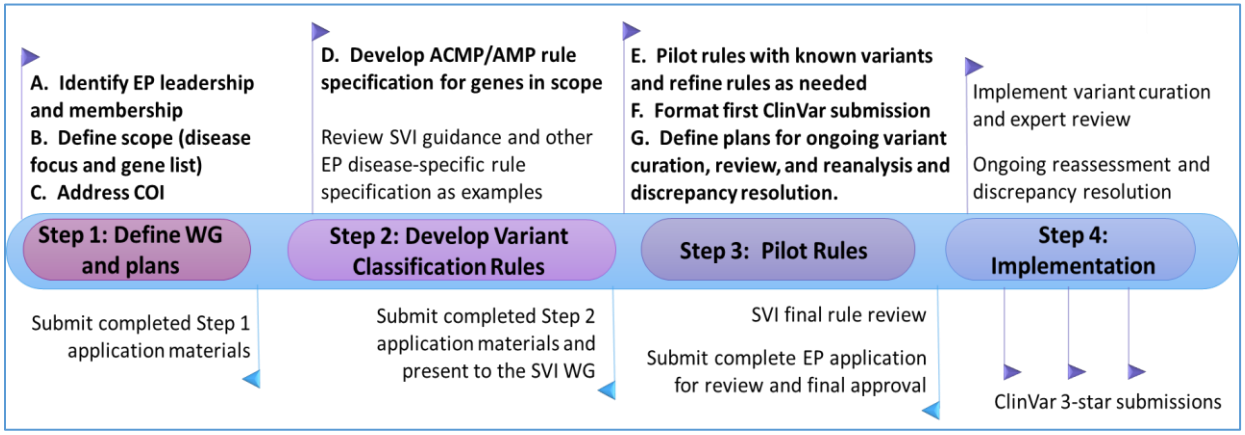
Appendix 1: Request for Expert Panel or Practice Guideline Designation for Submissions to ClinVar

Submitter Information	
Full Name of Submitting Source: ClinGen PTEN Variant Curation Expert Panel	
Acronym or other brief name for ClinVar data display: CG_PTEN_EP	
Expert Panel Member responsible for submission: Jessica Mester	
Email address: jmester@genedx.com	**Phone**: 216-399-6678
Expert Panel Coordinator and email address: Jessica Mester, jmester@genedx.com	

ClinGen – affiliated groups should compose their Expert Panel application in accordance to the below timeline. ClinGen affiliated groups are required to submit for Step 1 approval after completing items A-C. Similarly, after completing item D, ClinGen-affiliated groups are required to send their variant classification rules to the Sequence Variant Interpretation (SVI) WG. Finally, ClinGen groups will pilot and refine rules, format their first ClinVar submission, and define a protocol for ongoing variant curation (complete items E-G) and submit for Step 3 (final) approval by ClinGen's Steering Committee (SC).

External Expert Panel applicants are also suggested to complete their Expert Panel application in a stepwise manner, in accordance to the timeline shown below. We encourage these groups to begin communication with ClinGen's Clinical Domain WG Oversight Committee (after Step 1) and SVI (after Step 2) early in the application process. All Expert Panel applicants are required to submit for Step 3 (final) approval by ClinGen's Steering Committee.

Groups applying for **Practice Guideline** (4-star) status in ClinVar should contact ClinGen at clingen@clinicalgenome.org for the Practice Guideline application.



Expert Panel Submission Details

A. Composition of the Expert Panel

Expert Panels are expected to represent the diversity of expertise in the field, including all major areas of expertise (clinical, diagnostic laboratory, and basic research). Membership should include representation from three or more institutions and will encompass disease/gene expert members as well as biocurators. Biocurators do not have to be gene/disease experts and will be primarily responsible for assembling the available evidence for subsequent expert member review. For role, suggested examples include: primary biocurator, expert reviewer, etc.

Member List

Name	Institution	Area and Type of Expertise	Role
Charis Eng	Cleveland Clinic	Clinical, basic research	Co-chair, expert reviewer
Madhuri Hegde	Emory University	Diagnostic laboratory	Co-chair, expert reviewer
Jessica Mester	GeneDx	Diagnostic laboratory, clinical	Coordinator, expert reviewer, primary biocurator
Tina Pesaran	Ambry Genetics	Diagnostic laboratory	Expert reviewer, primary biocurator
Katherine Lachlan	Wessex Clinical Genetics Service	Clinical	Expert reviewer
Joanne Ngeow	National Cancer Centre Singapore	Clinical, basic research	Expert reviewer
Robert Huether	Tempus	Basic research	Expert reviewer
Kaitlin Sesock	Counsyl	Diagnostic laboratory, clinical	Expert reviewer, biocurator
Kathleen Hruska	GeneDx	Diagnostic laboratory	Expert reviewer
Helio Costa	Stanford University	Basic research	Expert reviewer
Jill Barnhotz-Sloan	Case Western Reserve University	Basic research	Expert reviewer
Rachid Karam	Ambry Genetics	Diagnostic laboratory	Expert reviewer
Liying Zhang	Memorial Sloan Kettering Cancer Center	Basic research	Expert reviewer
Felicia Hernandez	Ambry Genetics	Diagnostic laboratory	Biocurator
Melody Perpich	University of Chicago	Clinical	Biocurator

(Insert additional page if needed)

B. Scope of Work

Describe the scope of work of the Expert Panel (disease areas and gene(s) being addressed).

Pathogenic germline variants in PTEN are indicative of PTEN Hamartoma Tumor syndrome (PHTS, MIM+601728), an umbrella term used to describe any individual with a germline pathogenic *PTEN* variant regardless of clinical presentation. PHTS causes increased risk for benign and malignant tumors as well as neurodevelopmental disorders, and includes individuals with Cowden syndrome (MIM#158350), Bannayan-Riley-Ruvalcaba syndrome (MIM#153480), and other phenotypes such as Macrocephaly/Autism syndrome (#605309) found to have a germline pathogenic *PTEN* variant. This expert panel will provide assessment regarding the pathogenicity of variants in *PTEN* with respect to a PHTS phenotype.

C. Conflict of Interest Management

Expert Panels are expected to represent the diversity of expertise in the field and should be composed of a sufficient number of eligible expert reviewers to address academic and financial conflicts of interest that may arise.

- *Academic COI: Authors of literature about relevant variants may serve on the Expert Panel and are welcome to voice their opinion, but should not be the major arbiter of a variant classification when there is limited data available and it was provided by that individual or the individual's lab group.*
- *Financial COI: Commercial entities may participate on the Expert Panel, but should not be the major arbiter of a variant classification when there is limited data available and it was provided by that entity.*
- *No special measures are needed if there is group consensus on a variant classification; however, if a vote is needed, those with relevant conflicts of interest should recuse themselves.*
- *All conflicts will be declared publicly on the clinicalgenome.org website and reported in publications as appropriate.*

These guidelines were reviewed on our group call on June 14, 2017. The PTEN EP membership understands and agrees to abide by these guidelines.

Addendum dated April 6, 2018: all members of the PTEN EP have completed the ClinGen COI survey.

Note to Submitters: After completing Step 1 (application items A-C), please submit your draft Expert Panel application to the ClinGen Clinical Domain WG Oversight Committee (clingen@clinicalgenome.org) for review.

Date of Submission:

D. ACMG guideline specifications

Expert Panels are encouraged to use the ACMG/AMP variant assessment criteria as their starting point for a framework to adjudicate Mendelian variants according to the five class criteria (pathogenic, likely pathogenic, uncertain significance, likely benign, and benign). The Expert Panel process typically entails reviewing the evidence types and making gene-specific specifications to the ACMG/AMP guidelines, including consultation with the Sequence Variant Interpretation WG in order to facilitate harmonization of approaches across different expert panels.

Provide the gene-optimized rules for variant classification designed by the Expert Panel as an appendix. Documentation will be made publicly available and could consist of an unpublished document, manuscript pre-print, or published manuscript. The following items must be included in the submitted material:

- **Please attach a description of the specified ACMG/AMP guidelines for the gene(s) of interest, including evidence and rationale to support the rule specifications.**

- **Describe combinations of rules and evidence sources that could be used to classify any categories of variants (e.g. Benign or Likely Benign) in a batch:**

Please see the attached manuscript draft for a description of our ACMG/AMP guideline specifications and the evidence and rational supporting all adjustments and modifications made. We will utilize the rules for combining criteria for classification presented by Richards et al, 2015.

Note to Submitters: After completing Step 2 (application item D), please submit your draft Expert Panel application to the ClinGen Sequence Variant Interpretation Working Group (clingen@clinicalgenome.org) for review.

Date of Submission:

E. Validation of ACMG guideline specifications

Please provide a description of how your rules were validated with known variants.

The EP decided to first develop and test benign criteria on a set of PTEN variants defined as benign or likely benign (BEN/LBEN) per multiple ClinVar submitters, and then repeat the process for pathogenic criteria and a similar pathogenic/likely pathogenic (PATH/LPATH) PTEN variant test set. The ClinGen Genomic Variant Working Group, which provided preliminary review and feedback on the criteria during the development phase, recommended a minimum 80% concordance with the consensus ClinVar classifications prior to rule acceptance. Finally, variants with classifications of uncertain significance (VUS) or with conflicting interpretations by multiple submitters (CONF) were curated by two independent biocurators to assess inter-curator concordance and process workflow.

For the BEN/LBEN test set, 12/15 (80%) variants achieved a BEN/LBEN classification based on initial data accumulated from literature review, population databases, and in silico models. The three variants initially classified as VUS included c.-1026C>A, c.-1311T>C, and c.75G>A (p.L25=). Internal data was sought from group members to identify cases with homozygous occurrences, segregation data, or co-occurrence data that might be applied. In the case of c.-1026C>A, both homozygous observations (BS2) as well as co-occurrences with pathogenic variants in other genes explaining the patient's phenotype (BP5) were identified, leading to a final BEN classification. PTEN c.-1311T>C is present in gnomAD at an allele frequency of 1.42% (23/1618) within East Asian populations. Despite the denominator being less than 2000, BA1 was applied with approval from the Genomic Variant Working Group given the allele frequency would remain >1% if 2000 alleles were present in the studied population (23/2000 = 1.15%). PTEN c.75G>A is near a predicted U12-dependent splice donor for which *in silico* tools are not available. Although BP7 was applied based on the synonymous nature of this variant, no additional criteria apply at present, leaving this variant at VUS. Thus, the BEN/LBEN test set achieved a final concordance of 93.3% (14/15) with consensus ClinVar classifications.

Given that clinical laboratory data provided helpful evidence for the BEN/LBEN variants, these data were sought and incorporated for the initial review of the pathogenic criteria tested on 16 consensus ClinVar PATH/LPATH variants. On initial review, 15/16 (93.8%) variants achieved a PATH/LPATH classification. PTEN c.50_51delAA was present in 1/246,272 alleles in gnomAD, and the EP's initial PM2 language permitted use only when a variant was completely absent in sequenced populations. Thus the EP chose to modify PM2 as described below, permitting use for this variant and leading to a final 100% (16/16) concordance with consensus ClinVar classifications for the PATH/LPATH test set.

The final set of variants curated for pilot purposes included five with conflicting interpretations in ClinVar (CONF) and six with assertions of uncertain significance (VUS) by multiple submitters, with curation performed by two independent biocurators. Biocurators had complete concordance with respect to criteria and classification for five of the six VUS. For the five CONF variants, initial criteria applied by each biocurator differed slightly, but preliminary classifications did not differ by more than one "step" (VUS vs. LBEN, PATH vs. LPATH, etc.). Following brief discussion between biocurators and addition of internal laboratory data, complete agreement regarding criteria and preliminary classification were achieved for three of the five CONF variants, for a final inter-biocurator concordance of 72.7% (8/11) within the VUS and CONF variant sets. The PTEN EP criteria resolved classification for one of the VUS (c.235G>A, p.A79T was classified as LBEN) and two of the CONF variants (c.209+4_209+7delAGTA was classified as PATH, c.521A>G, p.Y174C was classified as LPATH).

F. Model ClinVar submission

Expert Panels are encouraged to make submissions to ClinVar through the ClinGen Variant Curation Interface (VCI) in order to standardize the content across expert panels.

Please provide a sample list of classified variants curated in the VCI or attached in the ClinVar submission template. The submission template can be downloaded here: ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/submission_templates/

PTEN variants curated in the VCI are as follows:

c.-9C>G
c.-903G>A
c.-1026C>A
c.-1059C>G
c.-1311T>C
c.18A>G (p.L6L)
c.75G>A (p.L25L)
c.132C>T (p.G44G)
c.360A>C (p.A120A)
c.1104T>C (p.D368D)
c.79+35C>T
c.165-13_165-10delGTTT
c.254-39G>T
c.801+23G>A
c.1026+32T>G
c.50_51delAA
c.511C>T (p.Q171X)
c.892C>T (p.Q298X)
c.964A>T (p.K322X)
c.987_990delTAAA
c.80-1G>C
c.165-1G>A
c.493-2A>G
c.801+1delG
c.802-2A>T
c.1026+1G>A
c.103A>G (p.M35V)
c.389G>A (p.R130Q)
c.407G>A (p.C136Y)
c.517C>T (p.R173C)
c.737C>T (p.P246L)
c.-1170C>T
c.209+3A>T
c.235G>A (p.A79T)

c.304_306dupAAA (p.K102_P103insK)
c.1052_1054delTAG (p.V351del)
c.1171C>T (p.P391S)
c.-764G>A
c.44G>A (p.R15K)
c.78C>T (p.T26=)
c.209+4_209+7delAGTA
c.521A>G (p.Y174C)

G. Define plans for ongoing variant curation, review, and reanalysis and discrepancy resolution

Expert Panels are expected to develop work schedules, review and resolve differences in interpretation, and provide standard procedures for variant assessment.

Standard Operating Procedures:

- **Meeting/call frequency:** Quarterly (every 3 months)

- **Curation/expert review/finalization process:**

  ☐ Version 1: One curator performs data entry and baseline curation; two domain experts perform blinded double review and classification. Discussions with the full EP are triggered if:

  a) the experts do not reach consensus,

  b) either expert raises concerns regarding the "fit" of a rule, or

  c) the strength of functional evidence needs further input.

  ☑ Version 2: Two curators perform independent assessments followed by full EP review and consensus classification.

  ☐ Other

Expert Panels are expected to keep their variant interpretations up-to-date and to expedite the re-review of variants that have a conflicting assertion submitted to ClinVar after the Expert Panel submission.

□ **Expert Panels are expected to contact the submitter of a newly submitted conflicting assertion in ClinVar from a one star submitter or above and attempt to resolve or address the conflict within 4 months of being notified about the conflict from ClinGen**

□ **Expert Panels are expected to re-review all VUS classifications made by the EP at least every 2 years to see if new evidence has emerged to re-classify the variants**

□ **Expert Panels are expected to re-review any LP or LB classifications when new evidence is available or when requested by the public via the ClinGen website.**

□ **If plans differ from the expectations above, please describe here:**

The PTEN EP understands and agrees to abide by these expectations to the best of our abilities.

Note to Submitters: Please send your completed Expert Panel application to ClinVar (clinvar@ncbi.nlm.nih.gov) and to the ClinGen Clinical Domain WG Oversight Committee (clingen@clinicalgenome.org) for review.

Date of Final Submission: