ClinGen Variant Curation Expert Panel (VCEP) Protocol

INTRODUCTION

A. All ClinGen Variant Curation Expert Panels (VCEPs) must fulfill all the requirements described below to become approved ClinGen VCEPs. Intermediate approval is required at several steps along the way. Frequent communication with the Clinical Domain Working Group Oversight Committee is encouraged.

B. Approved ClinGen VCEPs are part of the FDA Recognized ClinGen Human Variant Database. As such, this protocol represents the processes for VCEP formation, approval, curation, assessment and publication of variant classifications of pathogenicity. All final, approved variant classifications and the curated evidence supporting that classification is part of the ClinGen Human Variant Database.

C. Public access and transparency to the VCEP variant-level evidence, assessment process and classifications are central to the mission of ClinGen and a requirement of the ClinGen Human Variant Database. All final, approved variant classifications, accompanied by a description of the evidence supporting the classification will be submitted to ClinVar for public access. The VCEP's detailed method for classifying variants (specified criteria for evaluating pathogenicity and process for curation and expert review) must be provided with the application for final approval. This description of the variant classification criteria will accompany ClinVar submissions as will a summary of the individual assessment for each variant. The evidence assessed in the classification of individual variants will be available to external users via the public Evidence Repository on the ClinGen website. Lastly, the approved VCEP application materials will be available on the ClinGen website to ensure transparency about the ClinGen VCEP process.

1. Guidance for Application and Approval of ClinGen VCEP

1.1 Membership and Training

Variant Curation Expert Panel membership
VCEP membership is expected to represent individuals with diverse areas of expertise including medical professionals caring for patients relevant to the disease gene in question, medical geneticists, clinical laboratory diagnosticians and/or molecular pathologists who report such findings and appropriate researchers relevant to the disease, gene, functional assays and statistical analyses. It is expected that the individuals comprising the VCEP process will represent three or more academic or commercial institutions. ClinGen strongly supports inter-institutional collaboration and recommends reaching out to colleagues and collaborators within their area of focus to maximize the ability to represent experts in this disease area across multiple institutions and to build consensus and data sharing.

- One or more co-chairs who are respected authorities in the field based on publication record, or professional standing (for example, director of a molecular laboratory, tenured professor, eminent clinician) should be selected.
- There is no predefined number of members for a VCEP, though as described above the panel should represent the diversity of expertise in the field.
- Each panel should include independent participants from 3 or more institutions; inclusion of more than two senior members who are affiliated with the same institution is discouraged, though members may invite additional curation staff from their groups to support projects.

Biocurator Proficiency Training.
Each VCEP will consist of both domain experts and biocurators. Biocurators are not required to be gene/disease experts and will be primarily responsible for assembling the available evidence for subsequent expert member review. Trained ClinGen biocurators will be assigned to the VCEP to ensure understanding of and adherence to the ClinGen variant curation method and frameworks as outlined in the Variant Curation SOP (document in development). Additional biocurators may be recruited by the expert members of the developing VCEP. In addition, domain experts in the VCEP may choose to participate in biocurator training and biocuration activities.

- The VCEP is responsible for coordinating, monitoring, and recording the training and proficiency of new biocurators who have not been previously trained by ClinGen.
- An experienced ClinGen biocurator in the VCEP will assist and mentor biocurators during the training process. The biocurator mentor will guide the trainee through training curations and review all initial curations until the biocurator has demonstrated proficiency with the core competencies: evidence identification, transference of evidence into the Variant Curation Interface (VCI) for review, and assessment of the significance of evidence in accordance with the VCEP criteria. Proficiency is determined by the experienced biocurator.

- Once a biocurator has achieved proficiency, this is documented by the VCEP coordinator. Ongoing proficiency is evaluated through the regular variant curation review activities of the VCEP during variant assessment. Variant review always includes at least one VCEP expert or the full VCEP.
- Each VCEP may provide additional training specific to the gene(s) they are working on. Practice curations can be conducted in the VCI as a trainee delegate.
- The initial and on-going training of biocurators will be informed by the Biocurator Working Group.

The Biocurator Working Group (WG) is chaired by Jenny Goldstein (goldjen@email.unc.edu), and provides curation training assistance, as well as a forum for discussion of process questions, and relevant updates. The Biocurator WG also provides general training materials that outline approaches for evidence searches and data documentation required for each variant. Diverse topics are discussed on the calls such as Sequence Variant Interpretation (SVI) WG updates, use of the ClinGen interfaces (including the Variant Curation Interface (VCI)), and presentations from experts on relevant resources such as Ensembl, ClinVar, gnomAD etc. Recordings of all calls and relevant slide sets are archived on the Biocurator WG page on ClinGen's Confluence site and are accessible at any time. The listserv for the Biocurator WG providers a mechanism for disseminating updates, such as new guidance from the SVI, to ensure that biocurators stay current with ClinGen best practices.

- All VCEP biocurators are expected to join the ClinGen Biocurator WG. This group provides a forum for training and education of all ClinGen gene and variant biocurators. The group meets by conference call twice a month.

HIPAA Training.
All VCEP members are responsible for obtaining HIPAA and human subjects training based on their home institutional/affiliation guidelines and the level of access to human subject data. VCEP leadership and coordinators should ensure that members do not inadvertently share data that has not been stripped of protected health information or other identifiers. Identifiable and/or protected health information is not necessary for variant curation and evaluation and is not stored in the VCI or variant evidence summary.

1.2 Application Overview

VCEPs formed within ClinGen are required to fulfill the requirements outlined herein, and document them in the final application. The application is reviewed and approved in a stepwise manner (Figure 1) by the Clinical Domain WG Oversight Committee and the Rule Specification Review Committee of the SVI. ClinGen-affiliated VCEPs follow defined milestones to prepare submission materials and, as part of this process, are required to submit materials for approval after completing each of Steps 1-3. The VCEP coordinator records information on Confluence about each of the different steps as they are undertaken.

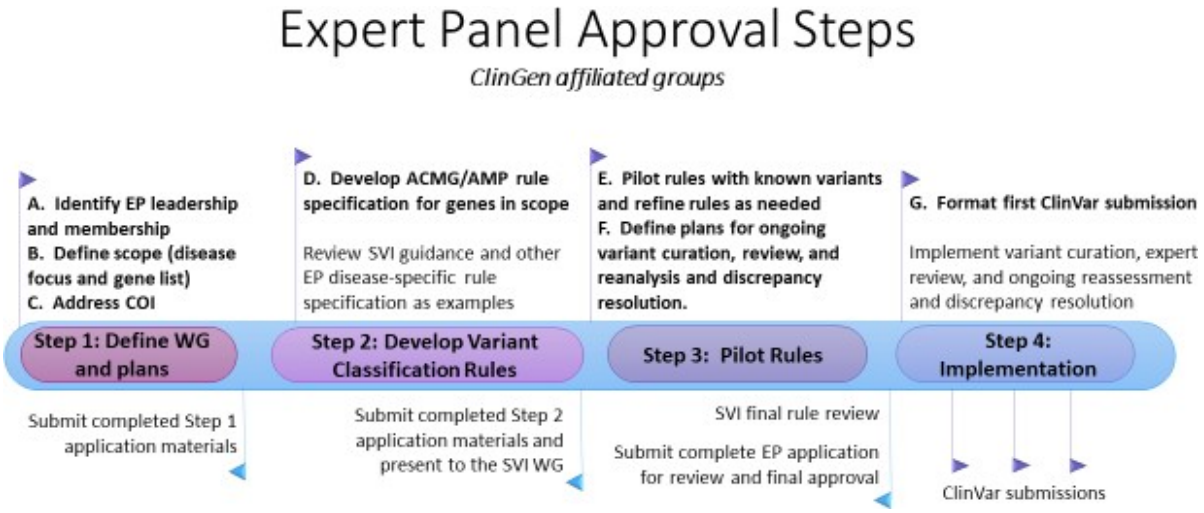

Fig. 1. Stepwise process for ClinGen Variant Curation Expert Panel application submission and review

Once approved by the Rule Specification Review Committee and the CDWG Oversight Committee, the full application document is made publicly available on the ClinVar Submitter page under "Expert panel documentation". See https://www.ncbi.nlm.nih.gov/clinvar/submitters/506439/ for an example. The newly approved ClinGen VCEP is encouraged to publish their gene/disease-specific ACMG/AMP criteria and validation in a peer-reviewed journal (see Data Sharing under section 2.3 below).

The final VCEP application submission packet (for Step 3) includes:

1. Composition of the group
2. Scope of work (genes/conditions)
3. Conflict of interest management
4. Detailed description of the specified ACMG/AMP criteria, including:
    a) Pathogenicity assertion criteria, and
    b) Description and explanation for any combinations of criteria and evidence sources that could be used to classify broad categories of variants (e.g. Benign or Likely Benign)
5. Validation of ACMG/AMP guideline specifications, including:

a) Evidence and rationale to support the rule specifications
b) Summary of validation process, including a list of the example variant set with evidence, assessment and asserted pathogenicity

6. Sample ClinVar submission data formatted appropriately
7. Indication of the method used for variant curation and review
   a) Select a ClinGen-approved methodology on the VCEP application form, or
   b) Describe an alternative method

1.3 Expert Panel Updates

All ClinGen Variant Curation Expert Panels are expected to submit an annual update form to the CDWG Oversight Committee (CDWG_OversightCommittee@clinicalgenome.org). These reports are due on May 1 of each year and should include a summary of the prior year's progress, plans for the coming year including any changes or addition to the scope of work and changes to the VCEP panel members. In addition, at any time during the year if there are significant changes such as changes in chairs, scope of work, or if the group is no longer able to follow the rules as written in the VCEP Protocol, the chair or coordinator should immediately notify the CDWG Oversight Committee of the situation.

2. Stepwise Process for VCEP Development

2.1 Step 1: Define the Expert Panel's composition and Scope of Work

Leadership and Member Recruitment

VCEP co-chairs and leadership should review this VCEP Protocol, the VCEP Application and the ACMG/AMP guidelines first. Then, with input from their parent Clinical Domain Working Group (CDWG), if applicable, VCEP leaders may nominate and invite members with appropriate expertise to accomplish their tasks (see section 1.1 Membership and Training). VCEPs should include members who have practical experience applying the ACMG/AMP guidelines. If the VCEP falls under a CDWG, the CDWG's Executive Committee should provide input on group composition (based on interactions within their professional societies, NIH institutes, etc.) The VCEP will establish and circulate expectations for their members in terms of attendance and accomplishments.

The CDWGs[1] will also provide guidance as needed for the creation, development, and direction of VCEPs within their domains (e.g. *PTEN* VCEP within the Hereditary Cancer CDWG). The overarching CDWG will lead the VCEP in focusing on specific areas and developing the policies and procedures necessary to accomplish certain tasks.

ClinGen VCEPs may also be formed outside of the current CDWGs if there is no relevant CDWG at the time; these VCEPs will receive guidance directly from the CDWG Oversight Committee.

Define Scope (disease focus and gene list)

In most cases, a VCEP will examine a single gene or a set of genes associated with a single condition or related conditions, and focus on the curation of variants in those genes (e.g., RASOpathy and specific genes: BRAF, HRAS, KRAS, MAP2K1, MAP2K2, PTON11, RAF1, SHOC2 and SOS1). We expect that a VCEP will begin with one or several of the most common or highly penetrant genes or gene families within their area of focus for variant curation and steadily enlarge the scope of the project over time.

[1] Milko L., Funke B., Hershberger R., *et al.* (2018). Development of Clinical Domain Working Groups for the Clinical Genome Resource (ClinGen): lessons learned and plans for the future. *Genetics in Medicine.*

Address Conflicts of Interest (COI)

VCEPs should be composed with COI in mind to ensure that there is a sufficient number of eligible expert reviewers across institutions to broaden perspective. ClinGen defines the following two types of COI:

I. Academic COI: Authors of literature about relevant variants may serve on the VCEP 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.

II. Financial COI: Commercial entities may participate on the VCEP 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.

- Curators should note potential conflicts of interest related to authorship for variants with limited evidence.
- Individual COI such as stock ownerships, patents, etc. are declared by authors in publications.
- VCEP coordinators will collect COI disclosures via the COI form (see appendix) and maintain a list along with the membership.
- VCEP members who are also ClinGen CDWG Oversight Committee members will not participate in the review of the VCEP application.

Completed Step 1 application materials should be submitted to the ClinGen CDWG Oversight Committee via (CDWG_OversightCommittee@clinicalgenome.org) for review in fulfillment of the requirements for Step 1.

2.2 Step 2: Develop Specified Variant Classification Rules

Develop ACMG/AMP rules specifications for genes in scope

Table 5 Rules for combining criteria to classify sequence variants

Pathogenic	(i) 1 Very strong (PVS1) *AND* (a) ≥1 Strong (PS1–PS4) *OR* (b) ≥2 Moderate (PM1–PM6) *OR* (c) 1 Moderate (PM1–PM6) and 1 supporting (PP1–PP5) *OR* (d) ≥2 Supporting (PP1–PP5) (ii) ≥2 Strong (PS1–PS4) *OR* (iii) 1 Strong (PS1–PS4) *AND* (a)≥3 Moderate (PM1–PM6) *OR* (b)2 Moderate (PM1–PM6) *AND* ≥2 Supporting (PP1–PP5) *OR* (c)1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
Likely pathogenic	(i) 1 Very strong (PVS1) *AND* 1 moderate (PM1–PM6) *OR* (ii) 1 Strong (PS1–PS4) *AND* 1–2 moderate (PM1–PM6) *OR* (iii) 1 Strong (PS1–PS4) *AND* ≥2 supporting (PP1–PP5) *OR* (iv) ≥3 Moderate (PM1–PM6) *OR* (v) 2 Moderate (PM1–PM6) *AND* ≥2 supporting (PP1–PP5) *OR* (vi) 1 Moderate (PM1–PM6) *AND* ≥4 supporting (PP1–PP5)
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)
Uncertain significance	(i) Other criteria shown above are not met OR (ii) the criteria for benign and pathogenic are contradictory

A primary task of the VCEPs is developing disease-gene specifications to the ACMG/AMP guidelines[2], and then determining how the specified rules are combined to classify sequence variants (as in Table 5 of Richards et al. 2015) according to the five Mendelian class criteria: pathogenic (P), likely pathogenic (LP), uncertain significance (VUS), likely benign (LB), and benign (B). Although most VCEPs use the general rules for combining criteria (as shown left), some VCEPS may modify them after obtaining approval from the VCI. An example is allowing a single strong piece of benign evidence to reach a Likely Benign classification. Any modifications to the rules for combining criteria must be included in the VCEP application along with the gene-disease specifications to the criteria and should be applied consistently.

In addition, VCEPs may make case-by-case variant-level decisions to override the calculated classification based on the full set of criteria. An example of this is in the case of VUSs with conflicting evidence. The VCEP may choose to discount certain pieces of evidence thereby allowing a P/LP/LB/B classification. The reasoning for these exceptions must be described in the relevant variant classification summary (see 2.4.D).

[2] Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., *et al.* & ACMG Laboratory Quality Assurance Committee. (2015). 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. *Genetics in Medicine.*

Sequence Variant Interpretation Working Group (SVI) Training and Guidance

To begin this process, the SVI provides an orientation to the ACMG/AMP guidelines and the approach to rule specification. This orientation includes presentation of rules that are broadly applicable across domains, such as applying statistical rigor to calculate allele frequency thresholds for benign classifications. The SVI will highlight other evidence categories where VCEPs are expected to develop unique rule specifications that require thorough literature searches and defining thresholds in the context of the gene/disease association.

SVI consults with and supports VCEPs to develop gene- and disease-specific refinements of the ACMG/AMP guidelines to increase the uniformity and consistency of the VCEP specifications across ClinGen. SVI general recommendations for using ACMG/AMP guidelines are publicly available at: https://www.clinicalgenome.org/svi and through publication. Topically focused presentations are available on request.

Variant Curation Interface (VCI) training

Representatives from the VCI development team and/or user group will provide an orientation to the VCEP. VCEP biocurators will receive further one-on-one, or small group training regarding the use of the VCI for variant curation as part of their proficiency training.

The VCI aggregates external evidence about variants and supports the manual curation of variant information. Supporting evidence is accessible to all VCI users. The data fields (tabs) within the VCI are defined by the evidence categories provided by the ACMG/AMP guidelines. Dynamic links to external information sources are also embedded within the relevant evidence category tabs.

External information sources are defined for each evidence category as outlined in the Richards et al. ACMG/AMP guidance document (see figure on next page) and the ClinGen Variant Curation SOP (currently under development), as well as additional resources identified as valid sources by the SVI. Evidence categories along with example sources are below.

Population, in-silico, and gene-centric evidence is aggregated and displayed for users.

- Population-based data (e.g. allele frequencies in various populations)
    - gnomAD, ExAC, PAGE, 1000 Genomes, Exome Sequencing Project and curated literature
- In silico prediction model data (e.g. evolutionary conservation, splicing predictors)
    - REVEL, SIFT, PolyPhen2, LRT, FATHMN, CADD, phyloP100way, GERP++, MaxEntScan, NNSPLICE
- Links to gene resources such as HGNC, Entrez Gene, Ensembl, UniProtKB and ClinVar
- Gene-centric clinical validity data
    - ExAC constraint scores

Experimental and case-level evidence is manually curated and entered by the user from published literature and other sources by the curator, as described in the ClinGen Variant Curation SOP (currently under development).

- Experimental data (e.g. animal model, tissue expression) Case-level data
    - Curated literature for functional domain and experimental evidence
- Case-level data

- Curated literature for case-control, healthy population observations
- Curated literature for segregation, cis-/trans-, de novo, and phenotypic specificity
- Curated non-published clinical data from laboratory testing)

Additional VCI training documents are publicly available for reference:

- Guidance for using the VCI is also available through a "Learn More" link on the interface homepage (https://curation.clinicalgenome.org/).
- Curation activities within the VCI are guided by the VCI Curation Help documents. These are available through the "Learn More" pages at this url https://github.com/ClinGen/clincoded/wiki/VCI-Curation-Help

In summary, all VCEPs receive VCEP-specific training and education along the following topics:

- ACMG/AMP criteria for variant interpretation
- ACMG/AMP criteria specification process, including education about which criteria are appropriate for a disease/gene-specific optimization process (e.g. establishment of allele frequency cut-offs)
- Variant curation and use of the VCI

Table 1 Population, disease-specific, and sequence databases

Population databases	
Exome Aggregation Consortium http://exac.broadinstitute.org/	Database of variants found during exome sequencing of 61,486 unrelated individuals sequenced as part of various disease-specific and population genetic studies. Pediatric disease subjects as well as related individuals were excluded.
Exome Variant Server http://evs.gs.washington.edu/EVS	Database of variants found during exome sequencing of several large cohorts of individuals of European and African American ancestry. Includes coverage data to inform the absence of variation.
1000 Genomes Project http://browser.1000genomes.org	Database of variants found during low-coverage and high-coverage genomic and targeted sequencing from 26 populations. Provides more diversity compared to the Exome Variant Server but also contains lower-quality data, and some cohorts contain related individuals.
dbSNP http://www.ncbi.nlm.nih.gov/snp	Database of short genetic variations (typically ≤50 bp) submitted from many sources. May lack details of the originating study and may contain pathogenic variants.
dbVar http://www.ncbi.nlm.nih.gov/dbvar	Database of structural variation (typically >50 bp) submitted from many sources.
Disease databases	
ClinVar http://www.ncbi.nlm.nih.gov/clinvar	Database of assertions about the clinical significance and phenotype relationship of human variations.
OMIM http://www.omim.org	Database of human genes and genetic conditions that also contains a representative sampling of disease-associated genetic variants.
Human Gene Mutation Database http://www.hgmd.org	Database of variant annotations published in the literature. Requires fee-based subscription to access much of the content.
Locus/disease/ethnic/other-specific databases	
Human Genome Variation Society http://www.hgvs.org/dblist/dblist.html Leiden Open Variation Database http://www.lovd.nl	The Human Genome Variation Society site developed a list of thousands of databases that provide variant annotations on specific subsets of human variation. A large percentage of databases are built in the Leiden Open Variation Database system.
DECIPHER http://decipher.sanger.ac.uk	A molecular cytogenetic database for clinicians and researchers linking genomic microarray data with phenotype using the Ensembl genome browser.
Sequence databases	
NCBI Genome http://www.ncbi.nlm.nih.gov/genome	Source of full human genome reference sequences.
RefSeqGene http://www.ncbi.nlm.nih.gov/refseq/rsg Locus Reference Genomic (LRG) http://www.lrg-sequence.org	Medically relevant gene reference sequence resource.
MitoMap http://www.mitomap.org/MITOMAP/HumanMitoSeq	Revised Cambridge reference sequence for human mitochondrial DNA.

Rules specification process

VCEPs may choose to proceed with rules specification using one or a combination of the following organizational methodologies.

- Approach 1: Subdividing the group and assigning a category from the guidelines to each subgroup and then each subgroup brings final decisions to the larger group for final approval through voting
- Approach 2: Discussing proposed changes and specifications to the guidelines within the larger panel and reaching consensus through voting.

ClinGen PI liaisons and coordinators will recommend when ClinGen VCEPs should request to join a Genomic Variant WG call with representatives from SVI via Danielle Azzariti (dazzarit@broadinstitute.org) to discuss difficult issues and for feedback and recommendations.

Completed Step 2 application materials, including draft criteria specifications should be submitted via email (dazzarit@broadinstitute.org)to the Rule Specification Review Committee of the SVI for review in partial fulfillment of the requirements for Step 2. **The VCEP also presents their draft specifications to the Rule Specification Review Committee of the SVI in fulfillment of Step 2.** The Review Committee will provide written feedback to the VCEP with a summary of recommendations to address prior to beginning the pilot of their specified rules. **After the VCEP responds in writing to any issues or recommendations raised by the Rule Specification Review Committee, a vote on Step 2 approval by the Rule Specification Review Committee is held. Approval is required prior to the start of the pilot process.**

2.3 Step 3: Pilot Specified Rules

Pilot rules with known variants and refine rules as needed

Apply specified variant classification rules to known variants for pilot testing and validation using the following criteria:

1. Use at least 10 to 12 Pathogenic/Likely Pathogenic variants, 10 to 12 Benign/Likely Benign variants and 10-12 Uncertain Significance variants or those with conflicting interpretations in ClinVar;
2. Use variants with a variety of different evidence types for the gene to test all relevant criteria codes;
3. Refine rule specifications based on test curations. All refinements should be discussed with SVI WG to determine if a formal revised ACMG/AMP criteria specifications should be resubmitted to the SVI WG.
4. All test variants must be curated on the final rules to confirm validation.

Rule Specification Review Committee

After completing the pilot, the VCEP submits its final specifications along with the variants used in the pilots and the results to the Rule Specification Review Committee via email (dazzarit@broadinstitute.org).

The Rule Specification Review Committee provides feedback through an interactive review process of any specific items that need to be addressed for approval. Communication continues until the Rule Specification Review Committee approves the VCEP rule specifications.

After the *Rule Specification Review Committee approves the final VCEP rule specifications, Completed Step 3 application materials should be submitted to the CDWG Oversight Committee (CDWG_OversightCommittee@clinicalgenome.org) for review in fulfillment of the requirements for Step 3* The Step 3 application must also be presented on a CDWG Oversight Committee call.

Approval from the CDWG Oversight Committee at this step signifies the group as a ClinGen VCEP. The newly approved ClinGen VCEP should format their first ClinVar submission, following the instructions outlined below and continue in preparation for submitting future variant classifications to ClinVar at the 3-star review level.

ClinGen Data Sharing Policy

All ClinGen-supported working groups must agree to disseminate their curation results via the ClinGen website and/or ClinVar immediately upon completion of expert review of each variant, gene, genomic region, or topic. Individual curation results cannot be held for publication. Data will be exported in either real time or on a regular basic (VCI to ClinVar). It is the responsibility of the author to confirm that, upon publication, the data he/she analyzed is available through ClinVar and/or ClinGen.

See below for information regarding ClinVar submission.

In addition, if the VCEP is planning to publish its rule specifications in a peer-reviewed journal, a copy of the paper must be provided to the SVI subcommittee with sufficient time for review before submission.

Rule specifications must also be posted on the ClinGen website in advance of paper submission so there are no copyright issues. A template for website posting is available from the SVI.

NHGRI Pre-print Policy

It is the expectation for all ClinGen-supported VCEPs that whenever possible, manuscripts will be pre-published on bioRxiv. If the authors do not anticipate submitting their manuscript to bioRxiv they must provide a written justification in their concept sheet.

3. ClinGen VCEP Implementation (Step 4) / On-going Curation

The following sections describe the processes for all ClinGen VCEPs for on-going variant assessments. VCEPs are expected to maintain ongoing curation and variant interpretation resulting in submissions to ClinVar at least once per year.

3.1 VCEP Variant Curation and Assessment

Selection of Variants

VCEPs are expected to maintain variant curation activities. Selection of variants for curation and evaluation will be made by consensus discussion by the VCEP membership. Variants are prioritized based on conflicting interpretations in ClinVar, frequent reports in current clinical databases such as ClinVar, variants that can be classified rapidly (predicted loss of function or those with high allele frequency), as well as those nominated by external parties.

Variant Curation and Preliminary Evaluation

Each VCEP will have defined their ongoing approach for variant curation review in their VCEP application. Most VCEPs will select to use one of the standard ClinGen approved methods as listed below. Otherwise another method must be described in detail in the VCEP application submission materials. All approaches must contain at least two reviewers.

Standard ClinGen Approved Method (provided for selection in the VCEP application):

- One curator performs biocuration, entering data into the VCI, and presents directly to the full EP for review and consensus classification.

The primary curator(s) will enter and review variant information for each evidence category in the VCI. The curator will utilize the VCEP-specified ACMG/AMP criteria to determine which of the ACMG/AMP rule codes to apply. Whenever a rule is evaluated, the evidence and/or reason for applying or not a particular evidence code should be entered. These evaluation statements should include reference to the supporting data including PMIDs as applicable.

The VCI programmatically aggregates all of the applied codes and calculates a tentative variant classification of pathogenicity based on the combining criteria outlined in Table 5 from the Richards et al. ACMG/AMP variant interpretation paper. The curator will either select the calculated classification or select a different pathogenicity classification to present to the VCEP for review. The curator will provide sufficient statements to support the selected classification, including a rationale if not applying the calculated classification. See Variant Evidence Summary 2.4.D below.

Final Variant Review and Assertion of Pathogenicity Classification (Approval)

Final decisions regarding variant classification are made by consensus of the VCEP membership. Consensus is achieved if there is either unanimous agreement by all members or if there is not unanimous agreement, there is majority vote in favor. Variant classifications require at least a majority vote (individual VCEPs may have higher quorum requirements) to be published as an approved classification decision.

If a majority vote is not obtained, the variant will either be considered an unclassified variant or VCEPs may decide to err on the side of conservativeness in these situations and classify using the more conservative class (e.g. agree that a variant will be considered VUS if a majority vote is not obtained for a LP or LB classification). “Unclassified variants” should be re-evaluated every 2 years to determine if additional evidence is available to support classification.

Final approval by the VCEP is required for all variant classifications. The approval of the classification is recorded in the VCI and the variant record is labeled as “Approved.”

Variant Evidence Summary

To promote understanding and enable external review, all finalized/approved variant classifications must be accompanied by brief, yet comprehensive evidence summaries. These evidence summaries will include the pathogenicity classification, the evidence category codes applied and the reasons for applying each code. These statements should include, where applicable, reference to external data sources such as PMIDs, ClinVar SCVs, gnomAD, etc.

These evidence summary statements should be provided by the VCEP within the summary evidence tab in the VCI. These summaries will be accessible within publicly accessible ClinGen databases such as the Evidence Repository.

Elements to include with a variant evidence summary include:

- The condition for which the variant is being assessed. This is generally defined in the scope of work of the VCEP. Of note, some variants may have evidence such as very high population allele frequencies that support the conclusion that they are benign for any possible monogenic condition associated with that gene. This additional information could be noted in the summary where applicable.

- Each evidence code applied. The evidence codes and the VCEP's modified rules may not be well known. Thus, VCEPs should include brief descriptions to support why a particular evidence code was applied. These can be initially entered during curation and review on the individual Evidence Tabs in the VCI. Reference supporting data with PMIDs, SCVs, or other indicators. These should be included within the final summary statement.

- Highlight exceptions. Statement(s) should be added to describe the instance when a piece of evidence or evidence category is "overruled" when applying or combining evidence codes. The reason for the exception should be clear and support the final conclusion regarding pathogenicity classification. For subjective pieces of evidence (e.g. the validity of a functional assay), this can be done by not applying the code and noting the reason it was not applied in the VCI, or, for objective pieces of evidence (e.g. allele frequency meets a threshold defined by the VCEP) where the code is automatically applied, describing in the final evidence summary why the evidence is not being considered (e.g. reduced penetrance variant). Another example is the case of an autosomal dominant condition with the reviewed variant has been reported to occur along with a second P/LP variant, but the VCEP chooses not to apply the benign code when calculating the final pathogenicity.

Example of Summary Description with exception highlighted:

The c.2167C>T (p.Arg723Cys) variant in MYH7 has been reported in >20 individuals with hypertrophic cardiomyopathy (PS4; PMID:1430197; PMID:27532257; PMID:9829907; PMID:16199542; PMID:20359594; PMID:12707239; ClinVar SCV000059423.5; ClinVar SCV000212630.1). Five of these probands carried additional variants in sarcomere genes (BP2; PMID:20359594; PMID:12707239; ClinVar SCV000059423.5). This variant has been identified as a de novo occurrence in 1 proband with hypertrophic cardiomyopathy (PM6; PMID:1430197). This variant segregated with disease in 7 affected individuals (PP1_Strong; PMID:9829907; ClinVar SCV000059423.5; ClinVar SCV000212630.1). This variant was identified in 2/66738 European chromosomes (PM2; http://exac.broadinstitute.org). This variant lies in the head region of the protein (aa 181-937) and missense variants in this region are statistically more likely to be disease-associated (PM1; PMID:27532257). Computational prediction tools and conservation analysis suggest that this variant may impact the protein (PP3). A different pathogenic missense variant has been previously identified at this codon which may indicate that this residue is critical to the function of the protein (PM5; c.2167C>G p.Arg723Gly - ClinVar Variation ID 42885). In summary, this variant meets criteria to be classified as pathogenic for hypertrophic cardiomyopathy in an autosomal dominant manner. The benign evidence code BP2 was not considered to be in conflict with this conclusion given that presence of a second variant can be seen in individuals with cardiomyopathy and may contribute to the severity of disease. MYH7-specific ACMG/AMP criteria applied (PMID:29300372): PS4; PP1_Strong; PM1; PM2; PM5; PM6; PP3; BP2

Format for submission to ClinVar

The VCEP is responsible for coordinating the ClinVar submission process. All variants curated, evaluated, summarized, and assigned a final pathogenicity assertion during the validation process are submitted to ClinVar. This process is initiated by the VCEP once a set of variant classifications has been approved within the VCI. The VCEP leadership alerts the VCI team and the Data Exchange team which triggers a data flow process of the variant classification, summary statement and curation evidence out of the VCI and into a ClinVar submission format. The ClinVar submission data is reviewed by the VCEP for accuracy against the variant records in the VCI. Once approved, the VCEP is responsible for submitting the formatted data to ClinVar.

- Submission instructions can be found at: https://www.ncbi.nlm.nih.gov/clinvar/docs/submit/
- The ClinVar variant submission template is available on ClinVar's FTP site ftp://ftp.ncbi.nlm.nih.gov/pub/clinvar/submission_templates/

VCEPs are expected to maintain ongoing curation and variant interpretation resulting in submissions to ClinVar at least once per year.

3.2 Public Access and Inquiry

Public access to variant classifications and evidence via ClinVar submission provides a means for encouraging inquiries and public comments regarding the evidence and rationale for classifications. To support the receipt of comments and inquiries, the contact information for each specific VCEP is made public on the ClinVar Submitter page. Users can quickly navigate to this contact information within ClinVar following embedded links from the variant assertion page.

Within ClinGen, each final, approved VCEP variant classification along with the curated variant-level evidence is available for external users review via public database such as the Evidence Repository. The classifications are linked to the individual VCEP web pages within ClinGen to aid inquiry. In addition, a mechanism to receive public feedback is available via a comment box present throughout the ClinGen website.

3.3 Reanalysis and Discrepancy Resolution

VCEPs are expected to keep their variant interpretations up-to-date and to expedite the reassessment of variants that have a conflicting assertion submitted to ClinVar after the VCEPs variant submission (see Table 1 below).

	Expert Panel Interpretation Involving a Discrepancy with a 'Single Submitter, Criteria Provided' SCV after EP submission or within the year prior				
Scenario	Medically Significant Difference P/LP vs. VUS/LB/B			VUS vs. LB/B Confidence Difference	
Reanalysis	Contact other submitter within 3 months, update as needed within 6 months			Within 2 yr	
	If a discrepancy is not reanalyzed in this time frame, the SCV will be dropped to "Single Submitter, Assertion criteria provided" status				
	Expert Panel Interpretation, No Discrepancy				
Scenario	P	LP	VUS	LB	B
Reanalysis	Not required	2y	2y	*	Not required
	If the Expert Panel does not maintain their interpretations in this timeframe, the Expert Panel may lose Expert Panel status and SCVs may be dropped to 'Single submitter, criteria provided' status				

*Review MAFs when new large population datasets are released

Table 1: VCEP Discrepancy resolution and resolution process

Medically Significant Discrepancy

VCEPs should expedite the reassessment of variants that have a conflicting assertion submitted to ClinVar that have a "one star" submitter level. The one star level in ClinVar corresponds to the submitter having provided some information with regard to variant classification criteria.

For discrepancies involving a medically significant assertion, P/LP vs VUS/LB/B, made by a one star submitter or above, VCEPs are expected to contact the other submitter within 3 months of being notified about the conflict. The VCEP will first contact the submitter of the discrepant classification based on the submitter details available in ClinVar. If the contact information is not correct in ClinVar, a reasonable effort will be made to contact the submitter. At least 3 attempts will be made to contact the submitter. If the submitter is not able to be contacted or does not reply to contact efforts after 1 month, the reassessment cannot proceed.

If the external submitter does provide criteria and evidence in support of the discrepant classification, then the VCEP and SVI will review the criteria for alignment with current standard practice outlined by Richards et al and specified by the VCEP. The VCEP will review the potentially new evidence in light of the evidence previously curated for the variant by the VCEP. Similarly, the VCEP will provide the evidence and criteria used by the VCEP to support the VCEP's classification to the external submitter.

Following re-evaluation, the VCEP and SVI will discuss with the external submitter their conclusions regarding the variant classification.

If a VCEP variant classification is revised from the original, the new VCEP classification will be submitted to ClinVar within 1 month of the new final classification. The updated ClinVar entry should be submitted within 6 months from the original discrepancy notification.

If the submitter of the discrepant classification does not provide the variant classification criteria to support their classification, ClinVar will be alerted to determine if the one star submitter level should be removed given the requirement to furnish evidence for classifications to be granted one star status.

Routine Reassessment

VCEPs are expected to support inquiries from ClinVar users to clarify evidence and rationale for classification as well as consideration of new evidence. Further, a mechanism to receive public feedback is available via a comment box present on every page of the ClinGen website.

VCEPs are expected to reassess all their LP and VUS classifications at least every 2 years and LB classifications when new large population datasets are released to see if new evidence has emerged to re-classify the variants.

If the VCEP does not maintain their interpretations in this timeframe, they may lose ClinGen Variant Curation Expert Panel status. If "VCEP status" is lost, ClinVar will be immediately notified of the change. ClinVar entries will be updated to reflect the name change as ClinGen VCEP will not apply. In addition, ClinVar will be notified that the variant classifications are not provided by a recognize Expert Panel and SCVs may be dropped to 'Single submitter, criteria provided' status.

Review of specified ACMG/AMP Criteria

The VCEP will undergo a review of their specified ACMG/AMP criteria every 2 years or as appropriate based on new gene-specific knowledge or SVI work group criteria guidance. This process will include consideration of any new guidance put forth by the SVI as well as updates to membership and scope, and in scientific and clinical knowledge about the characteristics of the particular disease/gene(s) group.

If the VCEP believes that a change/revision to the current approved specified ACMG/AMP criteria is needed, that change, and the justification must first be presented to the SVI and CDWG Oversight committee. This can occur at a regular SVI meeting or via an ad hoc meeting called for that purpose. The SVI and CDWG will provide guidance on whether to proceed with the proposed rule change during that meeting.

Any rule change must be validated based on an appropriate test set of variants. The results of this change validation should be presented to the SVI for approval.

- If the rule change would lead to a variant moving from a more certain state to a less certain state (e.g. LP to VUS or LB to VUS) then all previously curated variants would be queried to

determine if they are impacted by the rule change. All variants that are impacted would be re-curated based on the rule change and assessed for possible change in classification.

- If the rule change would lead to movement to a more certain state (VUS to LP or LB), then these changes may await routine re-evaluation as described in 3.2 Table 1.

Recurated and re-assessed variants will be resubmitted to ClinVar as an update. VCEPs are encouraged to resubmit the re-assessed variant classifications to ClinVar within 1 month of the re-evaluation or as soon as reasonably possible based on the number of impacted variants from approval of the rule change.

Revised rules will be published on the VCEP webpage on ClinGen as well as on the VCEP submitter page on ClinVar. Such changes would be announced as appropriate at professional conferences to aid in communication to relevant audiences.