CHEK2: CanVIG-UK Gene-Specific Guidance

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CanVIG-UK review of *CHEK2*: Consensus to use relevant recommendations from the ClinGen Hereditary Breast, Ovarian and Pancreatic Cancer Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for ***ATM* Version 1.4.0** (available at: https://clinicalgenome.org/affiliation/50039/, PDF attached below).
Additional points of specification are given below where applicable.

Additional Note: For diagnostic analysis and reporting for cancer indications, the scope of this test indication currently includes canonical protein truncating variants and *CHEK2* c.349A>G p.(Arg117Gly)[1].

Summary: Evidence towards Pathogenicity

Evidence element	Evidence strengths allowed				Use as per VCEP	Additional clarifications/thresholds/data-sources
PVS1	_VSTR	_STR	_MOD	_SUP	✗	**From CanVIG-UK:** Truncating variants prior to c.1493: use PVS1_vstr *(variants up to the last 50bp of the penultimate exon, therefore predicted to undergo nonsense mediated decay)* Truncating variants occurring from c.1494 to c.1566: use PVS1_str *(not predicted to undergo NMD, truncated/altered region includes nuclear localisation signal and therefore critical to protein function)* Truncating variants from c.1567: use PVS1_mod (*not predicted to undergo NMD, role of region unknown, variant removes <10% of protein*) Truncating variants within the first 100bp: use PVS1_mod Follow *ATM* VCEP guidance for PVS1(RNA), see below: • PVS1_Variable(RNA) shall be used for observed splice defects, whether from canonical +/-1,2 positions or other spliceogenic regions (including mid-exonic missense/synonymous variants that cause splice defects) with baseline weight as per the below decision tree. Weight can be further modified based on the quality of the RNA study including consideration of concepts such as: ○ Starting material (where patient material is preferable to in vitro minigene) ○ Use of NMD inhibitors where translation does occur such as cell lines ○ Primer design (to make sure it's comprehensive to capture possible multicassette events) ○ Method of quantification

						▪ where e.g. capillary electrophoresis is preferable to estimation by gel band density ▪ where SNP analysis is most preferred (where analysis of exonic SNPs and their relative presence in aberrant and WT transcripts is informative) ○ Quantification (where complete effects should have increased weight over incomplete effects) In the event that RNA data are available and they reflect a substantial variant-specific impact, do not use both PVS1(RNA) and PP3 or BP4. However, in the event that RNA data are available and they reflect no variant-specific impacts, PP3 or BP4 may be applied in conjunction with BP7(RNA).
PS1		_STR	_MOD	_SUP	✓	
PS2					✓	
PS3		_STR	_MOD	_SUP	✓	
PS4		_STR			✓	
PM1					✓	
PM2				_SUP	✓	**From VCEP:** Per correspondence: 30/07/2025: • If grpmax FAF ≤0.001% (regardless of n) - apply PM2_sup • If grpmax FAF not calculated (typically due to low n), look at highest ancestry AF: ○ If ≤0.001% (regardless of n) apply PM2_sup ○ If >0.001% but only n=1 in one population, apply PM2_sup ○ Otherwise, no criteria If grpmax FAF >0.001%, do not apply PM2_sup even if there is only one ancestry group with variant and ancestry AF is ≤0.001%
PM3					✗	**From CanVIG-UK:** No syndromic phenotype, do not use
PM4			_MOD		✓	
PM5				_SUP	✗	**From CanVIG-UK:** PM5_sup can be used for truncating variants after the first 100bp and prior to c.1493.
PP1					✗	**From CanVIG-UK:** No syndromic phenotype, do not use
PP2					✓	
PP3				_SUP	✓	
PP4					✓	
PP5					✓	

Summary: Evidence towards Benignity

BA1/BS1	_SA	_STR			✓	
BS2					✓	
BS3			_MOD	_SUP	✓	
BS4					✓	
BP1					✓	
BP2		_STR	_MOD	_SUP	✓	
BP3					✓	
BP4				_SUP	✓	
BP5					✓	
BP7		_STR	_MOD	_SUP	✓	

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.0		--	Initial Version	--	CStAG
1.1	25/05/23	--	Changed opening statement to clarify that these CanVIG guidelines should be used in conjunction with ClinGen VCEP guidelines.	Allen	CStAG
1.2	25/01/2024	Statement	Reference to current VCEP version (v1.3.0) and additional *CHEK2* missense variant reporting per UKCGG statement.	Allen	CStAG
1.2	23/05/2024	PM2	Updated to match statement for CanVIG ATM guidelines (to use gnomAD v2.1.1 per VCEP correspondence)	Allen	CStAG
1.3	07/10/2025	Statement	Update to reference current VCEP version (v1.4.0)	Allen	CStAG
1.3	07/10/2025	PM2	Added clarification for application of PM2_sup when n=1 in gnomAD v4.1	Allen	CStAG
1.3	07/10/2025	PM3, PP1	Added clarification to not use PP1 and PM3 (no syndromic phenotype)	Allen	CStAG

References:

1. Exceptional variants/gene-specific variant reporting, UKCGG website (available at: https://www.ukcgg.org/information-education/exceptional-variantsgene-specific-variant-reporting/).

Criteria Specification

ClinGen Hereditary Breast, Ovarian and Pancreatic Cancer Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for ATM Version 1.4.0

Affiliation: Hereditary Breast, Ovarian and Pancreatic Cancer VCEP
Type: Richards et.al., 2015 - Combining rules
Description : ACMG-modified rules specifications for ATM (autosomal dominant and autosomal recessive disorders)
Version : 1.4.0
Released : 7/14/2025
Release Notes :
Release notes v1.4

Removed n for PM2_Supporting and clarified use of gnomAD v4
Clarified when to assume in trans for PM3
Provided PP1 guidance for AR condition
Added SpliceAI thresholds for PP3 and BP4
Clarified use of PP3/BP4 in the presence of RNA data
Updated BP7 donor site cutoff from c.-40 to c.-21
Updated MONDO from hereditary breast carcinoma to ATM-related cancer predisposition
Minor formatting adjustments

Rules for ATM

General Comments: Release notes v1.4 Removed n for PM2_Supporting and clarified use of gnomAD v4 Clarified when to assume in trans for PM3 Provided PP1 guidance for AR condition Added SpliceAI thresholds for PP3 and BP4 Clarified use of PP3/BP4 in the presence of RNA data Updated BP7 donor site cutoff from c.-40 to c.-21 Updated MONDO from hereditary breast carcinoma to ATM-related cancer predisposition Minor formatting adjustments

Gene: ATM (HGNC:795)
Transcripts:
NM_000051.3

HGNC Name: ATM serine/threonine kinase
Disease:
ATM-related cancer predisposition (MONDO:0700270) **Mode of Inheritance:** Autosomal dominant inheritance
ataxia telangiectasia (MONDO:0008840) **Mode of Inheritance:** Autosomal recessive inheritance
ataxia - telangiectasia variant (MONDO:0018266) **Mode of Inheritance:** Autosomal recessive inheritance

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.

VCEP Specifications: Use ATM PVS1 Decision Tree.

- PVS1: Predicted splice defect
- PVS1_Strength(RNA): Observed splice defect
- The default RefSeq transcript for nucleotide (c.) annotation is **NM_000051.3/ENST00000278616.8.** All exons from this transcript can be considered constitutive exons without major alternate splice isoforms that could potentially rescue presumed LoF events (ENIGMA unpublished data).
- Of note, ATM is occasionally annotated with multiple non-coding first exons so exon numbering must be carefully reviewed for variant interpretation using literature sources of data.
- **The FAT/PI3K/FATC (collectively the FATKIN)** domains are considered *critical* for ATM protein function (PMID 28508083, 31740029, 31320732). PVS1 alterations that are predicted to escape NMD, but that adversely affect these domains can be granted PVS1 (as opposed to PVS1_Strong as the recommended base-line (PMID 30192042).
- **The HEAT repeat domain** is considered *important* for protein function based on the appearance of many A-T affected individuals harboring a variant resulting in an in-frame, single exon loss in this domain (PMID 10980530, 19535770, 30819809, 15054841, 22927201, 19691550, 10330348, 17124347, 8845835, 16266405, 9463314, 24090759, 22213089). PVS1-eligible alterations that are predicted to escape NMD, but that adversely affect the HEAT repeat domain can be granted PVS1_Strong. They are limited to strong due to a lack of known missense pathogenic alterations in this domain.
- The most 3’/C-Terminal residue considered to be pathogenic is p.R3047 (PMIDs: 8755918, 19691550, 18560558, 10980530,

26628246)

- NOTE: Many diagrams for ATM show the FAT, PI3-K and FATC domains as separated by spacers, however these are not empirically derived and there is evidence of missense pathogenic alterations in the ‘spacer’ regions. This VCEP considers them a contiguous domain (PMID 28508083).
- PVS1 can be applied as per the PVS1 decision tree.
  - PVS1_Variable(RNA) shall be used for observed splice defects, whether from canonical +/-1,2 positions or other spliceogenic regions (including mid-exonic missense/synonymous variants that cause splice defects) with baseline weight as per the below decision tree. Weight can be further modified based on the quality of the RNA study including consideration of concepts such as:
  - Starting material (where patient material is preferable to in vitro minigene)
  - Use of NMD inhibitors where translation does occur such as cell lines[56]
  - Primer design (to make sure it’s comprehensive to capture possible multicassette events)
  - Method of quantification
    - where e.g. capillary electrophoresis is preferable to estimation by gel band density
    - where SNP analysis is most preferred (where analysis of exonic SNPs and their relative presence in aberrant and WT transcripts is informative)
  - Quantification (where complete effects should have increased weight over incomplete effects)
  - Specific guidance on the use of RNA evidence in variant assessment is not a gene-specific consideration for PALB2 at this time, therefore discretion is left to assessors until further guidance is provided for this general concept from the Sequence Variant Interpretation group.
- In the event that RNA data are available and they reflect a substantial variant-specific impact, do not use both PVS1(RNA) and PP3 or BP4. However, in the event that RNA data are available and they reflect no variant-specific impacts, PP3 or BP4 may be applied in conjunction with BP7(RNA).

Very Strong

Use ATM PVS1 Decision Tree

Modification Type: Gene-specific,Strength

Strong

Use ATM PVS1 Decision Tree.

Modification Type: Gene-specific,Strength

Moderate

Use ATM PVS1 Decision Tree.

Modification Type: Gene-specific,Strength

Supporting

Use ATM PVS1 Decision Tree

Modification Type: Gene-specific,Strength

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.

VCEP Specifications:

- Use as ascribed for missense changes as long as a splice defect is ruled out for both variants;
- Use ATM PS1 Splicing table for splicing variants with similar predictions or observations of splice defect. (PMID: 36865205)

Strong

- Use for missense changes as long as splicing is ruled-out for both alterations.
- Use ATM PS1 Splicing table for splicing variants with similar predictions or observations of splice defect.

Modification Type: General recommendation

Moderate

Use ATM PS1 Splicing table for splicing variants with similar predictions or observations of splice defect.

Modification Type: General recommendation,Strength

Supporting

Use ATM PS1 Splicing table for splicing variants with similar predictions or observations of splice defect.

Modification Type: General recommendation,Strength

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.

Not Applicable

Comments: Do not use for AD or AR disease: Informative de novo occurrences have not yet been observed and de novo AR conditions are unlikely to be informed by phase

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.

VCEP Specifications: For protein, see detailed notes on ATM-specific assays; For RNA use code PVS1_Strength(RNA) and modulate strength based on assay quality and quantity (curator discretion).

Strong

Do not use as strong.

Modification Type: Gene-specific

Moderate

Use when a variant fails to rescue both an ATM specifc feature (e.g. phosphorylation of ATM-specific targets) AND radiosensitivity.

Modification Type: Gene-specific,Strength

Supporting

Use when a variant fails to rescue an ATM specifc feature, only (e.g. phosphorylation of ATM-specific targets). Do not use for radiosensitivity-only as that is not a feature specific to ATM deficiency

Modification Type: Gene-specific,Strength

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.

VCEP Specifications: PS4_Moderate: Do not use. Proband counting for genes causing a common disorder need to be calibrated in a population-specific way before use.

Strong

Case-control studies; p-value ≤.05 AND (Odds ratio, hazard ratio, or relative risk ≥2 OR lower 95% CI ≥1.5).

Modification Type: General recommendation

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.

Not Applicable

Comments: Do not use: Benign and pathogenic variants are known to occur within the same domains and germline mutational hotspots are not well defined at this time

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.

VCEP Specifications:

- Is not considered a conflicting piece of evidence for variants that otherwise are likely benign/benign
- Use as **PM2_Supporting** (not moderate)

Supporting

Frequency **≤.001%** in gnomAD v4 dataset

If n=1 in a single sub population, that is sufficiently rare and PM2_supporting would apply.

Modification Type: Gene-specific,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.

VCEP Specifications: See **ATM PM3/BP2 table** for approach to assign points per proband, and final PM3 code assignment based on the sum of PM3-related points.

Ataxia Telangiectasia (A-T) is a rare, severe, early-onset disease with some exceptions denoted ‘variant’ or ‘atypical’ A-T in which cases phenotypes are more mild with slower progression. Phenotypes associated with A-T are very specific and do not generally require differential diagnosis. Therefore, publications that claim a ‘clinical diagnosis of A-T’ are taken at face value and granted a ‘confident diagnosis. Specific phenotype criteria may qualify for ‘confident or ‘consistent’ diagnosis of A-T based on the below criteria. No additional weight modifications are made for ‘atypical’ cases if they meet ‘confident or ‘consistent’ criteria as although the disease progression is different, the clinical features are the same.

Variant may not exceed general population frequency >0.01%.

If the variant under assessment has co-occurred with at least 2 different P/LP variants, one co-occurrence must be weighed as phase unknown while the remaining can be assumed in *trans*

Multiple unrelated cases are additive.

- For example, one individual with a ‘confident A-T phenotype’ is homozygous for a variant scores 2.0 points. Another individual who has a ‘consistent A-T phenotype’ and has the same variant and another phase-unknown truncating ATM variant scores 1.0 points. The total points towards PM3 are 3.0 points leading to PM3 used as its baseline moderate strength.

CONFIDENT PHENOTYPE (must include Laboratory result)

- Presence of ≥2 Laboratory results 1-4 (see notes) -OR-
- Presence of Clinical feature 1a or 1b **AND** presence of Laboratory result 1 or 2 -OR-
- Presence of Clinical feature 2 or 3 **AND** Laboratory result 1 or 2

CONSISTENT PHENOTYPE (does not require laboratory result)

- Presence of two or more Clinical features of ataxia (1a-1e) -OR-
- Presence of one Clinical feature 1a or 1b **AND** either Clinical feature 2 or 3

Clinical features (Neurological and MRI findings):

1. Progressive cerebellar ataxia, manifesting as:
    - a: Progressive truncal/limb ataxia
    - b: Cerebellar degeneration (atrophy of the frontal and posterior vermis and both hemispheres by MRI).
    - c: Oculomotor apraxia (inability to follow an object across visual fields) or abnormal ocular saccades (rapid refixation from one object to another).
    - d: Choreoathetosis or dystonia (involuntary movements; twisting and repetitive movements, abnormal postures).
    - e: Peripheral axonal neuropathy OR Anterior horn cell neuronopathy
2. Oculocutaneous telangiectasia of the conjunctivae, ears, or face.
3. Immunodeficiency (often frequent infections) and/or leukemia/lymphoma.

Laboratory Results:

1. ATM protein levels ≤ 15% of controls in patient fibroblast or lymphoblastoid cell lines. If ATM protein levels are slightly greater than 15%, the ATM kinase activity must be shown to be "negative or low or residual" (see notes).
2. Elevated serum alpha-fetoprotein (AFP) levels >65ug/L in a patient ≥ 2 years old.
3. Increased sensitivity to ionizing radiation in patient fibroblast or lymphoblastoid cell lines.
4. Presence of a 7;14 chromosomal translocation in patient peripheral blood cells (≥ 5% of cells).

Notes:

1. ATM protein levels ≤15% of control levels show >95% sensitivity and >98% specificity for diagnosing ataxia-telangiectasia (A-T). Protein levels >15% may arise due to a missense variant, a leaky splicing variant, a variant resulting in a kinase-dead protein (where protein levels may not be affected), or a diagnosis other than A-T.
2. When assigning case report criteria based solely on laboratory results (i.e., presence of TWO or more of laboratory results 1-4), there is a greater likelihood that the most specific laboratory results #1 and #2 will be available, and that there will be some clinical indication that the individual(s) has A-T.

Very Strong

PM3_VeryStrong **≥ 8** points

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

Strong

PM3_Strong = **4** points

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

Moderate

PM3 = **2** points

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

Supporting

PM3_Supporting = **1** point

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

PM4

Original ACMG Summary

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

loss variants.

VCEP Specifications: Do not use for in-frame insertions or deletions less than a single exon; Use for stop-loss variants, only.

Moderate

Use for stop-loss variants.

Modification Type: General recommendation,Gene-specific

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.

VCEP Specifications:

- Based on location of the most C-terminal known pathogenic variant, **p.Arg3047*.**
- Use as **PM5_Supporting** (not moderate)
- Do not use for start-loss variants
- Do not use for missense changes: Multiple amino acid substitutions at the same residue can be pathogenic or benign and bioinformatic tools cannot yet confidently distinguish them

Supporting

- Apply to frameshifting or truncating variants with premature termination codons upstream of p.Arg3047.
- Apply to splice variants as with premature termination codons upstream of p.Arg3047 where PVS1_VS(RNA) is applied based on high quality observed splicing impact and must be NMD prone.

Modification Type: Gene-specific,Strength

PM6

Original ACMG Summary

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

Not Applicable

Comments: Do not use for AD or AR disease: Informative de novo occurrences have not yet been observed and de novo AR conditions are unlikely to be informed by phase

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.

VCEP Specifications:

- AR Condition: Affected relatives must have both variants identified in proband.
- AD Condition – Do not use: Co-segregation analysis in lower-penetrance genes can lead to false positive results (PMID 32773770)

Strong

AR Condition: Segregation in ≥3 affected relatives

Modification Type: Gene-specific

Moderate

AR Condition: Segregation in 2 affected relatives

Modification Type: Gene-specific

Supporting

AR Condition: Segregation in 1 affected relative

Modification Type: Gene-specific

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: Do not use: ATM does not have a defined low rate of missense benign variation.

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.

VCEP Specifications:

- NOTE: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
- NOTE: PP3 for splice predictions may not be applied in addition to PVS1 or PVS1_Variable(RNA) codes.
- Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.
- The VCEP uses SpliceAI as a sole predictor due to its ability to accurately predict loss of native splice sites and creation of cryptic sites (Jaganathan et al., 2019). This VCEP recommends SpliceAI thresholds set forth by the SVI in applying PP3 and BP4 to non-canonical splice variants: Apply PP3 for SpliceAI scores ≥0.2 and apply BP4 for SpliceAI scores ≤0.1 (Walker et al., 2023).
- In the event that RNA data are available and they reflect a substantial variant-specific impact, do not use both PVS1(RNA) and PP3 or BP4. However, in the event that RNA data are available and they reflect no variant-specific impacts, PP3 or BP4 may be applied in conjunction with BP7(RNA).

Supporting

- Missense: REVEL **>.7333**
- Splicing: Predicted impact via splicing (SpliceAI **≥0.2**) for silent, missense/in-frame and for intronic variants outside of donor and acceptor 1,2 sites.

Modification Type: Gene-specific

PP4

Original ACMG Summary

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

Not Applicable

Comments: Autosomal Dominant: do not use as breast cancer is a disease with multiple genetic etiology (genetic heterogeneity) and there are no features that can readily distinguish hereditary from sporadic causes. Autosomal Recessive: do not use as a separate line of evidence. Such evidence is built into the Ataxia Telangiectasia PM3|BP2 table

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.

VCEP Specifications: Follow all SVI general guidance on applying population filters.

Stand Alone

Grpmax Filtering AF **>.5%** in gnomAD v4 dataset

Modification Type: Disease-specific

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

VCEP Specifications: Follow all SVI general guidance on applying population filters.

Strong

Grpmax Filtering AF **>.05%** in gnomAD v4 dataset

Modification Type: Disease-specific

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.

Not Applicable

Comments: Do not use: ATM has incomplete penetrance.

BS3

Original ACMG Summary

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

VCEP Specifications: For protein, see detailed notes on ATM-specific assays;

For RNA use code BP7_RNA and modulate strength based on assay quality and quantity (curator discretion).

Moderate

Use when a variant rescues both an ATM specifc feature (e.g. phosphorylation of ATM-specific targets) AND radiosensitivity.

Modification Type: Disease-specific,Gene-specific,Strength

Supporting

Use when a variant rescues EITHER an ATM specifc feature OR rescues radiosensitivity.

Modification Type: Disease-specific,Gene-specific,Strength

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.

Not Applicable

Comments: AD Condition: Co-segregation analysis in low penetrance genes can lead to false positive results (PMID 32773770) AR Condition: Informative instances of lack of co-segregation in A-T families are too rare to be considered for weight at this time and can also be considered for BP2 if biallelic unaffected patients are observed in an A-T family.

BP1

Original ACMG Summary

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

Not Applicable

Comments: Do not use: Missense pathogenic variants are known for ATM

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.

VCEP Specifications: See **ATM PM3/BP2 table** for approach to assign points per proband, and final BP2 code assignment based on the sum of BP2-related points.

- When assessing homozygous or *in trans* variants (with a likely pathogenic or pathogenic ATM variant) for possible downgrade in an unaffected individual, the individual should be 18 years or older with no evidence of A-T.

Strong

BP2_Strong **≤ -4** points

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

Moderate

BP2_Moderate = **-2** points

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

Supporting

BP2 = **-1** point

See ATM PM3/BP2 table for approach to assign points per proband.

Modification Type: Disease-specific,General recommendation,Gene-specific,Strength

BP3

Original ACMG Summary

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

Not Applicable

Comments: Do not use.

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.

VCEP Specifications:

- NOTE: Splice analysis needs to be considered for all variant types (including missense, frameshift, nonsense, etc. as any variant has the potential to impact splicing which may preclude any expected protein effects)
- NOTE: BP4 for splice predictions may not be applied in conjunction with BP7_Variable(RNA) (a lack of observed RNA defect)
- Use caution in applying the wrong type of computational evidence (protein vs. RNA) towards the cumulative body of evidence for the opposite mechanism.
- The VCEP uses SpliceAI as a sole predictor due to its ability to accurately predict loss of native splice sites and creation of cryptic sites (Jaganathan et al., 2019). This VCEP recommends SpliceAI thresholds set forth by the SVI in applying PP3 and BP4 to non-canonical splice variants: Apply PP3 for SpliceAI scores ≥0.2 and apply BP4 for SpliceAI scores ≤0.1 (Walker et al., 2023).

- In the event that RNA data are available and they reflect a substantial variant-specific impact, do not use both PVS1(RNA) and PP3 or BP4. However, in the event that RNA data are available and they reflect no variant-specific impacts, PP3 or BP4 may be applied in conjunction with BP7(RNA).

Supporting

- Missense: REVEL score **≤.249**
- Splicing: No predicted impact via splicing (SpliceAI **≤0.1**).

Modification Type: General recommendation

BP5

Original ACMG Summary

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

Not Applicable

Comments: Do not use: Cases with multiple pathogenic variants have been observed with no noticeable difference in phenotype (e.g. BRCA1 and BRCA2). In addition, ATM has low penetrance and will naturally occur with other pathogenic variants more frequently due to higher tolerance/presence in the general population.

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.

VCEP

- BP7: Synonymous and deep intronic

Specifications:

- Can be used for deep intronic variants beyond (but not including) +7 (donor) and -21 (acceptor)
- May also apply BP4 to achieve Likely Benign
- Is not considered a conflicting piece of evidence against a body of evidence supporting a pathogenic splice defect

- BP7_Variable(RNA): RNA functional studies
  - Lack of aberrant splice defect: Please see PVS1_Variable(RNA) section (above) for guidance on baseline weights and modifications of weight based on quality for RNA assays
  - In the event that RNA data are available and they reflect a substantial variant-specific impact, do not use both PVS1(RNA) and PP3 or BP4. However, in the event that RNA data are available and they reflect no variant-specific impacts, PP3 or BP4 may be applied in conjunction with BP7(RNA).

Strong

BP7_Strong(RNA): Observed lack of aberrant RNA defect for silent substitutions and intronic variants. Variable weight applied depending on curator discretion of assay quality.

Modification Type: General recommendation

Moderate

BP7_Moderate(RNA): Observed lack of aberrant RNA defect for silent substitutions and intronic variants. Variable weight applied depending on curator discretion of assay quality.

Modification Type: General recommendation

Supporting

- BP7: Use for synonymous and deep intronic variants defined as further than (but not including) +7 and further than (but not including) -21 at donor and acceptor sites, respectively.
- BP7(RNA): Observed lack of aberrant RNA defect for silent substitutions and intronic variants. Variable weight applied depending on curator discretion of assay quality.

Modification Type: General recommendation

Rules for Combining Criteria

Pathogenic

1 Very Strong AND ≥ 1 Strong

1 Very Strong AND ≥ 2 Moderate

1 Very Strong AND 1 Moderate AND 1 Supporting

1 Very Strong AND ≥ 2 Supporting

≥ 2 Strong

1 Strong AND ≥ 3 Moderate

1 Strong AND 2 Moderate AND ≥ 2 Supporting

1 Strong AND 1 Moderate AND ≥ 4 Supporting

Likely Pathogenic

1 Very Strong AND 1 Moderate

1 Strong AND 1 Moderate

1 Strong AND ≥ 2 Supporting

≥ 3 Moderate

2 Moderate AND ≥ 2 Supporting

1 Moderate AND ≥ 4 Supporting

1 Very Strong *(PVS1, PM3_Very Strong)* **AND 1 Supporting** *(PVS1_Supporting, PS1_Supporting, PS3_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3)*

Benign

≥ 2 Strong

1 Stand Alone

Likely Benign

1 Strong AND 1 Supporting

≥ 2 Supporting

1 Strong *(BS1, BP2_Strong, BP7_Strong)*

Files & Images

ATM supplementary Tables 1 and 2 :

ClinGen HBOP ACMG Specifications ATM version 1.4: