MAYO CLINIC

CLINICAL VARIANT INTERPRETATION

USING ACMG/AMP CRITERIA TO CLASSIFY VARIANTS

Joe Farris, Ph.D.

Meeting Name here
Date and Location here

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Part 1: ACMG Criteria

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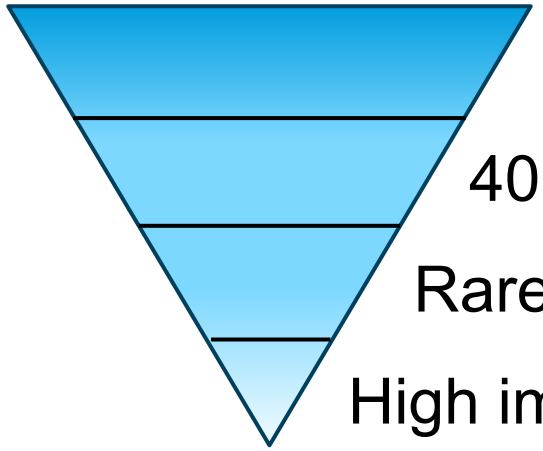
Genome Sequencing Yields Many Variants



4.1 – 5.0 million total variants

40,000 – 250,000 rare variants

Rare variants in disease relevant genes (n = ?)

High impact, rare variants in disease relevant genes (n = ?)

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

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Review: Terminology …

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

Variants

- **Missense variant:** variant leading to a protein change (ex. Arg515Ser)
- **Nonsense variant:** variant leading to the introduction of a premature stop codon (ex. Ser44Ter)
- **Silent variant:** variant leading to no protein change (but may have an effect on splicing)
- **Indel:** an insertion and/or deletion
- **Loss of function (LOF) variant**: a variant leading to truncation of the gene / protein.

Other terms

- **Proband:** the individual presenting with disease
- **Penetrance:** the proportion of individuals with a pathogenic variant in a given gene who express the associated trait (disease).

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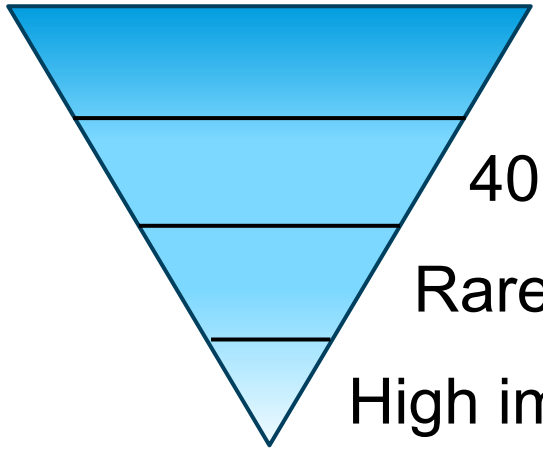
Genome Sequencing Yields Many Variants



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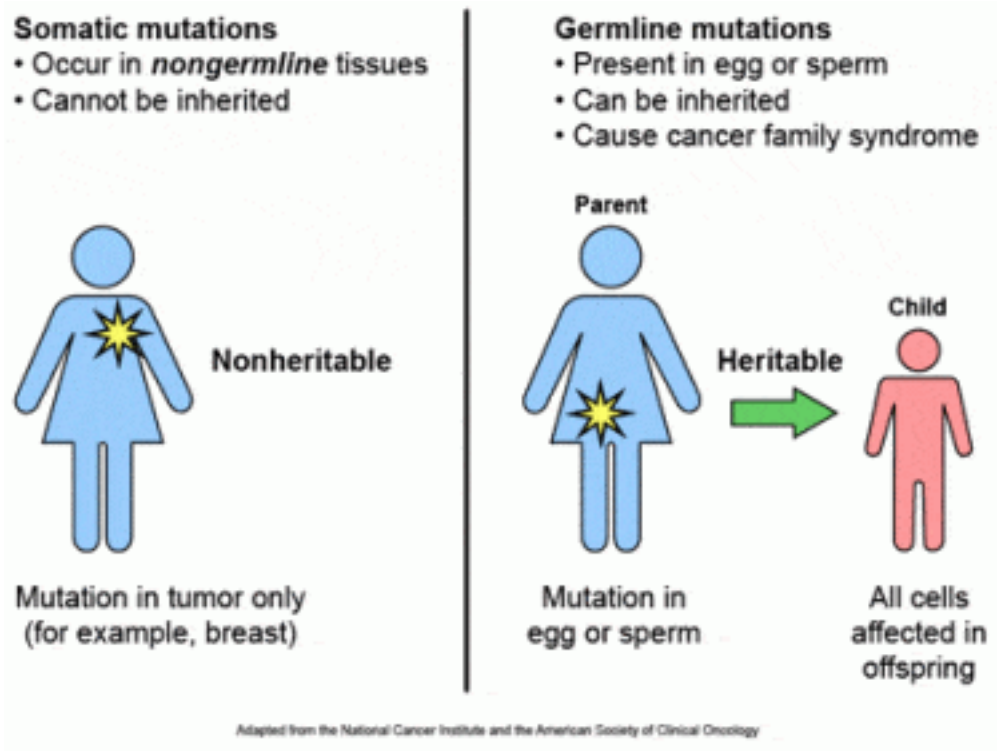
ACMG Criteria 2015

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

Sue Richards, 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.



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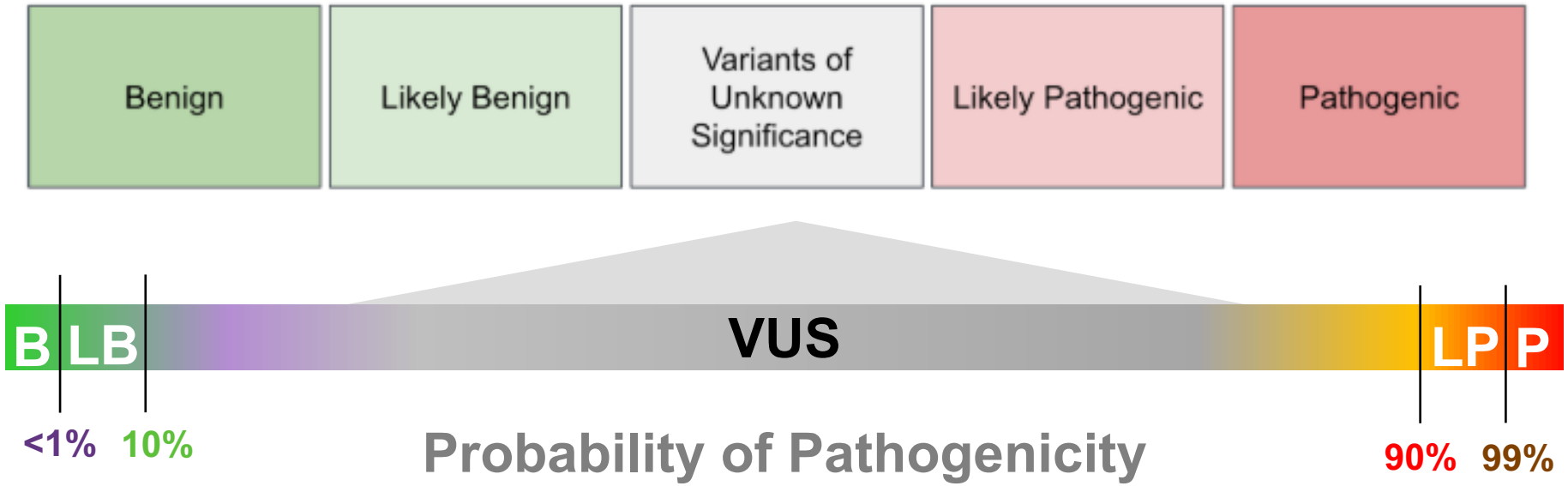
ACMG Criteria 2015



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ACMG Criteria 2015

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

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

- The American College of Medical Genetics (ACMG) criteria provide a common language for variant classification.
- 8 categories of evidence for either benignity or pathogenicity
- Evidence is ranked in different “strengths”

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Strengths of ACMG Criteria

The strength of most criteria is no longer static:

Criteria code	Brief Description	Strength 2015	Strength Range 2024
PVS1	Loss of function	very strong	moderate – very strong
PS1	Same AA change	strong	strong
PS2	*De novo*	strong	supporting – very strong
PS3	Functional evidence	strong	supporting – very strong
PS4	Prevalence in affected pop.	strong	supporting – strong
PM1	Functional domain	moderate	supporting – strong
PM2	Rare in pop. controls	moderate	supporting
PM3	*In trans*	moderate	supporting – very strong
PM4	Length changing	moderate	supporting – moderate
PM5	Same position, different AA	moderate	supporting – strong
PM6	Assumed *de novo*	moderate	supporting – very strong
PP1	Cosegregation	supporting – strong	supporting – strong
PP2	Intolerant to missense	supporting	supporting
PP3	*In silico*	supporting	supporting – moderate
PP4	Specific phenotype	supporting	supporting – moderate
PP5	Reputable source	supporting	discontinued

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Strengths of ACMG Criteria

The strength of most criteria is no longer static:

Criteria code	Brief Description	Strength 2015	Strength Range 2024
BA1	Population prevalence	stand alone	stand alone
BS1	MAF is too high	strong	supporting – strong
BS2	Present in healthy adults	strong	supporting – strong
BS3	Functional evidence	strong	supporting – strong
BS4	Non-segregation	strong	supporting – strong
BP1	Missense in a LOF gene	supporting	supporting
BP2	In cis with recessive / in trans with dominant	supporting	supporting
BP3	Indel in a repeat region	supporting	supporting
BP4	*In silico*	supporting	supporting – moderate
BP5	Alternative cause found	supporting	supporting
BP6	Reputable source	supporting	discontinued
BP7	Splice variant with no prediction	supporting	supporting

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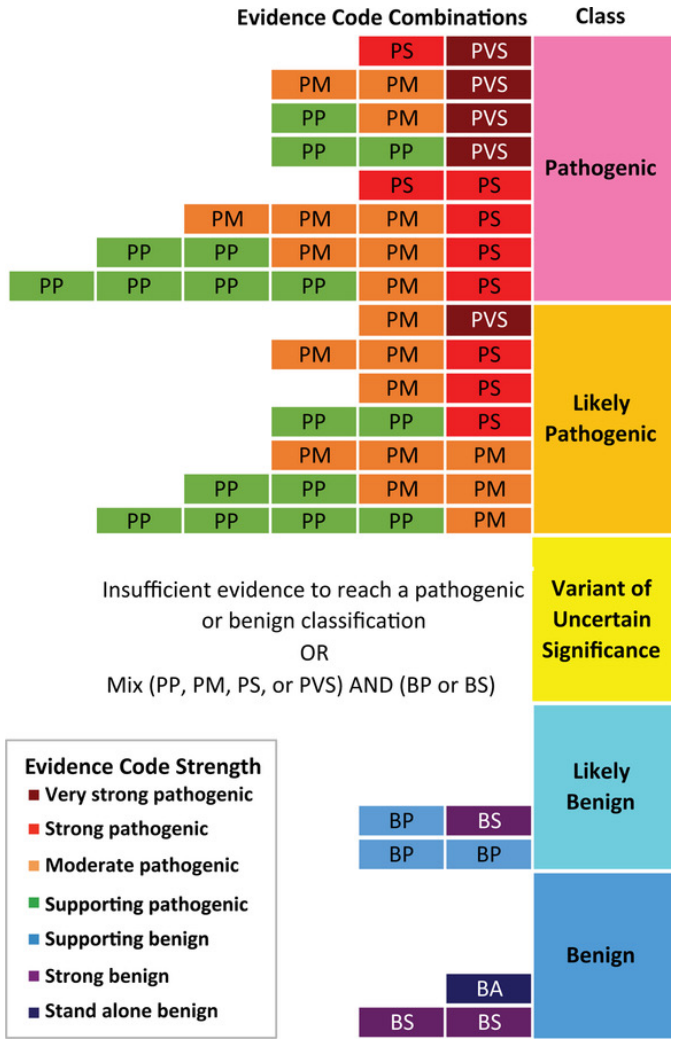
ACMG Point System

Being Phased Out



Now

Type	Strength	Bayesian points
Pathogenic	very strong	+8
	strong	+4
	moderate	+2
	supporting	+1
Benign	strong	- 4
	moderate	- 2
	supporting	- 1

Score Range	Class
≤ -6	Benign
-5 to -1	Likely benign
0 to 5	VUS
6 to 9	Likely pathogenic
≥ 10	Pathogenic

- Rather than combinations of codes, classifications are now assigned with a Bayesian classification framework (Tavtigian 2018)

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Summary of Overview

- ACMG criteria give us a common language with which we can characterize variants
- Variant interpretation involves both **assigning criteria** and **determining the strength** of the criteria assigned
  - Strength of criteria has evolved over time
- Summation of the assigned criteria's associated Bayesian points yields a final classification

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Specific Criteria

In this portion of the class, you will learn:

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

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

Criteria assignment:

- The reference control database is gnomAD, but other databases are available

- PM2: variant is absent/rare in control populations.
  - “Rarity” is specific for the gene, inheritance pattern, and penetrance of the disorder.
  - Max MAF < 0.0005 is a good “general” cutoff

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

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

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

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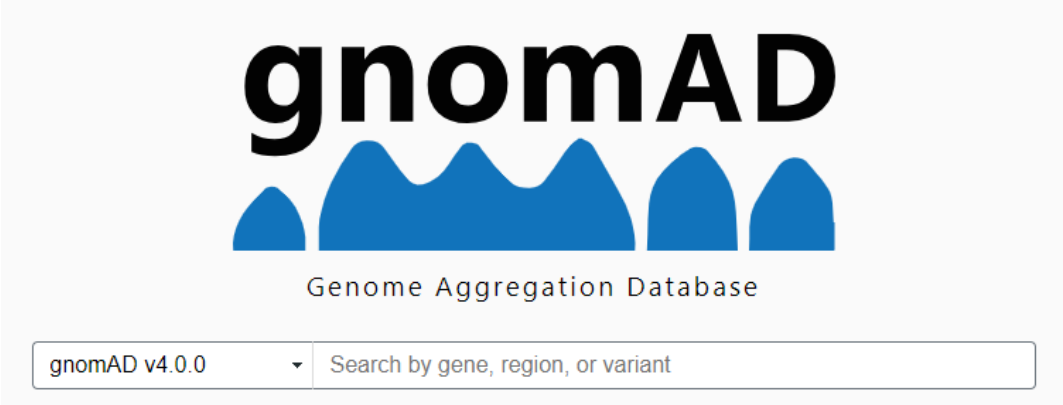
Population Data: gnomAD



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

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Population Data: gnomAD

Example: *ARID1B* variants:

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

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

Strength determination:

- PM2 is recommended to be applied only at supporting
  - Rare variants are common
  - See ClinGen PM2 recommendation 2020

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

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

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

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Population Data: Exceptions to BA1

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

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Population Data:
Prevalence in Affected Population (PS4)

Criteria assignment & strength:

- Variant is associated with an odds ratio > 5.0 of causing disease with a lower bounded confidence interval > 1.0.

- For many disease-causing variants (which very rare causing very rare diseases) you don't get enough data to calculate a significant odds ratio. In this case, ACMG allows "proband counting" thresholds determined for a particular gene / disorder:

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

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

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

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

Criteria assignment:

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

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

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

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

Strength determination:

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

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

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

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

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

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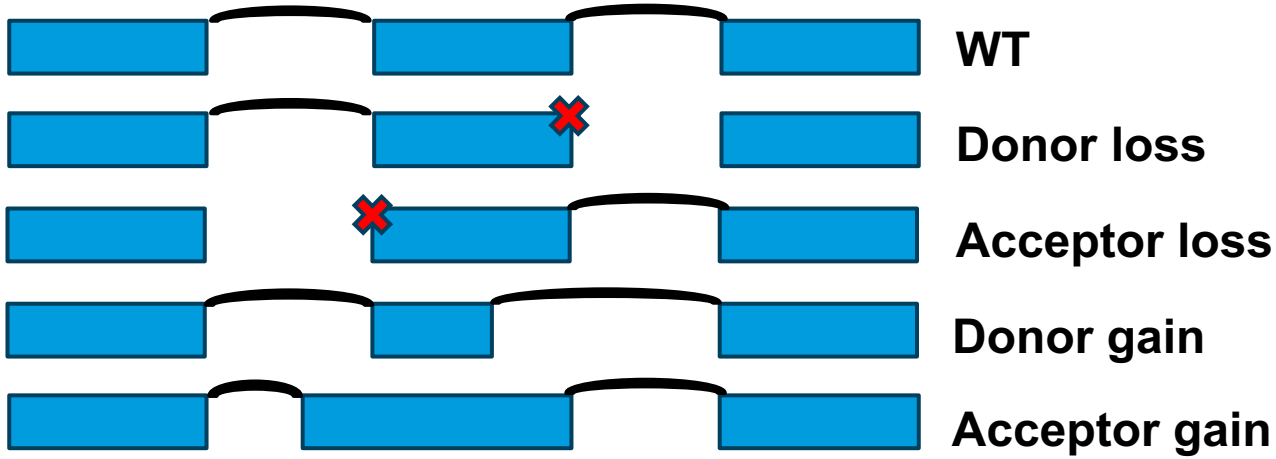
Computational and Predictive Data: Splicing *in silico* predictions (BP7, BP4, PP3)



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

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

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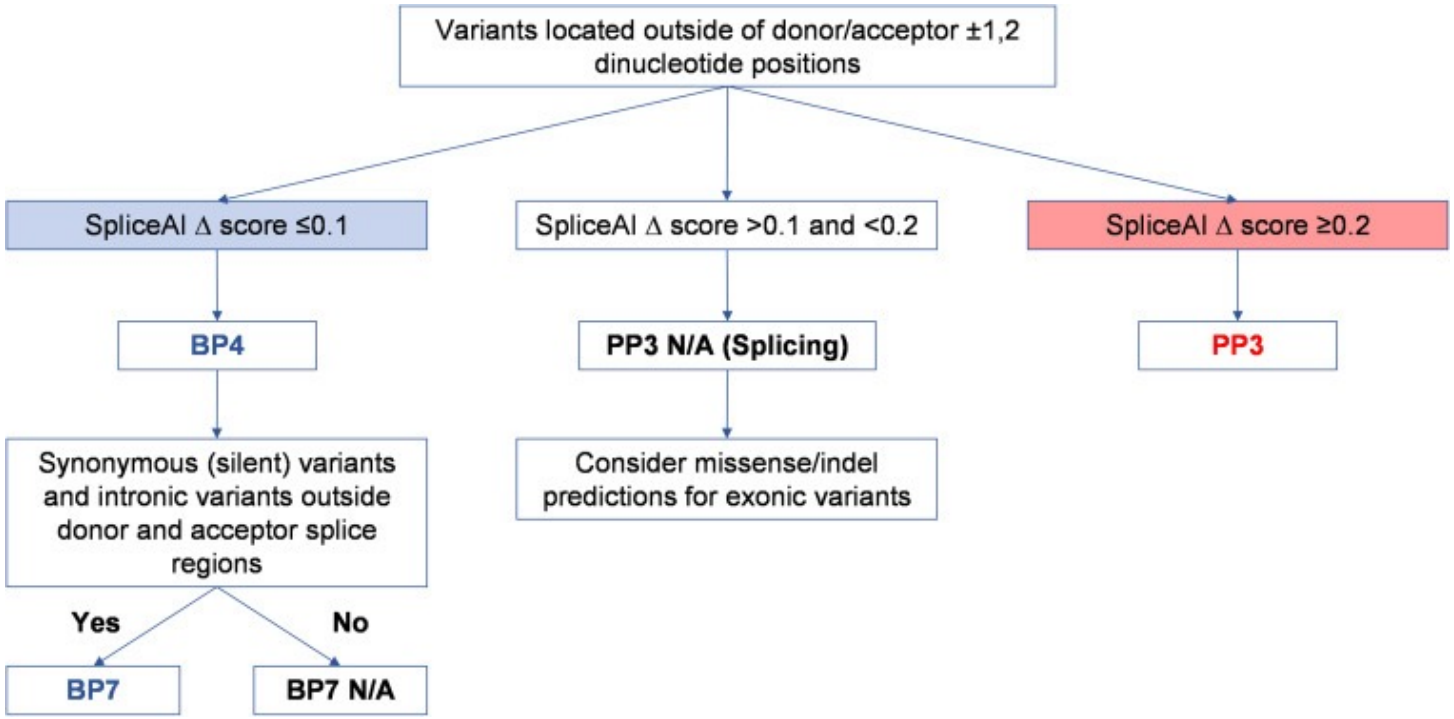
Computational and Predictive Data: Splicing *in silico* predictions (BP7, BP4, PP3)



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

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

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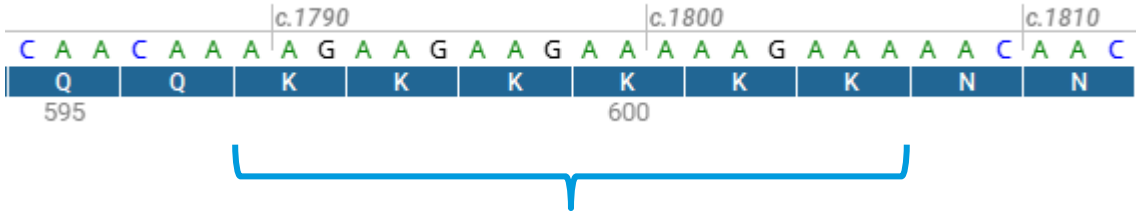
Computational and Predictive Data: In-frame indels (BP3, PM4)

Criteria assignment:

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

Strength:

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



Example of a repetitive region in *CHD7*

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

Criteria assignment:

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

Strength:

- BP1 is assigned at supporting.

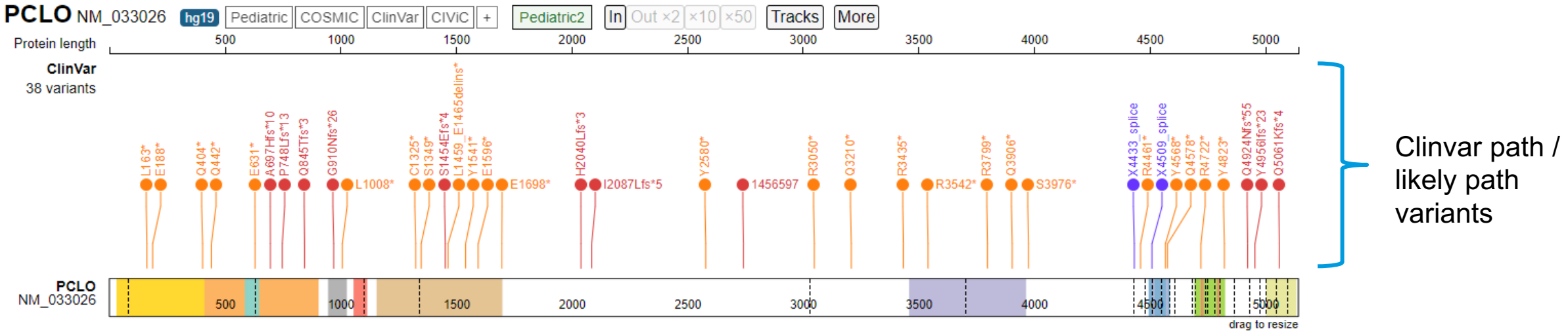

Image made in ProteinPaint (https://proteinpaint.stjude.org/)

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Computational and Predictive Data (PM5, PS1)

Criteria assignment:

- PM5 is assigned when a novel missense change is seen at the same position as a likely pathogenic or pathogenic variant.
  - LDLR: c.1721G>A (p.Arg574His)
    Likely pathogenic
  - LDLR: c.1721G>T (p.Arg574Leu)
    Variant under curation → **PM5**

- PS1 is assigned when a novel nucleotide change leads to the same missense variant previously classified as likely pathogenic or pathogenic.
  - Major caveat: do NOT apply if either variant is predicted to have a different splice effect

Strength:

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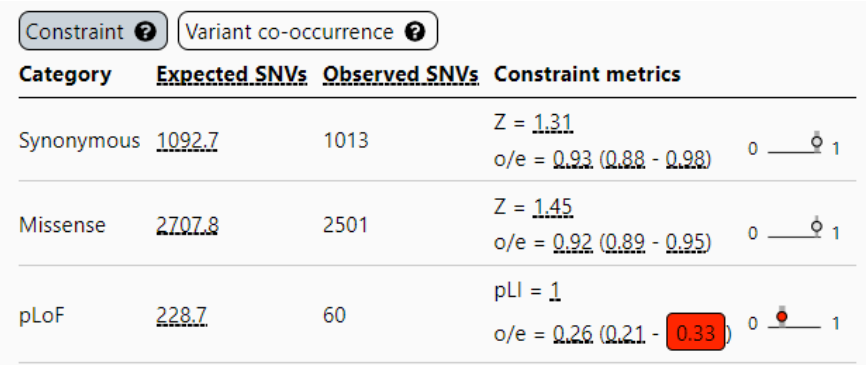
Computational and Predictive Data (PVS1)

Criteria assignment:

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



Constraint | Variant co-occurrence

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

Strength determination:

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

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

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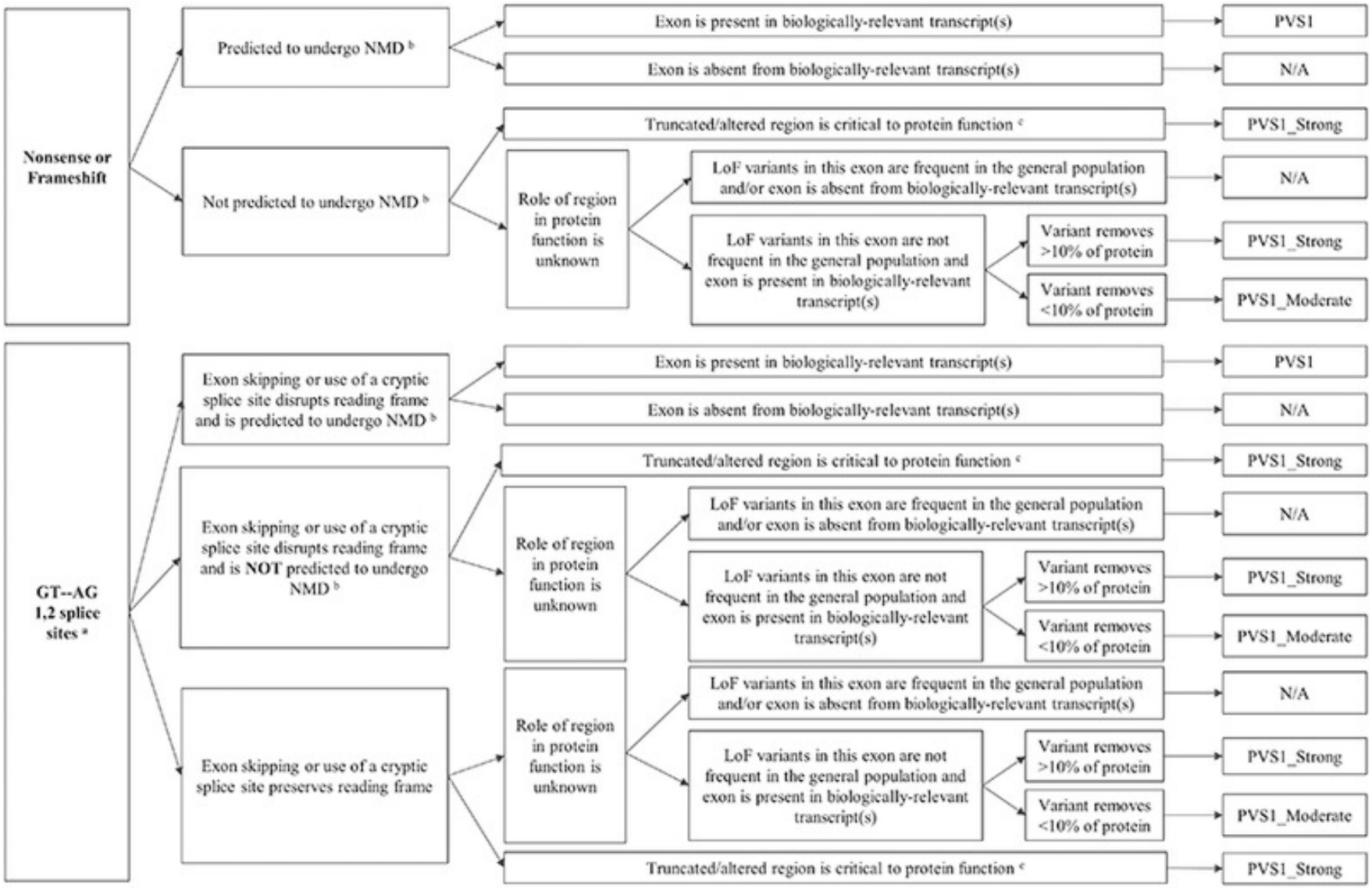
Computational and Predictive Data (PVS1)



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

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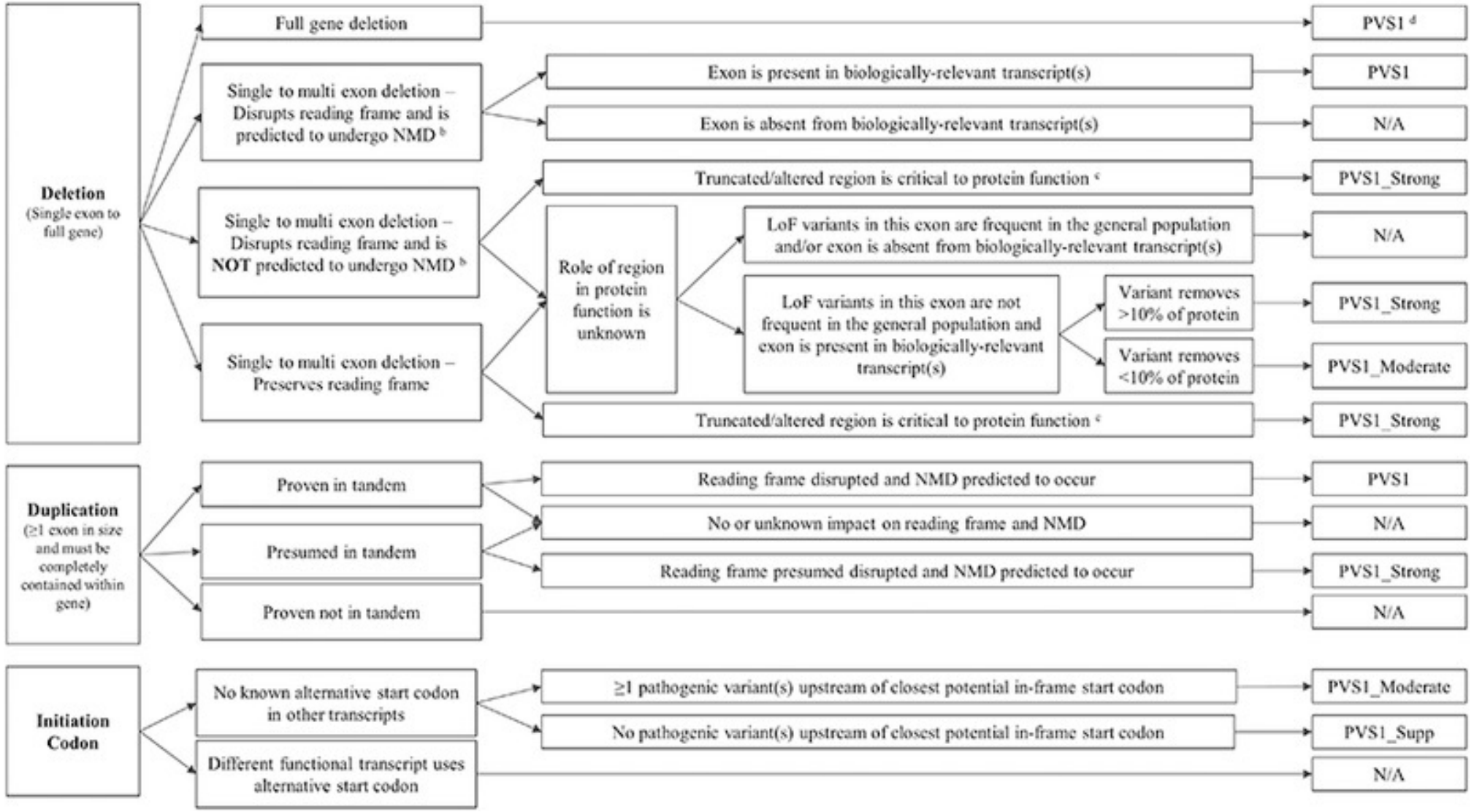
Computational and Predictive Data (PVS1)



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

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Functional Data (BS3, PS3)

Criteria assignment:

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

- New guidance (https://pubmed.ncbi.nlm.nih.gov/31892348/) outlines 4 steps for applying PS3 and BS3, including what strengths are allowed:

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

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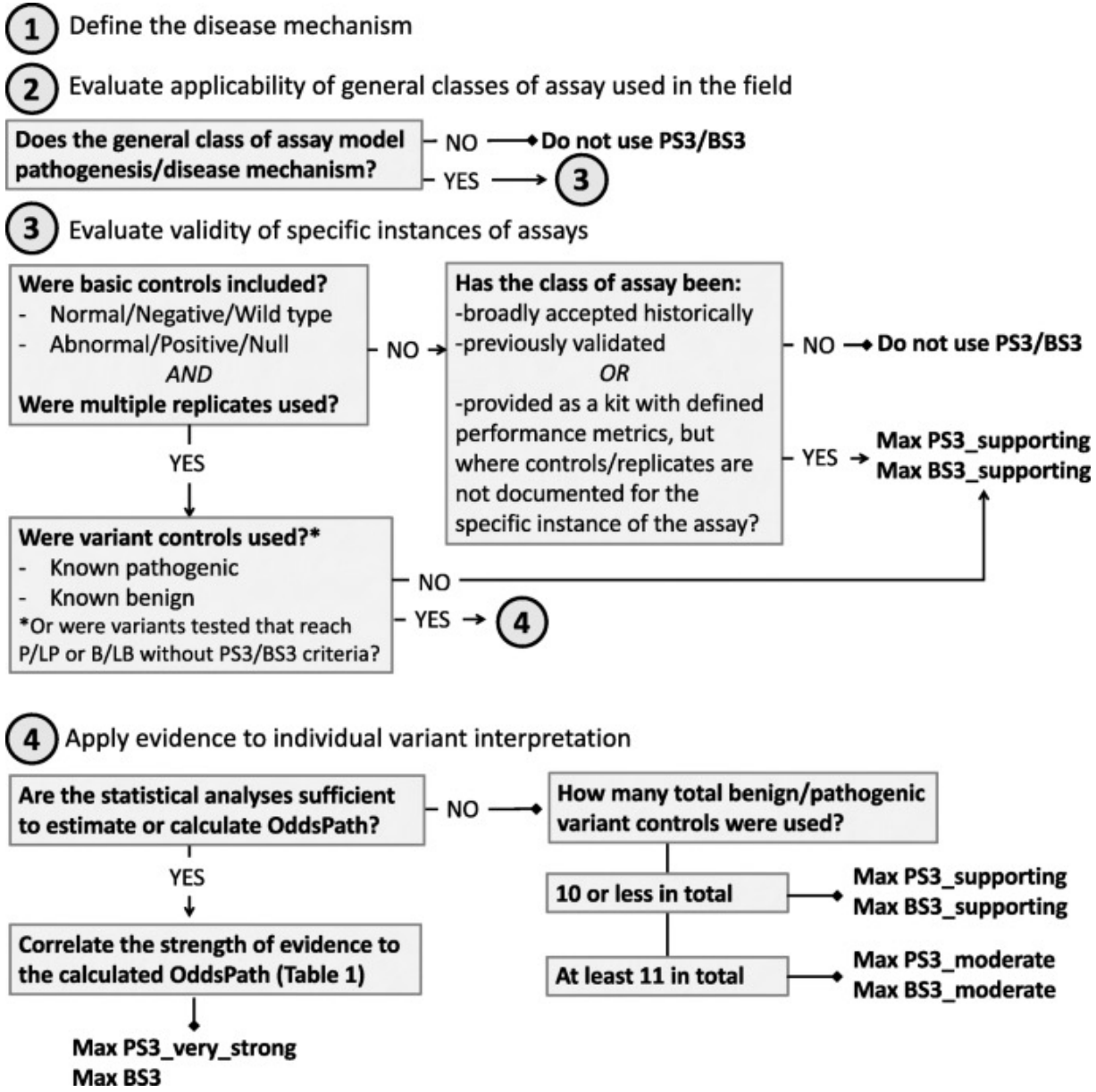
Functional Data (BS3, PS3)



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

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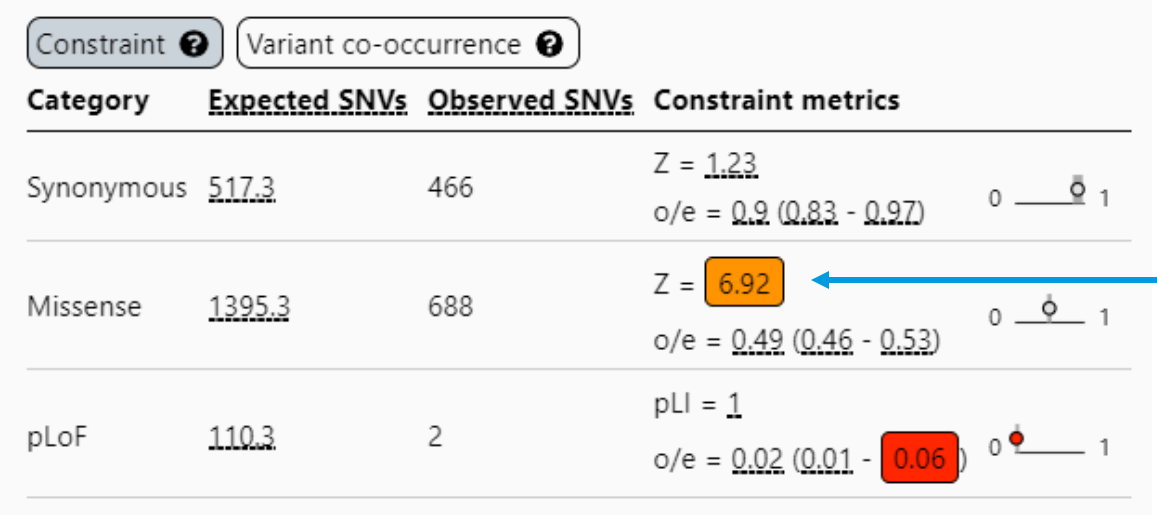
Functional Data:
Gene intolerant to missense: (PP2)



Missense z-score

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

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

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Functional Data:
Functional Region: (PM1)

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

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

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

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

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

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

Previous 1 Next

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

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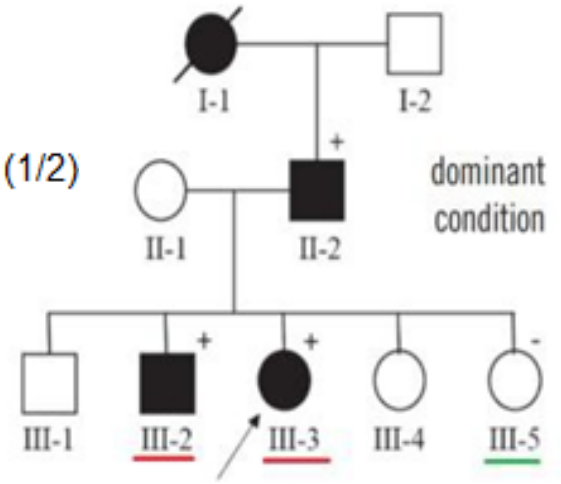
Segregation Data (BS4, PP1)

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

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

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

=Weak segregation
(≤ 3 affected individual)



Criteria application:

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

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

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De Novo Data (PM6, PS2)

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

Strength determination: see tables.

Notes:

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

Table 1. Points awarded per de novo occurrence

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

*Maximum allowable value of 1 may contribute to overall score

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

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

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Allelic Data (BP2, PM3)

Assignment:

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

Strength determination: see table.

Notes:

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

Table 1. Points awarded per in *trans* proband

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

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

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

PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
0.5	1.0	2.0	4.0

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

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Other Data (BP5)

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

Strength determination: supporting.

Notes:

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

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Phenotypic Data (PP4)

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

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

For example:

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

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

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

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

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

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

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Part 2: Resources and Databases

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TYPES OF INFORMATION

Gene-level information:
- Gene-disease associations
- Biological function
- Tissue-specific expression

} Is this gene a “good fit” for the patient?

Variant-level information:
- Population frequency
- Presence in the affected population
- *In-silico* predictions
- Location within the gene

} Does this variant have the potential to be pathogenic?

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GENE-DISEASE ASSOCIATION

Why it's useful: Understanding whether a gene is capable of causing your proband's phenotype is one of the most important parts of your analysis

Resources I will review:

- OMIM
- Gene Reviews

Other resources:

- Malacards

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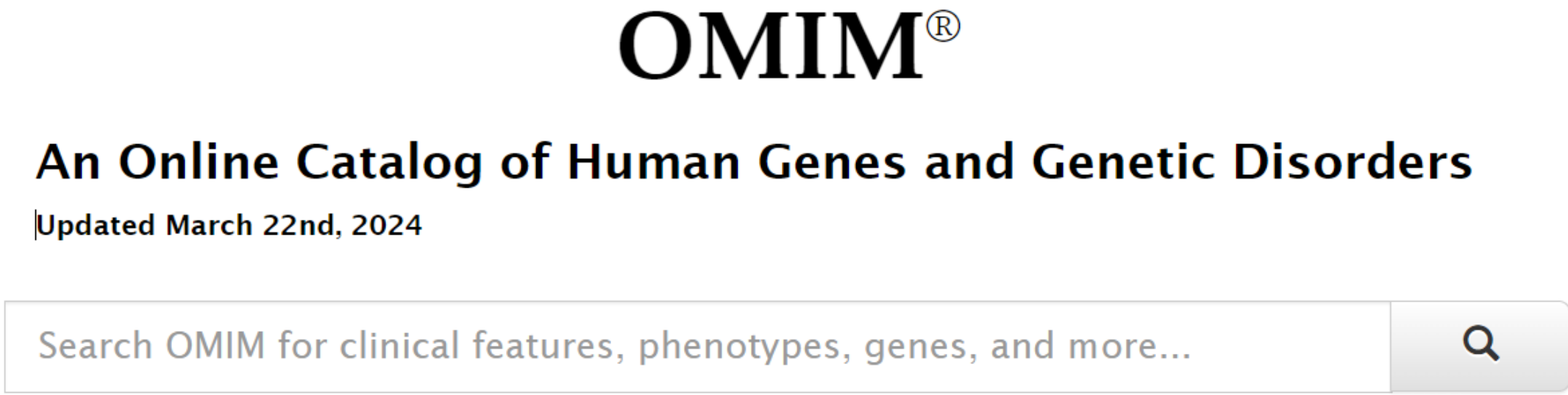
GENE-DISEASE ASSOCIATION: OMIM



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GENE-DISEASE ASSOCIATION: OMIM

1: * 238300. GLYCINE DECARBOXYLASE; GLDC

Cytogenetic location: 9p24.1, Genomic coordinates (GRCh38): 9:6,532,467-6,645,729

Matching terms: gldc

▼ Gene-Phenotype Relationships ▶ ICD+ ▶ Links

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key
9p24.1	Glycine encephalopathy1	605899	AR	3

▲ Close

Associated conditions

Inheritance patterns

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GENE-DISEASE ASSOCIATION: OMIM

605899

GLYCINE ENCEPHALOPATHY 1; GCE1

INHERITANCE

- Autosomal recessive

NEUROLOGIC

Central Nervous System

- Absent corpus callosum (variable)
- Lethargy
- Seizures
- Hiccups
- Hypotonia
- Hyporeflexia to hyperreflexia
- Myoclonic jerks
- Mental retardation
- Burst suppression pattern on neonatal EEG
- Expressive speech deficit

Behavioral Psychiatric Manifestations

- Hyperactivity
- Impulsivity
- Aggressiveness
- Irritability
- Restlessness

- OMIM phenotype lists should not be taken as exhaustive
- Can be hard to distinguish between features that will ALWAYS be present vs those that are SOMETIMES present

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GENE-DISEASE ASSOCIATION: GENE REVIEWS

GeneReviews®

Editors: Margaret P Adam, Editor-in-Chief, Jerry Feldman, Medical Editor, Ghayda M Mirzaa, Medical Editor, Roberta A Pagon, Medical Editor, Stephanie E Wallace, Medical Editor, Lora JH Bean, Molecular Genetics Editor, Karen W Gripp, Molecular Genetics Editor, and Anne Amemiya, Genetic Counseling Editor.

Seattle (WA): University of Washington, Seattle; 1993-2024.
ISSN: 2372-0697

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Search GeneReviews

GeneReviews Advanced Search Help

Establishing the Diagnosis

The diagnosis of NKH **is established** in a proband with elevated glycine in plasma and CSF (Table 1), a compatible pattern on brain imaging, and **either** biallelic pathogenic (or likely pathogenic) variants in one of the genes encoding the protein subunits of the GCS identified on molecular genetic testing (Table 2) **or** deficient activity of the GCS (without deficiency of cofactors such as enzyme-bound lipoate or pyridoxal phosphate). Today, confirmatory testing is primarily by molecular genetic testing; enzymatic testing is used only in select cases.

In contrast to OMIM, Gene Reviews gives you:

- Approximate prevalence of phenotypes
- Specific diagnostic criteria for a disorder

Gene Reviews, however, only catalogs well-established gene-disease associations, and is thus much less exhaustive

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BIOLOGICAL FUNCTION

Why it's useful: Knowing what the function of your gene is can help you understand whether it's capable of causing your probands phenotype. It also is essential if you begin to think about functional assays or therapeutic options for a gene.

Resources I will review:

- Uniprot
- GeneCards

Other resources:

- PubMed

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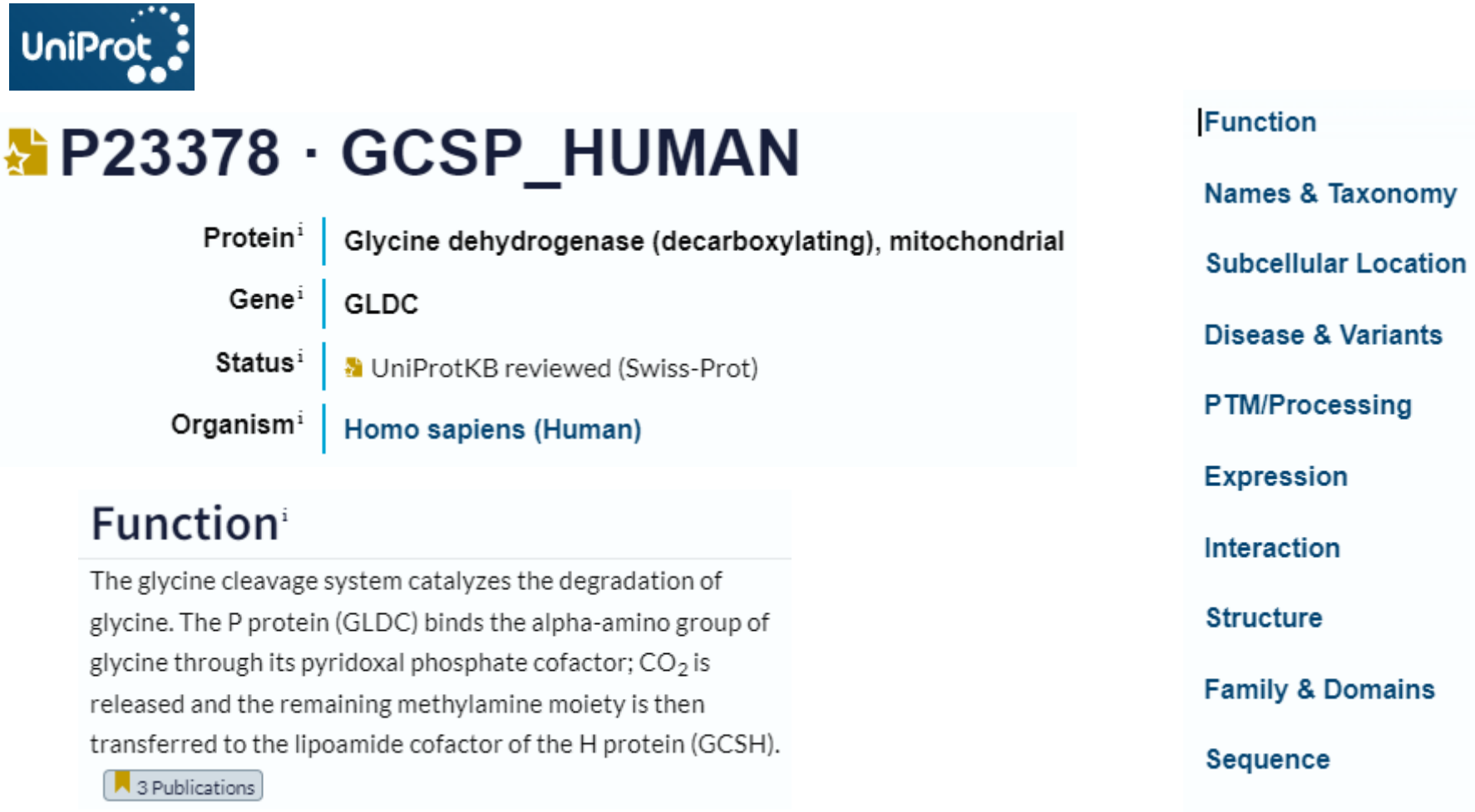
BIOLOGICAL FUNCTION: UNIPROT



- UniProt contains large amounts of gene / protein information

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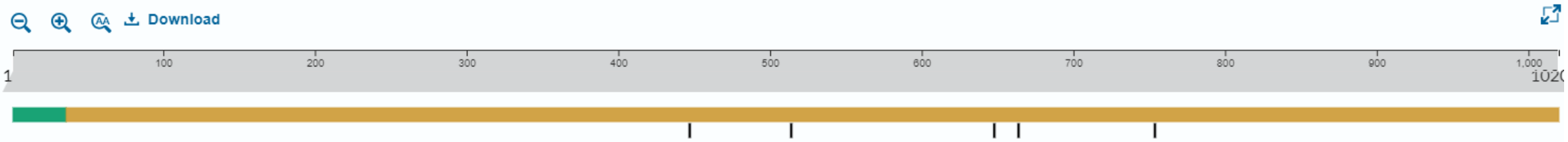
BIOLOGICAL FUNCTION: UNIPROT

PTM/Processing

Features

Showing features for transit peptide, chain, modified residue.



TYPE	ID	POSITION(S)	DESCRIPTION	
-- Select --				
Transit peptide		1-35	Mitochondrion Sequence Analysis	BLAST Add
Chain	PRO_0000010740	36-1020	Glycine dehydrogenase (decarboxylating), mitochondrial	BLAST Add
Modified residue		447	N6-acetyllysine By Similarity	
Modified residue		514	N6-acetyllysine By Similarity	
Modified residue		648	N6-acetyllysine By Similarity	
Modified residue		664	N6-acetyllysine By Similarity	
Modified residue		754	N6-(pyridoxal phosphate)lysine By Similarity	

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BIOLOGICAL FUNCTION: GENECARDS

NUMA1 Gene - Nuclear Mitotic Apparatus Protein 1

Protein Coding (Updated: Apr 3, 2024 ; GC11M072002 ; GIFtS: 52)

Aliases for NUMA1 Gene

GeneCards Symbol: *NUMA1* [2]

Nuclear Mitotic Apparatus Protein 1 [2 3 4 5]

NUMA [2 3 4 5]

Nuclear Matrix Protein-22 [3 4]

SP-H Antigen [3 4]

NMP-22 [3 4]

Centrophilin Stabilizes Mitotic Spindle In Mitotic Cells [3]

Nuclear Mitotic Apparatus Protein [4]

Structural Nuclear Protein [3]

NuMA Protein [4]

NMP22 [4]

External Ids for NUMA1 Gene

HGNC: 8059 NCBI Gene: 4926 Ensembl: ENSG00000137497 OMIM®: 164009 UniProtKB/Swiss-Prot: Q14980

Previous GeneCards Identifiers for NUMA1 Gene

GC11M074046, GC11M073253, GC11M071936, GC11M071440, GC11M071391, GC11M071713, GC11M068007

Search aliases for NUMA1 gene in PubMed and other databases

- Gene aliases are useful during lit searches on a gene

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BIOLOGICAL FUNCTION: GENECARDS

NUMA1 Gene - Nuclear Mitotic Apparatus Protein 1

Protein Coding (Updated: Apr 3, 2024 ; GC11M072002 ; GIFtS: 52)

NCBI Gene Summary for NUMA1 Gene

This gene encodes a large protein that forms a structural component of the nuclear matrix. The encoded protein interacts with microtubules and plays a role in the formation and organization of the mitotic spindle during cell division. Chromosomal translocation of this gene with the RARA (retinoic acid receptor, alpha) gene on chromosome 17 have been detected in patients with acute promyelocytic leukemia. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Nov 2013]

GeneCards Summary for NUMA1 Gene

NUMA1 (Nuclear Mitotic Apparatus Protein 1) is a Protein Coding gene. Diseases associated with NUMA1 include Acute Promyelocytic Leukemia and Bladder Calculus. Among its related pathways are Cell Cycle, Mitotic and Apoptosis and survival FAS signaling cascades. Gene Ontology (GO) annotations related to this gene include *nucleotide binding* and *microtubule binding*. An important paralog of this gene is GOLGA3.

UniProtKB/Swiss-Prot Summary for NUMA1 Gene

Microtubule (MT)-binding protein that plays a role in the formation and maintenance of the spindle poles and the alignement and the segregation of chromosomes during mitotic cell division (PubMed:7769006, 17172455, 19255246, 24996901, 26195665, 27462074). Functions to tether the minus ends of MTs at the spindle poles, which is critical for the establishment and maintenance of the spindle poles (PubMed:12445386, 11956313). Plays a role in the establishment of the mitotic spindle orientation during metaphase and elongation during anaphase in a dynein-dynactin-dependent manner (PubMed:23870127, 24109598, 24996901, 26765568). In metaphase, part of a ternary complex composed of GPSM2 and G(i) alpha proteins, that regulates the recruitment and anchorage of the dynein-dynactin complex in the mitotic cell cortex regions situated above the two spindle poles, and hence regulates the correct oritentation of the mitotic spindle (PubMed:23027904, 22327364, 23921553). During anaphase, mediates the recruitment and accumulation of the dynein-dynactin complex at the cell membrane of the polar cortical region through direct association with phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2), and hence participates in the regulation of the spindle elongation and chromosome segregation (PubMed:22327364, 23921553, 24996901, 24371089). Binds also to other polyanionic phosphoinositides, such as phosphatidylinositol 3-phosphate (PIP), lysophosphatidic acid (LPA) and phosphatidylinositol triphosphate (PIP3), in vitro (PubMed:24996901, 24371089). Also required for proper orientation of the mitotic spindle during asymmetric cell divisions (PubMed:21816348). Plays a role in mitotic MT aster assembly (PubMed:11163243, 11229403, 12445386). Involved in anastral spindle assembly (PubMed:25657325). Positively regulates TNKS protein localization to spindle poles in mitosis (PubMed:16076287). Highly abundant component of the nuclear matrix where it may serve a non-mitotic structural role, occupies the majority of the nuclear volume (PubMed:10075938). Required for epidermal differentiation and hair follicle morphogenesis (By similarity). (NUMA1_HUMAN,Q14980)

Gene Wiki entry for NUMA1 Gene

Additional gene information for NUMA1 Gene

HGNC (8059) NCBI Gene (4926) Ensembl (ENSG00000137497) OMIM® (164009) UniProtKB/Swiss-Prot (Q14980) Open Targets Platform(ENSG00000137497)

- Includes many short descriptions of the gene
- The disease associations listed in the GeneCards summary are rather sketchy.

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TISSUE EXPRESSION

Why it's useful: Gene expression in the affected tissue is another indicator of a gene's potential to cause a phenotype. It's also very useful if you're thinking about biosample collection from a proband.

Resources I will review:

- GTex
- Protein Atlas

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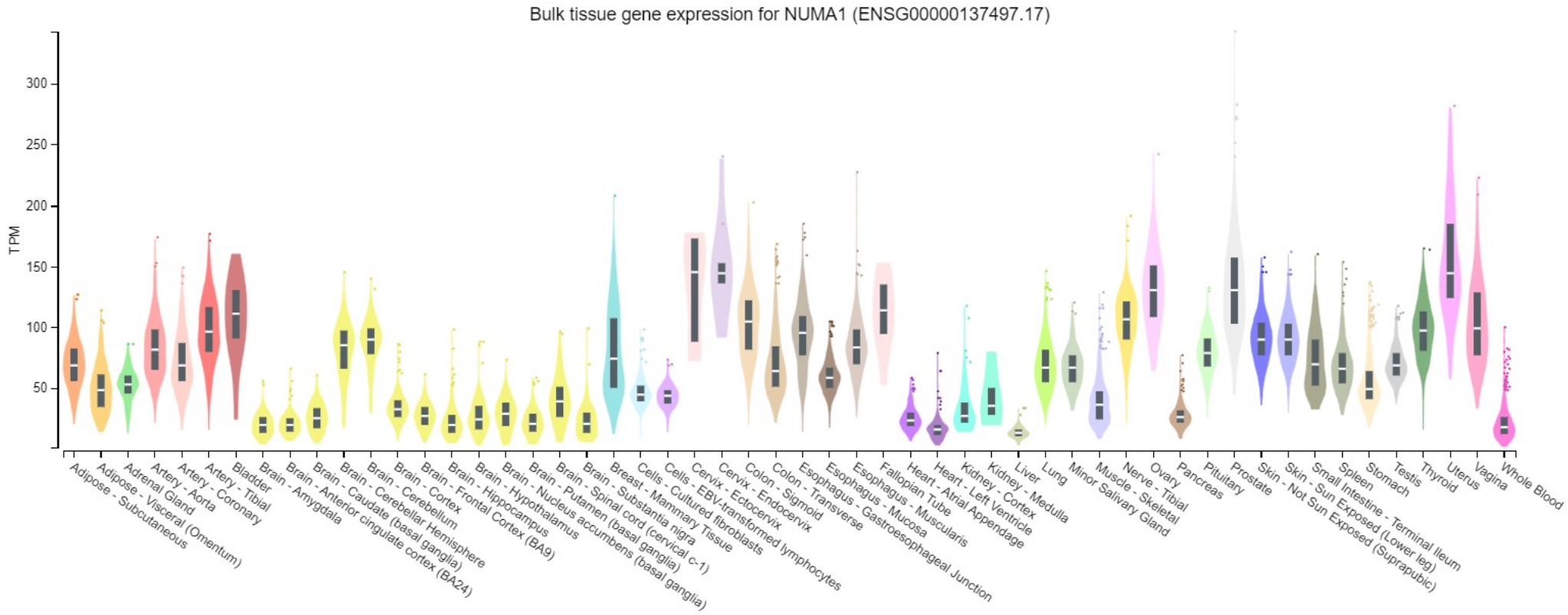
TISSUE EXPRESSION: GTEX

- Top
- Bulk Tissue Expression
- Single Cell Expression
- Exon Expression
- Single-Tissue eQTLs
- Single-Tissue sQTLs
- Single-Tissue ieQTLs
- Single-Tissue isQTLs



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TISSUE EXPRESSION: GTEX

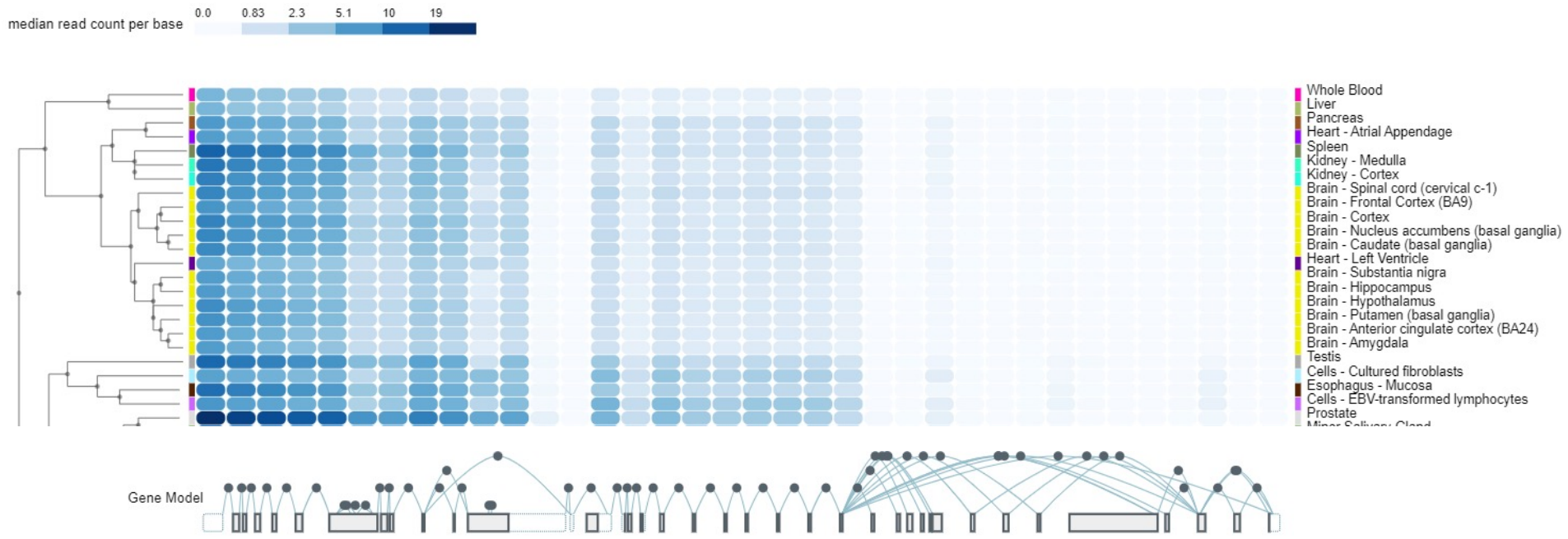
- Top
- Bulk Tissue Expression
- Single Cell Expression
- Exon Expression
- Single-Tissue eQTLs
- Single-Tissue sQTLs
- Single-Tissue ieQTLs
- Single-Tissue isQTLs

Exon expression for NUMA1 (ENSG00000137497.17)

Data Source: GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)

Exon Expression | Junction Expression | Isoform Expression

Exon Expression of NUMA1: ENSG00000137497.17 nuclear mitotic apparatus protein 1 [Source:HGNC Symbol;Acc:HGNC:8059]



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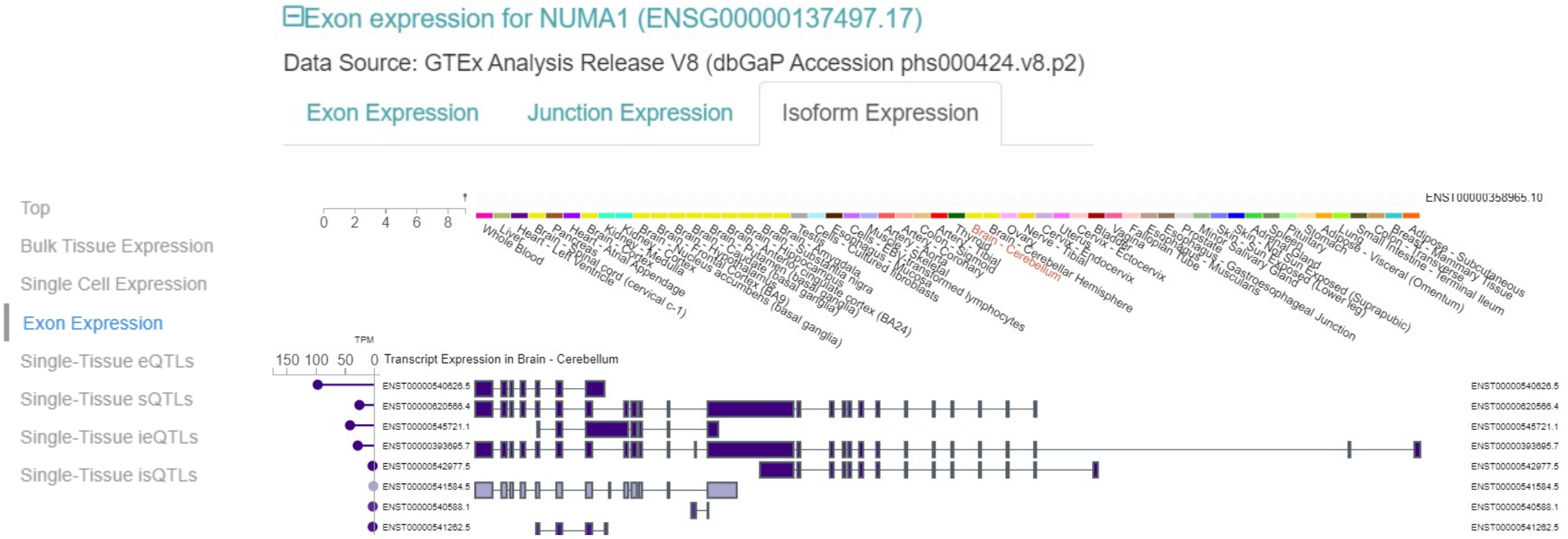
TISSUE EXPRESSION: GTEX



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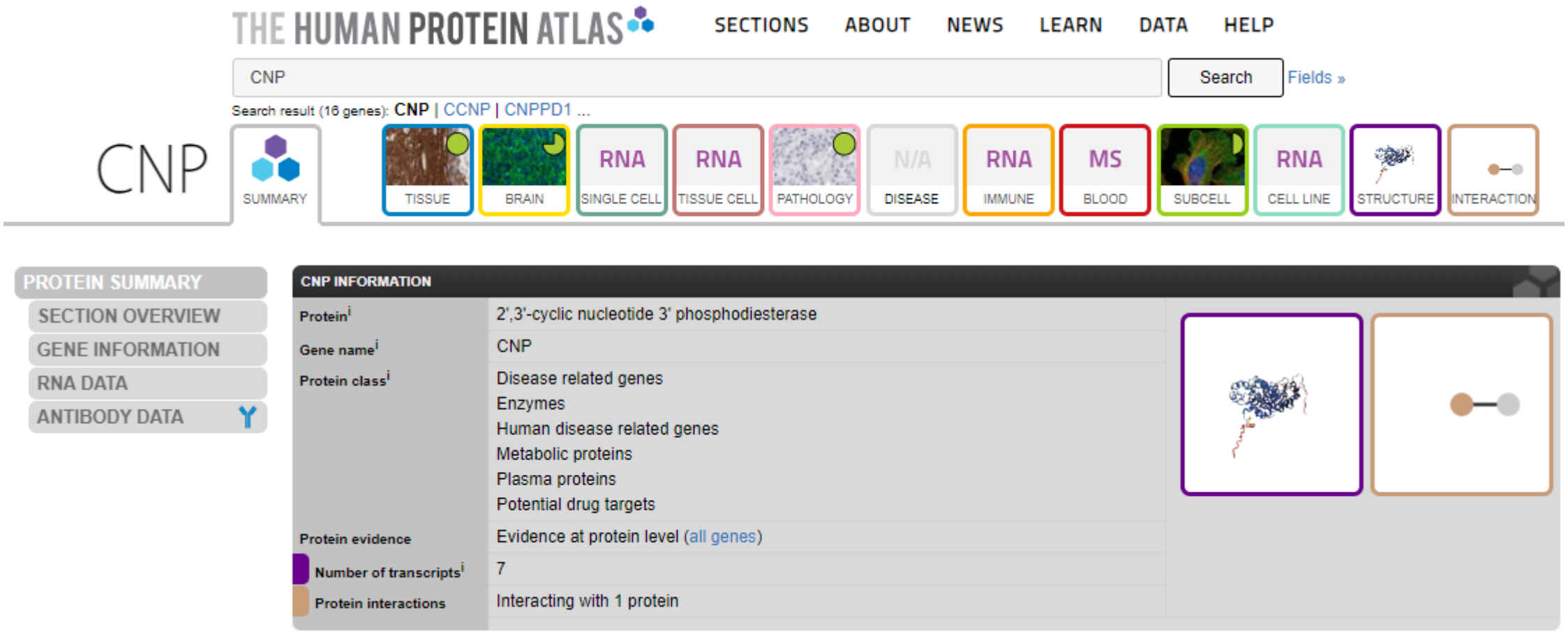
TISSUE EXPRESSION: PROTEIN ATLAS



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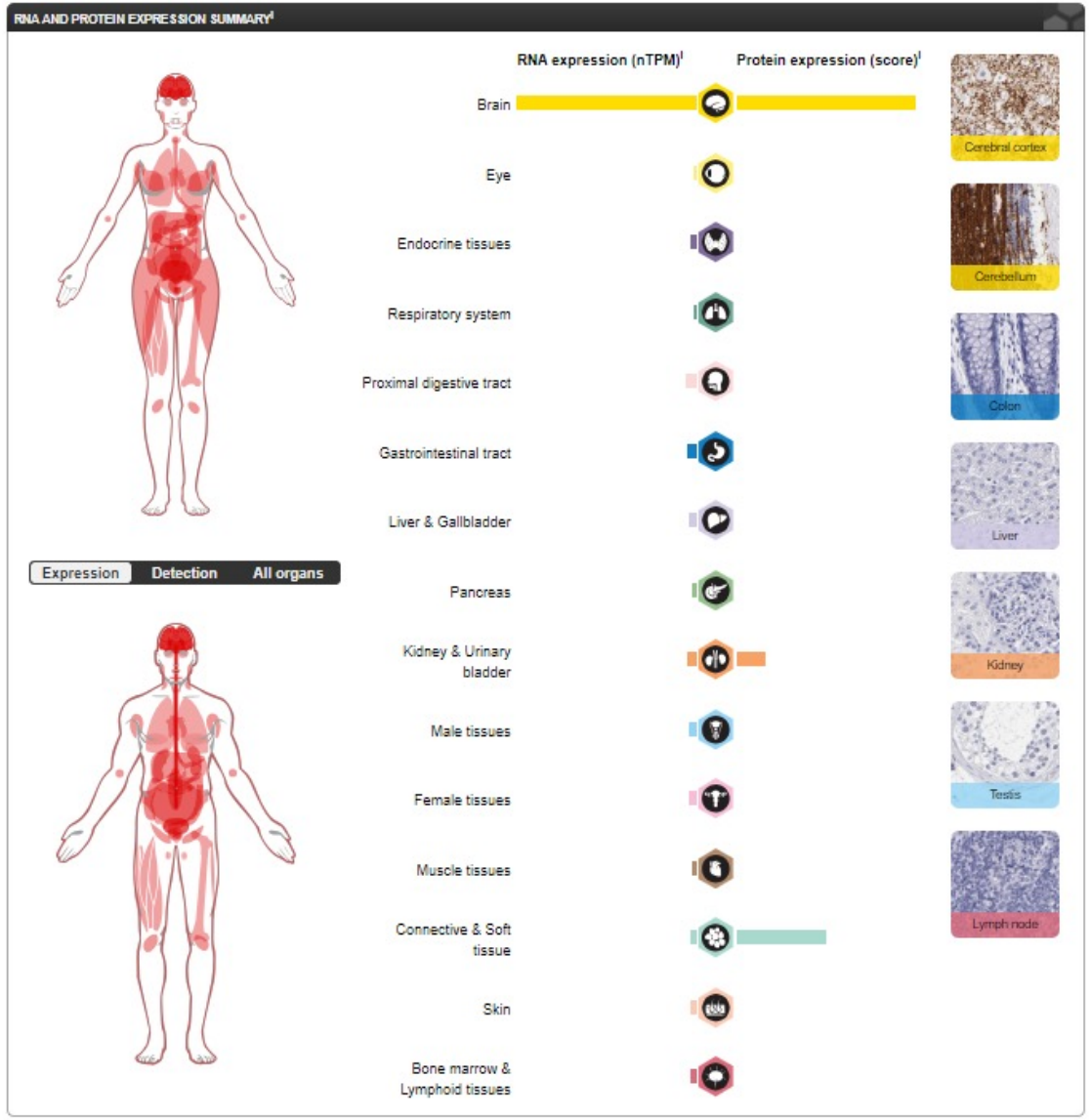
TISSUE EXPRESSION: PROTEIN ATLAS

HUMAN PROTEIN ATLAS INFORMATION[i]	
Tissue expression cluster (RNA)[i]	Oligodendrocytes - Myelination (mainly)
Tissue specificity (RNA)[i]	Tissue enriched (brain)
Tau specificity score (RNA)[i]	0.50
Tissue distribution (RNA)[i]	Detected in all
Protein evidence[i]	Evidence at protein level
Protein expression[i]	Highly specific expression in oligodendrocytes in CNS and peripheral nerves.



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TYPES OF INFORMATION

Gene-level information:
- Gene-disease associations
- Biological function
- Tissue-specific expression

} Is this gene a “good fit” for the patient?

Variant-level information:
- Population frequency
- Presence in the affected population
- *In-silico* predictions
- Location within the gene

} Does this variant have the potential to be pathogenic?

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POPULATION FREQUENCY

Why it's useful: Frequency of a variant in the healthy population is the frontline filter we use to weed out variants.

Resources I will review:

- gnomAD

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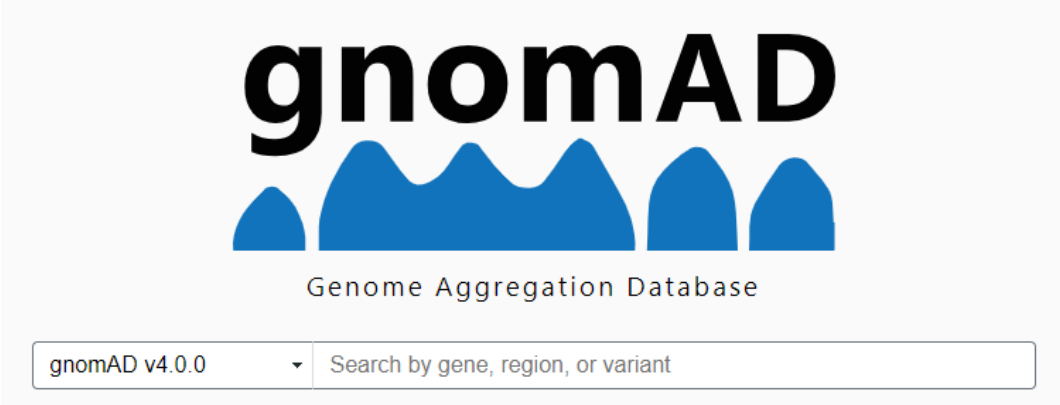
POPULATION FREQUENCY: GNOMAD



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

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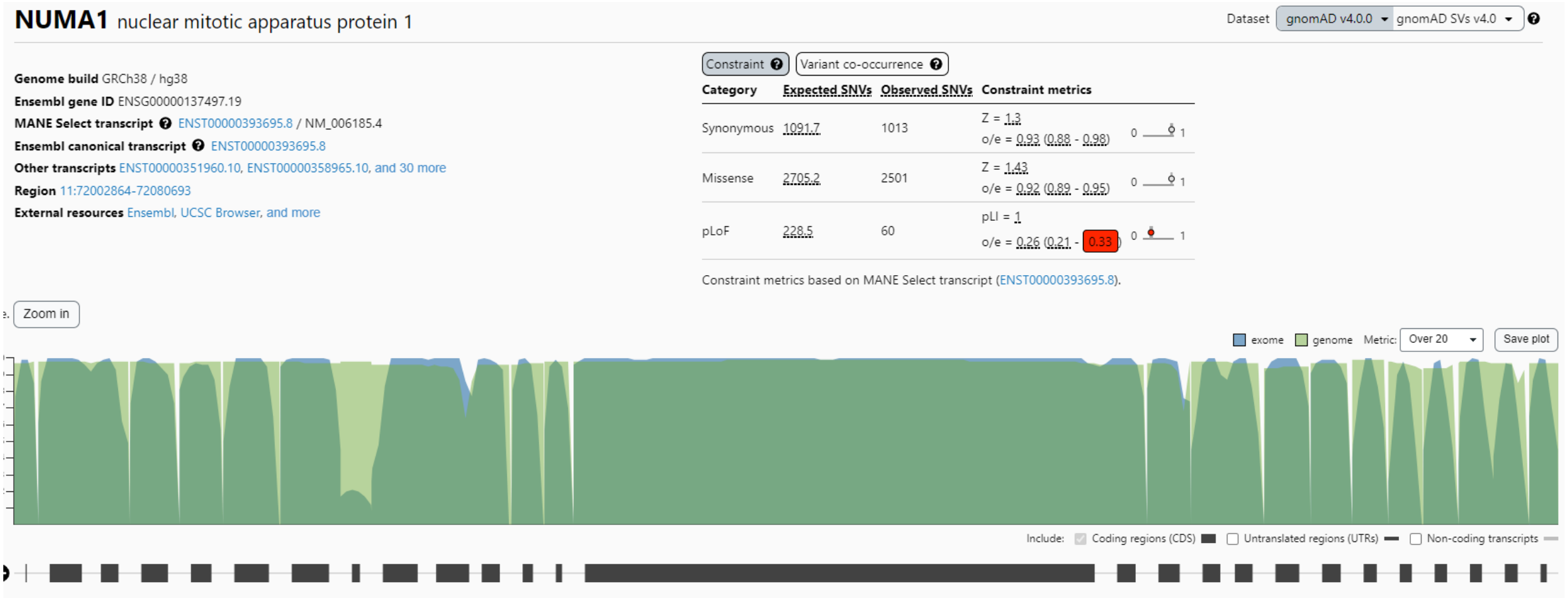
POPULATION FREQUENCY: GNOMAD



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POPULATION FREQUENCY: GNOMAD

Expected number of variants of a certain type based on the size of the gene and average number of that variant type across the genome

Actual number of that variant type observed in the gene

Category	Expected SNVs	Observed SNVs	Constraint metrics
Synonymous	485	481	Z = 0.14 o/e = 0.99 (0.92 - 1.07)
Missense	1205.1	1119	Z = 0.88 o/e = 0.93 (0.88 - 0.98)
pLoF	106.4	15	pLI = 1 o/e = 0.14 (0.09 - 0.22)

Missense z-score. Gene considered intolerant to missense variants if z-score is > 3.07

LOEUF score. Gene considered intolerant to LOF if the score is < 0.35 in gnomAD v2 or < 0.6 in gnomAD v4

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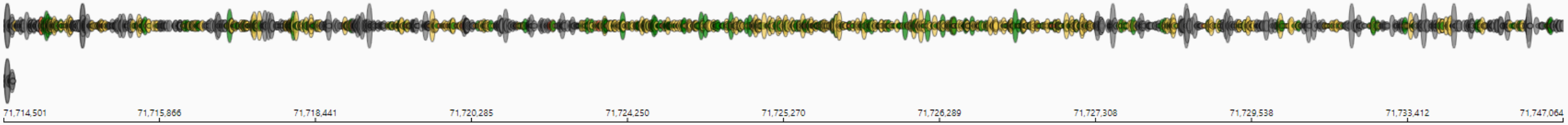
POPULATION FREQUENCY: GNOMAD

gnomAD variants



pLoF only | Missense / Inframe indel only | Synonymous only | Other only | all

Exomes | Genomes | SNVs | Indels | Filtered variants | Display neighboring variants

Search variant table

Export variants to CSV | Configure table

Note Only variants located in or within 75 base pairs of a coding exon are shown here. To see variants in UTRs or introns, use the region view.

The table below shows the HGVS consequence and VEP annotation for each variant's most severe consequence across all transcripts in this gene. Cases where the most severe consequence occurs in a non-canonical transcript are denoted with †. To see consequences in a specific transcript, use the transcript view.

Variant ID	Source	HGVS Consequence	VEP Annotation	LoF Curation	Clinical Significance	Flags	Allele Count	Allele Number	Allele Frequency	Number of Homozygotes
11-71714512-G-A	G	c.*61C>T	3' UTR				2	31378	6.37e-5	0
11-71714516-G-A	G	c.*57C>T	3' UTR				1	31378	3.19e-5	0
11-71714524-G-GAC	E	c.*48_*49insGT	3' UTR				2	251108	7.96e-6	0
11-71714526-GGT-G	E	c.*45_*46delAC	3' UTR				2	250892	7.97e-6	0
11-71714526-G-A	E G	c.*47C>T	3' UTR				2	282170	7.09e-6	0
11-71714526-G-T	E G	c.*47C>A	3' UTR				4	282170	1.42e-5	0
11-71714526-G-C	E G	c.*47C>G	3' UTR				261971	282170	9.28e-1	122051
11-71714528-T-G	E G	c.*45A>C	3' UTR				262004	282196	9.28e-1	122070
11-71714536-G-A	E	c.*37C>T	3' UTR				9	251194	3.58e-5	0
11-71714546-G-A	E G	c.*27C>T	3' UTR				19	282764	6.72e-5	0
11-71714547-T-TGGG	E	c.*23_*25dupCCC	3' UTR				1	251358	3.98e-6	0
11-71714550-G-A	E G	c.*23C>T	3' UTR				2	282748	7.07e-6	0
11-71714552-C-A	E G	c.*21G>T	3' UTR				17	282748	6.01e-5	0
11-71714554-A-G	G	c.*19T>C	3' UTR				1	31382	3.19e-5	0

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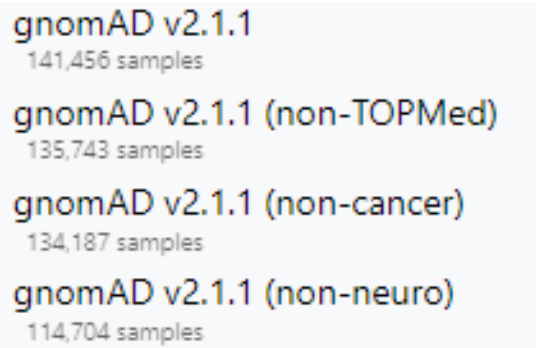
POPULATION FREQUENCY: GNOMAD

Some general comments:

- Many homozygotes / hemizygotes for a variant is a strong indicator for a benign variant
- A substantial number of heterozygotes for a gene that causes an autosomal dominant condition is a strong indicator for a benign variant
- Consider the age of onset of the disorder … these individuals are mostly healthy, but some with adult-onsent conditions may be present
    - gnomAD website: “As such, the gnomAD resource should serve as useful reference sets of allele frequencies for **severe pediatric disease** studies”



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POPULATION FREQUENCY: GNOMAD

SNV: X-53196818-A-G(GRCh38)

Copy variant ID | Gene page | Dataset: gnomAD v4.0.0

	Exomes	Genomes	Total
Filters	Pass	AS_VQSR	
Allele Count	3	1	4
Allele Number	640688	113463	754151
Allele Frequency	0.000004682	0.000008813	0.000005304
Grpmax Filtering AF (95% confidence)	0.000001360	0	0.000001460
Number of homozygotes	0	0	0
Number of hemizygotes	0	1	1
Fraction of individuals with >20x coverage	1.0	0.6	

External Resources

- dbSNP (rs1415009010)
- UCSC
- All of Us

Feedback

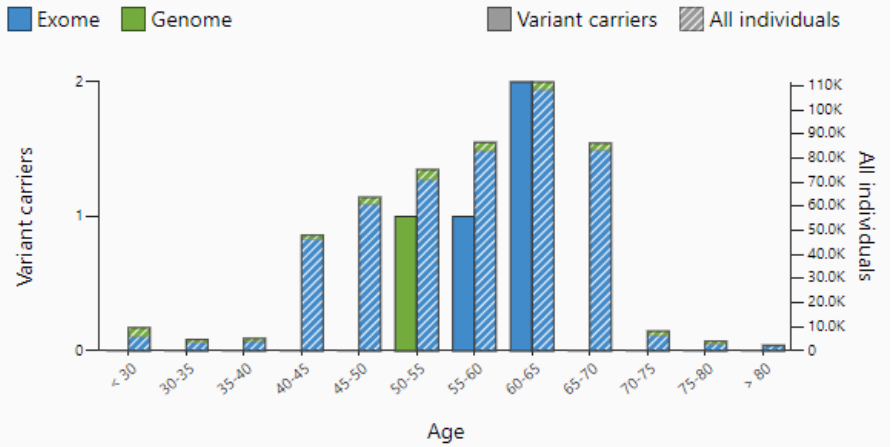
Report an issue with this variant

Genetic Ancestry Group Frequencies

gnomAD | HGDP | 1KG | Local Ancestry

Genetic Ancestry Group	Allele Count	Allele Number	Number of Homozygotes	Number of Hemizygotes	Allele Frequency
European (non-Finnish)	4	639302	0	1	0.000006257
Remaining	0	22704	0	0	0.000
Admixed American	0	11688	0	0	0.000
European (Finnish)	0	6631	0	0	0.000
Middle Eastern	0	1345	0	0	0.000
South Asian	0	14835	0	0	0.000
Ashkenazi Jewish	0	6632	0	0	0.000
East Asian	0	6605	0	0	0.000
African/African American	0	43722	0	0	0.000
Amish	0	687	0	0	0.000
XX	3	526242	0	0	0.000005701
XY	1	227909	0	1	0.000004388

Age Distribution



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PRESENCE IN THE AFFECTED POPULATION

Why it's useful: Multiple observations of the same variant in affected individuals is a strong indicator of pathogenicity.

Resources I will review:

- ClinVar
- LitVar

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PRESENCE IN THE AFFECTED POPULATION: CLINVAR

NM_000170.3(GLDC):c.1545G>C (p.Arg515Ser) Cite Follow Print Download

We've updated the ClinVar website to better support classifications of somatic variants!

Read more about changes to the website in our web release notes; more information about somatic variants in ClinVar is available on GitHub.

Germline

Classification (15)

Pathogenic
criteria provided, multiple submitters, no conflicts

Somatic

No data submitted for somatic clinical impact

Somatic

No data submitted for oncogenicity

Conditions - Germline

Condition	Classification (# of submissions)	Review status	Last evaluated	Variation/condition record
Non-ketotic hyperglycinemia	Pathogenic (12)	★★☆☆	Jan 16, 2024	RCV000012765.44
Generalized epilepsy Global developmental delay Obesity	Pathogenic (1)	★☆☆☆	Nov 28, 2016	RCV000449527.1
not provided	Pathogenic (1)	★☆☆☆	May 2, 2022	RCV001582476.5
GLDC-related condition	Pathogenic (1)	★☆☆☆	Mar 31, 2023	RCV003398490.4

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PRESENCE IN THE AFFECTED POPULATION: CLINVAR

The comments can sometimes contain useful information about how the variant was curated

Submissions - Germline

Classification (Last evaluated)	Review status (Assertion criteria)	Condition	Submitter	More information
Pathogenic (Oct 31, 2018)	★☆☆☆ (ICSL Variant Classification Criteria 09 May 2019) Method: clinical testing	Glycine encephalopathy 1 Affected status: unknown Allele origin: germline	Illumina Laboratory Services, Illumina Accession: SCV000916266.1 First in ClinVar: May 24, 2019 Last updated: May 24, 2019	Publications: PubMed (6) Comment: The GLDC c.1545G>C (p.Arg515Ser) variant has been reported in five studied and was found in a total of 60 probands including four in a homozygous ... (more)
Pathogenic (Apr 26, 2021)	★☆☆☆ (ACMG Guidelines, 2015) Method: research	Glycine encephalopathy 1 Affected status: yes Allele origin: paternal	HudsonAlpha Institute for Biotechnology, HudsonAlpha Institute for Biotechnology Study: AGHI WGS Accession: SCV001870362.1 First in ClinVar: Sep 19, 2021 Last updated: Sep 19, 2021	Comment: ACMG codes:PS3, PS4, PM2, PM3, PP3, PP5
Pathogenic (May 02, 2022)	★☆☆☆ (GeneDx Variant Classification Process June 2021) Method: clinical testing	Not Provided Affected status: yes Allele origin: germline	GeneDx Accession: SCV001812841.3 First in ClinVar: Sep 08, 2021 Last updated: Mar 04, 2023	Comment: Published functional studies demonstrate a damaging effect with significant impairment of enzyme function (Swanson et al., 2015); In-silico analyses, including protein predictors and evolutionary conservation, ... (more)

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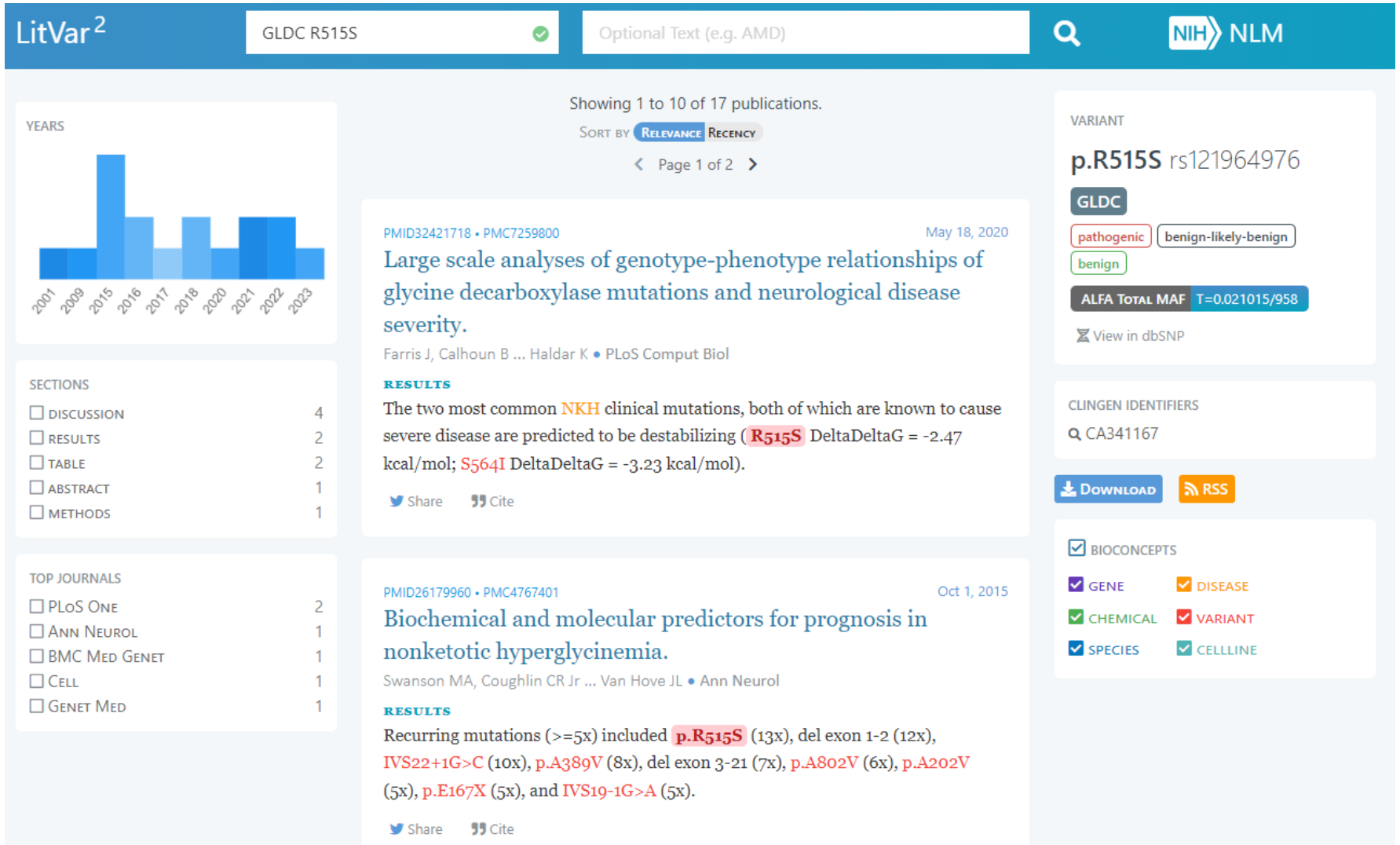
PRESENCE IN THE AFFECTED POPULATION: LITVAR



- Litvar searches Pubmed for specific references to a variant
- Very helpful for finding other affected probands or functional assays that have been done

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IN SILICO PREDICTIONS

Why it's useful: *In silico* tools are good indicators of whether a particular variant is damaging.

Resources I will review:

- REVEL – missense predictions
- Splice-AI – splicing predictions

Other resources:

- **NOTE:** ACMG recommends you pick one missense scoring tool and stick with that one to avoid confirmation bias. TOP Team as a group has chosen to use REVEL. But there are other tools like
  - CADD
  - AlphaMissense

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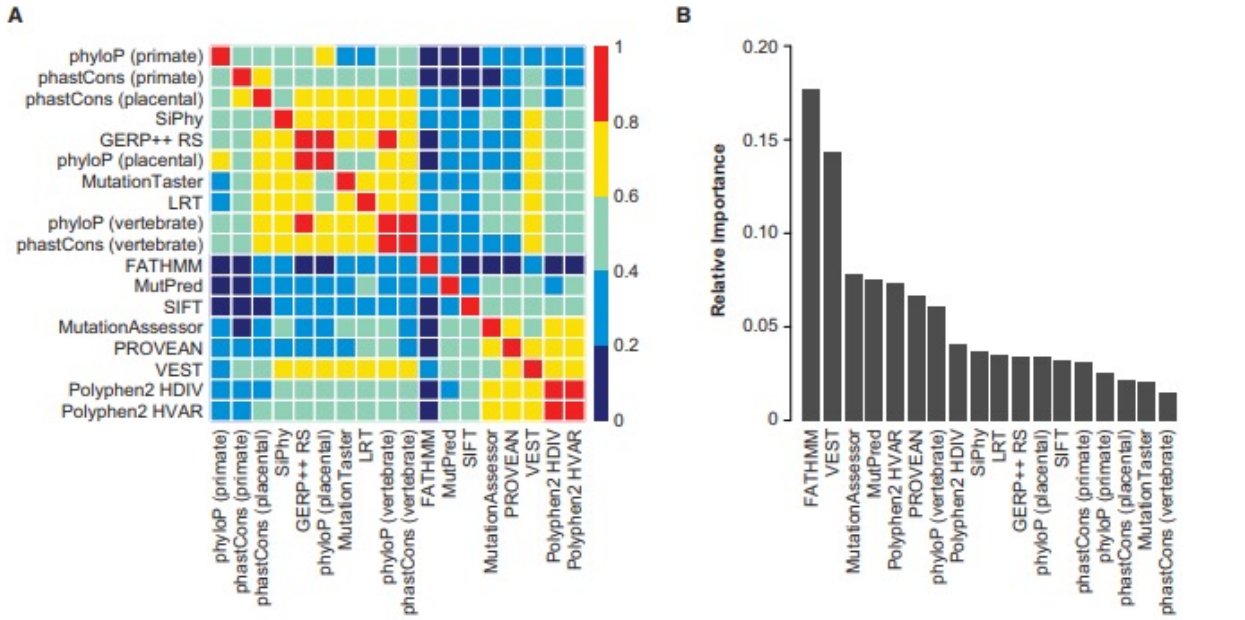
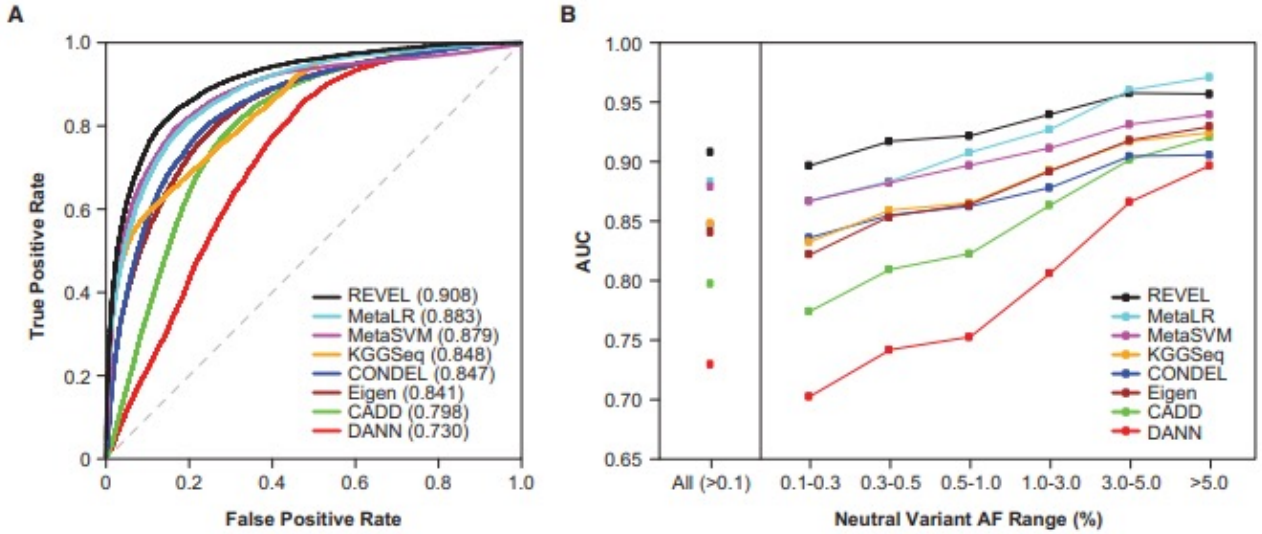
IN SILICO PREDICTIONS: REVEL





- REVEL is a conglomeration of 18 *in silico* tools that assess how damaging a particular missense variant is

- REVEL gives you a 0 to 1 score of how "damaging" a variant is:
  - < 0.29 Benign
  - 0.29 – 0.643 Uncertain
  - > 0.644 Pathogenic
  - See PMID:36413997 for where these boundaries come from

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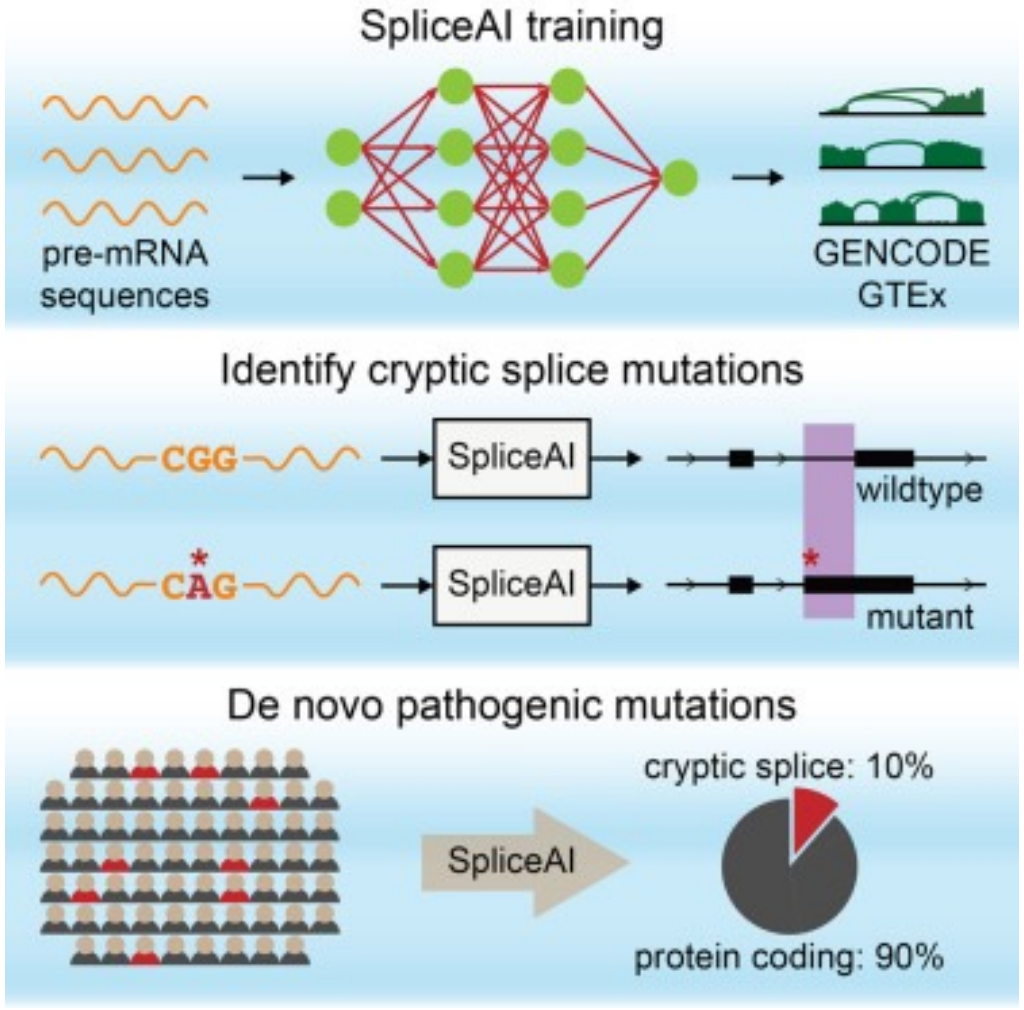
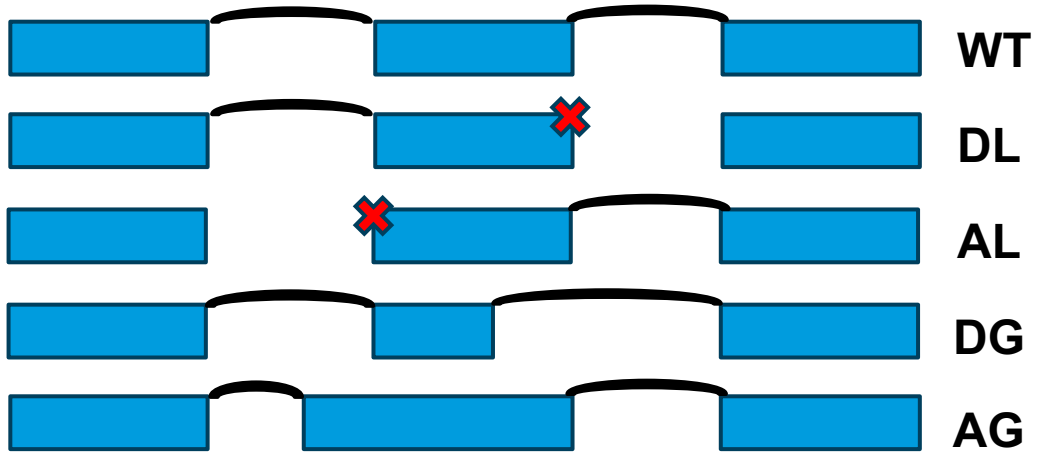
IN SILICO PREDICTIONS: SPLICE-AI





- Splice AI predicts:
  - Loss of canonical donors (DL)
  - Loss of canonical acceptors (AL)
  - Creation / strengthening of cryptic donors (DG)
  - Creation / strengthening of cryptic acceptors (AG)

- Any Splice-AI prediction > 0.2 is considered to likely have at least some effect

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LOCATION WITHIN THE GENE

Why it's useful: For certain genes, pathogenic variants can cluster in particular functional domains or affect specific residues.

Resources I will review:

- ProteinPaint
- Useful info also in Uniprot which I've already discussed

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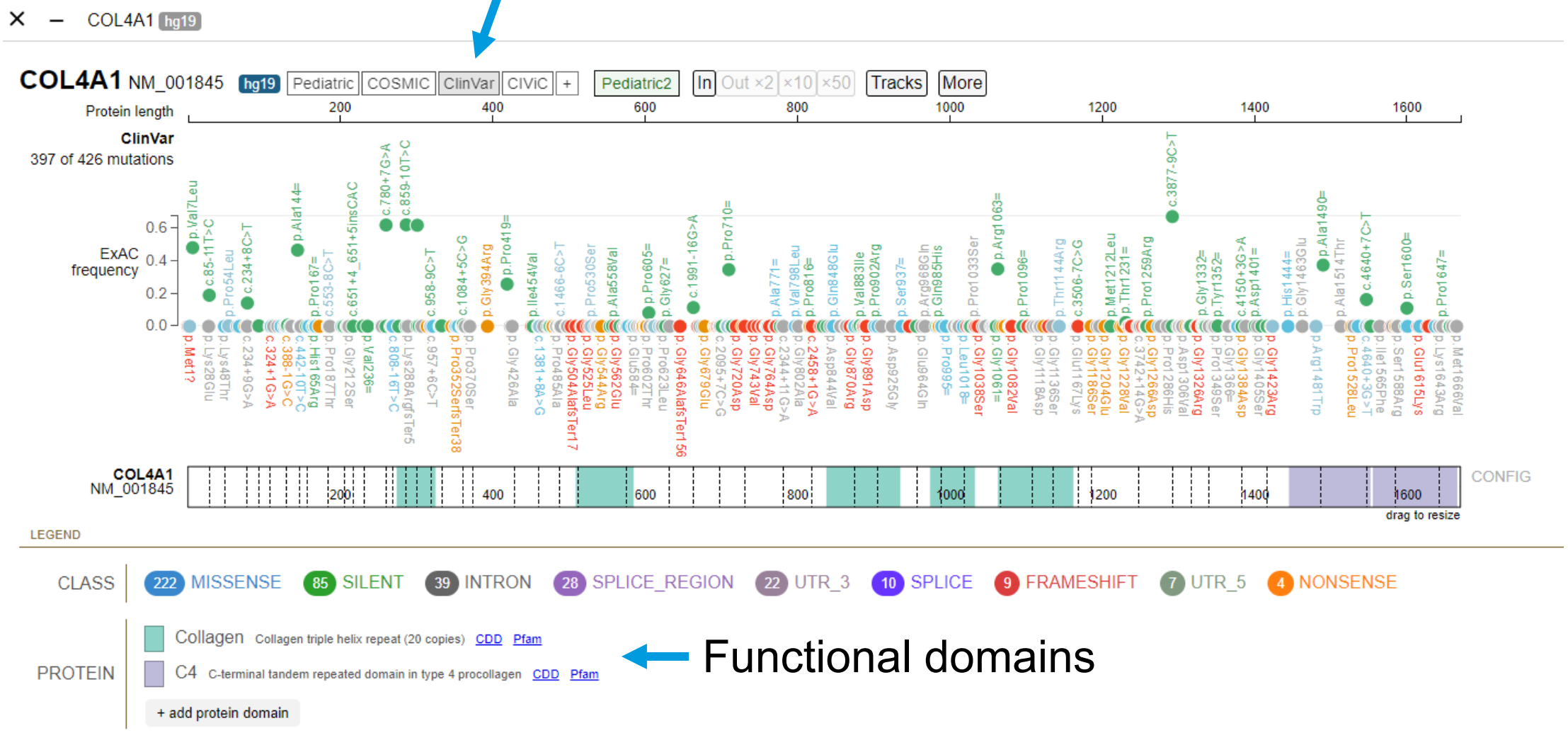
LOCATION WITHIN THE GENE: PROTEINPAINT

Press ClinVar to import all ClinVar variants



← Functional domains

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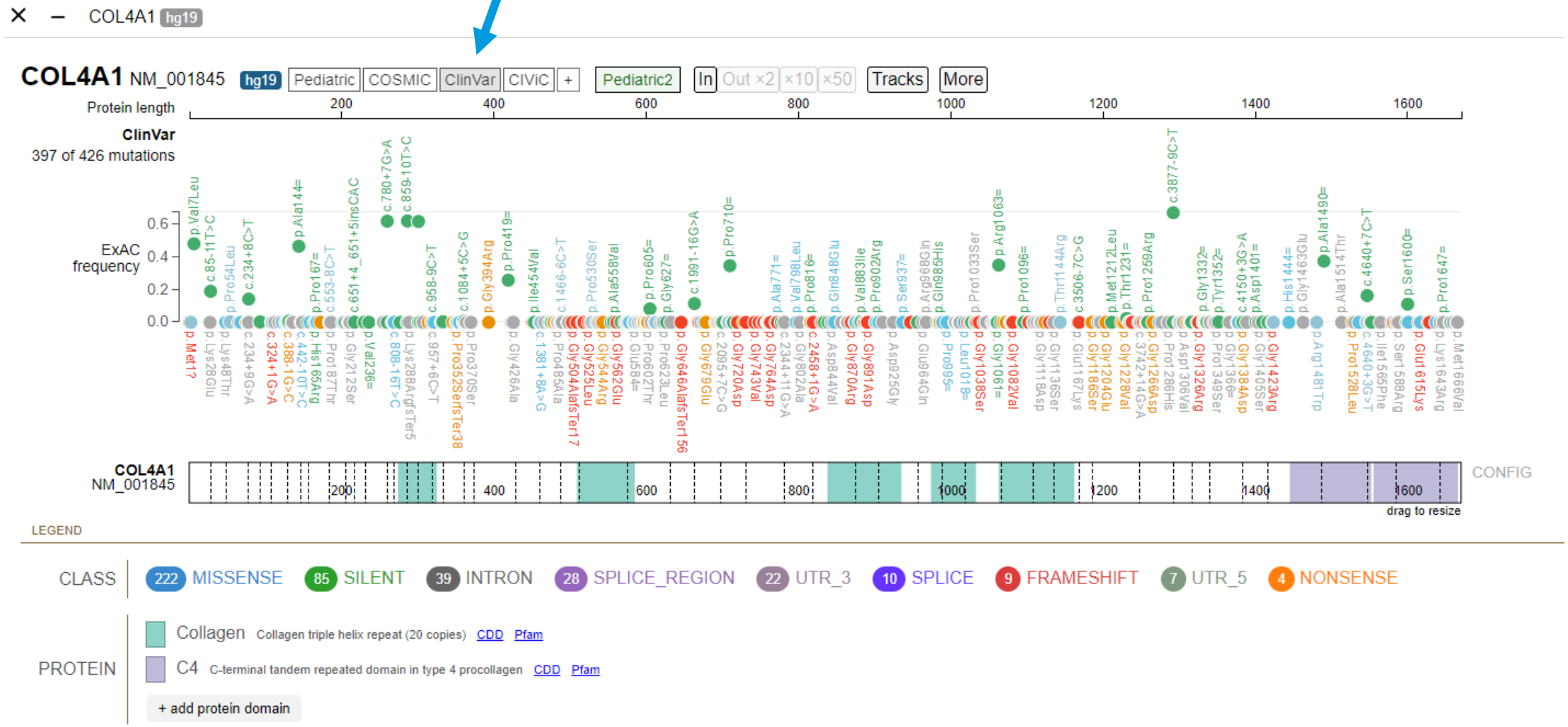
LOCATION WITHIN THE GENE: PROTEINPAINT

Press ClinVar to import all ClinVar variants



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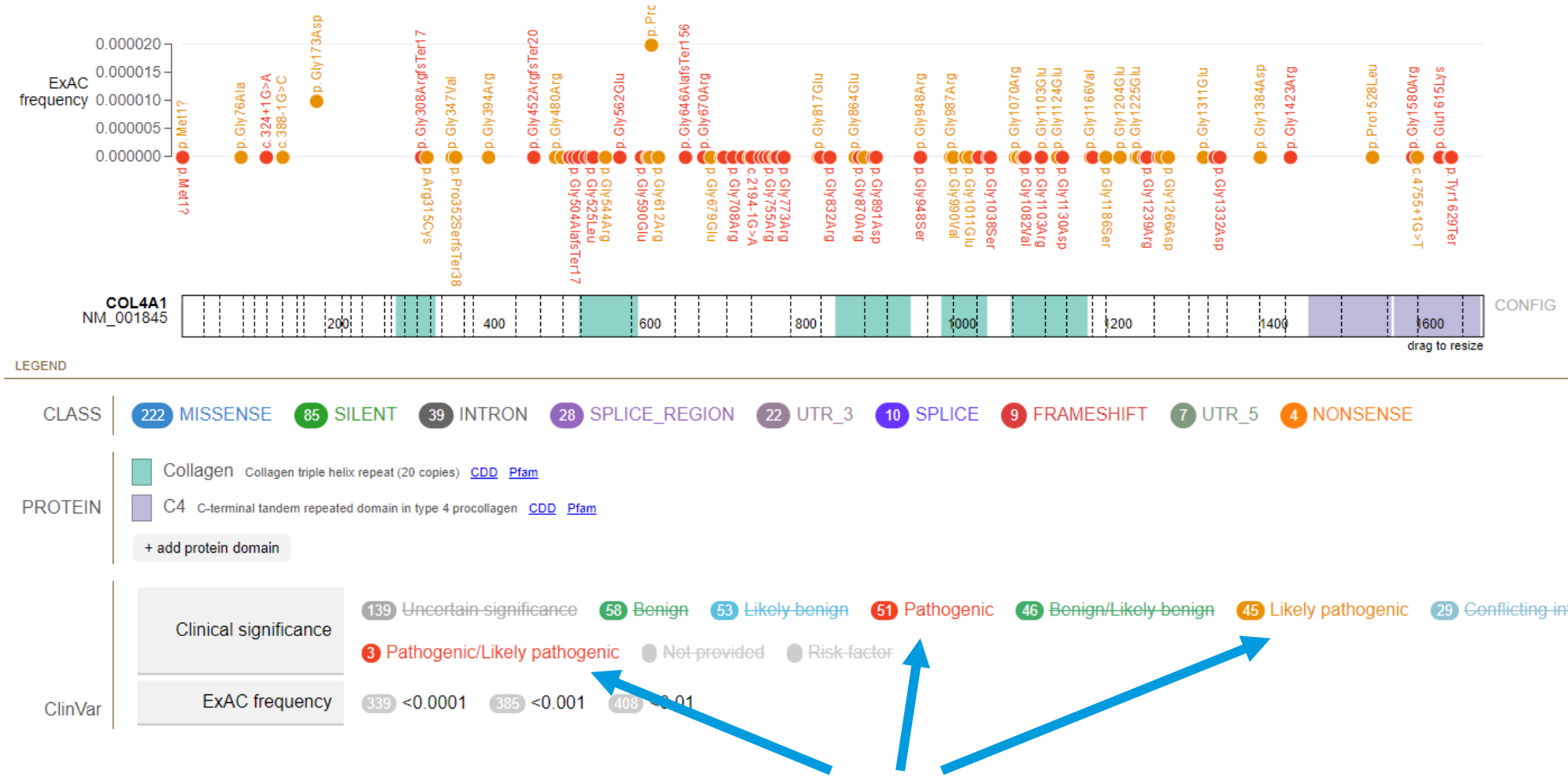
LOCATION WITHIN THE GENE: PROTEINPAINT



You can toggle which variant classification is shown

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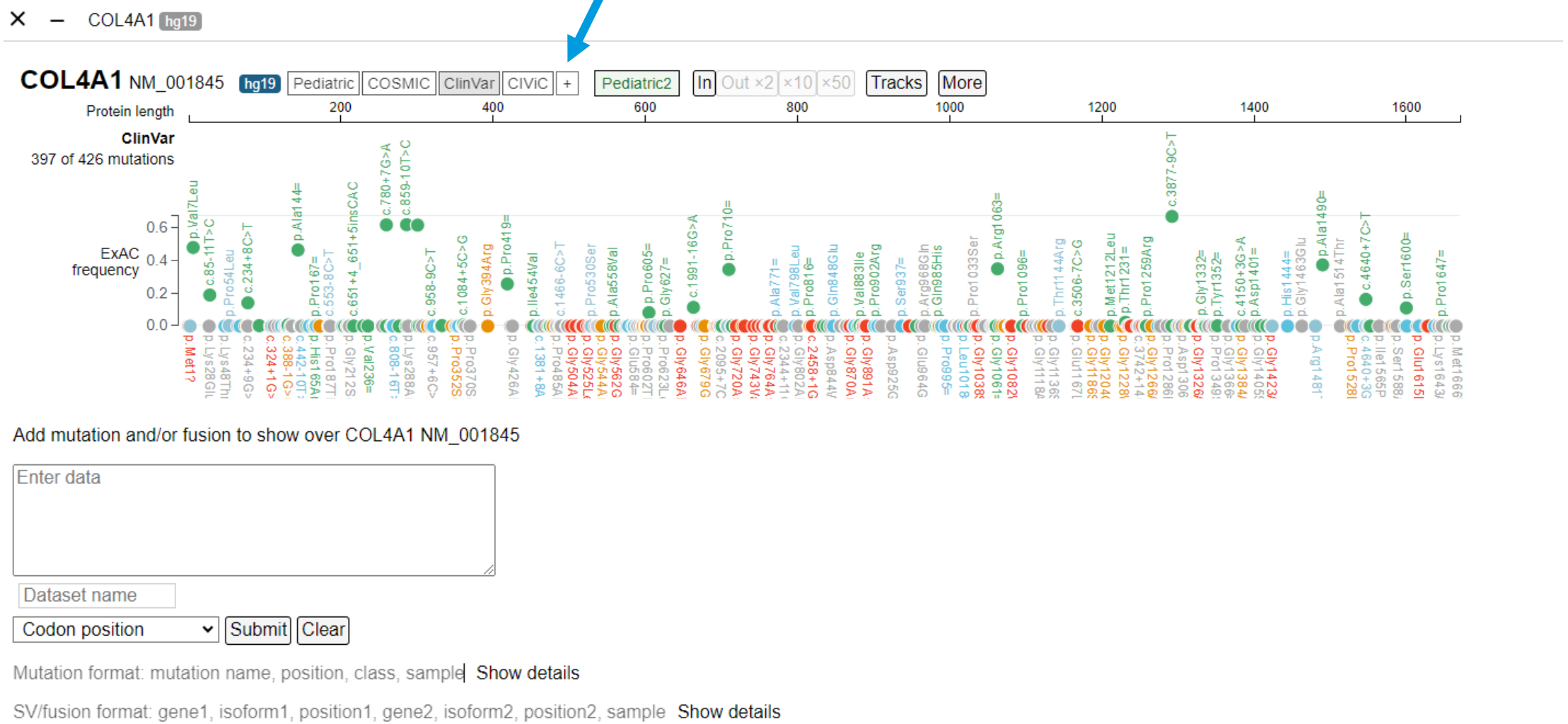
LOCATION WITHIN THE GENE: PROTEINPAINT

You can add your own variant



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GENERAL TOOLS

These tools are helpful for a general overview on a variant and pull together information from a lot of the resources I've discussed

Resources I will review:

- Franklin
- Mobi Details

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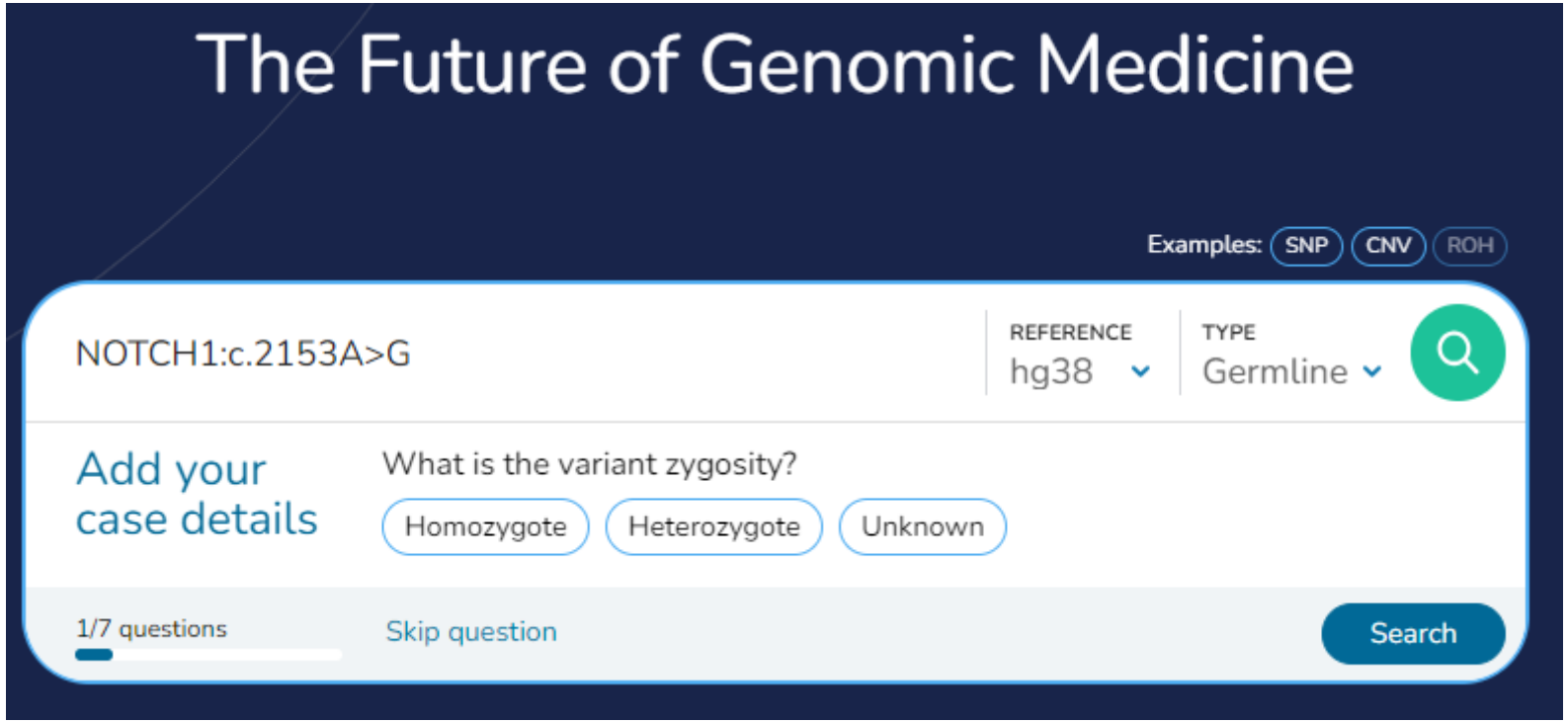
GENERAL TOOLS: FRANKLIN



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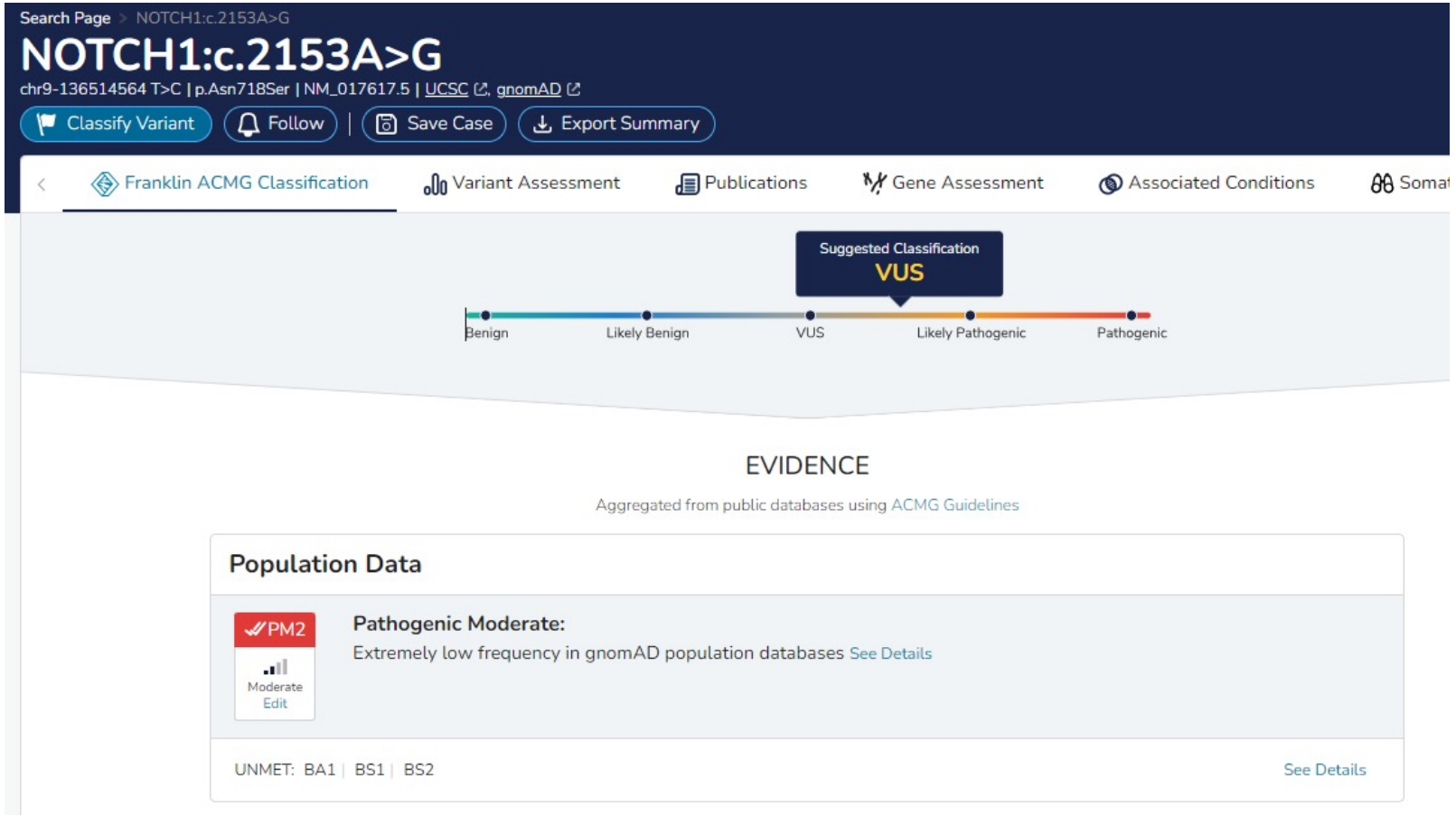
GENERAL TOOLS: FRANKLIN



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

- They don't apply all ACMG criteria correctly

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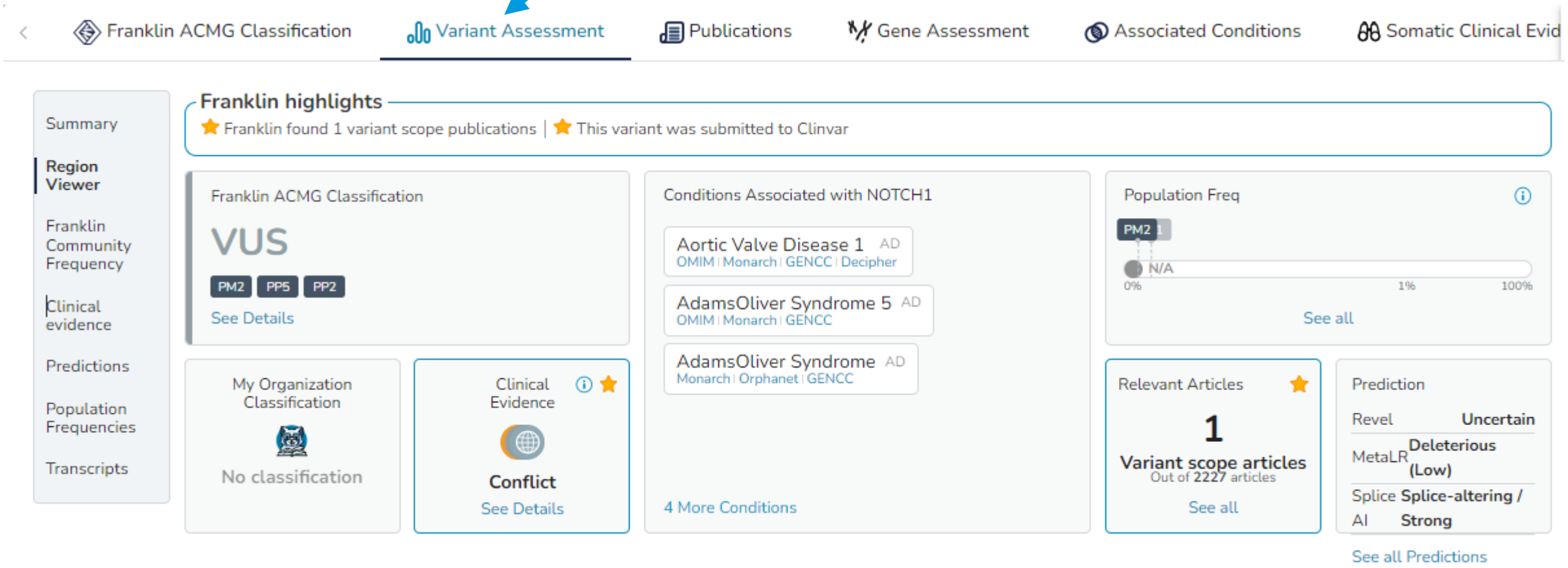
GENERAL TOOLS: FRANKLIN

Most helpful tab



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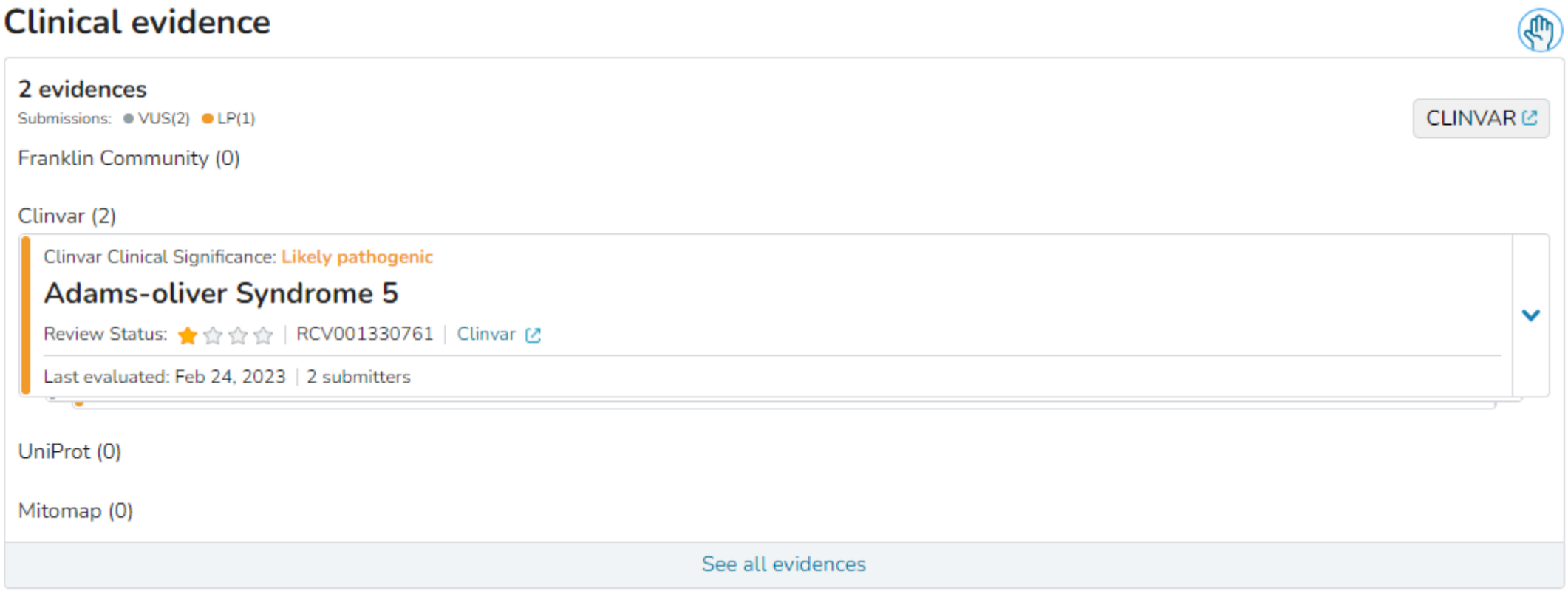
GENERAL TOOLS: FRANKLIN



- They mine and link to ClinVar

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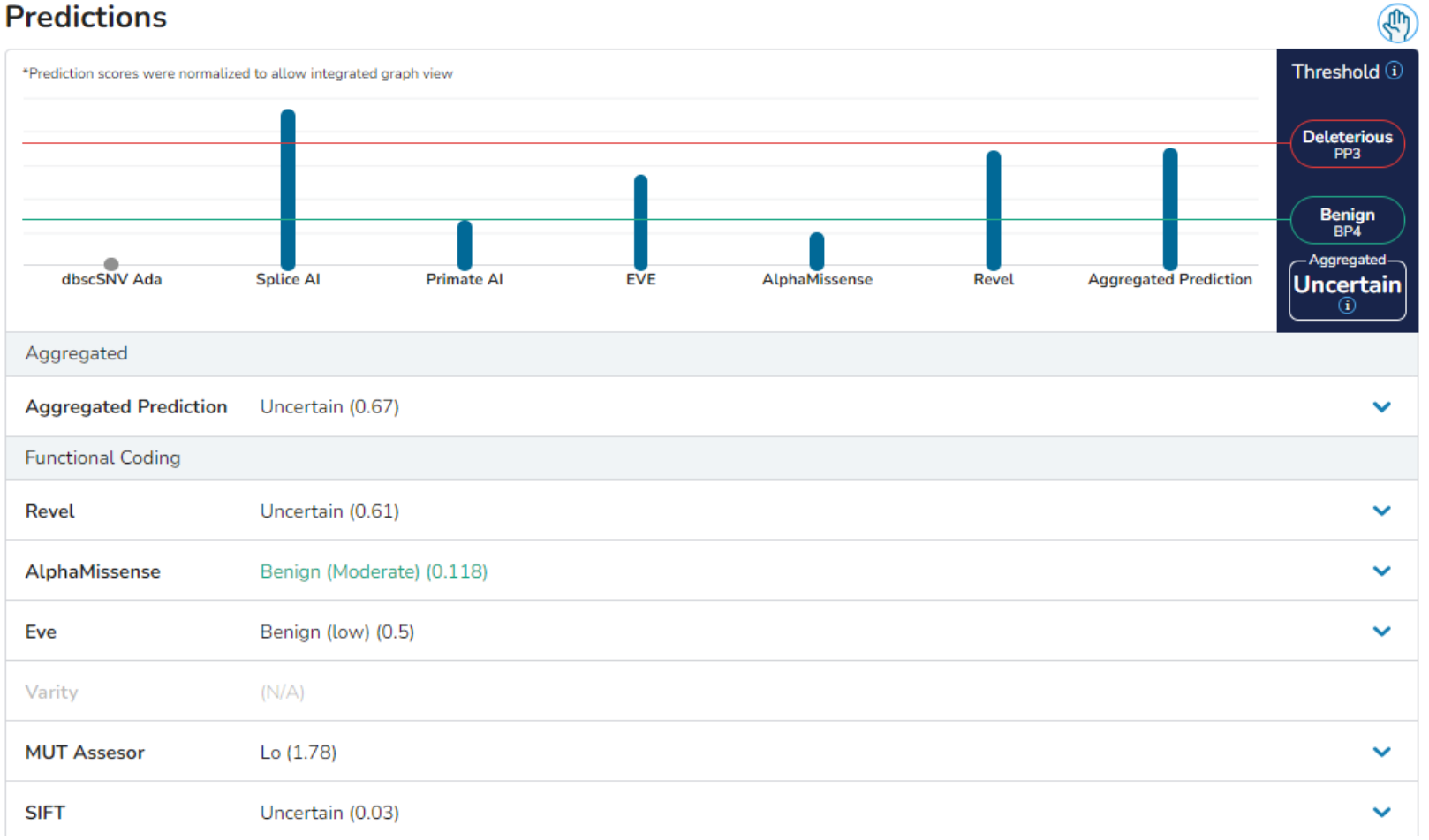
GENERAL TOOLS: FRANKLIN



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

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GENERAL TOOLS: FRANKLIN

- They pull frequency data from gnomAD

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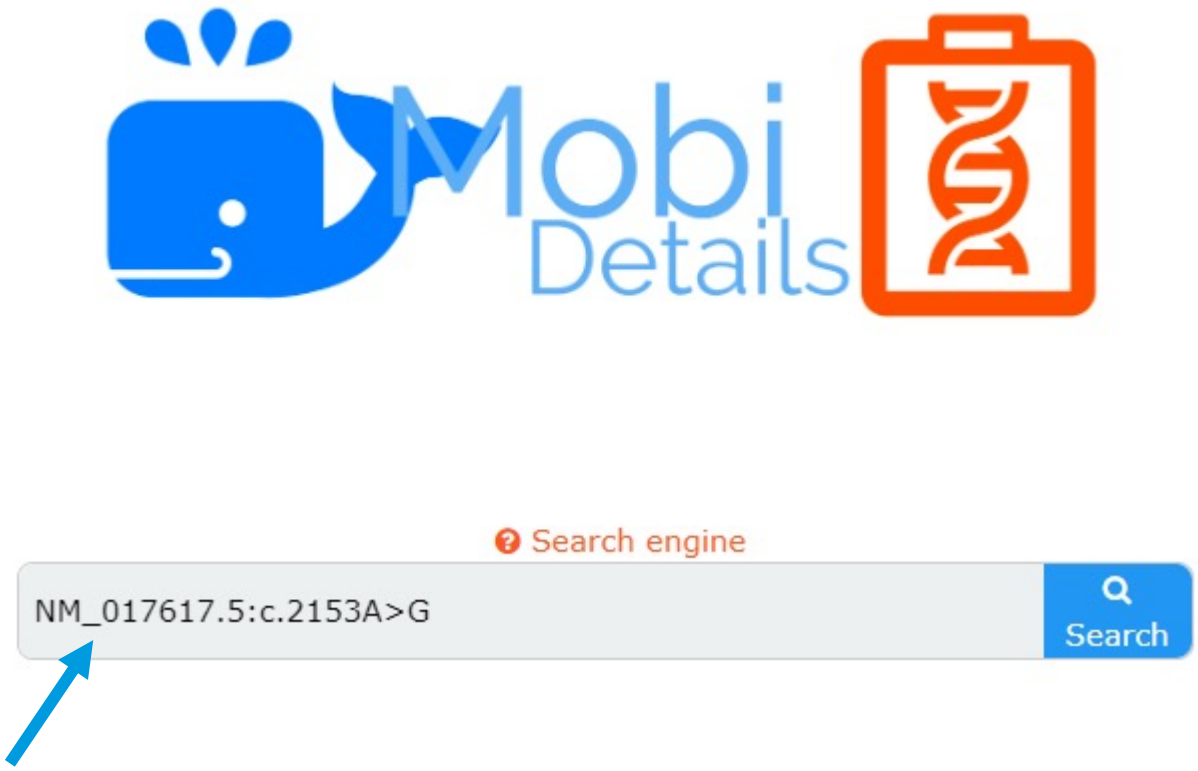
GENERAL TOOLS: MOBI DETAILS



Mobi Details requires that you use the NM transcript code (which you can get from Franklin)

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GENERAL TOOLS: MOBI DETAILS

Features	Values	Descriptions
HGNC gene symbol (ID):	*NOTCH1* (7881)	*HGNC gene symbol and corresponding ID*
HGVS DNA on transcript:	NM_017617.5:c.2153A>G MD canonical	*HGVS full nomenclature at DNA level on transcript*
HGVS RNA:	r.(?)	*HGVS full nomenclature at RNA level*
HGVS Protein:	NP_060087.3:p.(Asn718Ser)	*HGVS full nomenclature at protein level*
HGVS genomic (hg19):	chr9:g.139409016T>C	*HGVS full nomenclature at genomic level (hg19)*
HGVS strict genomic (hg19):	NC_000009.11:g.139409016T>C	*HGVS full strict nomenclature at genomic level (hg19)*
pseudo VCF (hg19):	9-139409016-T-C	*chr-pos-ref-alt (hg19)*
HGVS genomic (hg38):	chr9:g.136514564T>C	*HGVS full nomenclature at genomic level (hg38)*
HGVS strict genomic (hg38):	NC_000009.12:g.136514564T>C	*HGVS full strict nomenclature at genomic level (hg38)*
pseudo VCF (hg38):	9-136514564-T-C	*chr-pos-ref-alt (hg38)*

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GENERAL TOOLS: MOBI DETAILS

Positions

Features	Values	Descriptions
Position in transcript:	Exon 13	*Exon/intron position in NM_017617.5*
Position / splice site	55 bp from donor	*Position relative to the nearest splice site*
Position / protein	718 / 2555	*Position relative to the protein*
Position / domain	EGF-like 19; calcium-binding (716 - 751)	*Position in a protein domain (UNIPROT: P46531)*
Position tolerance	0.18 : intolerant - Transcript view	*MetaDome score, the closer to 0, the more intolerant to variation*
Wild type sequence	GACCCCACCTGCCTGTCTGAGGTCA A TGAGTGCAACAGCAACCCCTGCGTC	*Wild type DNA sequence +/- 25 bp*
Mutant sequence	GACCCCACCTGCCTGTCTGAGGTCA G TGAGTGCAACAGCAACCCCTGCGTC	*Mutant DNA sequence +/- 25 bp*

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GENERAL TOOLS: MOBI DETAILS

Missense predictions: p.(Asn718Ser)

Features	Values	Prediction	Descriptions
SIFT:	0.03	Damaging	*Threshold < 0.05 for Damaging - single score*
Polyphen 2 HumDiv:	0.658	Possibly Damaging	*Thresholds ≥ 0.454|0.957 for Possibly and Probably Damaging - single score*
Polyphen 2 HumVar:	0.345	Benign	*Thresholds ≥ 0.447|0.909 for Possibly and Probably Damaging - single score*
Fathmm:	-2.84	Damaging	*Threshold ≤ -1.5 for Damaging - single score*
AlphaMissense:	0.118	Likely Benign	*Thresholds 0.34|0.564 for Likely Benign, Ambiguous, Likely Pathogenic - single score*
REVEL:	0.61	Damaging	*Thresholds 0.2|0.5 for Benign, Uncertain, Damaging - meta score*
ClinPred:	0.948	Damaging	*Threshold ≥ 0.5 for Damaging - meta score*
Meta SVM:	0.4763 (10)	Damaging	*Threshold ≥ 0 for Damaging (reliabilty index: 0-10), 10:high - meta score*
Meta LR:	0.6883 (10)	Damaging	*Threshold ≥ 0.5 for Damaging (reliabilty index: 0-10), 10:high - meta score*
Mistic:	0.84	Damaging	*Threshold ≥ 0.5 for Damaging - meta score*

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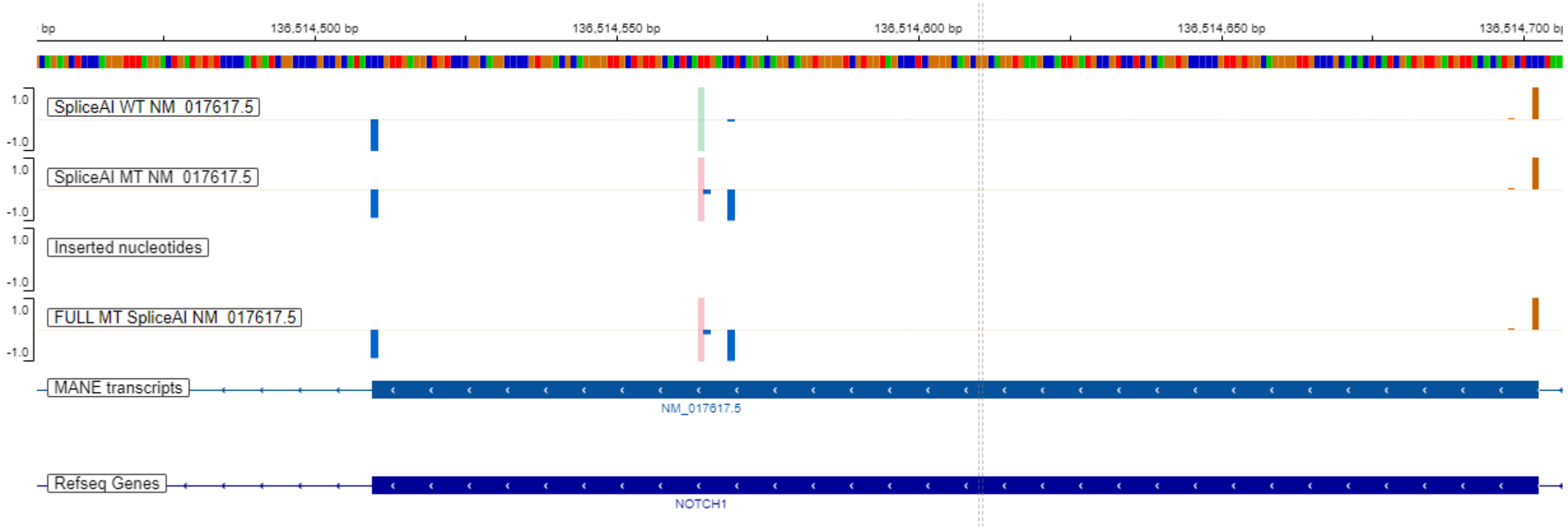
GENERAL TOOLS: MOBI DETAILS



- Blue bars = predicted donors
- Orange bars = predicted acceptors

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LINKS ON LINKS ON LINKS

Gene-level information:

- Gene-disease associations
  - OMIM: https://www.omim.org/
  - Gene Reviews: https://www.ncbi.nlm.nih.gov/books/NBK1116/
- Biological function
  - Uniprot: https://www.uniprot.org/
  - GeneCards: https://www.genecards.org/
- Tissue-specific expression
  - GTex: https://gtexportal.org/home/
  - Protein Atlas: https://www.proteinatlas.org/

Variant-level information:

- Population frequency
  - gnomAD: https://gnomad.broadinstitute.org/
- Presence in the affected population
  - ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/
  - LitVar:
- *In-silico* predictions
  - REVEL and Splice-AI
    - Franklin: https://franklin.genoox.com/clinical-db/home
    - Mobi Details: https://mobidetails.iurc.montp.inserm.fr/MD/
    - Splice-AI hosted at Broad: https://spliceailookup.broadinstitute.org/
- Location within the gene
  - Protein paint: https://proteinpaint.stjude.org/

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QUESTIONS & ANSWERS