ClinGen Variant Curation Expert Panel Application

Submitter Information	
Full Base Name of Expert Panel: Malignant Hyperthermia Susceptibility	
Short Base Name of Expert Panel (≤15 characters): MHS	
Expert Panel Coordinator: Jennifer Johnston	
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Expert Panel Member Responsible for ClinVar Submission: Jennifer Johnston	
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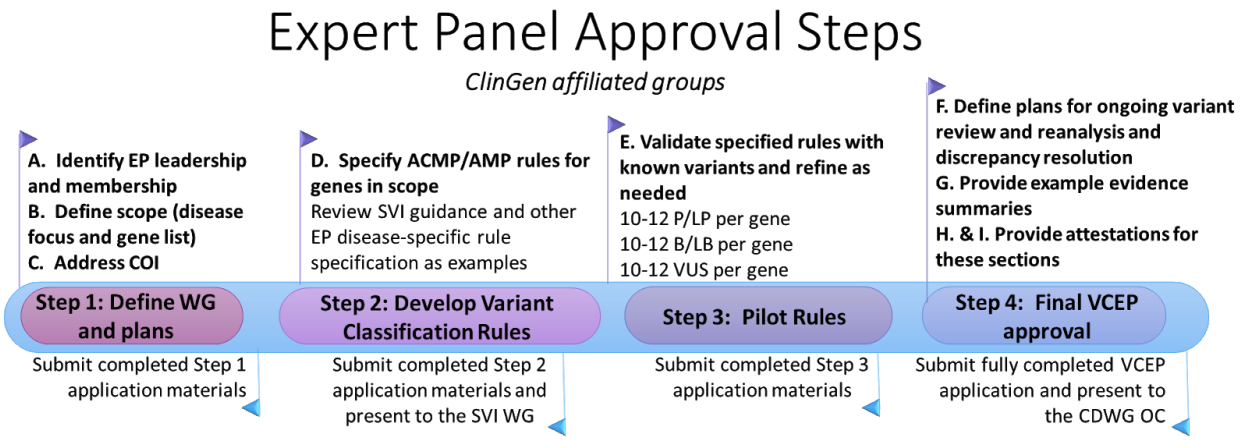
ClinGen Variant Curation Expert Panels (VCEPs) must fulfill the stepwise requirements in accordance with the diagram shown below to become an approved ClinGen VCEP with the resulting classified variants meeting FDA recognition. Refer to the ClinGen VCEP Protocol which can be found at https://clinicalgenome.org/site/assets/files/3263/vcep_protocol_v_8.pdf for detailed guidance on completing the ClinGen VCEP application and approval process. Additional pages may be added for all Sections A-I, if needed.

External VCEP applicants (not subject to FDA requirements but wishing to obtain ClinVar 3-star status) are also encouraged to use the same stepwise process for their VCEP application. We encourage these groups to begin communication early in the application process (prior to submitting Step 1) with the Sequence Variant Interpretation WG (SVI) and Clinical Domain Working Group (CDWG) Oversight Committee (OC). All VCEP applicants are required to submit for final VCEP approval by the CDWG OC.



Expert Panel Submission Details

A Composition of the Expert Panel (STEP 1)

Expert Panels are expected to represent the diversity of expertise in the field and should refer to Section 2.1 of the VCEP Protocol for guidance to complete the Member List below. Please list the VCEP Chair(s) and Coordinator(s) first.
*The column on the far right should be completed in Step 4 prior to the final approval presentation to the CDWG OC. Refer to Section 2.4 of the VCEP Protocol for a definition of core approval members.

Member List

Name, credentials, and email	Institution	Area and Type of Expertise	VCEP role	Indicate Step 4 core approval members*
Joanna Doe, PhD, FACMG jsmith@clingen.edu	ClinGen University	Clinical Molecular Geneticist; Inherited Cardiovascular Disorders	Chair	☐
Leslie Biesecker, MD, lesb@mail.nih.gov	NHGRI NIH	Genomics specialist	Co-chair Scientific lead Expert Reviewer	X

Henry Rosenberg, MD, henryrosenbergmd@gmail.com	St Barnabas Med Ctr, Livingston, NJ & MH Assoc. of US	Anesthesiologist, MH Association of United States, president	Co-chair	☐
Jennifer Johnston, PhD, jjohnsto@mail.nih.gov	NHGRI NIH	Clinical Molecular Geneticist, CLIA director, ABMGG board certified	Coordinator Biocurator Biocurator trainer	X
Robert Dirksen, PhD, Robert_Dirksen@URMC.Rochester.edu	Univ Rochester Med Sch, Dept Pharmacol & Physiol	Pharmacologist, Disease expert	Expert Reviewer	☐
Steven Gonsalves, PhD, Stephen.Gonsalves@hhs.gov	NHGRI NIH	Genomics specialist	Primary biocurator	☐
Sheila Riazi, MD, Sheila.Riazi@uhn.ca	Univ Toronto, Dept Anesthesiology	Anesthesiologist, Director Malignant Hyperthermia Investigation Unit, MH Association of United States, board member	Expert Reviewer	☐
Louis A. Saddic, III MD, lousaddic@gmail.com	Department of Anesthesiology, University of California Los Angeles	Anesthesiologist, Disease expert	ASA representative	☐
Nyamkhishig Sambuughin, PhD, nyamkhishig.sambuughin.ctr@usuhs.edu	Uniformed Svcs Univ Health Sci, Consortium for Health and Military Performance	Geneticist, Disease expert	Expert Reviewer	☐
Richa Saxena, PhD, SAXENA@molbio.mgh.harvard.edu	Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School	Physiologist, Disease expert	Expert Reviewer ASA representative	☐
James Weber, PhD, jim.weber@preventiongenetics.com	Prevention Genetics, Marshfield, WI	Clinical Molecular Geneticist, CEO Prevention Genetics	Expert Reviewer	X
Thierry Girard, MD, Thierry.Girard@usb.ch	University of Basel, Anesthesiology	Anesthesiologist, Member European Malignant Hyperthermia Group	Expert Reviewer	☐
Kathryn Stowell, PhD, K.M.Stowell@massey.ac.nz	School of Fundamental Sciences, Massey University, Palmerston North, New Zealand	Biochemist, Member European Malignant Hyperthermia Group	Expert Reviewer	☐

Phil Hopkins, MD, P.M.Hopkins@leeds.ac.uk	MH Unit, Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK	Anesthesiologist, Member European Malignant Hyperthermia Group	Expert Reviewer	☐
Rachel Robinson, PhD rachell.robinson@nhs.net	MH Unit, Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK	Molecular Geneticist, Variant curation expert	Biocurator Expert Reviewer	X
Sarah Shepherd, BSc sarah.shepherd14@nhs.net	MH Unit, Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK	Registered Clinical Scientist, Variant curation expert	Biocurator	X
Natalia Kraeva, PhD natalia.kraeva@uhn.ca	Univ Toronto, Dept Anesthesiology	Molecular Geneticist, Variant curation expert	Biocurator Expert Reviewer	X
Mungunsukh Ognoon, PhD mungunsukh.ognoon.ctr@usuhs.edu	Uniformed Svcs Univ Health Sci, Consortium for Health and Military Performance	Molecular Geneticist	Biocurator	☐

Describe the expertise of VCEP members who regularly use the ACMG/AMP guidelines to classify variants and/or review variants during clinical laboratory case sign-out**.**

Jim Weber is the CEO of Prevention Genetics and uses the ACMG/AMP criteria at Prevention. Jennifer Johnston runs a CLIA laboratory where she routinely uses the ACMG/AMP criteria for variant analysis. Leslie Biesecker is an expert in the ACMG/AMP criteria and is regularly involved in case conferences discussing variant analysis. Sarah Shepherd, Rachell Robinson, and Natalia Kraeva are highly familiar with clinical *RYR1* variant analysis and are familiar with the ACMG/AMP criteria. Natalia Kraeva also directs a clinical lab.

B. Scope of Work

For this section please define and list: 1) any specific rationale for choosing the condition or related conditions and/or the gene(s) of interest; 2) the specific gene or set of genes on which the VCEP is requesting approval to initiate work (each gene should have a Strong or Definitive disease association); 3) optional inclusion of future plans, possibly including an expanded list of genes, for the VCEP (requires an updated application before pursuing).

Refer to Section 2.1 of the VCEP Protocol for guidance and the established VCEP webpages on clinicalgenome.org for examples.

Our current effort is focused on assessing variants in *RYR1* that have been associated with malignant hyperthermia susceptibility (MHS). Future work will include variant assessment in *CACNA1S*.

C. Conflict of Interest (COI) and Competing Activities Management

The ClinGen Expert Panel Conflict of Interest Policy can be viewed here: https://www.clinicalgenome.org/docs/clingen-expert-panel-conflict-of-interest-policy/. Refer to Section 2.1 of the VCEP Protocol for additional information.

Contact *CDWG_OversightCommittee@clinicalgenome.org* to create a COI SurveyMonkey for your EP and to access results before submission of the Step 1 application.

☑ Check the box to attest that each member has completed a COI survey and an Excel file of results has been included with this application.

End of Step 1 VCEP application

Stop here and submit completed Step 1 application materials to (CDWG_OversightCommittee@clinicalgenome.org) for review in fulfillment of the requirements for Step 1.

Note: After Step 1 approval, you will be contacted to set up an affiliation in the Variant Curation Interface and an VCEP webpage on clinicalgenome.org. At this time, you will be ready to begin the ACMG/AMP specification process

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D. ACMG/AMP Guideline Specifications (STEP 2)

ClinGen Expert Panels are required to use the ACMG/AMP variant assessment criteria as their starting point for a framework to classify variants into the five categories (pathogenic, likely pathogenic, uncertain significance, likely benign, and benign).

Follow the detailed instructions and the Step 2 checklist found in Section 2.2 of the VCEP Protocol to draft your ACMG/AMP rule specifications for the genes/disease pairs within your scope of work and present them to representatives from the Sequence Variant Interpretation WG (SVI) VCEP Review Committee.

See attached manuscript. Supplemental Information includes gene-optimized rules for variant classification for *RYR1*.

We will be using the Bayesian framework for combining criteria as explained in Tavtigian et al. This is discussed in the manuscript.

End of Step 2 VCEP application

Stop here and submit completed Step 2 application materials to (CDWG_OversightCommittee@clinicalgenome.org) for review in fulfillment of the requirements for Step 2.

Note: The SVI VCEP Review Committee provides written feedback to the VCEP with a summary of recommendations to address prior to beginning the pilot. The VCEP responds in writing to the SVI VCEP Review Committee points. Finally, the SVI co-chairs approve the VCEP to move on to Step 3 and piloting the specified rules once all feedback has been addressed.

E. Validation of ACMG/AMP Guideline Specifications (STEP 3)

Apply specified variant classification rules to known variants for pilot testing and validation and submit pilot results and final, refined specifications for review following detailed instructions in Section 2.3 of the VCEP Protocol.

Note that VCEPs are required to use the ClinGen Variant Curation Interface (VCI) according to the detailed ClinGen General Sequence Variant Curation Process Standard Operating Procedures, though tracking your pilot variant classifications in a spreadsheet for ease of submission and review is recommended. A template spreadsheet with sample data can be found here.

Forty-four variants in *RYR1* considered "diagnostic mutations" by the European Malignant Hyperthermia Group (EMHG) were used to pilot draft rules. Rules were then applied to a further 40 variants with a range of pathogenicity assessments. The full VCEP discussed the classification of variants. This is included in the manuscript.

The following variants were classified in the VCI (this is a subset of classified variants, see manuscript for additional variants). These variants are currently in progress and are waiting on SVI to allow for finalizing assessments.

NM_000540.3(RYR1):c.103T>C p.(Cys35Arg)

NM_000540.3RYR1):c.488G>T p.(Arg163Leu)

NM_000540.3(RYR1):c.487C>T p.(Arg163Cys)

NM_000540.3(RYR1):c.130C>T p.(Arg44Cys)

NM_000540.3(RYR1):c.742G>C p.(Gly248Arg)

(See attached summaries)

End of Step 3 VCEP application

Stop here and submit completed Step 3 VCEP application materials to CDWG_OversightCommittee@clinicalgenome.org for review in fulfillment of the requirements for Step 3.

Note: The SVI VCEP Review Committee reviews the updated specifications and pilot results. The SVI VCEP Review Committee may request additional information on pilot variants. The VCEP should respond in writing to any SVI VCEP Review Committee points. Finally, the SVI VCEP Review Committee approves the VCEP's specifications and the VCEP can move on to Step 4.

F. Define Plans for Ongoing Variant Review and Reanalysis and Discrepancy Resolution (STEP 4)

VCEPs are expected to define and present their plans for sustained variant curation and review (after Step 4 approval), including work schedules, standard review process in Part I and reanalysis and discrepancy resolution in Part II. A detailed description of ClinGen-approved processes are outlined in Section 2.4 of the VCEP Protocol.

Part I: Ongoing Variant Curation and Review:

- **Meeting/call frequency:** Monthly calls.
- **VCEP Standardized Review Process:** (check one)
  - ☐ Process #1: Biocurator review followed by VCEP discussion
  - ☑ Process #2: Paired biocurator/expert review followed by expedited VCEP approval

For all variants approved by either of the processes described above, a summary of approved variants should be sent to ensure that any members absent from a call have an opportunity to review each variant. The summary should be emailed to the full VCEP after the call and should summarize decisions that were made and invite feedback within a week.

Part II: Reanalysis and Discrepancy Resolution:

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. Please check all 3 boxes below to attest that the VCEP will follow the ClinGen-approved schedule described below -or- describe other plans at the bottom of the section.

☑ VCEPs are expected to reassess any newly submitted conflicting assertion in ClinVar from a one star submitter or above and attempt to resolve or address the conflict within 6 months of being notified about the conflict from ClinGen. Please reach out to the submitter if you need additional information about the conflicting assertion.

☑ VCEPs are expected to re-review all LP and VUS classifications made by the EP at least every 2 years to see if new evidence has emerged to re-classify the variants

☑ VCEPs are expected to re-review any LB classifications when new evidence is available or when requested by the public via the ClinGen website.

☐ Check box if plans differ from the expectations above, and describe below:

G. Example Evidence Summaries

Provide at least 5 written evidence summaries that represent examples of the content that will be submitted to ClinVar to support variant classifications as described in Section 2.4 of the VCEP Protocol.

NM_000540.3(RYR1):c.103T>C; p.(Cys35Arg)

This sequence variant predicts a substitution of cysteine with arginine at codon 35 of the RYR1 protein, p.(Cys35Arg). The maximum allele frequency for this variant among the six major gnomAD populations is NFE: 0.000009, a frequency consistent with pathogenicity for MHS. This variant has been reported in 3 unrelated individuals who have a personal or family history of a malignant hyperthermia reaction and positive *in vitro* contracture test (IVCT) or caffeine halothane contracture test (CHCT) result (when the proband was unavailable for testing a positive diagnostic test result in a mutation positive relative was counted) PS4_Moderate(PMID:17710899, PMID:20681998, PMID:9066328). Functional studies in HEK293 cells show conflicting data regarding sensitivity to RYR1 agonists (PMID:9334205, PMID:26115329). This variant resides in a region of RYR1 considered to be a hotspot for pathogenic variants that contribute to MHS, PM1 (PMID: 21118704). This variant segregates with MHS in 11 individuals, PP1_Strong (PMID:9066328). A REVEL score > 0.85 supports pathogenicity, PP3_Moderate. Criteria implemented: PS4_Moderate, PM1, PP3_Moderate, PP1_Strong. Based on using Bayes to combine criteria this variant is assessed as Pathogenic, (PMID: 29300386).

NM_000540.3RYR1):c.488G>T; p.(Arg163Leu)

This sequence variant predicts a substitution of arginine with leucine at codon 163 of the RYR1 protein p.(Arg163Leu). This variant is absent from a large population databases (gnomAD). This variant has been reported in three unrelated individuals who have a personal or family history of a malignant hyperthermia reaction, two of these individuals had a positive *in vitro* contracture test (IVCT) or caffeine halothane contracture test (CHCT) result (when the proband was unavailable for testing a positive diagnostic test result in a mutation positive relative was counted), PS4_Moderate (PMID:30236257, PMID:16163667). Functional studies in HEK293 cells show an increased sensitivity to RYR1 agonists, PS3_Moderate (PMID:16163667, PMID:26115329). This variant resides in a region of RYR1 considered to be a hotspot for pathogenic variants that contribute to MHS, PM1 (PMID: 21118704). Another variant assessed as pathogenic occurs at this codon, p.(Arg163Cys), however PM5 was not applied as Arg→Cys is predicted to be more disruptive based on a higher Grantham score as compared to Arg→Leu. A REVEL score > 0.85 supports pathogenicity PP3_Moderate. Criteria implemented: PS4_Moderate, PS3_Moderate, PM1, PP3_Moderate. Based on using Bayes to combine criteria this variant is assessed as Likely Pathogenic, (PMID: 29300386).

NM_000540.3(RYR1):c.487C>T; p.(Arg163Cys)

This sequence variant predicts a substitution of arginine with cysteine at codon 163 of the RYR1 protein p.(Arg163Cys). This variant is not present in a large population databases (gnomAD). This variant has been reported in over 40 unrelated individuals who have a personal or family history of a malignant hyperthermia reaction and a positive *in vitro* contracture test (IVCT) or caffeine halothane contracture test (CHCT) result (when the proband was unavailable for testing a positive diagnostic test result in a mutation positive relative was counted) PS4 (PMID:30236257, PMID:12151923, PMID:16163667, PMID:8220423, PMID:11575529, PMID:12059893, PMID:23558838 and others). Functional studies in HEK293 cells show an increased sensitivity to RYR1 agonists. A knock-in mouse model supports pathogenicity of this variant demonstrating a malignant hyperthermia reaction in response to agonist, as well ex vivo studies show increased response to agonist with increased calcium release, PS3 (PMID:20461000, PMID:9334205, PMID:17122579). This variant resides in a region of RYR1 considered to be a hotspot for pathogenic variants that contribute to MHS, PM1 (PMID: 21118704). This variant segregates with MHS in 5 individuals, PP1_Moderate (PMID:12059893, PMID:7889656). A REVEL score > 0.85 supports pathogenicity, PP3_Moderate. Criteria implemented: PS4, PS3, PM1, PP1_Moderate, PP3_Moderate. This variant has been classified as Pathogenic.

NM_000540.3(RYR1):c.130C>T; p.(Arg44Cys)

This sequence variant predicts a substitution of cysteine at codon 44 of the RYR1 protein, p.(Arg44Cys). The maximum allele frequency for this variant among the six major gnomAD populations is AMR: 0.000029, a frequency consistent with pathogenicity for MHS. This variant has been reported in 2 unrelated individuals who have a personal or family history of a malignant hyperthermia reaction and a positive *in vitro* contracture test (IVCT) or caffeine halothane contracture test (CHCT) result (when the proband was unavailable for testing a positive diagnostic test result in a mutation positive relative was counted), PS4_Moderate (PMID:12709367, PMID:24433488). Functional studies in HEK293 cells show an increased sensitivity to RYR1 agonists, PS3_Moderate (PMID:23459219). This variant resides in a region of RYR1 considered to be a hotspot for pathogenic variants that contribute to MHS, PM1 (PMID: 21118704). A REVEL score > 0.85 supports pathogenicity, PP3_Moderate. Criteria implemented PS4_Moderate, PS3_Moderate, PM1, PP3_Moderate. Based on using Bayes to combine criteria this variant is assessed as Likely Pathogenic, (PMID: 29300386).

NM_000540.3(RYR1):c.742G>C; p.(Gly248Arg)

This sequence variant predicts a substitution of glycine with arginine at codon 248 of the RYR1 protein c.742G>C; p.(Gly248Arg). The maximum allele frequency for this variant among the six major gnomAD populations is EAS: 0.00005), a frequency consistent with pathogenicity for MHS. This variant has been reported in four unrelated individuals who have a personal or family history of a malignant hyperthermia reaction and a positive *in vitro* contracture test (IVCT) or caffeine halothane contracture test (CHCT) result (when the proband was unavailable for testing a positive diagnostic test result in a mutation positive relative was counted), PS4_Moderate (PMID:30236257, PMID:19346234). Functional studies in HEK293 cells show an increased sensitivity to RYR1 agonists, PS3_Moderate (PMID:26115329,

PMID:27857962). This variant resides in a region of RYR1 considered to be a hotspot for pathogenic variants that contribute to MHS, PM1 (PMID: 21118704). Another variant assessed as pathogenic occurs at this codon, c.742G>A; p.(Gly248Arg), PS1. A REVEL score > 0.85 supports pathogenicity, PP3_Moderate. Criteria implemented: PS4_Moderate, PS3_Moderate, PM1, PS1, PS3_Moderate. Based on using Bayes to combine criteria this variant is assessed as Pathogenic, (PMID: 29300386).

H. Designation of Biocurators, Biocurator Trainer(s) and Core Approval Members

Trained variant biocurators (*list below*)

The following VCEP members will be designated biocurators following Step 4 approval and have completed Level 1 and Level 2 training, filled out an attestation, and are enrolled in the ClinGen Community Curation Database:

Jennifer Johnston

Mungunsukh Ognoon

Natalia Kraeva

In training process:

Stephen Gonsalves

Rachell Robinson

Sarah Shepherd

Biocurator Trainer(s) (*list below*)

The following VCEP members will be the designated biocurator trainer(s) following Step 4 approval:

Jennifer Johnston

Core Approval Members (*check boxes in Step 1: Section A*)

Prior to submitting the Step 4 application, please return to Section A. “Composition of the Expert Panel” and designate via the checkboxes which VCEP members will serve as core approval members for ongoing final approval of variant classifications following Step 4 approval.

I. NHGRI Data Availability

Curated variants and genes are expected to be approved and posted for the community as soon as possible as described in Section 2.4 of the VCEP Protocol. Note that upon approval, a VCEP must finalize their set of variants for upload to the ClinGen Evidence Repository within 30 days.

☑ Check box to confirm your understanding that once a variant is approved in the VCI it will become publicly available in the Evidence Repository. They should not be held for publication.

Please review the ClinGen Publication Policy. It is expected that whenever possible, Expert Panel manuscripts will be submitted preprints to a preprint server (e.g. bioRxiv or medRxiv).

☑ Check box to confirm plans to submit preprints or provide a written justification for not posting pre-print.

End of Step 4 VCEP application

Stop here and submit completed Step 4 VCEP application materials to CDWG_OversightCommittee@clinicalgenome.org for review in fulfillment of the requirements for Step 4

Note: Fully completed VCEP applications (Section A and Sections F-I) must be presented for Step 4 final approval to the CDWG OC. If possible, contact CDWG_OversightCommittee@clinicalgenome.org with at least two months' notice for scheduling to avoid delays. Send the fully completed VCEP application materials (including Steps 1-4 of the application) at least two weeks prior to the call for circulation to the OC.