Variant Classification using ACMG/AMP Interpreting Sequence Guidelines

Steven M. Harrison
Associate Director, Clinical Research Sequencing Platform, Broad Genomics Platform

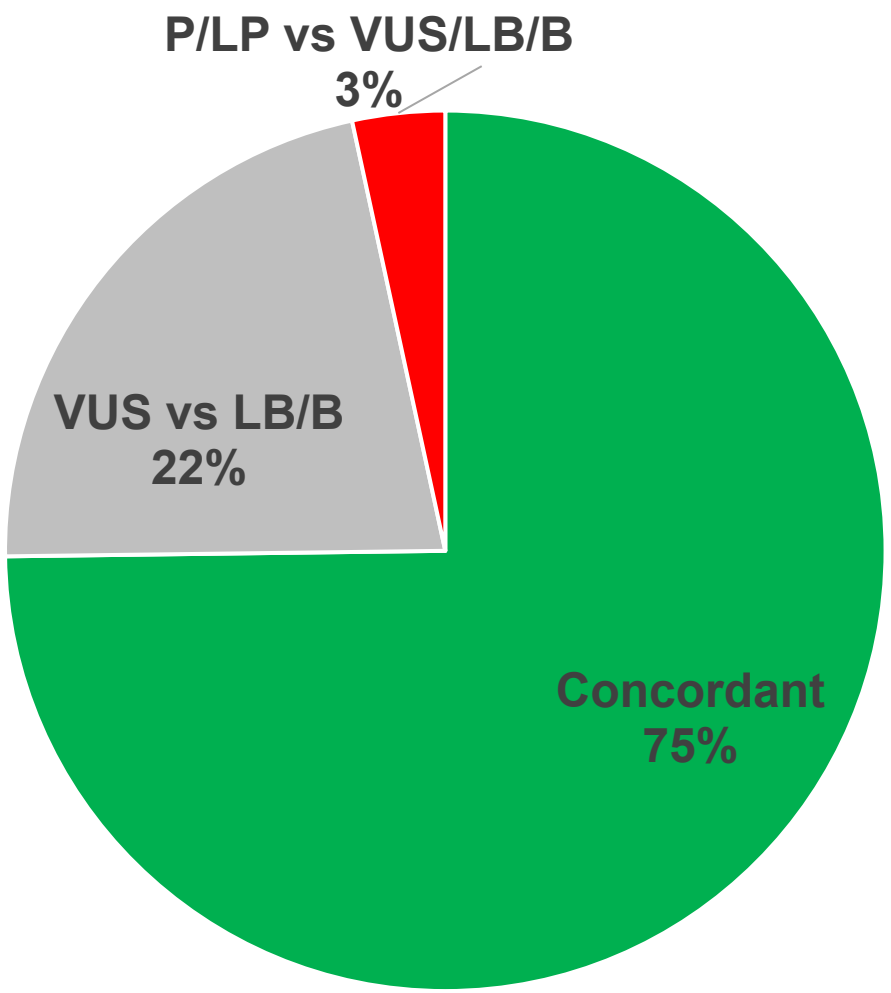
Email: Steven@broadinstitute.org

BROAD INSTITUTE

ClinGen Clinical Genome Resource

Variant Interpretation

- Differences in variant classification:
  - Laboratories interpreting the same variants
  - Data sharing through ClinVar and other sources



ClinVar Variants with >= 2 submitters
(164,226)

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

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

Table 5 Rules for combining criteria to classify sequence variants

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

PAH - NM_000277.2:c.1147C>T (p.Gln383Ter)

	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			

PAH established causal gene for phenylketonuria (PKU)

- Variant is loss-of-function and in a gene in which loss-of-function causes disease
  - **PVS1** applied
- Variant found in 1/16,254 (0.006%) in African chromosomes in gnomAD
  - **PM2** applied
- Variant observed in 1 proband with classic PKU who also carried variant p.Arg408Trp in trans (variant called Pathogenic by 18 labs in ClinVar)
  - **PM3** applied
- In total have PVS1, PM2, and PM3 applied = 1 Pathogenic VeryStrong and 2 Pathogenic Moderate

PAH - NM_000277.2:c.1147C>T (p.Gln383Ter)

	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			

Table 5 Rules for combining criteria to classify sequence variants

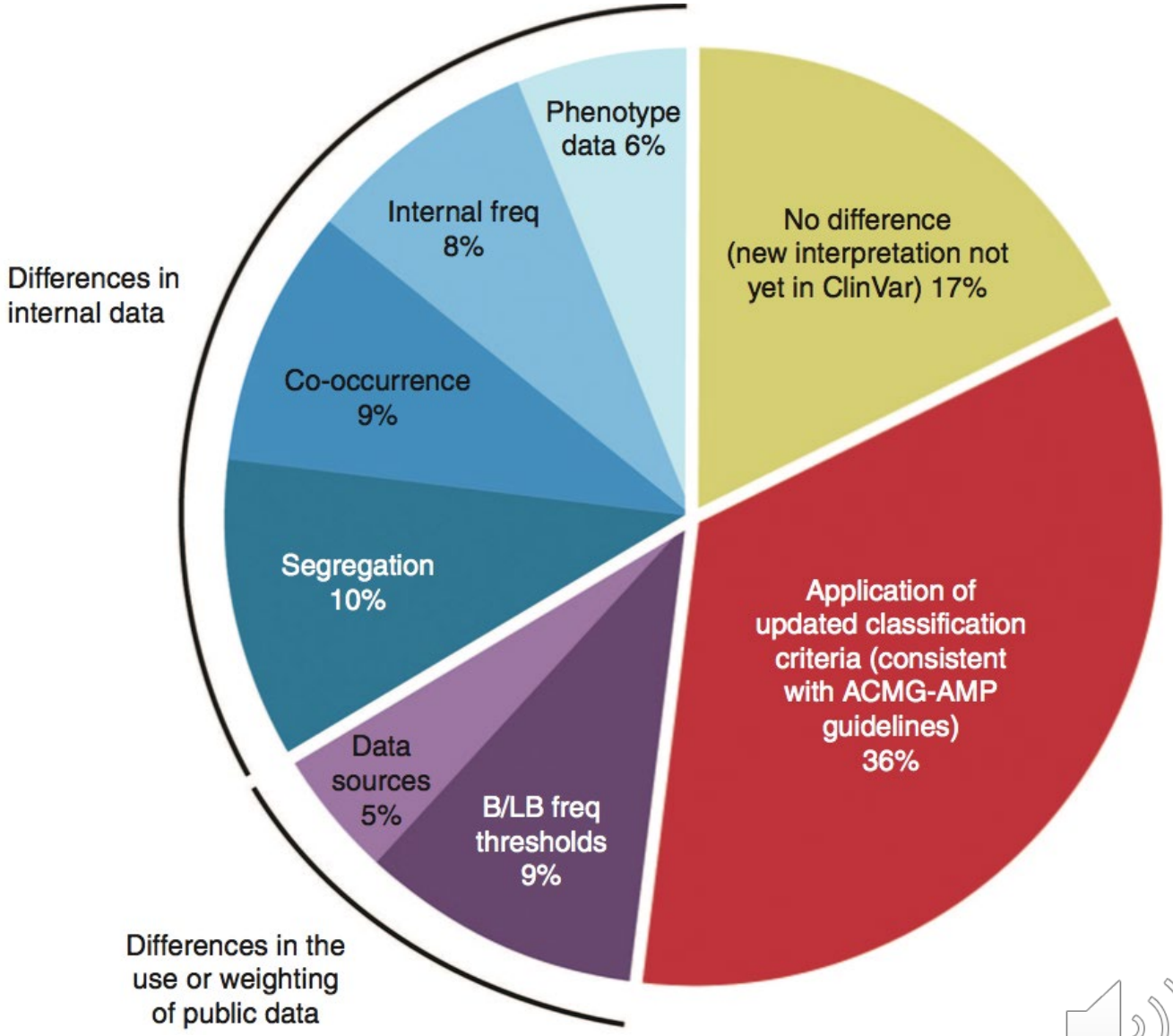
Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)

- In total have PVS1, PM2, and PM3 applied = 1 Pathogenic VeryStrong and 2 Pathogenic Moderate

Outcome of Discrepancy Resolution

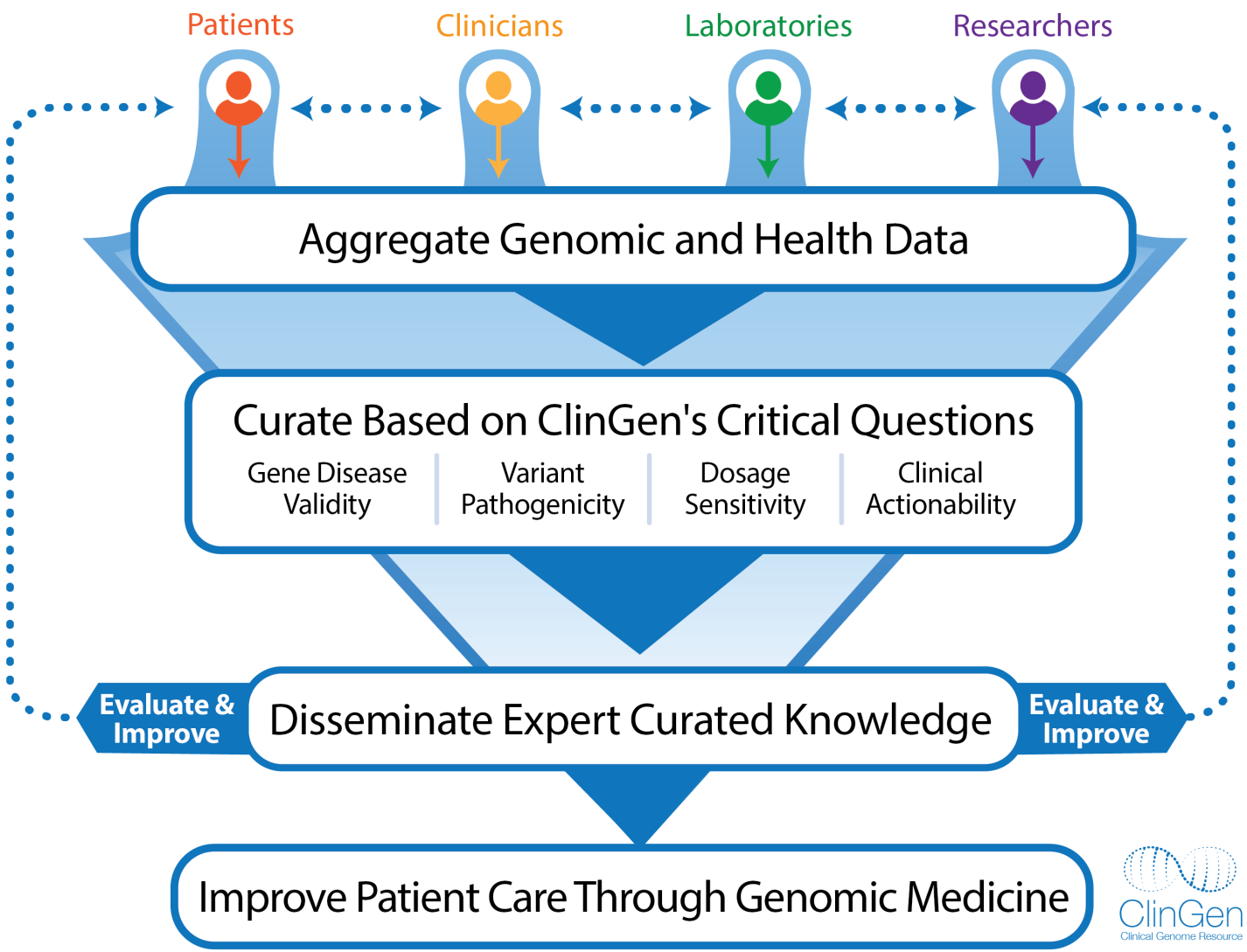
- Implementation of ACMG/AMP guidelines has increased interpretation concordance between labs
  - >1/3 discrepancies due to differences in classification algorithms

- Inconsistencies in application of ACMG/AMP criteria



PMID: 28301460

The Clinical Genome Resource (ClinGen)



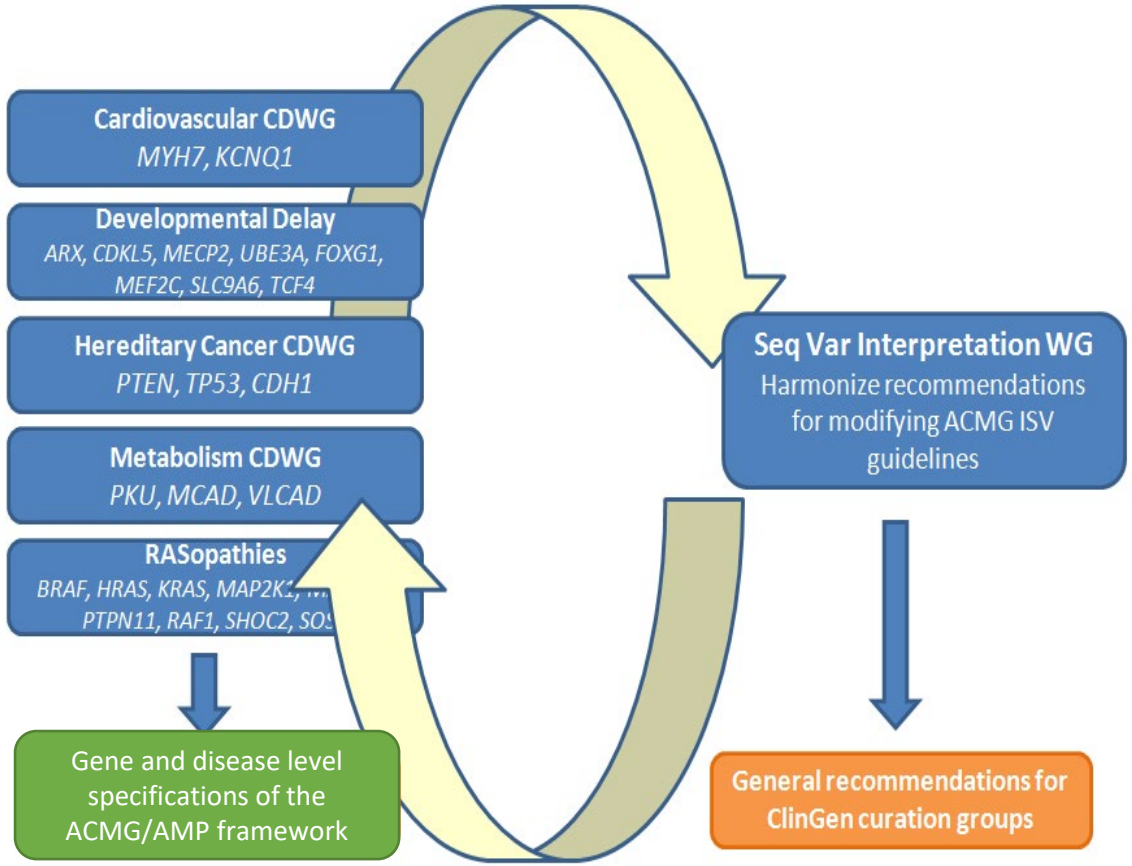
Purpose: Create an authoritative central resource that defines the clinical relevance of genes and variants for use in precision medicine and research.

- Funded by NIH/NHGRI
- >1400 people from >200 institutions worldwide

ClinGen Sequence Variant Interpretation WG

ACMG/AMP Rules

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



- Support Variant Curation Expert Panel groups developing gene- and disease-specific refinements of the ACMG/AMP guidelines
- Develop general recommendations for using the ACMG/AMP guidelines to improve consistency in usage and transparency in classification rationale

Sequence Variant Interpretation (SVI) Approval of EPs

Criteria requiring gene/disease specification

Criteria with completed SVI recommendation

Criteria with SVI recommendation in progress

	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			

SVI Webpage – clinicalgenome.org/svi

Sequence Variant Interpretation

The goal of the Sequence Variant Interpretation Working Group (SVI WG) is to support the refinement and evolution of the ACMG/AMP Interpreting Sequence Variant Guidelines to develop quantitative approaches to variant interpretation.

Subgroups | Documents | Tools | Membership

The Sequence Variant Interpretation WG also consults with and supports Expert Panel groups to develop gene- and disease-specific refinements of the ACMG/AMP Interpreting Sequence Variant Guidelines to increase the uniformity and consistency of the Expert Panel recommendations.The SVI WG has representation from the Biocurators WG, CNV Interpretation WG and Variant Curation Interface development team and all ClinGen Expert Panels.

SVI General Recommendations for Using ACMG/AMP Criteria

SVI provides general recommendations for using the ACMG/AMP criteria to improve consistency in usage and transparency in classification rationale.

- Guidance on how to rename criteria codes when strength of evidence is modified
- BA1: Updated Recommendation for the ACMG/AMP Stand Alone Pathogenicity Criterion for Variant Classification
  - BA1 Exception List (July 2018)
  - BA1 Exception List Nomination Form
- PVS1: Recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria
- PS2/PM6: Recommendation for de novo PS2 and PM6 ACMG/AMP criteria (Version 1.0)
- PS3/BS3: Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework
- PM2: Recommendation for Absence/Rarity Criterion PM2 (Version 1.0)
- PM3: Recommendation for in trans Criterion PM3 (Version 1.0)
- PP5/BP6: Recommendation for reputable source PP5 and BP6 ACMG/AMP criteria

Chairs

Leslie G. Biesecker, MD

Steven Harrison, PhD

Coordinators

Please contact a coordinator if you have questions.

Danielle Azzariti, MS, CGC
dazzarit@broadinstitute.org

ClinGen
Clinical Genome Resource

SVI Approved Expert Panel Specified ACMG/AMP Variant Interpretation Guidelines

- ClinGen Cardiomyopathy Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1
- ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines

ClinGen
Clinical Genome Resource

General SVI Publications

- Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion
- Updated recommendation for the benign stand-alone ACMG/AMP criterion
- The ACMG/AMP Reputable Source Criteria for the Interpretation of Sequence Variants
- Modeling the ACMG/AMP variant classification

- General SVI guidance
- All approved VCEP specifications
- Publications
- Membership

SVI Webpage – clinicalgenome.org/svi

Sequence Variant Interpretation

The goal of the Sequence Variant Interpretation Working Group (SVI WG) is to support the refinement and evolution of the ACMG/AMP Interpreting Sequence Variant Guidelines to develop quantitative approaches to variant interpretation.

Subgroups | Documents | Tools | Membership

The Sequence Variant Interpretation WG also consults with and supports Expert Panel groups to develop gene- and disease-specific refinements of the ACMG/AMP Interpreting Sequence Variant Guidelines to increase the uniformity and consistency of the Expert Panel recommendations.The SVI WG has representation from the Biocurators WG, CNV Interpretation WG and Variant Curation Interface development team and all ClinGen Expert Panels.

SVI General Recommendations for Using ACMG/AMP Criteria

SVI provides general recommendations for using the ACMG/AMP criteria to improve consistency in usage and transparency in classification rationale.

- Guidance on how to rename criteria codes when strength of evidence is modified
- BA1: Updated Recommendation for the ACMG/AMP Stand Alone Pathogenicity Criterion for Variant Classification
  - BA1 Exception List (July 2018)
  - BA1 Exception List Nomination Form
- PVS1: Recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria
- PS2/PM6: Recommendation for de novo PS2 and PM6 ACMG/AMP criteria (Version 1.0)
- PS3/BS3: Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework
- PM2: Recommendation for Absence/Rarity Criterion PM2 (Version 1.0)
- PM3: Recommendation for in trans Criterion PM3 (Version 1.0)
- PP5/BP6: Recommendation for reputable source PP5 and BP6 ACMG/AMP criteria

Chairs

Leslie G. Biesecker, MD
Steven Harrison, PhD

Coordinators

Please contact a coordinator if you have questions.

Danielle Azzariti, MS, CGC
dazzarit@broadinstitute.org

ClinGen
Clinical Genome Resource

SVI Approved Expert Panel Specified ACMG/AMP Variant Interpretation Guidelines

- ClinGen Cardiomyopathy Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1
- ClinGen CDH1 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines

ClinGen
Clinical Genome Resource

General SVI Publications

- Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion
- Updated recommendation for the benign stand-alone ACMG/AMP criterion
- The ACMG/AMP Reputable Source Criteria for the Interpretation of Sequence Variants
- Modeling the ACMG/AMP variant classification

- General SVI guidance
- All approved VCEP specifications
- Publications
- Membership

To document strength-modified evidence, ClinGen recommends ***using the original criteria code followed by an underscore and new level of strength***

Pathogenic

	Supporting	**Moderate**	**Strong**
Segregation Data	Co-segregation with disease in multiple affected family members ***PP1***		

To document strength-modified evidence, ClinGen recommends ***using the original criteria code followed by an underscore and new level of strength***

Pathogenic

	Supporting	**Moderate**	**Strong**
Segregation Data	Co-segregation with disease in multiple affected family members ***PP1***	# Co-segregation with disease in multiple affected family members ***PP1_Moderate***	## Co-segregation with disease in multiple affected family members ***PP1_Strong***

Workshop Outline

- **Recommendations for 3 key areas**:
  - ***Allele frequency data***
  - Variant type and location
  - Patient data

- Criteria combinations

Allele Frequency Data

	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	

- **BA1** (Benign StandAlone) = Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

- **BS1** (Benign Strong) = Allele frequency is greater than expected for disorder

- **PM2** (Pathogenic Moderate) = Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Proposed BA1 (from ClinGen's SVI WG):

Allele frequency is >5% in any general continental population dataset of at least 2,000 alleles for a gene without a gene- or variant-specific recommendation

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Proposed BA1:

Allele frequency is >5% in ***any general continental population dataset of at least 2,000 alleles*** for a gene without a gene- or variant-specific recommendation

- ***Can compare to individual, continental populations (>2,000 alleles)***
- ***Tested individual does not need to match ethnic origin of population dataset used***

Population	Allele Count	Allele Number	Allele Frequency
Latino	649	11522	0.05633
East Asian	361	8618	0.04189
Other	10	904	0.01106
European (Finnish)	22	6606	0.00333
South Asian	42	16440	0.002555
European (Non-Finnish)	73	66602	0.001096
African	6	10294	0.00058
Total	**1163**	**120986**	**0.009613**

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Proposed BA1:

Allele frequency is >5% in any general continental population dataset of at least 2,000 alleles ***for a gene without a gene- or variant-specific recommendation***

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Proposed BA1:

Allele frequency is >5% in any general continental population dataset of at least 2,000 alleles ***for a gene without a gene- or variant-specific recommendation***

- ***For most disorders, >5% is an order of magnitude higher than necessary***
- ***Exception list for non-benign alleles >5% in continental populations***

https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/

The Sequence Variant Interpretation WG also consults with and supports Expert Panel groups to develop gene- and disease-specific refinements of the ACMG/AMP Interpreting Sequence Variant Guidelines to increase the uniformity and consistency of the Expert Panel recommendations.The SVI WG has representation from the Biocurators WG, CNV Interpretation WG and Variant Curation Interface development team and all ClinGen Expert Panels.

SVI General Recommendations for Using ACMG/AMP Criteria

SVI provides general recommendations for using the ACMG/AMP criteria to improve consistency in usage and transparency in classification rationale.

- Guidance on how to rename criteria codes when strength of evidence is modified
- BA1: Updated Recommendation for the ACMG/AMP Stand Alone Pathogenicity Criterion for Variant Classification
  - BA1 Exception List (July 2018)
  - BA1 Exception List Nomination Form
- PVS1: Recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria
- PS2/PM6: Recommendation for de novo PS2 and PM6 ACMG/AMP criteria (Version 1.0)
- PM3: Recommendation for in trans Criterion PM3 (Version 1.0)
- PP5/BP6: Recommendation for reputable source PP5 and BP6 ACMG/AMP criteria

Chairs

Leslie G. Biesecker, MD

Steven Harrison, PhD

Coordinators

Please contact a coordinator if you have questions.

Danielle Azzariti, MS, CGC
dazzarit@broadinstitute.org

BA1 Exception List (variants >5% but not Benign)

Gene	Variant	Classification	ACMG/AMP Criteria 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

https://www.clinicalgenome.org/working-groups/sequence-variant-interpretation/

The Sequence Variant Interpretation WG also consults with and supports Expert Panel groups to develop gene- and disease-specific refinements of the ACMG/AMP Interpreting Sequence Variant Guidelines to increase the uniformity and consistency of the Expert Panel recommendations.The SVI WG has representation from the Biocurators WG, CNV Interpretation WG and Variant Curation Interface development team and all ClinGen Expert Panels.

SVI General Recommendations for Using ACMG/AMP Criteria

SVI provides general recommendations for using the ACMG/AMP criteria to improve consistency in usage and transparency in classification rationale.

- Guidance on how to rename criteria codes when strength of evidence is modified
- BA1: Updated Recommendation for the ACMG/AMP Stand Alone Pathogenicity Criterion for Variant Classification
  - BA1 Exception List (July 2018)
  - BA1 Exception List Nomination Form
- PVS1: Recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria
- PS2/PM6: Recommendation for de novo PS2 and PM6 ACMG/AMP criteria (Version 1.0)
- PM3: Recommendation for in trans Criterion PM3 (Version 1.0)
- PP5/BP6: Recommendation for reputable source PP5 and BP6 ACMG/AMP criteria

Email address *

Your email

Please provide your name *

Your answer

Please provide your affiliation *

Your answer

Provide a hyperlink to the variant in a population database. *

For example, HFE c.845G>A (p.Cys282Tyr) variant: http://exac.broadinstitute.org/variant/6-26093141-G-A

Your answer

Provide a hyperlink to the variant in ClinVar *

For example, HFE c.845G>A (p.Cys282Tyr) variant: https://www.ncbi.nlm.nih.gov/clinvar/variation/9/

Your answer

Provide evidence for variant to be excluded from BA1 filtration *

Free text. Please provide ACMG/AMP criteria applied and any PMIDs

Your answer

Evidence of benign impact	Category
Stand-alone	BA1 Allele frequency is >5% in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Proposed BA1:

Allele frequency is >5% in any general continental population dataset of at least 2,000 alleles ***for a gene without a gene- or variant-specific recommendation***

- ***How to determine a gene-specific threshold??***

Great tool to help determine AF thresholds:

https://cardiodb.org/allelefrequencyapp/

Whiffin et al 2017; PMID: 28518168

$$\frac{\text{Prevalence} \times \textbf{Heterogeneity}}{\text{Penetrance}}$$

= MAXIMUM CREDIBLE POP FREQ FOR DISEASE

https://cardiodb.org/allelefrequencyapp/

Frequency Filter | HOME | calculate AF | calculate AC | explore architecture | inverse AF | penetrance | about

Inheritance:

- (●) monoallelic
- ( ) biallelic

Prevalence = 1 in ... (people)

500

Allelic heterogeneity:

0 — 0.5 — 1

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Genetic heterogeneity:

0 — 1

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Penetrance:

0 — 0.5 — 1

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

Maximum credible population AF:

0.001

Confidence:

( ) 0.9 (●) 0.95 ( ) 0.99 ( ) 0.999

Reference population size (alleles)

121412

Maximum tolerated reference AC:

140

Approach for conditions with genetic heterogeneity

BA1

$$\left(\frac{[\text{disease prevalence}] \times [\text{\% gene contribution}]}{[\text{penetrance}]} \right)$$

Pick most common condition = threshold can be applied to all variants

Pick gene with highest contribution = threshold can be applied to all genes associated w/ condition

BS1

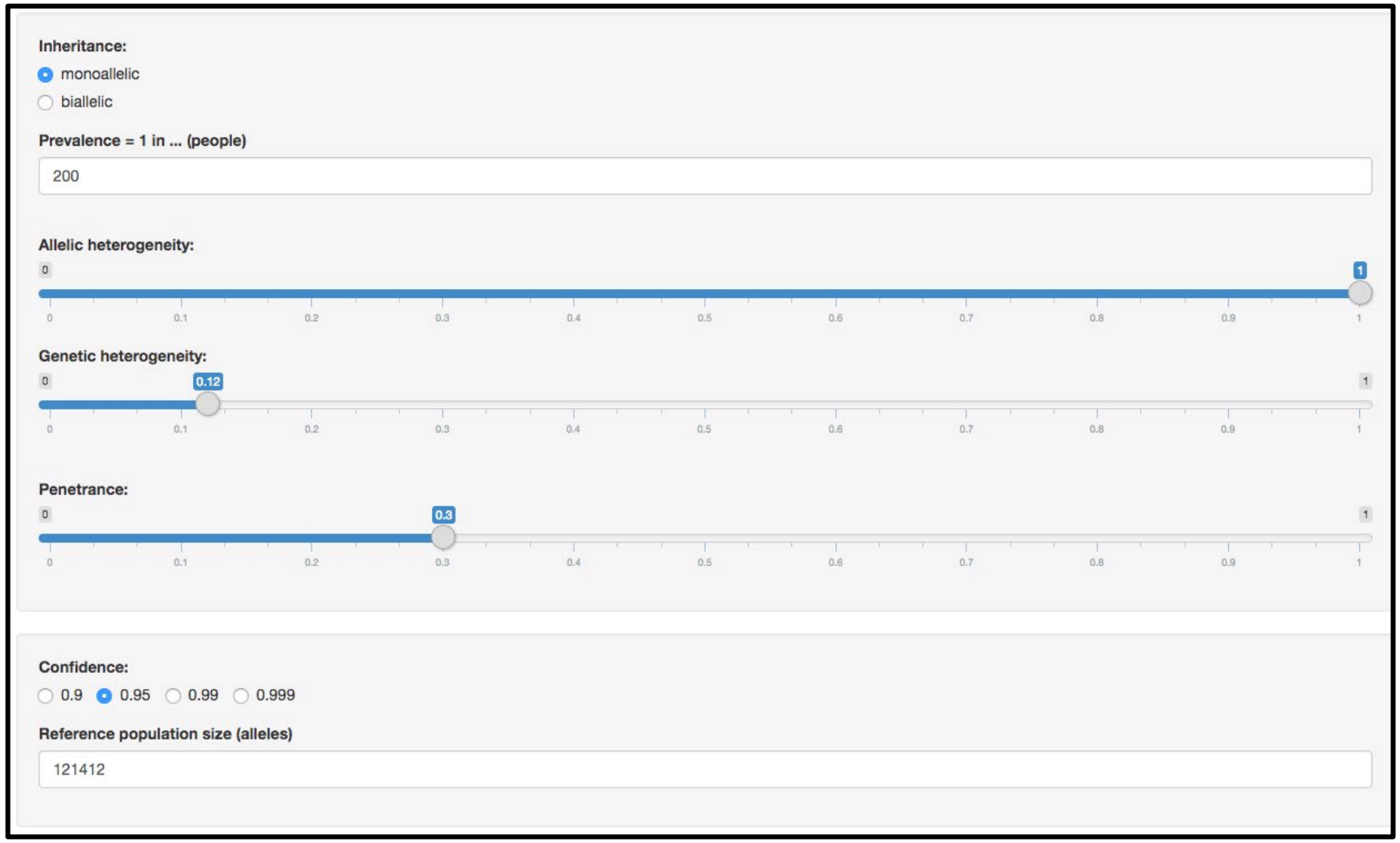
$$\left(\frac{[\text{disease prevalence}] \times [\text{\% max path. variant }]}{[\text{penetrance}]} \right)$$

Hypertrophic Cardiomyopathy – Determination of BA1 threshold

Allele Frequency Data Collection	
Prevalence	
Most common estimate (individuals):	1/200
Least common estimate (individuals):	1/500
Heterogeneity	
Top gene contributor to disease:	MYH7
Gene accounts for X%:	12%
Top variant contributor to disease:	R502W (MYBPC3)
Variant accounts for X%:	2%
Penetrance	
Highest estimate:	70%
Lowest estimate:	30%

Hypertrophic Cardiomyopathy – Determination of BA1 threshold

Allele Frequency Data Collection	
Prevalence	
Most common estimate (individuals):	1/200
Least common estimate (individuals):	1/500
Heterogeneity	
Top gene contributor to disease:	MYH7
Gene accounts for X%:	12%
Top variant contributor to disease:	R502W (MYBPC3)
Variant accounts for X%:	2%
Penetrance	
Highest estimate:	70%
Lowest estimate:	30%



Maximum credible populatio AF:

0.001

Population Database Resources

- Exome Aggregation Consortium (**ExAC**) - http://exac.broadinstitute.org/
  - **60,706 individuals** (121,412 alleles)
  - ~60% of ESP samples are in ExAC (3,936/6,503)
  - ~74% of 1000G samples are in ExAC (1,851/2,504)

- Genome Aggregation Database (**gnomAD**) - http://gnomad.broadinstitute.org/
  - **138,632 individuals** (277,264 alleles)
    - 123,136 exomes (includes all of ExAC)
    - 15,496 genomes

Filtering Allele Frequency “FAF”

Single nucleotide variant: 17-78092070-C-T

	Exomes	Genomes	Total
Filter	Pass	Pass	
Allele Count	43	15	58
Allele Number	246224	31342	277566
Allele Frequency	African 6	0.0004786	0.0002090
Popmax Filtering AF (95% confidence)	0.001441	0.001063	

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African	32	16166	0	0.001979
Latino	7	33954	0	0.0002062
European (Finnish)	1	21348	0	0.00004684
South Asian	1	29850	0	0.00003350
European (non-Finnish)	2	111156	0	0.00001799
Ashkenazi Jewish	0	9488	0	0.000
East Asian	0	18312	0	0.000
Other	0	5950	0	0.000
Total	**43**	**246224**	**0**	**0.0001746**

Include: ☑ Exomes ☐ Genomes

Filtering Allele Frequency “FAF”

Single nucleotide variant: 17-78092070-C-T

	Exomes	Genomes	Total
Filter	Pass	Pass	
Allele Count	43	15	58
Allele Number	[illegible]	31342	277566
Allele Frequency	[illegible] African [illegible]6	0.0004786	0.0002090
Popmax Filtering AF (95% confidence)	0.001441	0.001063	

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African	32	16166	0	0.001979
Latino	7	33954	0	0.0002062
European (Finnish)	1	21348	0	0.00004684
South Asian	1	29850	0	0.00003350
European (non-Finnish)	2	111156	0	0.00001799
Ashkenazi Jewish	0	9488	0	0.000
East Asian	0	18312	0	0.000
Other	0	5950	0	0.000
Total	**43**	**246224**	**0**	**0.0001746**

Include: ☑ Exomes ☐ Genomes

FAF functions as equivalent to a lower bound estimate for the true allele frequency of an observed variant

(i.e., the FAF for a given variant will always be lower than the calculated allele frequency)

Filtering Allele Frequency "FAF"

Single nucleotide variant: 17-78092070-C-T

	Exomes	Genomes	Total
Filter	Pass	Pass	
Allele Count	43	15	58
Allele Number	[illegible]	31342	277566
Allele Frequency	[illegible]	0.0004786	0.0002090
Popmax Filtering AF (95% confidence)	0.001441	0.001063	

African

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
African	32	16166	0	0.001979
Latino	7	33954	0	0.0002062
European (Finnish)	1	21348	0	0.00004684
South Asian	1	29850	0	0.00003350
European (non-Finnish)	2	111156	0	0.00001799
Ashkenazi Jewish	0	9488	0	0.000
East Asian	0	18312	0	0.000
Other	0	5950	0	0.000
Total	**43**	**246224**	**0**	**0.0001746**

Include: ☑ Exomes ☐ Genomes

Compare FAF to calculated **Maximum Credible Population AF**

If the variant's FAF is **GREATER** than **Max Credible Pop AF**, the variant is too common to cause disease

Allele Frequency Data

	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	

- PM2 (Pathogenic Moderate) = **Absent** from controls (**or at extremely low frequency if recessive**) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

- For dominant disorders – “absent” or “within pathogenic range”?

ExAC/gnomAD Data

"We have made every effort to **exclude individuals with severe pediatric diseases from the gnomAD data set**, and certainly do not expect our data set to be *enriched* for such individuals, but we typically cannot rule out the possibility that some of our participants do actually suffer from your disease of interest."

-gnomAD & ExAC FAQ page

ExAC individual age range:

- >70% of individuals between the ages of 40-79
- <10% below the age of 40

Allele Frequency Data

	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	

- PM2 (Pathogenic Moderate) = **Absent** from controls (**or at extremely low frequency if recessive**) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

- For dominant disorders – “absent” or “within pathogenic range”?

- Adult-onset disorders, ExAC/gnomAD individuals could be viewed as “general population” instead of “controls”

Allele Frequency Data

	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	

ClinGen WG specifications of PM2 threshold:

RASopathy - AD	Absent
CDH1 (gastric cancer / lobular breast cancer) - AD	<0.00001 (0.001%)
PTEN (PTEN hamartoma tumor syndrome) - AD	<0.00001 (0.001%)
Hearing loss - AD	<0.00002 (0.002%)
Cardiomyopathy - AD	<0.00004 (0.004%)
Hearing loss - AR	<0.00007 (0.007%)
PAH (Phenylketonuria) - AR	<0.0002 (0.02%)

Allele Frequency Data

	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	

SVI Recommendation for Absence/Rarity (PM2) - Version 1.0

The ClinGen Sequence Variant Interpretation (SVI) Working Group proposes decreasing the weight of criterion PM2 ("Absent from controls, or at extremely low frequency if recessive, in Exome Sequencing Project, 1000Genomes Project, or Exome Aggregation Consortium") from a Moderate strength level to a Supporting strength level (PM2_Supporting).

Workshop Outline

- **Recommendations for 3 key areas**:
  - Allele frequency data
  - ***Variant type and location***
  - Patient data

- Criteria combinations

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*) • Use caution interpreting LOF variants at the extreme 3′ end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*) • Use caution interpreting LOF variants at the extreme 3′ end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts

1. For this gene, do loss-of-function variants cause disease?
2. Does the variant actually cause loss-of-function?

SVI Guidance - https://www.clinicalgenome.org/svi/

SVI General Recommendations for Using ACMG/AMP Criteria

SVI provides general recommendations for using the ACMG/AMP criteria to improve consistency in usage and transparency in classification rationale.

- Guidance on how to rename criteria codes when strength of evidence is modified
- BA1: Updated Recommendation for the ACMG/AMP Stand Alone Pathogenicity Criterion for Variant Classification
  - BA1 Exception List (July 2018)
  - BA1 Exception List Nomination Form
- PVS1: Recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria
- PS2/PM6: Recommendation for de novo PS2 and PM6 ACMG/AMP criteria (Version 1.0)
- PS3/BS3: Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework
- PM2: Recommendation for Absence/Rarity Criterion PM2 (Version 1.0)
- PM3: Recommendation for in trans Criterion PM3 (Version 1.0)
- PP5/BP6: Recommendation for reputable source PP5 and BP6 ACMG/AMP criteria

SPECIAL ARTICLE

WILEY HGVS

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

Ahmad N. Abou Tayoun[1,2] | Tina Pesaran[3] | Marina T. DiStefano[4] | Andrea Oza[4] | Heidi L. Rehm[4,5,6] | Leslie G. Biesecker[7] | Steven M. Harrison[4] | ClinGen Sequence Variant Interpretation Working Group (ClinGen SVI)

Recommendations:
Is LoF is a mechanism?
Is variant truly LoF?

Is LoF a mechanism of disease??

- **Follow PVS1 Flowchart if:**
  - Clinical validity classification of gene is STRONG or DEFINITIVE

    AND
  - 3 or more LOF variants are Pathogenic without PVS1 AND >10% of variants associated with the phenotype are LOF (must be across more than 1 exon)

- **Decrease final strength by *one* level (i.e. VeryStrong to Strong) if:**
  - Clinical validity classification of gene is at least MODERATE

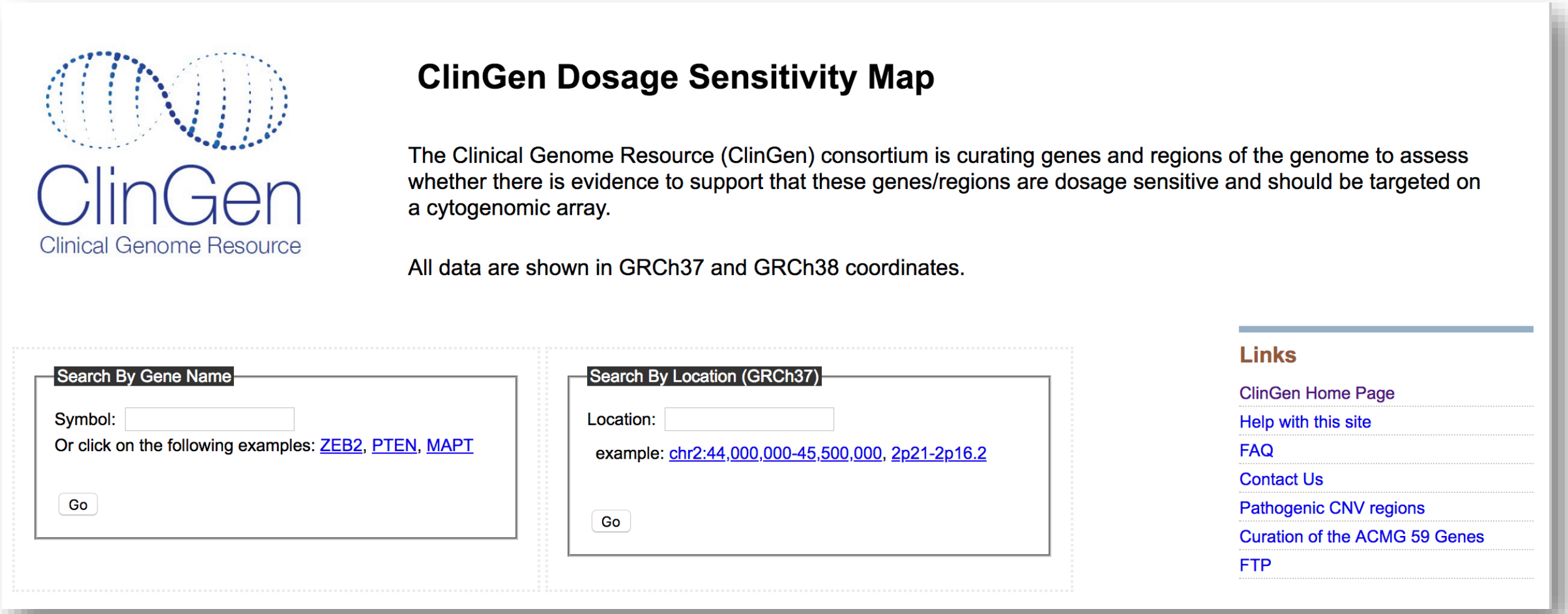
    AND
  - 2 or more LOF variants have been previously associated with the phenotype (must be across more than 1 exon)

    AND
  - Null mouse model recapitulates disease phenotype

- **Decrease final strength by *two* levels (i.e. VeryStrong to Moderate) if:**
  - Clinical validity classification is at least MODERATE

    AND EITHER
  - 2 or more LOF variants have been previously associated with the phenotype (must be across more than 1 exon)

    OR
  - Null mouse model recapitulates disease phenotype



Scores Haplioinsufficienty and Triplosensitively on 0-3 pt scale:

- **3:Sufficient** evidence suggesting dosage sensitivity is associated with clinical phenotype
- **2:Emerging** evidence suggesting dosage sensitivity is associated with clinical phenotype
- **1:Little** evidence suggesting dosage sensitivity is associated with clinical phenotype
- **0:No evidence** to suggest that dosage sensitivity is associated with clinical phenotype

https://www.clinicalgenome.org

Data Sharing Resources | GenomeConnect | Events | Contact

ClinGen
Clinical Genome Resource

Get Started | About Us | Curation Activities | Working Groups | Expert Panels | Documents & Announcements | Tools

Explore the clinical relevance of genes & variants

ClinGen is a National Institutes of Health (NIH)-funded resource dedicated to building a central resource that defines the clinical relevance of genes and variants for use in precision medicine and research.

Learn the new features available on the ClinGen website - Learn more

Gene | MYBPC3 | Search

All Curated Genes | Gene-Disease Validity | Dosage Sensitivity | Clinical Actionability | Curated Variants | More

ClinGen is defining the clinical relevance of genes and variants

ClinGen was founded in 2013 by the National Human Genome Research Institute, ClinGen is a growing collaborative effort, involving three grants, nine principal investigators and over 1,500 contributors from more than 35 countries. Below are a series of recent updates that ClinGen has been working on.

MYBPC3

View Gene Facts

3	1	3
Gene-Disease Validity Classifications	Dosage Sensitivity Classifications	Clinical Actionability Assertions

Curation Summaries | External Genomic Resources | ClinVar Variants

Group By Activity | Group By Gene-Disease Pair

Gene-Disease Validity

Gene	Disease	MOI	Classification	Report & Date
MYBPC3	arrhythmogenic right ventricular cardiomyopathy MONDO:0016587	Autosomal Dominant	Limited	08/06/2019
MYBPC3	hypertrophic cardiomyopathy 4 MONDO:0007268	Autosomal Dominant	Definitive	09/05/2017
MYBPC3	dilated cardiomyopathy MONDO:0005021	Autosomal Dominant	Limited	09/04/2020

Dosage Sensitivity

Gene	Disease	HI Score & TS Score	Report & Date
MYBPC3	hypertrophic cardiomyopathy 4 MONDO:0007268	3 (Sufficient Evidence for Haploinsufficiency)	11/12/2015
MYBPC3		0 (No Evidence for Triplosensitivity)	11/12/2015

Clinical Actionability

Gene	Disease	Adult & Pediatric Reports	Report & Date
MYBPC3	hypertrophic cardiomyopathy 4 MONDO:0007268	Adult - View Details	08/14/2017
MYBPC3	hypertrophic cardiomyopathy MONDO:0005045	Adult - View Details	08/14/2017
MYBPC3	familial hypertrophic cardiomyopathy MONDO:0024573	Adult - View Details	08/14/2017

Tolerant of Loss of Function Variants – Population Data

- pLI score = probability that a given gene is extremely intolerant of heterozygous loss-of-function variation
  - Closer to 1.0 – more LOF intolerant the gene appears to be
  - pLI > 0.9 are extremely LOF intolerant

- Observed/expected (*oe*) ratio (0-1):
  - **Expected** number of LOF variants
  - **Observed** number of LOF variants
  - Lower *oe* values (i.e., closer to 0) are indicative of strong LoF intolerance
  - Threshold of <0.35 for the upper bound of the *oe* confidence interval was suggested as a measure of significant LoF depletion

- However, pLI and *oe* are **predictors** – will not always be accurate
  - Applicable for DOMINANT disorders
  - ***Selection is largely blind to phenotypes emerging after reproductive age***

MYBPC3 myosin binding protein C3

Dataset gnomAD v2.1.1 gnomAD SVs v2.1

Genome build GRCh37 / hg19
Ensembl gene ID ENSG00000134571.6
Ensembl canonical transcript ENST00000545968.1
Other transcripts ENST00000544791.1, ENST00000256993.4, and 1 more
Region 11:47352957-47374253
References Ensembl, UCSC Browser, and more

Constraint

Category	Exp. SNVs	Obs. SNVs	Constraint metrics
Synonymous	329.9	335	Z = -0.22 o/e = 1.02 (0.93 - 1.11)
Missense	794.1	679	Z = 1.45 o/e = 0.86 (0.8 - 0.91)
pLoF	67.3	29	pLI = 0 o/e = 0.43 (0.32 - 0.59)

exome genome Metric: Mean Save plot

ZEB2 zinc finger E-box binding homeobox 2

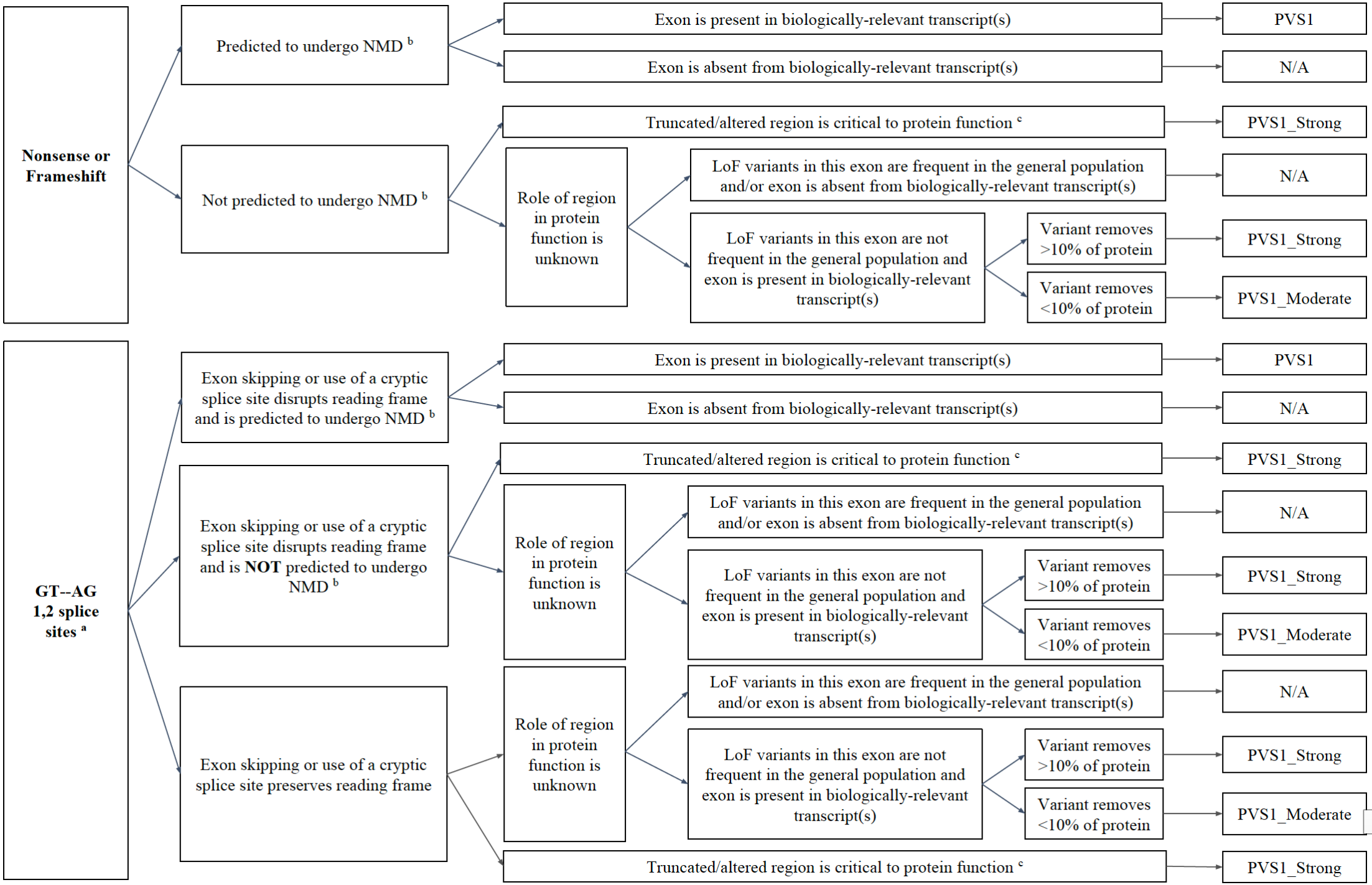
Dataset gnomAD v2.1.1 gnomAD SVs v2.1

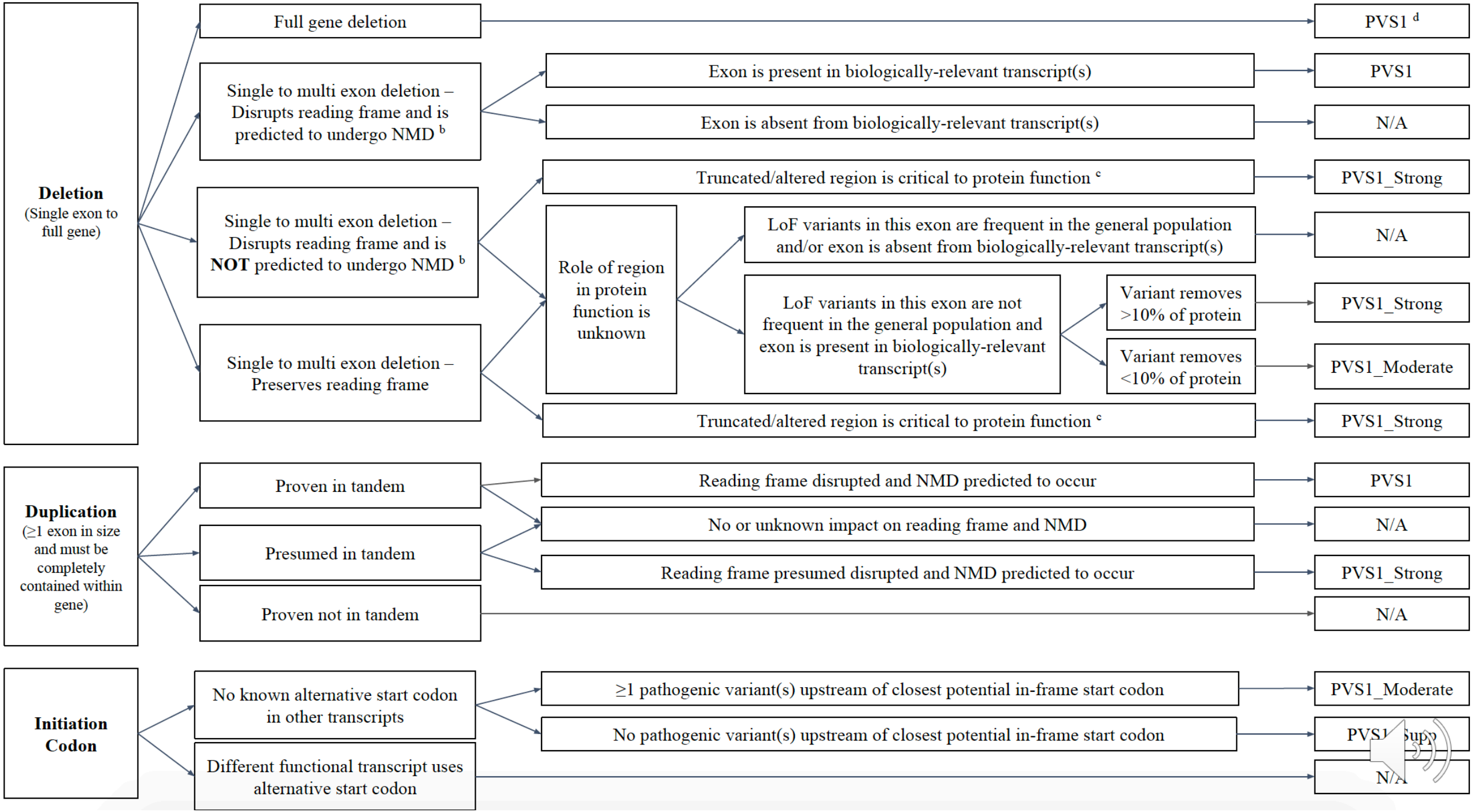
Genome build GRCh37 / hg19
Ensembl gene ID ENSG00000169554.12
Ensembl canonical transcript ENST00000558170.2
Other transcripts ENST00000392861.2, ENST00000419938.1, and 25 more
Region 2:145141648-145282147
References Ensembl, UCSC Browser, and more

Constraint

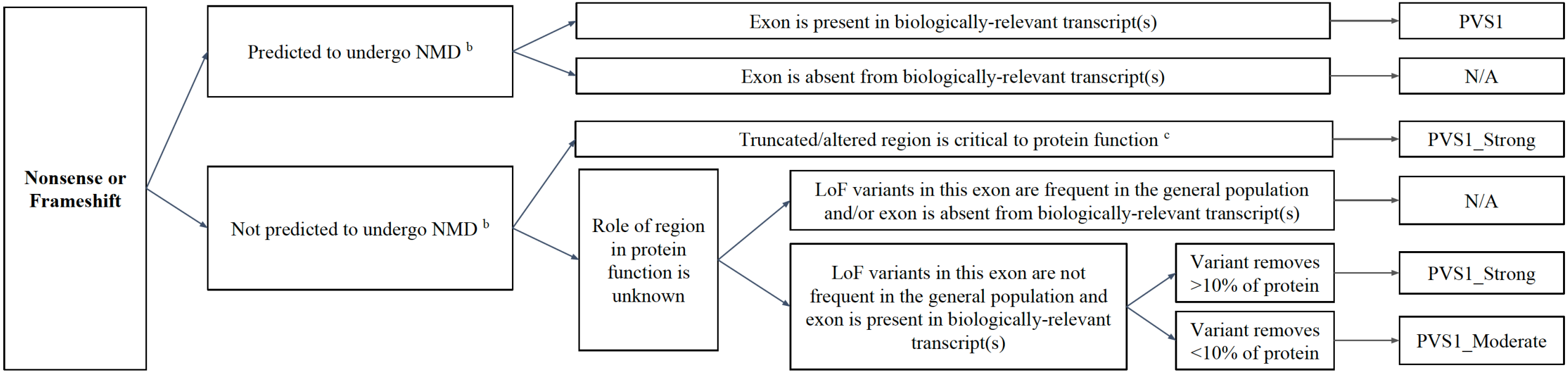
Category	Exp. SNVs	Obs. SNVs	Constraint metrics
Synonymous	259.2	264	Z = -0.23 o/e = 1.02 (0.92 - 1.13)
Missense	663.9	378	Z = 3.94 o/e = 0.57 (0.52 - 0.62)
pLoF	44.3	1	pLI = 1 o/e = 0.02 (0.01 - 0.11)

Evidence of pathogenicity	Category
Very strong	PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease Caveats: • Beware of genes where LOF is not a known disease mechanism (e.g., *GFAP*, *MYH7*) • Use caution interpreting LOF variants at the extreme 3′ end of a gene • Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact • Use caution in the presence of multiple transcripts

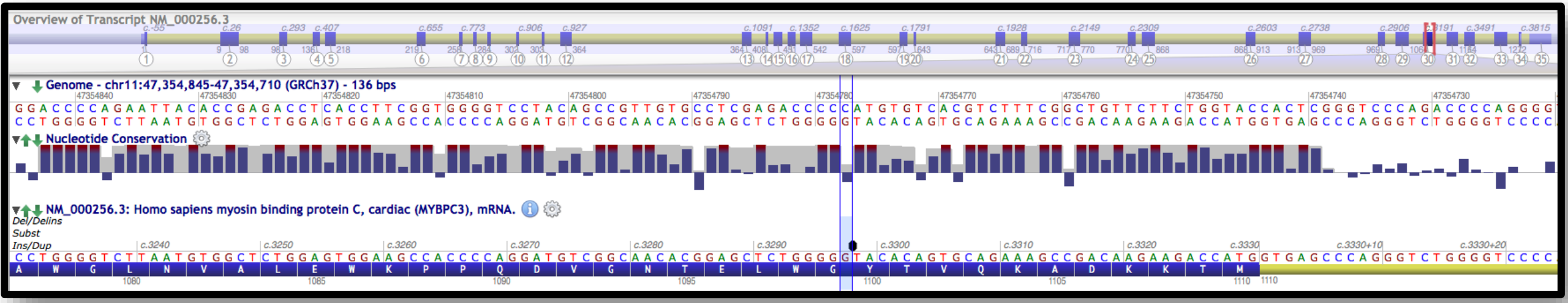

Nonsense or Frameshift
Predicted to undergo NMD b
Exon is present in biologically-relevant transcript(s)
PVS1
Exon is absent from biologically-relevant transcript(s)
N/A
Not predicted to undergo NMD b
Truncated/altered region is critical to protein function c
PVS1_Strong
Role of region in protein function is unknown
LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s)
N/A
LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
Variant removes >10% of protein
PVS1_Strong
Variant removes <10% of protein
PVS1_Moderate
GT--AG 1,2 splice sites a
Exon skipping or use of a cryptic splice site disrupts reading frame and is predicted to undergo NMD b
Exon is present in biologically-relevant transcript(s)
PVS1
Exon is absent from biologically-relevant transcript(s)
N/A
Exon skipping or use of a cryptic splice site disrupts reading frame and is NOT predicted to undergo NMD b
Truncated/altered region is critical to protein function c
PVS1_Strong
Role of region in protein function is unknown
LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s)
N/A
LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
Variant removes >10% of protein
PVS1_Strong
Variant removes <10% of protein
PVS1_Moderate
Exon skipping or use of a cryptic splice site preserves reading frame
Role of region in protein function is unknown
LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s)
N/A
LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
Variant removes >10% of protein
PVS1_Strong
Variant removes <10% of protein
PVS1_Moderate
Truncated/altered region is critical to protein function c
PVS1_Strong

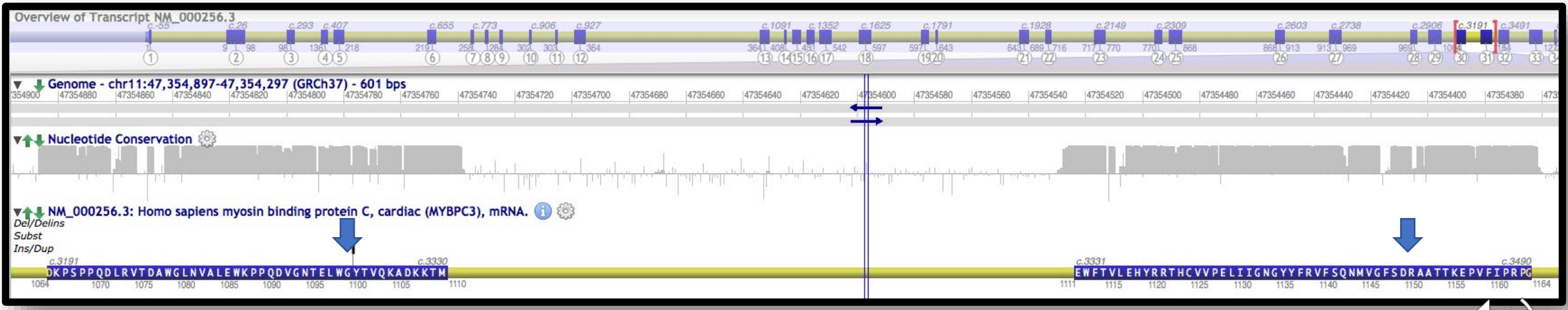

Deletion
(Single exon to full gene)
Full gene deletion
PVS1 d
Single to multi exon deletion – Disrupts reading frame and is predicted to undergo NMD b
Exon is present in biologically-relevant transcript(s)
PVS1
Exon is absent from biologically-relevant transcript(s)
N/A
Single to multi exon deletion – Disrupts reading frame and is NOT predicted to undergo NMD b
Truncated/altered region is critical to protein function c
PVS1_Strong
Role of region in protein function is unknown
LoF variants in this exon are frequent in the general population and/or exon is absent from biologically-relevant transcript(s)
N/A
LoF variants in this exon are not frequent in the general population and exon is present in biologically-relevant transcript(s)
Variant removes >10% of protein
PVS1_Strong
Variant removes <10% of protein
PVS1_Moderate
Single to multi exon deletion – Preserves reading frame
Truncated/altered region is critical to protein function c
PVS1_Strong
Duplication
(≥1 exon in size and must be completely contained within gene)
Proven in tandem
Reading frame disrupted and NMD predicted to occur
PVS1
Presumed in tandem
No or unknown impact on reading frame and NMD
N/A
Reading frame presumed disrupted and NMD predicted to occur
PVS1_Strong
Proven not in tandem
N/A
Initiation Codon
No known alternative start codon in other transcripts
≥1 pathogenic variant(s) upstream of closest potential in-frame start codon
PVS1_Moderate
No pathogenic variant(s) upstream of closest potential in-frame start codon
Different functional transcript uses alternative start codon
N/A

NM_000256.3(MYBPC3):c.3297dup (p.Tyr1100Valfs*49)

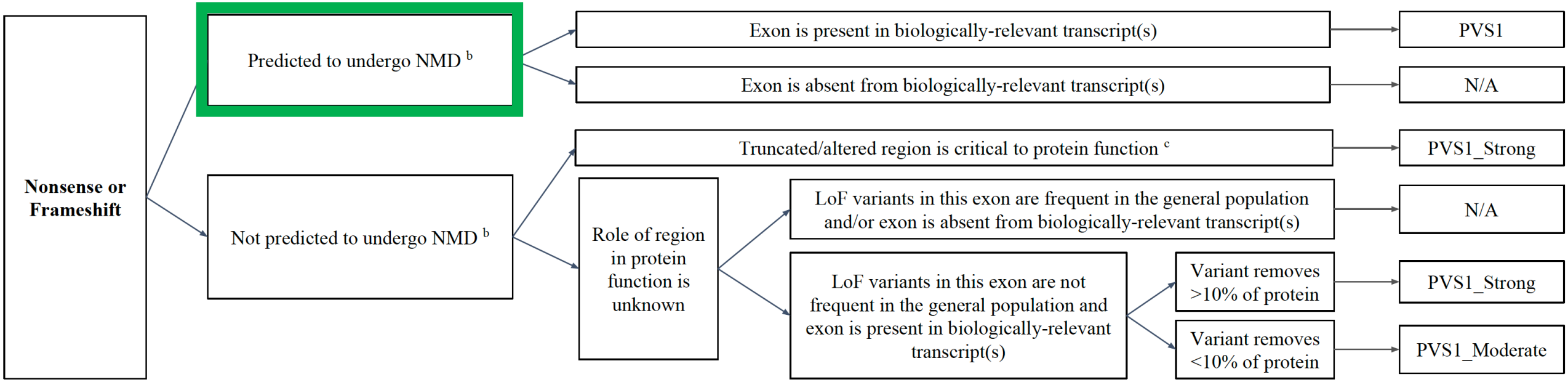


NM_000256.3(MYBPC3):c.3297dup (p.Tyr1100Valfs*49)





NM_000256.3(MYBPC3):c.3297dup (p.Tyr1100Valfs*49)



We are aware of the loading issues and are working to address them. The old version is available at http://gnomad-old.broadinstitute.org

MYBPC3 myosin binding protein C, cardiac

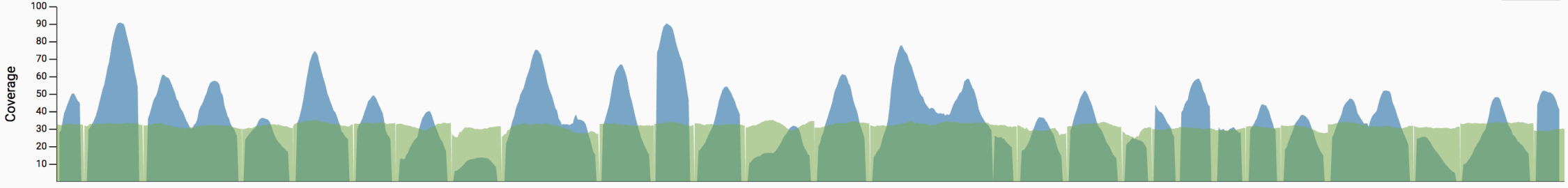
Dataset gnomAD v2.1.1 gnomAD SVs

Ensembl gene ID	ENSG00000134571
Ensembl transcript ID	ENST00000545968 (canonical)
UCSC Browser	11:47352958-47374254
GeneCards	MYBPC3
OMIM	600958

Gene Constraint

Category	Exp. no. SNVs	Obs. no. SNVs	Constraint metrics	
Synonymous	329.9	335	Z = -0.22	o/e = 1.02 (0.93 - 1.11)
Missense	794.1	679	Z = 1.45	o/e = 0.86 (0.80 - 0.91)
LoF	67.3	29	pLI = 0	o/e = 0.43 (0.32 - 0.59)

exome genome Save plot

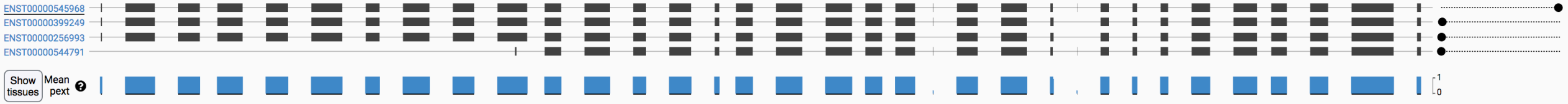


Include: CDS UTR Non-coding transcript

Hide transcripts

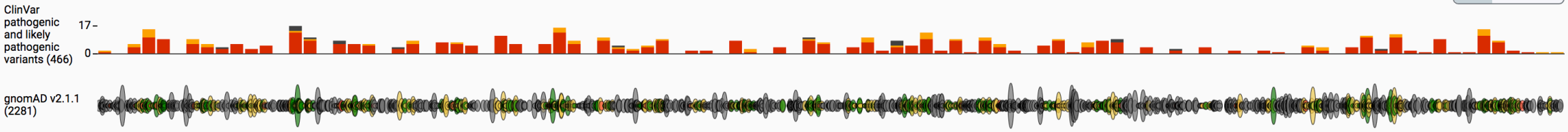
Isoform expression
Mean (23.592)

Save plot

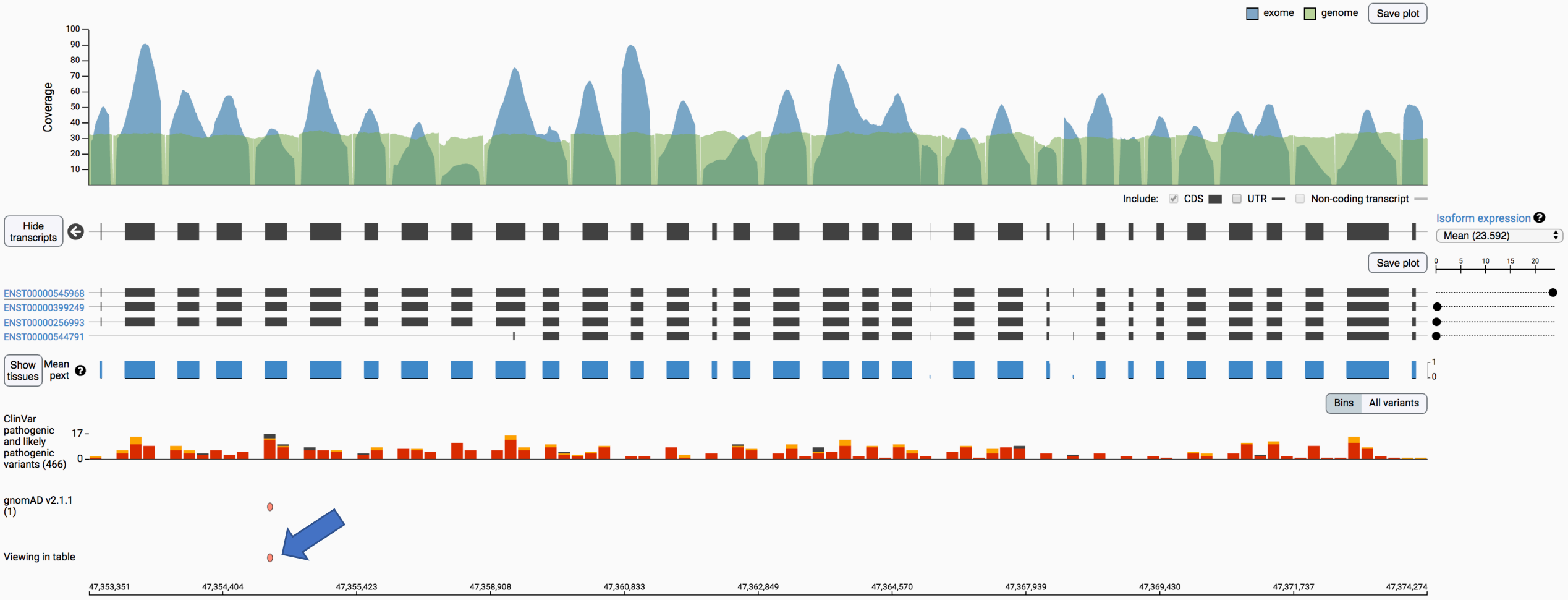


Show tissues Mean pext

Bins All variants



NM_000256.3(MYBPC3):c.3297dup (p.Tyr1100Valfs*49)



LoF only | Missense only | Synonymous only | Other only

Include filtered variants
SNVs
Indels

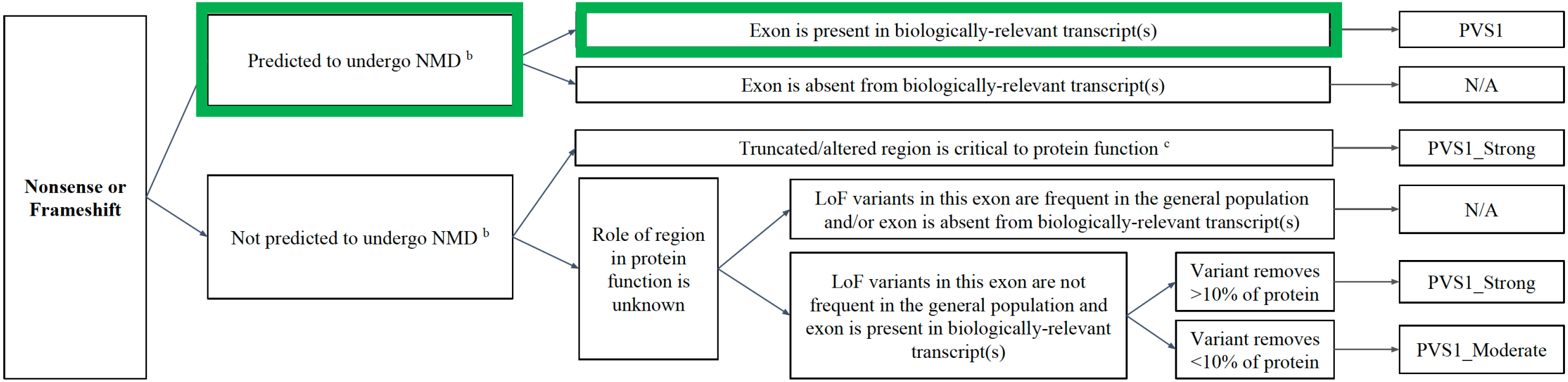
tyr1100

Export variants to CSV

† denotes a consequence that is for a non-canonical transcript

Variant ID	Source	Consequence	Annotation	Flags	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
11-47354777-A-AC	E	p.Tyr1100ValfsTer49	frameshift		3	216706	1.38e-5	0

NM_000256.3(MYBPC3):c.3297dup (p.Tyr1100Valfs*49)



Missense Variants (PP2 and PM1)

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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	

<u>Gene Missense z-score</u>
Calculated by comparing the expected versus observed number of missense variants

<u>Regional constraint score</u>
Compares expected versus observed for specific regions of a gene

MYH7 – **Gene** Constraint

MYH7 myosin, heavy chain 7, cardiac muscle, beta

Dataset gnomAD v2.1.1 gnomAD SVs

Ensembl gene ID	ENSG00000092054
Ensembl transcript ID	ENST00000355349 (canonical)
UCSC Browser	14:23881948-23904928
GeneCards	MYH7
OMIM	160760

Suggests **GENE** is missense constrained (z > 3.09)

Gene Constraint

Category	Exp. no. SNVs	Obs. no. SNVs	Constraint metrics	
Synonymous	471.7	552	Z = -2.91	o/e = 1.17 (1.09 - 1.26)
Missense	1110.9	742	Z = 3.93	o/e = 0.67 (0.63 - 0.71)
LoF	96.5	43	pLI = 0	o/e = 0.45 (0.35 - 0.57)

exome genome Save plot

Coverage 100 90 80 70 60 50 40 30 20 10

Include: CDS UTR Non-coding transcript

Show transcripts

Show tissues Mean pext

Bins All variants

ClinVar pathogenic and likely pathogenic variants (330) 22 0

gnomAD v2.1.1 (791)

MYH7 – **Regional** Constraint (only in ExAC)

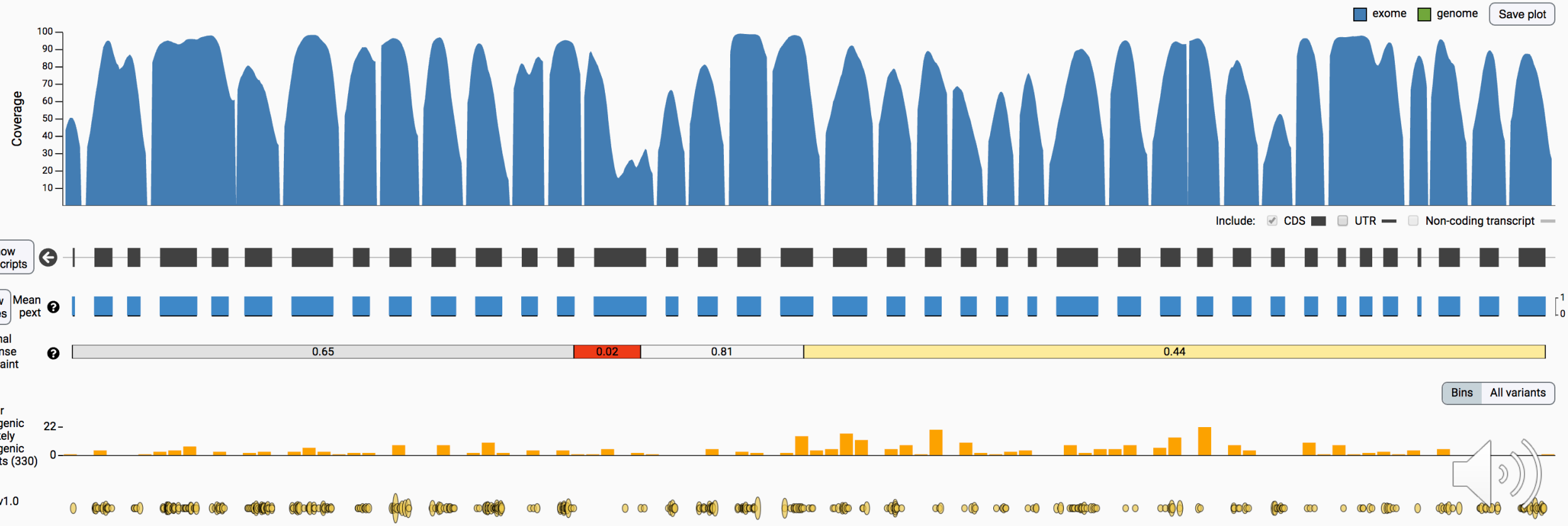
MYH7 myosin, heavy chain 7, cardiac muscle, beta

Dataset: ExAC v1.0 | gnomAD SVs

Ensembl gene ID	ENSG00000092054
Ensembl transcript ID	ENST00000355349 (canonical)
UCSC Browser	14:23881948-23904928
GeneCards	MYH7
OMIM	160760

Gene Constraint

Category	Exp. no. SNVs	Obs. no. SNVs	Constraint metric
Synonymous	378	391	Z = -0.41
Missense	797.4	420	Z = 6.54
LoF	70.2	21	pLI = 0



MYH7 – **Regional** Constraint (only in ExAC)

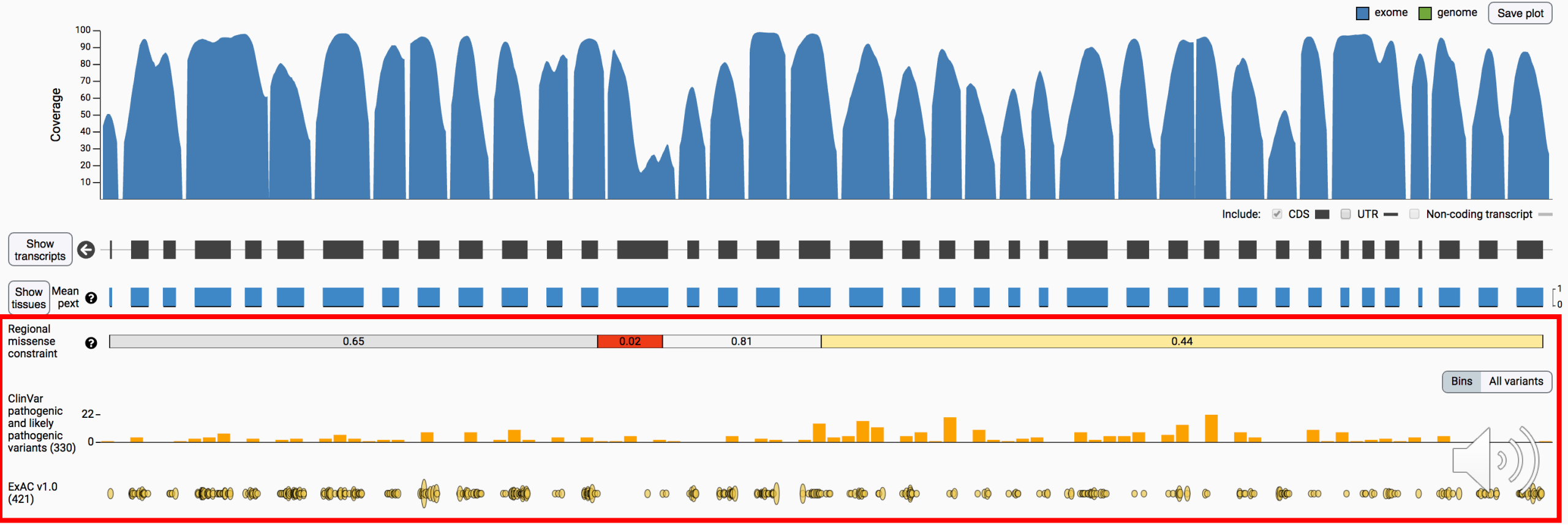
MYH7 myosin, heavy chain 7, cardiac muscle, beta

Dataset: ExAC v1.0 | gnomAD SVs

Ensembl gene ID	ENSG00000092054
Ensembl transcript ID	ENST00000355349 (canonical)
UCSC Browser	14:23881948-23904928
GeneCards	MYH7
OMIM	160760

Gene Constraint

Category	Exp. no. SNVs	Obs. no. SNVs	Constraint metric
Synonymous	378	391	Z = -0.41
Missense	797.4	420	Z = 6.54
LoF	70.2	21	pLI = 0



MYH7 – **Regional** Constraint (only in ExAC)

MYH7 myosin, heavy chain 7, cardiac muscle, beta

Dataset ExAC v1.0 gnomAD SVs

Ensembl gene ID	ENSG00000092054
Ensembl transcript ID	ENST00000355349 (canonical)
UCSC Browser	14:23881948-23904928
GeneCards	MYH7
OMIM	160760

Gene Constraint

Category	Exp. no. SNVs	Obs. no. SNVs	Constraint metric
Synonymous	378	391	Z = -0.41
Missense	797.4	420	Z = 6.54
LoF	70.2	21	pLI = 0

exome genome Save plot

Include: CDS UTR Non-coding transcript

Regional constraint

Important: Currently regional constraint is only available / displayed when selecting the ExAC dataset. In addition, it is only displayed for gene that exhibit regional missense constraint.

Overall interpretation

We searched for regions within transcripts that were intolerant of missense variation within the ExAC dataset. We used the observed and expected missense variation in each transcript in a likelihood ratio test to identify those transcipts that had two or more regions with significantly different levels of missense constraint (as measured by depletion of expected missense variation). Missense constraint values closer to zero indicate increased intolerance against missense variation.

Regional missense constraint: 0.65 | 0.02 | 0.81 | 0.44

Bins All variants

ClinVar pathogenic and likely pathogenic variants (330): 22 – 0

ExAC v1.0 (421)

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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	

Gene Missense z-score

Calculated by comparing the expected versus observed number of missense variants

DRAFT PROPOSAL:
Applicable if Z score > 3.00

Regional constraint score

Compares expected versus observed for specific regions of a gene

CAUTION: Missense constraint z-score could be high simply because one region of the gene is highly constrained

Workshop Outline

- **Recommendations for 3 key areas**:
  - Allele frequency data
  - Variant type and location
  - ***Patient data***

- Criteria combinations

How to account for multiple probands with consistent phenotypes?

	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			

How to account for multiple probands with consistent phenotypes?

disease in a given case. Pathogenicity should be determined by the entire body of evidence in aggregate, including all cases studied, arriving at a single conclusion.

Application of case-level evidence does not need to be met by case currently in front of you

ANY case that meets criteria can be used

De Novo Data

Benign		Pathogenic			
Strong	Supporting	Supporting	Moderate	Strong	Very Strong
			De novo **(without paternity & maternity confirmed)** ***PM6***	***De novo*** **(paternity & maternity confirmed)** ***PS2***	

PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, and so on, can contribute to nonmaternity.

PM6 Assumed de novo, but without confirmation of paternity and maternity

Pathogenic	
Moderate	**Strong**
De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2

PS2 PM6 de novo variants

A variant observed to have arisen de novo (parental samples testing negative) is considered strong support for pathogenicity if the following conditions are met:

(i) Both parental samples were shown through identity testing to be from the biological parents of the patient. Note that PM6 applies if identity is assumed but not confirmed.

(ii) The patient has a family history of disease that is consistent with de novo inheritance (e.g., unaffected parents for a dominant disorder). It is possible, however, that more than one sibling may be affected because of germ-line mosaicism.

(iii) The phenotype in the patient matches the gene's disease association with reasonable specificity. For example, this argument is strong for a patient with a de novo variant in the *NIPBL* gene who has distinctive facial features, hirsutism, and upper-limb defects (i.e., Cornelia de Lange syndrome), whereas it would be weaker for a de novo variant found by exome sequencing in a child with nonspecific features such as developmental delay.

SVI Guidance - https://www.clinicalgenome.org/svi/

SVI General Recommendations for Using ACMG/AMP Criteria

SVI provides general recommendations for using the ACMG/AMP criteria to improve consistency in usage and transparency in classification rationale.

- Guidance on how to rename criteria codes when strength of evidence is modified
- BA1: Updated Recommendation for the ACMG/AMP Stand Alone Pathogenicity Criterion for Variant Classification
  - BA1 Exception List (July 2018)
  - BA1 Exception List Nomination Form
- PVS1: Recommendations for Interpreting the Loss of Function PVS1 ACMG/AMP Variant Criteria
- PS2/PM6: Recommendation for de novo PS2 and PM6 ACMG/AMP criteria (Version 1.0)
- PS3/BS3: Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework
- PM2: Recommendation for Absence/Rarity Criterion PM2 (Version 1.0)
- PM3: Recommendation for in trans Criterion PM3 (Version 1.0)
- PP5/BP6: Recommendation for reputable source PP5 and BP6 ACMG/AMP 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

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

A de novo *NIPBL* variant in 3 probands….

- **Confirmed** *de novo* in one patient with Cornelia de Lange syndrome (**2 pt**)
- **Assumed** *de novo* in one patient with Cornelia de Lange syndrome (**1 pt**)
- **Assumed** *de novo* in another patient with Cornelia de Lange syndrome (**1 pt**)

Total of 4 points (2+1+1) = **VeryStrong**

Recessive Disorders – PM3

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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		

SVI revision to PM3: For recessive disorders, detected in trans with a pathogenic ***or likely pathogenic*** variant in ***an affected patient***

Recessive Disorders – Elevate PM3 strength

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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		

PM3	for recessive disorders, variant in *trans* with a pathogenic variant	invoke this rule as supporting if the phase is not established
		can upgrade if more than one proband is reported

Amendola et al; Table 1

Recessive Disorders – Elevate PM3 strength

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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		
			PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
			0.5 points	1.0 points	2.0 points	4.0 points

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

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

PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
0.5	1.0	2.0	4.0

Variant found 5 probands:

2 homozygous occurrences (0.5 pts each; 1 pt)

3 probands with confirmed pathogenic variant in trans (1.0 pts each; 3 pts)

TOTAL: 4.0 pts
PM3_VeryStrong

Dominant Disorders – Modify Strength of PS4

	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	

PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls

Note 1: Relative risk or OR, as obtained from case–control studies, is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0. See the article for detailed guidance.

Note 2: In instances of very rare variants where case–control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Dominant Disorders – Modify Strength of PS4

Supporting	Moderate	Strong
	Absent in population databases PM2	Prevalence in affecteds statistically increased over controls (OR method)
Variant identified in # probands with consistent phenotypes PS4_Supporting	Variant identified in ## probands with consistent phenotypes PS4_Moderate	Variant identified in ### probands with consistent phenotypes PS4

PS4 could be used for typical case-control studies with an OR

OR

PS4 could be used for multiple unrelated probands with consistent phenotypes

Comparison of PS4 specification across disease areas

	PS4 Specification			PM2 Specification
	Supporting	Moderate	Strong	
RASopathy	1 proband	3 probands	5 probands	Strictly absent
PTEN	1 proband	2 probands	4 probands	<0.001%
CDH1	1 proband	2 probands	4 probands	<0.001%
Hearing Loss	2 probands	6 probands	15 probands	<0.002%
Cardiomyopathy	2 probands	6 probands	15 probands	<0.004%

Patient phenotype highly specific - PP4

Benign		Pathogenic			
Strong	Supporting	Supporting	Moderate	Strong	Very Strong
	Found in case with an alternative cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

Patient phenotype highly specific - PP4

Benign		Pathogenic			
Strong	Supporting	Supporting	Moderate	Strong	Very Strong
	Found in case with an alternative cause *BP5*	Patient's phenotype or FH highly specific for gene *PP4*			

PP4 – applicable for non-genetic confirmation testing

- PAH group:
  - Plasma phenylalanine > 120 umol/L
- Mitochondrial:
  - Reduction in electron transport chain activity in patient cells

How to account for multiple probands with consistent phenotypes?

	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			

How to account for multiple probands with consistent phenotypes?

	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			

PS4 spectrum – count probands (dominant)

PM3 spectrum – count probands (recessive)

How to account for multiple probands with consistent phenotypes?

	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			

PS4 spectrum – count probands (dominant)

PM6/PS2 spectrum – ≥1 de novo occurrence

PM3 spectrum – count probands (recessive)

How to account for multiple probands with consistent phenotypes?

	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			

PS4 spectrum – count probands (dominant)

PM6/PS2 spectrum – ≥1 de novo occurrence

PM3 spectrum – count probands (recessive)

PP4 – ≥1 proband meeting criteria (should NOT be used to count probands)

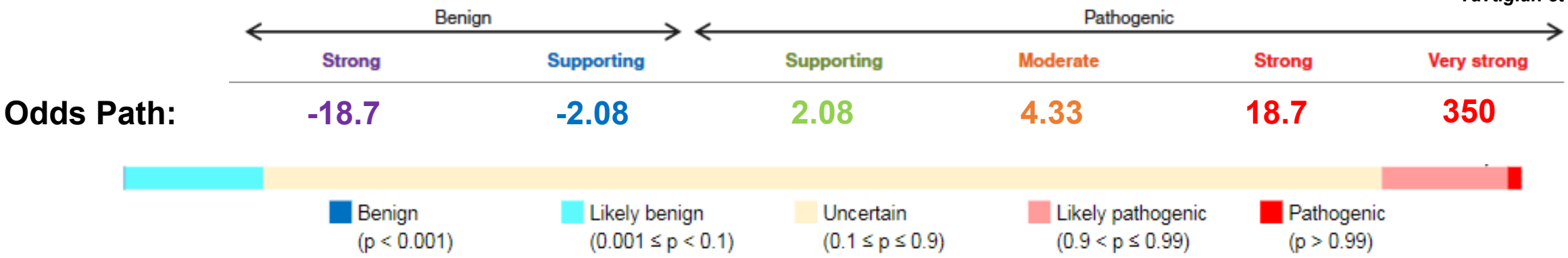
Workshop Outline

- Recommendations for 3 key areas:
  - Allele frequency data
  - Variant type and location
  - Patient data

- ***Criteria combinations***

Bayes formulation of ACMG/AMP guidelines

Tavtigian et al 2018



- Help determine the relative weight of a piece of evidence
  - Is “absence” (PM2) really 4.33:1 odds of pathogenicity?
  - For PP3, at what threshold does a REVEL score reach 2.08:1 odds of pathogenicity?

Bayes formulation of ACMG/AMP guidelines

Tavtigian et al 2018

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Odds Path:	-18.7	-2.08	2.08	4.33	18.7	350

Benign	Likely benign	Uncertain	Likely pathogenic	Pathogenic
($p < 0.001$)	($0.001 \le p < 0.1$)	($0.1 \le p \le 0.9$)	($0.9 < p \le 0.99$)	($p > 0.99$)

ACMG/AMP – compatible point system

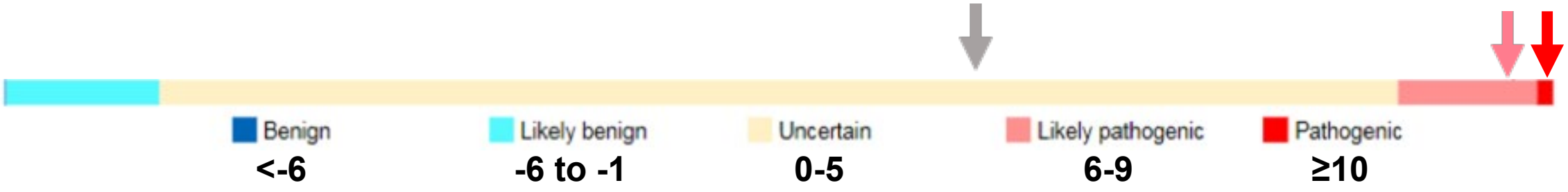
Tavtigian et al 2020

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Odds Path:	-4	-1	1	2	4	8

Benign	Likely benign	Uncertain	Likely pathogenic	Pathogenic
≤ -7	-1 to -6	0 to 5	6 to 9	≥ 10

Advantages of point approach to ACMG/AMP guidelines

- Better understanding of variants with conflicting evidence



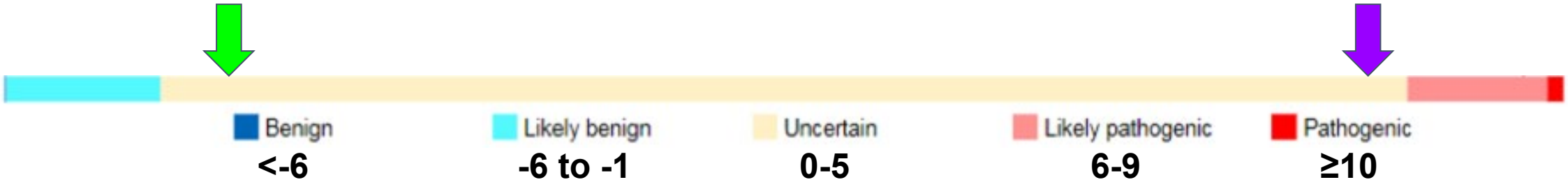
- PS4 + PM1 + PM6 + PP1_Moderate = **Pathogenic (10 pts)**
- PS4 + PM1 + PM6 + PP1_Moderate + ***BP2*** = **Likely pathogenic (9 pts)**
- PS4 + PM1 + PM6 + PP1_Moderate + ***BP2* + *BS3*** = **Uncertain significance (5 pts)**

Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

Advantages of point approach to ACMG/AMP guidelines

- Allows for criteria combinations not listed in the 2015 ACMG guidelines
  - LoF variant (PVS1; 8 pts) + absent from controls (PM2_Supporting; 1 pt) = **Likely pathogenic (9 pts)**

- Differentiation of VUS category (0-5 pts)
  - In silico damaging impact (PP3) = **Uncertain significance (1 pt)**
  - In silico damaging impact (PP3) + in critical function domain (PM1) + found in trans with pathogenic variant (PM3) = **Uncertain significance (5 pts)**



Acknowledgements

- ClinGen Sequence Variant Interpretation WG
  - Les Biesecker, Steven Harrison, Jonathan Berg, Steven Brenner, Gary Cutting, Sian Ellard, Marc Greenblatt, Peter Kang, Izabela Karbassi, Rachel Karchin, Jessica Mester, Anne O'Donnell-Luria, Tina Pesaran, Sharon Plon, Heidi Rehm, Natasha Strande, Sean Tavtigian, Scott Topper

- Expert Panels working on specifying the ACMG/AMP guidelines