Criteria Specification

ClinGen ENIGMA BRCA1 and BRCA2 Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for BRCA2 Version 1.1.0

Affiliation: ENIGMA BRCA1 and BRCA2 VCEP
Description : ClinGen Internal VCEP ACMG/AMP specifications for BRCA1 and BRCA2
Version : 1.1.0
Released : 12/21/2023
Release Notes :
Updates in v1.1:

Updated web-link for suggested co-segregation analysis tool (COOL)

Updated assigned PVS1 codes for some variants in Specifications Table 4

Updated assigned PS3/BS3 codes for some variants in Specifications Table 9, including results updates from interim Findlay report

Corrected typos and errors

Added Supplementary table 16 - mRNA assay evidence used to highlight IVS1/2 variants eligible for PVS1 (RNA) code

Clarified code combinations table in Specifications document: any conflicting pathogenic and benign evidence codes should be resolved using approach 2 (points system)

Rules for BRCA2

Gene: BRCA2 (HGNC:1101)
Transcripts:
NM_000059.4

HGNC Name: BRCA2 DNA repair associated
Disease:
breast-ovarian cancer, familial, susceptibility to, 2 (MONDO:0012933) **Mode of Inheritance:** Autosomal dominant inheritance

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

Null variant (nonsense, frameshift, splice site (donor/acceptor +/−1,2), initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease. Apply at appropriate strength according to PVS1 flowchart, which considers knowledge of clinically important functional domains. See Specifications Table 4 and Appendix D for details.

Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect *as measured by effect on mRNA transcript profile (mRNA assay only).* Apply as PVS1 (RNA) at appropriate strength. See Specifications Figure1B and Appendix E for details.

Modification Type: Gene-specific

Strong

Null variant (nonsense, frameshift, splice site (donor/acceptor +/−1,2), initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease. Apply at appropriate strength according to PVS1 flowchart, which considers knowledge of clinically important functional domains. See Specifications Table 4 and Appendix D for details.

Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect *as measured by effect on mRNA transcript profile (mRNA assay only).* Apply as PVS1 (RNA) at appropriate strength. See Specifications Figure1B and Appendix E for details.

Modification Type: Gene-specific

Moderate

Null variant (nonsense, frameshift, splice site (donor/acceptor +/−1,2), initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease. Apply at appropriate strength according to PVS1 flowchart, which considers knowledge of clinically important functional domains. See Specifications Table 4 and Appendix D for details.

Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect *as measured by effect on mRNA transcript profile (mRNA assay only).* Apply as PVS1 (RNA) at appropriate strength. See Specifications Figure1B and Appendix E for details.

Modification Type: Gene-specific

Supporting

Null variant (nonsense, frameshift, splice site (donor/acceptor +/−1,2), initiation codon,

single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease. Apply at appropriate strength according to PVS1 flowchart, which considers knowledge of clinically important functional domains. See Specifications Table 4 and Appendix D for details.

Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect *as measured by effect on mRNA transcript profile (mRNA assay only).* Apply as PVS1 (RNA) at appropriate strength. See Specifications Figure1B and Appendix E for details.

Modification Type: Gene-specific

Instructions: In alignment with SVI recommendations for PVS1 code application, evidence strength and description has been separated for different variant types. Apply according to PVS1 flowchart, which considers knowledge of clinically important functional domains. For predicted protein termination codon (PTC) variants, apply with exon-specific weights derived for the PM5_PTC code (See Appendix D for details).

See Specifications Table 4, provided as a separate searchable excel file, for a comprehensive summary of codes applicable for all variants considered against the *BRCA1* and *BRCA2* PVS1 decision trees (initiation, nonsense/frameshift, deletion, duplication, splice site (donor/acceptor ±1,2)) – organized by exon. See Appendix Figure 5,6 and Table 5 for further justification.

See **Specifications Figure 1B** for process to apply codes for splicing data, in content of location and predicted bioinformatic impact of the variant, and adaptive weighting according to assay methodology and proportion of functional transcript retained.

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

Apply **PS1**, for predicted **missense** substitutions, where a previously classified **pathogenic** variant is considered to act via protein change (no confirmed or predicted effect on mRNA splicing (SpliceAI≤0.1)).

Apply **PS1**, for exonic and intronic variants with same predicted impact on **splicing,** as a previously classified **pathogenic** variant. Vary weight depending on relative positions,

and confidence in classification of the reference variant.

See Specifications Table 5 and Appendix E, J and K for details.

Modification Type: General recommendation

Moderate

Apply **PS1_Moderate**, for predicted **missense** substitutions, where a previously classified **likely pathogenic** variant is considered to act via protein change (no confirmed or predicted effect on mRNA splicing (SpliceAI≤0.1)).

Apply **PS1_Moderate**, for exonic and intronic variants with same predicted impact on **splicing**, as a previously classified **(likely) pathogenic** variant. Vary weight depending on relative positions, and confidence in classification of the reference variant.

See Specifications Table 5 and Appendix E, J and K for details.

Modification Type: General recommendation

Supporting

Apply **PS1**, for exonic and intronic variants with same predicted impact on **splicing**, as a previously classified **(likely) pathogenic** variant. Vary weight depending on relative positions, and confidence in classification of the reference variant.

See Specifications Table 5 and Appendix E, J and K for details.

Modification Type: General recommendation

Instructions: For both missense and splicing scenarios, (Likely) Pathogenic variant classification should be assigned using VCEP specifications.

For application of PS1 for splicing predictions, **see Specifications Table 5.** The predicted event of the VUA must precisely match the predicted event of the known (likely) pathogenic variant (e.g. both predicted to lead to exon A skipping, or both to enhanced use of cryptic site B), AND the strength of the prediction for the VUA must be of similar or higher strength than the strength of the prediction for the known (likely) pathogenic variant. For an exonic variant, predicted or proven functional effect of missense substitution/s encoded by the variant and the established pathogenic variant should also be considered before PS1 code application for splicing prediction.

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.

Not Applicable

Comments: BRCA1/2-related cancers occur relatively commonly. No information to calibrate the predictive capacity of de novo occurrences.

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. Apply PS3 for assays measuring effect via protein only OR mRNA and protein combined. See Specifications Table 9 for code recommendations from calibrated published assays. Also see Figure1C and Appendix E for details.

Well-established *in vitro* or *in vivo* functional studies supportive of a damaging effect *as measured by effect on mRNA transcript profile (mRNA assay only).* Apply as PVS1 (RNA) at appropriate strength. See Specifications Figure1B and Appendix E for details.

Modification Type: General recommendation

Instructions: See **Specifications Figure 1C** for simplified flowchart/s to advise application of codes for functional data, in content of variant type and location within a (potentially) clinically important functional domain. Do not apply when conflicting results are present from well-established assays with sufficient controls, which cannot be explained by experimental design.

See **Specifications Figure 1B** for process to apply codes for splicing data, in content of location and predicted bioinformatic impact of the variant, and adaptive weighting according to assay methodology and proportion of functional transcript retained.

See **Specifications Table 9,** provided as a separate file in excel format to facilitate searches and look-ups by variant c. and p. nomenclature. It includes PS3 and BS3 code recommendations and rationale for code application of published functional assays data that has been calibrated, and considered against predicted/reported splicing. This is not an

exhaustive list; there will be ongoing consideration of additional published functional assay results.

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.

Strong

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls. Case-control studies; p-value ≤0.05 and OR ≥4 (lower confidence interval excludes 2.0). See Appendix F for details.

Modification Type: Clarification,Gene-specific

Instructions: Case dataset should be ethnicity and country-matched to control dataset. If case-control LR estimates are available for a given dataset, these should be used in preference to case-control OR data, under code PP4 (or BP5, if appropriate). Do not use Proband Counting as originally described.

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.

Not Applicable

Comments: Considered as component of bioinformatic analysis (PP3/BP4).

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 from controls in an outbred population, from gnomAD v2.1 (non-cancer, exome only subset) and gnomAD v3.1 (non-cancer). Region around the variant must have an average read depth ≥25. See Appendix G for details.

Modification Type: Gene-specific

Instructions: Observation of a variant only once in a gnomAD outbred population is not informative. Do not apply for insertion, deletion or delins variants. Do not apply if read depth <25 at region around the variant.

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.

Strong

Apply for patient with phenotype consistent with BRCA1- or BRCA2-related Fanconi Anemia (FA), and co-occurrent variants in the same gene. Phenotype is considered consistent with BRCA1- or BRCA2-related FA if:

(i) Increased chromosome breakage (DEB, MMC, or spontaneous) and at least one clinical feature indicative of BRCA1/2-related FA, categorized under: physical features, pathology and laboratory findings, cancer diagnosis *≤5yr*.

(ii) Result unknown for chromosome breakage, and at least two clinical features indicative of BRCA1/2-related FA under at least two of the three categories: physical features, pathology and laboratory findings, cancer diagnosis ≤5yr.

See **Specifications Table 6** for approach to assign points per proband, and final PM3 code assignment based on the sum of PM3-related points. Also see Appendix H for additional details.

PM3_Strong = ≥4 points

Modification Type: Gene-specific

Moderate

Apply for patient with phenotype consistent with BRCA1- or BRCA2-related Fanconi Anemia (FA), and co-occurrent variants in the same gene. Phenotype is considered consistent with BRCA1- or BRCA2-related FA if:

(i) Increased chromosome breakage (DEB, MMC, or spontaneous) and at least one clinical feature indicative of BRCA1/2-related FA, categorized under: physical features, pathology and laboratory findings, cancer diagnosis *≤5yr*.

(ii) Result unknown for chromosome breakage, and at least two clinical features indicative of BRCA1/2-related FA under at least two of the three categories: physical features, pathology and laboratory findings, cancer diagnosis ≤5yr.

See **Specifications Table 6** for approach to assign points per proband, and final PM3 code assignment based on the sum of PM3-related points. Also see Appendix H for additional details.

PM3 = 2 points

Modification Type: Gene-specific

Supporting

Apply for patient with phenotype consistent with BRCA1- or BRCA2-related Fanconi Anemia (FA), and co-occurrent variants in the same gene.Phenotype is considered consistent with BRCA1- or BRCA2-related FA if:

(i) Increased chromosome breakage (DEB, MMC, or spontaneous) and at least one clinical feature indicative of BRCA1/2-related FA, categorized under: physical features, pathology and laboratory findings, cancer diagnosis *≤5yr*.

(ii) Result unknown for chromosome breakage, and at least two clinical features indicative of BRCA1/2-related FA under at least two of the three categories: physical features, pathology and laboratory findings, cancer diagnosis ≤5yr.

See **Specifications Table 6** for approach to assign points per proband, and final PM3 code assignment based on the sum of PM3-related points. Also see Appendix H for additional details.

PM3_Supporting = 1 point

Modification Type: Gene-specific

Instructions: Co-occurrent P or LP variant should be assigned classification using VCEP specifications.

Variant under assessment must be sufficiently rare (meet PM2_Supporting, or PM2 not applicable).

See **Specifications Table 6** for approach to assign points per proband, and final PM3 code assignment based on the sum of PM3-related points.

For related individuals score only most severe presentation.

Also see **Specifications Table 6** for additional stipulations

PM4

Original ACMG Summary

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

Not Applicable

Comments: Considered as component of bioinformatic analysis (PP3/BP4).

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.

Strong

Protein termination codon (PTC) variant in an exon where a different proven pathogenic PTC variant has been seen before. Use to justify additional weight for PTC variants annotated as PVS1. See Specifications Table 4 for PM5_PTC code strengths applicable per exon. See Appendix D for additional details.

Modification Type: Other

Moderate

Protein termination codon (PTC) variant in an exon where a different proven pathogenic PTC variant has been seen before. Use to justify additional weight for PTC variants annotated as PVS1. See Specifications Table 4 for PM5_PTC code strengths applicable per exon. See Appendix D for additional details.

Modification Type: Other

Supporting

Protein termination codon (PTC) variant in an exon where a different proven pathogenic PTC variant has been seen before. Use to justify additional weight for PTC variants annotated as PVS1. See Specifications Table 4 for PM5_PTC code strengths applicable per exon. See Appendix D for additional details.

Modification Type: Other

Instructions: Only applied to genomic PTC changes (not splicing). Weight determined by exon where the termination codon occurs (may not be the same exon as the variant position). See Specifications Table 4, provided as a separate searchable excel file, for PM5_PTC codes applicable for predicted termination codon variants - organized by exon.

PM6

Original ACMG Summary

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

Not Applicable

Comments: BRCA1/2-related cancers occur relatively commonly. No information to calibrate the predictive capacity of de novo occurrences.

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 in a gene definitively known to cause the disease, as measured by a quantitative co-segregation analysis method. See Appendix I for details.

Apply weight as per Bayes Score:

PP1_Strong – LR>18.7:1

PP1_Very Strong – LR>350:1

Modification Type: Gene-specific

Moderate

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease, as measured by a quantitative co-segregation analysis method. See Appendix I for details.

Apply weight as per Bayes Score:

PP1_Moderate – LR>4.3:1

Modification Gene-specific

Type:

Supporting

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease, as measured by a quantitative co-segregation analysis method. See Appendix I for details.

Apply weight as per Bayes Score:

PP1 - LR >2.08:1

Modification Type: Gene-specific

Instructions: Recommend use of online tool COOL located on this website: http://bjfenglab.org/

Additional information, including pedigree formatting, is currently available at: https://fenglab.chpc.utah.edu/cool3/manual.html

Stipulation: to apply code as Pathogenic Very Strong, VUS should have bioinformatically predicted (or experimentally proven) effect on protein or mRNA splicing. If co-segregation score is from a single family, or several families from an isolated population, assess the possibility of a different causative pathogenic variant.

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.

Not Applicable

Comments: High frequency of benign missense variants.

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

Apply PP3 for missense or in-frame insertion, deletion or delins variants inside a (potentially) clinically important functional domain and predicted impact via protein change (BayesDel no-AF score ≥0.30). As justified in the appendices, (potentially) clinically important functional domains are defined as: BRCA2 PALB2 binding domain aa 10-40; BRCA2 DNA binding aa 2481-3186.

Apply PP3 for predicted splicing (SpliceAI ≥0.2) for silent, missense/in-frame (irrespective of location in clinically important functional domain) and for intronic variants outside of donor and acceptor 1,2 sites.

See Specifications Figure1A and Appendix J for details.

Modification Type: Gene-specific

Instructions: See **Specifications Figure 1A** for process to apply codes according to variant type, location and predicted bioinformatic impact.

PP4

Original ACMG Summary

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

Strong

Breast cancer is very common and has a high degree of genetic heterogeneity (caused by pathogenic variants in numerous genes). Use ONLY to capture combined LR towards pathogenicity, based on multifactorial likelihood clinical data.

PP4_Strong – LR>18.7:1

PP4_Very Strong – LR>350:1

Combined LR 1.00-2.08 is not informative (PP4 not applicable).

See Specifications Table7 and Appendix B for details.

Modification Type: Gene-specific

Moderate

Breast cancer is very common and has a high degree of genetic heterogeneity (caused by pathogenic variants in numerous genes). Use ONLY to capture combined LR towards pathogenicity, based on multifactorial likelihood clinical data.

PP4_Moderate – LR>4.3:1

Combined LR 1.00-2.08 is not informative (PP4 not applicable).

See Specifications Table7 and Appendix B for details.

Modification Type: Gene-specific

Supporting

Breast cancer is very common and has a high degree of genetic heterogeneity (caused by pathogenic variants in numerous genes). Use ONLY to capture combined LR towards pathogenicity, based on multifactorial likelihood clinical data.

PP4 - LR >2.08:1

Combined LR 1.00-2.08 is not informative (PP4 not applicable).

See Specifications Table7 and Appendix B for details.

Modification Type: Gene-specific

Instructions: Use in the context of clinically calibrated evidence types, with sufficient detail to review data sources, types and weights. Published data points may include co-segregation with disease, co-occurrence with a pathogenic variant in the same gene, reported family history, breast tumor pathology, and case-control data. Can also apply for unpublished data, where there is no appropriate ACMG/AMP code. Assign weight based on combined LR for clinical data.

See Specifications Table7 for example application.

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

Filter allele frequency (FAF) is above 0.1% (FAF > 0.001) in gnomAD v2.1 (non-cancer, exome only subset) and/or gnomAD v3.1 (non-cancer), non-founder population(s). See

Appendix G for details.

Modification Type: Gene-specific

Instructions: Apply based on maximum filter allele frequency observed in a gnomAD non-founder population, considering exome and genome data separately.

Do not apply if read depth <20. Do not apply to well-established pathogenic founder variants.

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

Filter allele frequency (FAF) is above 0.01% (FAF > 0.0001) in gnomAD v2.1 (non-cancer, exome only subset) and/or gnomAD v3.1 (non-cancer), non-founder population(s). See Appendix G for details.

Modification Type: Gene-specific

Supporting

Filter allele frequency (FAF) is above 0.002% (FAF > 0.00002) and less than or equal to 0.01% (FAF ≤ 0.0001) in gnomAD v2.1 (non-cancer, exome only subset) and/or gnomAD v3.1 (non-cancer), non-founder population(s). See Appendix G for details.

Modification Type: Gene-specific

Instructions: Apply based on maximum filter allele frequency in a gnomAD non-founder population, considering exome and genome data separately.

Do not apply if read depth <20. Do not apply to well-established pathogenic founder variants.

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

Applied in absence of features of recessive disease, namely Fanconi Anemia phenotype. See **Specifications Table 8** for additional stipulations, and approach to assign points per proband, and final BS2 code assignment based on the sum of BS2-related points. See Appendix H for additional details.

BS2 = ≥ 4 points

Modification Type: Gene-specific

Moderate

Applied in absence of features of recessive disease, namely Fanconi Anemia phenotype. See **Specifications Table 8** for additional stipulations, and approach to assign points per proband, and final BS2 code assignment based on the sum of BS2-related points. See Appendix H for additional details.

BS2_Moderate = 2 points

Modification Type: Gene-specific

Supporting

Applied in absence of features of recessive disease, namely Fanconi Anemia phenotype. See **Specifications Table 8** for additional stipulations, and approach to assign points per proband, and final BS2 code assignment based on the sum of BS2-related points. See Appendix H for additional details.

BS2_Supporting = 1 points

Modification Type: Gene-specific

Instructions: Co-occurrent P or LP variant should be assigned classification using VCEP specifications.

See **Specifications Table 8** for approach to assign points per proband, and final BS2 code assignment based on the sum of BS2-related points.

Also see **Specifications Table 8** for additional stipulations.

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. Assay measures effect via protein only OR mRNA and protein combined. See Specifications Table 9 for code recommendations from calibrated published assays. Also see Figure1C and Appendix E for details.

Well-established *in vitro* or *in vivo* functional studies supportive of no damaging effect *as measured by effect on mRNA transcript profile (mRNA assay only).* Apply as BP7 (RNA) at appropriate strength. See Specifications Figure1B and Appendix E for details.

Modification Type: Gene-specific

Instructions: See **Specifications Figure 1C** for simplified flowchart/s to advise application of codes for functional data, in content of variant type and location within a (potentially) clinically important functional domain. Do not apply when conflicting results are present from well-established assays with sufficient controls, which cannot be explained by experimental design.

See **Specifications Figure 1B** for process to apply codes for splicing data, in content of location and predicted bioinformatic impact of the variant, and adaptive weighting according to assay methodology and proportion of functional transcript retained.

See **Specifications Table 9,** provided as a separate file in excel format to facilitate searches and look-ups by variant c. and p. nomenclature. It includes PS3 and BS3 code recommendations and rationale for code application of published functional assays data that has been calibrated, and considered against predicted/reported splicing. This is not an exhaustive list; there will be ongoing consideration of additional published functional assay results.

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 a family, as measured by a quantitative co-segregation analysis method. See Appendix I for details.

Apply weight as per Bayes Score:

BS4 - LR <0.05:1

BS4_VeryStrong – LR <0.00285:1

Modification Type: Gene-specific

Moderate

Lack of segregation in affected members of a family, as measured by a quantitative co-segregation analysis method. See Appendix I for details.

Apply weight as per Bayes Score:

BS4_Moderate - LR <0.23:1

Modification Type: Gene-specific

Supporting

Lack of segregation in affected members of a family, as measured by a quantitative co-segregation analysis method. See Appendix I for details.

Apply weight as per Bayes Score:

BS4_Supporting - LR 0.23-0.48:1

Modification Type: Gene-specific

Instructions: Recommend use of online tool COOL located on this website: http://bjfenglab.org/

Additional information, including pedigree formatting, is currently available at: https://fenglab.chpc.utah.edu/cool3/manual.html

BP1

Original ACMG Summary

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

Strong

Apply BP1_Strong for silent substitution, missense or in-frame insertion, deletion or delins variants outside a (potentially) clinically important functional domain AND no splicing predicted (SpliceAI ≤0.1). As justified in the appendices, (potentially) clinically important functional domains are defined as: BRCA2 PALB2 binding domain aa 10-40; BRCA2 DNA binding aa 2481-3186. See Specifications Figure1A and Appendix J for details.

Modification Gene-specific,Strength

Type:

Instructions: See **Specifications Figure 1A** for process to apply codes according to variant type, location and predicted bioinformatic impact. *Missense prediction not applicable*.

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.

Not Applicable

Comments: Applied only in the context of BS2.

BP3

Original ACMG Summary

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

Not Applicable

Comments: Captured by bioinformatic tool prediction, and domain analysis. See Appendix J for details

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

Missense or in-frame insertion, deletion or delins variants inside a (potentially) clinically important functional domain, and no predicted impact via protein change or splicing (BayesDel no-AF score ≤ 0.18 AND SpliceAI ≤0.1).

Silent variant inside a (potentially) clinically important functional domain, if no predicted impact via splicing (SpliceAI ≤0.1).

Intronic variants outside of the native donor and acceptor splice sites (i.e. not +/- 1,2 positions) AND no predicted impact via splicing (SpliceAI ≤0.1).

As justified in the appendices, (potentially) clinically important functional domains are defined as: BRCA2 PALB2 binding domain aa 10-40; BRCA2 DNA binding aa 2481-3186. See Specifications Figure1A and Appendix J for details.

Modification Type: Clarification,Gene-specific

Instructions: See **Specifications Figure 1A** for process to apply codes according to variant type, location and predicted bioinformatic impact.

BP5

Original ACMG Summary

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

Strong

Use ONLY to capture combined LR against pathogenicity, based on multifactorial likelihood clinical data.

BP5_VeryStrong – LR <0.00285:1

BP5_Strong - LR <0.05:1

Not applicable for co-observation: cases with pathogenic variants in two (or more) different known breast–ovarian cancer risk genes have no specific phenotype.

Modification Type: Gene-specific

Moderate

Use ONLY to capture combined LR against pathogenicity, based on multifactorial likelihood clinical data.

BP5_Moderate - LR <0.23:1

Not applicable for co-observation: cases with pathogenic variants in two (or more) different known breast–ovarian cancer risk genes have no specific phenotype.

Modification Type: Gene-specific

Supporting

Use ONLY to capture combined LR against pathogenicity, based on multifactorial likelihood clinical data.

BP5 - LR 0.23-0.48:1

Not applicable for co-observation: cases with pathogenic variants in two (or more) different known breast–ovarian cancer risk genes have no specific phenotype.

Modification Type: Gene-specific

Instructions: Use in the context of clinically calibrated evidence types, with sufficient detail to review data sources, types and weights. Published data points may include co-segregation with disease, co-occurrence with a pathogenic variant in the same gene, reported family history, breast tumor pathology, and case-control data. Can also apply for unpublished data, where there is no appropriate ACMG/AMP code. Assign weight based on combined LR for clinical data.

See Specifications Table7 for example application.

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.

Strong

Well-established in vitro or in vivo functional studies shows no damaging effect on protein function *as measured by effect on mRNA transcript profile – mRNA assay only.* Apply as BP7_Strong (RNA) for intronic and silent variants, as well as missense/in-frame variants located outside a (potentially) clinically important functional domain. See Specifications Figure1B and Appendix E for details.

As justified in the appendices, (potentially) clinically important functional domains are defined as: BRCA2 PALB2 binding domain aa 10-40; BRCA2 DNA binding aa 2481-3186. See Specifications Figure1A and Appendix J for details.

Modification Type: General recommendation,Gene-specific

Supporting

Silent variant inside a (potentially) clinically important functional domain, IF BP4 met.

Intronic variants located outside conserved donor or acceptor motif positions (at or beyond positions +7/-21) IF BP4 met.

See Specifications Figure1A and Appendix J for additional details.

As justified in the appendices, (potentially) clinically important functional domains are defined as: BRCA2 PALB2 binding domain aa 10-40; BRCA2 DNA binding aa 2481-3186. See Specifications Figure1A and Appendix J for details.

Modification Type: Clarification,General recommendation

Instructions: See **Specifications Figure 1B** for process to apply codes for splicing data, in content of location and predicted bioinformatic impact of the variant, and adaptive weighting according to assay methodology and proportion of functional transcript retained. Not applicable for missense variants inside a (potentially) clinically important functional domain as they may still impact protein function through the amino acid change.

Following convention, this code is applied in addition to BP4 (no splicing prediction, Splice AI ≤0.1) to capture the low prior probability of pathogenicity of silent variants. Nucleotide conservation is not considered relevant. See **Specifications Figure 1A** for process to apply codes according to variant type, location and predicted bioinformatic impact.

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)*

1 Very Strong *(PVS1)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1)* **AND ≥ 1 Moderate** *(PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)*

≥ 3 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)*

2 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)* **AND ≥ 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)*

2 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

Likely Pathogenic

1 Strong *(PVS1_Strong, PS1, PS3, PS4, PM3_Strong, PM5_Strong, PP1_Strong, PP4_Strong)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 3 Moderate *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)*

2 Moderate *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Moderate *(PVS1_Moderate, PS1_Moderate, PM3, PM5, PP1_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PVS1_Supporting, PS1_Supporting, PM2_Supporting, PM3_Supporting, PM5_Supporting, PP1, PP3, PP4)*

Benign

≥ 2 Strong *(BS1, BS2, BS3, BS4, BP1_Strong, BP5_Strong, BP7_Strong)*

1 Stand Alone *(BA1)*

1 Strong *(BS1, BS2, BS3, BS4, BP1_Strong, BP5_Strong, BP7_Strong)* **AND 2 Moderate** *(BS2_Moderate, BS4_Moderate, BP5_Moderate)*

1 Strong *(BS1, BS2, BS3, BS4, BP1_Strong, BP5_Strong, BP7_Strong)* **AND 1 Moderate** *(BS2_Moderate, BS4_Moderate, BP5_Moderate)* **AND ≥ 1 Supporting** *(BS1_Supporting, BS2_Supporting, BS4_Supporting, BP4, BP5, BP7)*

1 Strong *(BS1, BS2, BS3, BS4, BP1_Strong, BP5_Strong, BP7_Strong)* **AND ≥ 3 Supporting** *(BS1_Supporting, BS2_Supporting, BS4_Supporting, BP4, BP5, BP7)*

Likely Benign

1 Strong *(BS1, BS2, BS3, BS4, BP1_Strong, BP5_Strong, BP7_Strong)* **AND 1 Supporting** *(BS1_Supporting, BS2_Supporting, BS4_Supporting, BP4, BP5, BP7)*

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

1 Strong *(BP1_Strong)*

1 Strong *(BS1, BS2, BS3, BS4, BP1_Strong, BP5_Strong, BP7_Strong)* **AND 1 Moderate** *(BS2_Moderate, BS4_Moderate, BP5_Moderate)*

1 Moderate *(BS2_Moderate, BS4_Moderate, BP5_Moderate)* **AND ≥ 1 Supporting** *(BS1_Supporting, BS2_Supporting, BS4_Supporting, BP4, BP5, BP7)*

Files & Images

Appendices_V1.1_2023-11-22: Appendices document for V1.1

Specifications_V1.1_2023-11-22: Specifications document for V1.1

Specifications_Table4_V1.1_2023-11-22: Specifications Table 4 excel document for V1.1

Specifications_Table9_V1.1_2023-11-22: Specifications Table 9 excel document for V1.1

Supplementary_Tables_V1.1_2023-11-22: Supplementary Tables excel document for V1.1