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<br><br>Missense in gene where only truncating cause disease BP1<br><br>Silent variant with non predicted splice impact BP7<br><br>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<br><br>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<br><br>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 |
|---|---|---|---|
| | | | |
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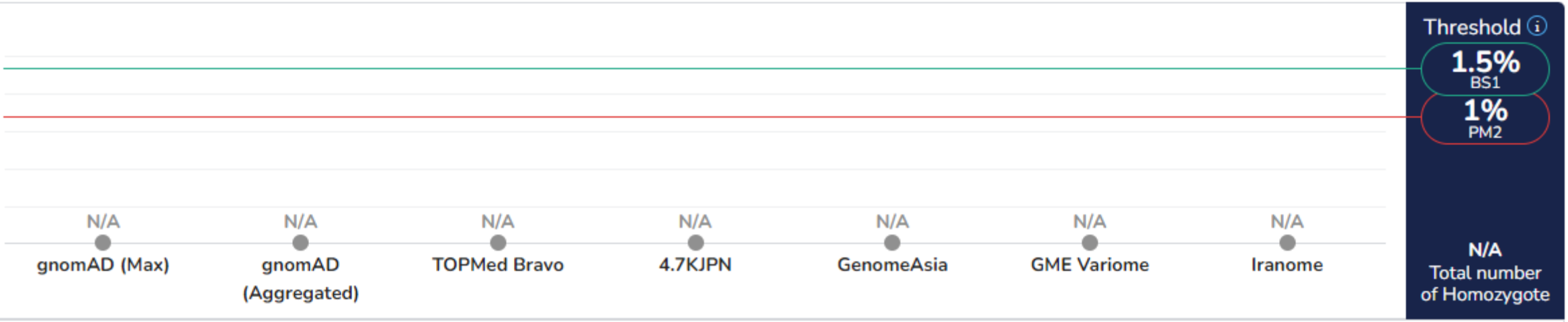
| | | | |

# PM2

## Population Frequencies

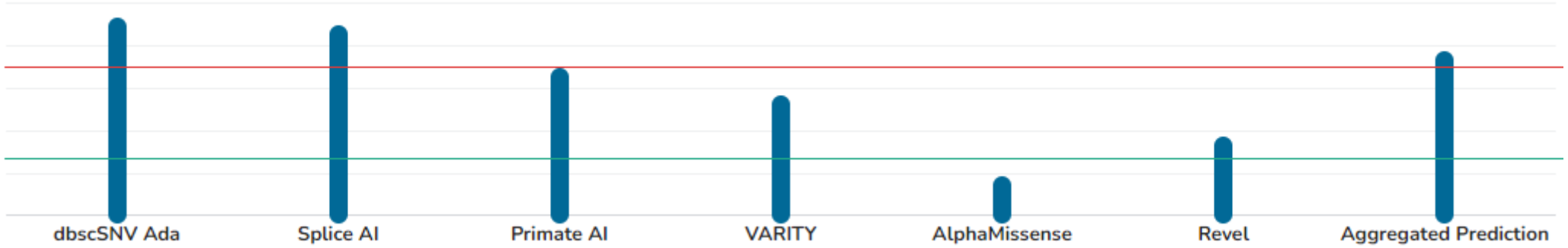



| | | |
|---|---|---|
| N/A | **gnomAD (Exome)**<br>**No Observation** for this variant in gnomAD (Exome) | **N/A** Alleles of **N/A**<br>**N/A** homozygote<br>**N/A** Individuals |
| N/A | **gnomAD (Genome)**<br>**No Observation** for this variant in gnomAD (Genome) | **N/A** Alleles of **N/A**<br>**N/A**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

# PP3

*Prediction scores were normalized to allow integrated graph view

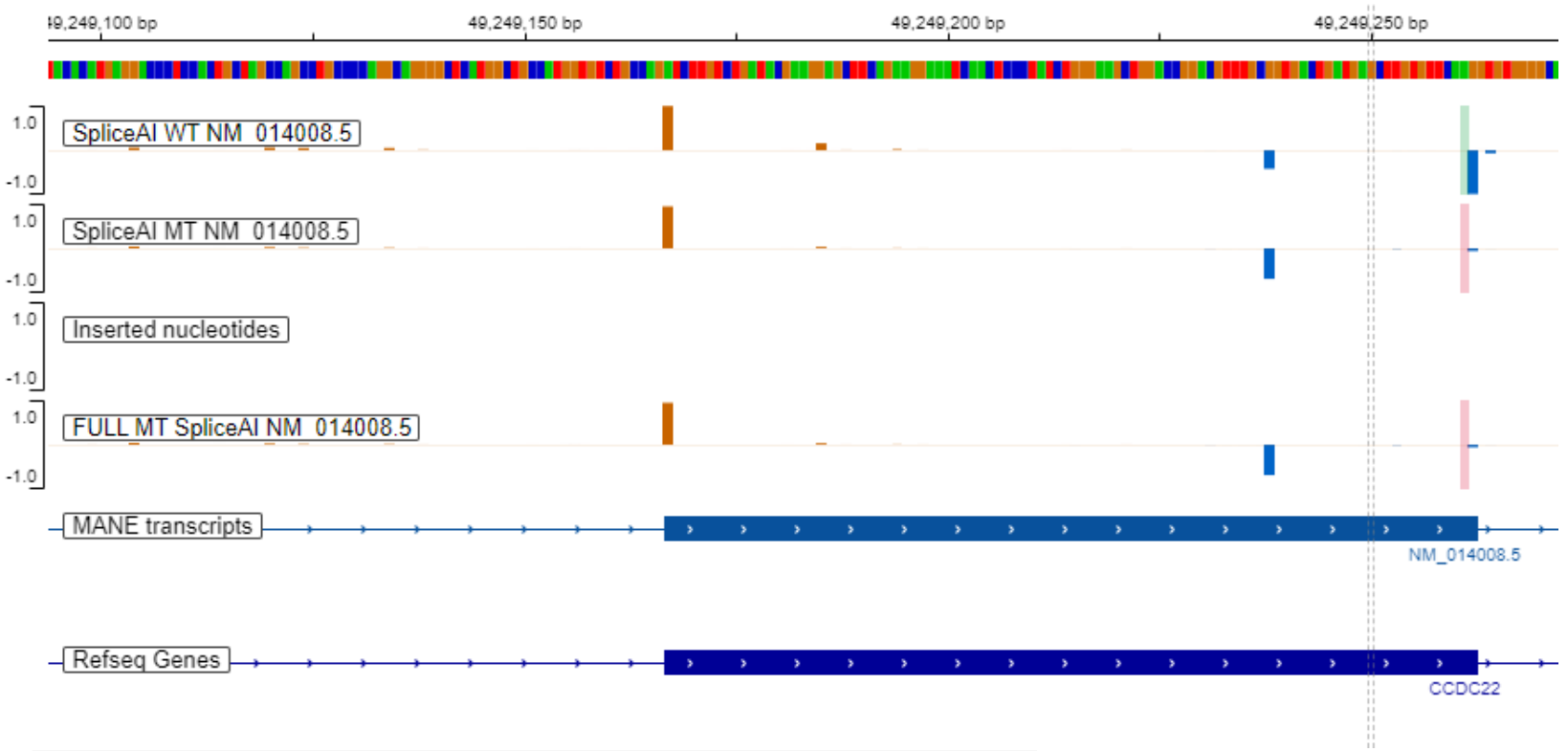



| Functional Coding | |
|---|---|
| **Revel** | Uncertain (0.38) |
| **AlphaMissense** | Benign (Moderate) (0.085) |
| Eve | (N/A) |
| **Varity** | Deleterious (low) (0.42) |
| **MUT Assesor** | Med (2.04) |
| **SIFT** | Benign (Supporting) (0.128) |

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

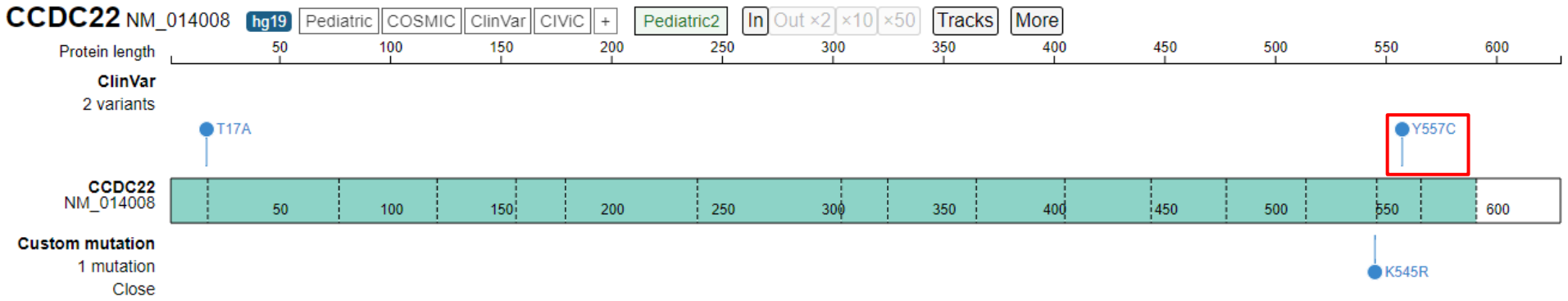
# PP3




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

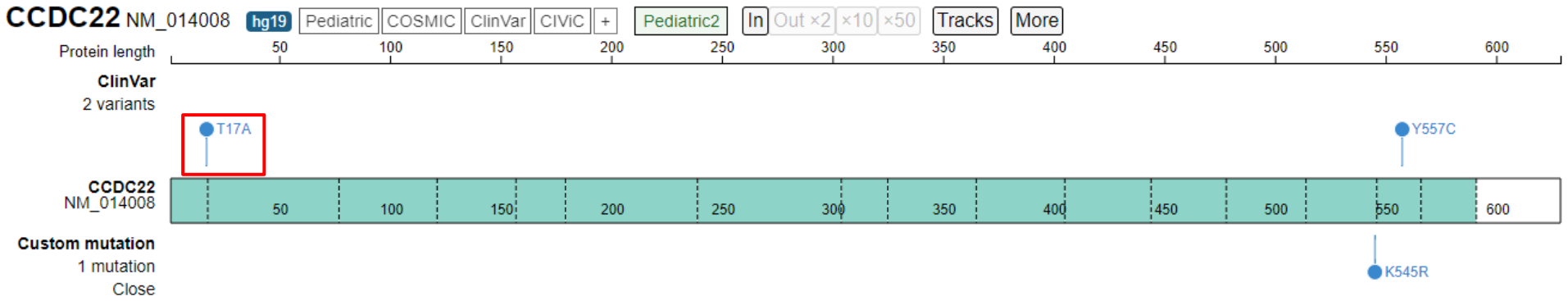
# PP3

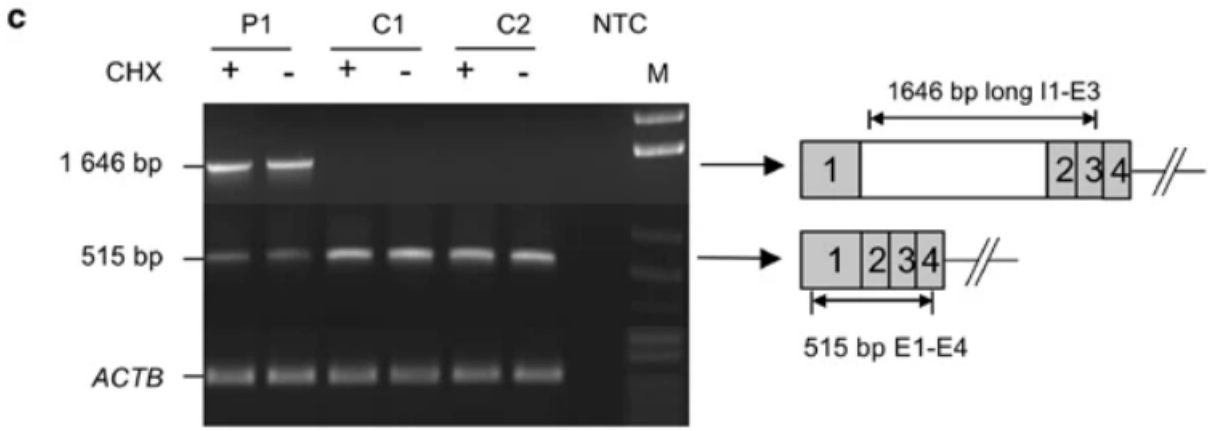



| variant | gene | = canonical transcript | = non-coding transcript | Δ type | Δ score ⓘ | pre-mRNA position ⓘ |
|---|---|---|---|---|---|---|
| X-49106004-A-G<br>UCSC, gnomAD | CCDC22 (ENSG00000101997.13_6 / ENST00000376227.4_3 / NM_014008.5)<br>biotype: protein coding<br>canonical transcript<br>OMIM, 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_005422.4
- Inheritance: Unknown, but variant is on the X-chromosome

| Criteria being considered | Strength being applied | Evidence | Points |
|---|---|---|---|
| | | | |
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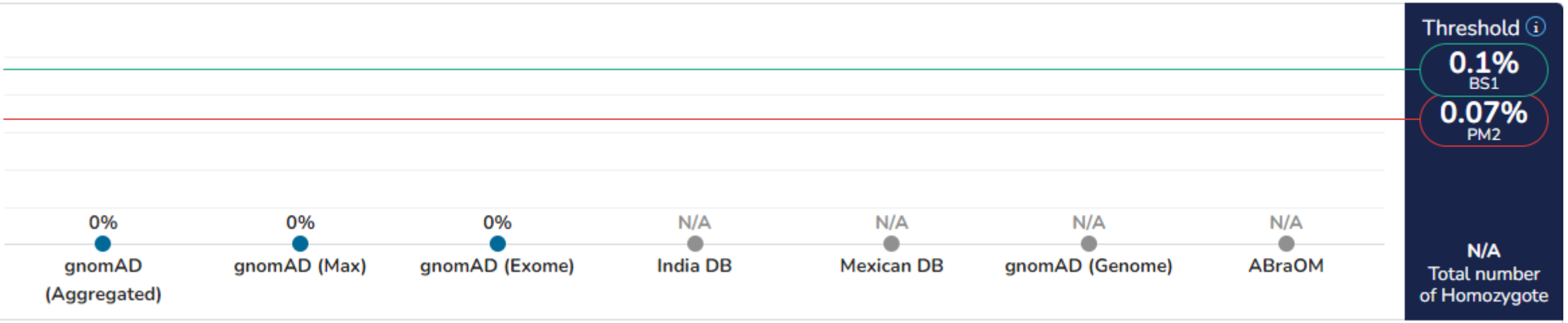
# PM2

## 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)

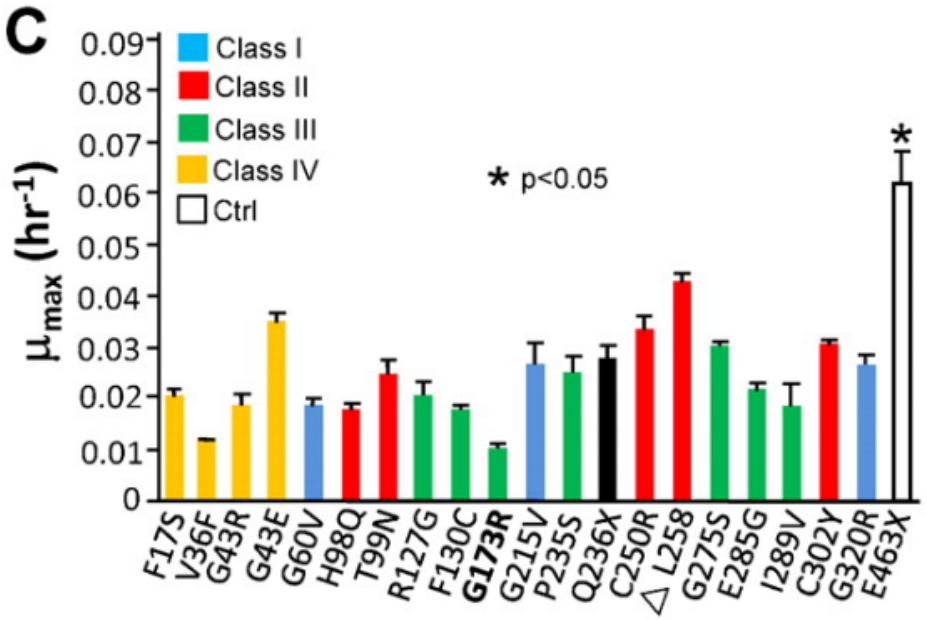
•The variant was observed in a 56 y/o male with retinitis pigmentosa in a Chinese cohort. Age of onset was 3 y/o. (PMID: 32100970)

•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

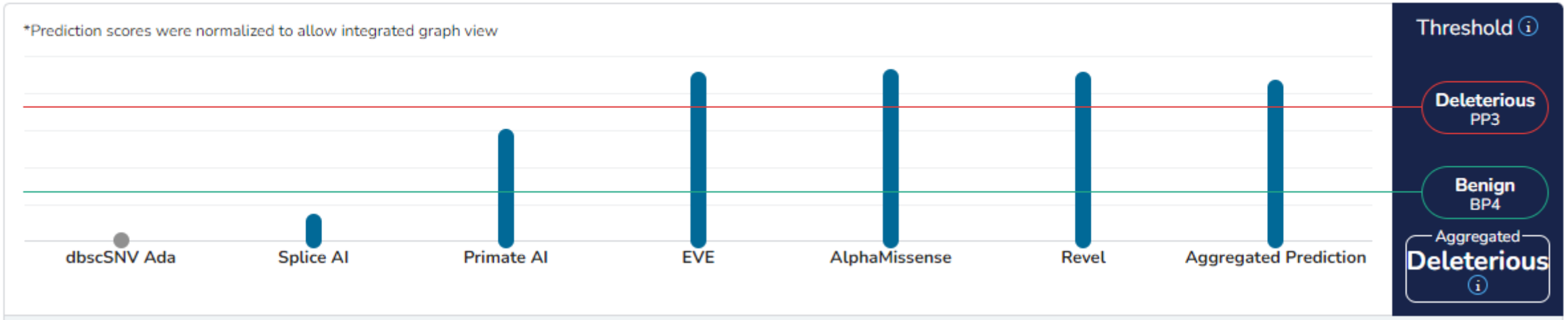
Cys302Tyr was shown to reduce the interaction between RPGR and RPGRIP1α by yeast hybridization assay (PMID: 23213406)



# PP3

## Predictions




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

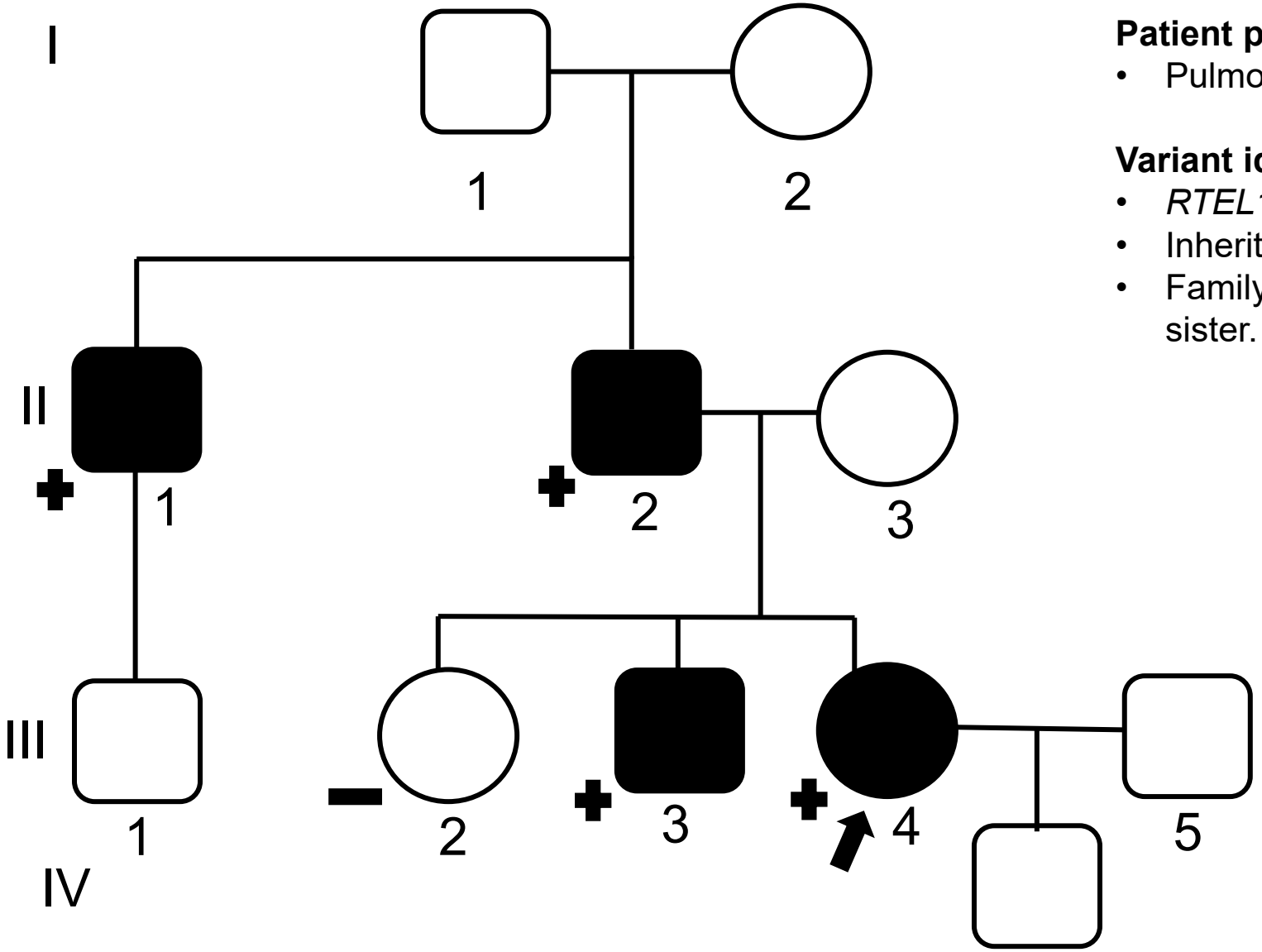
 

## Case 3: Group Walkthrough #2

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 |
|---|---|---|---|
| | | | |
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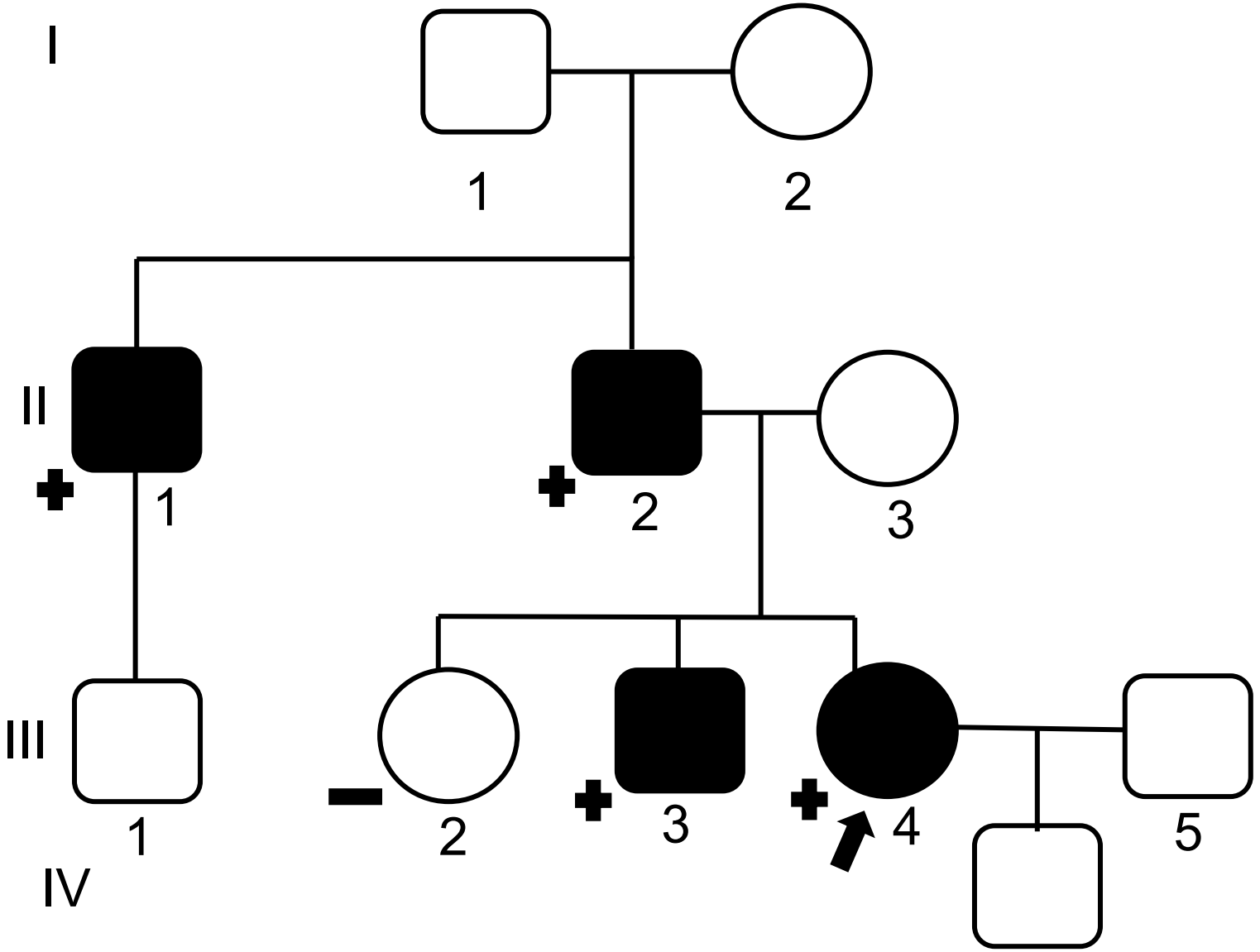
# PP1




## 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.

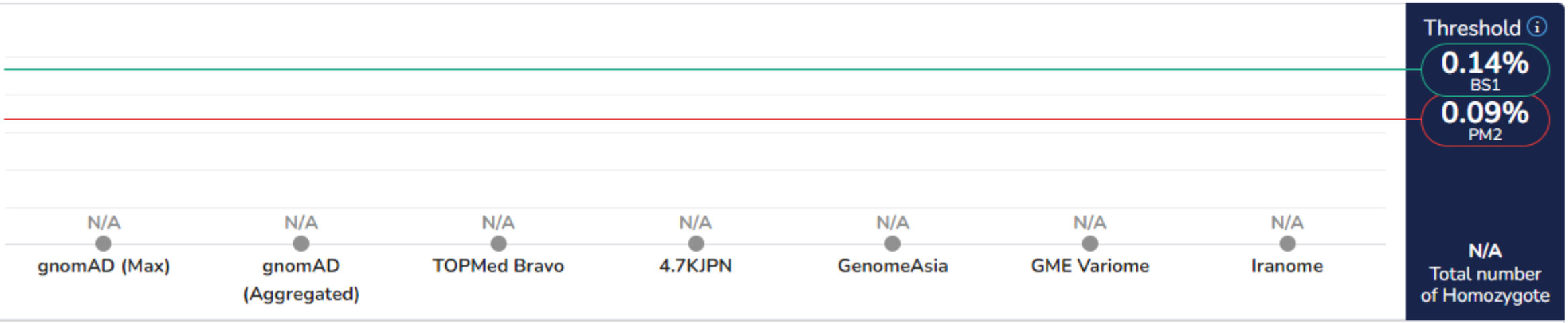
| PP1 Strength | # Affected Individuals | Meiosis Method $(1/2)^m$ |
|---|---|---|
| Weak (Supporting) | AD: ≥3<br>AR: ≥2 | ≤1/8 in 1 family<br>≤1/4 in >1 family |
| Moderate | AD: ≥6<br>AR: ≥3, ≥2 families | ≤1/16 in 1 family<br>≤1/8 in >1 family |
| Strong | AD: ≥10<br>AR: ≥5, ≥2 families | ≤1/32 in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

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

# PM2

## Population Frequencies




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

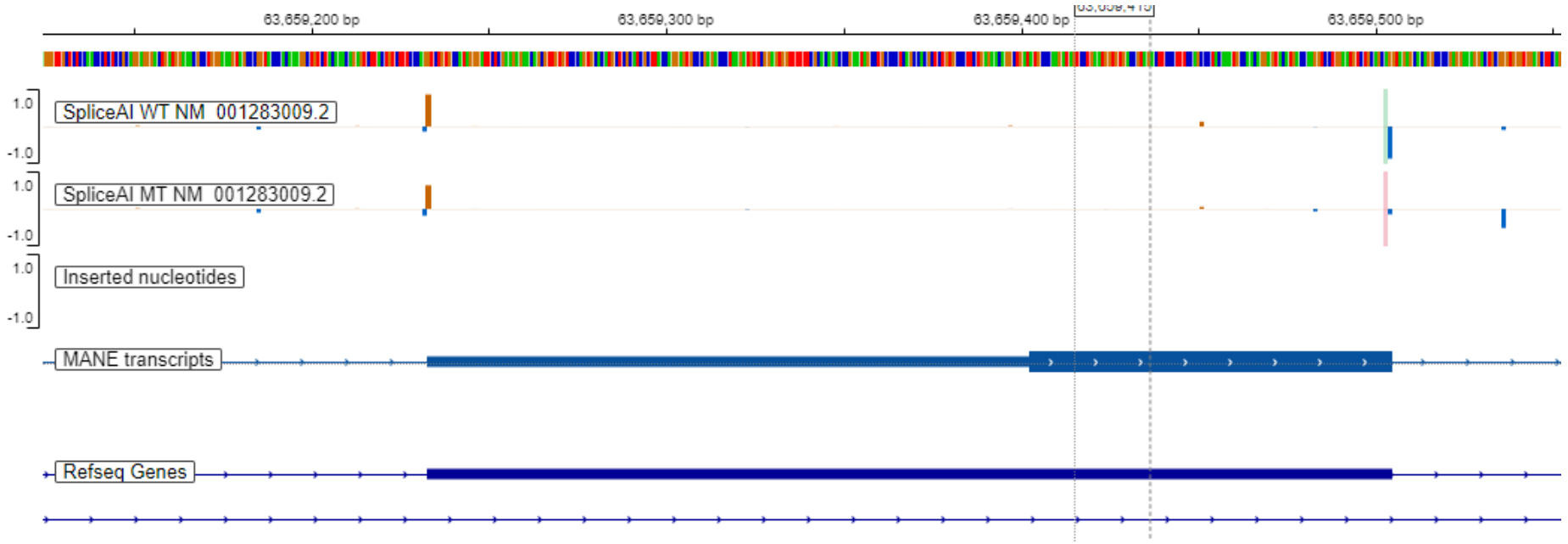
# PP3

| | | |
|---|---|---|
| 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* | | | |
| | | | |
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| | | | |
| Variant 2 R223C | | | |
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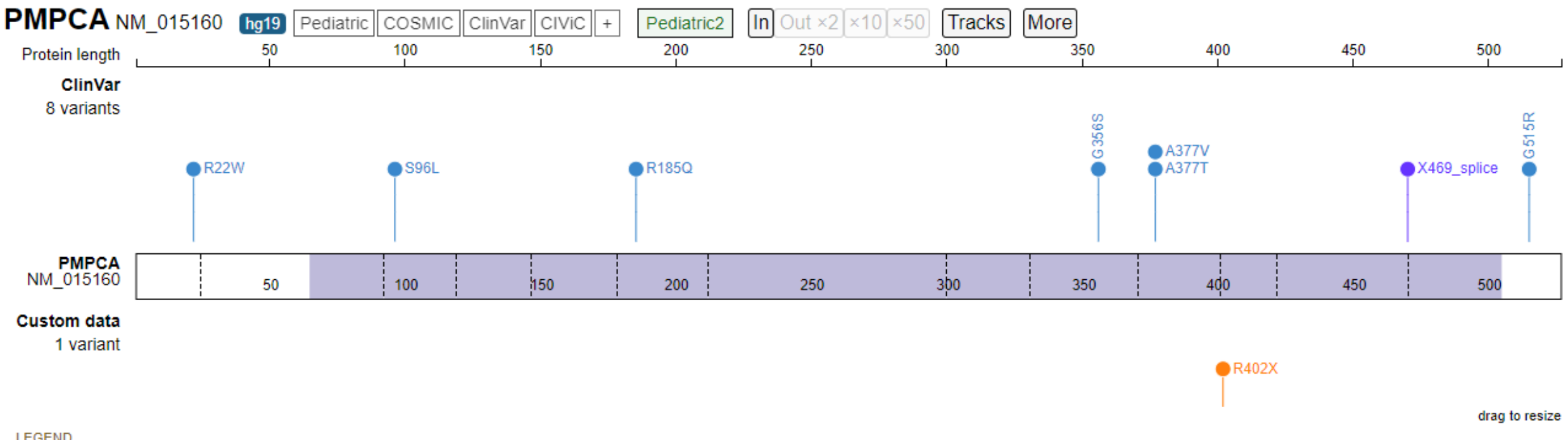
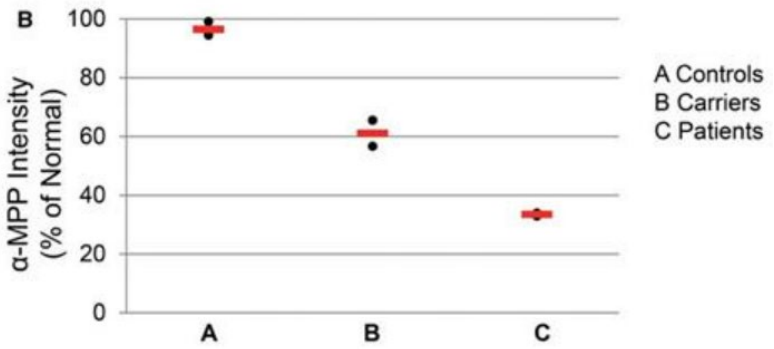
# Variant 1: PVS1

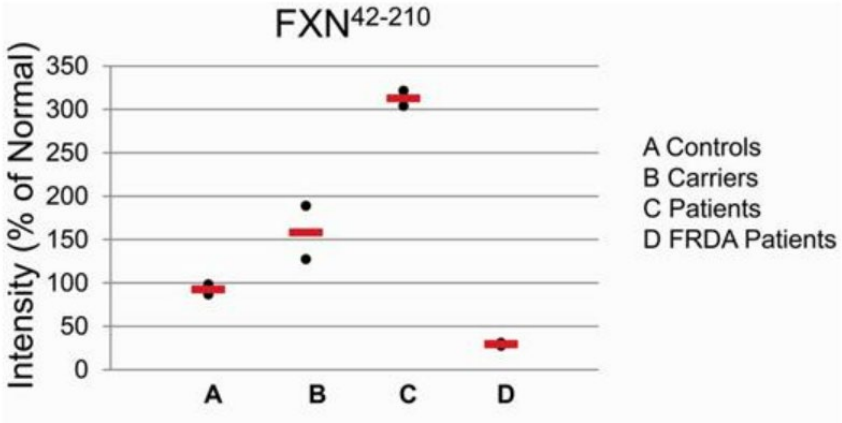


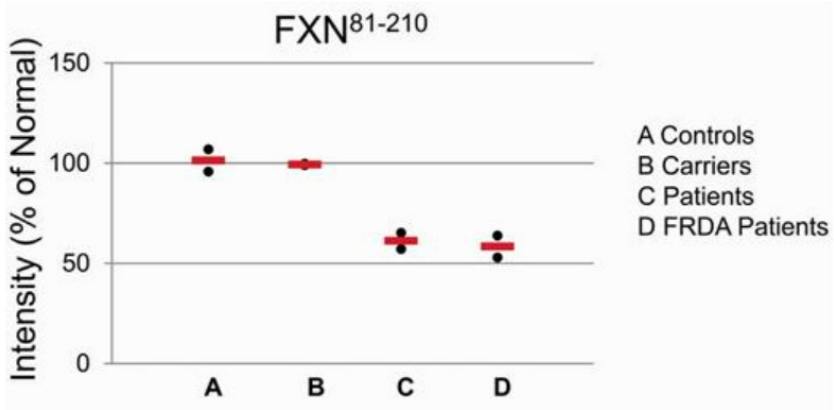

 

# Variant 1: PVS1




- 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

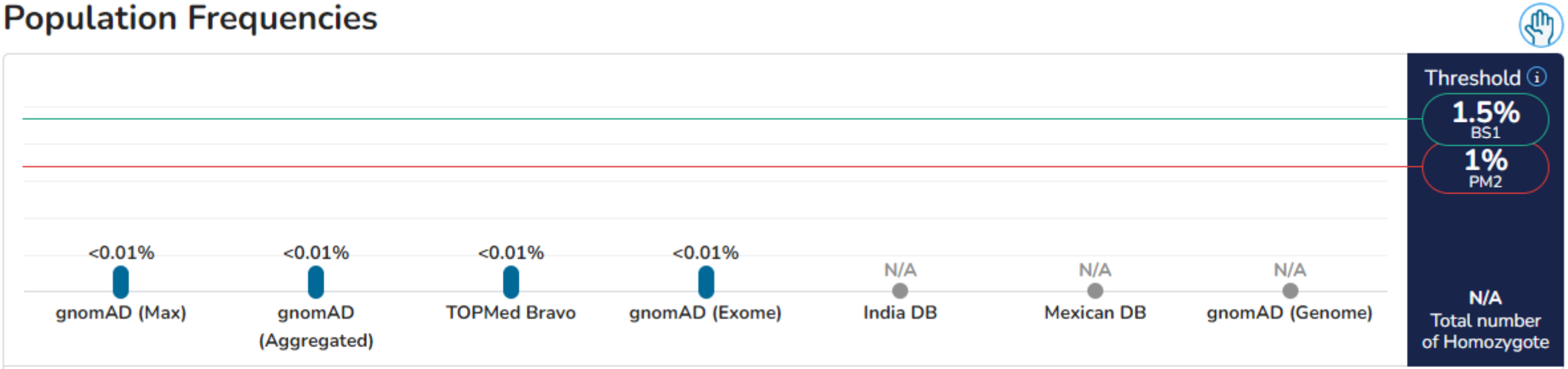


# Variant 1: PM2

## 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)<br>0.25 (LP) |
| Homozygous occurrence<br>*(max point 1.0)* | 0.5 | N/A |
| Uncertain significance variant<br>*(max point 0.5)* | 0.25 | 0.0 |

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

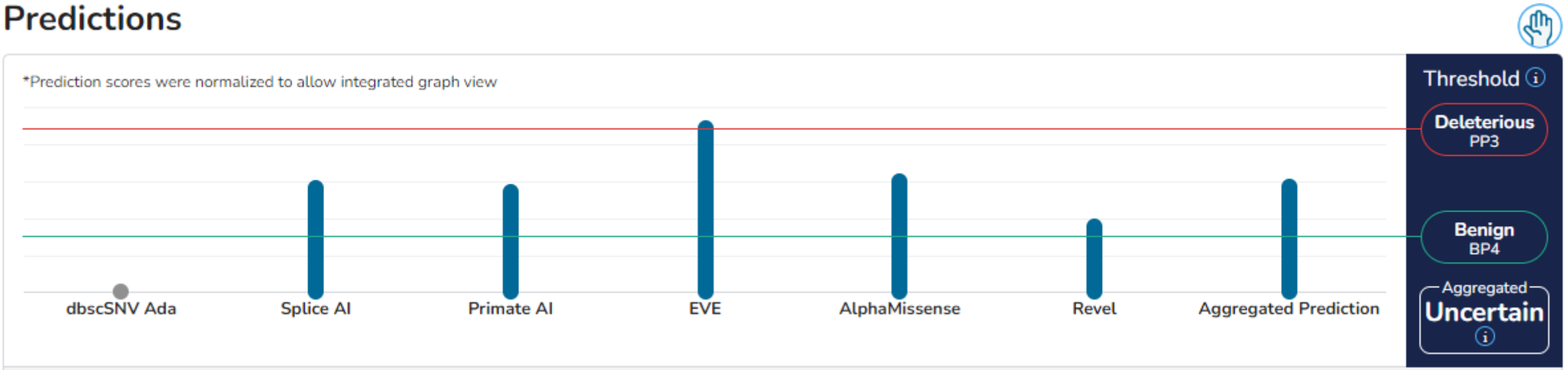
**Table 2. Recommendation for determining the appropriate evidence strength level for PM3**

| PM3_Supporting | PM3 | PM3_Strong | PM3_VeryStrong |
|---|---|---|---|
| 0.5 | 1.0 | 2.0 | 4.0 |

# Variant 2: PP3

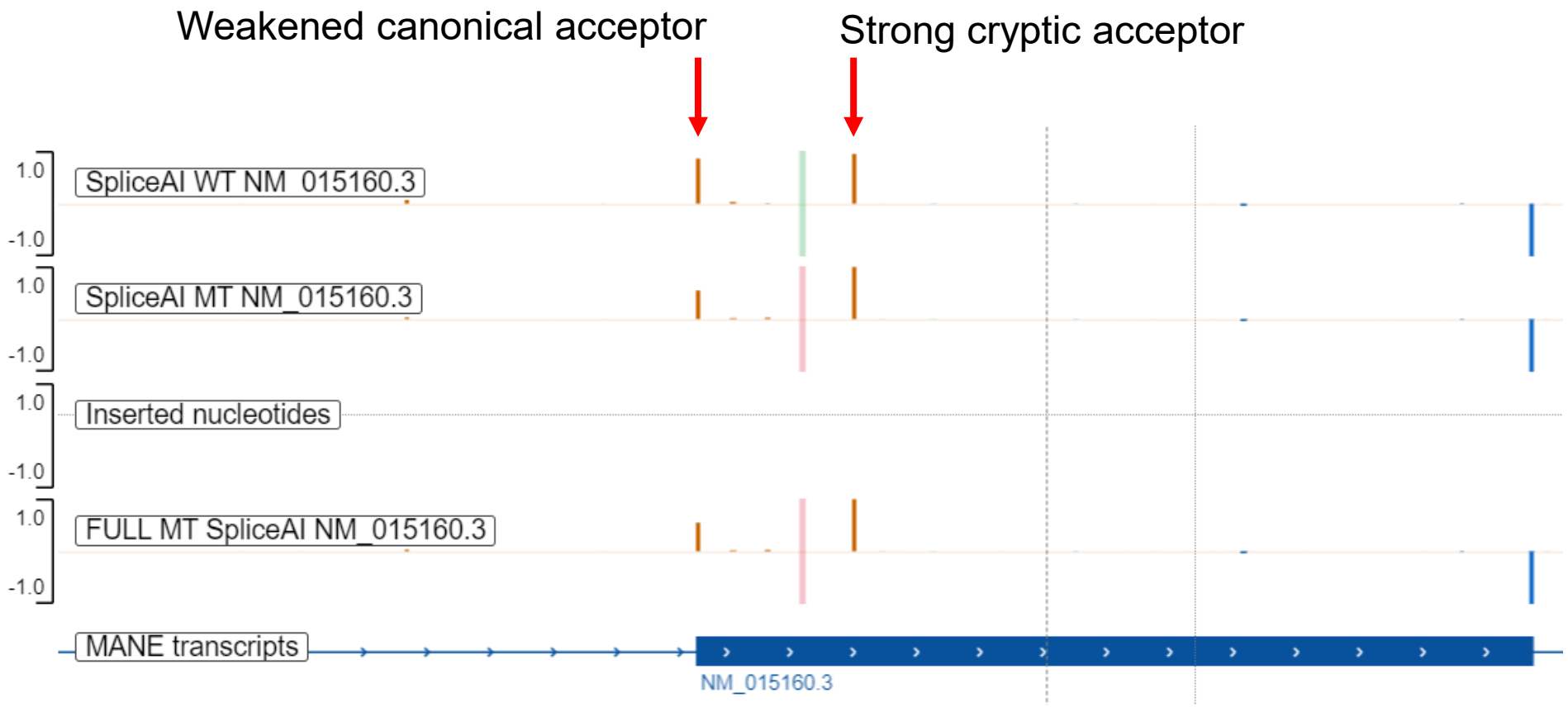
## Predictions




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

# QUESTIONS & ANSWERS