | Δ score ⓘ | pre-mRNA position ⓘ |
|----------------|---|--------------------------|---------------------------|---------------|-----------|---------------------|
| X-49106004-A-G | CCDC22 (ENSG00000101997.13_6 / ENST00000376227.4_3 / NM_014008.5) | | | Acceptor Loss | 0.01 | -4 bp |
| UCSC, gnomAD | biotype: protein coding canonical transcript | | | Donor Loss | 0.47 | 25 bp |
| | OMIM, GTEx, gnomAD, ClinGen, Ensembl, Decipher, GeneCards | | | Acceptor Gain | 0.07 | -25 bp |
| | | | | Donor Gain | 0.98 | -1 bp |

PP3



ACMG Criteria 2015

| | Benign | | | Pathogenic | | |
|-----------------------------------|---|--|--|--|---|--|
| | Strong | Supporting | Supporting | Moderate | Strong | Very strong |
| Population data | MAF is too high for disorder OR observed in controls incongruent with disease phenotype BS2 | | | Absent in population databases PM2 | Prevalence increased significantly in affected individuals compared to controls | |
| Computational and predictive data | | Multiple lines of computational evidence support a deleterious effect on the gene/gene product PP3 Missense change where only deleterious changes have been seen before PM5 Silent variant with an predicted splice impact BP7 In-frame insertion/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 no similar pathogenic missense change has been seen before PM5 Protein truncating changing variant I | Same amino acid change established pathogenic PS1 | Predicted to alter all variants where there is a known mechanism of disease PVS1 |
| Functional data | Well-established functional assays show no deleterious effect BS3 | | Missense in gene with low reported missense variants and pathogenic variants in common P2 | Mutational hot spot or well-known function with no variation PM1 | Well-established functional assays show a deleterious effect | |
| Segregation data | Nonsense variants with segregation | | Cosegregation with disease in all affected members | | | |
| De novo data | | | | De novo without paternal or maternity confirmation BP6 | De novo (paternal and maternity confirmed) PS2 | |
| Allelic data | | Observed in trans with a dominant allele BP2 Observed with a pathogenic variant BP2 | | For recessive disorders observed in trans with a pathogenic variant PM3 | | |
| Other database | | Reputable database w/out shared de novo benign BP6 | Reputable database = pathogenic P5 | | | |
| Other data | | Found in association with an alternative disease BP5 | Patient's phenotype or FH highly specific for gene PP4 | | | |

PS2

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 |

Conditions with X-linked inheritance: if the variant occurs *de novo* in an unaffected carrier mother, and family history is consistent - i.e., she has no affected brothers/other male relatives apart from her affected son(s) – *de novo* criteria may be applied despite the fact that she is unaffected.

PS2

Ritscher-Schinzel syndrome 2

INHERITANCE

- X-linked recessive

GROWTH

Other

- Growth delay, postnatal

HEAD & NECK

Head

- Large head circumference

Face

- Broad forehead
- Short philtrum

Eyes

- Uplanting palpebral fissures
- Hypertelorism

Mouth

- Protruding tongue
- Abnormal dentition (in some patients)

Neck

- Broad neck

CARDIOVASCULAR

Heart

- Ventricular septal defect
- Atrial septal defect

Vascular

- Patent ductus arteriosus

GENITOURINARY

External Genitalia (Male)

- Cryptorchidism

SKELETAL

Skull

- Large anterior fontanelles

Spine

- Scoliosis

Hands

- Distal digital anomalies

- Syndactyly

- Camptodactyly

- Clinodactyly

- Hypoplastic distal phalanges

Feet

- Overriding toes

- Broad halluces

SKIN, NAILS, & HAIR

Hair

- Low posterior hairline

- Aplasia cutis (in some patients)

MUSCLE, SOFT TISSUES

- Hypotonia

NEUROLOGIC

Central Nervous System

- Delayed psychomotor development

- Poor speech

- Dandy-Walker malformation

- Cerebellar hypoplasia

MISCELLANEOUS

- Variable features

- Two unrelated families have been reported (last curated November 2015)