Criteria Specification

ClinGen Rett and Angelman-like Disorders Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for UBE3A Version 3.0.0

Affiliation: Rett and Angelman-like Disorders VCEP
Version : 3.0.0
Released : 11/10/2022
Release Notes :
Added nuclear localization to UBE3A functional assay table. Reformatted previously-approved specifications to fit new CSpec format with no content changes made.

Rules for UBE3A

Gene: UBE3A (HGNC:12496)
Preferred Transcript: NM_130838.2
HGNC Name: ubiquitin protein ligase E3A
Disease: Angelman syndrome (MONDO:0007113)

Criteria & Strength Specifications

PVS1

Original ACMG Summary

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.
- 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.

Very Strong

Null variant in a gene where loss of function is a known mechanism of disease.

- Use as defined by ClinGen SVI working group (PMID:30192042).
- PVS1 is applicable for any truncating variant up to p.K841, for any frameshift variant that results in a read-through of the stop codon, for initiation codon variants, for canonical splice site variants predicted to result in an out-of-frame product, and for intragenic deletions/duplications predicted to result in an out-of-frame product.

Modification Type: Disease-specific

Strong

Null variant in a gene where loss of function is a known mechanism of disease.

- PVS1_Strong is applicable for any truncating variant from p.K842 to p.G850 and for canonical splice site variants that flank exons 7, 8 (in-frame exons).

Modification Type: Disease-specific

Moderate

Null variant in a gene where loss of function is a known mechanism of disease.

- PVS1_Moderate is applicable for any truncating variant distal of p.G850.

Modification Type: Disease-specific

Instructions: For intragenic deletions/duplications that are predicted to result in a product that preserves reading frame:

- For single exon in-frame deletions assign the same strength (PVS1, PVS1_strong, or PVS1_moderate) as for splice site variants that preserve reading frame indicated above.
- For multiple exon in-frame deletions PVS1 can be assigned to deletions that include single in-frame exons in the PVS1 category listed in the splice site section above OR if the exon contains a functionally important domain as specified in PM1.
- Given the extensive data available for UBE3A, classifications for single or multi-exon in-frame deletions are assigned as PVS1 or PVS1_strong. Refer to PVS1 flow chart for additional guidance.

PS1

Original ACMG Summary

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.

Strong

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

Modification Type: None

PS2

Original ACMG Summary

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 non-maternity.

Very Strong

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

- ≥2 independent occurrences of PS2.
- ≥2 independent occurrences of PM6 and one occurrence of PS2.
- Evidence from literature must be fully evaluated to support independent events.

Modification Type: None

Strong

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

- 1 occurrence of PS2.

Modification Type: None

Instructions: Applicable to all genes in affected individuals identified as mosaic for the variant (as the presence of a variant in the mosaic state is confirmatory of the variant being de novo). Because of the very high de novo rate of pathogenic variants in UBE3A, de novo observation can be attributed the highest value points per proband (2 points for confirmed de novo and 1 point for assumed de novo) if the patient is known to be affected with a neurodevelopmental phenotype consistent with the gene.

PS3

Original ACMG Summary

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.

Strong

Well-established in vitro or in vivo functional studies supportive of a damaging effect.

- RNA studies that demonstrate abnormal splicing and an out-offrame transcript.
- Do not use for canonical splice site variants and when PVS1 is used.

Modification Type: Disease-specific

Supporting

Well-established in vitro or in vivo functional studies supportive of a damaging effect.

- RNA studies that demonstrate abnormal splicing and an inframe product (unless it affects an in-frame exon specified in the PVS1 section).
- See included table for acceptable functional studies.

Modification Type: Disease-specific

PS4

Original ACMG Summary

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.

Strong

The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

- 5+ observations.

Modification Type: Strength

Moderate

The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

- 3-4 observations.

Modification Strength

Type:

Supporting

The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

- Use for 2nd independent occurrence.

Modification Type: Strength

Instructions:

- Detailed phenotype not needed. Need to confirm patient is 'affected with a neurodevelopmental phenotype consistent with the gene' at a minimum.
- Patient can be published OR an internal case OR observed at an outside lab (i.e. via ClinVar) OR described in the reputable databases [LOVD (UBE3A), RettBASE (MECP2, CDKL5, FOXG1)]. However independent case has to be confirmed to be a different patient than yours (compare gender/age).
- Do not use this criterion for variants where BS1 is applied or where PM2 does not apply.

PM1

Original ACMG Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Moderate

Located in a mutational hot spot and/or critical and well-established functional domain.

- 3' cysteine binding site: aa 820 (PMID 9887341)

Modification Type: Disease-specific

PM2

Original ACMG Summary

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.
Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

Absent/rare from controls in an ethnically-matched cohort population sample.

- Use if absent, zero observations in control databases.

Modification Type: Strength

PM3

Original ACMG Summary

For recessive disorders, detected in trans with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase.

Not Applicable

Comments: Not applicable for UBE3A.

PM4

Original ACMG Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Strong

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

- PM4_Strong is applicable to stop-loss variants in UBE3A, as several stop loss variants in this gene has been described in affected individuals PMID 25212744.

Modification Type: Disease-specific

Moderate

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Modification Type: Disease-specific

Supporting

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

- Smaller in-frame events (< 3 amino acid residues) unless they occur in a functionally important region (see PM1 for functionally important domain for this gene).

Modification Type: Strength

PM5

Original ACMG Summary

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.

Strong

Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

- ≥2 different missense changes affecting the amino acid residue.
- Do not apply PM1 in these situations.

Modification Type: Strength

Moderate

Missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.

- Applicable to all genes as written.
- A Grantham or BLOSUM score comparison can be used to determine if the variant is predicted to be as or more damaging than the established pathogenic variant.

Modification Type: None

PM6

Original ACMG Summary

Assumed de novo, but without confirmation of paternity and maternity.

Very Strong

Assumed de novo without confirmation of paternity and maternity.

- ≥4 independent occurrences of PM6. Evidence from literature must be fully evaluated to support independent events.

Modification Type: Strength

Strong

Assumed de novo without confirmation of paternity and maternity.

- ≥2 independent occurrences of PM6. Evidence from literature must be fully evaluated to support independent events.

Modification Type: Strength

Moderate

Assumed de novo without confirmation of paternity and maternity.

- 1 occurrence of PM6.

Modification Type: None

Instructions: Because of the very high de novo rate of pathogenic variants in UBE3A, de novo observation can be attributed the highest value points per proband (2 points for confirmed de novo and 1 point for assumed de novo) if the patient is known to be affected with a neurodevelopmental phenotype consistent with the gene.

PP1

Original ACMG Summary

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.

Strong

Co-segregation with disease in multiple affected family members.

- ≥5 informative meiosis.

Modification Type: Strength

Moderate

Co-segregation with disease in multiple affected family members.

- 3-4 informative meiosis.

Modification Type: Strength

Supporting

Co-segregation with disease in multiple affected family members.

- 2 informative meiosis.

Modification Type: Strength

Instructions: Note: individuals must have disease consistent with reported phenotype (even if on the mild end of spectrum of the disease).

PP2

Original ACMG Summary

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

Not Applicable

Comments: Not applicable for UBE3A.

PP3

Original ACMG Summary

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.

Supporting

- For missense variants use REVEL with a score ≥ 0.75.
- For splice site variants use MaxEntScan, NNSPLICE and SpliceSiteFinder-like when all of the prediction programs support significant splicing alteration (significant splicing alterations defined as ≥15% decrease to the natural splice site and ≥70% gain in prediction strength of cryptic splice site).

Modification Type: None

PP4

Original ACMG Summary

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

Supporting

Phenotype specific for disease with single genetic etiology.

- See gene specific clinical phenotype guidelines.

Modification Type: Disease-specific

PP5

Original ACMG Summary

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

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BA1

Original ACMG Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

Allele frequency above 0.05%.

- Use large population databases (i.e. gnomAD).
- Use if variant is present at ≥0.0003 (0.03%) in any sub-population.
- Use if allele frequency is met in any general continental population dataset of at least 2,000 observed alleles.

Modification Type: Disease-specific

Instructions: The frequency cutoff is based on summation of prevalence of genes covered in the Rett/Angelman-like working group. The prevalence values were determined using the most conservative numbers found in the literature.

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Allele frequency greater than expected for disorder.

- Use large population databases (i.e. gnomAD).
- Use if variant is present at ≥0.00008 (0.008%) and <0.0003 (0.03%) in any sub-population.
- Use if allele frequency is met in any general continental population dataset of at least 2,000 observed alleles.

Modification Type: Disease-specific

Instructions: The frequency cutoffs are based on MECP2 expected disease allele frequency (1 in 8500 females/1.5 alleles [assumes 50/50 male/female ratio]). MECP2 is the most prevalent of the genes covered in the Rett/Angelman-like working group and was chosen as most conservative number.

BS2

Original ACMG Summary

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.

Strong

Observed in the heterozygous/hemizygous state in a healthy adult.

- 4 unaffected (related and maternally inherited or unrelated) Het (UBE3A).

Modification Type: Strength

Supporting

Observed in the heterozygous/hemizygous state in a healthy adult.

- 2 unaffected (related and maternally inherited or unrelated) Het (UBE3A),

Modification Type: Strength

Instructions:

- Should be applied in cases where the healthy adult is devoid of neurodevelopmental phenotypes.
- Best to use with internal curated data that includes clinical information or published patients that have been phenotyped.

BS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Strong

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

- RNA functional studies that demonstrate no impact on splicing and transcript composition. It can be downgraded based on quality of data.
- Not applicable for other functional studies (see tables for other accepted functional studies).

Modification Type: Disease-specific

BS4

Original ACMG Summary

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.

Strong

Lack of segregation in affected members of a family.

- Absent in a similarly affected family member, when seen in two or more families.

Modification Type: Strength

Supporting

Lack of segregation in affected members of a family.

- Absent in a similarly affected family member.

Modification Type: Strength

Instructions: Need to confirm that the family member is ‘affected with a neurodevelopmental phenotype consistent with the gene’ at a minimum.

BP1

Original ACMG Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Not Applicable

Comments: Not applicable for UBE3A.

BP2

Original ACMG Summary

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.

Supporting

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.

- BP2 is not applicable for UBE3A in trans state.

Modification Type: Disease-specific

Instructions: Knock out of UBE3A results in disease but viable phenotype (PMID 9808466).

BP3

Original ACMG Summary

In frame-deletions/insertions in a repetitive region without a known function.

Not Applicable

Comments: Not applicable for UBE3A.

BP4

Original ACMG Summary

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.

Supporting

- For missense variants use REVEL with a score ≤ 0.15.
- For splice site variants use MaxEntScan, NNSPLICE and SpliceSiteFinder-like when the majority of the prediction programs do not support significant splicing alteration (significant splicing alterations defined as ≥15% decrease to the natural splice site and ≥70% gain in prediction strength of a cryptic splice site).

Modification Type: None

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Strong

Variant found in a case with an alternate molecular basis for disease.

- ≥3 cases with alternate molecular basis for disease.

Modification Type: Strength

Supporting

Variant found in a case with an alternate molecular basis for disease.

- ≥1 cases with alternate molecular basis for disease.

Modification Type: Disease-specific

Instructions:

- For example if a variant in UBE3A is identified in a patient with lissencephaly in whom a pathogenic variant is identified in the PAFAH1B1 gene.
- Variant should also be maternally inherited in the case with an alternate molecular basis for disease for this criteria to be used.
- Do not apply if variant is de novo.

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to

the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BP7

Original ACMG Summary

A synonymous 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.

Supporting

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.

- Defined 'not highly conserved' regions in BP7 as those with PhastCons score <1 and/or PhyloP score <0.1 and/or the variant is the reference nucleotide in one primate and/or three mammal species.

Modification Type: None

Instructions: For silent variants BP4 and BP7 can be added

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)*

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND 1 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

≥ 2 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)* **AND 1**

Moderate *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

Likely Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

≥ 3 Moderate *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

2 Moderate *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Moderate *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM4_Strong, PM5_Strong, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong, PM6_Very Strong)* **AND 1 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PM4_Supporting, PP1, PP3, PP4)*

Benign

≥ 2 Strong *(BS1, BS2, BS3, BS4, BP5_Strong)*

1 Stand Alone *(BA1)*

Likely Benign

1 Strong *(BS1, BS2, BS3, BS4, BP5_Strong)* **AND 1 Supporting** *(BS2_Supporting, BS4_Supporting, BP2, BP4, BP5, BP7)*

≥ 2 Supporting *(BS2_Supporting, BS4_Supporting, BP2, BP4, BP5, BP7)*

Files & Images

UBE3A Functional Assays:

Clinical Phenotype Guidelines: Phenotype guidelines for UBE3A mentioned in PP4.

PVS1 Flowchart for UBE3A:

References

1. Bienvenu T Carrié A et al. *MECP2 mutations account for most cases of typical forms of Rett syndrome.* **Hum Mol Genet** (2000) 9 (9) p. 1377-84. 10.1093/hmg/9.9.1377 10814719
2. Erlandson A Hallberg B et al. *MECP2 mutation screening in Swedish classical Rett syndrome females.* **Eur Child Adolesc Psychiatry** (2001) 10 (2) p. 117-21. 10.1007/s007870170034 11469283
3. Sadikovic B Fernandes P et al. *Mutation Update for UBE3A variants in Angelman syndrome.* **Hum Mutat** (2014) 35 (12) p. 1407-17. 10.1002/humu.22687 25212744

4. Fang P Lev-Lehman E et al. *The spectrum of mutations in UBE3A causing Angelman syndrome.* **Hum Mol Genet** (1999) 8 (1) p. 129-35. 10.1093/hmg/8.1.129 9887341