MAYO CLINIC

CLINICAL VARIANT INTERPRETATION LAB

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Introduction to Computational Genomics
June 25th, 2024

ACMG Criteria

	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			

Case 1: Guided Walkthrough

I will walk you through the variant classification with active discussion from the class

Case #1

Patient phenotype:

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

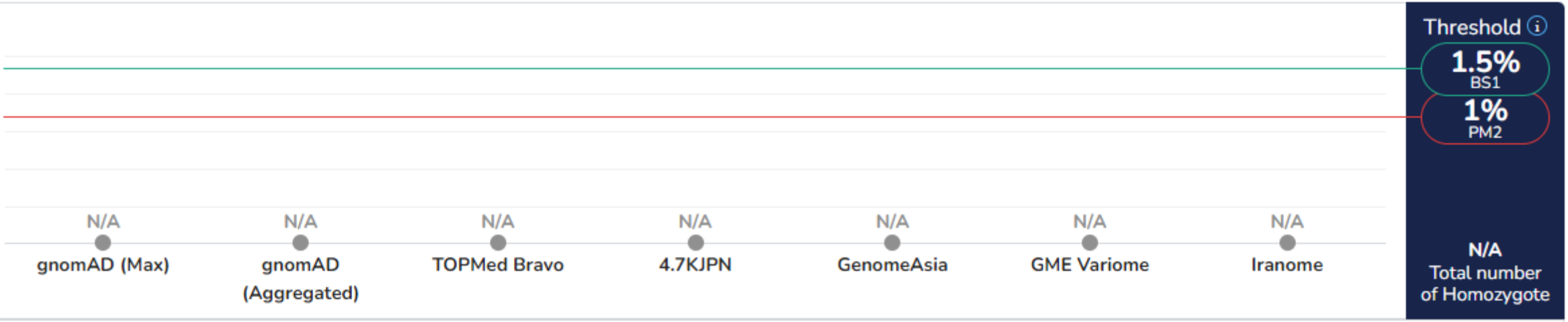
Variant identified via trio genome:

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

Criteria being considered	Strength being applied	Evidence	Points

PM2

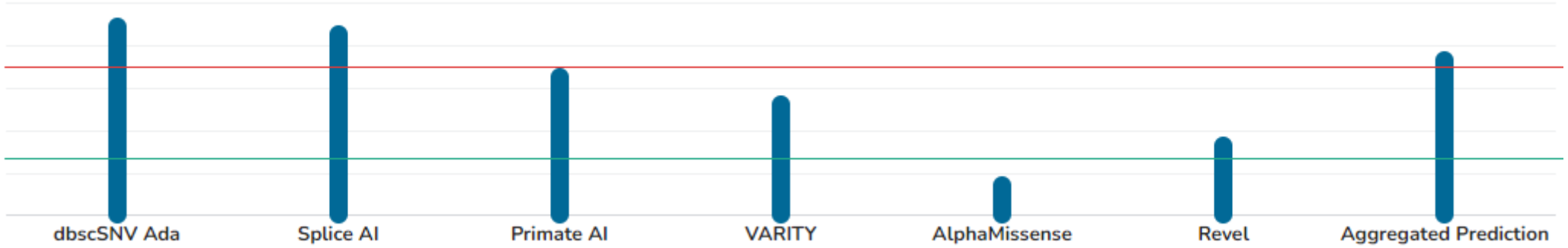
Population Frequencies



N/A	**gnomAD (Exome)** **No Observation** for this variant in gnomAD (Exome)	**N/A** Alleles of **N/A** **N/A** homozygote **N/A** Individuals
N/A	**gnomAD (Genome)** **No Observation** for this variant in gnomAD (Genome)	**N/A** Alleles of **N/A** **N/A** homozygote **N/A** Individuals
N/A	**gnomAD (Aggregated)** **No Observation** for this variant in gnomAD (Aggregated)	**N/A** Alleles of **N/A** **N/A** homozygote **N/A** Individuals

PP3

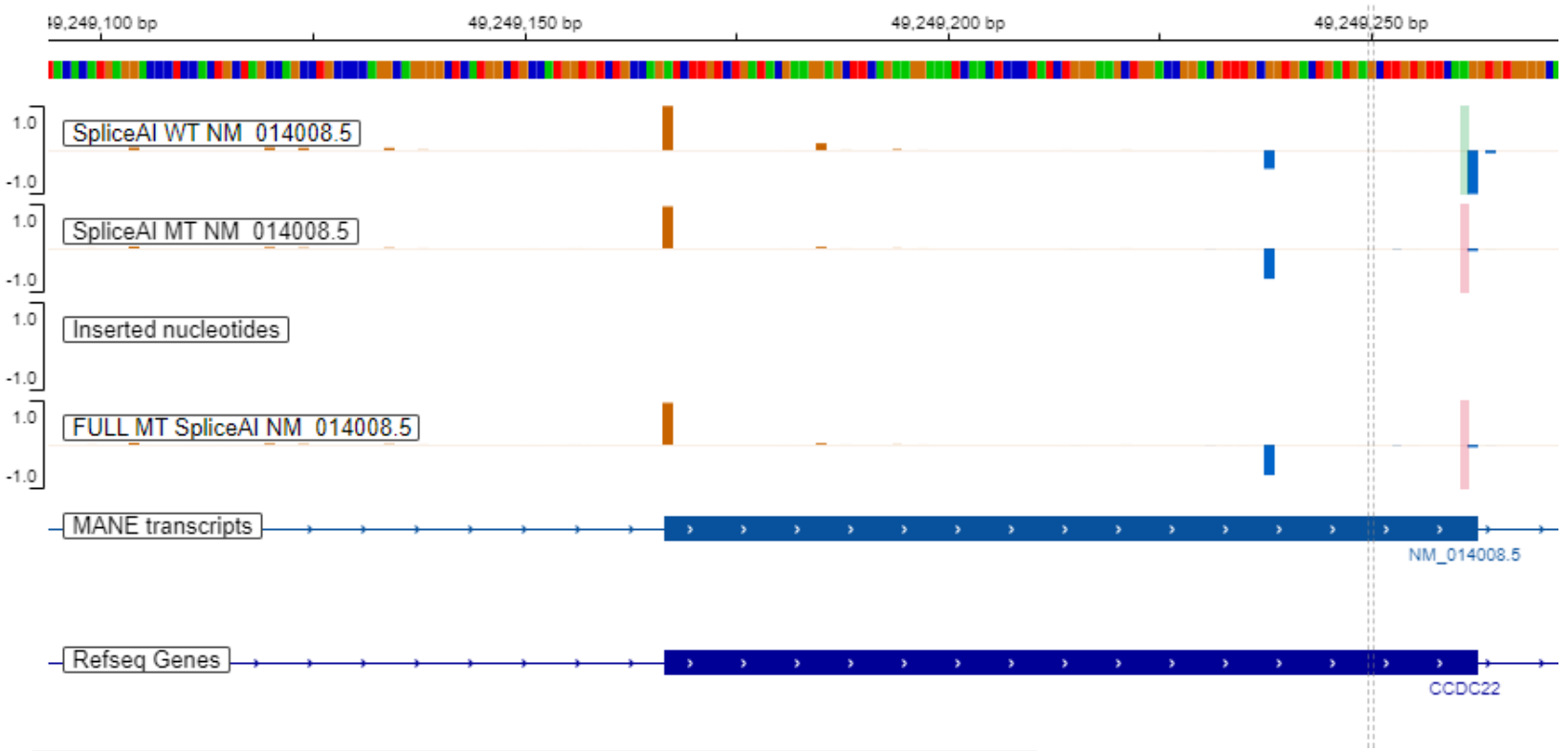
*Prediction scores were normalized to allow integrated graph view



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

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

PP3



spliceAI AG:	0.01 (1)
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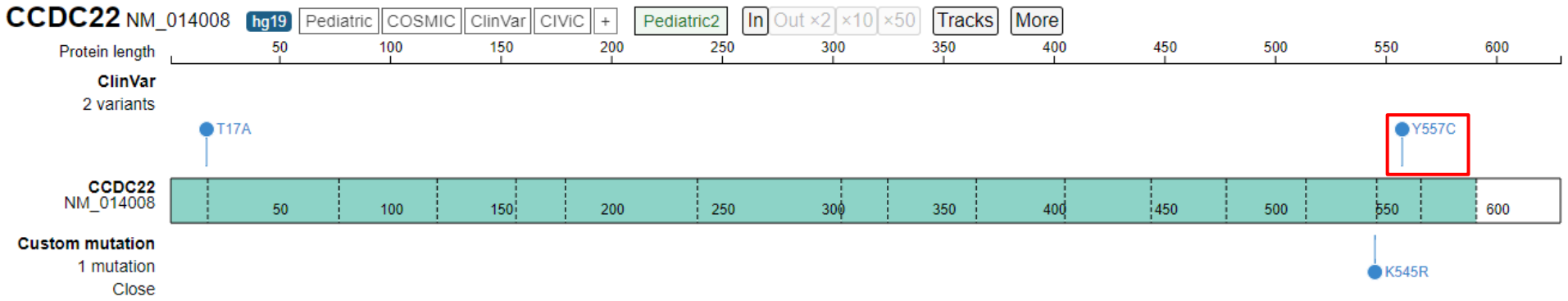
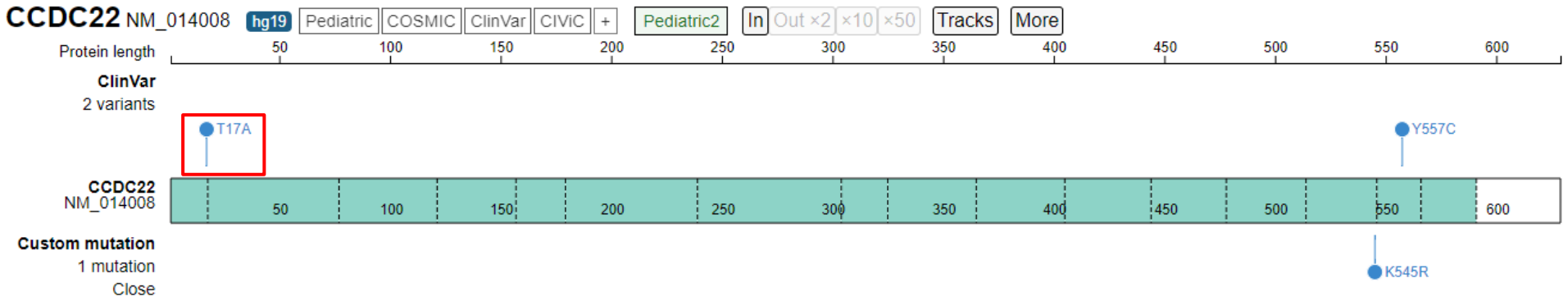
spliceAI DL:	0.92 (1)

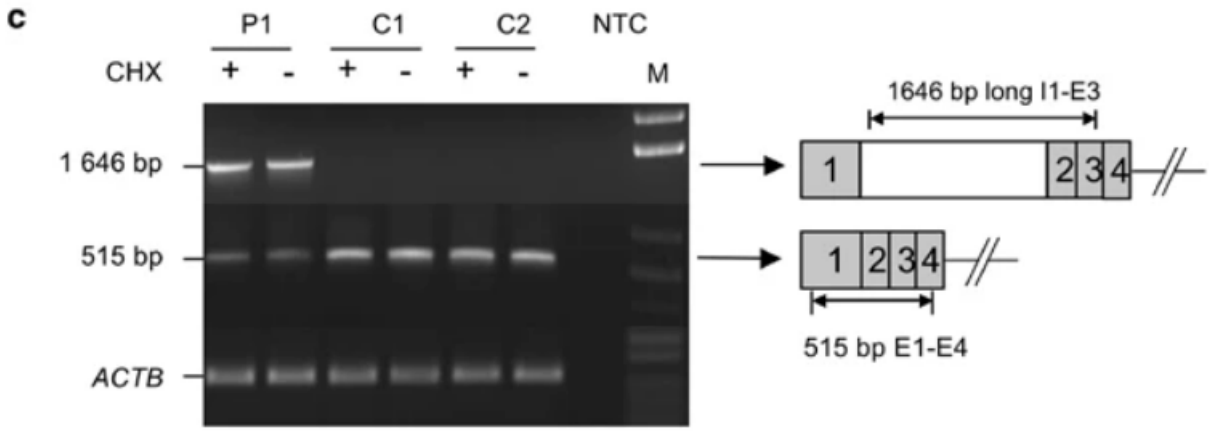
PP3



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

PP3





The second reported missense variant has been functionally proven to cause retention of intron 1.

PS2

Table 1. Points awarded per de novo occurrence

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

*Maximum allowable value of 1 may contribute to overall score

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

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

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

PS2

Ritscher-Schinzel syndrome 2

INHERITANCE
- X-linked recessive

GROWTH
Other
- Growth delay, postnatal

HEAD & NECK
Head
- Large head circumference

Face
- Broad forehead
- Short philtrum

Eyes
- Upslanting palpebral fissures
- Hypertelorism

Mouth
- Protruding tongue
- Abnormal dentition (in some patients)

Neck
- Broad neck

CARDIOVASCULAR
Heart
- Ventricular septal defect
- Atrial septal defect

Vascular
- Patent ductus arteriosus

GENITOURINARY
External Genitalia (Male)
- Cryptorchidism

SKELETAL
Skull
- Large anterior fontanelles

Spine
- Scoliosis

Hands
- Distal digital anomalies
- Syndactyly
- Camptodactyly
- Clinodactyly
- Hypoplastic distal phalanges

Feet
- Overriding toes
- Broad halluces

SKIN, NAILS, & HAIR
Hair
- Low posterior hairline
- Aplasia cutis (in some patients)

MUSCLE, SOFT TISSUES
- Hypotonia

NEUROLOGIC
Central Nervous System
- Delayed psychomotor development
- Poor speech
- Dandy-Walker malformation
- Cerebellar hypoplasia

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

Case 2: Group Walkthrough #1

In breakout groups, walk through your variants and apply criteria you think are appropriate based on publically available databases.

Case #2

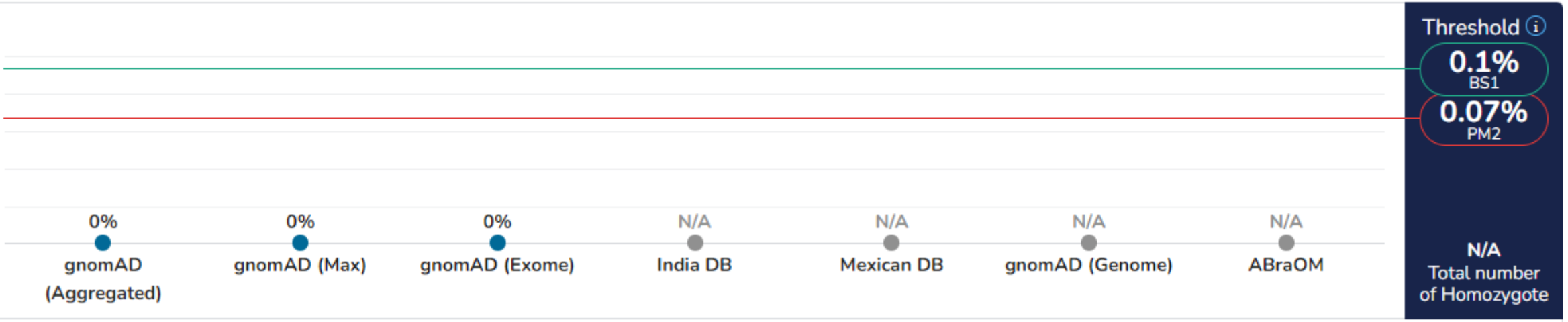
Patient phenotype:
- Retinitis pigmentosa

Variant identified via trio exome:
- *RPGR:*c.905G>A, Cys302Tyr
- Transcript: NM_001034853.2
- Inheritance: Unknown, but variant is on the X-chromosome

Criteria being considered	Strength being applied	Evidence	Points

PM2

Population Frequencies



0% **gnomAD (Aggregated)**
No Observation for this variant in gnomAD (Aggregated)
0 Alleles of **1,088,482**
0 homozygote
N/A Individuals

0% **gnomAD (Max)**
No Observation for this variant in gnomAD (Max)
0 Alleles of **833,458**
0 homozygote
N/A Individuals

0% **gnomAD (Exome)**
No Observation for this variant in gnomAD (Exome)
0 Alleles of **1,088,482**
0 homozygote
N/A Individuals

PS4

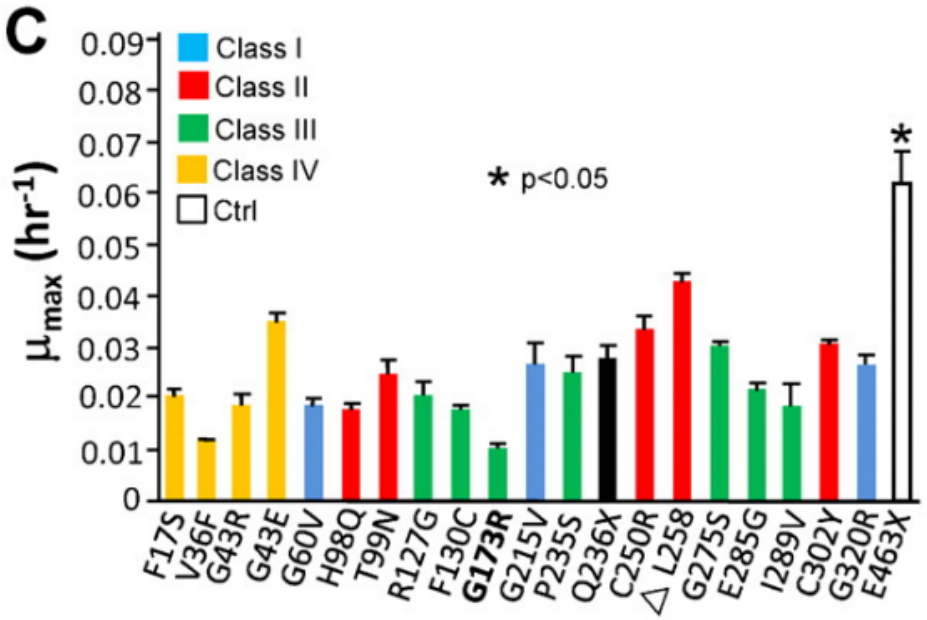
- The variant was observed in three males with retinitis pigmentosa in one family. (PMID: 10937588)

- The variant was observed in a 56 y/o male with retinitis pigmentosa in a Chinese cohort. Age of onset was 3 y/o. (PMID: 32100970)

- The variant was observed in at least one family member from a large retinal degeneration cohort (PMID: 32037395) (They applied PP1 indicating multiple family members were observed).

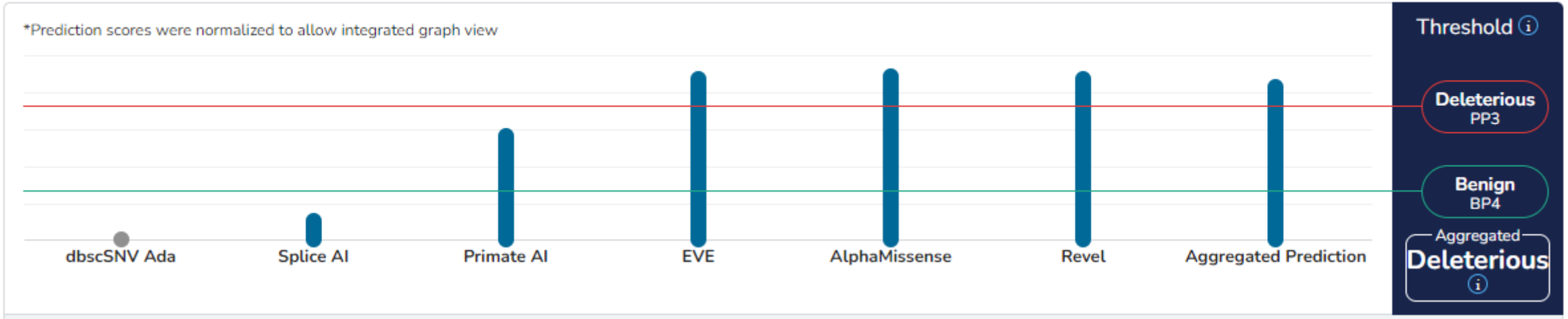
PS3

Cys302Tyr was shown to reduce the interaction between RPGR and RPGRIP1α by yeast hybridization assay (PMID: 23213406)



PP3

Predictions

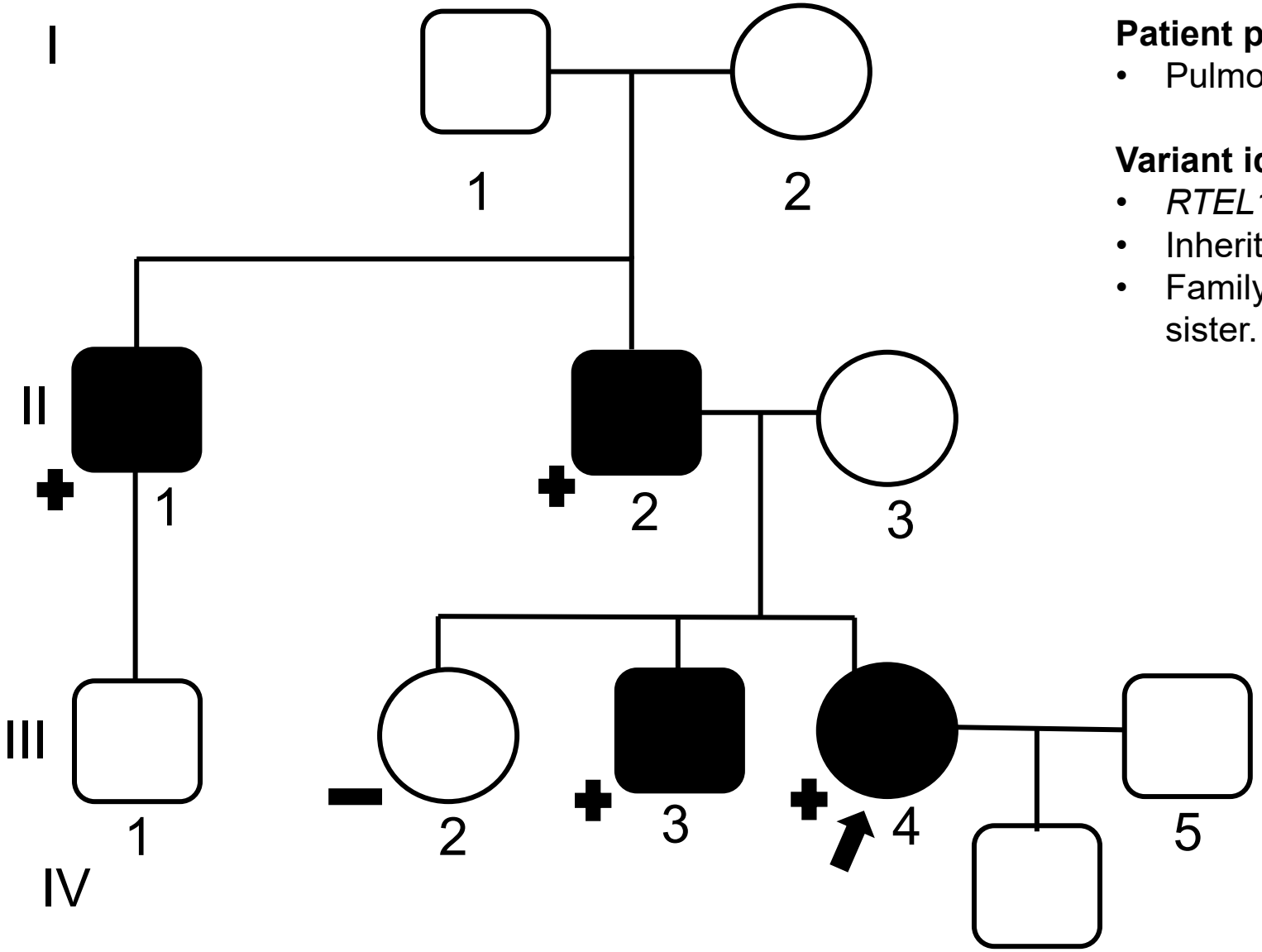


Functional Coding	
Revel	Deleterious (Moderate) (0.92)
AlphaMissense	Deleterious (Moderate) (0.959)
Eve	Deleterious (0.93)

Case 3: Group Walkthrough #2

In breakout groups, walk through your variants and apply criteria you think are appropriate based on publically available databases.

Variant #3



Patient phenotype:

- Pulmonary fibrosis, shorted telomeres

Variant identified via trio genome:

- *RTEL1:*c.101A>G, Q34R
- Inheritance: Paternal
- Family history: 1 affected brother, 1 unaffected sister. Father and paternal uncle are affected.

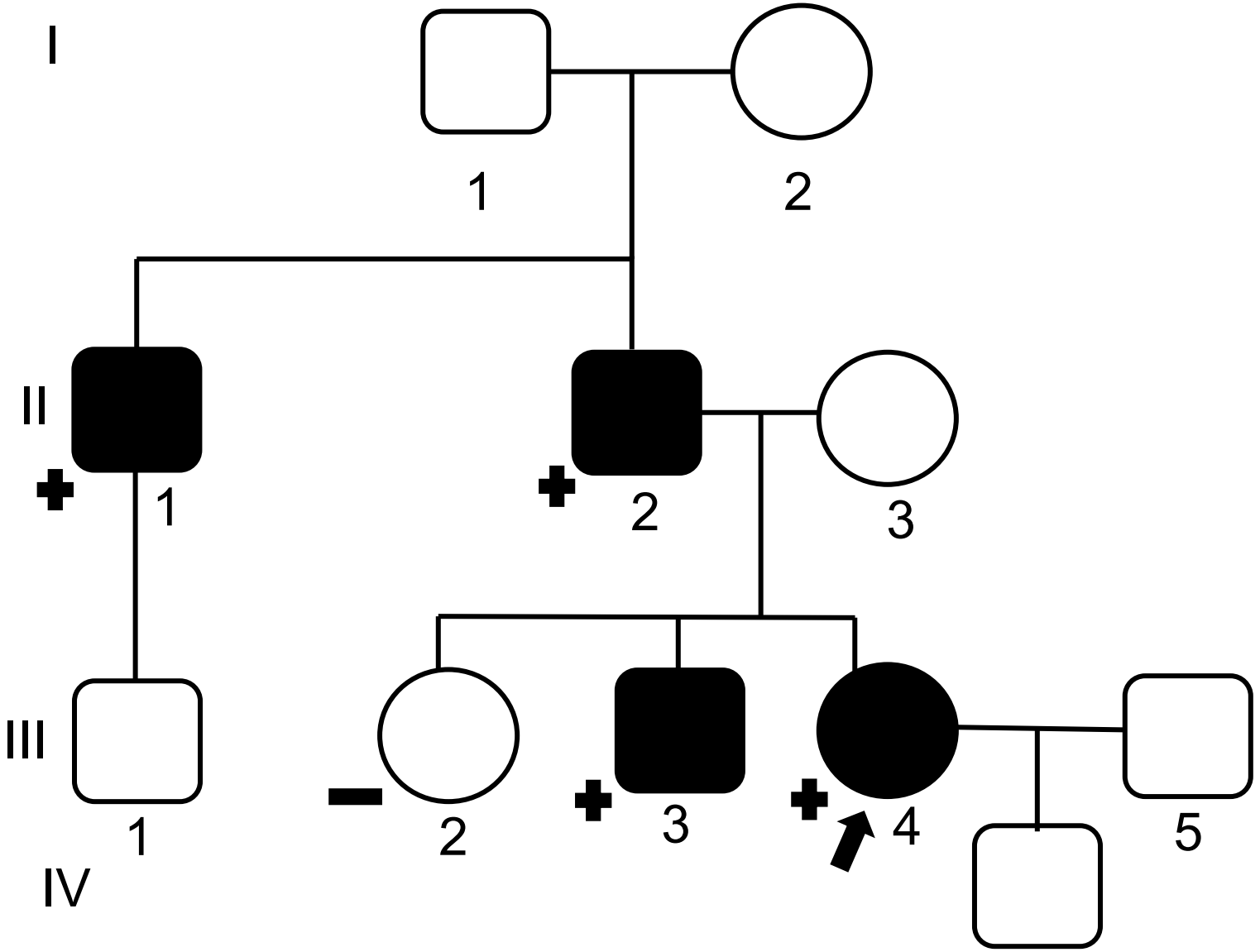
Variant #3

Patient phenotype:
- Pulmonary fibrosis, shorted telomeres

Variant identified via trio genome:
- *RTEL1:*c.101A>G, Q34R
- Inheritance: Paternal
- Family history: 1 affected brother, 1 unaffected sister. Father and paternal uncle are affected.

Criteria being considered	Strength being applied	Evidence	Points

PP1



Case Segregation Data (PP1) Important Considerations

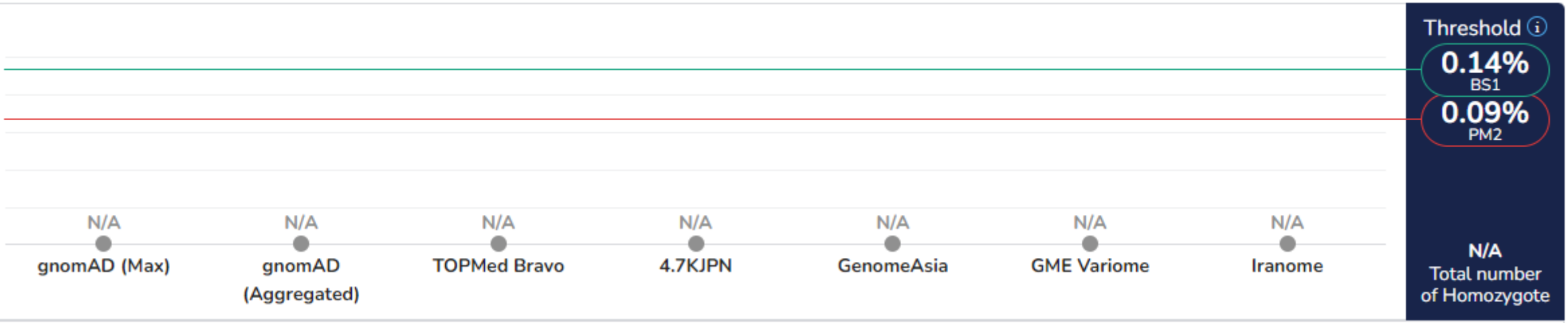
Can be incorporated as part of the assessment in case-level evidence. However, the evidence for pathogenicity should be carefully applied. Segregation signifies evidence for linkage of a locus, rather than direct variant-specific pathogenicity. There are two approaches to calculate or estimate the strength of evidence from a pedigree: (1) affected individuals per Sherloc estimates and (2) the probability of observed co-segregation (Meiosis Method $(1/2)^m$). The variable "m" is the number of meioses of the variant of interest in a family. See the example pedigree.

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

$(1/2)^3 \times (1/2) = 1/16$

PM2

Population Frequencies



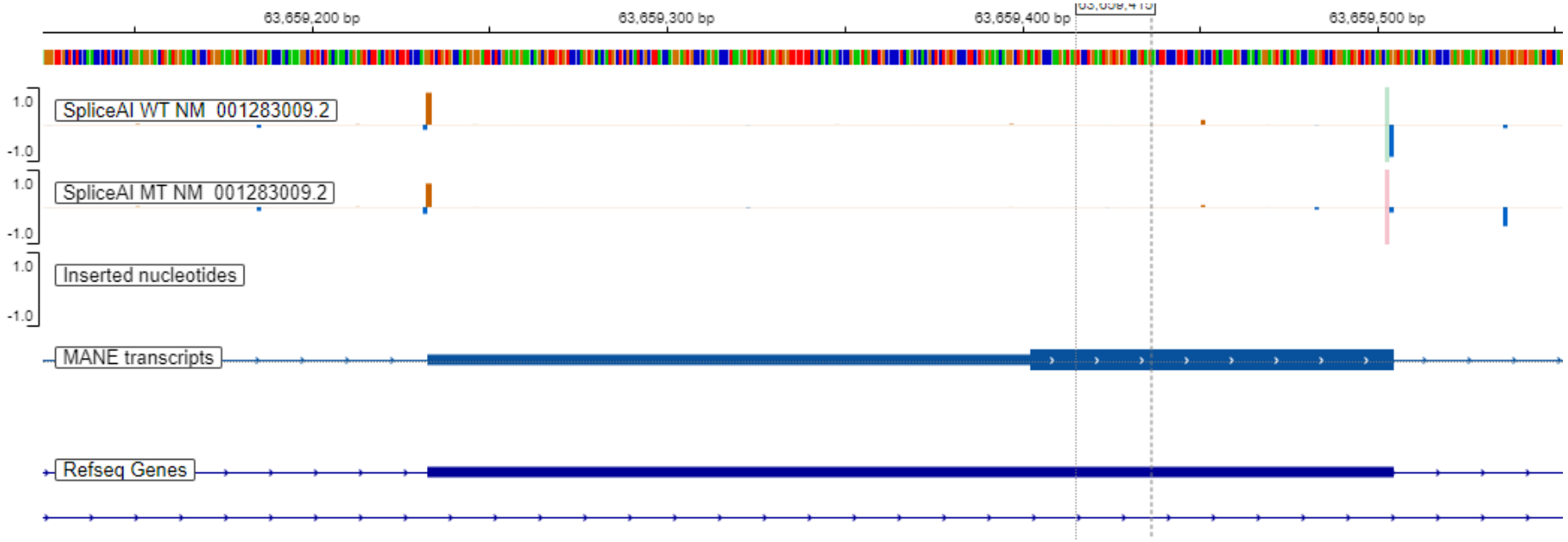
N/A	**gnomAD (Exome)** **No Observation** for this variant in gnomAD (Exome)	**N/A** Alleles of **N/A** **N/A** homozygote **N/A** Individuals
N/A	**gnomAD (Genome)** **No Observation** for this variant in gnomAD (Genome)	**N/A** Alleles of **N/A** **N/A** homozygote **N/A** Individuals
N/A	**gnomAD (Aggregated)** **No Observation** for this variant in gnomAD (Aggregated)	**N/A** Alleles of **N/A** **N/A** homozygote **N/A** Individuals

PP3

Revel	Benign (Moderate) (0.1)	⌄
AlphaMissense	Benign (Strong) (0.063)	⌄
Eve	(N/A)	
Varity	Benign (low) (0.12)	⌄
MUT Assesor	Neutral (0.46)	⌄

By REVEL score, you would apply BP4_moderate

PP3



spliceAI AG:	0.00 (33)
spliceAI AL:	0.00 (-34)
spliceAI DG:	0.42 (33)
spliceAI DL:	0.71 (1)

PP4

INHERITANCE
- Autosomal dominant

RESPIRATORY
Lung
- Pulmonary fibrosis
- Dyspnea
- Hepatopulmonary syndrome (in some patients)
- Dilated pulmonary vasculature with shunting

ABDOMEN
Liver
- Hepatopulmonary syndrome (in some patients)
- Portal hypertension
- Nodular regenerative hyperplasia seen on liver biopsy

Spleen
- Splenomegaly

SKELETAL
Hands
- Digital clubbing

MUSCLE, SOFT TISSUES
- Ascites

LABORATORY ABNORMALITIES
- Decreased telomere length in lymphocytes
- Elevated liver enzymes

MISCELLANEOUS
- Adult onset
- Variable manifestations
- Incomplete penetrance

Case 4: Group Walkthrough #3

In breakout groups, walk through your variants and apply criteria you think are appropriate based on publically available databases.

Case #4

Patient phenotype:

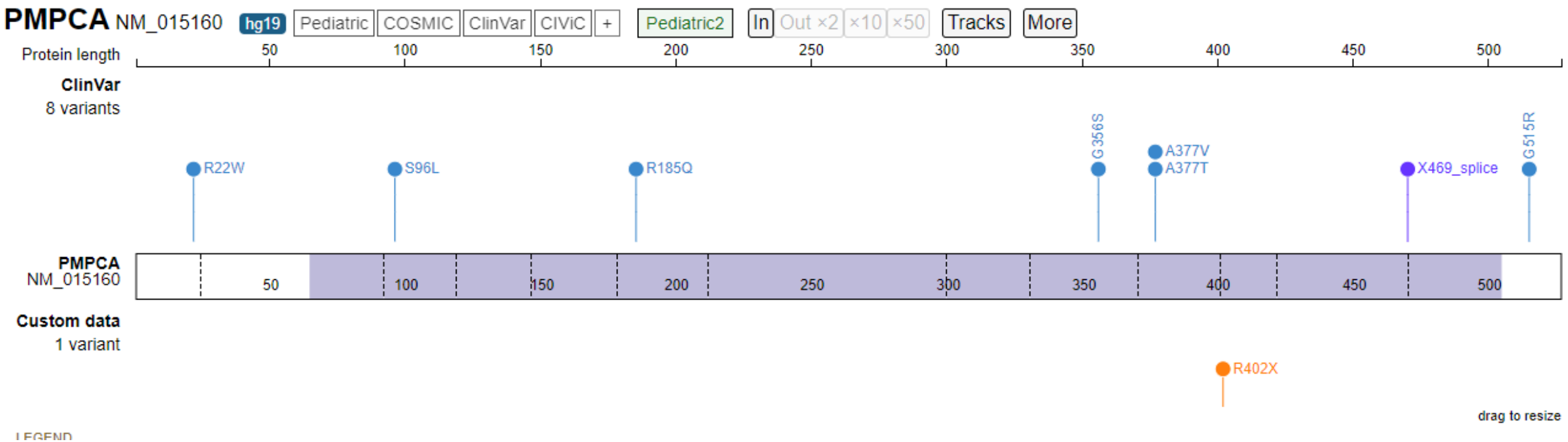
- Developmental regression, ataxia, seizures, cerebellar atrophy, nystagmus, Dandy-Walker malformation

Variant identified via trio genome:

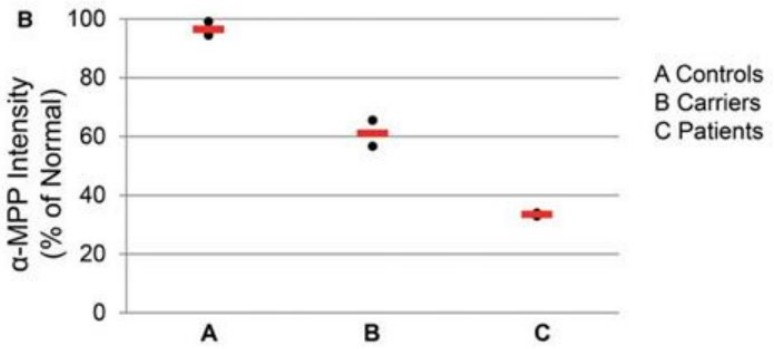
- *In trans* variants:
  - *PMPCA*:c.1204C>T, R402*
  - *PMPCA*:c.667C>T, R223C

Criteria being considered	Strength being applied	Evidence	Points
Variant 1 R402*			
Variant 2 R223C			

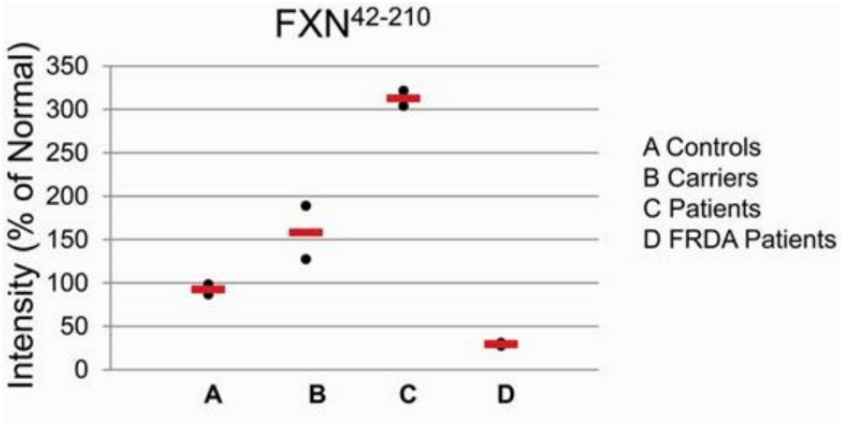
Variant 1: PVS1

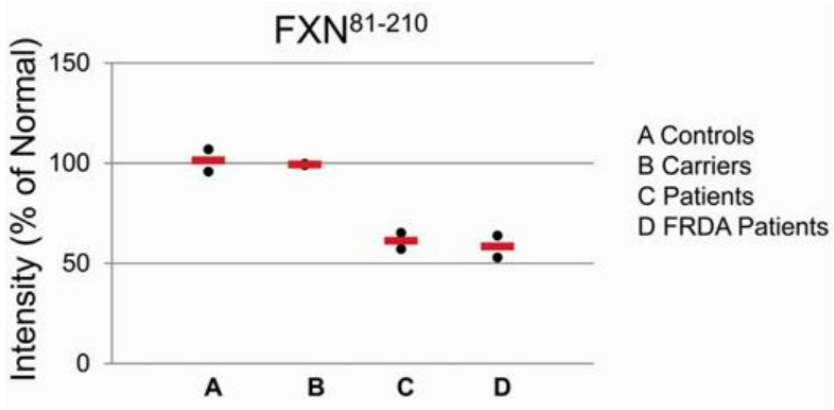


Variant 1: PVS1



- PMPCA is a peptidase which matures mitochondrial proteins
- A377T homozygote led to **decreased** PMPCA protein levels
- Led to **increased** pre-processed FXN and **decreased** mature FXN, similar to levels seen in FRDA patients





Variant 1: PM2

Population Frequencies

0.0012% **gnomAD (Max)**
Very Rare variant in gnomAD (Max)

1 Alleles of **86,242**
0 homozygote
N/A Individuals

0.0003% **gnomAD (Aggregated)**
Very Rare variant in gnomAD (Aggregated)

4 Alleles of **1,461,464**
0 homozygote
N/A Individuals

Variant 2: PM3

A severe form of autosomal recessive spinocerebellar ataxia associated with novel *PMPCA* variants

Yoko Takahashi [a,*], Masaya Kubota [b], Rika Kosaki [c], Kenjiro Kosaki [d], Akira Ishiguro [a]

Patient had *in trans* variants: R223C and D285lfs*16

- Same missense variant *in trans* with a LOF variant

Patient history:

- **birth to 7 months:** sitting & standing at 7 months
- **16 months:** developmental regression
- **4 years:** nystagmus, postural tremors, cogwheel rigidity, atrophy of the cerebellar vermis and T2 hyperintensities in the cerebellar cortex. *PLA2G6* was suspected until WES revealed bilateral *PMPCA* variants.

Highly similar genotype leading to a highly similar phenotype.

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

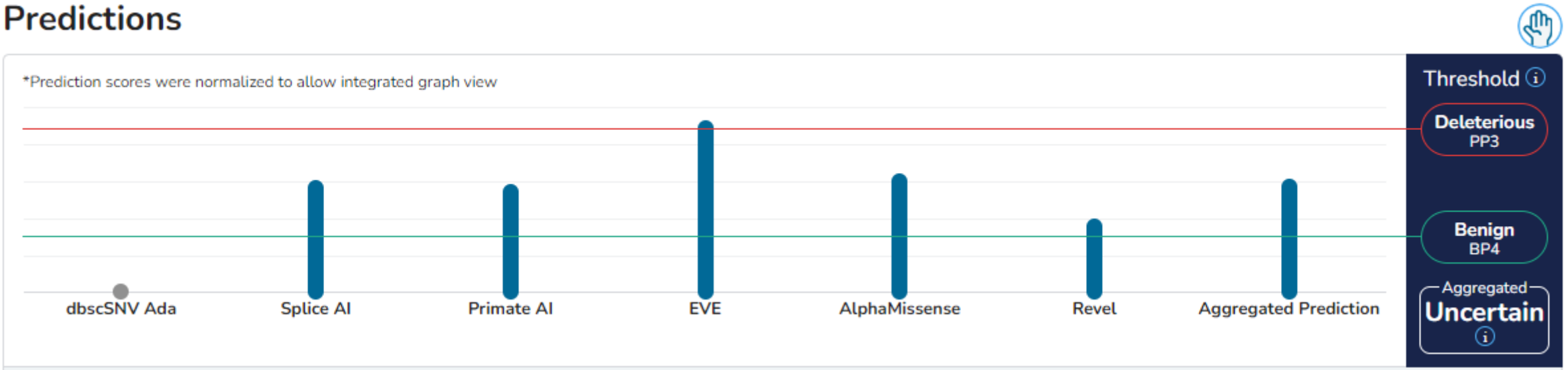
[1]All variants should be sufficiently rare (meet PM2 specification); P - Pathogenic; LP - Likely pathogenic

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

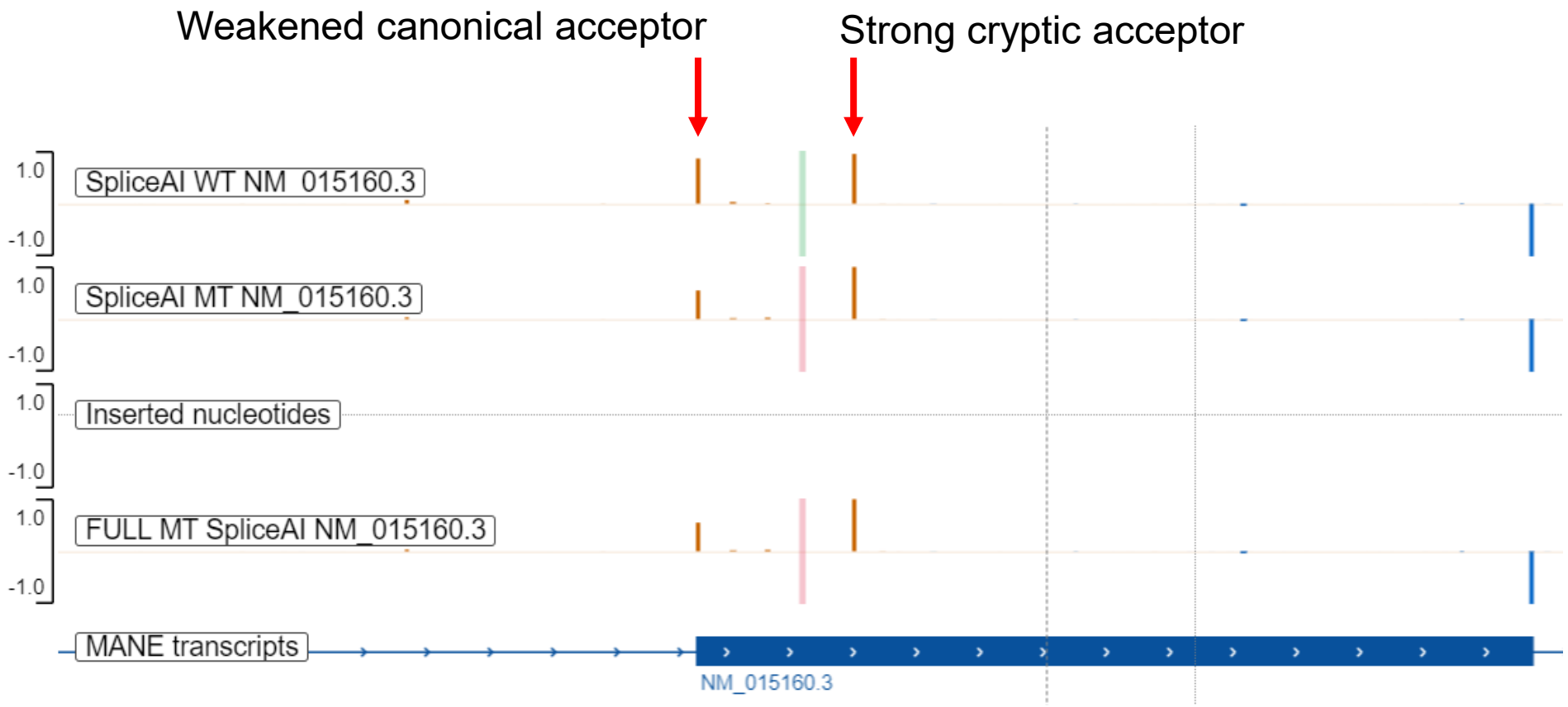
Variant 2: PP3

Predictions



Functional Coding	
Revel	Uncertain (0.35)
AlphaMissense	Uncertain (0.567)
Eve	Deleterious (0.75)

Variant 2: PP3



Splice-AI predicts weakening of canonical acceptor. If cryptic acceptor is used, the protein is thrown out of frame.

Variant 2: PM2

Population Frequencies

0.0022%	**gnomAD (Max)** **Very Rare** variant in gnomAD (Max)	**1** Alleles of **44,896** **0** homozygote **N/A** Individuals
0.0011%	**gnomAD (Aggregated)** **Very Rare** variant in gnomAD (Aggregated)	**17** Alleles of **1,612,006** **0** homozygote **N/A** Individuals
0.0013%	**gnomAD (Genome)** **Very Rare** variant in gnomAD (Genome)	**2** Alleles of **152,170** **0** homozygote **N/A** Individuals
0.001%	**gnomAD (Exome)** **Very Rare** variant in gnomAD (Exome)	**15** Alleles of **1,459,836** **0** homozygote **N/A** Individuals

QUESTIONS & ANSWERS