The PTEN Gene ClinGen Expert Panel: A model for creating a framework for gene specific criteria using ACMG guidelines

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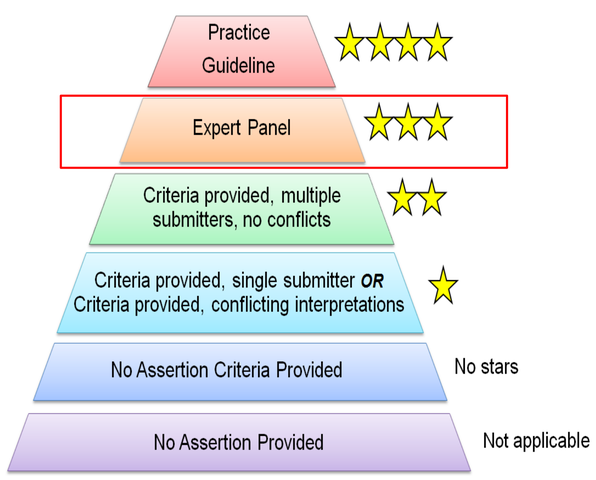
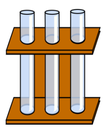
Introduction

ClinVar is a publicly available resource of genetic variants along with evidence supporting each assertion submitted by clinical and research laboratories. Different groups using their own approaches and available evidence for variant interpretation often results in conflicting assertions. To help ClinVar users understand the level of evidence behind each assertion, ClinGen developed a hierarchical 4-star rating system where more stars indicate a greater level of review, with ClinGen-designated Expert Panels at **3 stars** (figure courtesy of Heidi Rehm, PhD).



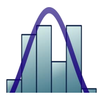
For some genes an expert group was already in existence and was able to apply for expert panel status; for others, ClinGen is facilitating expert panel development. **PTEN**, associated with Cowden, Bannayan-Riley-Ruvalcaba, and other syndromes caused by germline PTEN mutation[2], is the first gene in the Hereditary Cancer domain for which an expert panel has been developed within the ClinGen framework. Group members include:

Clinicians with experience caring for patients with PTEN mutations



Laboratory personnel who analyze and interpret PTEN variants in clinical and research settings

Basic and clinical researchers studying PTEN structure and function



Biostatisticians and personnel with population database expertise

The Task: PTEN-Specific Criteria

The group was tasked with developing gene-specific variant classification criteria, using the 2015 ACMG/AMP Variant Interpretation Guidelines[3] as a starting point.

	Benign		Pathogenic			
	Strong	Supporting	Supporting	Moderate	Strong	Very strong
Population data	MAF is too high for disorder BA1/BS1 OR observation in controls inconsistent with disease penetrance BS2			Absent in population databases PM2	Prevalence in affecteds statistically increased over controls PS4	
Computational and predictive data		Multiple lines of computational evidence suggest no impact on gene /gene product BP4 Missense in gene where only truncating cause disease BP1 Silent variant with non predicted splice impact BP7 In-frame indels in repeat w/out known function BP3	Multiple lines of computational evidence support a deleterious effect on the gene /gene product PP3	Novel missense change at an amino acid residue where a different pathogenic missense change has been seen before PM5 Protein length changing variant PM4	Same amino acid change as an established pathogenic variant PS1	Predicted null variant in a gene where LOF is a known mechanism of disease PVS1
Functional data	Well-established functional studies show no deleterious effect BS3		Missense in gene with low rate of benign missense variants and path. missenses common PP2	Mutational hot spot or well-studied functional domain without benign variation PM1	Well-established functional studies show a deleterious effect PS3	
Segregation data	Nonsegregation with disease BS4		Cosegregation with disease in multiple affected family members PP1	Increased segregation data →		
De novo data				De novo (without paternity & maternity confirmed) PM6	De novo (paternity and maternity confirmed) PS2	
Allelic data		Observed in *trans* with a dominant variant BP2 Observed in *cis* with a pathogenic variant BP2		For recessive disorders, detected in trans with a pathogenic variant PM3		
Other database		Reputable source w/out shared data = benign BP6	Reputable source = pathogenic PP5			
Other data		Found in case with an alternate cause BP5	Patient's phenotype or FH highly specific for gene PP4			

Working groups were assembled to analyze current knowledge related to PTEN regarding the following evidence types: population frequency, splicing, computational/predictive data, functional data, phenotype, and segregation/de novo data.

The Process

- Each working group presented its findings to the team. This generated discussion about whether the evidence type was relevant for PTEN and elevated the group's knowledge to ensure educated discussions.
- The co-chairs proposed the following process, which was agreed upon by the entire group:

Draft benign criteria for all evidence types

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Curate a set of test benign variants

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Make edits/adjustments as needed

↓

Repeat the process for pathogenic criteria

- The group discussed each criteria from the ACMG/AMP document separately, deciding whether to adopt as-is, tailor to fit PTEN, or discard if not relevant to PTEN.

Draft Benign Criteria for PTEN

Proposed benign criteria are listed below. Rules to combine criteria were kept as defined by ACMG/AMP.

Stand-Alone Criteria

BA1: Allele frequency ≥1% (≥2,000 alleles tested, present in ≥5 alleles)

Strong Criteria

BS1: Allele frequency 0.1%-1% (2,000 alleles tested, present in ≥5 alleles)

BS2: Homozygous observation, pt confirmed as healthy/unaffected
- N=1 observation with homozygous status confirmed
- N=2 without confirmation

BS3: Functional data
- Normal phosphatase activity
- RNA, mini-gene or other splicing assay demonstrates NO splicing impact (applies to intronic or synonymous variants)

BS4: Lack of segregation in >1 family

Benign	(i) 1 Stand-alone (BA1) *OR* (ii) ≥2 Strong (BS1–BS4)
Likely benign	(i) 1 Strong (BS1–BS4) and 1 supporting (BP1–BP7) *OR* (ii) ≥2 Supporting (BP1–BP7)

Supporting Criteria

BP1: Allelic data
- One observation *in trans* with a known pathogenic variant
- 3 observations *in cis* and/or phase unknown with different pathogenic variants

BP2: Functional data
- Normal in vitro cellular assay
- Transgenic model organism no different from WT

BP3: 2 homozygous observations, no clinical data provided

BP4: For synonymous/intronic variants: 2/3 in silico models predict no impact

BP5: 2 or more co-occurrences of pathogenic variants in another gene fully explaining the patient's phenotype
- Other gene must be considered highly penetrant
- Patient's phenotypic features explained by PTEN must not overlap with phenotype caused by the other gene

BP6: Lack of segregation in 1 family

BP7: Variant is synonymous or located at or beyond +7/-21 and nucleotide not conserved

Alterations to the ACMG/AMP criteria include:
- Lower allele frequency thresholds to account for the rarity of germline PTEN mutations
- BP1 was removed (pathogenic PTEN missense variants exist)
- Added criteria for homozygous observations and supporting evidence for functional data and segregation.

Curation Process: Beta Testing

- To test the criteria, **15 variants** classified as benign or likely benign by multiple ClinVar submitters were selected.
- Group members volunteered to assemble data for each variant.
- Findings will be reviewed and a final classification made by group consensus.

Future Directions

- Testing of the proposed benign criteria is currently underway.
- Pathogenic criteria will be developed and tested in a similar manner.
- After final edits are made to the gene-specific criteria and curation process, an application will be submitted for formal Expert Panel status.

References

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2. Eng C. PTEN: one gene, many syndromes. Hum Mutat. 2003 22(3):183-98.
3. Richards S 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 May 17(5):405-24.

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