MAYO CLINIC

CENTER FOR
INDIVIDUALIZED MEDICINE

Variant Interpretation
ACMG Guidelines part 1

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Objectives

1. Introduction to ACMG variant interpretation guidelines and updated recommendations.
2. Understand best-practices of pathogenicity evidence acquisition and integration for variant classification.
3. Discussion on the current limitations and the future of clinical variant interpretation.

Pretest questions:

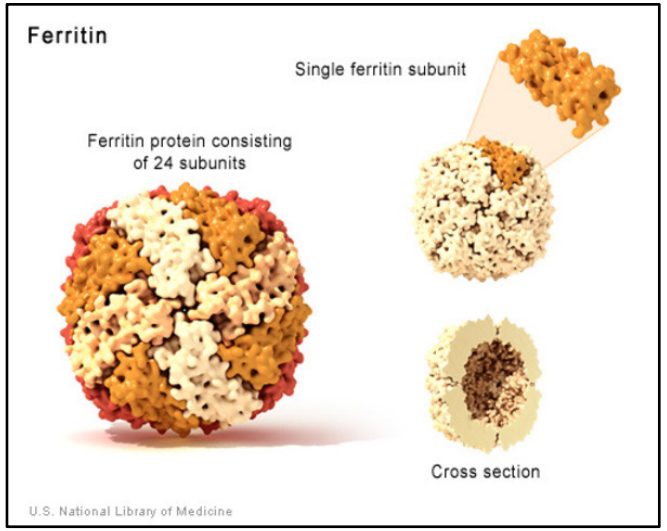
1) When we classify a variant, we do it ONLY in the context of the case we are working on, we classify `if the variant is causing the disease in the patient`.

A) TRUE
B) FALSE

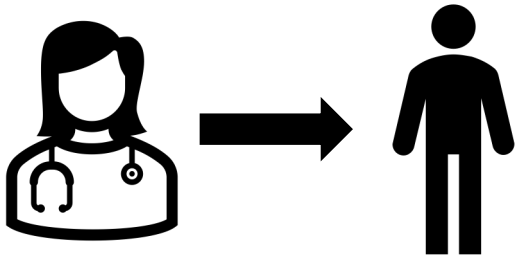
2) Retinoblastoma, the most malignant form of eye cancer, arises from a dominant pathogenic variant in one gene *RB1*, but only about 75% of people who carry this variant develop the disease. We are talking about:
A) Penetrance
B) Expressivity

3) A frequent variant (found in >5% in a population) will always be classified as `benign`
A) TRUE
B) FALSE

One quick story…



“ You have a pathogenic mutation in *HFE* which is responsible for autosomal dominant hemochromatosis”



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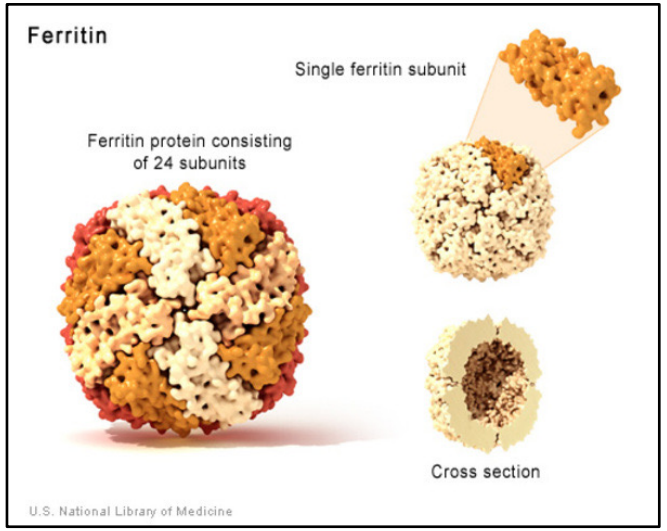
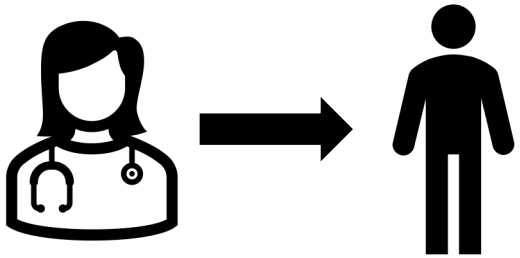


“ You have a pathogenic mutation in *HFE* which is responsible for autosomal dominant hemochromatosis”

Pesquisa da mutação c.187C>G (p.H63D): Mutação presente, em heterozigose

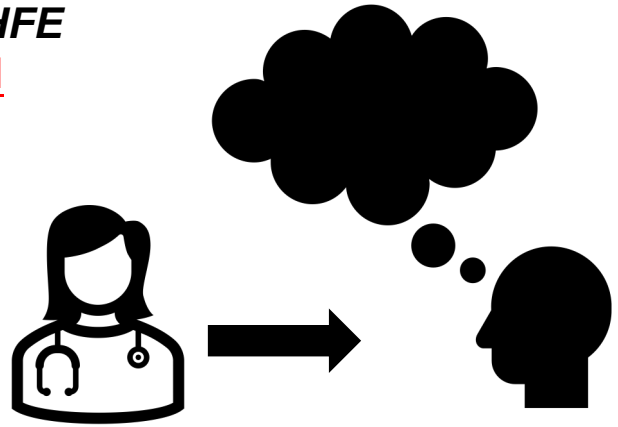
H63D is considered to be the “minor” variant, which seldom causes significant iron overload, even when it is present in compound heterozygosity with C282Y.



“RISK ALLELE”

One quick story…

“ You have a pathogenic mutation in *HFE* which is responsible for autosomal dominant hemochromatosis”



Wrong terminology
Wrong Inheritance pattern…

How can we all speak the same `language`?

Established a common framework and criteria for variant classification

ACMG

American College of Medical Genetics and Genomics

Translating Genes Into Health®

AMP ASSOCIATION FOR MOLECULAR PATHOLOGY

cap

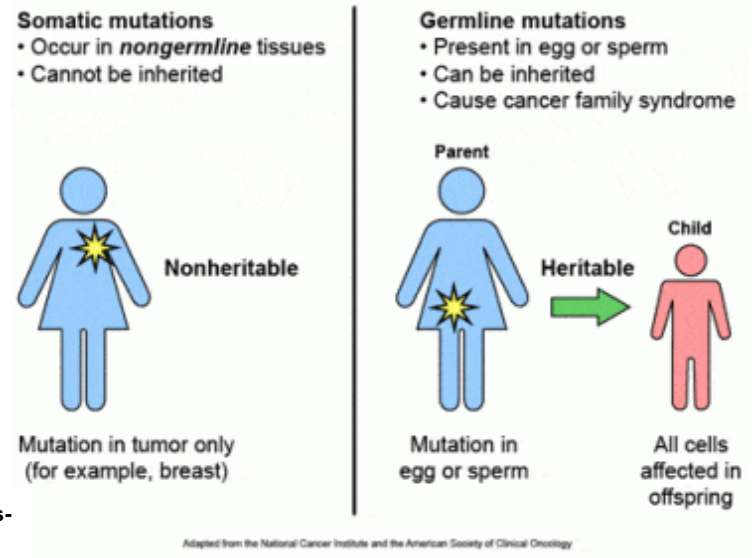
2015 ACMG Guidelines

PMID: 25741868

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

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

- 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.**



https://positivebioscience.com/somatic-mutations-vs-germline-mutations/

Class
Pathogenic
Likely Pathogenic
Variant of Uncertain Significance
Likely Benign
Benign

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- **It is not intended** for the interpretation of **somatic variation, pharmacogenomic variants, or variants in genes associated with multigenic non-Mendelian complex disorders.**
- Care must be taken when applying these rules to candidate genes ("genes of uncertain significance", **GUS**)
- This report recommends the use of specific standard terminology: 'pathogenic', 'likely pathogenic', 'uncertain significance', 'likely benign', and 'benign' to describe variants identified in **Mendelian disorders.**

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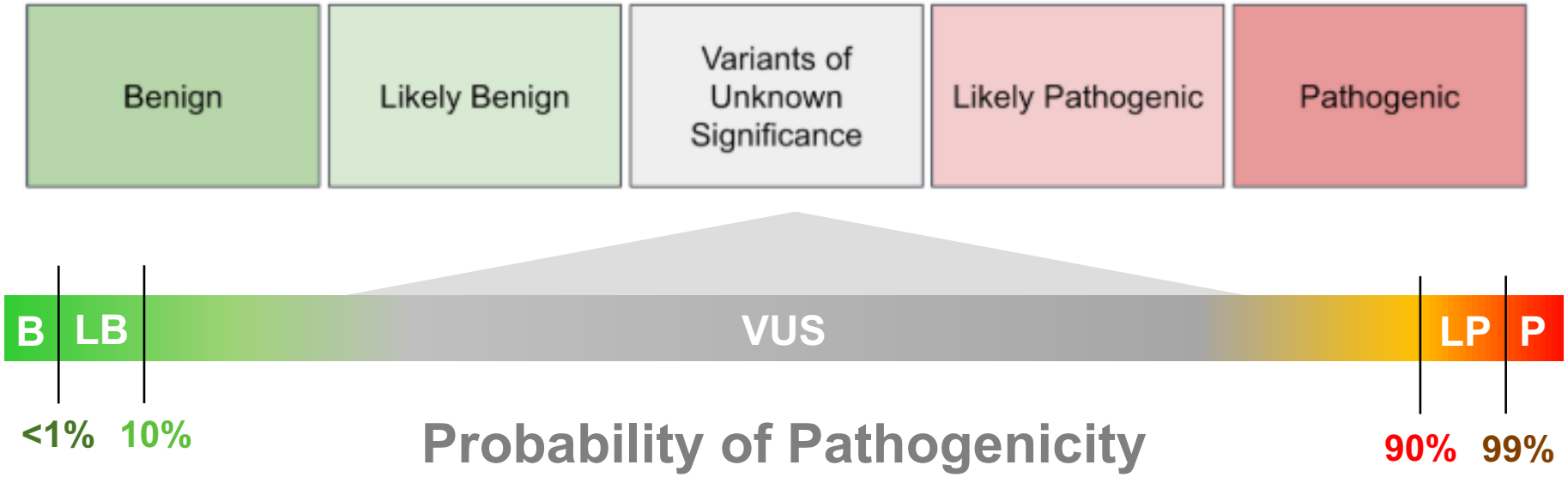
Class
Pathogenic
Likely Pathogenic
Variant of Uncertain Significance
Likely Benign
Benign

A variant which is proven to be deleterious to protein or gene function and is associated with a particular human disease phenotype.

A variant that does not appear to have a *deleterious* effect often associated with a "normal" human phenotype.

What is `Likely`?

The rules proposed to classify sequence variants follows is a heuristic system for variant classification that is compatible with a formal, quantitative, Bayesian classifier.



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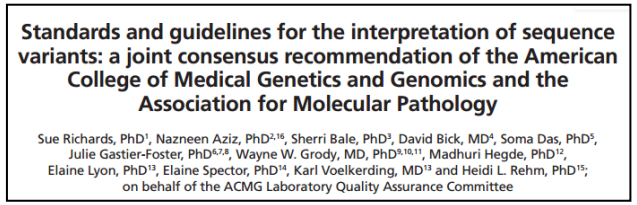

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Terminology

- In the past…

Mutation

Permanent change in the nucleotide sequence

Polymorphism

Variant with a frequency above 1%.

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- Terminology

Variant

Permanent change in the nucleotide sequence

Variant

Variant with a frequency above 1%.

the following modifiers: (1) pathogenic, (2) likely pathogenic, (3) uncertain significance, (4) likely benign, or (5) benign.

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Use of ClinGen

- They anticipated "that those working in specific disease groups should continue to develop more focused guidance regarding the classification of variants in specific genes given that the applicability and **weight assigned to certain criteria may vary by gene and disease" (Richards et al., 2015)**

ClinGen
Clinical Genome Resource

ClinGen - Clinical Genome Resource

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

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- The guideline defined 28 criteria, with codes that addressed types of variant evidence. Each evidence type or criterion code was assigned a direction, benign (B) or pathogenic (P), and a level of strength: stand-alone (A), very strong (VS), strong (S), moderate (M), or supporting (P).

	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			

ACMG 2015 guidelines discrete criteria's have a strong quantitative correlation with the odds of pathogenicity of a variant.

		BENIGN CRITERIA		PATHOGENIC CRITERIA			
Strength of Evidence		Strong	Supporting	Supporting	Moderate	Strong	Very Strong
Odds of Pathogenicity*		-18.7	-2.08	2.08	4.33	18.7	350.0
Evidence Category and Corresponding ACMG/AMP Codes	Population Data	*BA1*+ BS1 BS2			PM2	PS4	
	Allelic Evidence & Co-Segregation Data	BS4	BP2 BP5	PP1 →	PM3 PM6	PS2	
	Computation & Predictive Data		BP1 BP3 BP4 BP7	PP2 PP3	PM1 PM4 PM5	PS1	PVS1
	Functional Data	BS3				PS3	
	Other		BP6	PP4 PP5			

Table 5 Rules for combining criteria to classify sequence variants

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

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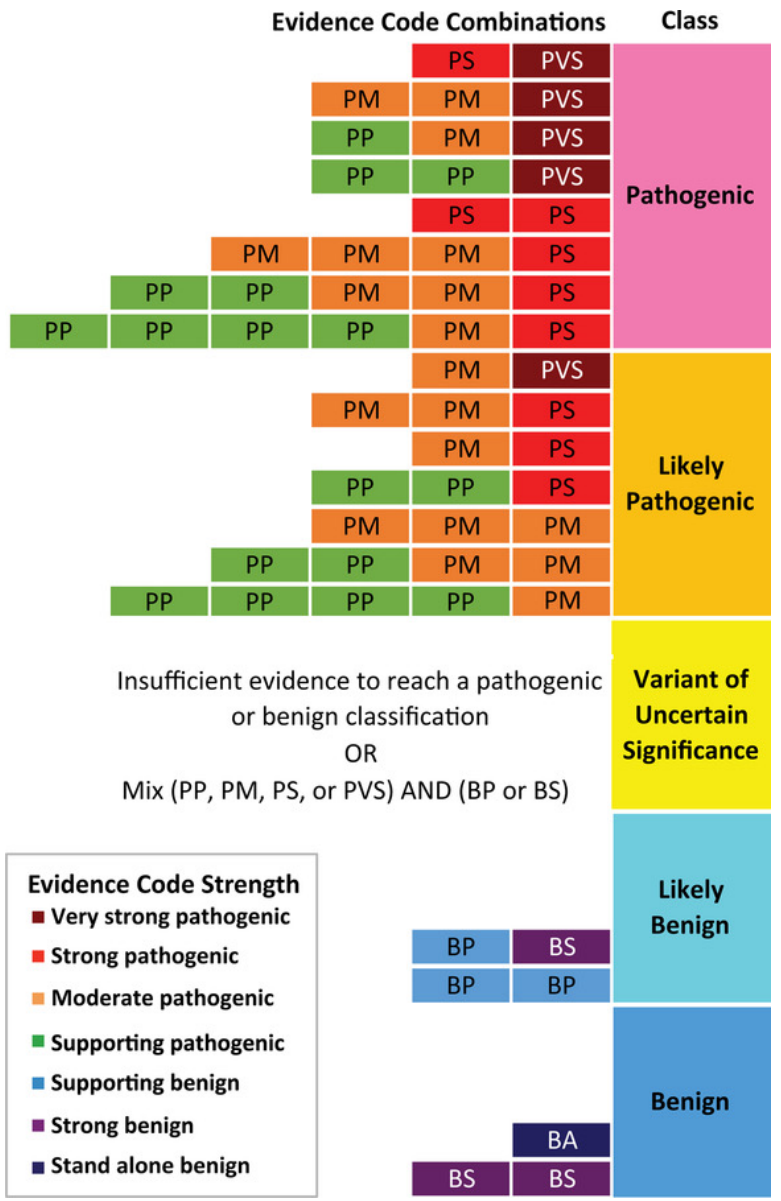

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Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

PS4 + PM2 + PP1

Pathogenic

Level of Evidence: *Strong*

= Likely Pathogenic

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PS4 + PM2 + PP1

Pathogenic

Level of Evidence: *Strong*

= Likely Pathogenic

BP4

Benign

Level of Evidence: *Supporting*

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Conflicting evidence example:

PS4 + PM2 + BP2 + BP4

= Variant of Uncertain Significance (VUS)

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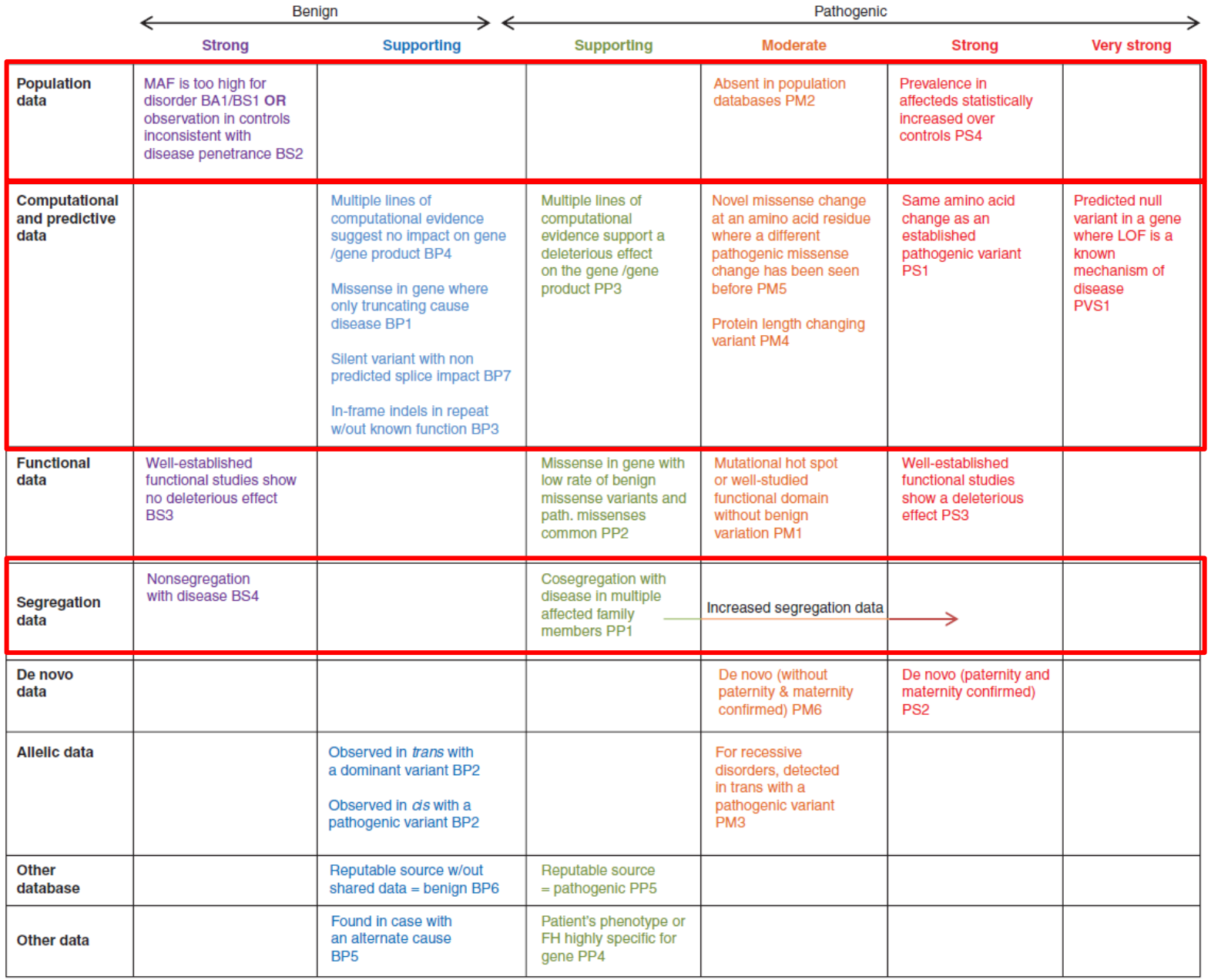

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Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
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1- Population Data

PMID: 25741868

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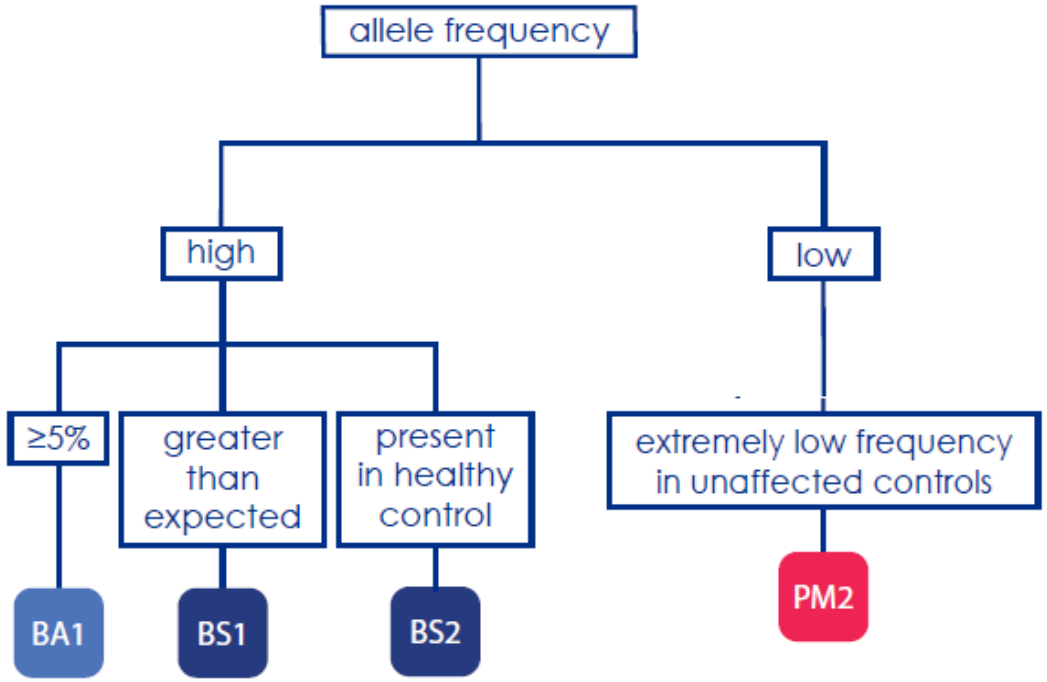

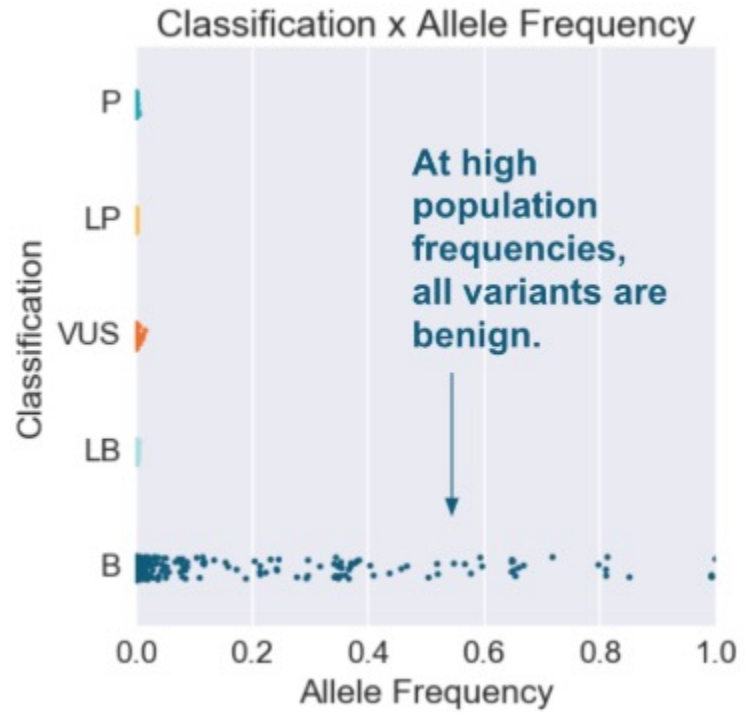

The cutoffs of each of these criteria depends on many factors such as: Prevalence of disease, age of onset, and penetrance

PMID: 25741868

Examples of Databases

Table 1

Population, Disease-Specific, and Sequence Databases

Population Databases	
Exome Aggregation Consortium http://exac.broadinstitute.org/	Database of variants found during exome sequencing of 61,486 unrelated individuals sequenced as part of various disease-specific and population genetic studies. Pediatric disease subjects as well as related individuals were excluded.
Exome Variant Server http://evs.gs.washington.edu/EVS	Database of variants found during exome sequencing of several large cohorts of individuals of European and African American ancestry. Includes coverage data to inform the absence of variation.
1000 Genomes http://browser.1000genomes.org	Database of variants found during low-coverage and high-coverage genomic and targeted sequencing from 26 populations. Provides more diversity compared to EVS but also contains lower quality data and some cohorts contain related individuals.
dbSNP http://www.ncbi.nlm.nih.gov/snp	Database of short genetic variations (typically 50 bp or less) submitted from many sources. May lack details of originating study and may contain pathogenic variants.
dbVar http://www.ncbi.nlm.nih.gov/dbvar	Database of structural variation (typically greater than 50 bp) submitted from many sources.
Disease Databases	
ClinVar http://www.ncbi.nlm.nih.gov/clinvar	Database of assertions about the clinical significance and phenotype relationship of human variation.
OMIM http://www.omim.org	Database of human genes and genetic conditions that also contains a representative sampling of disease-associated genetic variants.
Human Gene Mutation Database http://www.hgmd.org	Database of variant annotations published in the literature. Requires fee-based subscription for much of the content.
Locus/Disease/Ethnic/Other-Specific Databases http://www.hgvs.org/dblist/dblist.html http://www.lovd.nl	The HGVS site developed a list of thousands of different databases that provide variant annotations on specific subsets of human variation. A large percentage of databases are built in the LOVD system.
DECIPHER http://decipher.sanger.ac.uk	A molecular cytogenetic database for clinicians and researchers linking genomic microarray data with phenotype using the Ensembl genome browser.
Sequence Databases	
NCBI Genome http://www.ncbi.nlm.nih.gov/genome	Source of full human genome reference sequences.
RefSeqGene http://www.ncbi.nlm.nih.gov/refseq/rsg and Locus Reference Genomic (LRG) http://www.lrg-sequence.org	Medically relevant gene reference sequence resource
MitoMap http://www.mitomap.org/MITOMAP/HumanMitoSeq	Revised Cambridge reference sequence (rCRS) for the Human Mitochondrial DNA

Examples of Databases

Table 1

Population, Disease-Specific, and Sequence Databases

Population Databases	
Exome Aggregation Consortium http://exac.broadinstitute.org/	Database of variants found during exome sequencing of 61,486 unrelated individuals sequenced as part of various disease-specific and population genetic studies. Pediatric disease subjects as well as related individuals were excluded.
Exome Variant Server http://evs.gs.washington.edu/EVS	Database of variants found during exome sequencing of several large cohorts of individuals of European and African American ancestry. Includes coverage data to inform the absence of variation.
1000 Genomes http://browser.1000genomes.org	Database of variants found during low-coverage and high-coverage genomic and targeted sequencing from 26 populations. Provides more diversity compared to EVS but also contains lower quality data and some cohorts contain related individuals.
dbSNP http://www.ncbi.nlm.nih.gov/snp	Database of short genetic variations (typically 50 bp or less) submitted from many sources. May lack details of originating study and may contain pathogenic variants.
dbVar http://www.ncbi.nlm.nih.gov/dbvar	Database of structural variation (typically greater than 50 bp) submitted from many sources.
Disease Databases	
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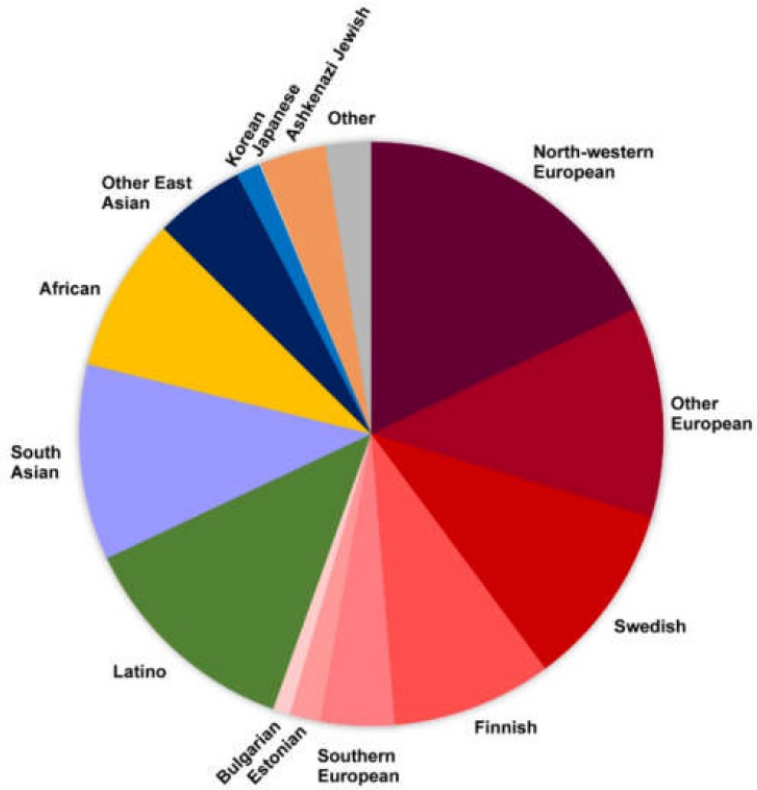
MitoMap http://www.mitomap.org/MITOMAP/HumanMitoSeq	Revised Cambridge reference sequence (rCRS) for the Human Mitochondrial DNA

PMID: 25741868

gnomAD

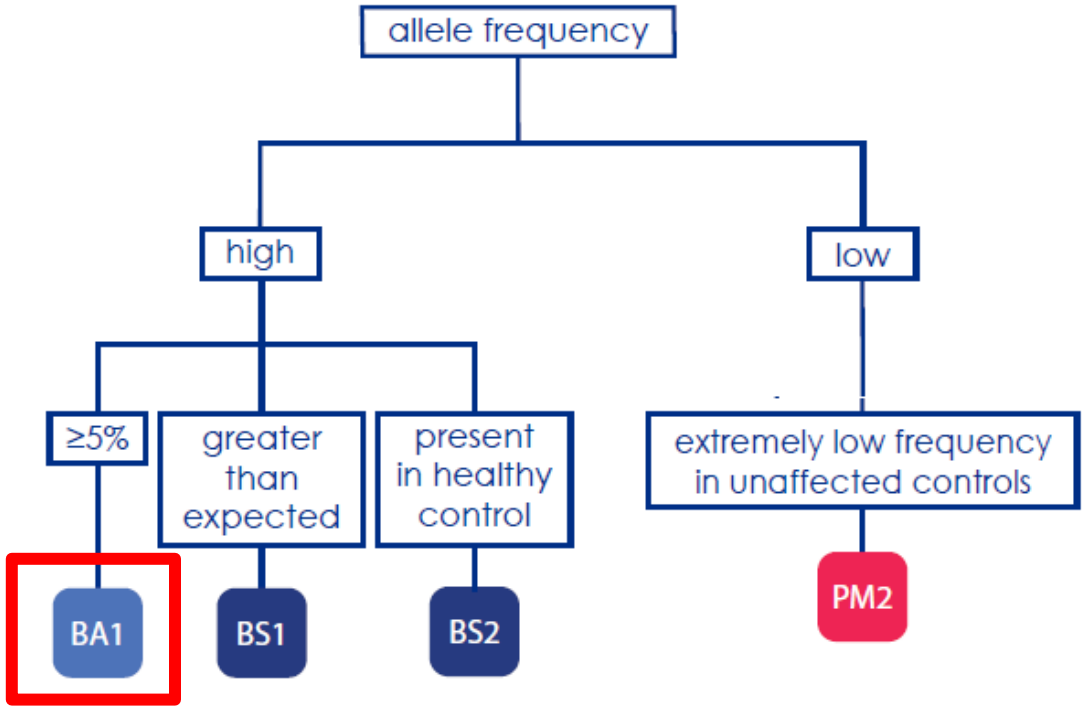
Genome Aggregation Database



https://doi.org/10.3390/genes10040275

Keep in mind if the database correctly assess the population of the proband !

	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	



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

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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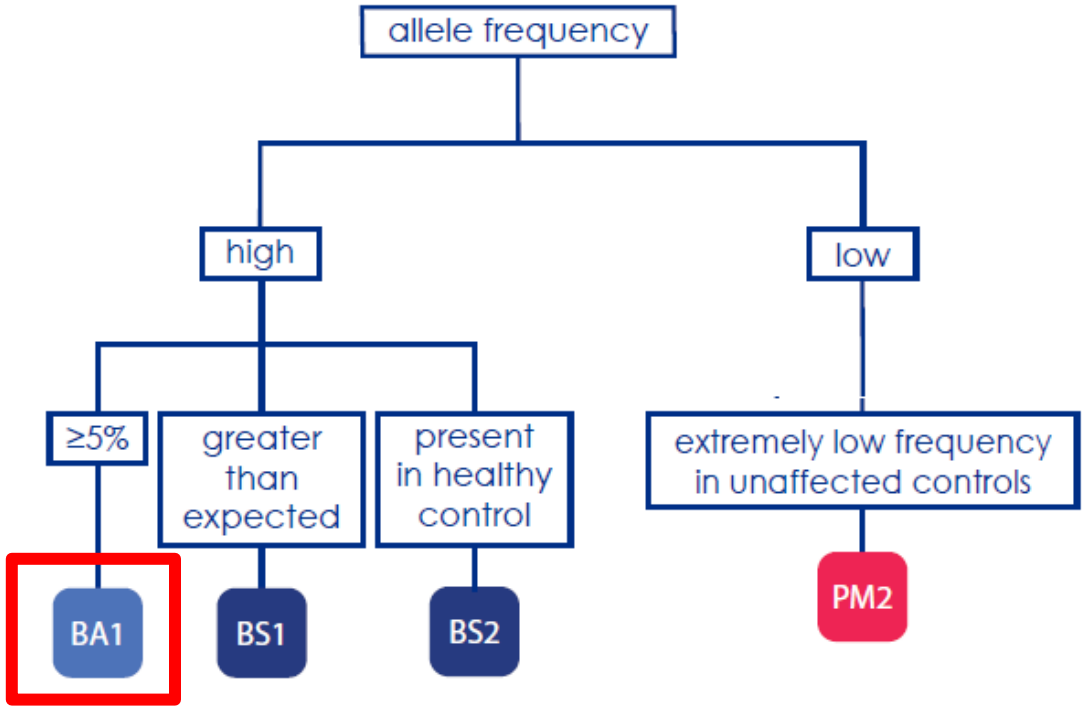


ClinGen
Clinical Genome Resource

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

List of nine variants for which there was some evidence of pathogenicity even though the MAF was high for these variants!

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

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

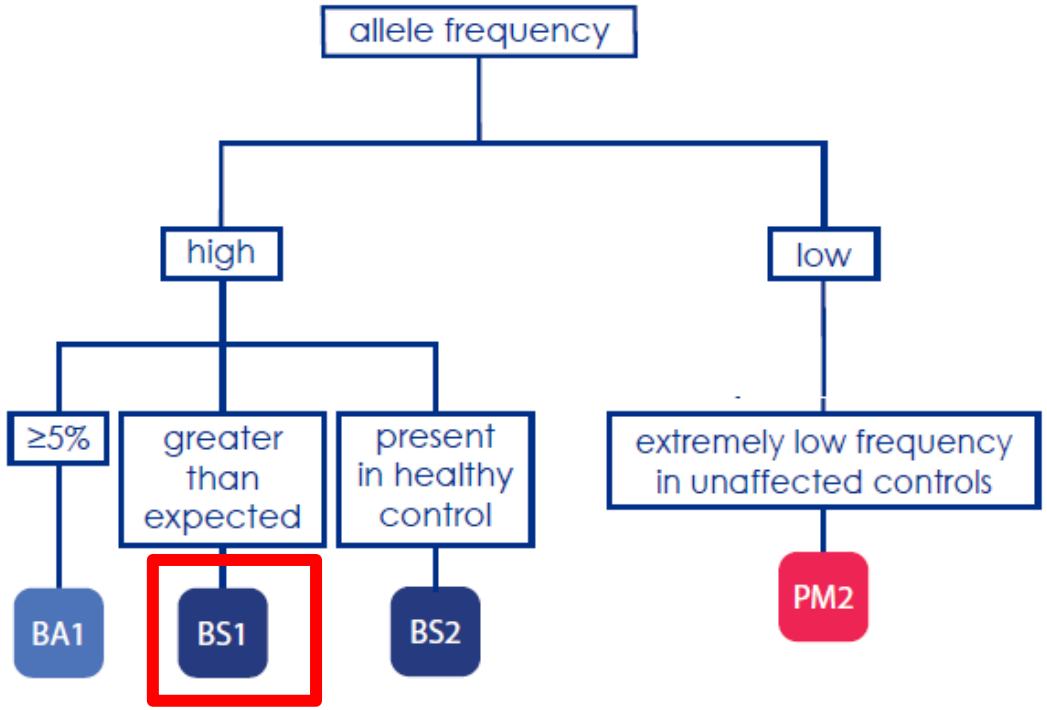
Detected at >5% MAF only in Finnish population (see text).

Genomic coordinates on build GRCh37

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

https://clinicalgenome.org/site/assets/files/3460/ba1_exception_list_07_30_2018.pdf

	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	



BS1 Allele frequency is greater than expected for disorder (see **Table 6**)

What `greater than expected` means?

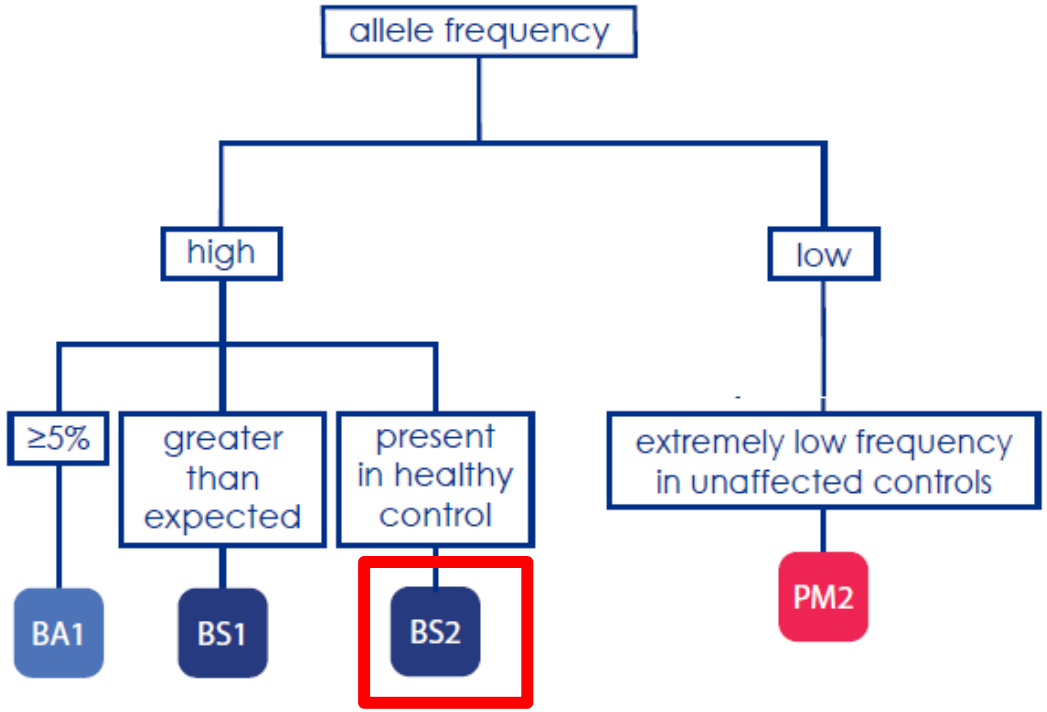
Different population frequency thresholds

Comparison of population frequency thresholds from ClinGen Variant Curation Expert Panels.

	Criteria	Prevalence	Heterogeneity	Penetrance	Threshold
Cardiomyopathy (AD)	**BA1**	1:200	10.60% [L]	30%	≥0.001 (0.1%)
	BS1		2% [A]		≥0.0002 (0.02%)
	PM2	1:500		50%	<0.00004 (0.004%)
RASopathy (AD)	**BA1**	1:2500	100%	40%	≥0.0005 (0.05%)
	BS1		50% [L]		≥0.00025 (0.025%)
	PM2	-	-	-	Absent [R]
***CDH1* (AD)**	**BA1**	1:800	100%	30%	≥0.002 (0.2%)
	BS1	1:1250			≥0.001 (0.1%)
	PM2	-	-	-	<0.00001 (0.001%) [R]
Hearing Loss (AD)	**BA1**	1:30	5% [L/A]	80%	≥0.001 (0.1%)
	BS1	1:150			≥0.0002 (0.02%)
	PM2	-	-	-	<0.00002 (0.002%) [M]
Hearing Loss (AR)	**BA1**	1:200	7.2% [A]	100%	≥0.005 (0.5%)
	BS1		4.4% [A]		≥0.003 (0.3%)
	PM2	-	-	-	<0.00007 (0.007%) [M]
***PAH* (AR)**	**BA1**	1:5000	90% [L]	80%	≥0.015 (1.5%)
	BS1		2% [A]		≥0.002 (0.2%)
	PM2	-	-	-	<0.0002 (0.02%) [M]
***PTEN** (AD)**	**BA1**	-	-	-	≥0.01 (1%)
	BS1	-	-	-	≥0.001 (0.1%)
	PM2	-	-	-	<0.00001 (0.001%) [R]

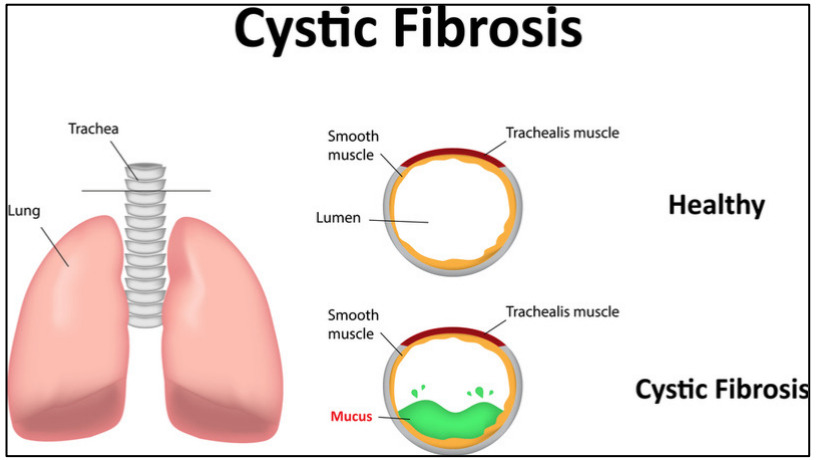
PMID: 31479589

	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	



BS2 Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age

CFTR - c.-8G>C



https://medlineplus.gov/genetics/condition/cystic-fibrosis/

	Exomes	Genomes	Total
Filters	Pass	Pass	
Allele Count	11486	1727	13213
Allele Number	251068	31346	282414
Allele Frequency	0.04575	0.05509	0.04679
Popmax Filtering AF (95% confidence)	0.05902	0.06839	
Number of homozygotes	364	64	428
Mean depth of coverage	97.2	29.1	

External Resources

- dbSNP (rs1800501)
- UCSC
- ClinVar (93148)
- ClinGen Allele Registry (CA146694)

Feedback

Report an issue with this variant

Population Frequencies

Population	Allele Count	Allele Number	Number of Homozygotes	Allele Frequency
European (Finnish)	2385	25068	106	0.09514
East Asian	1248	19910	44	0.06268
European (non-Finnish)	7524	128920	238	0.05836
Other	384	7218	17	0.05320
Ashkenazi Jewish	338	10364	6	0.03261
Latino/Admixed American	652	35416	6	0.01841
South Asian	456	30606	10	0.01490
African/African American	226	24912	1	0.009072
XX	6119	129256	203	0.04734
XY	7094	153158	225	0.04632
Total	**13213**	**282414**	**428**	**0.04679**

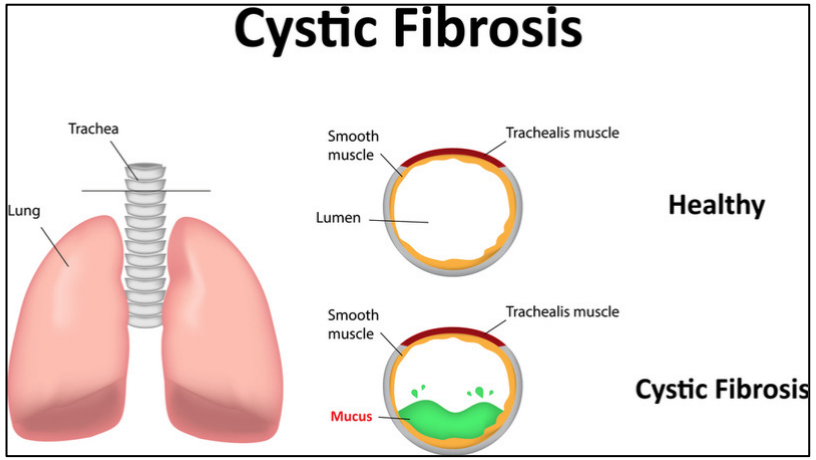
* Allele frequencies for some sub-continental populations were not computed for genome samples.

Include: Exomes Genomes

Related Variants

https://gnomad.broadinstitute.org/

CFTR - c.-8G>C



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Include: Exomes Genomes

Related Variants

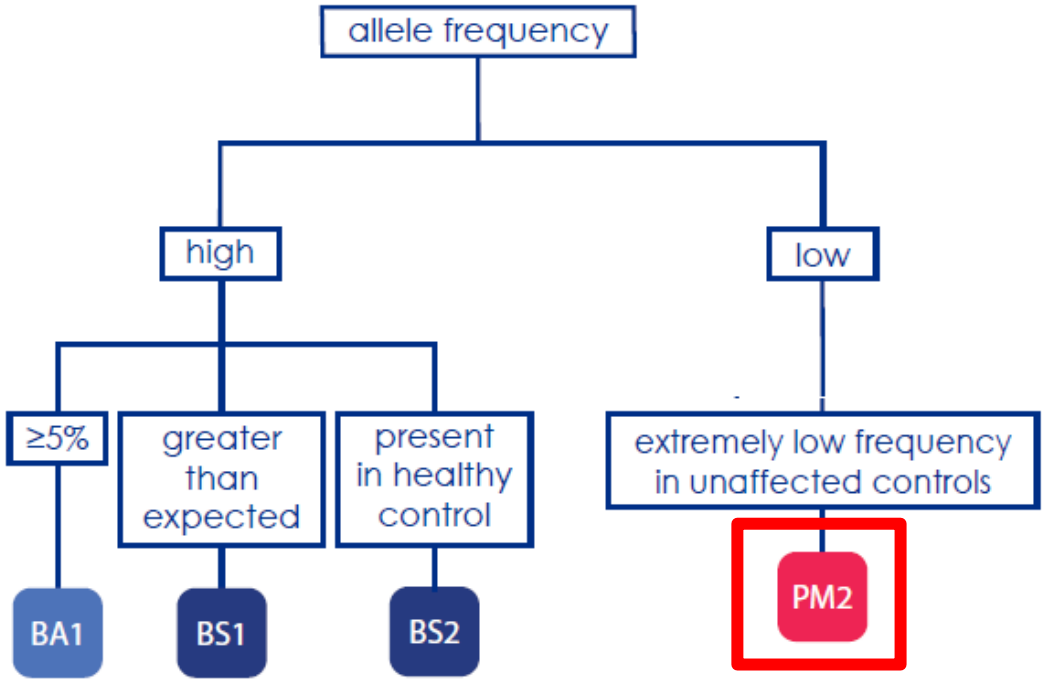
https://gnomad.broadinstitute.org/

https://www.fitnessforhealth.org/healthy-living-for-older-adults-tips-and-resources/

Observed in healthy adult individuals

BS2

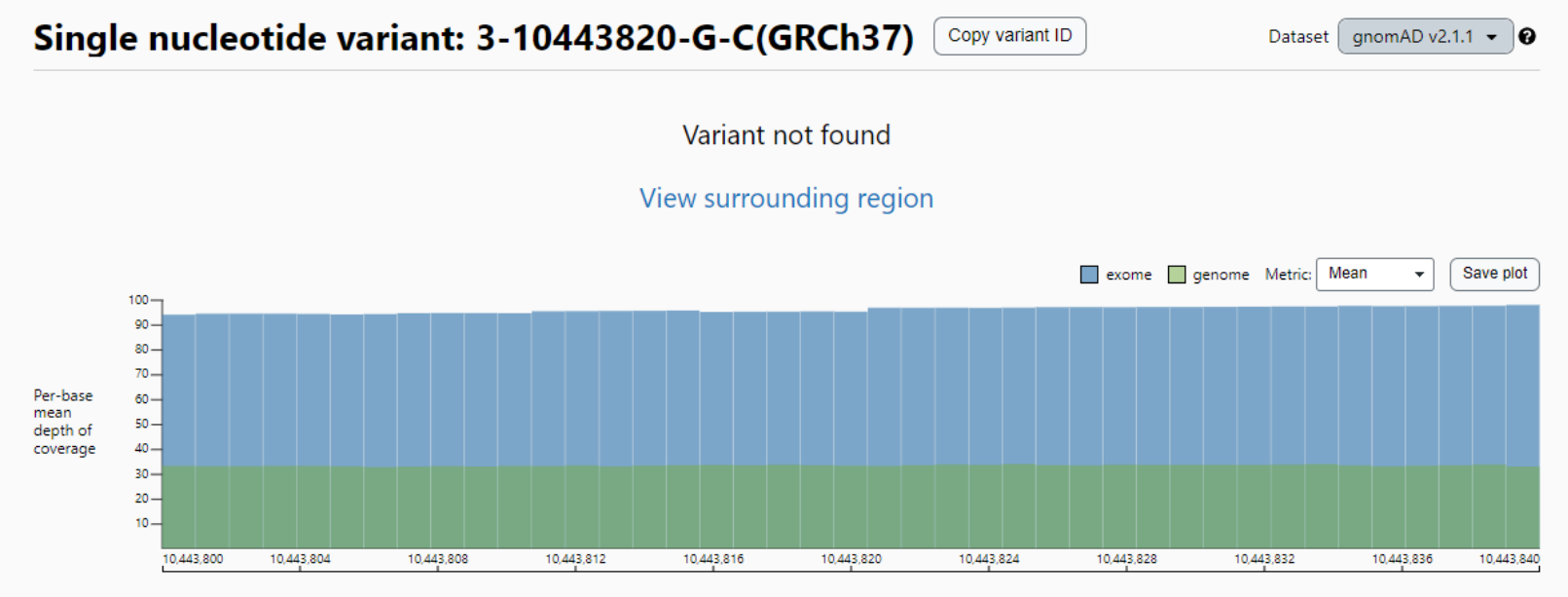
	Benign			Pathogenic		
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	



PM2 Absent from controls (or at extremely low frequency if recessive) (Table 6) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium

Caveat: Population data for insertions/deletions may be poorly called by next-generation sequencing.

ATP2B2 HET c.610C>G



https://gnomad.broadinstitute.org/

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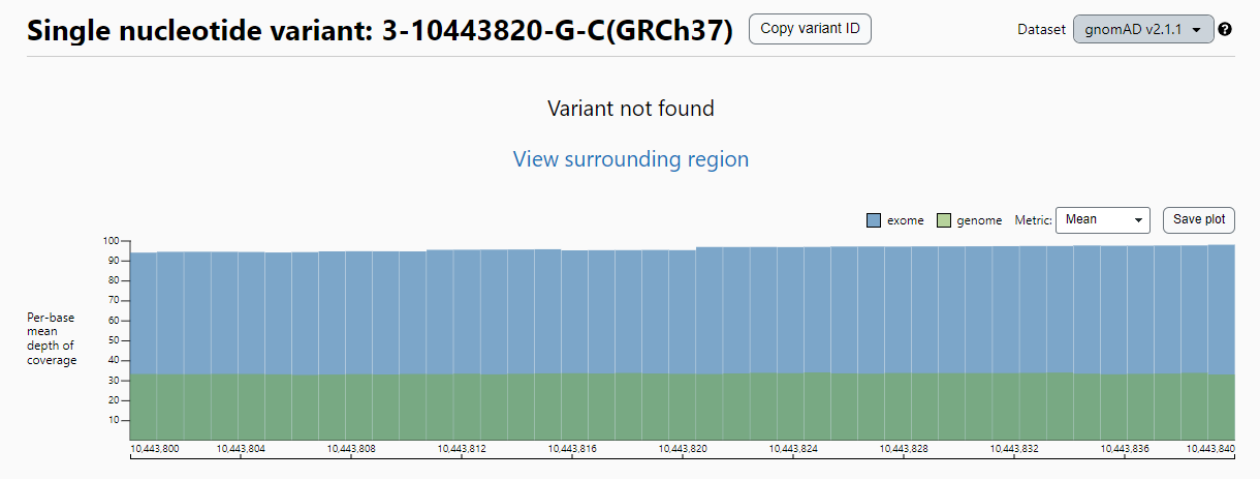
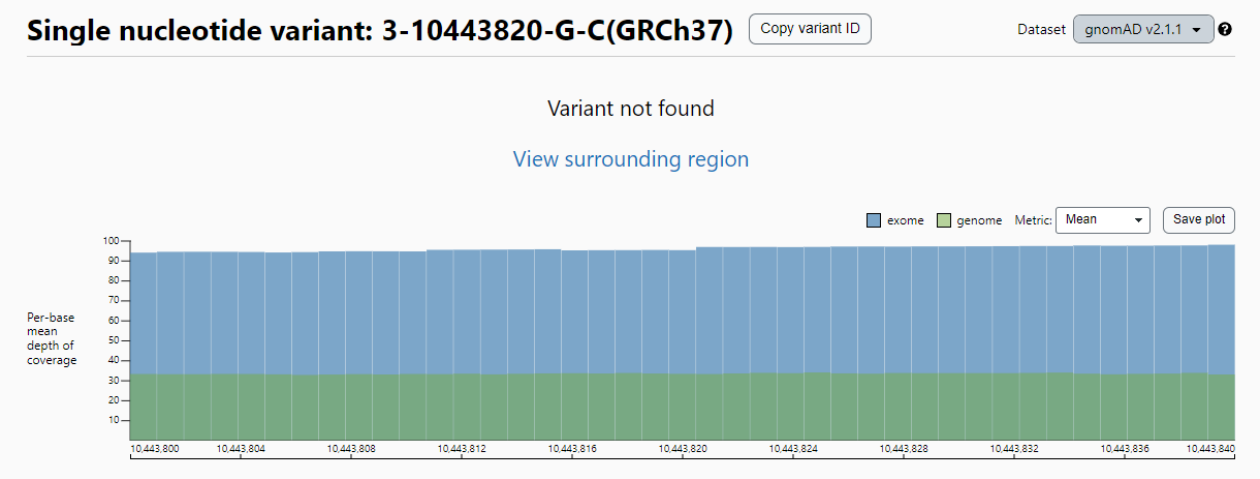


https://gnomad.broadinstitute.org/

`M` as moderate level of evidence?

ATP2B2 HET c.610C>G



https://gnomad.broadinstitute.org/

PM2

`M` as moderate level of evidence?

ClinGen Sequence Variant Interpretation Recommendation for PM2 - Version 1.0
Working Group Page: https://clinicalgenome.org/working-groups/sequence-variant-interpretation/
Date Approved: September 4, 2020

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).

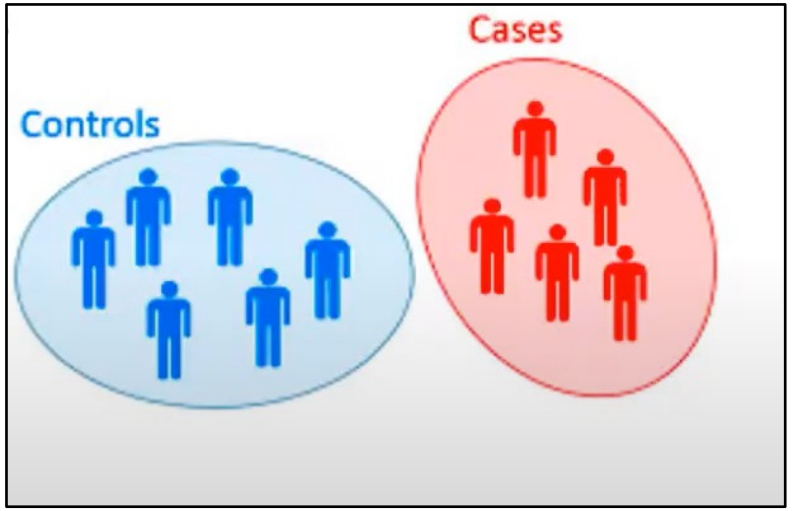
Now PM2_Suppoting

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

	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 is increased in affected individuals is significantly increased compared with the prevalence in controls.
- Relative risk (RR) or odds ratio (OR) in a case-control study is >5.0, and the confidence interval around the estimate of relative risk or OR does not include 1.0.

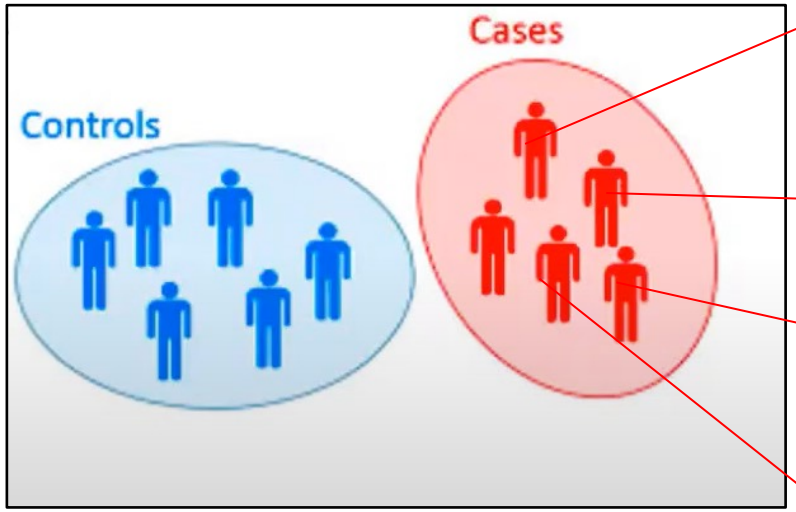


What if some genetic diseases have a very low prevalence (1: 1,000,000)?

	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	

The prior observation of the variant in multiple unrelated patients with the same phenotype

This approach requires PM2 to be applicable → Absent in controls



Dr. Klee et al. 2015

Rory et al. 2022

Filippo et al. 2008

Dr. Schimmenti et al. 2018

May be used as "moderate" or lower level of evidence.

Examples of Case Prevalence or Previously Reported Cases (PS4)
High Prevalence or Multiple Unrelated Patients Observed with Variant and Phenotype

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

1- Population Data

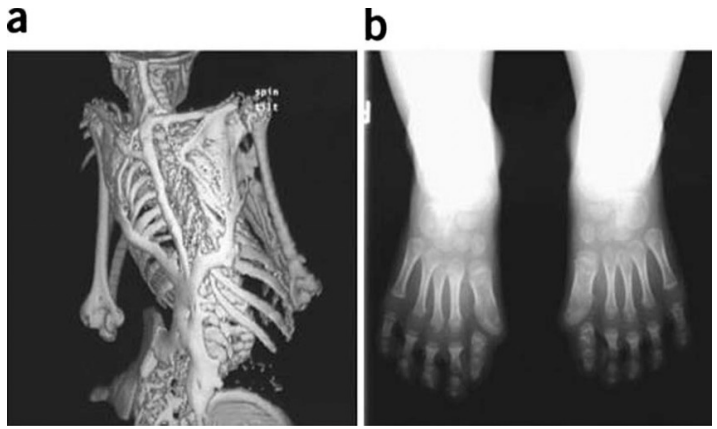
2- Computational and Predictive Data

Before we continue…

Does it explain the phenotype?

Good phenotype overlap?

Does the type of mutation make sense for the gene/phenotype?



https://doi.org/10.1186/1750-1172-6-80

+ Variant in *ACVR1*

Does it explain the phenotype?

Good phenotype overlap?

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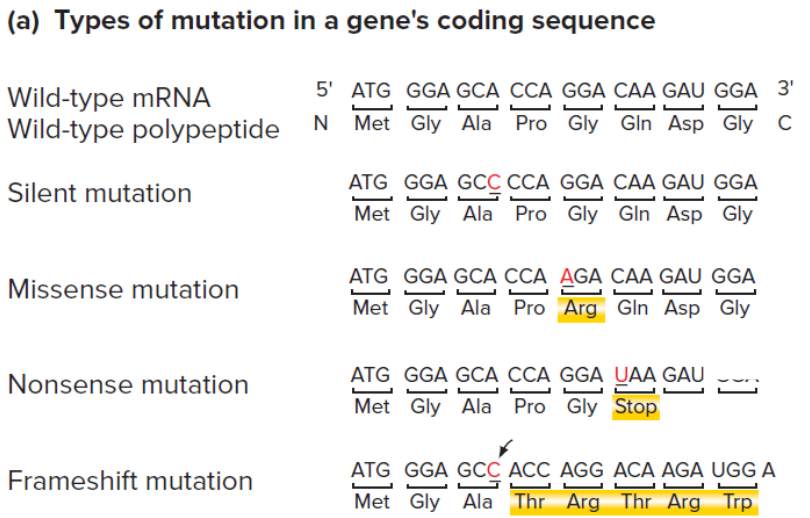

DOI:10.1002/mma.4764

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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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			

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Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

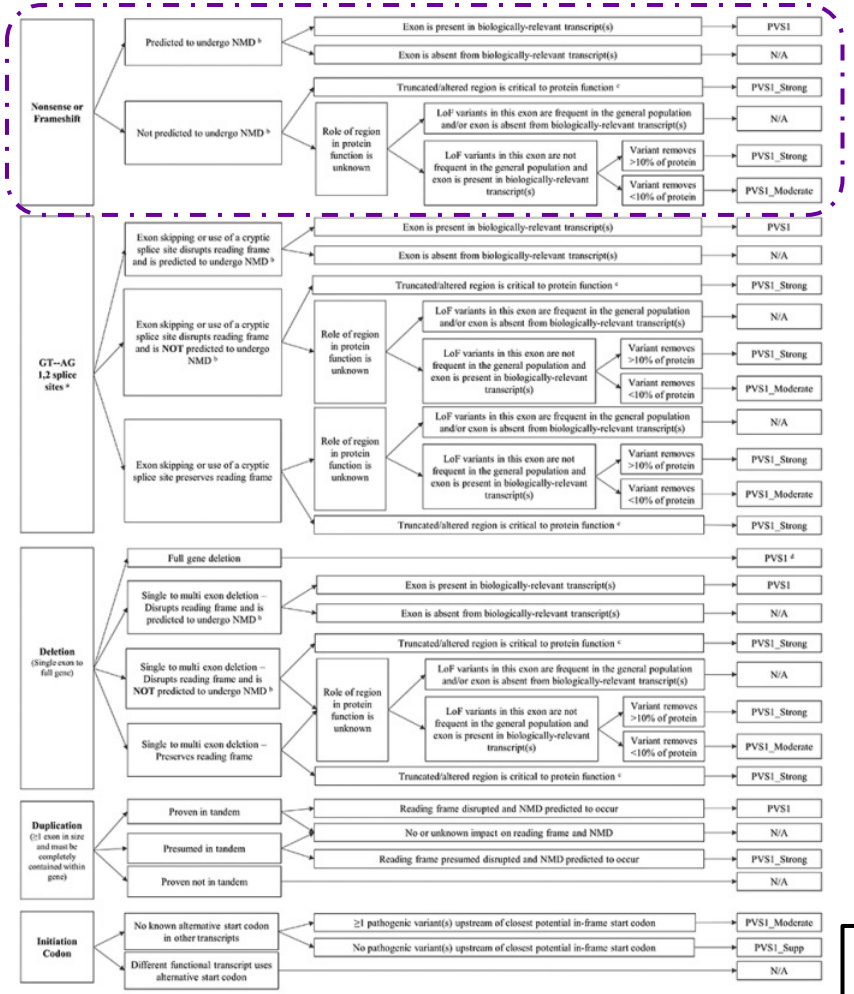
- Focus on the "Pathogenic criteria"

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

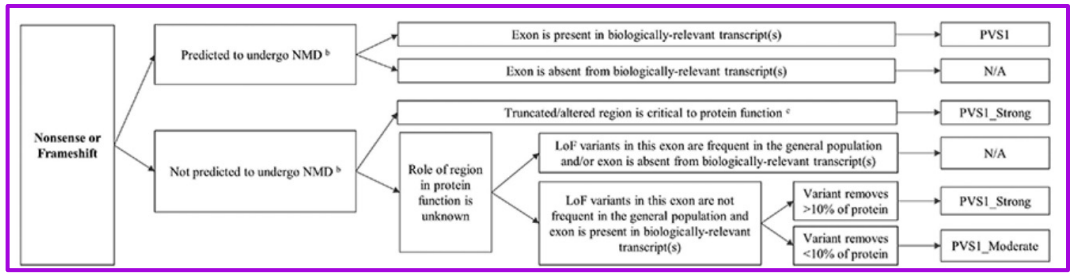
Loss of Function Criteria (PVS1) (only "very strong" level of evidence)

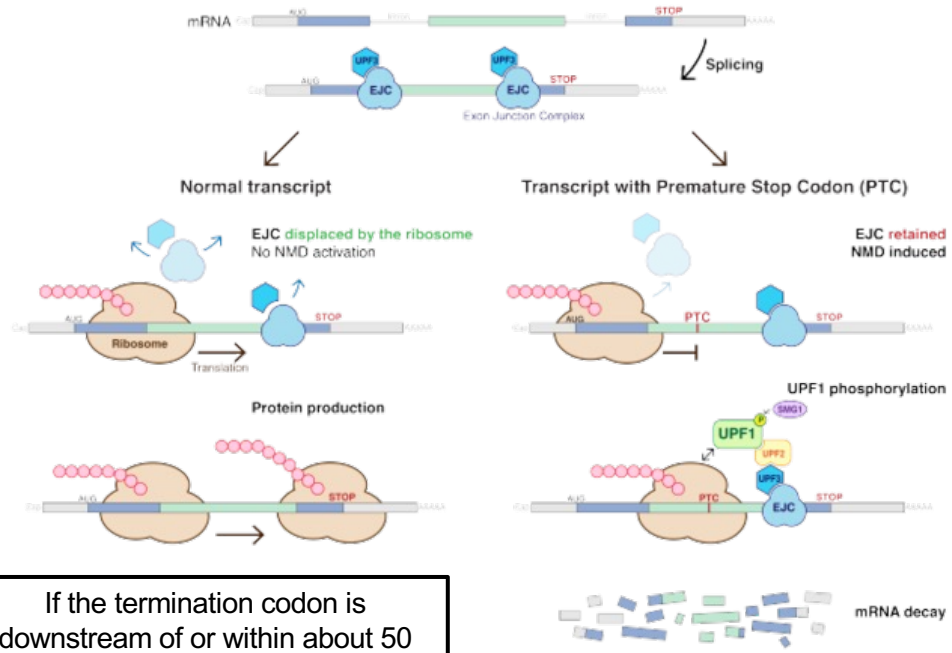
- Null variant in a gene where loss of function (LoF) is a known mechanism of disease.

PVS1



https://www.clinicalgenome.org/site/assets/files/3677/clingen_variant-curation_sopv1.pdf

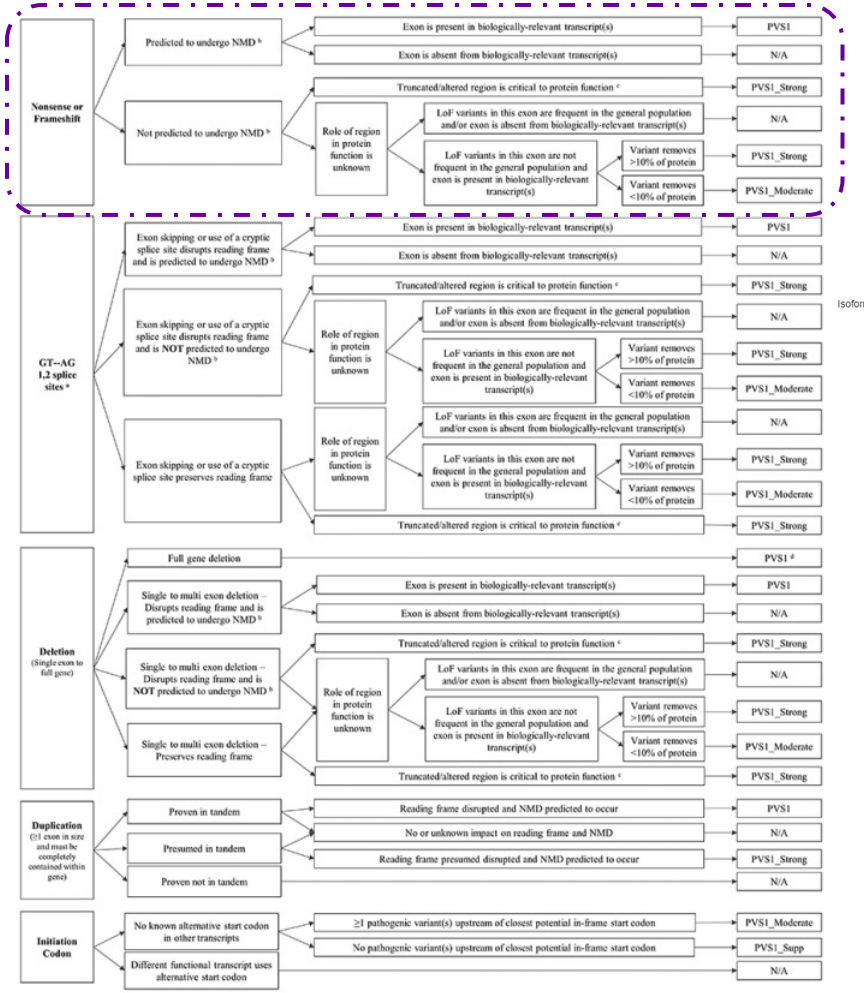




https://en.wikipedia.org/wiki/Nonsense-mediated_decay

If the termination codon is downstream of or within about 50 nucleotides of the final exon-junction complex then the transcript is translated normally.

PVS1



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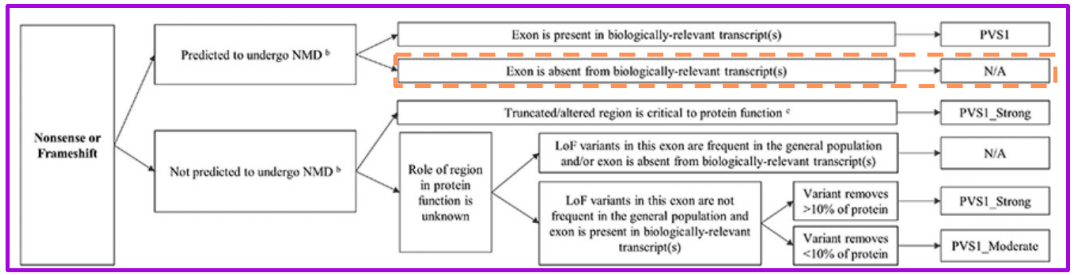
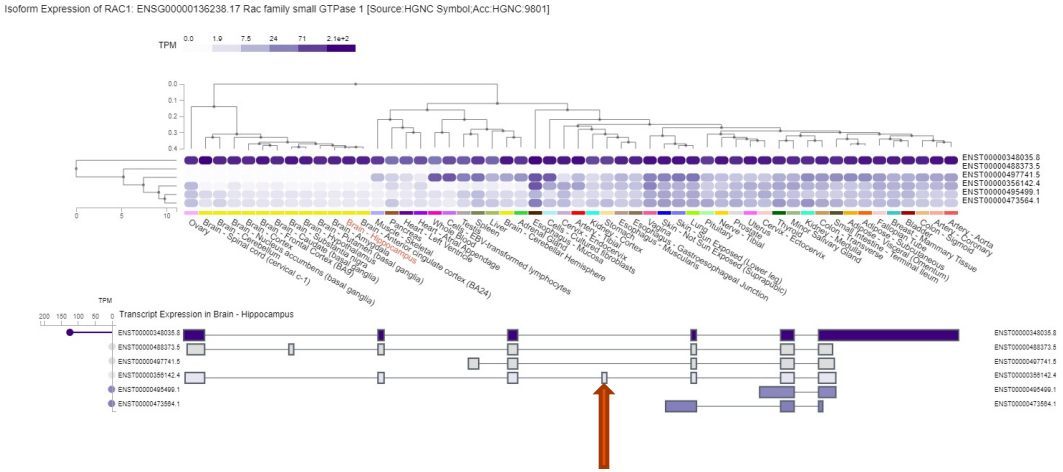
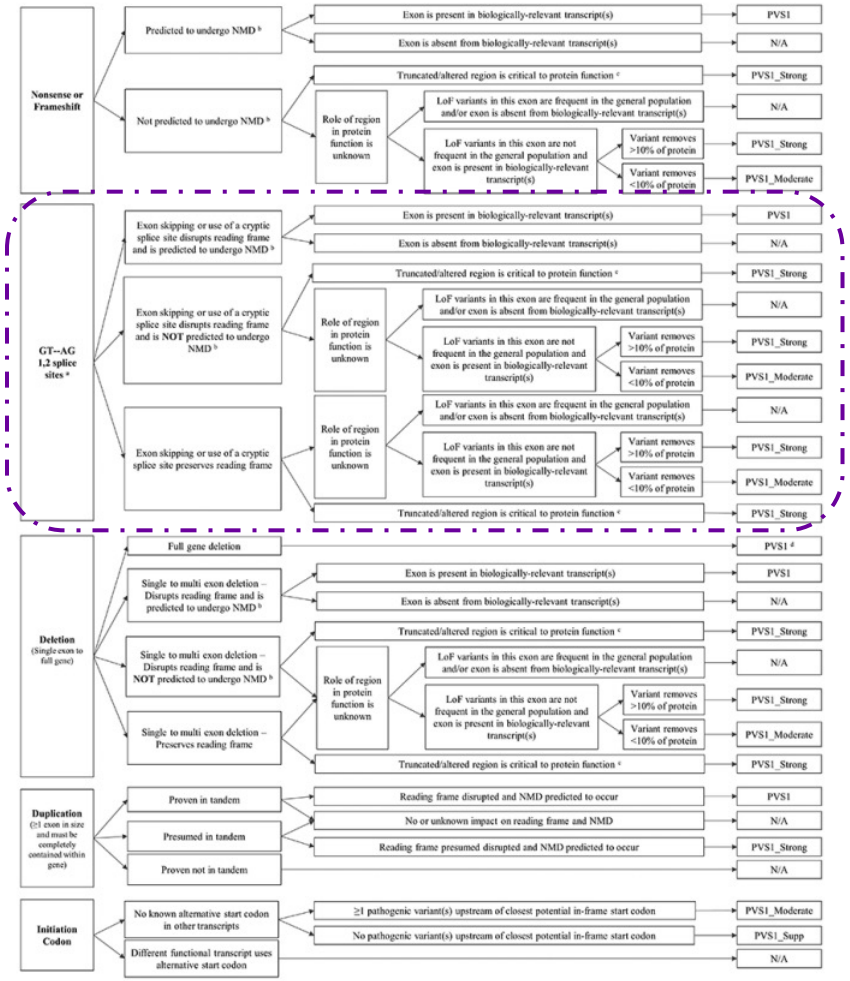




https://gtexportal.org/home/

PVS1



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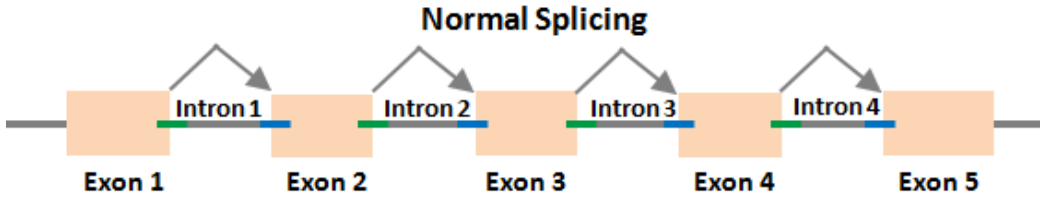

PVS1

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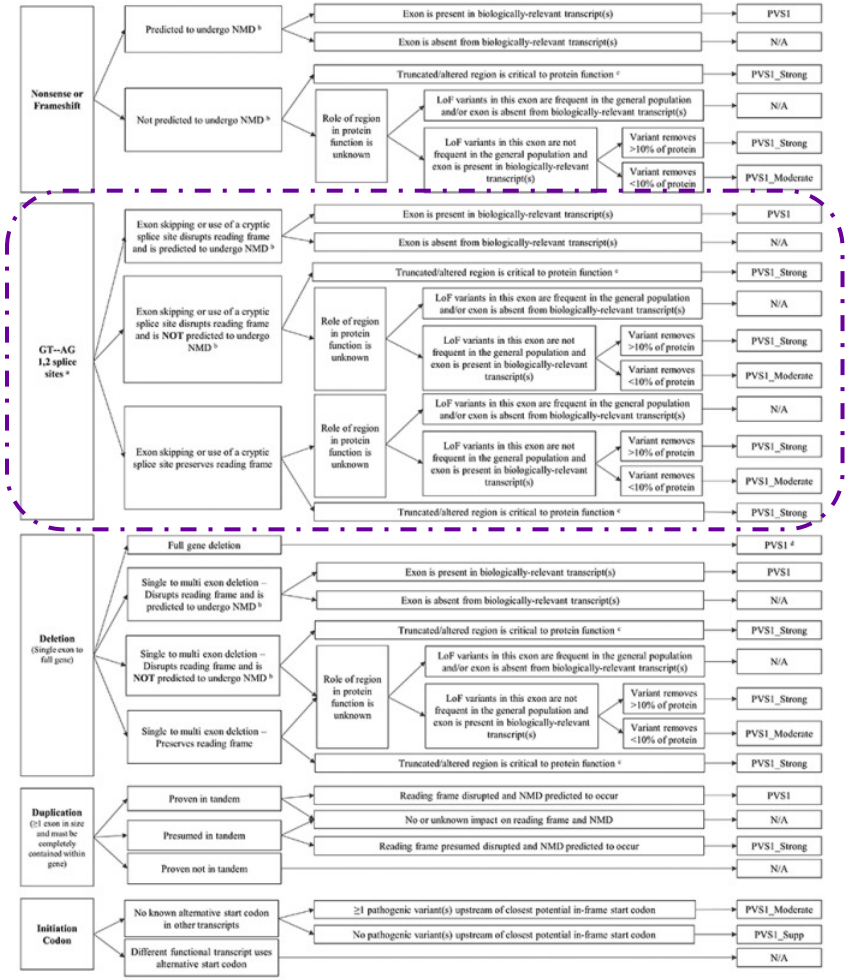
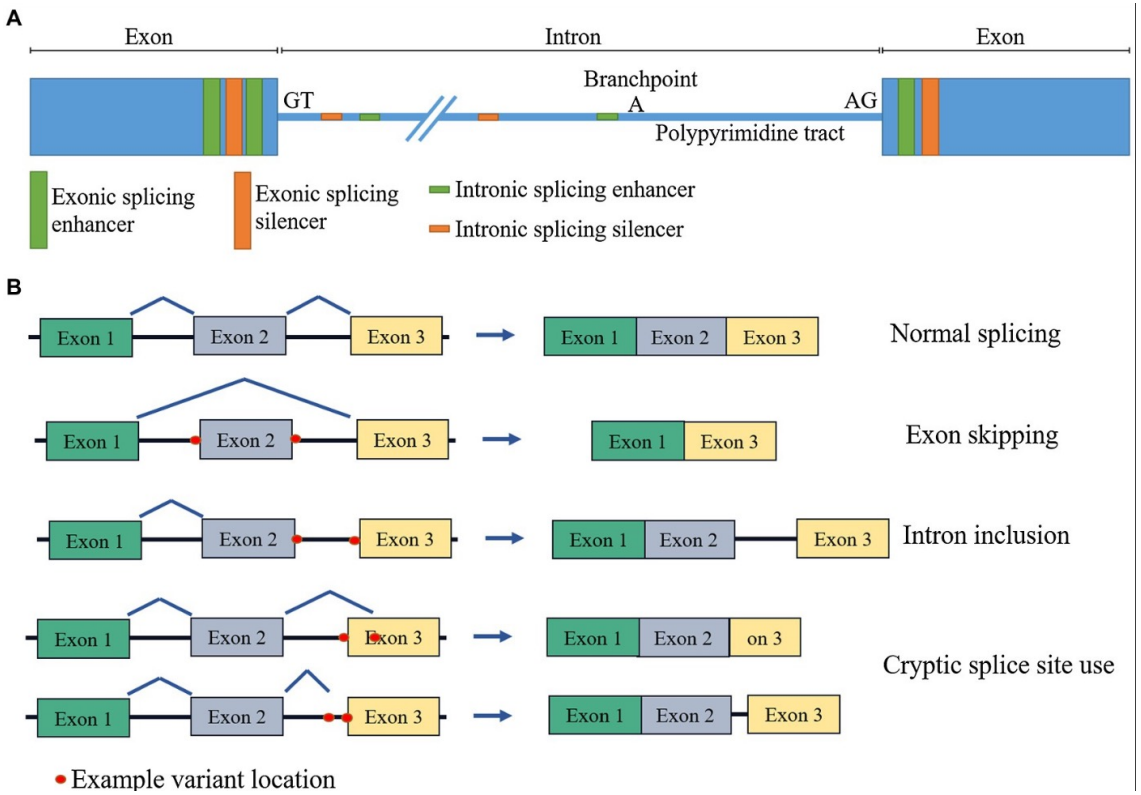
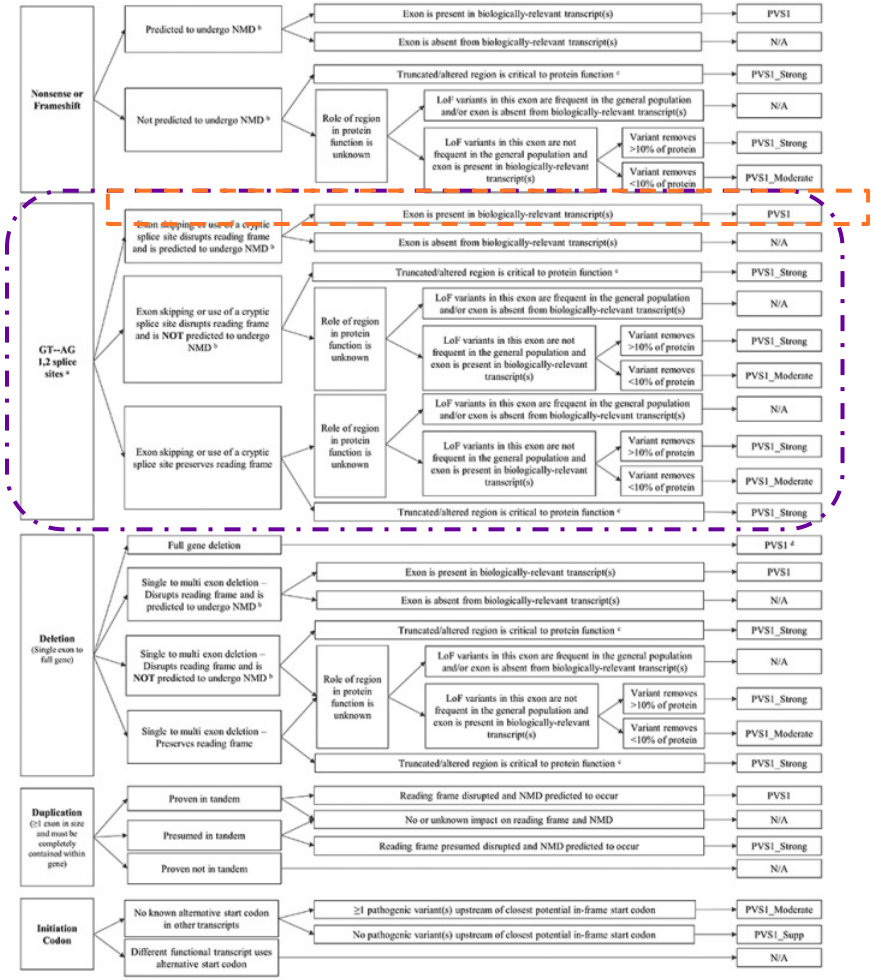




https://doi.org/10.3389/fgene.2021.689892

PVS1



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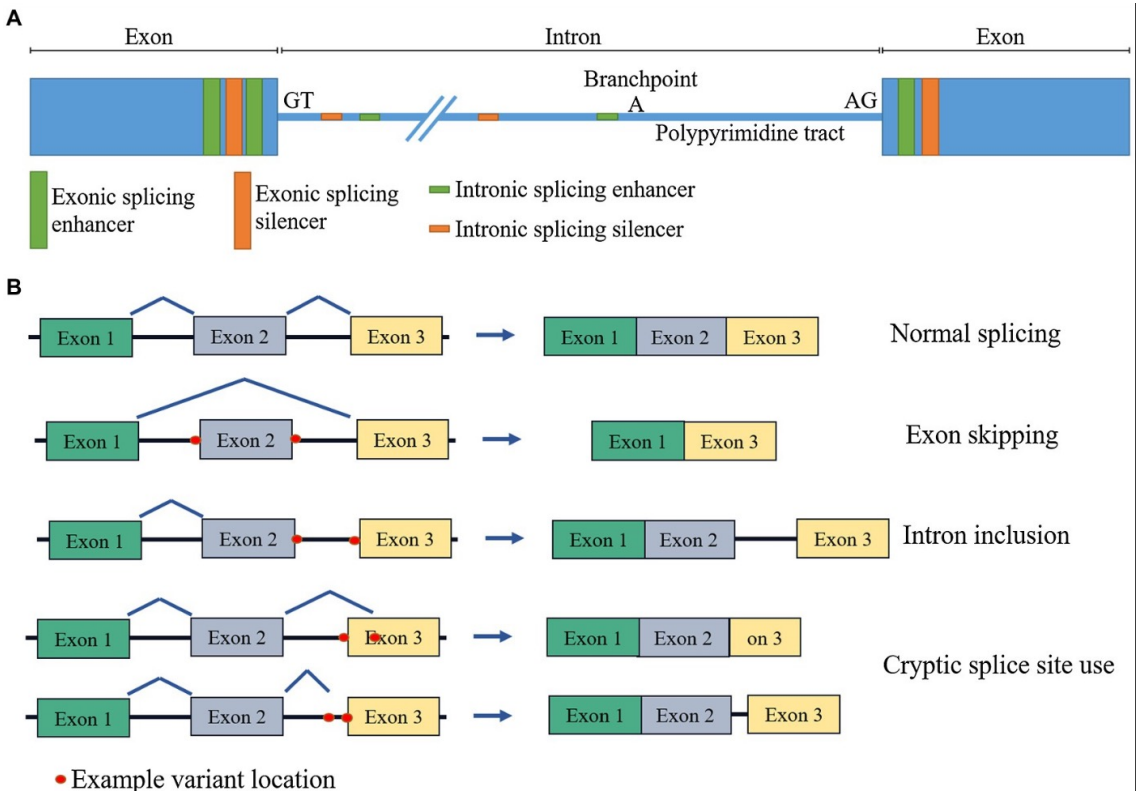

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PVS1- How to investigate if LOF is a `known mechanism of disease`

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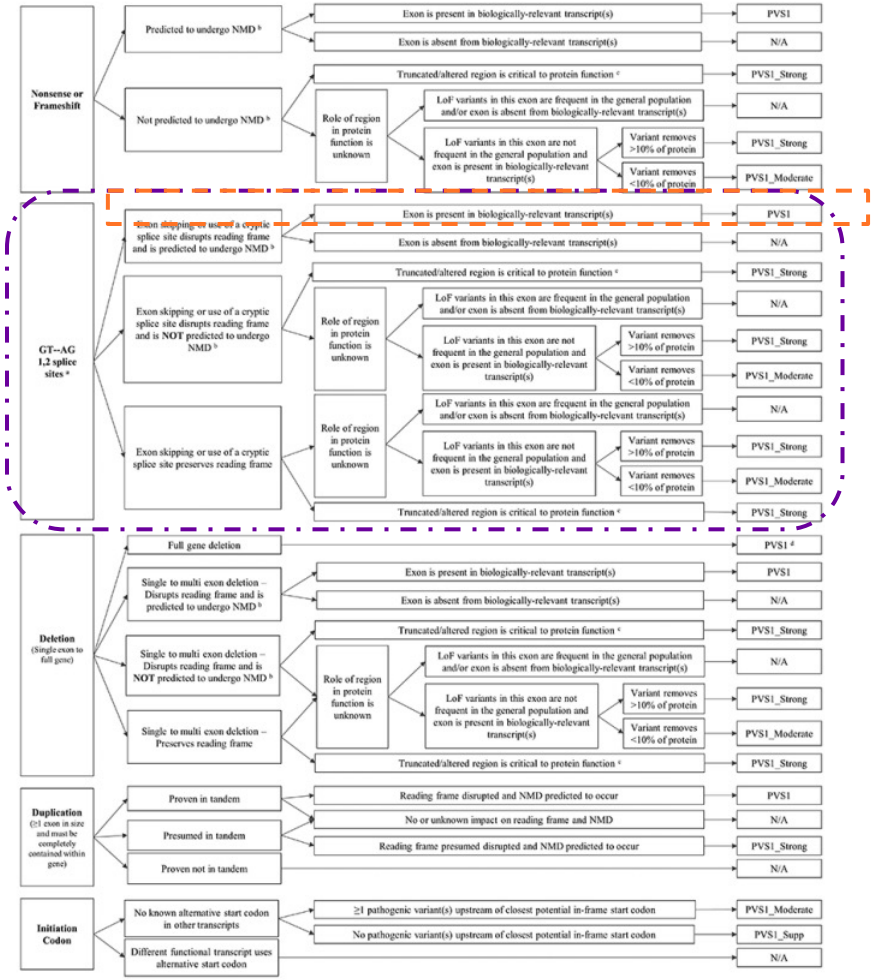
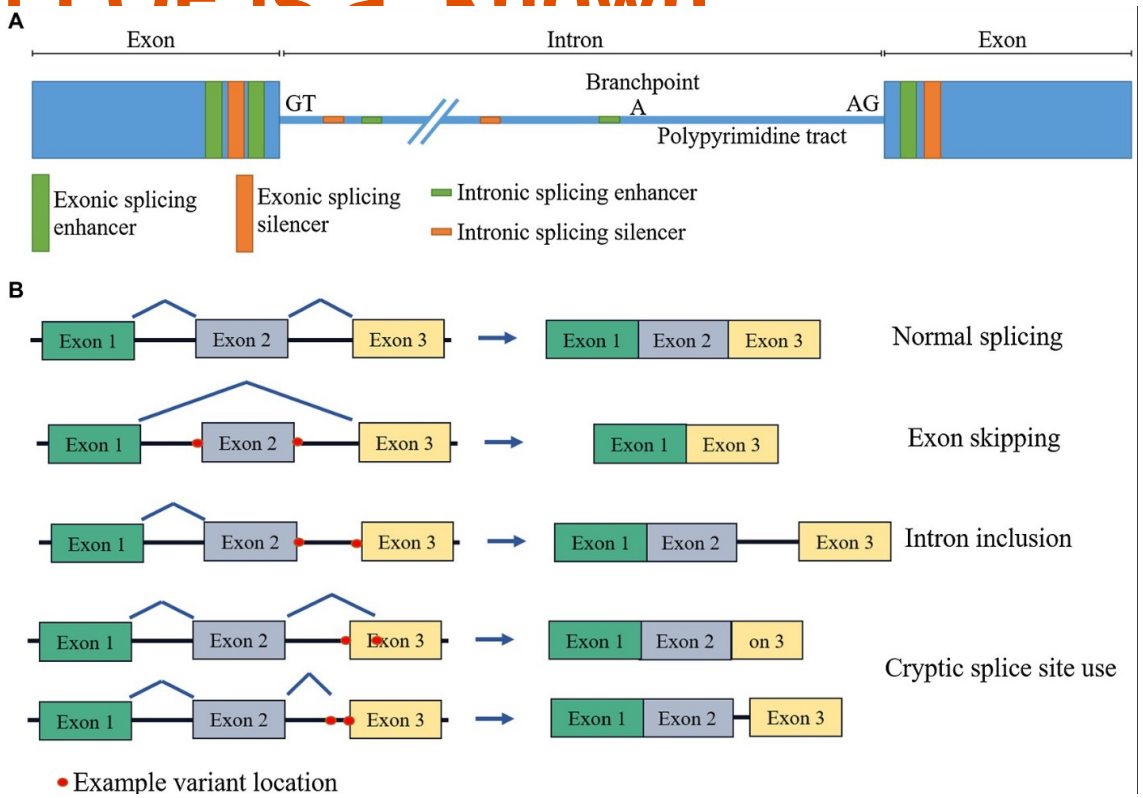


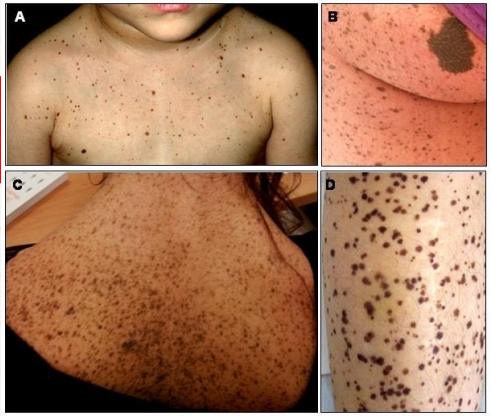


https://doi.org/10.3389/fgene.2021.689892

Table 3.

Missense and LoF annotations and curations per gene from ClinGen Variant Curation Expert Panels

Gene	Disease Area (MOI)	HI Score	gnomAD LoF *oe* metric (90% CI)	PVS1?	Missense Z score (ExAC / gnomAD)	PP2?
MYH7	Cardio (AD)	0	0.45 (0.35–0.57)	Yes (Mod)	6.54 / 3.93	No
BRAF		1	0.1 (0.05–0.21)	No	3.99 / 3.72	Yes
HRAS		0	0.36 (0.16–0.93)	No	2.69 / 1.51	Yes
KRAS		0	0.63 (0.34–1.24)	No	1.36 / 2.32	Yes
MAP2K1		0	0.15 (0.07–0.38)	No	3.43 / 3.11	Yes
MAP2K2	RAS (AD)	1	0.1 (0.04–0.33)	No	1.48 / 1.87	Yes
PTPN11		3	0.03 (0.01–0.14)	No	3.43 / 3.13	Yes
RAF1		0	0.19 (0.11–0.35)	No	2.82 / 2.46	Yes
SHOC2		-	0 (0.00–0.14)	No	2.57 / 2.97	Yes
SOS1		0	0.07 (0.03–0.14)	No	2.18 / 3.05	Yes
PTEN	PHTS (AD)	3	0.24 (0.13–0.51)	Yes	3.71 / 3.49	Yes
CDH1	HDGC (AD)	3	0.25 (0.15–0.43)	Yes	0.81 / 0.71	No
PAH	PKU (AR)	30	1.12 (0.84–1.50)	Yes	−1.54 / −0.65	No
CDH23	HL (AR)	30	0.38 (0.26–0.57)	Yes	−0.24 / 0.71	No
GJB2		-	2.62 (1.39–1.98)	Yes	−1.07 / 1.17	No
MYO7A		-	0.7 (0.58–0.85)	Yes	−1.44 / 1.07	No
SLC26A4		-	0.89 (0.68–1.18)	Yes	−3.23 / −2.01	No
TECTA		30	0.45 (0.35–0.58)	Yes	2.3 / 1.61	No
USH2A		30	0.76 (0.67–0.86)	Yes	−5.12 / −2.47	No
COCH	HL (AD)	-	0.59 (0.40–0.91)	No	0.34 / 0.68	No
KCNQ4		-	0.22 (0.12–0.41)	Yes	2.73 / 1.83	No
MYO6		-	0.3 (0.22–0.42)	Yes	1.02 / 1.39	No
TECTA		30	0.45 (0.35–0.58)	No	2.3 / 1.61	No



https://doi.org/10.1186/1750-1172-3-13

doi:10.1002/cphg.93

PVS1- How to investigate if LOF is a `known mechanism of disease`

*176876
Table of Contents

ICD+

* 176876

PROTEIN-TYROSINE PHOSPHATASE, NONRECEPTOR-TYPE, 11; PTPN11

Alternative titles; symbols

PROTEIN-TYROSINE PHOSPHATASE 2C; PTP2C
TYROSINE PHOSPHATASE SHP2; SHP2

https://www.omim.org/

HGNC Approved Gene Symbol: PTPN11

Cytogenetic location: 12q24.13 Genomic coordinates (GRCh38): 12:112,418,946-112,509,917 (from NCBI)

Gene-Phenotype Relationships

Location	Phenotype Clinical Synopses	Phenotype MIM number	Inheritance	Phenotype mapping key
12q24.13	LEOPARD syndrome 1	151100	AD	3
	Leukemia, juvenile myelomonocytic, somatic	607785		3
	Metachondromatosis	156250	AD	3
	Noonan syndrome 1	163950	AD	3

OMIM - Online Mendelian Inheritance in Man®

ICD+

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PROTEIN-TYROSINE PHOSPHATASE, NONRECEPTOR-TYPE, 11; PTPN11

Allelic Variants (36 Selected Examples) :

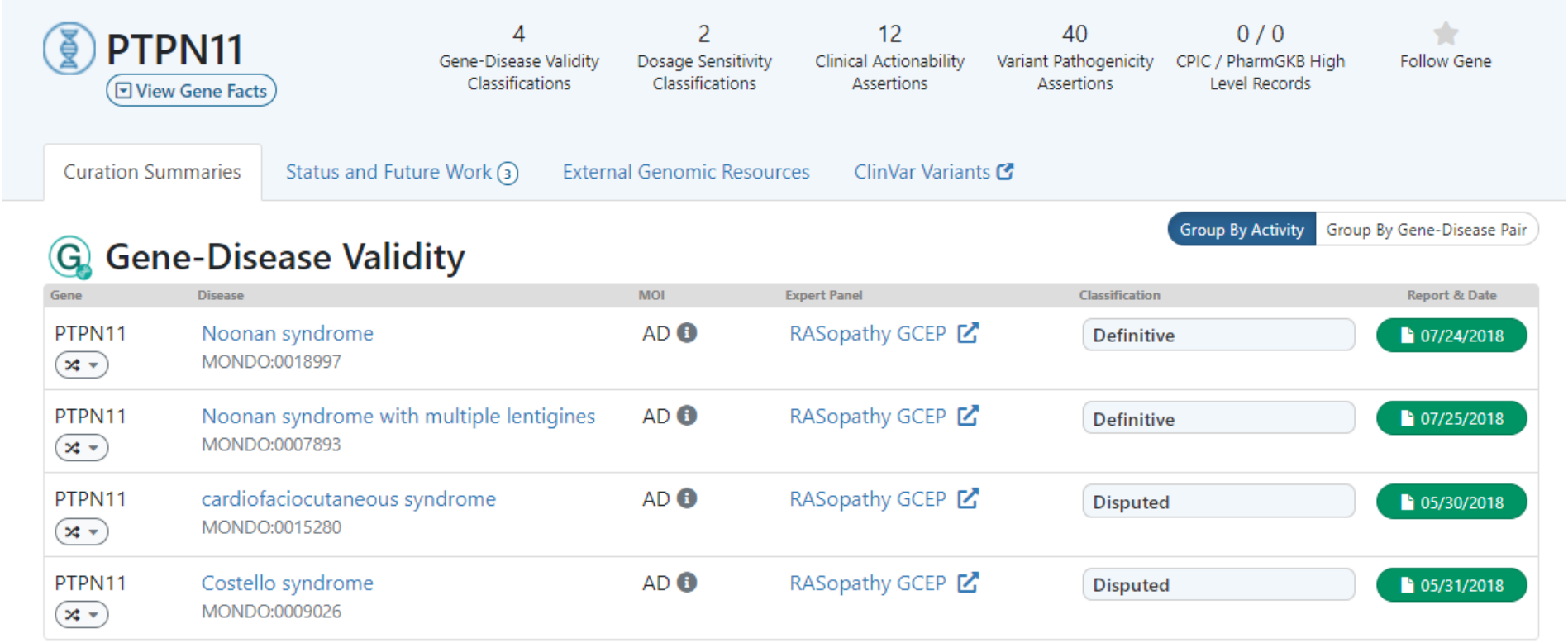
All ClinVar Variants

Number	Phenotype	Mutation	SNP	gnomAD	ClinVar
.0001	NOONAN SYNDROME 1	PTPN11, ALA72SER	rs121918453	-	RCV000014252...
.0002	NOONAN SYNDROME 1	PTPN11, ALA72GLY	rs121918454	-	RCV000014253...
.0003	NOONAN SYNDROME 1	PTPN11, ASN308ASP	rs28933386	rs28933386	RCV000014254...
.0004	NOONAN SYNDROME 1	PTPN11, ASN308SER	rs121918455	-	RCV000014255...
.0005	LEOPARD SYNDROME 1	PTPN11, TYR279CYS	rs121918456	-	RCV000030620...
.0006	LEOPARD SYNDROME 1	PTPN11, THR468MET	rs121918457	rs121918457	RCV000033533...
.0007	NOONAN SYNDROME 1	PTPN11, SER502THR	rs121918458	-	RCV000014260...
.0008	NOONAN SYNDROME 1	PTPN11, TYR63CYS	rs121918459	rs121918459	RCV000014261...
.0009	NOONAN SYNDROME 1	PTPN11, TYR62ASP	rs121918460	rs121918460	RCV000014257...
.0010	NOONAN SYNDROME 1	PTPN11, ASP61GLY	rs121918461	-	RCV000014258...
.0011	NOONAN SYNDROME 1	PTPN11, THR73ILE	rs121918462	-	RCV000014262...
.0012	NOONAN SYNDROME 1	PTPN11, PHE285SER	rs121918463	-	RCV000014263...
.0013	MOVED TO 176876.0011	-	-	-	-
.0014	LEUKEMIA, JUVENILE MYELOMONOCYTIC, SOMATIC	PTPN11, GLU76LYS	rs121918464	-	RCV000014264...
.0015	LEUKEMIA, JUVENILE MYELOMONOCYTIC, SOMATIC	PTPN11, GLU76VAL	rs121918465	-	RCV000014265...
.0016	LEUKEMIA, JUVENILE MYELOMONOCYTIC, SOMATIC	PTPN11, GLU76GLY			
.0017	LEUKEMIA, JUVENILE MYELOMONOCYTIC, SOMATIC	PTPN11, GLU76ALA			
.0018	NOONAN SYNDROME	PTPN11, GLN79ARG			
.0019	NOONAN SYNDROME	PTPN11, THR411MET			
.0020	LEOPARD SYNDROME 1	PTPN11, ALA461THR			
.0021	LEOPARD SYNDROME 1	PTPN11, GLY464ALA			
.0022	LEOPARD SYNDROME 1	PTPN11, GLN510PRO			
.0023	NOONAN SYNDROME	PTPN11, GLN510ARG	rs121918470	rs121918470	RCV000014273...

https://www.omim.org/

- Most of the variants associated with the phenotype are Missense

ClinGen



PTPN11

View Gene Facts

4	2	12	40	0 / 0	
Gene-Disease Validity Classifications	Dosage Sensitivity Classifications	Clinical Actionability Assertions	Variant Pathogenicity Assertions	CPIC / PharmGKB High Level Records	Follow Gene

Curation Summaries | Status and Future Work (3) | External Genomic Resources | ClinVar Variants

Group By Activity | Group By Gene-Disease Pair

Gene-Disease Validity

Gene	Disease	MOI	Expert Panel	Classification	Report & Date
PTPN11	Noonan syndrome MONDO:0018997	AD	RASopathy GCEP	Definitive	07/24/2018
PTPN11	Noonan syndrome with multiple lentigines MONDO:0007893	AD	RASopathy GCEP	Definitive	07/25/2018
PTPN11	cardiofaciocutaneous syndrome MONDO:0015280	AD	RASopathy GCEP	Disputed	05/30/2018
PTPN11	Costello syndrome MONDO:0009026	AD	RASopathy GCEP	Disputed	05/31/2018

- Noonan syndrome is believed to be caused **by gain-of-function** defects in *PTPN11* (PMID:11992261), and LEOPARD syndrome is believed to be caused by **dominant-negative mechanisms** (PMID: 16358218). Evidence gathered for the haploinsufficiency rating for this gene is related to the metachondromatosis phenotype.

PMID: 25741868

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

PS1

PS1	Same amino acid change as a previously established pathogenic variant regardless of nucleotide change Example: Val->Leu caused by either G>C or G>T in the same codon

(b)

Second letter

First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G

https://rsscience.com/codon-chart/

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First letter	U	C	A	G	Third letter
U	UUU Phe UUC Phe	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr	UGU Cys UGC Cys	U
	UUA Leu UUG Leu		UAA Stop UAG Stop	UGA Stop UGG Trp	C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G

CUU → CUC

Both are Leucine

Caveat:	Beware of changes that impact splicing rather than at the amino acid/protein level

https://rsscience.com/codon-chart/

PMID: 25741868

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PM5	Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before Example: Arg156His is pathogenic; now you observe Arg156Cys

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(b) Second letter

	U	C	A	G	
U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G

First letter / Third letter

Leu257Pro - Pathogenic

CUU → CCU

https://rsscience.com/codon-chart/

PMID: 25741868

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U	UUU Phe UUC Phe UUA Leu UUG Leu	UCU Ser UCC Ser UCA Ser UCG Ser	UAU Tyr UAC Tyr UAA Stop UAG Stop	UGU Cys UGC Cys UGA Stop UGG Trp	U C A G
C	CUU Leu CUC Leu CUA Leu CUG Leu	CCU Pro CCC Pro CCA Pro CCG Pro	CAU His CAC His CAA Gln CAG Gln	CGU Arg CGC Arg CGA Arg CGG Arg	U C A G

Leu257Pro - Pathogenic

CUU → CCU

Leu257His - ???

CUU → CAU

Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

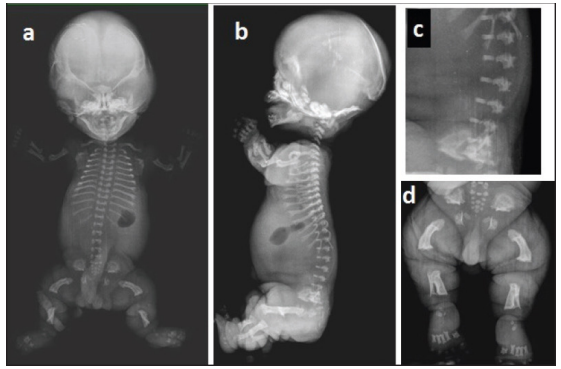
https://rsscience.com/codon-chart/

An interesting example… *FGFR3*

Lys650

Lys650Glu

Type II thanatophoric dysplasia



DOI: 10.4103/0974-5009.165584

An interesting example… *FGFR3*

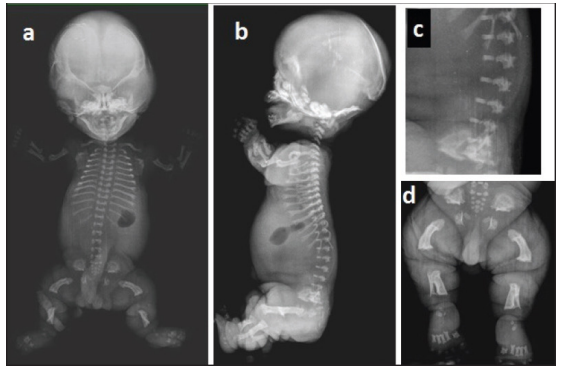
Lys650

Lys650Glu

Lys650Met

Type II thanatophoric dysplasia

SADDAM



https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fibroblast-growth-factor-receptor-3

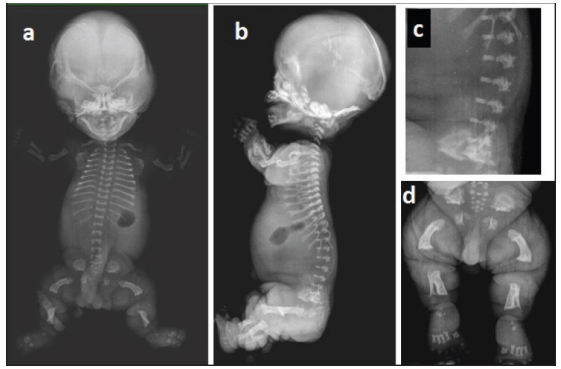
DOI: 10.4103/0974-5009.165584

An interesting example… *FGFR3*

Lys650

Lys650Glu

Type II thanatophoric dysplasia



DOI: 10.4103/0974-5009.165584

Lys650Met

SADDAM

https://www.sciencedirect.com/topics/agricultural-and-biological-sciences/fibroblast-growth-factor-receptor-3

Lys650Asn

Hypochondroplasia

https://www.hss.edu/LLcase19.asp

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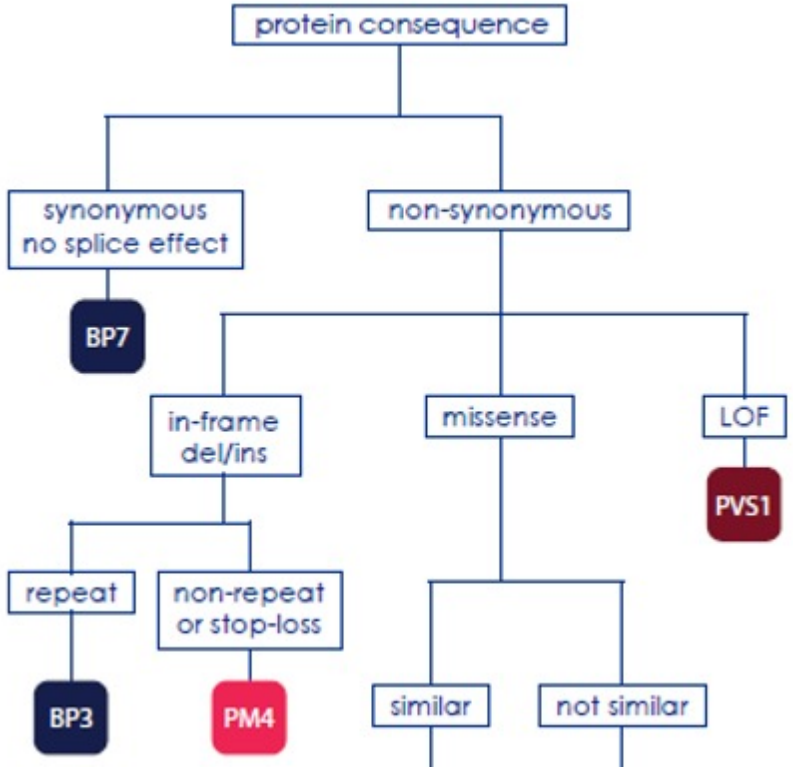
PM4

- Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.
- To prevent double-counting of this evidence type, we recommend that PM4 should not be applied for any variant in which PVS1, at any strength level, is also applied.



Example – *CTNNB1* c.1021_1026del, p.(Ser341_Arg342del)

615075

NEURODEVELOPMENTAL DISORDER WITH SPASTIC DIPLEGIA AND VISUAL DEFECTS; NEDSDV

Alternative titles; symbols

MENTAL RETARDATION, AUTOSOMAL DOMINANT 19, FORMERLY; MRD19, FORMERLY

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus
3p22.1	Neurodevelopmental disorder with spastic diplegia and visual defects	615075	AD	3	CTNNB1

https://www.omim.org/

.0017	NEURODEVELOPMENTAL DISORDER WITH SPASTIC DIPLEGIA AND VISUAL DEFECTS	CTNNB1, 4-BP DEL, NT1272	rs398122907
.0018	NEURODEVELOPMENTAL DISORDER WITH SPASTIC DIPLEGIA AND VISUAL DEFECTS	CTNNB1, ARG515TER	rs397514554
.0019	NEURODEVELOPMENTAL DISORDER WITH SPASTIC DIPLEGIA AND VISUAL DEFECTS	CTNNB1, GLN309TER	rs376393123
.0020	NEURODEVELOPMENTAL DISORDER WITH SPASTIC DIPLEGIA AND VISUAL DEFECTS	CTNNB1, 1-BP DUP, NT705	rs587777412
.0021	NEURODEVELOPMENTAL DISORDER WITH SPASTIC DIPLEGIA AND VISUAL DEFECTS	CTNNB1, ARG535TER	rs886039332

Example – *CTNNB1* c.1021_1026del, p.(Ser341_Arg342del)

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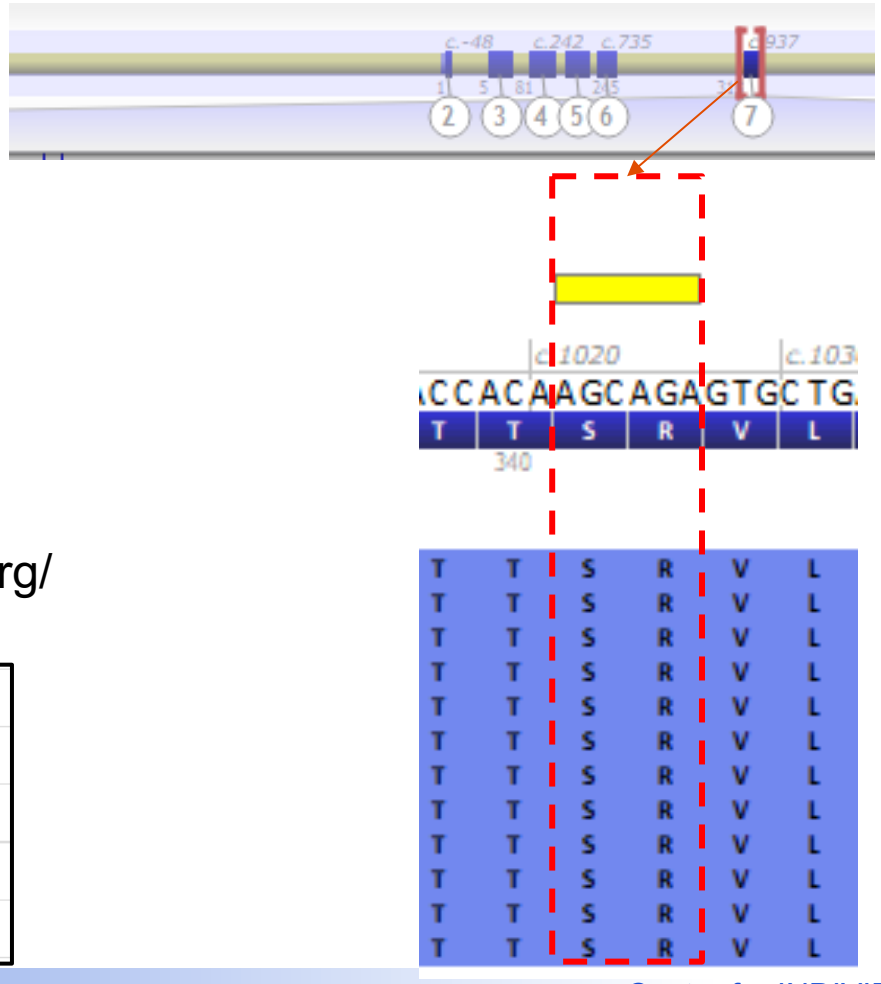
Images from Alamut software:



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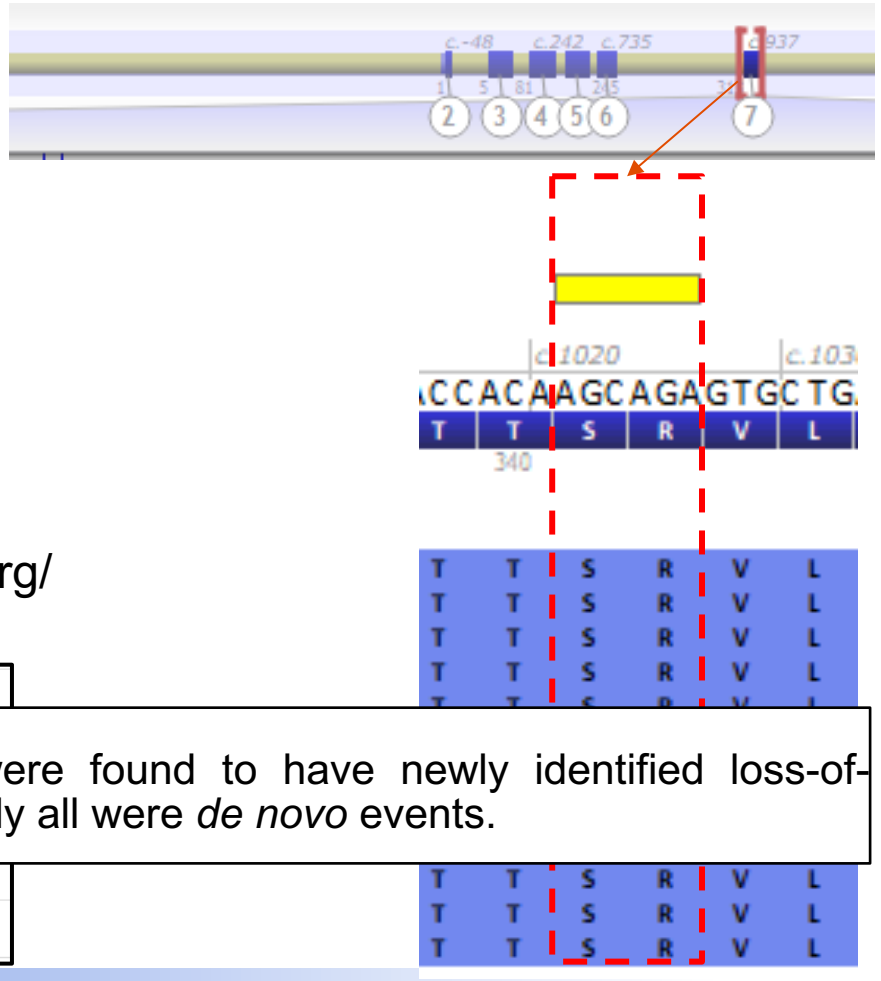

- 16 individuals from 15 families were found to have newly identified loss-of-function *CTNNB1* mutations. Virtually all were *de novo* events.

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

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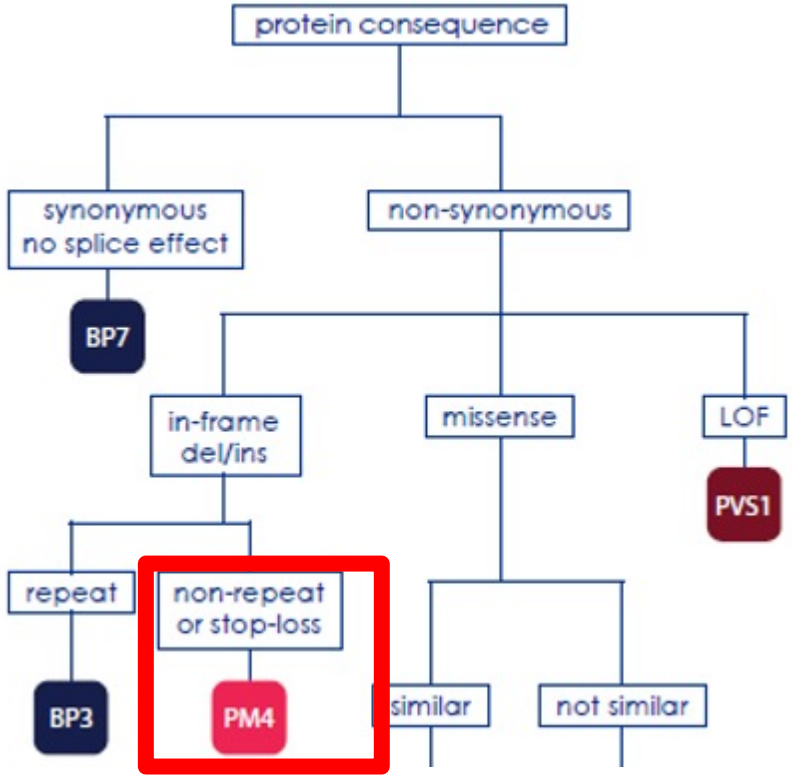

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- PP3

PP3	Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

…Some of the *In Silico* tools mentioned

Table 2

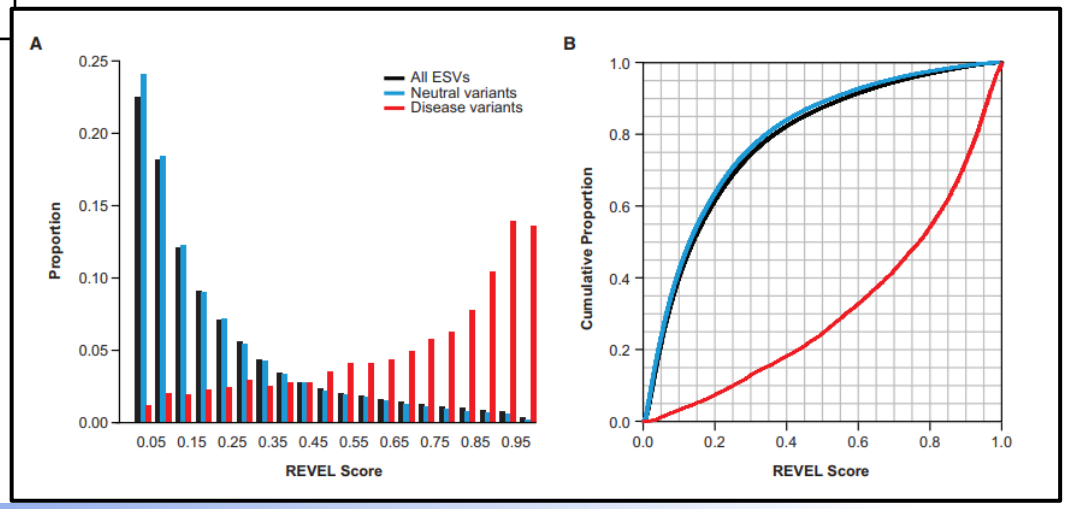
In Silico Predictive Algorithms

	nsSNPAnalyzer	http://snpanalyzer.uthsc.edu
	Condel	http://bg.upf.edu/condel/home
	CADD	http://cadd.gs.washington.edu

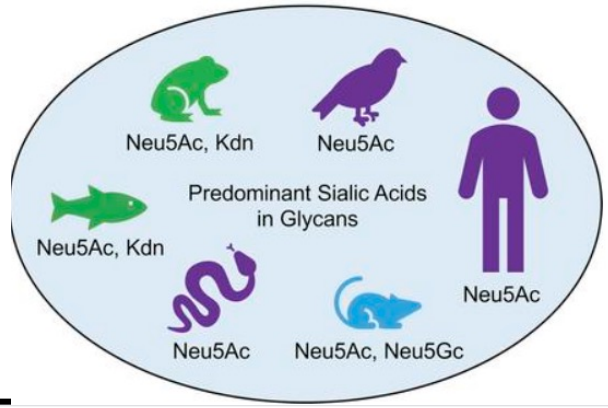
REVEL

ARTICLE

REVEL: An Ensemble Method for Predicting the Pathogenicity of Rare Missense Variants



Example



phyloP scores measure evolutionary conservation at individual alignment sites. Interpretations of the scores are compared to the evolution that is expected under neutral drift.

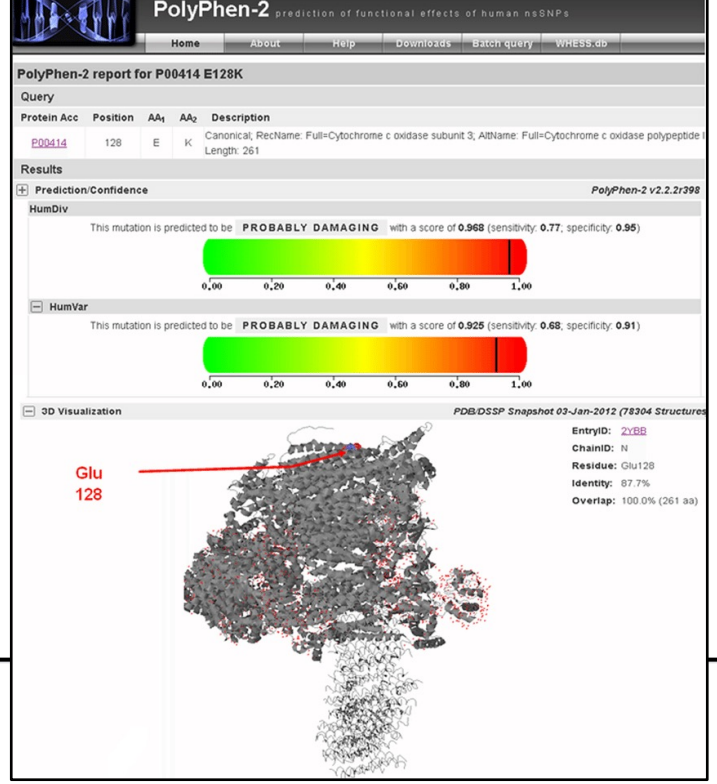
https://www.jci.org/articles/view/137681

Revel Deleterious (low) (0.6)

REVEL is an ensemble method for predicting the pathogenicity of missense variants based on a combination of scores from 13 individual tools: MutPred, FATHMM v2.3, VEST 3.0, PolyPhen-2, SIFT, PROVEAN, MutationAssessor, MutationTaster, LRT, GERP++, SiPhy, phyloP, and phastCons. REVEL was trained using recently discovered pathogenic and rare neutral missense variants, excluding those previously used to train its constituent tools. The REVEL score for an individual missense variant can range from 0 to 1, with higher scores reflecting greater likelihood that the variant is disease-causing.

https://franklin.genoox.com/

Example



Impact of amino acid allelic variants on protein structure/function can be reliably predicted via analysis of multiple sequence alignments and protein 3D-structures.

http://genetics.bwh.harvard.edu/pph2/

Revel

REVEL is an ensemble method for predicting the pathogenicity of missense variants based on a combination of scores from 13 individual tools: MutPred, FATHMM v2.3, VEST 3.0, PolyPhen-2, SIFT, PROVEAN, MutationAssessor, MutationTaster, LRT, GERP++, SiPhy, phyloP, and phastCons. REVEL was trained using recently discovered pathogenic and rare neutral missense variants, excluding those previously used to train its constituent tools. The REVEL score for an individual missense variant can range from 0 to 1, with higher scores reflecting greater likelihood that the variant is disease-causing.

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PMID: 25741868

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Scores are not deterministic of biological effect/deleteriousness, they are used as "supporting evidence"

gDNA: Chr6(GRCh37):g.51720765A>G
cDNA: NM_138694.3(*PKHD1*):c.7837T>C
p.Trp2613Arg

Polyphen-2: Probably damaging
CADD: 29
M-CAP: Probably
PredictSNP2: Deleterious

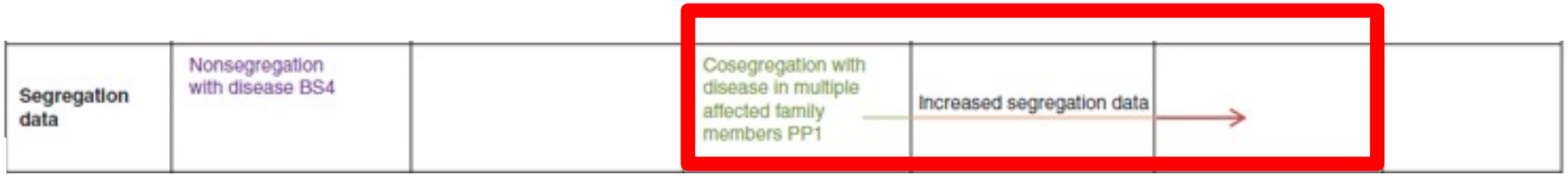
Scores agree towards SNV being deleterious

1- Population Data

2- Computational and Predictive Data

3- Segregation Data

PMID: 25741868

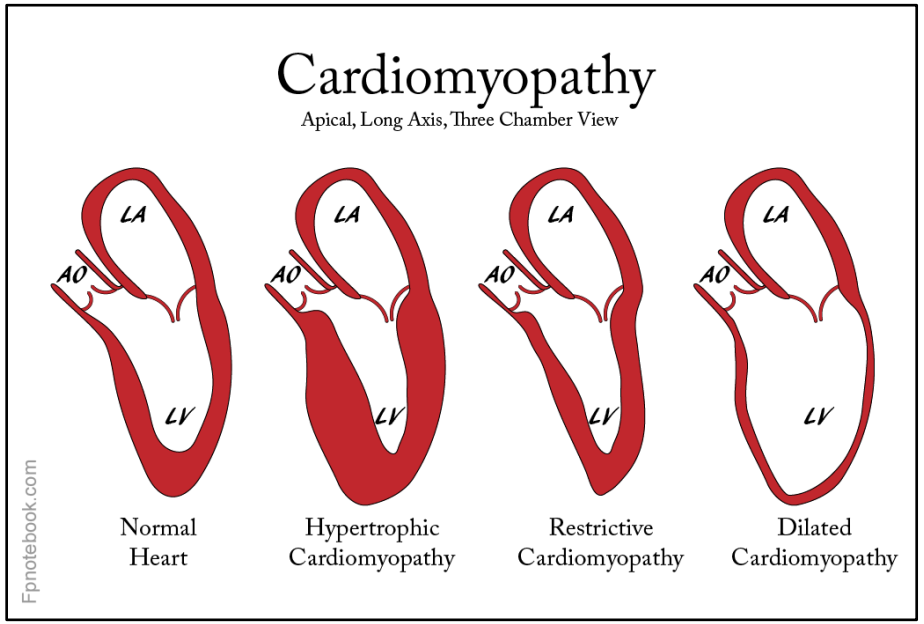


PP1

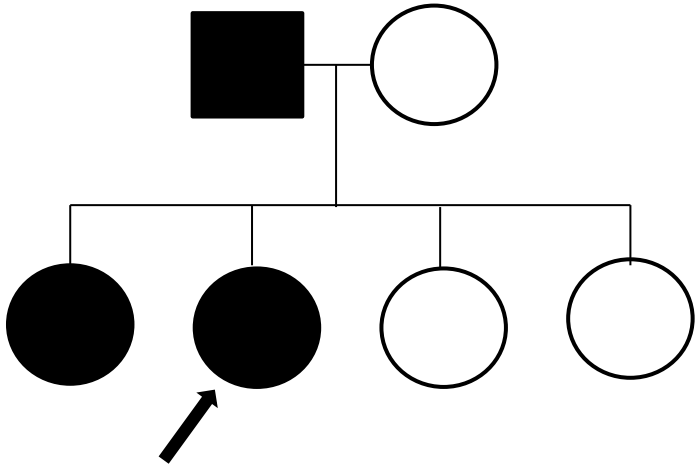
- Co-segregation with disease in multiple affected family members of a single family in a gene definitively known to cause the disease.
  - ***Note:*** May be used as stronger evidence with increasing segregation data.

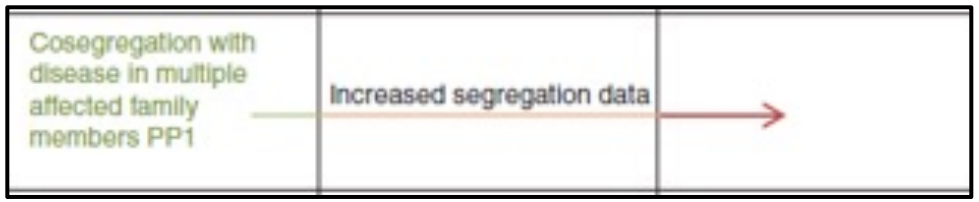
…Example of segregation

- Restrictive cardiomyopathy; Variant of uncertain significance in *FLNC*



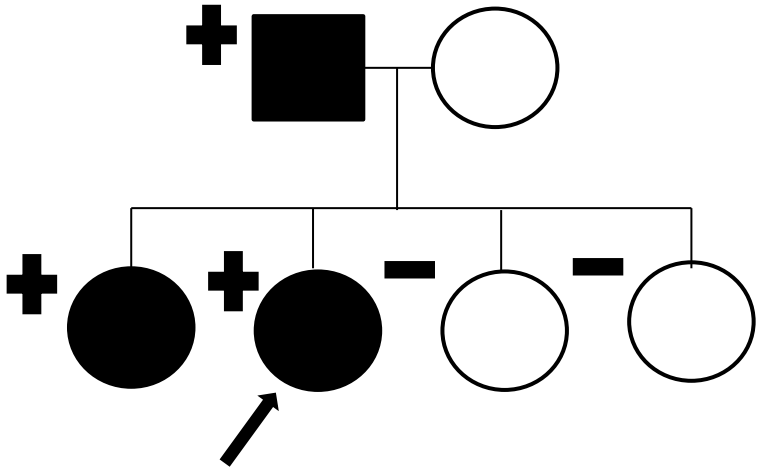
https://fpnotebook.com/CV/Myocardium/Crdmypthy.htm

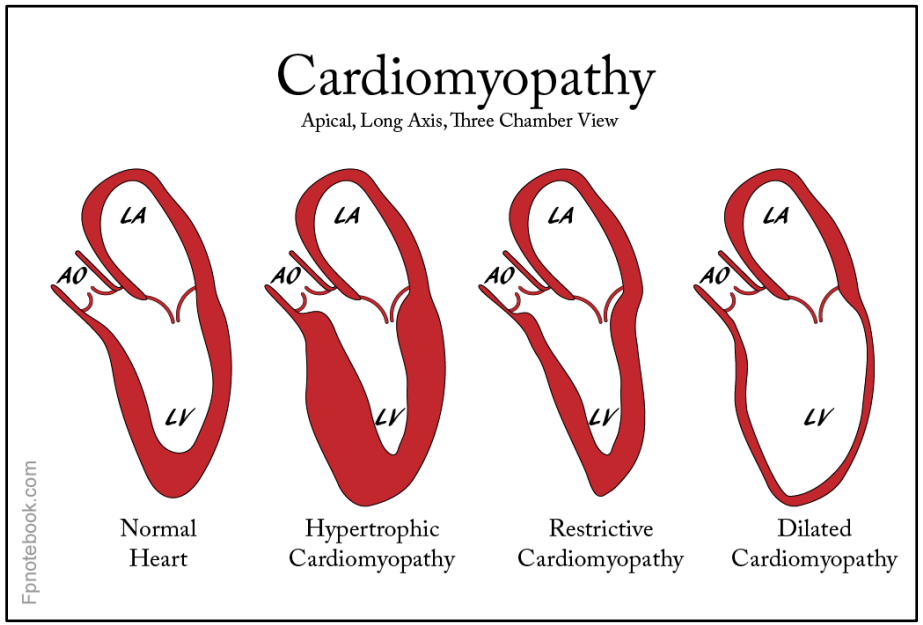




…Example of segregation

- Restrictive cardiomyopathy; Variant of uncertain significance in *FLNC*





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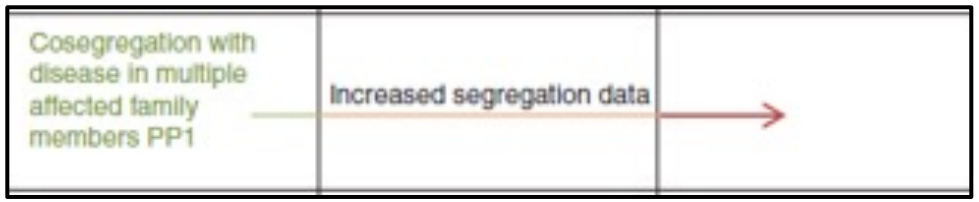


…Example of segregation

- Restrictive cardiomyopathy; Variant of uncertain significance in *FLNC*

ARTICLE

Consideration of Cosegregation in the Pathogenicity Classification of Genomic Variants

Gail P. Jarvik[1,*] and Brian L. Browning[1]

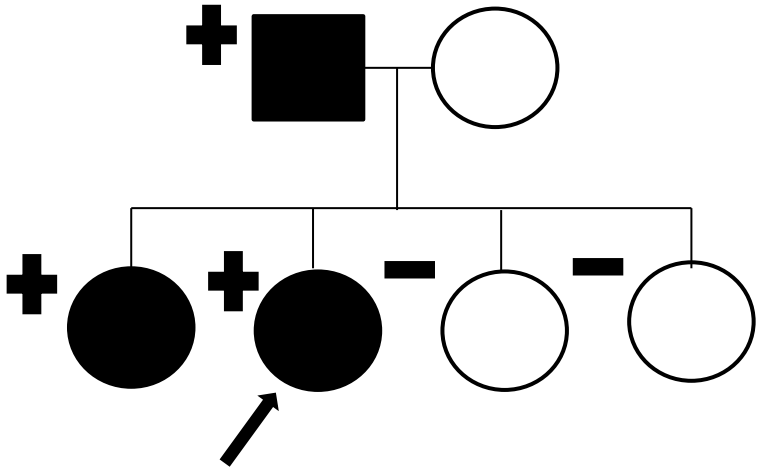
Table 1

Proposed Cosegregation Evidence to Support Each ACMG-AMP[1] Pathogenicity Evidence Level

	Single Family	>1 Family
Strong evidence	≤1/32 (≤0.03)	≤1/16 (≤0.06)
Moderate evidence	≤1/16 (≤0.06)	≤1/8 (≤0.125)
Supporting evidence	≤1/8 (≤0.125)	≤1/4 (≤0.25)

Open in a separate window

N, probability of observed cosegregation if not pathogenic, totaled over all families (or 1/BF). Note that the strongest evidence level supported by a given N is selected.



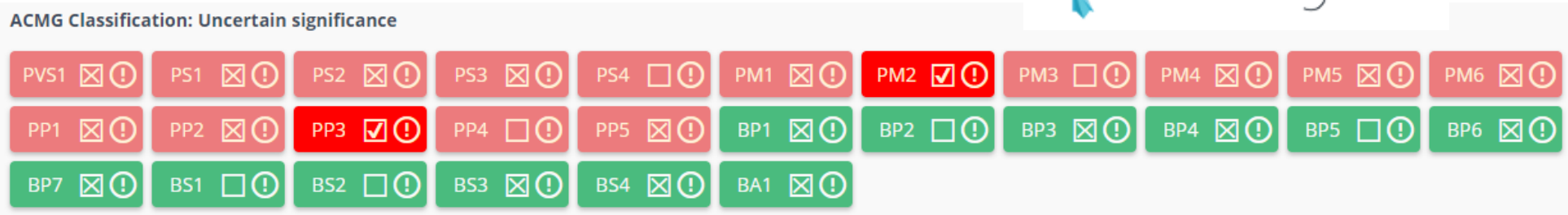
Under a dominant model, this probability is $N = (1/2)^m$, where m is the number of meioses of the variant of interest that are informative for cosegregation.

Watch out for different penetrance, expressivity and phenocopies!

Publicly Available Calculators and Workflows

- Publically available tools that will help add up your "points"
  - https://varsome.com/
  - http://wintervar.wglab.org/
  - http://www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/
- Several analysis software integrate guidelines into their workflow

emedgene



Post-test questions:

1) When we classify a variant, we do it ONLY in the context of the case we are working on, we classify `if the variant is causing the disease in the patient`.

A) TRUE
B) FALSE

2) Retinoblastoma, the most malignant form of eye cancer, arises from a dominant pathogenic variant in one gene *RB1*, but only about 75% of people who carry this variant develop the disease. We are talking about:
A) Penetrance
B) Expressivity

3) A frequent variant (found in >5% in a population) will always be `benign`
A) TRUE
B) FALSE

Post-test questions:

1) When we classify a variant, we do it ONLY in the context of the case we are working on, we classify `if the variant is causing the disease in the patient`.

A) TRUE
B) FALSE

WE CLASSIFY THE VARIANT

2) Retinoblastoma, the most malignant form of eye cancer, arises from a dominant pathogenic variant in one gene *RB1*, but only about 75% of people who carry this variant develop the disease. We are talking about:

A) Penetrance
B) Expressivity

3) A frequent variant (found in >5% in a population) will always be `benign`

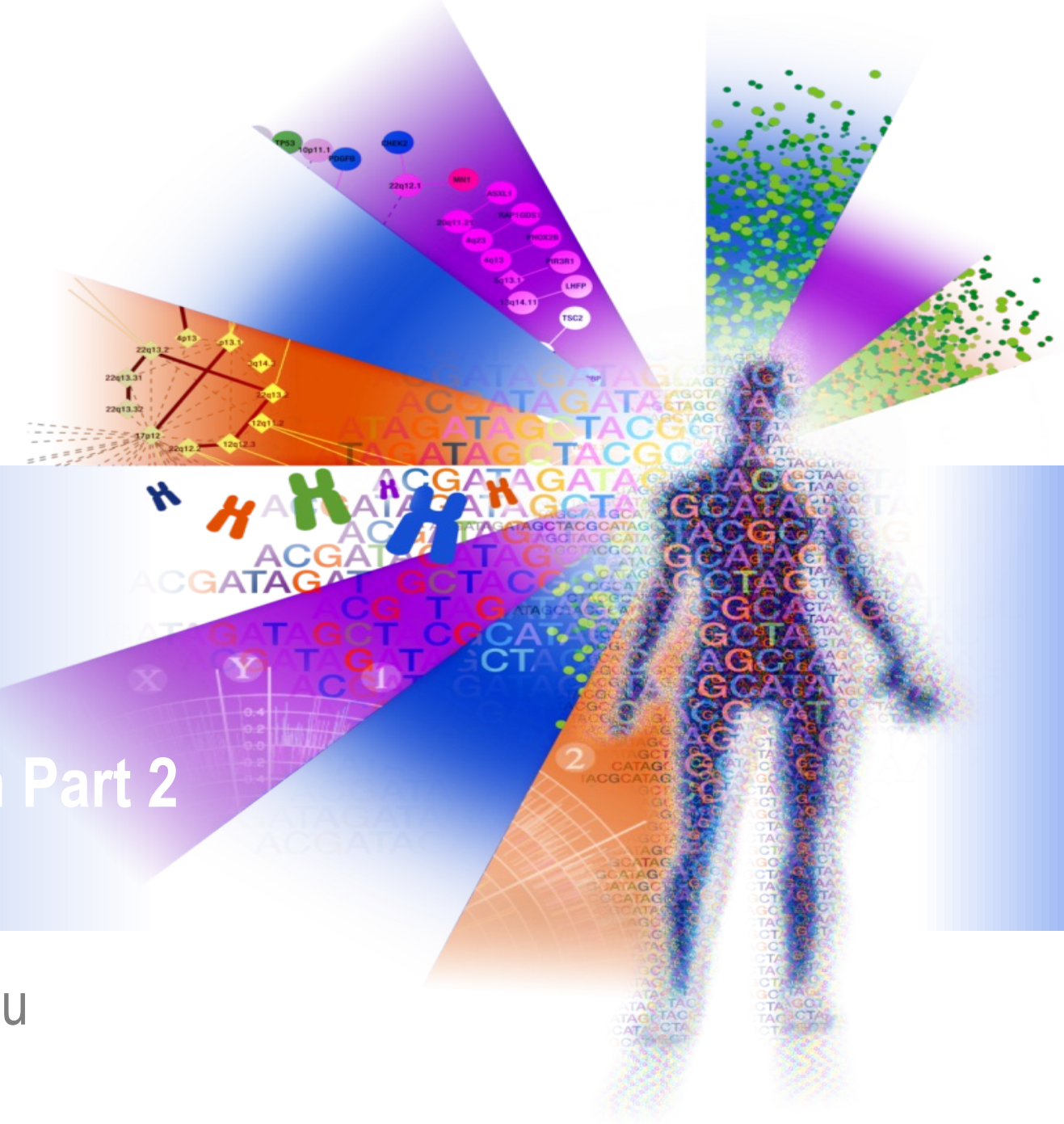
A) TRUE
B) FALSE

To be continued…

Questions?

MAYO CLINIC

CENTER FOR
INDIVIDUALIZED MEDICINE



Variant Interpretation Part 2
ACMG Guidelines

Wilke.Matheus@mayo.edu

 |

	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			

Impact Prediction: Computational or Knowledge-based

"In-Silico" Tools

PP3

Predicted Damaging

Criteria for classifying pathogenic variants

Multiple lines of computational evidence support a deletrious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).

Caveats:

- Because many *in silico* algorithims use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion.
- PP3 can be used only once in any evaluation of a variant.

BP4

Computationally Inert

Criteria for classifying benign variants

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.).

Caveats:

- Because many *in silico* algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion.
- BP4 can be used only once in any evaluation of a variant.

In-Silico Tool	Prediction
Cadd Phred	22.9
Mutation Taster	D;D;D;D;D;D
MetaSVM	T
REVEL	0.580

Impact Prediction: Computational or Knowledge-based

- 2022 new guidelines update. Will be implemented in the future

Method	Pathogenic (PP3)				Benign (BP4)			
	Very Strong	Strong	Moderate	Supporting	Supporting	Moderate	Strong	Very Strong
BayesDel	-	≥ 0.50	[0.27, 0.50)	[0.13, 0.27)	(-0.36, -0.18]	≤ -0.36	-	-
CADD	-	-	≥ 28.1	[25.3, 28.1)	(17.3, 22.7]	(0.15, 17.3]	≤ 0.15	-
EA	-	-	≥ 0.821	[0.685, 0.821)	(0.069, 0.262]	≤ 0.069	-	-
FATHMM	-	-	≤ -5.04	[-5.04, -4.14)	(3.32, 4.69]	≥ 4.69	-	-
GERP++	-	-	-	-	(-4.54, 2.70]	≤ -4.54	-	-
MPC	-	-	≥ 1.828	[1.360, 1.828)	-	-	-	-
MutPred2	-	≥ 0.932	[0.829, 0.932)	[0.737, 0.829)	(0.197, 0.391]	(0.010, 0.197]	≤ 0.010	-
PhyloP	-	-	≥ 9.741	[7.367, 9.741)	(0.021, 1.879]	≤ 0.021	-	-
PolyPhen2	-	-	≥ 0.999	[0.978, 0.999)	(0.009, 0.113]	≤ 0.009	-	-
PrimateAI	-	-	≥ 0.867	[0.790, 0.867)	(0.362, 0.483]	≤ 0.362	-	-
REVEL	-	≥ 0.932	[0.773, 0.932)	[0.644, 0.773)	(0.183, 0.290]	(0.016, 0.183]	(0.003, 0.016]	≤ 0.003
SIFT	-	-	≤ 0.000	[0.000, 0.001)	(0.080, 0.327]	≥ 0.327	-	-
VEST4	-	≥ 0.965	[0.861, 0.965)	[0.764, 0.861)	(0.302, 0.449]	≤ 0.302	-	-

Calibration of computational tools for missense variant pathogenicity classification and ClinGen recommendations for PP3/BP4 criteria.

Pejaver V[1], Byrne AB[2], Feng BJ[3], Pagel KA[4], Mooney SD[5], Karchin R[6], O'Donnell-Luria A[7], Harrison SM[8], Tavtigian SV[9], Greenblatt MS[10], Biesecker LG[11], Radivojac P[12], Brenner SE[13], ClinGen Sequence Variant Interpretation Working Group

Table 2. Estimated threshold ranges for all tools in this study corresponding to the four pathogenic and four benign intervals. A "–" implies that the given tool did not meet the posterior probability (likelihood ratio) threshold. See Supplemental 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.

PMID: 36413997

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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Functional Evidence:

PS3

Functional Consequence

Criteria for classifying pathogenic variants

Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product.

Note:

Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well established.

BS3

No Functional Consequence

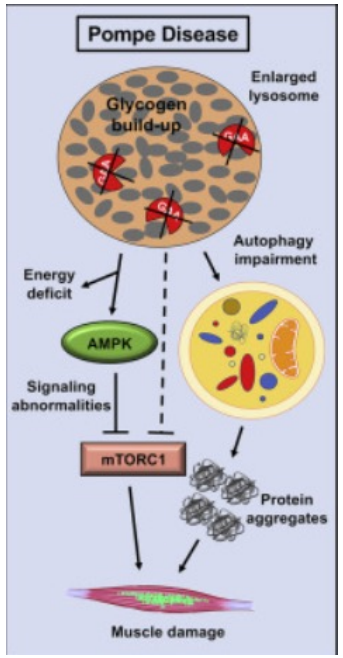
Criteria for classifying benign variants

Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing.

What defines a "well established" functional study or assay? How reliable? This is not simple.

GAA example

From ClinGen Lysosomal Storage Disorders Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 2



PS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Strong

Well-established in vitro or in vivo functional studies supportive of a damaging effect.

- RT-PCR evidence of mis-splicing for non-canonical intronic variants with no evidence of normal splice products.

Modification Type: None

Moderate

Well-established in vitro or in vivo functional studies supportive of a damaging effect.

- <5% wild type GAA activity when the variant is expressed in a heterologous cell type and evidence of abnormal GAA synthesis and/or processing.
- RT-PCR evidence of mis-splicing for non-canonical intronic variants with evidence of normal splice products.

Modification Type: Strength,Disease-specific

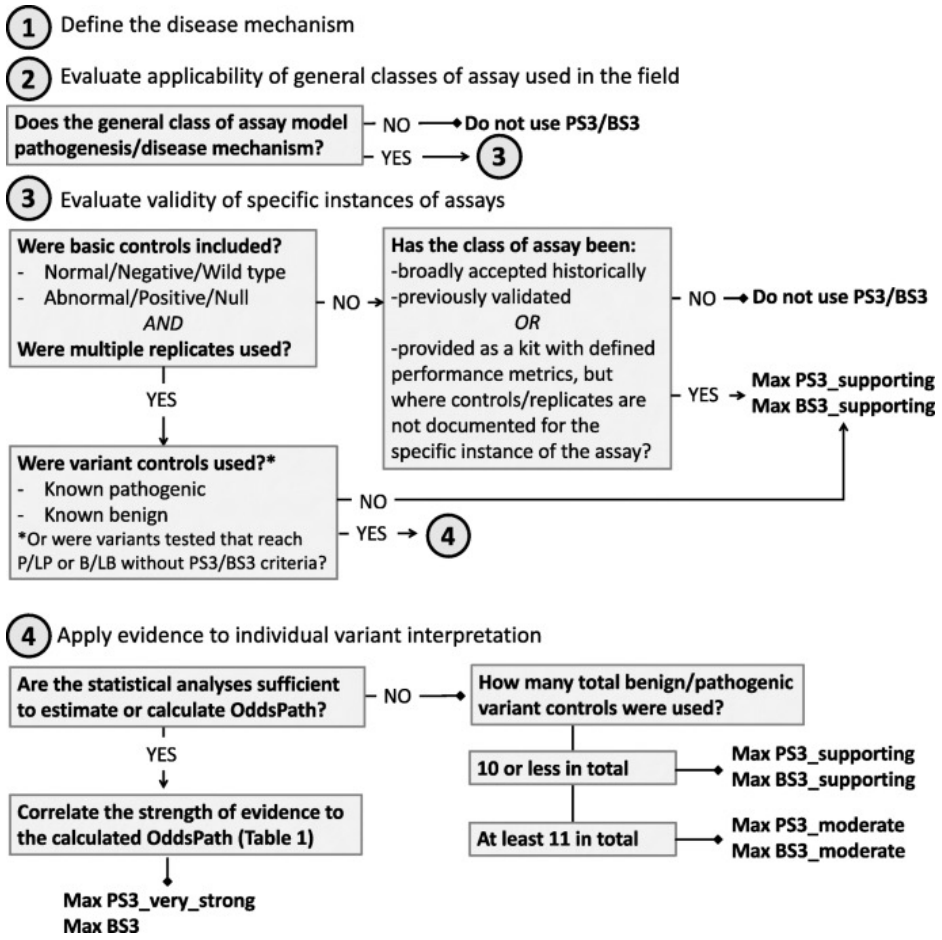
Supporting

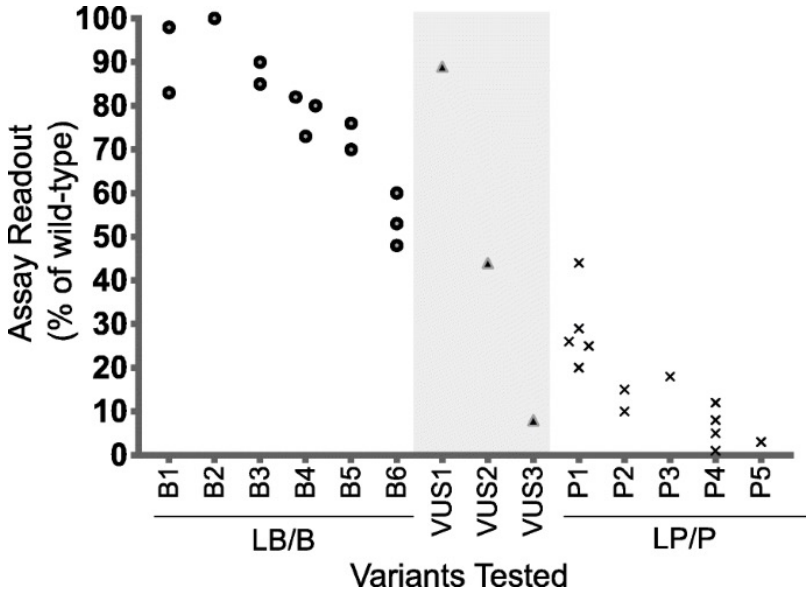
Well-established in vitro or in vivo functional studies supportive of a damaging effect.

- <30% wild type GAA activity when the variant is expressed in a heterologous cell type.
- RT-PCR evidence of mis-splicing for non-canonical intronic variants with evidence of normal splice products.

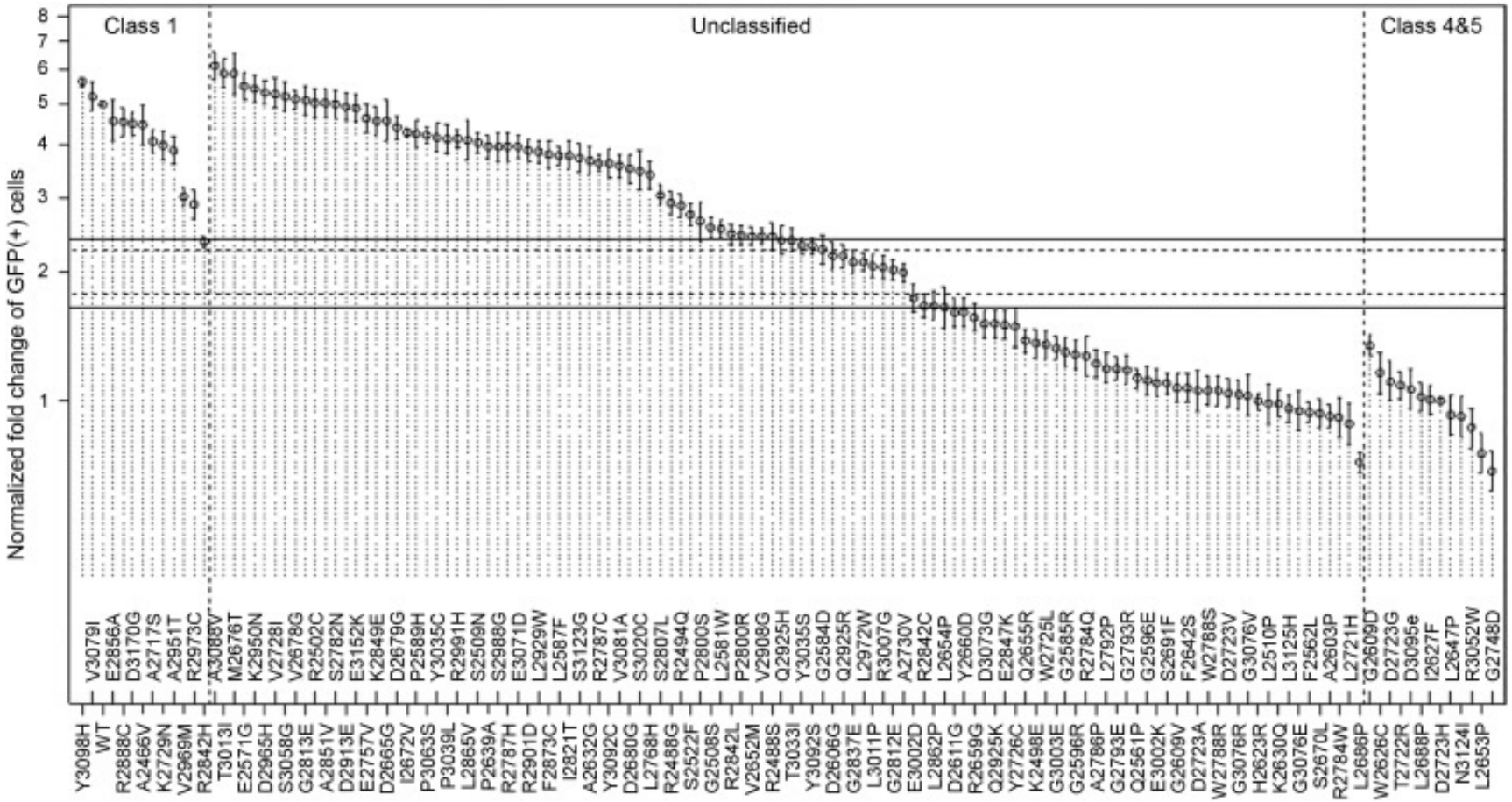
Modification Type: Strength,Disease-specific

Decision Tree to guide PS3/BS3 criterion

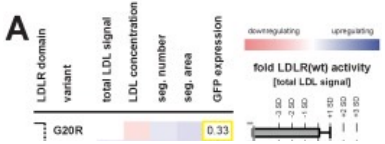




PS3/BS4



PS3/BS4



exome sequencing
of 3,235 subjects
1,716 MI cases and 1,519 MI controls

LDLR genotypes
194 subjects with rare LDLR alleles

12 clear **LoF** variants

70 missense variants
with allele frequencies between 1.5E-04 and 6.3E-03

over-expression | comple-mentation

effect on **LDL-uptake**
assessed for each variant

plasma LDL-C levels
251 subjects >190mg/dl
1,901 subjects <190mg/dl

validation:
* FC levels
* localization
* maturation

disruptive-missense
(14 variants)

D131G	F282L
G137V	N316S
S177L	D472Y
C197R	P526S
D221G	G549D
C222Y	H583D
C276S	P685L

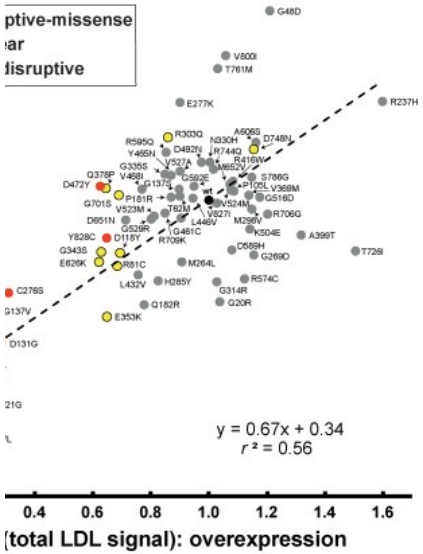
unclear
(10 variants)

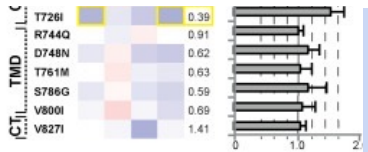
R81C	Q378P
D118Y	E626K
R303Q	G701S
G343S	D748N
E353K	V859M

non-disruptive
(46 variants)

G20R	E277K	L446V	G529R	T726I
G48D	H285Y	G461C	R574C	R744Q
T62M	M298V	Y465N	D589H	T761M
P105L	G314R	V468I	G592E	S786G
G137S	N330H	D492N	R595Q	V800I
P181R	G335S	K504E	A606S	V827I
Q182R	V369M	G516D	D651N	
R237H	A399T	V523M	M652V	
M264L	R416W	V524M	R706G	
G269D	L432V	V527A	R709K	

improved association testing for plasma LDL-C and MI





	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	with disease BS4		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			

"Functional" Impact Prediction: Computational or Knowledge-based

MASTERMIND®
COMPREHENSIVE GENOMIC SEARCH ENGINE

Variant Interpretation Cards

mastermind.genomenon.com

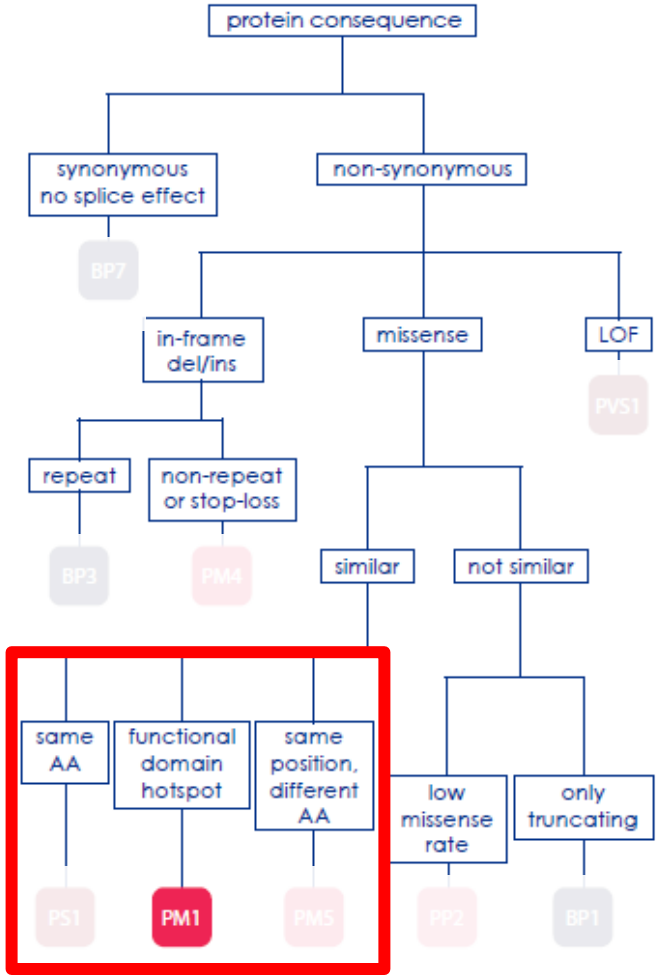
PM1

Hotspot

Criteria for classifying pathogenic variants

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

https://www.genomenon.com/mastermind-variant-interpretation-cards-download/



Richards CS et al. *Gen Med.* 2015;17:405-423

“Functional” Impact Prediction: Computational or Knowledge-based

PM1

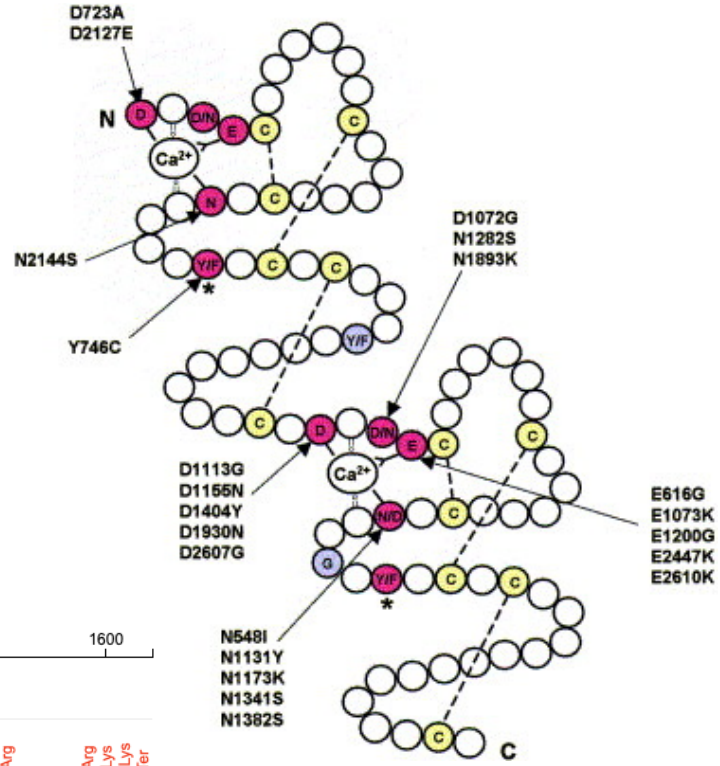
Hotspot

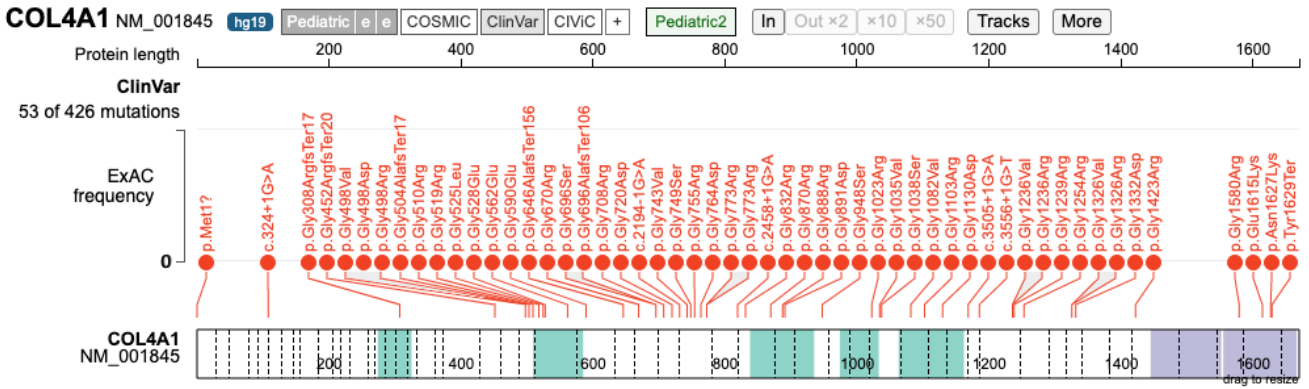
Circulation: Genomic and Precision Medicine
Volume 11, Issue 6, June 2018
https://doi.org/10.1161/CIRCGEN.117.002039

ORIGINAL ARTICLE

Tailoring the American College of Medical Genetics and Genomics and the Association for Molecular Pathology Guidelines for the Interpretation of Sequenced Variants in the *FBN1* Gene for Marfan Syndrome

Proposal for a Disease- and Gene-Specific Guideline





Richards CS et al. *Gen Med.* 2015;17:405-423

“Functional” Impact Prediction: Computational or Knowledge-based

PM1
Hotspot

Reference laboratories are very conservative in the use of this criteria because of its subjectivity

Automated criteria

Rule	Explanation
PM1 Moderate	UniProt protein CLN6_HUMAN trans-membrane region 'Helical' has 6 non-VUS missense/in-frame/non-synonymous, variants (6 pathogenic and 0 benign), pathogenicity = 100.0% which is more than threshold 50.0%.

“Functional” Impact Prediction: Computational or Knowledge-based

Gene-specific ClinGen expert panels

Gene-specific criteria for PTEN variant curation: Recommendations from the ClinGen PTEN Expert Panel

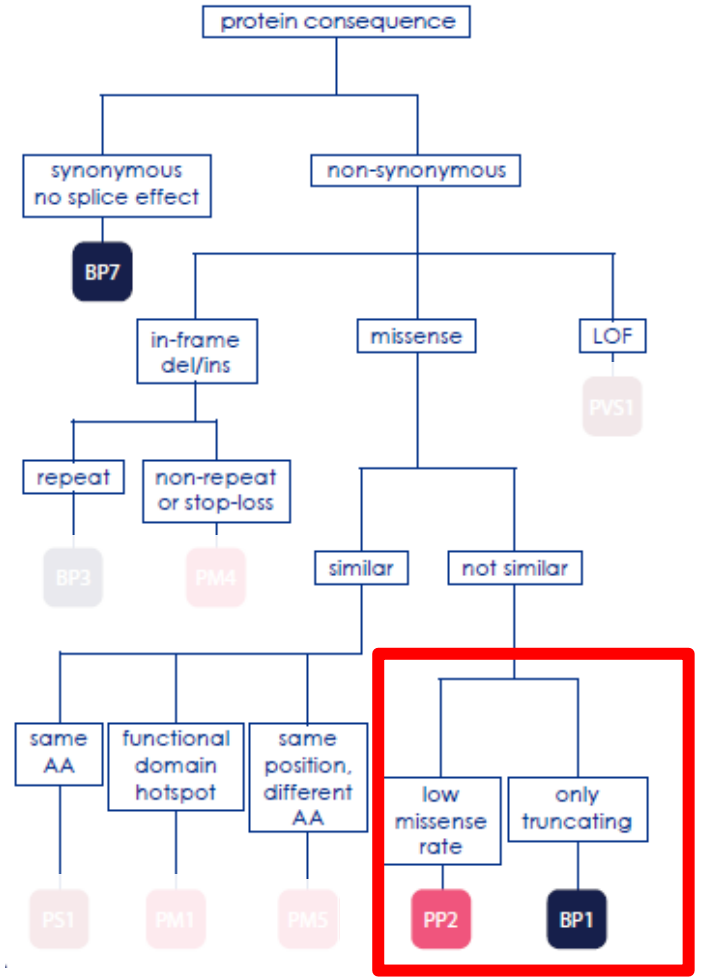
Mester JL, Ghosh R, Pesaran T, Huether R, Karam R, Hruska KS, Costa HA, Lachlan K, Ngeow J, Barnholtz-Sloan J, Sesock K, Hernandez F, Zhang L, Milko L, Plon SE, Hegde M, Eng C.

Recommendations for PM1 specified in guidelines if applicable

Moderate	PM1	DS	Located in a mutational hot spot and/or critical and well-established functional domain. Defined to include residues in catalytic motifs: 90-94, 123-130, 166-168 (NP_000305.3).

Impact Prediction: Computational or Knowledge-based

https://www.genomenon.com/mastermind-variant-interpretation-cards-download/

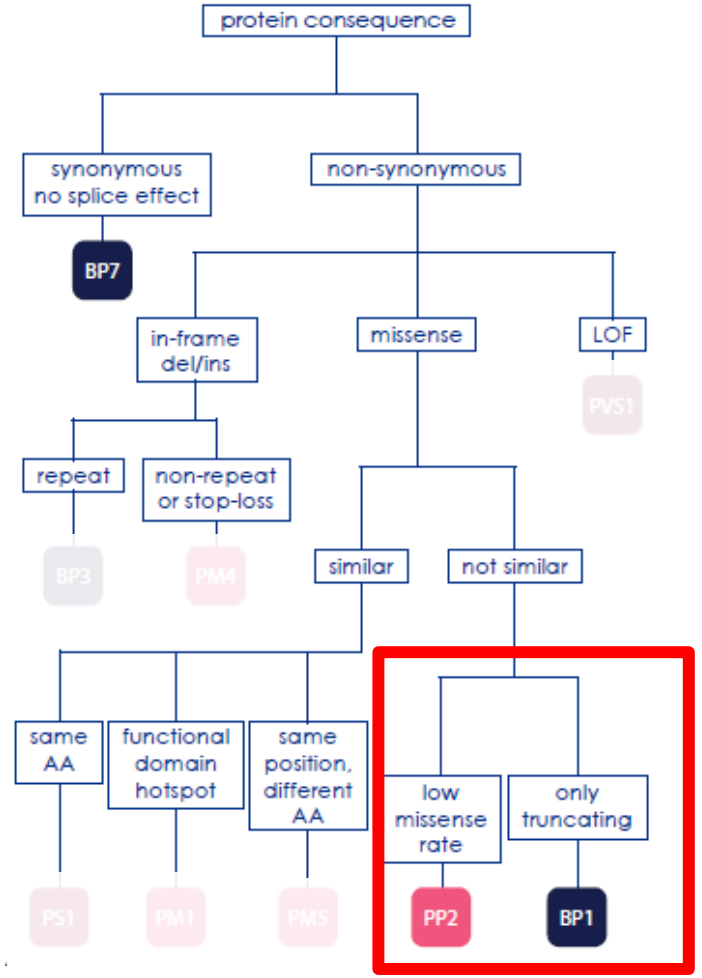


Impact Prediction: Computational or Knowledge-based

Constraint

Category	Exp. SNVs	Obs. SNVs	Constraint metrics	
Synonymous	163.3	147	Z = [illegible] o/e = [illegible] (0.79 - 1.03)	0 — 1
Missense	498.1	262	Z = 3.76 o/e = 0.53 (0.47 - 0.58)	0 — 1
pLoF	45.6	6	pLI = 1 o/e = 0.13 (0.07 - 0.26)	0 — 1

Z-score “bigger” than 4
(Z > 3 in some literature)



Richards CS et al. *Gen Med.* 2015;17:405-423

Impact Prediction: Computational or Knowledge-based

"Gene-wide" score

Constraint

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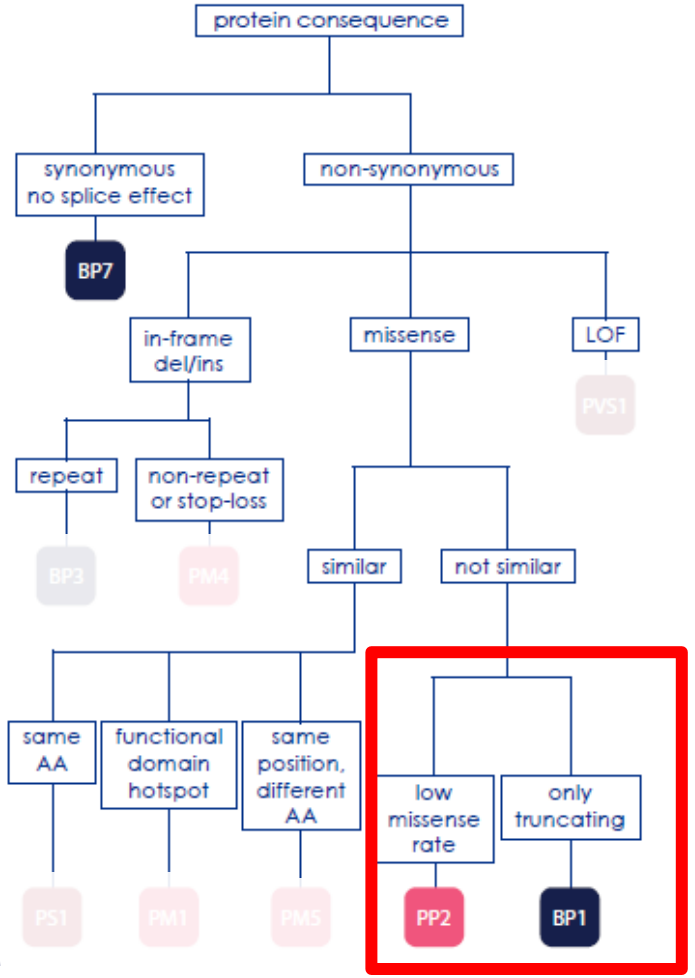


Impact Prediction: Computational or Knowledge-based

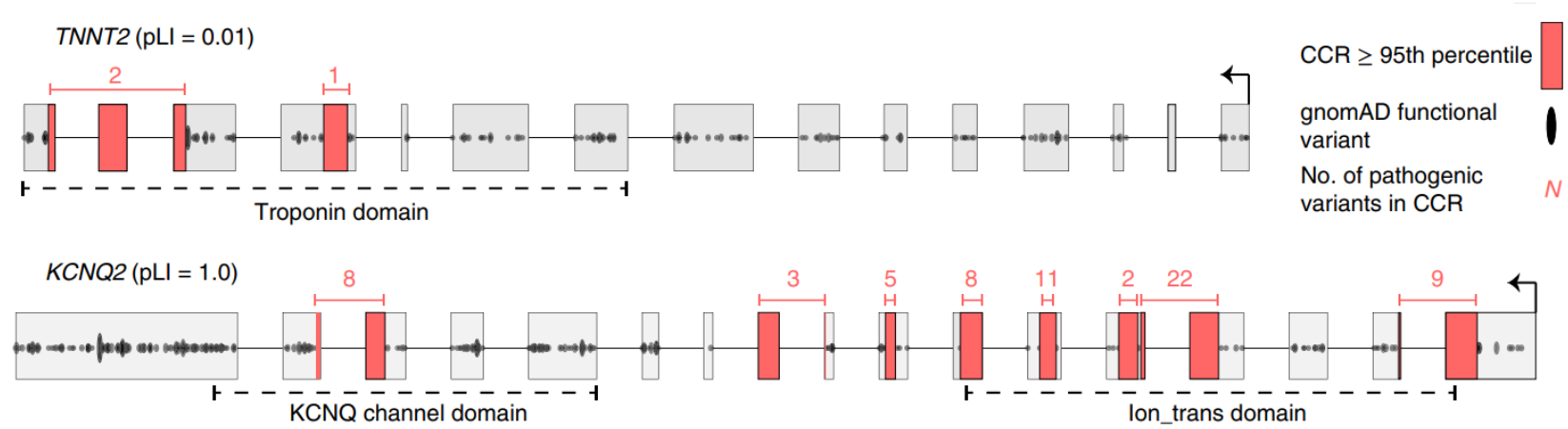
- Gene-wide summary measures of constraint are prone to overstating and understating constraint within specific regions of protein-coding genes

A map of constrained coding regions in the human genome

James M. Havrilla[1,2], Brent S. Pedersen[1,2], Ryan M. Layer[3,4] and Aaron R. Quinlan[1,2,5*]

nature genetics

Regional intolerance correlates with important functional domains



Impact Prediction: Computational or Knowledge-based

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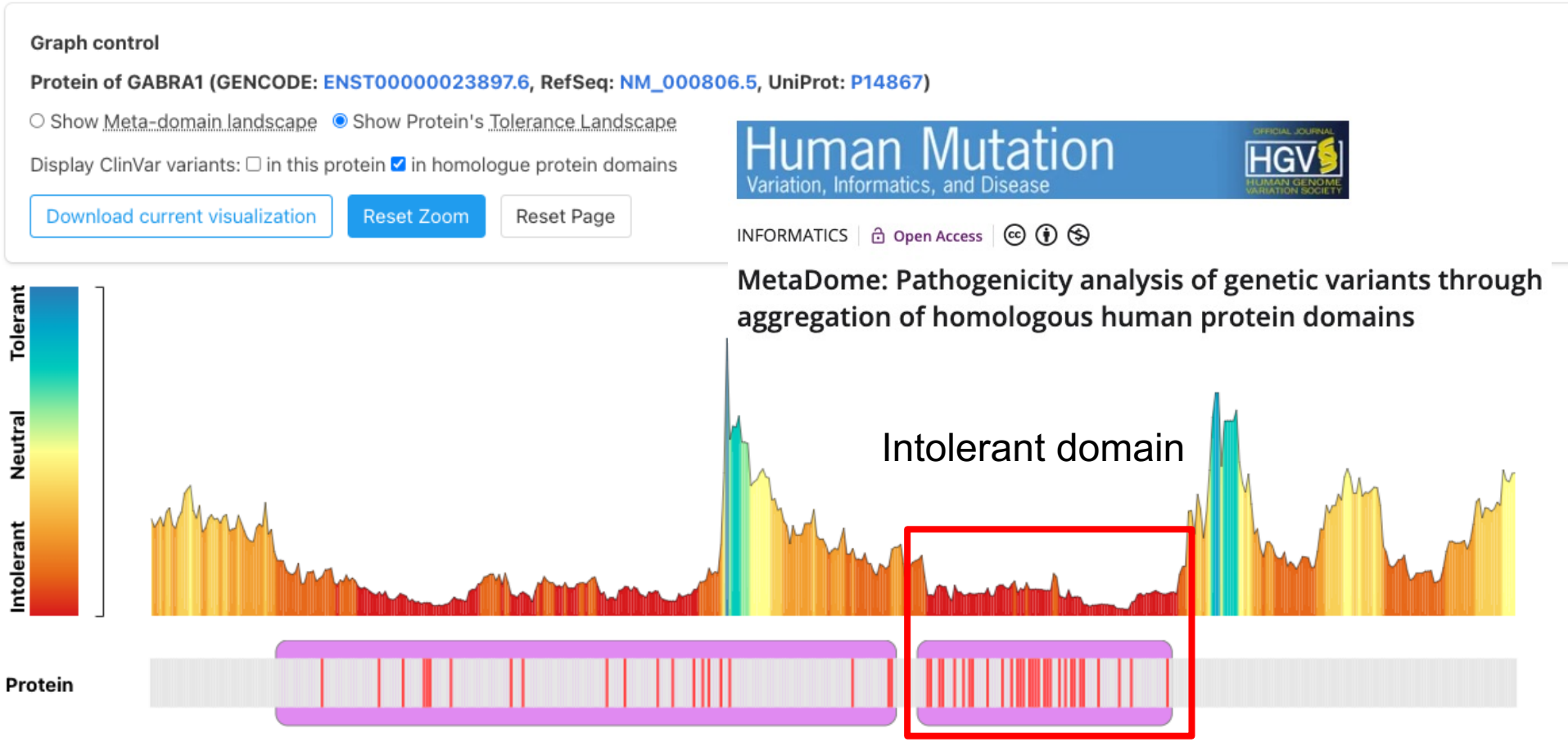

https://stuart.radboudumc.nl/metadome/dashboard

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	Strong	Supporting	Supporting	Moderate	Strong	Very strong
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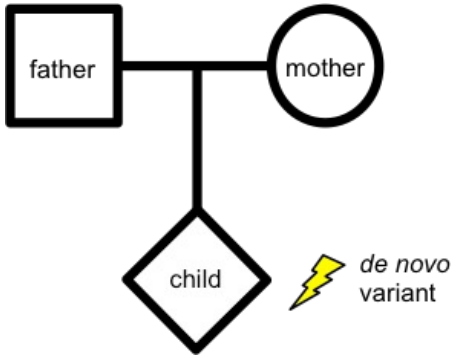
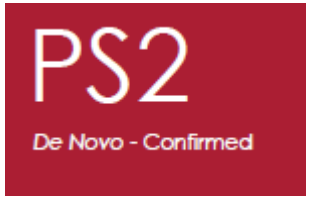
Case-specific data to consider

Case-Specific Evidence - Segregation Data



https://www.genomenon.com/mastermind-variant-interpretation-cards-download/



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

Confirm parental status through validated test

PS2/PM6

- parental confirmed
- phenotype consistency
- number of *de novo* observations

Phenotypic consistency	Points per Proband	
	***de novo* with confirmed parental relationships**	***de novo* with unconfirmed parental relationships**
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

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

https://clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_1.pdf

PS2/PM6

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- phenotype consistency
- number of *de novo* observations

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0.5	1	2	4

https://clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_1.pdf

If a NIPBL variant was de novo in one patient with Cornelia de Lange syndrome, with confirmed parental relationships and de novo in two additional unrelated patients with Cornelia de Lange syndrome with unconfirmed parental relationships, then …

PS2/PM6

https://clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_1.pdf

- parental confirmed
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Phenotypic consistency	Points per Proband	
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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

If a NIPBL variant was de novo in one patient with Cornelia de Lange syndrome, with confirmed parental relationships and de novo in two additional unrelated patients with Cornelia de Lange syndrome with unconfirmed parental relationships, then …

..Very Strong evidence level is applied (PS2_Very Strong) based on combined point value of 4 (Table 2).

PS2/PM6 – Additional considerations

- A patient with early infantile epileptic encephalopathy and a de novo *SIK1* variant with confirmed parental relationships is awarded 1 point (as the patient's phenotype is consistent with the gene but not highly specific and the variant is de novo with confirmed parental relationships). If this patient is the only de novo occurrence for the variant, then a Moderate strength level (PS2_Moderate) is applied.
- A patient with nonsyndromic intellectual disability and a de novo *ASH1L* variant is awarded 0.5 points (as the variant is de novo with confirmed parental relationships and patient's phenotype is consistent with the gene but not highly specific and there is significant evidence of genetic heterogeneity). If this patient is the only de novo occurrence for the variant, then a Supporting strength level (PS2_Supporting) is applied.
- A patient with developmental delay but no other features of Cornelia de Lange syndrome and a de novo *NIPBL* variant with unconfirmed parental relationships is awarded zero points as this phenotype is not consistent with the gene/disease association. If this patient was the only de novo occurrence for the variant, then no de novo criteria are applied.

	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	
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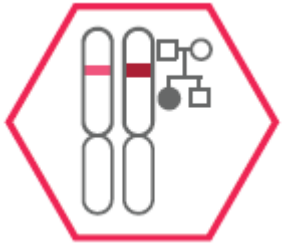
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation 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			

Case-specific data to consider

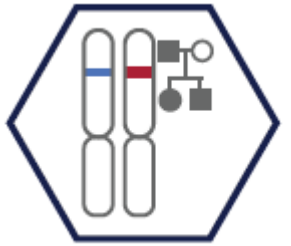
Case-Specific Evidence – Allelic Data

https://www.genomenon.com/mastermind-variant-interpretation-cards-download/



For recessive disorders, detected in trans with a pathogenic variant.

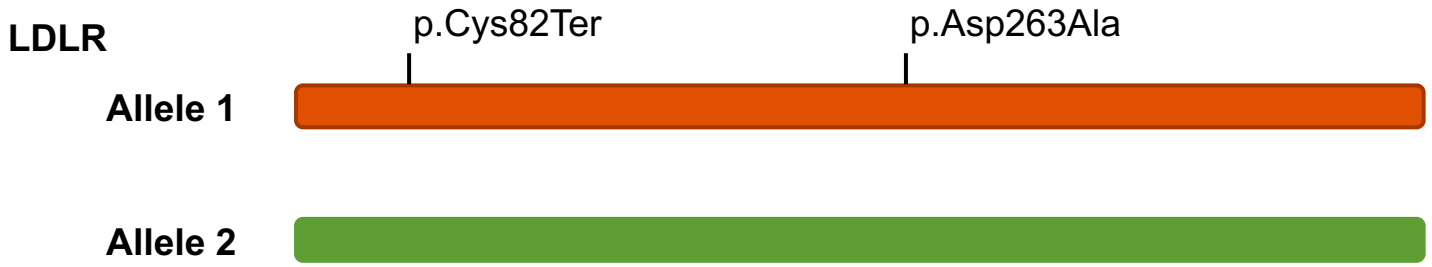
Note:
This requires testing of parents (or offspring) to determine phase.



Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

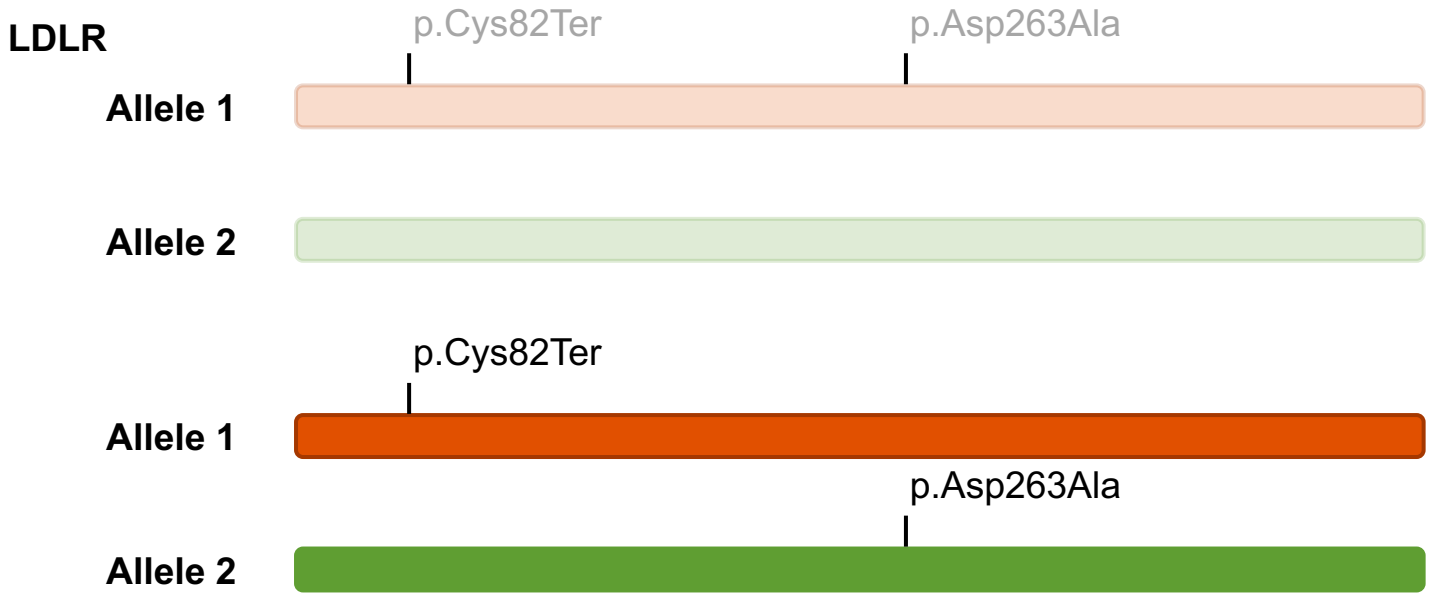
PM3/BP2

- Patient presents with Familial Hypercholesterolemia (AD)



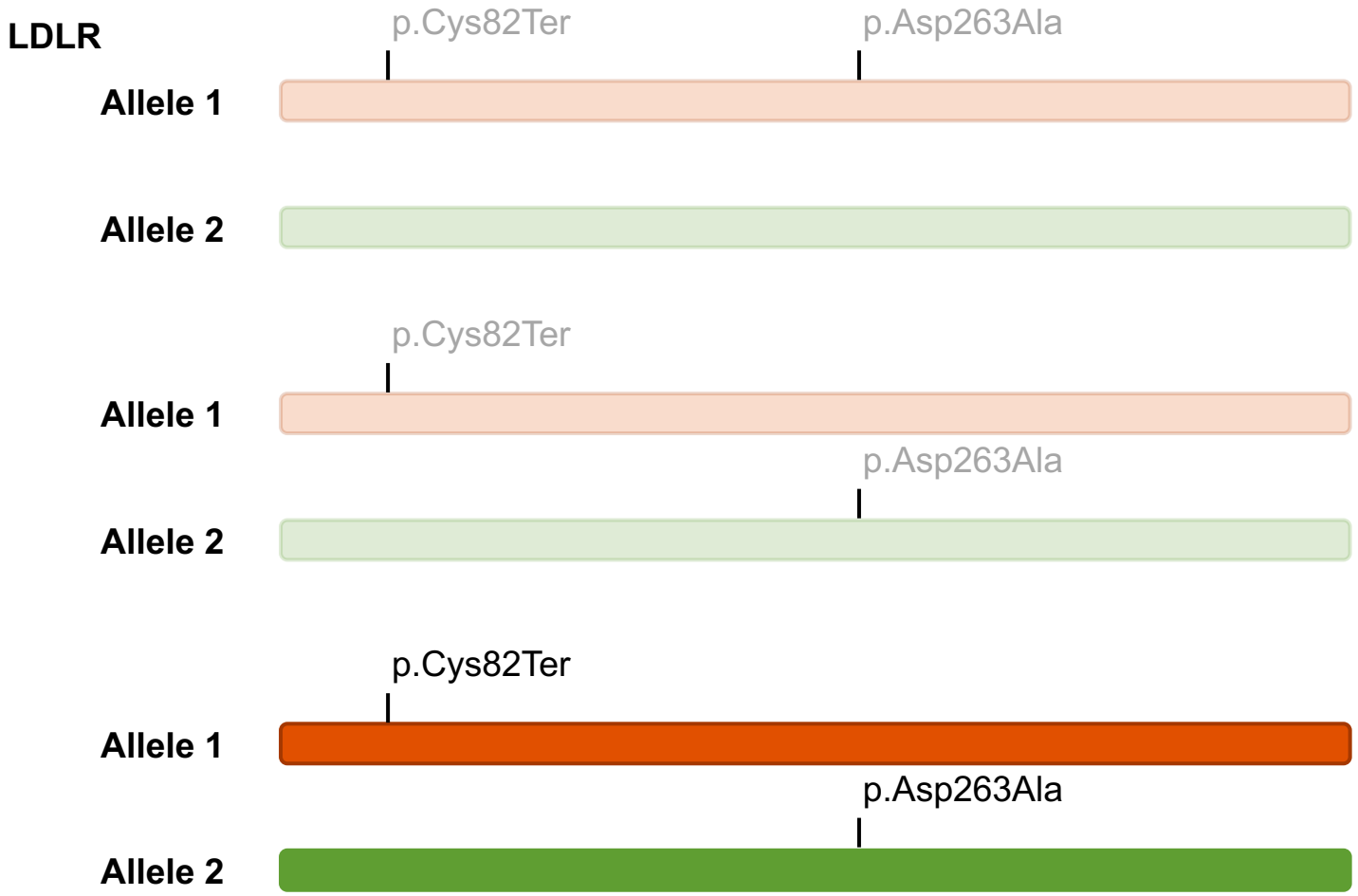
PM3/BP2

- Patient presents with Familial Hypercholesterolemia



PM3/BP2

- Patient presents with HoFH



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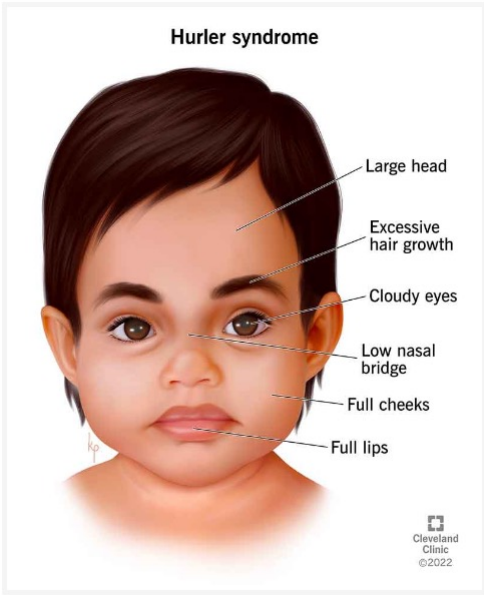
Other		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Case-specific data to consider

Case-Specific Evidence – Phenotype Specificity

PP4

Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.



https://my.clevelandclinic.org/health/diseases/24000-hurler-syndrome

+

enzyme α-L-iduronidase (IDUA)

	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		
Reputable sources		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			

PP5/BP6

NM_000249.4(MLH1):c.931A>G (p.Lys311Glu)

Interpretation:	**Likely pathogenic**
Review status:	★★★☆ reviewed by expert panel
Submissions:	5 (Most recent: Sep 24, 2021)
Last evaluated:	Mar 9, 2018
Accession:	VCV000230595.10
Variation ID:	230595
Description:	single nucleotide variant

Who is reputable?

Expert panel curation takes precedence (if available)

Submitted interpretations and evidence

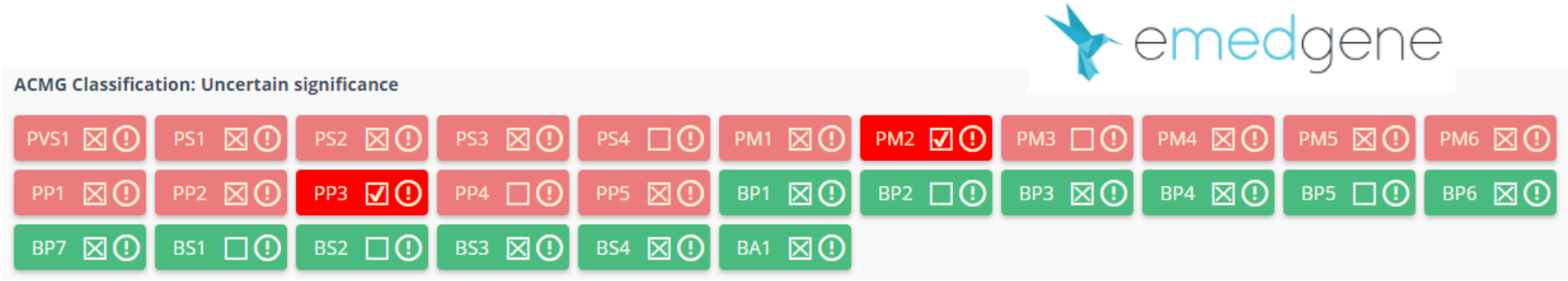
Interpretation (Last evaluated)	Review status (Assertion criteria)	Condition (Inheritance)	Submitter	More information
Likely pathogenic (Mar 09, 2018)	reviewed by expert panel (Guidelines v2.3) Method: curation	Lynch syndrome I Affected status: yes Allele origin: germline	International Society for Gastrointestinal Hereditary Tumours (InSiGHT) Accession: SCV000740673.1 Submitted: (Mar 23, 2018)	Other databases http://www.insight-database.org/... Comment: Multifactorial probability: 0.999 but with conflicting data. Reduced classification to class 4 pending somatic information.
Uncertain significance (Dec 06, 2019)	criteria provided, single submitter (Ambry Autosomal Dominant and X-Linked criteria (3/2017)) Method: clinical testing	Hereditary cancer-predisposing syndrome Affected status: unknown Allele origin: germline	Ambry Genetics Accession: SCV000274194.5 Submitted: (Nov 30, 2020)	Comment: The p.K311E variant (also known as c.931A>G), located in coding exon 11 of the MLH1 gene, results from an A to G substitution at nucleotide ... (more)
Uncertain significance (Apr 24, 2019)	criteria provided, single submitter (LabCorp Variant Classification Summary - May 2015) Method: clinical testing	not specified Affected status: unknown Allele origin: germline	Women's Health and Genetics/Laboratory Corporation of America, LabCorp Accession: SCV000696173.3 Submitted: (Sep 24, 2019)	Comment: Variant summary: MLH1 c.931A>G (p.Lys311Glu) results in a conservative amino acid change located in the N-terminal domain (IPR002099) of the encoded protein sequence. Four of ... (more)
Uncertain significance (Jun 21, 2020)	criteria provided, single submitter (Invitae Variant Classification Sherloc (09022015)) Method: clinical testing	Hereditary nonpolyposis colorectal neoplasms Affected status: unknown Allele origin: germline	Invitae Accession: SCV000543638.6 Submitted: (Jan 07, 2021)	Publications: PubMed (4) Comment: This sequence change replaces lysine with glutamic acid at codon 311 of the MLH1 protein (p.Lys311Glu). The lysine residue is highly conserved and there is ... (more)
Uncertain significance (Jun 11, 2020)	criteria provided, single submitter (GeneDx Variant Classification Process June 2021) Method: clinical testing	Not Provided Affected status: yes Allele origin: germline	GeneDx Accession: SCV000565923.3 Submitted: (Sep 24, 2021)	Comment: Not observed at a significant frequency in large population cohorts (Lek et al., 2016); In silico analysis supports that this missense variant has a deleterious ... (more)

https://my.clevelandclinic.org/health/diseases/24000-hurler-syndrome

Publicly Available Calculators and Workflows

- Publically available tools that will help tally up your “points”
  - https://varsome.com/
  - http://wintervar.wglab.org/
  - http://www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/
  - https://mobidetails.iurc.montp.inserm.fr/MD/

- Several analysis software integrate guidelines into their workflow



Publicly Available Calculators and Workflows

- http://www.medschool.umaryland.edu/Genetic_Variant_Interpretation_Tool1.html/

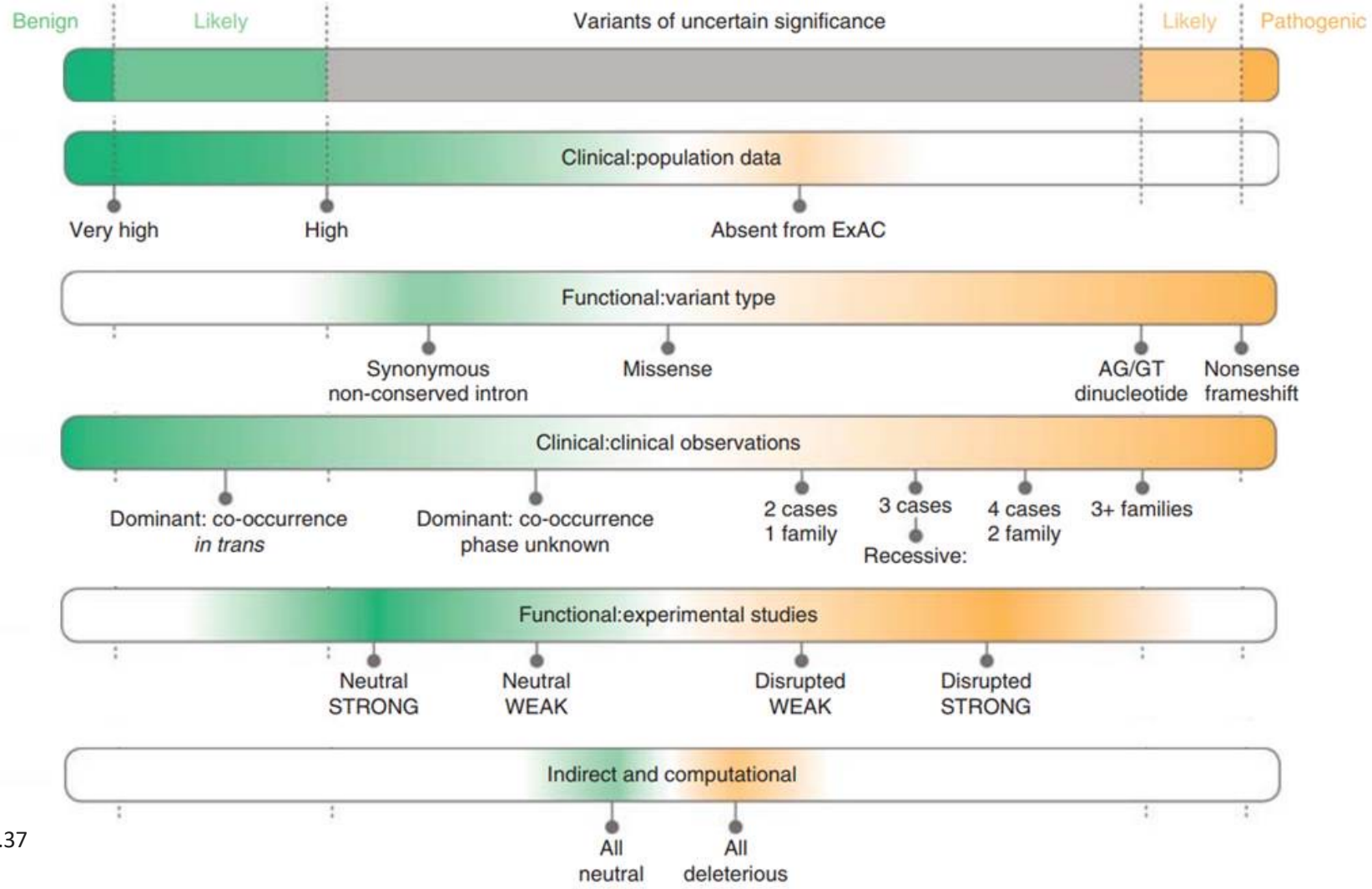
X PVS1 null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where LOF is a known mechanism of disease

_ PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change
_ PS2 De novo (both maternity and paternity confirmed) in a patient with the disease and no family history
_ PS3 Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product
_ PS4 The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls
_ PP1 (Strong evidence) Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

_ PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
X PM2 Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
_ PM3 For recessive disorders, detected in trans with a pathogenic variant
_ PM4 Protein length changes as a result of in-frame deletions/insertions in a nonrepeat region or stop-loss variants
_ PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
_ PM6 Assumed de novo, but without confirmation of paternity and maternity
_ PP1 (Moderate evidence) Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease

Variant Classification:
Likely pathogenic (I)

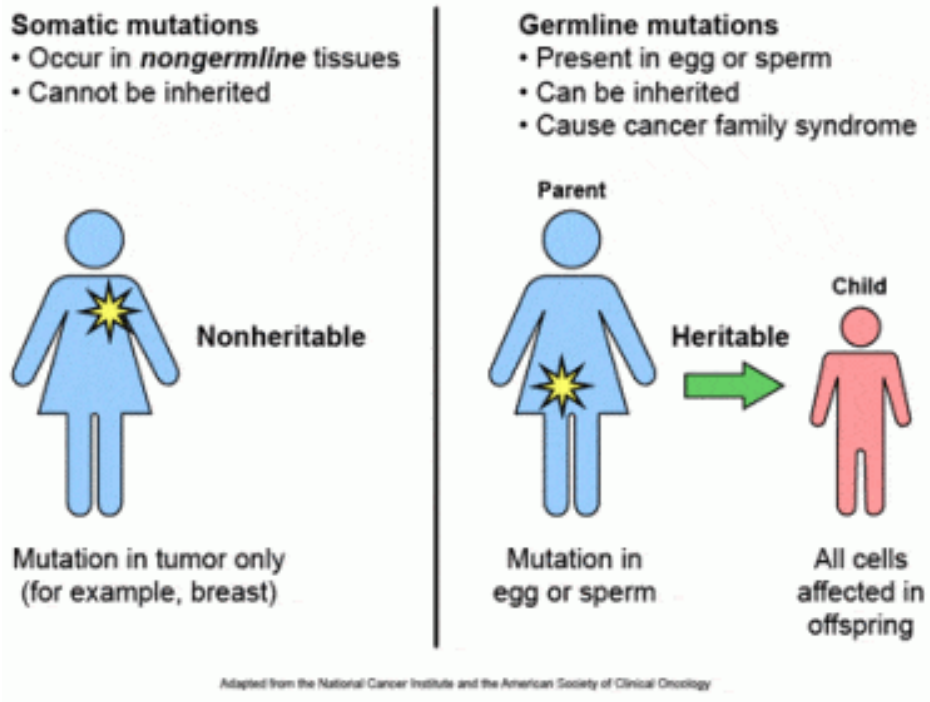
The ACMG guidelines are not mandatory, or the only ones used



https://doi.org/10.1038/gim.2017.37

Warning!

Germline and Somatic Classification and Catalogue Differences



OncoKB

ClinVar

COSMIC
Catalogue Of Somatic Mutations In Cancer

human phenotype ontology

OMIM®

Warning!

Germline and Somatic Classification and Catalogue Differences

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

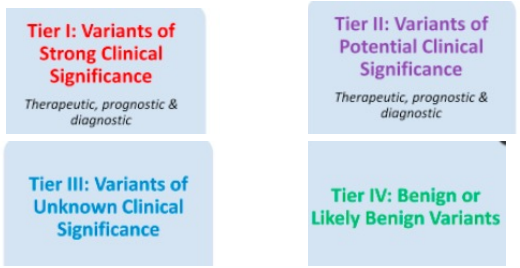
Germline mutations
- Present in egg or sperm
- Can be inherited
- Cause cancer family syndrome

Categories:

Diagnostic

Prognostic

Therapeutic



Categories:

Pathogenic

Likely Pathogenic

VUS – Variant of Uncertain Significance

Likely Benign

Benign

Framework Summary for Variant Interpretation – 6 key questions

- Allele Frequency?
- What is the mechanism of disease?***
- Known or predicted impact?
- Do we have functional evidence? How reliable?
- Phenotype overlaps with gene-disease association described?
- Does it segregate with disease?

Questions?

VUS examples

Gene	Genomic position	Coding DNA	Variant	Inheritance
DDX41	Chr5:176941942G>A	c.773C>T	p.Pro258Leu	Mother Neg (Healthy) Father Neg (Healthy)

Age: 71 y
Sex: Male
RFR: Pancytopenia
Family History: Negative

Gene	Genomic position	Coding DNA	Variant	Inheritance
RTEL1	Chr20:622908596A>G	c.101A>G	p.Gln34Arg	Mother is Neg (Healthy) Father is Het (Affected)

Age: 62 y
Sex: Female
RFR: Idiopathic pulmonary fibrosis and short telomeres
Family History: Father and Brother are affected and carry the mutation.
Sister is affected and does not carry the mutation.
Cousin is unaffected and carries the variant

VUS examples

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