Variant Curation Evidence and Assertion Criteria

What is variant curation?

To determine whether a genetic variant is pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, or benign, genetics professionals **perform an assessment of available data** about the variant of interest. Upon reviewing the evidence, they **apply assertion criteria** to ultimately **determine the pathogenicity** of the variant.

Benign	Likely Benign	Variant of Uncertain Significance (VUS)	Likely Pathogenic	Pathogenic

To curate small sequence changes, genetics providers and professionals generally utilize criteria outlined in joint guidelines from the **American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP),** which were published in 2015. These criteria include weighted **codes**, which have various strengths ranging from Supporting to Very Strong. Each code represents a type of evidence that can be applied. Over time, modifications have been applied to these guidelines, and laboratories also can vary in how they apply criteria from these guidelines. Additionally, for some specific genes or diseases, there are ClinGen Variant Curation Expert Panels (VCEPs) which have more refined criteria to determine pathogenicity of variants.

What are the main types of evidence?

Population Frequency

How frequently is the variant seen in healthy populations?

Computational Data

Do in silico tools predict that the variant has an impact on the protein?

Predictive Data

Does the variant affect important domains or cause loss of protein function?

Functional Data*

Do functional assays show a pathogenic effect on the protein?

Proband and Family Data

Has the variant been found in patients with the associated disease and in their family members?

Each of these types of evidence have specific codes that are assigned. Codes can be applied at **four different levels: Supporting (P), Moderate (M), Strong (S), Very Strong (VS)**. Below, you can find commonly used codes that are associated with each of these pieces of evidence:

	 Population Frequency	 Computational Data	 Predictive Data	 Functional Data	 Proband and Family Data
Pathogenic	PM2_P	PP3	PVS1, PS1, PM5, PM4	PS3	PS4 (Dominant), PM3 (Recessive), PP1 (Segregations), PM6 (de novo)
Benign	BS1, BA1	BP4	BP7, BP1	BS3	BP2, BP5, BS4

* **Functional Data will not be detailed in this handout**

How are these types of evidence used in variant curation?

Certain types of evidence provide more support for or against pathogenicity than others. Furthermore, a combination of several types of evidence is required to prove whether a variant is pathogenic. In general:

Examples of Strong Evidence

- ✓ If a variant is **found in many patients and family members** with a specific health condition, this can provide up to a strong line of evidence that the variant may be pathogenic.
- ✓ If a variant occurs **more frequently in population databases** than the prevalence of disease, this is a very strong indicator that the variant may be benign.
- ✓ If a variant occurs in a gene for which loss of protein function has been established in literature and by experts as a mechanism of disease, and the **variant introduces an early stop codon or causes a frameshift**, this may provide strong evidence that the variant is pathogenic.

- ✓ If an **animal model of the variant** exhibits a similar phenotype to the associated disease, this provides strong evidence that the variant may be pathogenic. If not, it may provide strong evidence that the variant may be benign.

Examples of Moderate Evidence

- ✓ If a few **amino acids are predicted to be removed in-frame**, this provides a moderate line of evidence that the variant may be pathogenic.
- ✓ Some **functional studies** provide up to a moderate line of evidence that the variant may be pathogenic.

Examples of Supporting Evidence

- ✓ If **computational tools** predict that the variant causes/does not cause an impact to the protein, this provides a lower level of support that a variant may be pathogenic/benign.
- ✓ If a variant is **absent from population databases**, this provides a lower level of support that a variant may be pathogenic.

How much evidence is needed for a variant to be considered Likely Pathogenic or Pathogenic?

To reach **Likely Pathogenic**, a variant must have:

- ✓ 1 Moderate AND ≥4 Supporting
- ✓ 2 Moderate AND ≥2 Supporting
- ✓ 1 Strong AND 1-2 Moderate
- ✓ 1 Very Strong AND 1 Moderate

To reach **Pathogenic**: a variant must have:

- ✓ 1 Strong and either: ≥3 Moderate, 2 Moderate and ≥2 Supporting, 1 Moderate and ≥4 Supporting
- ✓ ≥2 Strong
- ✓ 1 Very Strong and either: ≥1 Strong, ≥2 Moderate, 1 Moderate and 1 Supporting, ≥2 Supporting

Where can I learn more about variant curation guidelines?

1. *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* (PMID: 25741868): **https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4544753/**
    a. These are the general guidelines for interpretation of small sequence changes.
2. Criteria Specification Registry: **https://cspec.genome.network/cspec/ui/svi/**
    a. Guidelines for specific genes or diseases, as developed by corresponding Variant Curation Expert Panels, are available through this resource.

Population Frequency & gnomAD database

An important part of variant curation is determining whether or not a variant has been found in the general population and at what **allele frequency (AF)**. If a variant is found at a frequency that is higher than expected for the disease, the variant is most likely not causing disease. The most common way to determine the AF of the variant is by using gnomAD. Below, find information on how to use gnomAD and apply the codes associated with AF.

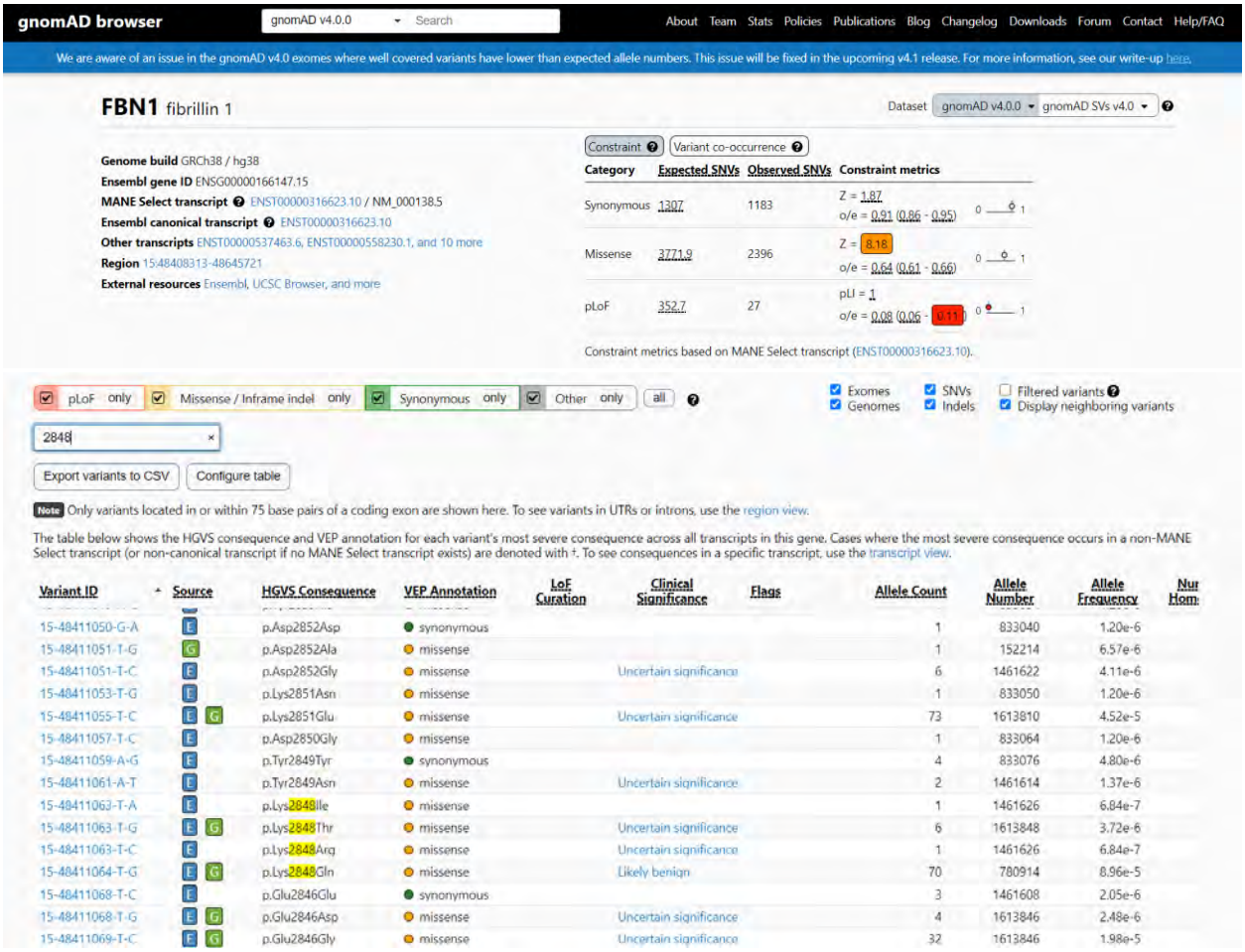
gnomAD Database
Website: https://gnomad.broadinstitute.org/

Overview: The gnomAD database is a collection of variants that have been found in a multi-ethnic population, which presumably are free of severe childhood onset disease (PMID: 32461654). We use the allele frequency of the highest population (MaxAF) to determine whether a pathogenic or benign code should be applied to the variant. Typically, do not use populations which have less than 2,000 alleles (1,000 individuals) for consideration of MaxAF.

How to search for a variant:
Since variants can differ in nomenclature depending on the build and transcript used, the best way to get to a gnomAD variant is by using Franklin or Varsome (see below). However use the following guide for searching directly on gnomAD.

You can search using the genomic coordinate directly from the first page (for example "1-55051215-G-GA", which includes: "chromosome-genomic coordinate-ref allele-alt allele"). However the easier way to do this is by first going to the gene page, and then scrolling or searching to find the variant of interest. If your variant is not present, that means that it was not identified in the control population.
Please note:

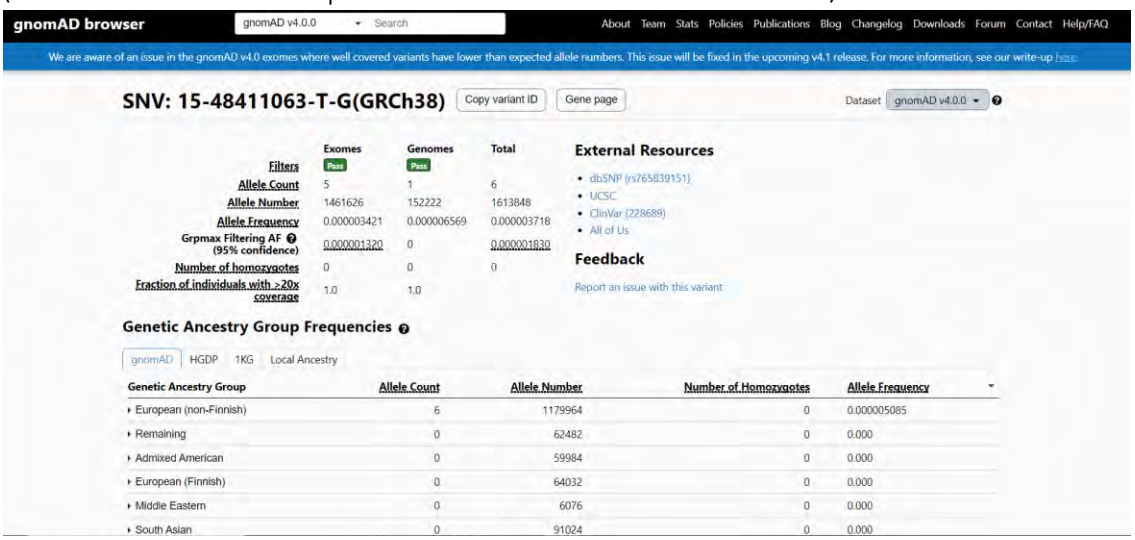
- ✓ It is important to confirm that the build and the transcript that is being used by gnomAD is the same as your variant. If the transcript you were given for the variant is different than the one listed in gnomAD (MANE select transcript), you can instead use the genomic coordinate (g.). However the genomic coordinate will differ based on the genomic build that was used. gnomAD version 2 is on build37 (hg19), gnomAD version 3 and 4 are on build38 (hg38).
- ✓ A variant may be present in one version, but absent in another. To toggle between the different versions of gnomAD, click on the arrow next to the dataset version (Can be found at the top right of the screen) and click on the version you would like to view. Typically, the newest version of gnomAD should be used as it has the biggest sample size.



You can use the "Search variant table" feature located above the "Export variants to CSV" button to find an amino acid p. Or c. (Please note: this variant table may appear on a different place of the page depending on the size of your browser).

If you click on the link to the variant page (blue coordinates in the Variant ID column), you will be able to see population frequencies. We would use the frequency of the most common population

(in the case below - "European" at an AF of 0.000005 or 0.0005%).



gnomAD can also give you other information about the gene or variant:

- ✓ External resources - It provides links to ClinVar or UCSC if available
- ✓ Variant Effect Predictor - it can show you what effect the variant has on alternative transcripts or in other genes
- ✓ In silico predictions - it will give you REVEL scores and other computational predictors
- ✓ Gene constraint scores - it can show you if the gene is constrained for loss of function or missense variants (i.e. it has a higher or lower than expected number of those variants)
- ✓ Age distribution: gnomAD will sometimes show you the ages of the individuals who have that variant. This may be helpful to understand the likelihood that those individuals would already have the disease in question. For example, if the individuals are under the age that you would expect to see the disease in question, you may not consider those individuals as helpful to your curation.

How to determine if the allele frequency is high or low: Allowed AF is different for each gene and condition, and depends on a number of things including penetrance, prevalence, mode of inheritance and allelic/genetic heterogeneity (whether or not multiple genes or variants can cause the condition). We have compiled a list of AF cutoffs for different conditions below. If the allele frequency of your variant is *below* the **PM2_Supporting** cutoff, you can apply that code. If the AF is *above* the **BS1 or BA1** cutoff, the respective code can be applied. Please note that BA1 is a stand alone code. Therefore if your variant reached BA1 it is automatically considered benign. (Please note that there are exceptions to this rule such as hypomorphic or low penetrance variants, etc.)

How each factor influences the allele frequency of a gene/disease pair:

- ✓ *Mode of inheritance*: The allowed AF of a recessive condition will be higher than a dominant condition, because unaffected carriers will be present in the database and are expected

- ✓ *Penetrance*: If a condition has low penetrance, the allowed AF will be higher, as there will be more "healthy" individuals with a pathogenic variant that will not develop disease.
- ✓ *Prevalence*: If a disease is more common, we expect a higher allele frequency compared to a condition that is extremely rare
- ✓ *Allelic/genetic heterogeneity*: If many genes and variant exist that cause the same condition, the allowed AF should be reduced, as only some of the prevalence of the disease can be attributed to that specific variant.

How to calculate an allowed allele frequency if your gene does not have a VCEP guideline: For autosomal recessive conditions, the carrier frequency of a variant should not exceed the Hardy-Weinberg equilibrium based on the prevalence ($p^2 + 2pq + q^2 = 1$) where q^2 is the prevalence of the condition and 2pq is the carrier frequency (to calculate we set $p^2 = 1$).
An online calculator such as https://www.perinatology.com/calculators/Hardy-Weinberg.htm or https://www.cardiodb.org/allelefrequencyapp/ can be used to find the maximum allowed carrier frequency if you know the prevalence of the condition.

TIP: Caution should be taken in the case of hypomorphic alleles, which may not cause disease in the homozygous state, however may cause disease when in trans with a severe variant. These may be at higher frequencies in the general population or found in the homozygous state in healthy individuals, but still impact disease risk.

PM2_Supporting/BS1/BA1 Cutoffs

Please note: gnomAD will provide you the allele frequency and not the percentage. To use the below percentages, make sure to convert.

Gene/Disease	PM2_Supporting	BS1	BA1
ACADVL	0.1%	0.35%	0.7%
Brain Malformations		0.0185%	0.0926%
Cardiomyopathy (*MYH7*, etc.)	0.004%	0.02%	0.1%
CDH1	0.001%	0.1%	0.2%
Cerebral Creatine Deficiency (*GAMT*)	0.04%	0.1%	0.3%
Cerebral Creatine Deficiency (*GATM*)	0.0055%	0.01%	0.05%
Cerebral Creatine Deficiency (*SLC6A8*)	0.002%	0.02%	0.2%
F8	Absent in males	0.003%	0.03%

F9	Absent in males	0.00056%	0.0056%
DICER1	<0.0005%	0.03%	0.3%
BRCA1	absent	0.01%	0.1%
BRCA2	absent	0.02%	0.1%
Epilepsy - *SCN1A*	0-1 alleles	0.0004%	0.02%
Epilepsy - *SCN2A*	0-1 alleles	0.0002%	0.01%
Epilepsy - *SCN3A*	0-1 alleles	0.0002%	0.01%
Epilepsy - *SCN8A*	0-1 alleles	0.0002%	0.01%
Epilepsy - *SCN1B*	0-1 alleles	0.01%	0.3%
Familial Hypercholesterolemia (*LDLR*)	0.02%	0.2%	0.5%
FBN1	0.0005%	0.005%	0.1%
Glaucoma- *MYOC*	0.01%	0.1%	1%
Hearing Loss (Autosomal Recessive)	0.007%	0.3%	0.5%
Hearing Loss (Autosomal Dominant)	0.002%	0.02%	0.1%
ATM	0.001%	0.05%	0.5%
PALB2	0.0003%	0.01%	0.1%
Hereditary Hemorrhagic Telangiectasia - *ENG/ACVRL1*	0.004%	0.08%	0.2%
Colorectal Cancer - *APC*	0.0003%	0.001%	0.1%
LCA/early onset retinal dystrophy	0.02%	0.08%	0.8%
Lysosomal Diseases (*GAA*)	0.1%	0.5%	1%
RYR1 (Malignant	Not used	0.08%	0.38%

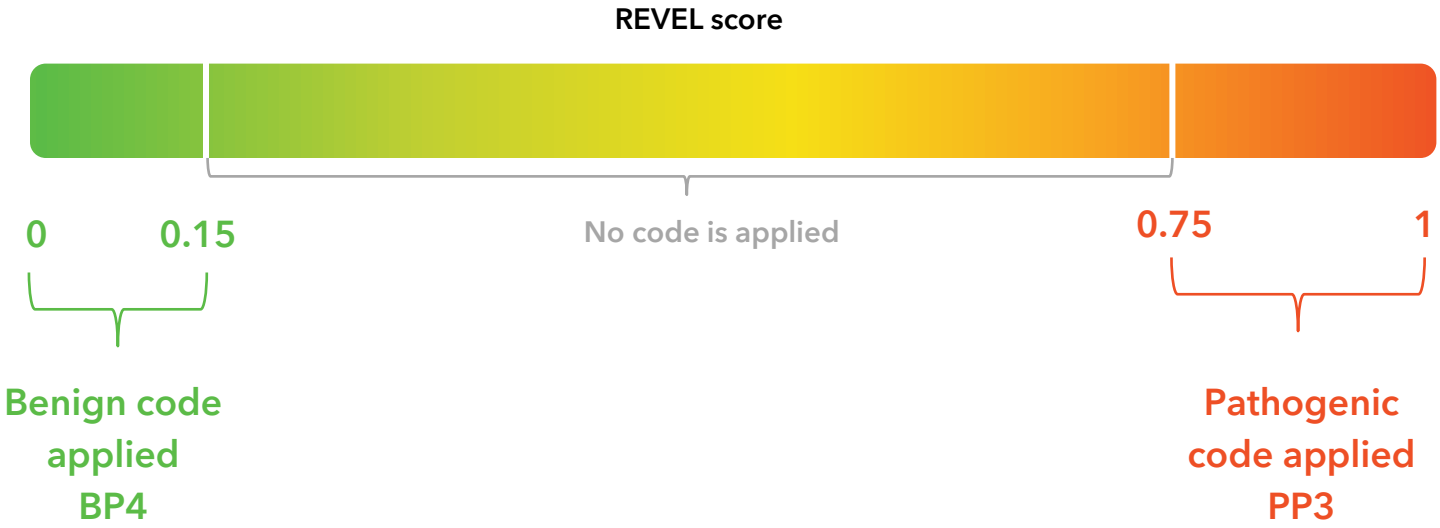
hyperthermia)			
SLC19A3	0.005%	0.05%	0.1%
PDHA1	0.00092%	0.092%	
POLG	0.05%	.5%	1%
ETHE1	0.002%	0.02%	0.1%
mtDNA genes	0.002%	0.5%	1%
Monogenic diabetes (*HNF1A*, *HNF4A*, *GCK*)	0.0003%*	0.003% (0.004% for *GCK*)	0.01%
RUNX1	absent	0.015%	0.15%
PAH	0.02%	0.2%	1.5%
Platelet disorders (*ITGA2B*, *ITGB3*)	0.01%	0.158%	0.24%
PTEN	0.001%	0.0043%	0.056%
Pulmonary Hypertension (*BMPR2*)	0.01%	0.1%	1%
RASopathy (*SHOC2, NRAS, RAF1, SOS1, SOS2, PTPN11, KRAS, MAP2K1, HRAS, MAP2K2, BRAF*)	absent	0.025%	0.05%
Rett and Angelman-like disorders (*MECP2, CDKL5, FOXG1, UBE3A, SLC9A6, TCF4*)	absent	0.008% (0.025% for *CDKL5*)	0.03% (0.05% for TCF4)
Severe Combined Immunodeficiency (SCID) - *ADA*	0.017%	0.16%	0.7%
SCID - *DLCRE1C*	0.003% and no homozygotes	0.078%	0.35%
SCID - *IL7R*	0.004%	0.13%	0.57%
SCID -*JAK3*	0.01%	0.1%	0.45%
SCID - *RAG1*	0.01%	0.19%	0.87%

SCID - *RAG2*	0.0059% and no homozygotes	0.19%	0.87%
SCID - *IL2RG*	0.01%	0.25%	1.1%
Thrombosis (*SERPINC1*)	0.002%	0.02% (be careful of frequent founder variants)	0.2%
TP53	Absent	0.03%	0.1%
VHL	0.00016%	0.0016%	0.016%

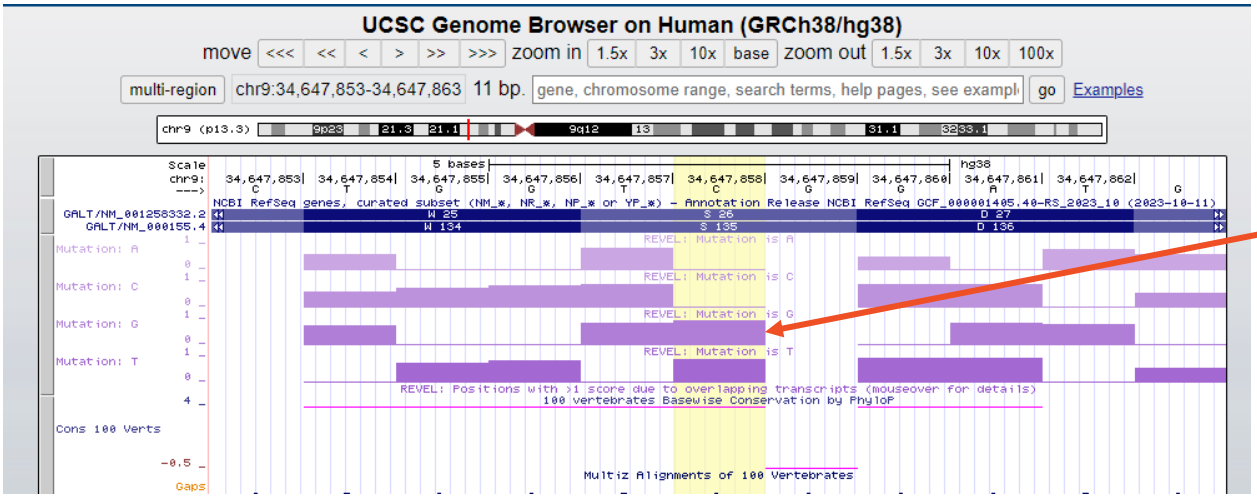
Computational Data

In silico prediction tools can be used as part of the variant curation process. Typically, these will only be assigned a supporting weight code (**PP3**). While there are many in silico tools (SIFT, PolyPhen, etc.) that can be used, typically the meta-predictor REVEL is used. The following REVEL scores are used:

*Please note that ClinGen VCEPs sometimes change the REVEL cutoffs. Please look at the VCEP for the disease you are curating to see if this aligns. If there is no VCEP, use the below.

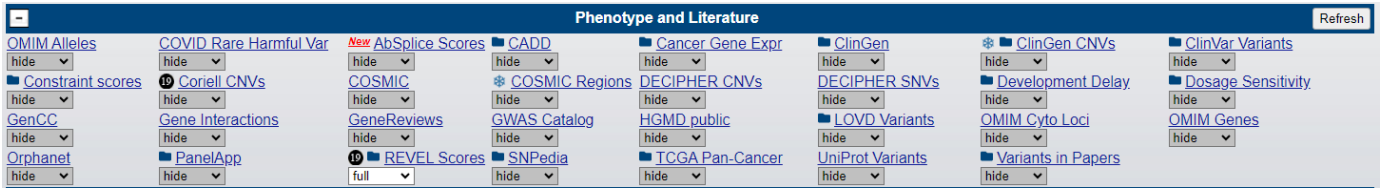


Where to find REVEL scores: REVEL scores can be found on the gnomAD website or on the curation tools detailed below (Franklin and Varsome). If your variant is not present in gnomAD, you can also add the REVEL score track on UCSC, which will give you the REVEL score for each variant at a specific location. See below for an example:



Hover over this bar to show the revel score if the variant changes the base from C>G.

If you do not have the REVEL track present on your view of UCSC, you can add it using the track feature at the bottom of the website. When you scroll down, you will see "REVEL Scores" under the "Phenotype and Literature" category:



Click here and change to "full". Then use the refresh button to add it to your current page.

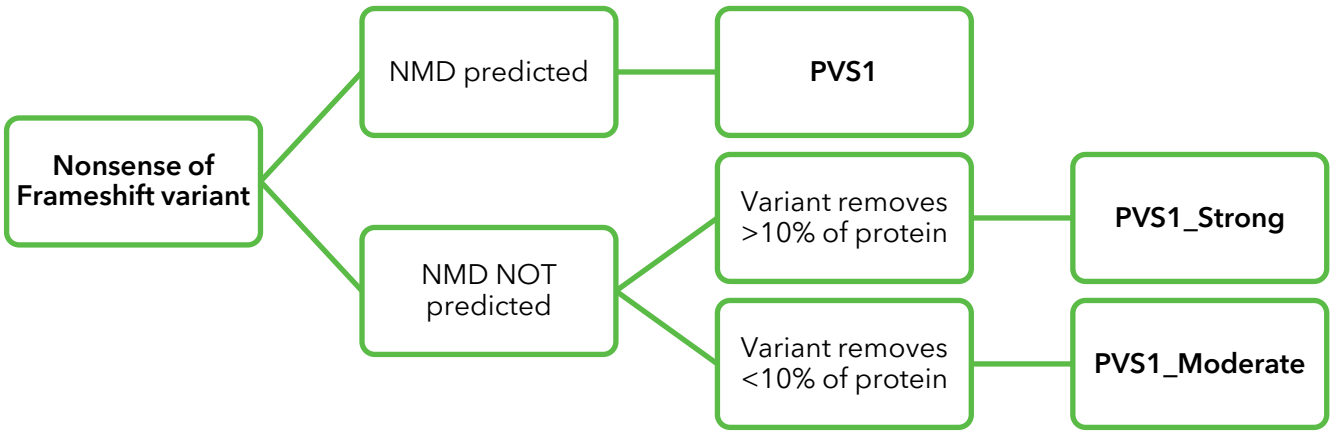
Predictive Data (PVS1, Loss of function)

Variants which predict a **loss of function** (such as frameshift, nonsense and splice site variants) can have a very strong code (PVS1) applied. This means that if the variant qualifies for PVS1 at the very strong level, the variant only needs to be found infrequently in the population (PM2_Supporting) to reach Likely Pathogenic. Please see the ClinGen specifications for using these codes here: https://clinicalgenome.org/site/assets/files/5182/pm2_-_svi_recommendation_-_approved_sept2020.pdf. Since these variants can make it to Likely Pathogenic without any other evidence (proband, functional, segregations, etc.), it is important to make sure that the loss of function variant qualifies for PVS1. For a full explanation of using PVS1, please see PMID: 30192042. Below find an overview of how to determine if PVS1 should be applied and at what level.

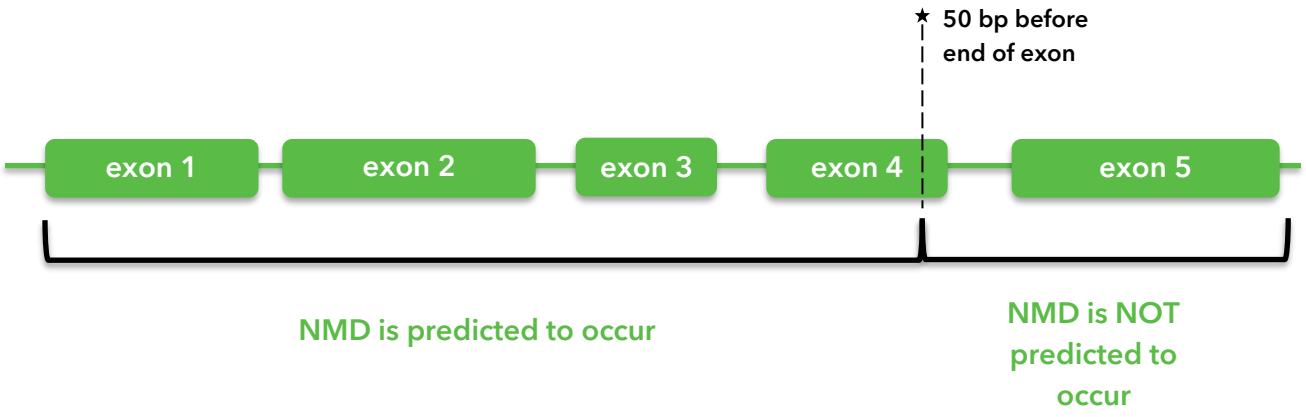
The following flowcharts can be used if:

- Loss of function is a proven mechanism of disease for the gene
- The exon that the variant is in is in biologically relevant transcripts and is not alternatively spliced (it is present in transcripts that are expressed in the body)

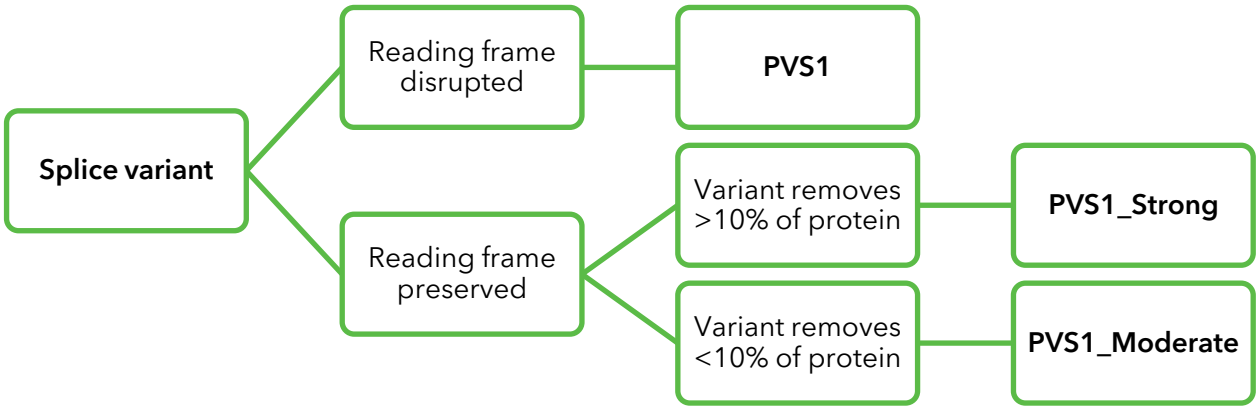
For **nonsense and frameshift variants**, the weight of PVS1 is determined by whether **nonsense mediated decay (NMD)** is predicted to occur and how much of the protein is removed.



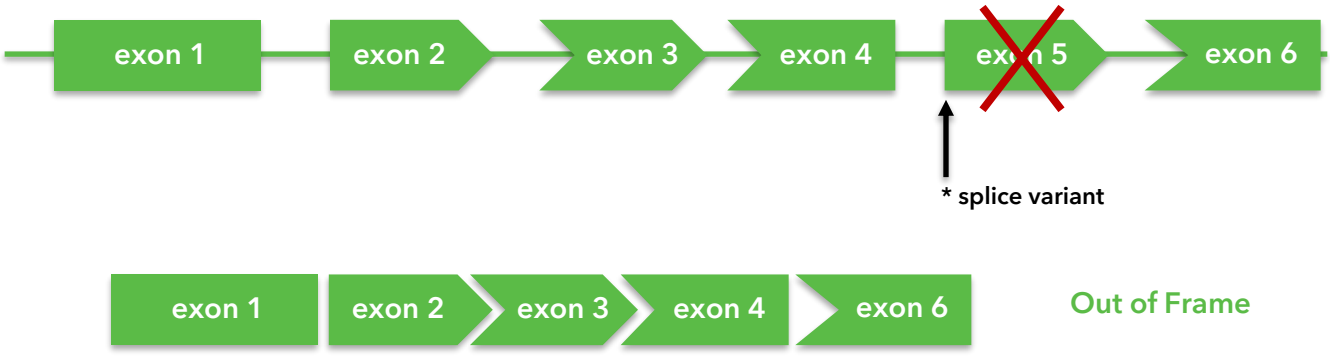
Nonsense Mediated Decay is a mechanism used by cells to degrade mRNAs that contain premature stop codons caused by nonsense or frameshift variants. However typically, **stop codons which are in the final exon or the 50bp of the second to last exon** are NOT predicted to have NMD occur.

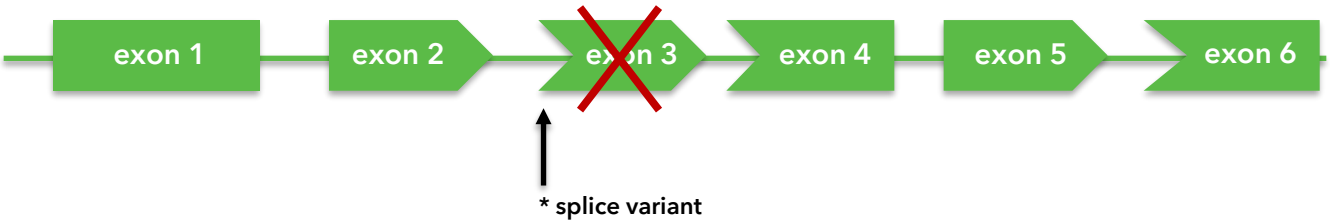


For splice variants, the weight of PVS1 applied is dependent on whether the removal of the exon that the splice variant impacts is out of frame or in frame.



An exon is **out of frame** (the reading frame is disrupted) if the number of nucleotides in the exon removed is NOT a multiple of 3. In this case, when the exon before and after are spliced together, the reading frame shifts causing frameshift or premature stops in the mRNA. An exon is **in frame** if the number of nucleotides is a multiple of 3. In this case, the exons before and after will splice together in a way that preserves the reading frame. In that case, the level of PVS1 is dependent how much of the protein is removed (less or more than 10%).




exon 1
exon 2
exon 3
exon 4
exon 5
exon 6
* splice variant


exon 1
exon 2
exon 4
exon 5
exon 6

In Frame

Proband and Family Data

A significant part of variant curation is looking in the literature for individuals with the variant of interest who are affected with the corresponding condition. The number of probands needed to reach a supporting, moderate or strong level of evidence depends on the disease inheritance and specificity. Below find general guidelines on how many probands to count.

Dominant Conditions:

Category 1: Common diseases (ex. Cardiomyopathy, BRCA1/2)

Probands needed	Strength of code
2-5	Supporting (PS4_P)
6-14	Moderate (PS4_M)
15+	Strong (PS4)

Category 2: Rare disease, but the phenotype is not specific (ex. Neurodevelopmental disorder)

Probands needed	Strength of code
1-2	Supporting (PS4_P)
3-4	Moderate (PS4_M)
5+	Strong (PS4)

Category 3: Rare disease with specific phenotypes (ex. Pheochromocytoma)

Probands needed	Strength of code
1	Supporting (PS4_P)
2-3	Moderate (PS4_M)
4+	Strong (PS4)

Recessive Conditions:

For recessive conditions, the points per proband depends on the zygosity of the variant. For homozygous variants, the allotted number of points below is assigned. For compound heterozygotes, the points assigned depend on whether the other variant found is pathogenic and whether it was confirmed to be *in trans*. and, for compound heterozygotes, the pathogenicity of the variant that is found along with your variant. Probands who have the variant in question in the heterozygous state WITHOUT another variant identified do not receive any points. Points are summed across all probands identified in the literature to determine the strength of the code.

Zygosity	Points assigned (per proband)
Homozygous variant in a proband with consanguineous parents	0.25
Homozygous variant	0.5
Compound heterozygous with a pathogenic variant found **in trans**	1
Compound heterozygous with a pathogenic variant of **unknown phase**	0.5
Compound heterozygous with a rare VUS **in trans**	0.25

Total points (summed across all probands found in literature)	Strength of code
0.5	Supporting (PM3_P)
1	Moderate (PM3)
2	Strong (PM3_S)
4	Very Strong (PM3_VS)

Segregations:

Segregations are the number of affected family members with the same variant as the proband and the same phenotype. For more information on how to count segregations please see ClinGen's training tool here: https://clinicalgenome.org/docs/segregation-analysis/

Dominant		Recessive	
Total segregations	**Strength of code**	**Total Segregations**	**Strength of code**
3	Supporting (PP1)	1	Supporting (PP1)
5	Moderate (PP1_M)	2	Moderate (PP1_M)
7	Strong (PP1_S)	3	Strong (PP1_S)

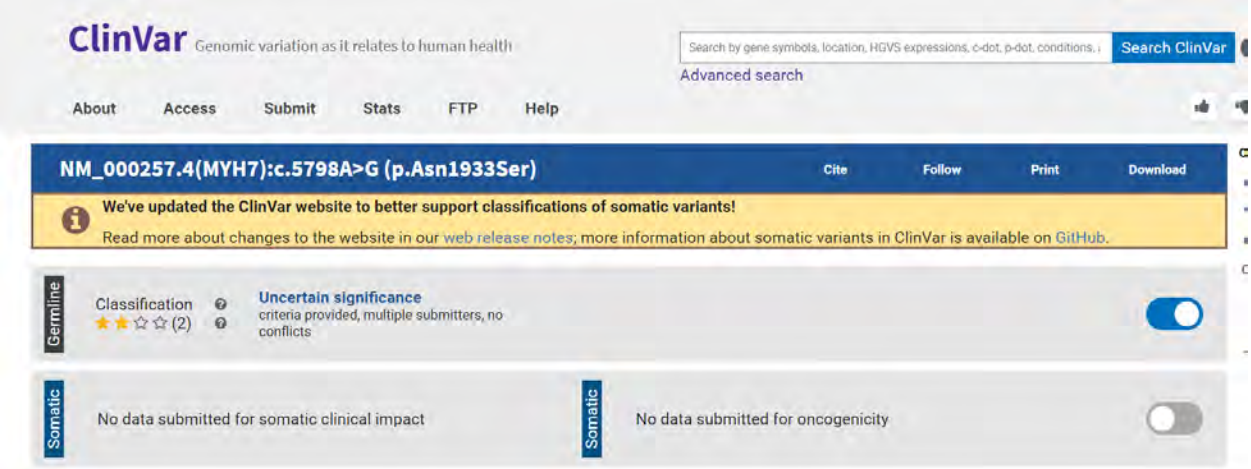
Variant Curation Databases

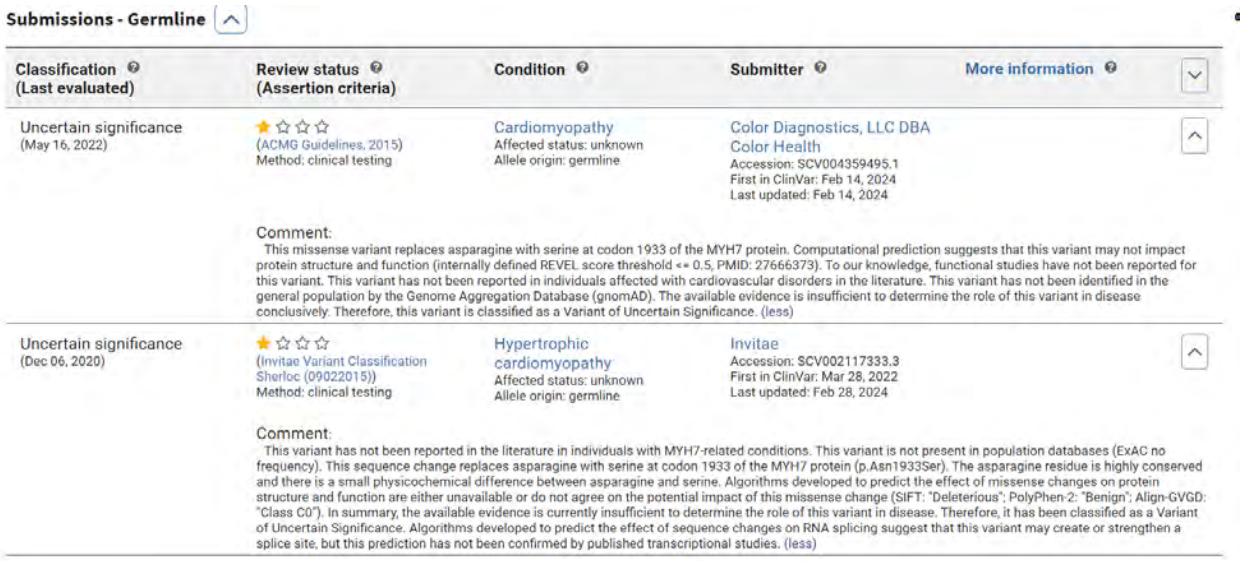
ClinVar

Website: https://www.ncbi.nlm.nih.gov/clinvar/

Overview: ClinVar contains entries for specific variants from various submitters (clinical labs, research labs, providers, etc.) asserting a pathogenicity. For instance, Invitae or GeneDx, may submit a variant as Pathogenic and may include information about why they have classified it that way.

How to use: Search for a variant of interest using the transcript and c. or by going first to the gene page and then scrolling to find your variant.
Note: Please make sure that the transcript that you are using matches the one used in ClinVar. Alternatively you can use the genomic coordinate.





Submissions - Germline

Classification (Last evaluated)	Review status (Assertion criteria)	Condition	Submitter	More information
Uncertain significance (May 16, 2022)	★☆☆☆ (ACMG Guidelines, 2015) Method: clinical testing	Cardiomyopathy Affected status: unknown Allele origin: germline	Color Diagnostics, LLC DBA Color Health Accession: SCV004359495.1 First in ClinVar: Feb 14, 2024 Last updated: Feb 14, 2024	
	Comment: This missense variant replaces asparagine with serine at codon 1933 of the MYH7 protein. Computational prediction suggests that this variant may not impact protein structure and function (internally defined REVEL score threshold <= 0.5, PMID: 27666373). To our knowledge, functional studies have not been reported for this variant. This variant has not been reported in individuals affected with cardiovascular disorders in the literature. This variant has not been identified in the general population by the Genome Aggregation Database (gnomAD). The available evidence is insufficient to determine the role of this variant in disease conclusively. Therefore, this variant is classified as a Variant of Uncertain Significance. (less)			
Uncertain significance (Dec 06, 2020)	★☆☆☆ (Invitae Variant Classification Sherloc (09022015)) Method: clinical testing	Hypertrophic cardiomyopathy Affected status: unknown Allele origin: germline	Invitae Accession: SCV002117333.3 First in ClinVar: Mar 28, 2022 Last updated: Feb 28, 2024	
	Comment: This variant has not been reported in the literature in individuals with MYH7-related conditions. This variant is not present in population databases (ExAC no frequency). This sequence change replaces asparagine with serine at codon 1933 of the MYH7 protein (p.Asn1933Ser). The asparagine residue is highly conserved and there is a small physicochemical difference between asparagine and serine. Algorithms developed to predict the effect of missense changes on protein structure and function are either unavailable or do not agree on the potential impact of this missense change (SIFT: "Deleterious"; PolyPhen-2: "Benign"; Align-GVGD: "Class C0"). In summary, the available evidence is currently insufficient to determine the role of this variant in disease. Therefore, it has been classified as a Variant of Uncertain Significance. Algorithms developed to predict the effect of sequence changes on RNA splicing suggest that this variant may create or strengthen a splice site, but this prediction has not been confirmed by published transcriptional studies. (less)			

You can read through the comments section of the lab submissions to determine if there are any publications of interest or whether or not there are functional or genetic data available.

Why labs may differ in their classification:

- ✓ Different interpretations of the guidelines used for variant interpretation (ACMG)
- ✓ Internal patient data and proprietary databases/tools
- ✓ Timing: Variant classification may change over time due to updated information/more publication data. Make sure to look at the date assessed to determine if a classification may be out of date

Franklin

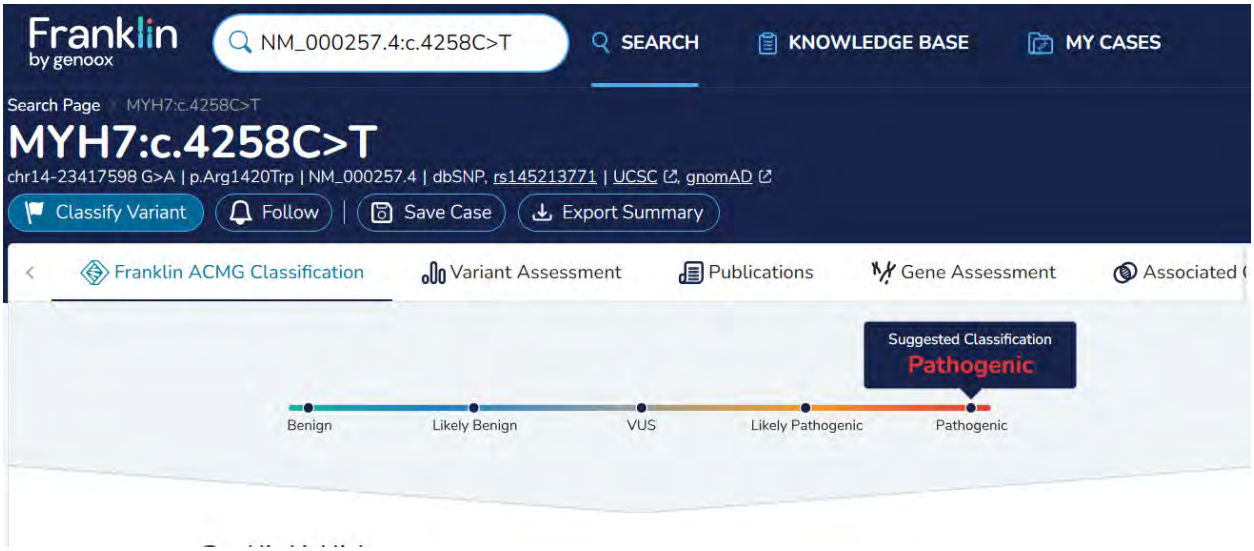
Website: https://franklin.genoox.com/clinical-db/home

Description: A variant curation tool, which compiles information about the variant from multiple other databases (such as gnomAD/ClinVar).

PLEASE NOTE: The initial automated classification that is given with Franklin should not be taken as the actual classification. Instead, use the tools provided to add your own codes and use Franklin only to provide information from relevant databases.

How to Use: Enter your variant into the search tool. (Suggested nomenclature is "Transcript : c." such as: NM_000368.5:c.359T>C)

Franklin will give you a suggested classification, along with which codes they are using. If you disagree with the codes, you can downgrade or add your own.

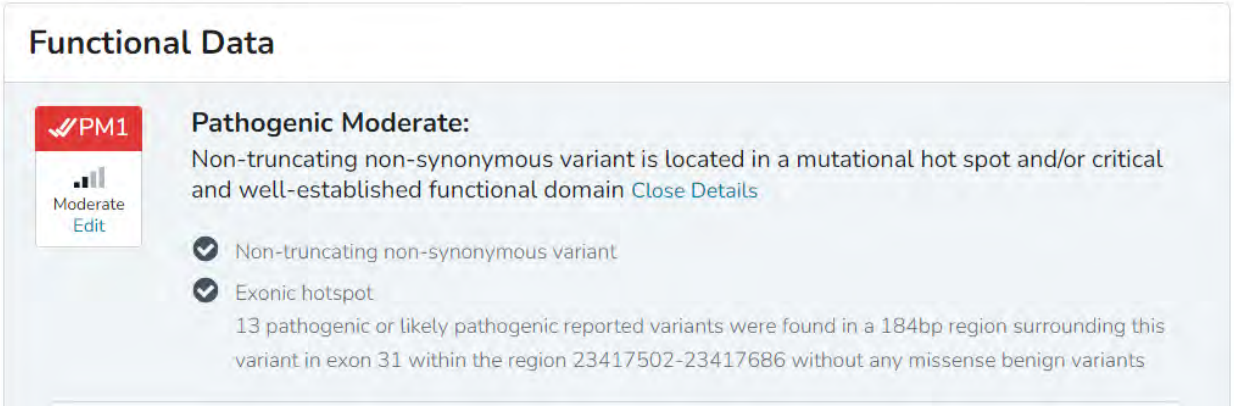


Franklin can tell you:

- ✓ If the gene has ClinGen specific guidelines
- ✓ Population frequency from multiple databases, including gnomAD
- ✓ ClinVar entries
- ✓ Summary of proband data that has been submitted to ClinVar
- ✓ Publications associated with variant (not always correct - publications may be missed or may not include the variant in question)
- ✓ If there are other variants at this location published in ClinVar
- ✓ Many in silico predictions

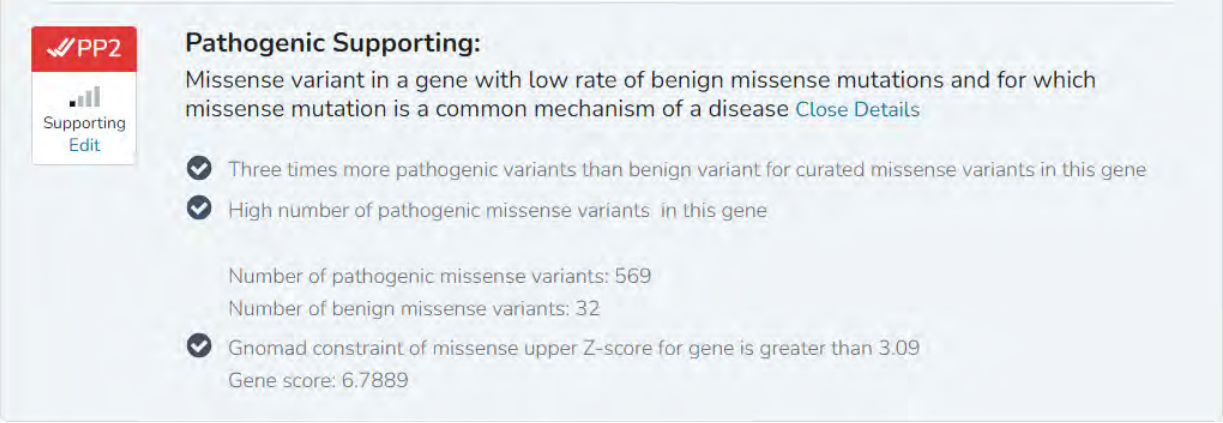
Things to consider with Franklin:

- o They use PM2 at the moderate level, which is no longer suggested by ClinGen Guidelines. PM2 should only be used at the supporting level (please see: https://clinicalgenome.org/site/assets/files/5182/pm2_-_svi_recommendation_-_approved_sept2020.pdf)
- o They may overcall PM1 (mutational hotspots). They just look at if there are other reported LP/P variants in the area instead of if there are published functional domains. Example (MYH7 p, NM_000257.4:c.4258C>T/p.Arg1420Trp :



The MYH7 gene has a ClinGen-approved mutational hotspot at amino acids 181-937. This would not qualify for PM1 based on the ClinGen rules.

- They may also overcall PP2, as it is just based on statistical analysis of ClinVar reports (which may not be accurate)



- They may use PP5 and BP6 based on ClinVar reports (which may not be accurate)

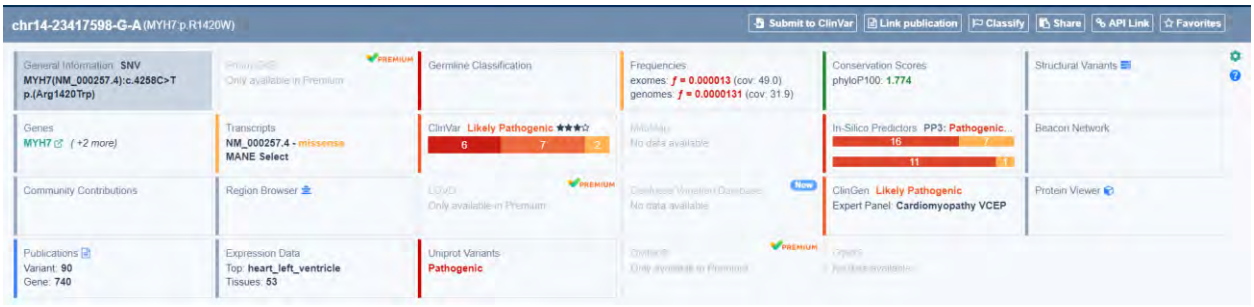
VarSome

Website: https://varsome.com/

Description: A variant curation tool, which compiles information about the variant from multiple other databases (such as gnomAD/ClinVar).

How to use: Enter your variant into the search tool. (Suggested nomenclature is "Transcript : c." such as: NM_000368.5:c.359T>C).

Varsome will then give you links and information from many different databases. If you click on "Germline Classification" it will suggest codes to use.

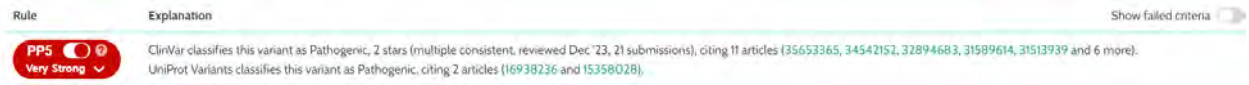


Varsome can tell you:

- ✓ Population frequency from databases (gnomAD)
- ✓ ClinVar entries
- ✓ Publications associated with variant (not always correct - publications may be missed or may not include the variant in question)
- ✓ Many in silico predictions

Things to consider with VarSome:

- o May overuse PP5 (reputable source calls Pathogenic)
- o They may overcall PM1 (mutational hotspots). They just look at if there are other reported LP/P variants in the area instead of if there are published functional domains (similarly to Franklin above). Example: it uses a Very Strong code just based off of ClinVar Submissions:



Glossary

Genotype	A score of the type of variants present at a specific location within the genome (i.e. What variants are present in a specific gene in a patient, and how many copies of each are there?) ❖ *Example: e3/e3 is the most common genotype for* APOE.
Phenotype	The manifestation of the disease of interest in the patient. ❖ *Example: The patient's phenotype involves ectopia lentis, aortic dilatation, pectus carinatum, and arachnodactyly. They would be a good candidate for genetic testing for Marfan syndrome.*
Affected	A descriptor of an individual with the phenotype. ❖ *Example: Has this variant been found in individuals affected with cystic fibrosis?*
Unaffected	A descriptor of an individual without the phenotype. For some phenotypes, evaluations may need to be done to confirm whether someone is truly unaffected. ❖ *Example: Although the patient's mother passed away from Huntington's disease, he was very surprised that the cause of her death was genetic because all three of his mother's siblings were unaffected, and his maternal grandparents died in a car crash when they were younger than the age of onset for Huntington's disease.*
Allele Frequency	The frequency at which a specific genetic variant is seen in the general population or in certain ancestral groups ❖ *Example: The allele frequency of this benign variant in European populations is 5%.*
Proband	A patient that has the variant of interest and the phenotype associated with that variant or the gene that the variant is in. There can only be one proband within each affected family, regardless of whether there are other individuals with both the variant and phenotype of interest. ❖ *Example: The proband is a 45 year old woman, assigned female at birth, with a personal history of bilateral breast cancer, and a family history of breast and ovarian cancer. She was identified to have the p.Ala295Thr variant in* BRCA2.
Segregations	Refers to family members of a proband who also have the variant and phenotype of interest. Can also be used as a verb to describe whether the variant has been identified in affected individuals in the family. ❖ *Example: The proband's mother has a personal history of breast cancer, and her maternal aunt was recently diagnosed with epithelial ovarian cancer. Both of them underwent genetic testing and were identified to have the p.Ala295Thr variant in* BRCA2, *which had previously been identified in the proband.*

	Therefore, this variant segregated with disease in two affected individuals in this family.
in cis	A descriptor for variants inherited from the same parent of a proband. ❖ *Example: The two variants in the CFTR gene identified in our patient were inherited from the patient's father. Those variants were therefore inherited* in cis.
in trans	A descriptor for variants inherited from different parents of a proband. Typically, for autosomal recessive conditions, two variants must be inherited *in trans* to cause the phenotype. ❖ *Example: One variant in the CFTR gene was inherited from the patient's father, and another variant in the CFTR gene was inherited from the patient's mother; therefore, the variants were inherited* in trans.
De novo	A descriptor for variants that were not identified in either of a patient's parents. A variant is assumed *de novo* if parentage (confirmation of whether an individual's parents are genetically their parents) has not been confirmed, and it is confirmed *de novo* if parentage has been confirmed. ❖ *Example: Both parents were tested for the novel variant identified in our patient, and neither of them have the variant. The lab also confirmed parentage of both parents. This variant was identified* de novo *in our patient.*
Phasing	A verb to describe the process of determining whether variants are *in cis, in trans*, or if a variant is *de novo.* Also a general descriptor for how variants are inherited in reference to a proband's parents. ❖ *Example: To help us determine the likelihood that this* CFTR *VUS may be pathogenic, we should determine the phasing of this variant in reference to the other pathogenic variant that our patient has. Are the parents available for testing?*

If you have any questions or feedback on this handout, would like to suggest changes or inquire about additional training, please contact the Variant Curation Task Force at variantcurationrequests@mgb.org.

Thank you to the following people for helping in the development of this handout: Lauren Briere, MS, CGC, Helen Chen, MS, CGC, Emma Henricks, MS, CGC, Anna Nagy, MS, CGC, Chelsea Stevens, MS, CGC, the Variant Curation Task Force members