PTEN CanVIG-UK Gene-Specific Guidance

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CanVIG-UK review of *PTEN* 08/03/2024: Consensus to use relevant recommendations from the **PTEN Expert Panel Specifications for PTEN Version 3.1.0** (available at: https://clinicalgenome.org/affiliation/50012/, PDF attached below), with additional points of specification given below where applicable.

Summary: Evidence towards Pathogenicity

Evidence element	Evidence strengths allowed				Thresholds/data-sources/applications specifically relevant to PTEN
PS4	_VSTR	_STR	_MOD	_SUP	**AMENDED FROM VCEP GUIDANCE** Using the Cleveland Clinic (CC) scoring system[1] for phenotype specificity: • Adults: ○ 1 point per proband with a Cleveland Clinic (CC) score of ≥25. ○ 0.5 points per proband with a CC score of 20-24. Where family members of a proband attain a CC score as described above, their point score may be used in addition to achieve higher evidence strength for PS4. When combining phenotype scores from additional family members for PS4, at least one family member must have one of the following three critical phenotypes: • Macrocephaly • Lhermitte-Duclos Disease • GI Hamartoma **If multiple members from the same family are used for PS4, <u>do not apply PP1</u> where the same family is used as evidence for PP1 and PS4.** Otherwise sum points towards the phenotypic specificity score, and use phenotypic specificity score ranges as per VCEP guidance. For paediatric cases, in addition to the scoring as specified in VCEP guidance, a paediatric phenotype score of 5 points may attain 1 point towards PS4. *Note: CC and paediatric scoring tables are available within the VCEP guidance.*

PP4					Not applicable as per VCEP
PM2			_MOD	_SUP	**PM2_supporting: As per VCEP.** PM2_moderate may be applied if the variant is absent from gnomAD v2.1.1 and UKBiobank (per CanVIG-UK consensus specification).
PVS1	_VSTR	_STR	_MOD	_SUP	**As per VCEP, with the following addition**: • PVS1_vstr may be applied for truncating variants in the first 100bp of the gene as there is no alternative start codon.
PS1		_STR			As per VCEP specification
PM4			_MOD		As per VCEP specification
PM5			_MOD		As per VCEP specification
PP3				_SUP	**As per VCEP with the following modifications:** • SpliceAI threshold: >0.2 • SpliceAI alone may be used to attain PP3
PM1			_MOD		As per VCEP specification
PP2				_SUP	As per VCEP specification
PS3		_STR	_MOD	_SUP	As per VCEP specification
PP1		_STR	_MOD	_SUP	**As per VCEP.** See PS4 regarding co-usage where there are multiple affected family members.
PS2	_VSTR	_STR			As per VCEP specification
PM6	_VSTR	_STR	_MOD		As per VCEP specification
PM3					Not applicable as per VCEP
PP5					Not applicable (code discontinued)

Summary: Evidence towards Benignity

BA1/BS1	_SA	_STR	_SUP	As per VCEP specification
BS2		_STR	_SUP	As per VCEP specification
BP4			_SUP	**As per VCEP with the following modifications:** • SpliceAI alone may be used to attain BP4
BP1				Not applicable as per VCEP
BP7			_SUP	As per VCEP specification
BP3				Not applicable as per VCEP
BS3		_STR	_SUP	As per VCEP specification
BS4		_STR	_SUP	As per VCEP specification
BP2			_SUP	As per VCEP specification
BP6				Not applicable (code discontinued)
BP5			_SUP	As per VCEP specification

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.0	01/11/2022	All	Initial Version	Allen	CStAG
1.1	08/03/2024	PP3/BP4	REVEL thresholds removed as incorporated into VCEP guidance. SpliceAI thresholds added to match CanVIG-UK consensus/analyses and VarSeak requirement removed.	Allen	CStAG
1.1	08/03/2024	PS4	Added clarification re: Paediatric score of 5 in paediatric cases (can attain 1 point for PS4)	Allen	CStAG
1.1	08/03/2024	PVS1	Added application of PVS1_vstr for truncating variants in first 100bp (due to no known alternative start codon)	Allen	CStAG
1.1	08/03/2024	PM2	Added that PM2_moderate may be applied per CanVIG-UK consensus specification	Allen	CStAG

References

1) Tan MH, Mester J, Peterson C, et al. A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands. Am J Hum Genet. 2011;88(1):42-56. doi:10.1016/j.ajhg.2010.11.013

Criteria Specification

ClinGen PTEN Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for PTEN Version 3.1.0

Affiliation: PTEN VCEP
Description : ACMG Classification Rules Specified for PTEN Variant Curation
Version : 3.1.0
Released : 3/14/2024
Release Notes :
Minor Changes:
1. Correct SpliceAI cutoff for BP4 rule
2. Correct the Rules for Combining Criteria
3. Add BLOSUM matrix, Cleveland Clinic core and Pediatric score tables

Rules for PTEN

General Comments: Minor Changes: 1. Correct SpliceAI cutoff for BP4 rule 2. Correct the Rules for Combining Criteria 3. Add BLOSUM matrix, Cleveland Clinic core and Pediatric score tables

Gene: PTEN (HGNC:9588)
HGNC Name: phosphatase and tensin homolog
Transcripts:

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

Use PTEN PVS1 decision tree.

Modification Type: Disease-specific

Strong

Use PTEN PVS1 decision tree.

Modification Type: Disease-specific

Moderate

Use PTEN PVS1 decision tree.

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** Follow SVI guidance, using PTEN-specific information. Per the PVS1 workflow guidance provided in Tayoun et al. 2018 (PMID 30192042), the following will apply:

1. Nonsense, frameshift variants:
    - PVS1 applies to variants predicted to result in nonsense-mediated decay (NMD); the predicted NMD cutoff for PTEN occurs at c.1121 (p.D375).
    - For nonsense or frameshift variants at the 3’ end of the gene NOT predicted to result in nonsense-mediated decay, PVS1 may still be applied if the protein is disrupted at or 5’ to c.1121 (NM_000314.6). Please see supplementary information in manuscript for evidence supporting this cutoff.
    - PVS1_Moderate applies to variants resulting in protein truncation 3’ of this cutoff.
2. Splicing variants (+/- 1,2 intronic positions):
    - Only apply to the variants resulting NMD (please refer to decision tree) OR entire exon deletion:
        - Exons 1,2,4,5,6 OR 7 deletions OR multi-exon deletion: PVS1 (Resulting frameshift)
        - Exons 3,8 OR 9 deletions: PVS1_Strong (in-frame but truncated/altered region is critical to protein function).
3. Deletion (Single/multi exon to full gene): Please refer to decision tree.
4. Duplication: Please refer to decision tree.
5. Initiation codon: PVS1 applies to initiation codon variants.

PTEN EP Commentary: No known alternative start codon in other transcripts. There are sufficient patients’ data from literature and labs support the pathogenicity of initiation codon variants.

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 OR different variant at same nucleotide position as a pathogenic splicing variant, where in silico models predict impact equal to or greater than the known pathogenic variant.

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** PS1 will be applied as described and expanded to include a different nucleotide substitution for an intronic splice site variant if the predicted impact is equal to or greater than the known pathogenic variant per *in silico* splicing tools. Caution should be used when applying this criteria to exonic variants causing aberrant splicing.

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

Two proven OR four assumed OR one proven + two assumed de novo observations in a patient with the disease and no family history.

Modification Type: Strength

Strong

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

Modification Type: None

Instructions: PS2_Very Strong: Two or more occurrences of PS2 OR two or more occurrences of PM6 AND one occurrence of PS2.

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 on the gene or gene product.

- RNA, mini-gene, or other assay shows impact on splicing

Modification Type: Disease-specific

Moderate

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

- Phosphatase activity ≤ -1.11 per Mighell et al. 2018, PMID: 29706350.

Modification Type: Disease-specific

Supporting

Phosphatase activity <50% of wild-type or abnormal *in vitro* cellular assay or transgenic model with phenotype different from wild type that does not meet PS3_moderate.

Modification Type: Disease-specific,Strength

Instructions:

PTEN EP Specification:

PS3 may be applied to the following assays:

- RNA, mini-gene, or other assay demonstrating an impact on splicing.

PS3_Moderate:

- Mighell et al. 2018 (PMID: 29706350): Massively parallel functional assay interrogating phosphatase activity.
  - In the supplementary material (Table S2) search for the variant in columns A or B and make sure the variant in question is listed as TRUE under column I (high confidence). If not, do not use as evidence.

- Under column G, the cumulative score is listed. Apply PS3_moderate for all variants with scores < -1.11.

PS3_Supporting: Other studies demonstrating lipid phosphatase activity <50% of wild-type or abnormal *in vitro* cellular assay or transgenic model with phenotype different from wild-type that does not meet PS3_moderate. Examples of *in vitro* cellular assays to be considered for PS3_supporting evidence may include:

- *In vitro* assay demonstrating >50% reduction in phosphatase activity compared to wild type control. Phosphatase assays for which criteria may be applied must include a catalytic dead control, such as p.C124S, as well as at least three biological replicates: Myers et al. 1998 (PMID: 9811831), Stambolic et al. 1998 (PMID: 9778245), Han et al. 2000 (PMID: 10866302), Rodriguez-Escudero et al. 2011 (PMID: 21828076), Costa et al. 2015 (PMID: 26504226), Malek et al. 2017 (PMID: 29056325).
- Decreased PTEN or increased pAKT expression: Tan 2011 (PMID: 21194675), Spinelli 2015 (PMID: 25527629).
- Disruption of protein cellular localization: Lobo et al. 2009 (PMID: 19457929), He et al. 2012 (PMID: 22962422), Gil et al. 2015 (PMID: 25875300)
- Aberrant cellular phenotypes, including defective cell migration, proliferation, and invasion: Costa et al. 2015 (PMID: 26504226), Malek et al. 2017 (PMID: 29056325)

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.

Very Strong

Probands with specificity score ≥16 (see text).

Modification Type: Strength

Strong

Probands with specificity score 4-15.5 (see text) OR The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls.

Modification Type: Strength

Moderate

Probands with specificity score of 2-3.5 (see text).

Modification Type: Strength

Supporting

Phenotype specific for disease with single genetic etiology. Proband(s) with specificity score of 1-1.5 (see text).

Modification Type: Disease-specific

Instructions: **PTEN EP Commentary:** This criterion is unlikely to be used in this manner for a condition as rare as PHTS. However, if sufficiently powered, a case-control study finding an odds ratio >2 for a PHTS component phenotype with $p<0.05$ and 95% confidence interval with lower limit >1.5, this criteria may be applied. However, this criterion may *not* be applied in combination with PP4.

- Use 2: Patient's phenotype or family history is highly specific for a disease with a single genetic etiology.

PTEN EP Specifications: This criterion may not be applied if BS1 applies. Phenotype specificity scores are added across independent probands and calculated as follows:

Adults:

- 1 point per proband with Cleveland Clinic (CC) score >30 (Tan 2011)
- 0.5 points per proband with CC score of 25-29.

Children:

- 1 point per proband with pediatric phenotype score >5 (please see supplementary information in manuscript for scoring rubric).
- 0.5 points per proband with pediatric phenotype score of 4, but autism/developmental delay/intellectual disability may not contribute to the score.

PS4_Very Strong: Probands with specificity score >16.

PS4: Probands with specificity score of 4-15.5.

PS4_Moderate: Probands with specificity score of 2-3.5.

PS4_Supporting: Proband(s) with specificity score of 1-1.5.

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. Defined to include residues in catalytic motifs: 90-94, 123-130, 166-168 (NP_ 000305.3)

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** Defined to include residues in one of PTEN's catalytic motifs, which include the WPD loop (residues 90-94), P-loop (also described as phosphatase core, residues 123-130), and the TI-loop (residues 166-168) (NP_ 000305.3) (Lee 1999).

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 in population

- Databases present at <0.00001 (0.001%) allele frequency in gnomAD or another large sequenced population. If multiple alleles are present within any subpopulation, allele frequency in that subpopulation must be <0.00002 (0.002%).

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** Criteria may be applied if a variant is present at <0.00001 (0.001%) allele frequency in gnomAD or another large sequenced population. If multiple alleles are present within a subpopulation, allele frequency in that subpopulation must be <0.00002 (0.002%). Please see supplementary information in manuscript supporting application of PM2 for ultra-rare alleles.

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: This rule is not applicable to PTEN.

PM4

Original ACMG Summary

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

Moderate

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants. Applies to in-frame insertions or deletions impacting at least one residue in a catalytic motif (see PM1), and variants causing protein extension.

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** For in-frame insertions or deletions, criteria may apply only if the variant impacts at least one residue in one of the catalytic motifs specified in the PM1 criteria. Criteria will also apply for variants resulting in protein extension.

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.

Moderate

Missense change at an amino acid residue where a different missense change determined to be pathogenic or likely pathogenic has been seen before. In addition, variant being interrogated must have BLOSUM62 score equal to or less than the known variant.

Modification Type: Disease-specific

Instructions: **PTEN EP Specifications:**

- This rule may be applied when the known variant is likely pathogenic unless applying would lead to a higher (pathogenic) classification for the variant being assessed.
- The variant in question need not be novel but must have a BLOSUM62 (Henikoff 1992) score equal to or less than the known variant.

PM6

Original ACMG Summary

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

Very Strong

Two proven OR four assumed OR one proven + two assumed de novo observations in a patient with the disease and no family history.

Modification Type: Strength

Strong

Two probands with presumed *de novo* occurrence (maternity/ paternity not confirmed) with the disease and no family history.

- May also be used for a proband with presumed de novo occurrence for an individual with a highly specific phenotype (meets criteria to count towards PS4)

Modification Type: Strength

Moderate

Assumed *de novo,* but without confirmation of paternity and maternity, in proband with the disease and no family history.

Modification Type: None

Instructions: PM6_Very Strong: Four or more occurrences of PM6 OR two occurrences of PM6 AND one occurrence of PS2.

PM6_Strong: Two occurrences of PM6 OR occurrence of PM6 for an individual with a highly specific phenotype (meets criteria to count towards PS4).

- Of note, when PM6_S is applied for a single individual with phenotype specificity, the individual will not be counted towards PS4 as well.

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, with ≥7 meioses observed across at least two families.

Modification Type: Strength

Moderate

Co-segregation with disease in multiple affected family members, with 5 or 6 meioses observed.

Modification Type: Strength

Supporting

Co-segregation with disease in multiple affected family members, with 3 or 4 meioses observed.

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:**

PP1: Requires 3 or 4 meioses in order to apply.

PP1_Strong: At least 7 meioses required across at least two families.

PP1_Moderate: Requires 5 or 6 meioses in order to apply.

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.

Supporting

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

Modification Type: None

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

Multiple lines of computational evidence support a deleterious effect on the gene or gene product.

- Splicing variants: Concordance of SpliceAI and VarSeak
- Missense variants: REVEL score > 0.7

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** To be applied to synonymous or intronic variants where SpliceAI and VarSeak *in silico* models predict a splicing impact (SpliceAI: scores 0.5-1 are consider evidence of pathogenic. VarSeak: Class 4 and 5 are consider evidence of pathogenic). May also be applied to missense variants with REVEL score > 0.7.

PTEN EP Commentary: Per Bayesian adaptation of the ACMG/AMP variant interpretation framework (Tavtigian et al., 2018), odds of pathogenicity (OddsPath) were estimated for various numbers of previously classified controls. When REVEL scores > 0.7 were used as evidence of pathogenic and < 0.5 were used as evidence of benign, the oddsPath was equated with moderate evidence strength for pathogenic conditions. Given that the VCEP also applies PP2 for missense variants, we decided to downgrade the evidence strength to be used at a supporting level.

PP4

Original ACMG Summary

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

Not Applicable

Comments: PTEN EP Commentary: Phenotype specificity has been incorporated into the rule specifications for PS4 Use 2.

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

gnomAD Filtering allele frequency >0.00056 (0.056%)

Modification Type: Disease-specific

Instructions: To be applied for variants with filtering allele frequency >0.00056 (>0.056%) in gnomAD. Please see information in BS1 section for data supporting this cutoff.

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

gnomAD Filtering allele frequency from 0.000043 (0.0043%) up to 0.00056 (0.056%)

Modification Type: Disease-specific

Supporting

Allele frequency from 0.0000043 (0.00043%) up to 0.000043 (0.0043%).

Modification Type: Disease-specific,Strength

Instructions: **PTEN EP Specification:**

BS1: To be applied for variants with filtering allele frequency of 0.000043 up to 0.00056 (0.0043% up to 0.056%) in gnomAD.

BS1_Supporting: To be applied for variants with filtering allele frequency of 0.0000043 up to 0.000043 (0.00043% up to 0.0043%) in gnomAD.

BA1, BS1, and BS1_P thresholds are based on the approach published by Whiffin et al. (PMID 28518168) using the following values:

- Prevalence: 1 in 9,000 (based on 15 disease-associated alleles present among the gnomAD population of ~135,000 individuals)
- Allelic heterogeneity: 22/282 (based on prevalence of most common pathogenic *PTEN* variants, p.R130X and p.R335X, per Tan et al. PMID 21194675 and Bubien 2013 PMID 23335809)
- Penetrance: 10% (overall cancer by age 40 for men with pathogenic germline *PTEN* variants is approximately 20% per Bubien 2013 PMID 23335809)

Using these data points results in a BS1 value of 0.000043. BA1 was calculated by setting allelic heterogeneity to 1, and BS1_P by reducing BS1 by an order of magnitude.

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 homozygous state in a healthy or PHTS-unaffected individual. One observation if homozygous status confirmed, two if not confirmed. To be applied at supporting evidence level if BS1 is also applied.

Modification Type: Disease-specific

Supporting

Two homozygous observations with no clinical data provided, or meets criteria for BS2 but

BS1 is also applied.

Modification Type: Disease-specific,Strength

Instructions: **PTEN EP Specifications:**

BS2: Variant must be observed in the homozygous state in a healthy or PHTS-unaffected individual. Two independent observations are required if the homozygous status is not confirmed via parental testing. If BS1 is also applied, this criteria will be applied at the supporting evidence level to avoid a variant reaching benign status solely based on homozygous occurrences due to high population frequency (BS1+BS2).

BS2_Supporting: Two homozygous observations with no clinical data provided, or meets criteria for BS2 but BS1 is also applied.

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. To be applied to intronic or synonymous variants, RNA, mini-gene or other splicing assay demonstrating no splicing impact.

Modification Type: Disease-specific

Supporting

In vitro or *in vivo* functional study or studies showing no damaging effect on protein function.

- Phosphatase activity >0 per Mighell et al. 2018, PMID: 29706350.

Modification Type: Disease-specific,Strength

Instructions: **PTEN EP Specifications:**

BS3: For intronic or synonymous variants: RNA, mini-gene, or other assay demonstrate no impact on splicing.

BS3_Supporting: *In vitro* or *in vivo* functional study or studies showing no damaging effect on protein function.

PTEN EP Specifications: BS3_supporting may be applied to the following assays:

- Mighell et al. 2018 (PMID: 29706350): Massively parallel functional assay interrogating phosphatase activity.
  - In the supplementary material (Table S2) search for the variant in columns A or B and make sure the variant in question is listed as TRUE under column I (high confidence). If not, do not use as evidence.
  - Under column G, the cumulative score is listed. Apply BS3_supporting for all variants with scores > 0.
- For missense variants: Other studies showing lipid phosphatase activity comparable to wild type in addition to a second assay appropriate to the protein domain demonstrating no statistically significant difference from wild type. Phosphatase assays for which criteria may be applied must include a catalytic dead control, such as p.C124S (NP_ 000305.3), as well as at least three biological replicates: Myers et al. 1998 (PMID: 9811831), Stambolic et al. 1998 (PMID: 9778245), Han et al. 2000 (PMID: 10866302), Rodriguez-Escudero et al. 2011 (PMID: 21828076), Costa et al. 2015 (PMID: 26504226), Malek et al. 2017 (PMID: 29056325)
- Examples of second assays may include:
  - Decreased PTEN or increased pAKT expression: Tan et al. 2011 (PMID: 21194675), Spinelli et al. 2015 (PMID: 25527629).
  - Disruption of protein cellular localization: Lobo et al. 2009 (PMID: 19457929), He et al. 2012 (PMID: 22962422), Gil et al. 2015 (PMID: 25875300).
  - Aberrant cellular phenotypes, including defective cell migration, proliferation, and invasion: Costa et al. 2015 (PMID: 26504226)
  - Malek et al. 2017 (PMID: 29056325).

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 two or more families.

Modification Type: Disease-specific

Supporting

Lack of segregation in affected members of one family.

Modification Type: Disease-specific,Strength

Instructions: **PTEN EP Specification:**

BS4: Two or more families are require for strong evidence level.

BS4_Supporting: Lack of segregation in one family.

BP1

Original ACMG Summary

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

Not Applicable

Comments: This rule is not applicable to PTEN.

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 or likely pathogenic PTEN variant OR at least three observations in cis and/or phase unknown with different pathogenic/likely pathogenic PTEN variants.

Modification Type: Disease-specific

Instructions: **PTEN EP Specifications:** The other variant may be either pathogenic or likely pathogenic. This rule may also be applied for at least three observations of the variant *in cis* or unknown phase with different pathogenic or likely pathogenic *PTEN* variants.

BP3

Original ACMG Summary

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

Not Applicable

Comments: This rule is not applicable to PTEN.

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

Multiple lines of computational evidence suggest no impact on gene or gene product.

- Splicing variants: Concordance of SpliceAI and VarSeak
- Missense variants: REVEL scores < 0.5

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** To be applied to synonymous or intronic variants where SpliceAI and VarSeak *in silico* models predict no splicing impact (SpliceAI: scores 0-0.2 are considered evidence of benign. VarSeak: Class 1 and 2 are considered evidence of benign). Not to be applied for variants which may impact the intron 1 splice donor or acceptor sites, and to be used cautiously for variants which may impact the intron 6 splice acceptor. May also be applied to missense variants with REVEL score < 0.5.

PTEN EP Commentary: Per Bayesian adaptation of the ACMG/AMP variant interpretation framework (Tavtigian et al., 2018), odds of pathogenicity (OddsPath) were estimated for various numbers of previously classified controls. When REVEL scores > 0.7 were used as evidence of pathogenic and < 0.5 were used as evidence of benign, the oddsPath was equated with moderate evidence strength for benign conditions. Given that the VCEP also applies PP2 for missense variants, we decided to downgrade the evidence strength to be used at a supporting level.

BP5

Original ACMG Summary

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

Supporting

Variant found in a case with an alternate molecular basis for disease. Other gene/disorder must be considered highly penetrant AND patient's personal/family history must demonstrate no overlap between other gene and PTEN.

Modification Type: Disease-specific

Instructions: **PTEN EP Specifications:** At least two such cases are required for criteria to apply. In addition, the other gene/disorder must be considered highly penetrant AND the patient's personal/family history must demonstrate no overlap between the other gene and *PTEN*.

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) or intronic variant at or beyond +7/-21 for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice.

Modification Type: Disease-specific

Instructions: **PTEN EP Specification:** Intronic variants must be positioned at or beyond +7/-21.

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong, PS4_Very Strong, PM6_Very Strong)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)*

1 Very Strong *(PVS1, PS2_Very Strong, PS4_Very Strong, PM6_Very Strong)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong, PS4_Very Strong, PM6_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND 1 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

1 Very Strong *(PVS1, PS2_Very Strong, PS4_Very Strong, PM6_Very Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

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

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

Likely Pathogenic

1 Very Strong *(PVS1, PS2_Very Strong, PS4_Very Strong, PM6_Very Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

≥ 3 Moderate *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

2 Moderate *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

1 Moderate *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PS4, PM6_Strong, PP1_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS3_Moderate, PS4_Moderate, PM1, PM4, PM5, PM6, PP1_Moderate)*

1 Very Strong *(PVS1, PS2_Very Strong, PS4_Very Strong, PM6_Very Strong)* **AND 1 Supporting** *(PS3_Supporting, PS4_Supporting, PM2_Supporting, PP1, PP2, PP3)*

Benign

≥ 2 Strong *(BS1, BS2, BS3, BS4)*

1 Stand Alone *(BA1)*

Likely Benign

≥ 2 Supporting *(BS1_Supporting, BS2_Supporting, BS3_Supporting, BS4_Supporting, BP2, BP4, BP5, BP7)*

1 Strong *(BS1, BS2, BS3, BS4)*

Files & Images

PVS1_DecisionTree:

RULES FOR COMBINING CRITERIA FOR CLASSIFICATION:

RULES FOR COMBINING CRITERIA FOR CLASSIFICATION

Variants will be classified per Richards et al., 2015 with the following exceptions:

- 1 Benign Strong (BS) = Likely Benign.
- 1 Pathogenic Very Strong (PVS) + 1 Pathogenic Supporting (PP) = Likely Pathogenic.
- Variants with conflicting evidence may be classified using the Bayesian points system (Tavtigian et al., 2018).

BLOSUM matrix:

	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val
Ala	4																			
Arg	−1	5																		
Asn	−2	0	6																	
Asp	−2	−2	1	6																
Cys	0	−3	−3	−3	9															
Gln	−1	1	0	0	−3	5														
Glu	−1	0	0	2	−4	2	5													
Gly	0	−2	0	−1	−3	−2	−2	6												
His	−2	0	1	−1	−3	0	0	−2	8											
Ile	−1	−3	−3	−3	−1	−3	−3	−4	−3	4										
Leu	−1	−2	−3	−4	−1	−2	−3	−4	−3	2	4									
Lys	−1	2	0	−1	−3	1	1	−2	−1	−3	−2	5								
Met	−1	−1	−2	−3	−1	0	−2	−3	−2	1	2	−1	5							
Phe	−2	−3	−3	−3	−2	−3	−3	−3	−1	0	0	−3	0	6						
Pro	−1	−2	−2	−1	−3	−1	−1	−2	−2	−3	−3	−1	−2	−4	7					
Ser	1	−1	1	0	−1	0	0	0	−1	−2	−2	0	−1	−2	−1	4				
Thr	0	−1	0	−1	−1	−1	−1	−2	−2	−1	−1	−1	−1	−2	−1	1	5			
Trp	−3	−3	−4	−4	−2	−2	−3	−2	−2	−3	−2	−3	−1	1	−4	−3	−2	11		
Tyr	−2	−2	−2	−3	−2	−1	−2	−3	2	−1	−1	−2	−1	3	−3	−2	−2	2	7	
Val	0	−3	−3	−3	−1	−2	−2	−3	−3	3	1	−2	1	−1	−2	−2	0	−3	−1	4

Cleveland Clinic score:

Feature	Score	Feature	Score
Neurological		**Breast**	
Macrocephaly	6	Cancer dx <40 yrs	4
Extreme macrocephaly (men: ≥63 cm, women: ≥60 cm)	10	Cancer dx 40-49 yrs	2
Lhermitte-Duclos disease	10	Cancer dx ≥50 yrs	1
Autism/developmental delay	1	Fibrocystic breast disease	1
Skin		**Thyroid**	
Trichilemmoma (biopsy-proven)	10	Cancer dx <20 yrs	10
Oral papillomas	6	Cancer dx 20-49 yrs	4
Penile freckling	6	Cancer dx ≥50 yrs	1
Acral keratoses	1	Goiter, nodules, or Hashimoto's thyroiditis	4
Lipoma	1	**Genitourinary**	
Arteriovenous malformation	6	Endometrial cancer dx 20-29 yrs	10
Gastrointestinal		Endometrial cancer dx 30-49 yrs	6
≥5 gastrointestinal polyps, any type	6	Endometrial cancer dx ≥50 yrs	1
Hamartoma or ganglioneuroma	10	Uterine fibroids	1
Glycogenic acanthosis	10	Renal cell carcinoma	1

PTEN Phenotype Scoring for Pediatric Patients:

TABLE 2 PTEN phenotype scoring for pediatric patients

Feature	Score (points)
Macrocephaly of >2 SD to <4 SD	2
Extreme macrocephaly (≥4 SD)	3
PTEN-specific MRI characteristics (dilated Virchow-Robin, prominent perivascular spaces)	2
Autism/developmental delay (DD)/intellectual disability (ID)	2
Penile freckling	3
Lipoma	1
Oral papilloma	3
Hamartomatous polyp(s)	3
Arteriovenous malformation/hemangioma	2
Thyroid cancer	3
Thyroid nodule/Hashimoto's thyroiditis	2

References

1. Costa HA Leitner MG et al. *Discovery and functional characterization of a neomorphic PTEN mutation.* **Proc Natl Acad Sci U S A** (2015) 112 (45) p. 13976-81. 10.1073/pnas.1422504112 26504226
2. Gil A Rodríguez-Escudero I et al. *A functional dissection of PTEN N-terminus: implications in PTEN subcellular targeting and tumor suppressor activity.* **PLoS One** (2015) 10 (4) p. e0119287. 10.1371/journal.pone.0119287 25875300
3. Han SY Kato H et al. *Functional evaluation of PTEN missense mutations using in vitro phosphoinositide phosphatase assay.* **Cancer Res** (2000) 60 (12) p. 3147-51. 10866302
4. He X Saji M et al. *PTEN lipid phosphatase activity and proper subcellular localization are necessary and sufficient for down-regulating AKT phosphorylation in the nucleus in Cowden syndrome.* **J Clin Endocrinol Metab** (2012) 97 (11) p. E2179-87. 10.1210/jc.2012-1991 22962422
5. Henikoff S Henikoff JG *Amino acid substitution matrices from protein blocks.* **Proc Natl Acad Sci U S A** (1992) 89 (22) p. 10915-9. 10.1073/pnas.89.22.10915 1438297
6. Lobo GP Waite KA et al. *Germline and somatic cancer-associated mutations in the ATP-binding motifs of PTEN influence its subcellular localization and tumor suppressive function.* **Hum Mol Genet** (2009) 18 (15) p. 2851-62. 10.1093/hmg/ddp220 19457929
7. Malek M Kielkowska A et al. *PTEN Regulates PI(3,4)P(2) Signaling Downstream of Class I PI3K.* **Mol Cell** (2017) 68 (3) p. 566-580.e10. 10.1016/j.molcel.2017.09.024 29056325
8. Mighell TL Evans-Dutson S et al. *A Saturation Mutagenesis Approach to Understanding PTEN*

Lipid Phosphatase Activity and Genotype-Phenotype Relationships. **Am J Hum Genet** (2018) 102 (5) p. 943-955. 10.1016/j.ajhg.2018.03.018 29706350

9. Myers MP Pass I et al. *The lipid phosphatase activity of PTEN is critical for its tumor supressor function.* **Proc Natl Acad Sci U S A** (1998) 95 (23) p. 13513-8. 10.1073/pnas.95.23.13513 9811831
10. Richards S Aziz N et al. *Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.* **Genet Med** (2015) 17 (5) p. 405-24. 10.1038/gim.2015.30 25741868
11. Rodríguez-Escudero I Oliver MD et al. *A comprehensive functional analysis of PTEN mutations: implications in tumor- and autism-related syndromes.* **Hum Mol Genet** (2011) 20 (21) p. 4132-42. 10.1093/hmg/ddr337 21828076
12. Spinelli L Black FM et al. *Functionally distinct groups of inherited PTEN mutations in autism and tumour syndromes.* **J Med Genet** (2015) 52 (2) p. 128-34. 10.1136/jmedgenet-2014-102803 25527629
13. Stambolic V Suzuki A et al. *Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN.* **Cell** (1998) 95 (1) p. 29-39. 10.1016/s0092-8674(00)81780-8 9778245
14. Tavtigian SV Greenblatt MS et al. *Modeling the ACMG/AMP variant classification guidelines as a Bayesian classification framework.* **Genet Med** (2018) 20 (9) p. 1054-1060. 10.1038/gim.2017.210 29300386
15. Tan MH Mester J et al. *A clinical scoring system for selection of patients for PTEN mutation testing is proposed on the basis of a prospective study of 3042 probands.* **Am J Hum Genet** (2011) 88 (1) p. 42-56. 10.1016/j.ajhg.2010.11.013 21194675