Normal population data (PM2, BA1, BS1, BS2)

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

- PM2: Absent from controls (or at extremely low frequency if recessive)
- BA1: Allele frequency is >5%
- BS1: Allele frequency is greater than expected for disorder
- BS2: Observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age

Genome Aggregation Database (gnomAD)

- v2 release is composed of 125,748 exomes and 15,708 genomes (GRCh37)
- gnomAD structural variant (SV) v2.1 represents 10,847 genomes (GRCh37)
- v3.1 spans 76,156 genomes (GRCh38)

- Contributed by worldwide genome projects
  Full list available at
  https://gnomad.broadinstitute.org/about

What populations are represented in the gnomAD data?

gnomAD v3

Population	overall	controls/biobanks	non-cancer	non-neuro	non-TOPMed	non-v2
African/African American	20,744	4,554	20,583	16,253	12,431	14,377
Amish	456	30	456	431	56	455
Latino/Admixed American	7,647	2,345	7,553	7,424	6,460	6,878
Ashkenazi Jewish	1,736	68	1,651	1,694	499	1,538
East Asian	2,604	1,215	2,486	2,604	1,883	1,414
European (Finnish)	5,316	2,750	5,316	3,495	5,270	3,662
Middle Eastern	158	123	152	155	136	154
European (non-Finnish)	34,029	3,427	32,411	31,966	10,533	25,988
South Asian	2,419	1,558	2,403	2,418	2,405	1,946
Other	1,047	395	1,012	1,002	760	932
XX	38,947	6,717	38,060	35,271	16,438	30,110
XY	37,209	9,748	35,963	32,171	23,995	27,234
Total	**76,156**	**16,465**	**74,023**	**67,442**	**40,433**	**57,344**

gnomAD v2

Population	overall		controls		non-cancer		non-neuro		non-TOPMed	
	exomes	genomes	exomes	genomes	exomes	genomes	exomes	genomes	exomes	genomes
African/African American	8,128	4,359	3,582	1,287	7,451	4,359	8,109	1,694	6,013	4,278
Amish	0	0	0	0	0	0	0	0	0	0
Latino/Admixed American	17,296	424	8,556	123	17,130	424	15,262	277	17,229	405
Ashkenazi Jewish	5,040	145	1,160	19	4,786	145	3,106	123	4,999	69
East Asian	9,197	780	4,523	458	8,846	780	6,708	780	9,195	761
European (Finnish)	10,824	1,738	6,697	581	10,816	1,738	8,367	582	10,823	1,738
European (non-Finnish)	56,885	7,718	21,384	2,762	51,377	7,718	44,779	6,813	55,840	5,547
South Asian	15,308	*	7,845	*	15,263	*	15,304	*	15,308	*
Other	3,070	544	957	212	2,810	544	2,433	367	3,032	506
XX	57,787	6,967	25,645	2,508	53,850	6,967	47,831	4,799	55,662	6,299
XY	67,961	8,741	29,059	2,934	64,629	8,741	56,237	5,837	66,777	7,005
Total	**125,748**	**15,708**	**54,704**	**5,442**	**118,479**	**15,708**	**104,068**	**10,636**	**122,439**	**13,304**

* For v2 genomes, we have a total of only 31 South Asian samples so they are grouped with Other.

- useful reference sets of allele frequencies for severe pediatric diseases
- can be used for adult-onset diseases (e.g. neurological diseases, caners) with additional filters

▼ I have identified a rare variant in gnomAD that I believe is associated with a specific clinical phenotype. What phenotype data are available for these individuals?

Most of the individuals who have contributed data to gnomAD were not fully consented for phenotype data sharing, and unfortunately at this time we are typically unable to provide any information about the clinical status of variant carriers. We have made every effort to exclude individuals with severe pediatric diseases from the gnomAD data set, and certainly do not expect our data set to be *enriched* for such individuals, but we typically cannot rule out the possibility that some of our participants do actually have your disease of interest.

Asian-specific reference databases

- The Chinese Millionome Database(CMDB)
  - 141,431 WGS data from unrelated healthy Chinese individuals
- TogoVar
  - 15,989 WGS and 1,333 WES data from Japanese individuals
- KOVA
  - 1,896 WGS and 3,409 WES data from healthy Korean individuals
- SG10K
  - 10,000 whole-genome sequences from healthy Chinese, Indian, and Malay

vs 2,604 genomes of East Asian in gnomAD v3

Update from ClinGen

Updated recommendation for the benign stand-alone ACMG/AMP criterion

Ghosh R, Harrison SM, Rehm HL, Plon SE, Biesecker LG; ClinGen Sequence Variant Interpretation Working Group.

https://pubmed.ncbi.nlm.nih.gov/30311383/

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

SVI Recommendation for Absence/Rarity (PM2) - Version 1.0

https://clinicalgenome.org/docs/pm2-recommendation-for-absence-rarity/

BA1

- Exception List (July 2018)
- e.g. NM_004004.5(*GJB2*): c.109G>A (p.Val37Ile)
  - a common AR variant for hearing loss
  - reduced penetrance

PM2

- Downgrade to PM2_Supporting
- Unless gene has ClinGen Expert Panel Specifications

Disease population data (PS4)

- PS4: The prevalence of the RARE variant (must also meet PM2) in affected individuals is significantly increased compared with the prevalence in controls
  - 1. Relative risk or odd ratios >5.0, and the confidence interval does not include 1.0.
  - 2. For very rare variants, the prior observation of the variant in multiple unrelated patients with the same phenotype (only applicable to dominant diseases)
    - Can adjust from PS4 to PS4_Supporting
    - Exact threshold varies depending on the disease prevalence
    - E.g. ClinGen Hearing Loss vs RASopathy Expert Panel Specifications
    - Strong: ≥15 vs ≥5 probands
    - Moderate: ≥6 vs ≥3 probands
    - Supporting: ≥2 vs ≥1 probands

Hong Kong Genome Institute 香港基因組中心

Useful databases

- Disease variant databases
  - ClinVar
  - Human Gene Mutation Database
  - DECIPHER
  - Leiden Open Variation Database

Limited phenotype information

- Literature resources
  - PubMed
  - Google Scholar
  - Mastermind
    - Publication list at variant-level

Labor intensive

De novo occurrence (PS2, PM6)

- PS2: *De novo* (both maternity AND paternity confirmed) in a patient with the disease and no family history
- PM6: Assumed *de novo*, but without confirmation of paternity OR maternity
- Combined into one criteria in later ClinGen Recommendations

Update from ClinGen

ClinGen Sequence Variant Interpretation Recommendation for de novo Criteria (PS2/PM6) - Version 1.1
Working Group Page: https://clinicalgenome.org/working-groups/sequence-variant-interpretation/
Date Approved: March 18, 2018, updated May 5, 2021
Changes from v1: Clarified that confirmed/assumed is with regards to parental relationships and not de novo status

SVI Recommendation for *De Novo* Criteria (PS2 & PM6) - Version 1.1

Table 1. Points* awarded per *de novo* occurrence

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

*Note that these points are *not* equivalent to the points used to classify a variant per the Tavtigian et al 2020 "Fitting a naturally scaled point system to the ACMG/AMP variant classification guidelines"
**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

https://clinicalgenome.org/docs/ps2-pm6-recommendation-for-de-novo-ps2-and-pm6-acmg-amp-criteria-version-1.0/-

- Can adjust from PS2_Supporting to PS2

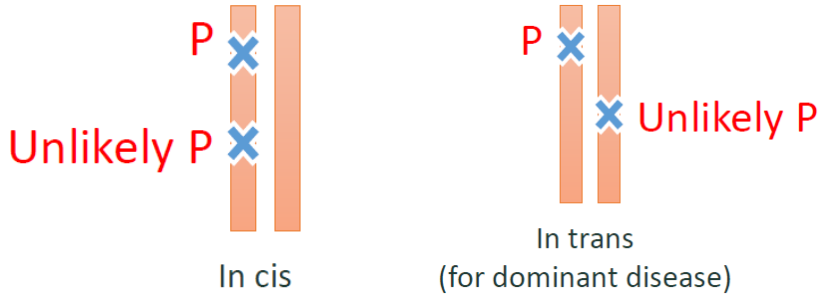
Phenotype in the patient must be consistent with the gene/disease

- Phenotype highly specific for gene
  - increased levels of plasma branched-chain amino acids (*BCKDHB*)
- Phenotype consistent with gene but not highly specific:
  - early infantile epileptic encephalopathy (*SIK1*)
- Phenotype consistent with gene but not highly specific and high genetic heterogeneity
  - non-syndromic intellectual disability (*ASH1L*)

Allelic data (PM3, BP2)

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very Strong
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*		

- PM3: For recessive disorders, detected in trans with a pathogenic variant
- BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern



Update from ClinGen

ClinGen Sequence Variant Interpretation Recommendation for in *trans* Criterion (PM3) - Version 1.0
Working Group Page: https://clinicalgenome.org/working-groups/sequence-variant-interpretation/
Date Approved: May 2, 2019

SVI Recommendation for in *trans* Criterion (PM3) - Version 1.0

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

- Can adjust from PM3_VeryStrong to PM3_Supporting
- Variant must meet PM2
- Phasing may be confirmed by sequencing one patient
- Avoid double counting of evidence
  - Applying PM3 to both v1 and v2 (v1+v2 observed in patient) ✖
  - Applying PM3 to v1 only (v1+v2 observed in patient) ○
  - Applying PM3 to v1 and v2 (v1+v2 and v2+v3 observed in different patients) ○