BRCA1/BRCA2: CanVIG-UK Gene-Specific Guidance

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For use in conjunction with CanVIG-UK Consensus Specification for Cancer susceptibility Genes of ACGS Best Practice Guidelines for Variant Classification. Evidence lines for which there are no gene-specific recommendations should be reviewed in context of CanVIG-UK Consensus Specification for Cancer Susceptibility Genes.

Evidence towards Pathogenicity

Evidence element and evidence strengths allowed		Thresholds/data-sources/applications specifically relevant to BRCA1/BRCA2
PS4: Case-control: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	_VSTR _STR _MOD _SUP	NHSD case control data can be used for case-control analysis: • Controls should represent appropriate ethnicity and sex matching (i.e. female non-cancer NFE controls should be used if the case series consists predominantly of females, as with the current NHSD case series) and series denominator • As this is an enriched series, OR≥10 is required • Current data/denominator counts for base substitutions are available at CanVar-UK • For non-base-substitutions i.e. deletions/duplications/insertions, NHSD counts can be accessed from CanVIG-UK If there are insufficient data to perform case-control analyses, PS4 can be applied: • at PS4_sup or PS4_mod if there are observations of the variant in ≥5 or ≥10 different families respectively with a pattern of diagnoses consistent with a hereditary breast and ovarian cancer syndrome • **and** the variant is very rare or absent in control populations (i.e. PM2 has been applied)
PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC	_MOD _SUP	Cancer-free female controls (of any/all ethnicities) should be used (due to low penetrance in male pathogenic variant carriers). Otherwise, the main CanVIG-UK consensus guidance should be followed.

PVS1: **Predicted null variant** (in a gene where LOF is a known mechanism of disease)

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It is predicted that truncating variants occurring at the 3' end of the gene will not undergo NMD. The residues below demarcate the consensus boundary, 3' of which protein truncating variants are not established to result in NMD and/or impairment of function of residual protein.
BRCA1 (NM_007294.3): 1855[1]
BRCA2 (NM_000059.3): 3309[2]
Based on ENIGMA recommendations, as re-initiation sites have also been shown to result in the loss of important functional domains in BRCA1 and BRCA2, it is acceptable to use PVS1 at a very strong level for variants identified within the first 100bp of both BRCA1 and BRCA2[1].
A number of variants at canonical splice sites are predicted or known to lead to naturally occurring in-frame RNA isoforms that may rescue gene functionality. ENIGMA has compiled the below list of splice variants for which the variant transcript may be functional and for some of which PVS1 may not be applicable.

Gene	Region	Bases	Strength
BRCA1	intron 5	c.301+1	Sup
		c.301+2	Sup
	intron 6	c.442-1	Sup
		c.442-2	Sup
	intron 7	c.548-1	N/A
		c.548-2	N/A
	intron 8	c.593+1	N/A
		c.593+2	N/A
		c.594-1	N/A
		c.594-2	N/A
	intron 9	c.670+1	N/A
		c.670+2	N/A
		c.671-1	Mod
		c.671-2	Mod
	intron 10	c.4096+1	Mod
		c.4096+2	Mod
		c.4097-1G>C, G>T	Sup
		c.4097-2A>C, A>T	Sup
	intron 11	c.4186-1	Sup
		c.4186-2A>C, A>T	Sup
	intron 12	c.4358-1	Sup
		c.4358-2A>C, A>T	Sup

Gene	Region	Bases	Strength
BRCA2	intron 2	c.68-1	Sup
		c.68-2	Sup
	intron 3	c.317-1	N/A
		c.317-2	N/A
	intron 4	c.425+1	N/A
		c.425+2	N/A
	intron 6	c.517-1G>C, G>T	N/A
		c.517-2	N/A
	intron 7	c.631+1	N/A
		c.631+2	N/A
	intron 9	c.794-1G>C, G>T	N/A
		c.794-2	N/A
	intron 10	c.1909+1	N/A
		c.1909+2	N/A
	intron 11	c.6842-1	N/A
		c.6842-2	N/A
	intron 12	c.6937+1	N/A
		c.6937+2	N/A
	intron 19	c.8488-1	Sup
		c.8488-2	Sup
	intron 23	c.9118-1G>A	Sup
	intron 24	c.9257-1G>C, G>T	Sup
		c.9257-2	Sup

Adapted from ENIGMA, 2017[1] and forthcoming ENIGMA guidelines.

PS1: Same amino acid change as an established variant

_STR

PM4: Protein-length-changing variant

_MOD
_SUP

PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic seen before

_MOD
_SUP

PP3: In silico: Multiple lines of computational evidence support a deleterious effect on the gene or gene product

_SUP

PM1, PP2: Enrichment/constraint:
PP2: Missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease
PM1: Located in a

_STR
_MOD
_SUP

Within forthcoming ACMG guidance, it is anticipated that these elements will all be incorporated within PP3 and only awarded to variants within key domains.

In the interim, we recommend:

- Use of PM1_sup and/or PM4_sup for any variant within BRCA1 RING (aa 2-101), BRCT (aa 1650-1857) COILED-COIL DOMAIN (aa 1391-1424), BRCA2 DNA-binding domain (aa 2481-3186), and BRCA2 PALB2 binding domain (aa 10-40).
- Use of PM1_mod for missense at specific residues[3]:
  RING: 18, 22, 37, 39, 41, 44, 47, 61, 64, 71
  BRCT: 1685, 1688, 1697, 1699, 1706, 1708, 1715, 1736, 1738, 1739, 1748, 1764, 1766, 1770, 1775, 1786, 1837, 1838, 1839, 1853
  DBD: 2607, 2626, 2627, 2663, 2722, 2723, 2748, 3052, 3124
- PM1 cannot be used where functional data are being used for PS3, as per main CanVIG-UK guidance
- PP2 should not be used for BRCA1/BRCA2
- Use of PM5, PS1, PP3 otherwise as per CanVIG-UK Consensus Specification

mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation		
PS3: Functional: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product	_VSTR _STR _MOD _SUP	**BRCA1:** Findlay et al, 2018[4]: Strong Bouwman et al, 2020[5]: Strong Starita et al, 2018[15]: Strong Fernandes et al, 2019[6]: Supporting Petitalot et al, 2019[7]: Supporting **BRCA2:** Guidugli et al, 2018[8]/Hart et al, 2019[9]/Richardson et al, 2021[10]: Strong Mesman et al, 2019[16]: Moderate See CanVIG Functional Assays Scores See the table at the bottom of this document for guidance on combining assay results
PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	_VSTR _STR _MOD _SUP	Segregation evidence extracted from multifactorial analysis data can be used within PP1/BS4 using the thresholds specified in the PP5/BP6 guidance. Where combined with multiple evidence of other types, segregation evidence from multifactorial analysis data should be incorporated into the PP5/BP6 criteria Meiosis counting approaches may be used in addition if this evidence comes from families not already included in the multifactorial analyses. **Evidence cannot exceed 'Very strong'**
PS2/PM6: De novo (maternity and paternity confirmed/unconfirmed) in a patient with the disease and no family history	_STR _MOD _SUP	
PM3: in trans with a pathogenic variant (**recessive disorders)**	_STR _MOD _SUP	Frequency data regarding co-occurrence in trans extracted from multifactorial analyses should be incorporated into PM3 or BP2 using the thresholds described in the PP5/BP6 guidance. Where combined with multiple evidence of other types, frequency data regarding co-occurrence in trans from multifactorial analyses should be incorporated into PP5/BP6 ***In addition***, the SVI recommendations for in trans Criterion (PM3) can be used for either BRCA1 or BRCA2 for individuals with a Fanconi anaemia phenotype if this evidence comes from families not already included in the multifactorial analyses used for PP5. Evidence towards a Fanconi phenotype comprise: • **Clinical:** diagnosis of childhood cancer or skeletal/structural/developmental abnormalities • **Molecular/Cellular:** aberration on mitomycin-induced chromosomal breakage +/- depletion of BRCA2 in lymphocytes Both clinical and molecular/cellular aberrations must be present for a case to contribute to evidence **Evidence cannot exceed 'Strong'** ***Note:*** Caution is required in inferring the pathogenicity for the monoallelic phenotype, as variants may be hypomorphic (e.g. a variant contributing and causing a Fanconi anaemia phenotype may be low penetrance for breast cancer). Where the majority of evidence for variant pathogenicity comes from observations of the variant in cases of Fanconi Anaemia, it may be appropriate to comment on this in the clinical report

PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation

_VSTR
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_SUP

Published multifactorial analysis data providing likelihood ratios (LR) or log likelihood ratios (LLR) may be used as data sources encompassing:

- Segregation (PP1/BS4)
- Specificity of familial and/or tumour phenotype (PP4/BP5)
- Co-occurrence in trans (PM3/BP2)

Where individual likelihood ratios are insufficient to assign evidence strength, a **combined score** encompassing multiple evidence types can instead be applied within PP5 to represent the totality of evidence.

Suitable analyses:

- Easton et al, 2007[11]
- Vallée et al, 2012[12]
- Parsons et al, 2020[13]
- Caputo et al, 2021[14]

Evidence is presented as either a Likelihood Ratio (LR) or Log Likelihood Ratio (LLR).

If evidence is supplied as an LR: Use the table below to directly convert the LR to the applicable Evidence Strength.

If evidence is supplied as an LLR: First, convert the LLR to a Likelihood Ratio (LR) by finding the exponent of the LLR (for example, an LLR of 2.00 = an LR of 7.38); conversion of an LLR to an LR can be done using the =EXP() function within Excel. Once the LR is calculated, use the table below to directly convert the LR to the applicable Evidence Strength (LR of 7.38 = MOD).

Conversions from LR or LLR to Evidence (Exponent) points is also available for applicable variants at https://canvaruk.org/

Likelihood Ratio	Evidence (Exponent) Points	Evidence Strength
2.08 – 4.30	1	SUP
4.31 – 18.70	2	MOD
18.71 – 350.40	4	STR
≥ 350.41	8	VSTR

Explanatory Notes:

- Where multiple potentially valid LR/LLRs are available for a variant, the value from the most recent publication should be used.
- Evidence (Exponent) Points are calculated by applying the logarithm of the LR to base 2.08. A calculated Evidence Point that is between two categories (eg 3 points) is assigned the weaker strength of the two categories it lies between (eg MOD for 3 points)

Please note, use of classifications from other reputable sources (eg ClinVar) is no longer valid. Please refer to the consensus specification for further details.

PP4: Phenotypic specificity (Patient's phenotype or family history is highly specific for a

_STR
_MOD
_SUP

Tumour and family history phenotypic data extracted from multifactorial analyses should be incorporated into PP4 or BP5 using the thresholds described in the PP5/BP6 guidance. Where combined with multiple evidence of other types, tumour and family history phenotypic data from multifactorial analyses are

disease with a single genetic aetiology)		incorporated into the PP5 evidence criterion. Patient phenotypic evidence whose strength cannot be quantified should not be used. **Evidence cannot exceed 'Strong'**

Evidence towards Benignity

BA1/BS1: Allele frequency is "too high" in ExAC or gnomAD for disorder	_SA _STR	**BA1: MTAF = 0.001 (0.1%)** **BS1: MTAF = 0.0001 (0.01%)** The U95%CI should be used as the filtering allele count for the MTAF. This can be calculated using cardiodb or within gnomAD (see training resources from Miranda Durkie for methodology) Cancer-free **female controls** should be used (due to low penetrance in male pathogenic variant carriers)
BS2: Observation in controls inconsistent with disease penetrance. 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	_STR _SUP	
BP4: In silico: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	_SUP	
BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease	_SUP	Can be used for missense variants with no predicted splicing effect (as per main CanVIG-UK consensus specification) at non-conserved residues outside of BRCA1 RING (aa 2-101), BRCT (aa 1650-1857) COILED-COIL DOMAIN (aa 1391-1424) and BRCA2 DNA-binding domain (aa 2481-3186) and BRCA2 PALB2 binding domain (aa 10-40)
BP7: Synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence	_SUP	
BP3: In-frame deletions/insertions in a repetitive region	_SUP	
BS3: Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	_STR _MOD _SUP	
BS4: Non segregation with disease	_STR _SUP	*see PP1
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis	_STR _SUP	*see PM3
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the	_STR _SUP	*see PP5 Likelihood Ratio | Evidence (Exponent) | Evidence Strength

laboratory to perform an independent evaluation		<table><tr><th></th><th>Points</th><th></th></tr><tr><td>0.48 – 0.23</td><td>-1</td><td>SUP</td></tr><tr><td>0.22 – 0.05</td><td>-2</td><td>MOD</td></tr><tr><td>0.04 – 0.00285</td><td>-4</td><td>STR</td></tr><tr><td><0.00284</td><td>-8</td><td>VSTR</td></tr></table>
BP5: Alternate molecular basis for disease	_SUP	*see PP4

Recommendations for the management of conflicting functional assay results See table below for management of discrepancy for BRCA1 variants between Findlay et al, 2018[4] and Bouwman et al, 2020[5] discordant assay results. For more general guidance regarding conflicting results from other functional assays, refer to the table in the main CanVIG-UK consensus specification.

Findlay Class	Findlay Score	Bouwman Platinum	Bouwman Olaparib	Bouwman DR-GFP	PS3_STR	BS3_STR
LOF	<-1.328	All deleterious/ likely deleterious (1 intermediate allowed)			✓	×
LOF	<-1.328	Any are neutral/likely neutral			×	×
INT (towards LOF)	-1.328 to -1.038	All deleterious/ likely deleterious			✓	×
INT (towards FUNC)	-1.038 to -0.748	All neutral/likely neutral			×	✓
INT	-1.328 to -0.78	Conflicting results or any intermediate			×	×
FUNC	>-0.748	All neutral/likely neutral (1 intermediate allowed)			×	✓
FUNC	>-0.748	Any are deleterious/likely deleterious			×	×

N.B: Bouwman et al, 2020 “not clear” refers to opposite categorisation ± the standard deviation of repeat experiments and should be treated as conflicting assay results. Where a variant is LOF on the Findlay et al assay and has an RNA score of <-2, this indicates that LOF is due to interference with splicing and therefore should not be treated as conflicting evidence if the variant is neutral on the Bouwman et al assay.

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.12	***01/09/2021***	PP4	Guidance on use of LLRs from published epidemiological studies amended to account for the use of natural logs in the statistics presented	Garrett	Turnbull
1.12	***01/09/2021***	PM1	Addition of critical residues in the DNA binding domain of BRCA2. Critical residues in all listed functional domains updated to mirror draft 2021 ENIGMA guidance	Garrett	Turnbull
1.12	***01/09/2021***	BP1	Resolution of typo in BRCT region specification	Garrett	Turnbull
1.13	***15/10/2021***	PVS1	Clarification that PVS1 may not be applicable for some of the variants at ENGIMA specified positions	Garrett	Turnbull
1.14	***02/12/2021***	PS4	Terminology change to reflect transition of PHE to NHSD	Garrett	Turnbull
1.14	***02/12/2021***	PVS1	Addition of recommendations for variants within the first 100bp	Callaway	CStAG
1.15	***28/04/2022***	PM1/ PM4	Clarification that PM1_sup and PM4_sup may be used in combination but PM4 not to be used at moderate. Removal of mention	Garrett	CStAG

			that CanVIG-UK provide non-white ethnicity counts under PS4		
1.16	***28/07/2022***	PP5	Removal of Lindor *et al* 2011 paper from recommended genetic epidemiology papers to use in calculating Evidence (Exponent) Points.	Allen	Turnbull
1.17	***27/09/2022***	PS4	Addition of PS4_mod application where ≥10 HBOC families observed.	Garrett	CStAG
1.18	***31/03/2023***	PP5/ BP6	Rewording of application details for clarity. Removal of reputable source evidence from PP5 per consensus specification. Addition of recommended analyses papers.	Allen	CStAG
1.18	***31/03/2023***	PVS1/ PM1/ PS3	Incorporation of functional assays, hotspot, and splice sites from upcoming ENIGMA recommendations.	Allen	CStAG

References

1. Draft ACMG/AMP Classification Rules Specified for BRCA1 & BRCA2 ENIGMA Variant Curation Expert Panel, Classification Criteria V1.0 2021-06-21., 2021.
2. Mesman RLS, Calléja F, Hendriks G, et al. The functional impact of variants of uncertain significance in BRCA2. *Genetics in medicine : official journal of the American College of Medical Genetics* 2019;21(2):293-302. doi: 10.1038/s41436-018-0052-2 [published Online First: 2018/07/11]
3. ENIGMA. *BRCA1/2* Gene Variant Classification Criteria Version 2.5.1 2017 [Available from: https://enigmaconsortium.org/library/general-documents/enigma-classification-criteria/.
4. Findlay GM, Daza RM, Martin B, et al. Accurate classification of BRCA1 variants with saturation genome editing https://sge.gs.washington.edu/BRCA1/. *Nature* 2018;562(7726):217-22. doi: 10.1038/s41586-018-0461-z [published Online First: 2018/09/14]
5. Bouwman P, van der Heijden I, van der Gulden H, et al. Functional Categorization of BRCA1 Variants of Uncertain Clinical Significance in Homologous Recombination Repair Complementation Assays. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2020;26(17):4559-68. doi: 10.1158/1078-0432.Ccr-20-0255 [published Online First: 2020/06/18]
6. Fernandes VC, Golubeva VA, Di Pietro G, et al. Impact of amino acid substitutions at secondary structures in the BRCT domains of the tumor suppressor BRCA1: Implications for clinical annotation. *The Journal of biological chemistry* 2019;294(15):5980-92. doi: 10.1074/jbc.RA118.005274 [published Online First: 2019/02/16]
7. Petitalot A, Dardillac E, Jacquet E, et al. Combining Homologous Recombination and Phosphopeptide-binding Data to Predict the Impact of BRCA1 BRCT Variants on Cancer Risk. *Mol Cancer Res* 2019;17(1):54-69. doi: 10.1158/1541-7786.Mcr-17-0357 [published Online First: 2018/09/28]
8. Guidugli L, Shimelis H, Masica DL, et al. Assessment of the Clinical Relevance of BRCA2 Missense Variants by Functional and Computational Approaches. *American journal of human genetics* 2018;102(2):233-48. doi: 10.1016/j.ajhg.2017.12.013 [published Online First: 2018/02/06]
9. Hart SN, Hoskin T, Shimelis H, et al. Comprehensive annotation of BRCA1 and BRCA2 missense variants by functionally validated sequence-based computational prediction models. *Genetics in medicine : official journal of the American College of Medical Genetics* 2019;21(1):71-80. doi: 10.1038/s41436-018-0018-4 [published Online First: 2018/06/10]
10. Richardson ME, Hu C, Lee KY, et al. Strong functional data for pathogenicity or neutrality classify BRCA2 DNA-binding-domain variants of uncertain significance. *American journal of human genetics* 2021;108(3):458-68. doi: 10.1016/j.ajhg.2021.02.005 [published Online First: 2021/02/21]
11. Easton DF, Deffenbaugh AM, Pruss D, et al. A systematic genetic assessment of 1,433 sequence variants of unknown clinical significance in the BRCA1 and BRCA2 breast cancer-predisposition genes. *American journal of human genetics* 2007;81(5):873-83. doi: 10.1086/521032 [published Online First: 2007/10/10]

12. Vallée MP, Francy TC, Judkins MK, et al. Classification of missense substitutions in the BRCA genes: a database dedicated to Ex-UVs. *Hum Mutat*. 2012;33(1):22-28. doi:10.1002/humu.21629 [published Online First: 2011/11/03]
13. Parsons MT, Tudini E, Li H, et al. Large scale multifactorial likelihood quantitative analysis of BRCA1 and BRCA2 variants: An ENIGMA resource to support clinical variant classification. *Human mutation* 2019;40(9):1557-78. doi: 10.1002/humu.23818 [published Online First: 2019/05/28]
14. Caputo SM, Golmard L, Léone M, et al. Classification of 101 BRCA1 and BRCA2 variants of uncertain significance by cosegregation study: A powerful approach. *Am J Hum Genet.* 2021;108(10):1907-1923. doi:10.1016/j.ajhg.2021.09.003 [published Online First: 2021/09/30]
15. Starita LM, Islam MM, Banerjee T, et al. A Multiplex Homology-Directed DNA Repair Assay Reveals the Impact of More Than 1,000 BRCA1 Missense Substitution Variants on Protein Function. *Am J Hum Genet.* 2018;103(4):498-508. doi:10.1016/j.ajhg.2018.07.016 [published Online First: 2018/09/12]
16. Mesman RLS, Calléja FMGR, Hendriks G, et al. The functional impact of variants of uncertain significance in BRCA2. *Genet Med.* 2019;21(2):293-302. doi:10.1038/s41436-018-0052-2 [published Online First: 2018/07/10]