

Baylor Miraca Genetics Laboratories
Procedure Manual

Criteria for Variant Classification-Modified ACMG Criteria

BMGL approved criteria are labeled with “[BMGL]”

1. Criteria for Classifying Pathogenic Variants

This section is based on “Table 3. Criteria for Classifying Pathogenic Variants” of the 2015 ACMG guidelines

Notes [BMGL]

- Avoid applying similar evidence twice: e.g. PS2 and PM6 (*de novo* criteria) cannot be both invoked for a variant in a patient; if all evidences from the literature were considered and counted and related criteria are invoked, then PP5 or BP6 cannot be invoked again as an additional line of evidence.
- Criteria can be “upgraded” or “downgraded” based on the strength of the evidence using lab director’s professional judgements. Suggested classification in BMGL: “FINAL CLASSIFICATION_ORIGINAL CRITERION”, e.g. PM_PS3 (meaning an evidence considered using PS3 is downgraded to PM as the final classification).

Very strong evidence of pathogenicity

PVS1 Null variant (nonsense, frameshift, canonical +/-1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease

Caveats:

- Beware of genes where LOF is not a known disease mechanism (e.g. *GFAP*, *MYH7*)
- Use caution interpreting LOF variants at the extreme 3’ end of a gene.
- [BMGL]: Changes in the first and last exon will be auto-alerted in the analysis pipeline and considered on a case by case basis.
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact
- Use caution in the presence of multiple transcripts
- [BMGL]: For splice sites, the change should affect the invariant GT/AG (+/-1 or 2 position) sequences for U2 introns
- [BMGL]: A start codon may not be qualified as a *bona fide* pathogenic variant if there is another Methionine downstream and close by. Classification should be on a case-by-case basis

Strong evidence of pathogenicity

PS1 Same amino acid change as a previously established pathogenic variant regardless of nucleotide change

Example: Val->Leu caused by either G>C or G>T in the same codon
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level

PS2 *De novo* (both maternity and paternity confirmed) in a patient with the disease and no family history

Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, *etc.* can contribute to nonmaternity

Note [BMGL]: use this rule for AD, X-linked disorders or mitochondrial disorders. For AR disorder, PM3 should also be considered.

PS3 Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect on the gene or gene product *

Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established

* [BMGL]: Enzyme studies on the patient samples do not necessarily connect a particular change with biochemical results. Mutagenesis studies may be needed. For carrier, the report should only consider well established clinical tests that have confirmative value as valid functional study.

PS4 The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls

Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.

Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

PS5 [BMGL]: Mosaicism in a parent for AD or X-linked disorders

PS6 [BMGL]: ≥2 independent occurrences of PM6 (assumed *de novo*, but without confirmation of paternity and maternity), from internal and/or published data

- PS7 **[BMGL]**: ≥2 independent occurrences of PM7 (PVS1 variant in a gene in which a truncating variant or large deletion was previously reported in a patient) from internal and/or published data
- PS8 **[BMGL]**: in-frame single exon skipping, or gross deletion results in in-frame single exon deletion in a domain of known function

Moderate evidence of pathogenicity

- PM1 Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation
- PM2 Absent from controls (or at extremely low frequency if recessive) (see Table 6) in Exome Sequencing Project, 1000 Genomes or ExAC
Caveat: Population data for indels may be poorly called by next generation sequencing
- Note **[BMGL]**: Population specific data can be used
- PM3 For recessive disorders, detected in *trans* with a pathogenic variant
- Note: This requires testing of parents (or offspring) to determine phase
- PM4 Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants
- PM5 Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
- Example: Arg156His is pathogenic; now you observe Arg156Cys
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level
- PM6 Assumed *de novo*, but without confirmation of paternity and maternity
- PM7 **[BMGL]**: PVS1 variant in a gene in which a truncating variant or large deletion was previously reported in a patient
- PM8 **[BMGL]**: Small in-frame changes that are located in a region where similar in-frame change(s) were previously reported in patient(s)

Supporting evidence of pathogenicity

PP1 Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease

Note: May be used as stronger evidence with increasing segregation data

PP2 Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease

PP3 Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many *in silico* algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Note [BMGL]: Current consensus among CSER sites is “all or none”: apply this rule if all prediction programs used in a lab support the variant to be “deleterious” or “benign”. If there are conflicting predictions, then don’t invoke this criterion.

PP4 Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology

PP5 Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation

Note [BMGL]: only invoke when there is new evidence from the source that was not considered for other criteria during the variant evaluation.

2. Criteria for Classifying Benign Variants

This section is based on “Table 4. Criteria for Classifying Benign Variants” of the 2015 ACMG guidelines

Stand-Alone evidence of benign impact

BA1 Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes, or ExAC

Note [BMGL]: Population specific data can be used

Strong evidence of benign impact

BS1 Allele frequency is greater than expected for disorder (see table 6)

Note [BMGL]: Population specific data can be used

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

Note [BMGL]: per CSER, it is preferred not to apply both BS1 and BS2 based on the same set of data. e.g. if BS1 is applied based on ExAC data, then other data (such as parental data) is preferred to use if BS2 is to be applied too.

BS3 Well-established *in vitro* or *in vivo* functional studies shows no damaging effect on protein function or splicing

BS4 Lack of segregation in affected members of a family

Caveat: The presence of phenocopies for common phenotypes (*i.e.* cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Supporting evidence of benign impact

- BP1 Missense variant in a gene for which primarily truncating variants are known to cause disease
- 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
- BP3 In-frame deletions/insertions in a repetitive region without a known function
- BP4 Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)

Caveat: As many *in silico* algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Note [**BMGL**]: Current consensus among CSER sites is “all or none”: apply this rule if all prediction programs used in a lab support the variant to be “deleterious” or “benign”. If there are conflicting predictions, then don’t invoke this criterion.

- BP5 Variant found in a case with an alternate molecular basis for disease
- BP6 Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation

Note [**BMGL**]: only invoke when there is new evidence from the source that was not considered for other criteria during the variant evaluation

- BP7 A synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved

3. Rules for Combining Criteria

This section is based on “Table 5. Rules for Combining Criteria” of the 2015 ACMG guidelines

Pathogenic

- 1) 1 Very Strong (PVS1) *AND*
 - a) ≥ 1 Strong (PS1-PS4) *OR*
 - b) ≥ 2 Moderate (PM1-PM6) *OR*
 - c) 1 Moderate (PM1-PM6) and 1 Supporting (PP1-PP5) *OR*
 - d) ≥ 2 Supporting (PP1-PP5)
- 2) ≥ 2 Strong (PS1-PS4) *OR*
- 3) 1 Strong (PS1-PS4) *AND*
 - a) ≥ 3 Moderate (PM1-PM6) *OR*
 - b) 2 Moderate (PM1-PM6) *AND* ≥ 2 Supporting (PP1-PP5) *OR*
 - c) 1 Moderate (PM1-PM6) *AND* ≥ 4 Supporting (PP1-PP5)

Likely Pathogenic

- 1) 1 Very Strong (PVS1) *AND* 1 Moderate (PM1-PM6) *OR*
- 2) 1 Strong (PS1-PS4) *AND* 1-2 Moderate (PM1-PM6) *OR*
- 3) 1 Strong (PS1-PS4) *AND* ≥ 2 Supporting (PP1-PP5) *OR*
- 4) ≥ 3 Moderate (PM1-PM6) *OR*
- 5) 2 Moderate (PM1-PM6) *AND* ≥ 2 Supporting (PP1-PP5) *OR*
- 6) 1 Moderate (PM1-PM6) *AND* ≥ 4 Supporting (PP1-PP5)

Benign

- 1) 1 Stand-Alone (BA1) *OR*
- 2) ≥ 2 Strong (BS1-BS4)

Likely Benign

- 1) 1 Strong (BS1-BS4) and 1 Supporting (BP1-BP7) *OR*
- 2) ≥ 2 Supporting (BP1-BP7)

***Variants should be classified as Uncertain Significance if other criteria are unmet or the criteria for benign and pathogenic are contradictory.**