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 individuals from UKBiobank should be used if the case series consists predominantly of females, as with the current NHSD case series) • For unenriched cases, an OR threshold of >4 should be used based on the ENIGMA threshold for high-penetrance genes. However, as this is an enriched series, a dataset-specific enrichment factor should be used to calculate the odds ratio (OR) threshold where available. Otherwise, an OR threshold of >8 should be used • 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 if there are observations of the variant in ≥5 different families **and** the variant is seen in ≤ 1/50,000 individuals in UKBiobank • at PS4_mod if there are observations of the variant in ≥10 different families **and** the variant is absent from UKBiobank.

<table>
<tr><td></td><td></td><td>

- Families used must have a pattern of diagnoses consistent with a hereditary breast and ovarian cancer syndrome.

</td></tr>
<tr><td>PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC</td><td>_MOD
_SUP</td><td>Cancer-free female controls of any/all ethnicities from gnomAD v2.1.1 and UKBiobank (if not being used for PS4) should be used (due to low penetrance in male pathogenic variant carriers). Otherwise, the main CanVIG-UK consensus guidance should be followed.</td></tr>
<tr><td>PVS1: Predicted null variant (in a gene where LOF is a known mechanism of disease)</td><td>_VSTR
_STR
_MOD
_SUP</td><td>It is predicted that truncating variants occurring at the 3’ end of the gene will not undergo NMD. The residues that demarcate the boundary where 3' of this residue NMD does not occur are:

BRCA1: c.5419 p.1806
BRCA2: c.9600 p.3200

PVS1_vstr can be used for variants truncating 5' of these residues.
The last residue known to not undergo NMD but still be important for protein function is BRCA1 p.1857 and BRCA2 p.3308, therefore PVS1_vstr can also be used between BRCA1 p.1806 – p.1857 (although p1855-1857 data is equivocal) and BRCA2 p.3200 – p.3308.

PVS1 is n/a for residues including and 3' of BRCA1 p.1858 and BRCA2 p.3309.

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.
<table>
<tr><th>Gene</th><th>Region</th><th>Bases</th><th>Strength</th></tr>
<tr><td rowspan="20">BRCA1</td><td rowspan="2">intron 5</td><td>c.301+1</td><td>Sup</td></tr>
<tr><td>c.301+2</td><td>Sup</td></tr>
<tr><td rowspan="2">intron 6</td><td>c.442-1</td><td>Sup</td></tr>
<tr><td>c.442-2</td><td>Sup</td></tr>
<tr><td rowspan="2">intron 7</td><td>c.548-1</td><td>N/A</td></tr>
<tr><td>c.548-2</td><td>N/A</td></tr>
<tr><td rowspan="4">intron 8</td><td>c.593+1</td><td>N/A</td></tr>
<tr><td>c.593+2</td><td>N/A</td></tr>
<tr><td>c.594-1</td><td>N/A</td></tr>
<tr><td>c.594-2</td><td>N/A</td></tr>
<tr><td rowspan="4">intron 9</td><td>c.670+1</td><td>N/A</td></tr>
<tr><td>c.670+2</td><td>N/A</td></tr>
<tr><td>c.671-1</td><td>Mod</td></tr>
<tr><td>c.671-2</td><td>Mod</td></tr>
<tr><td rowspan="4">intron 10</td><td>c.4096+1</td><td>Mod</td></tr>
<tr><td>c.4096+2</td><td>Mod</td></tr>
<tr><td>c.4097-1G>C, G>T</td><td>Sup</td></tr>
<tr><td>c.4097-2A>C, A>T</td><td>Sup</td></tr>
<tr><td rowspan="2">intron 11</td><td>c.4186-1</td><td>Sup</td></tr>
<tr><td>c.4186-2A>C, A>T</td><td>Sup</td></tr>
<tr><td rowspan="2">intron 12</td><td>c.4358-1</td><td>Sup</td></tr>
<tr><td>c.4358-2A>C, A>T</td><td>Sup</td></tr>
</table>
<table>
<tr><th>Gene</th><th>Region</th><th>Bases</th><th>Strength</th></tr>
<tr><td rowspan="24">BRCA2</td><td rowspan="2">intron 2</td><td>c.68-1</td><td>Sup</td></tr>
<tr><td>c.68-2</td><td>Sup</td></tr>
<tr><td rowspan="2">intron 3</td><td>c.317-1</td><td>N/A</td></tr>
<tr><td>c.317-2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 4</td><td>c.425+1</td><td>N/A</td></tr>
<tr><td>c.425+2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 6</td><td>c.517-1G>C, G>T</td><td>N/A</td></tr>
<tr><td>c.517-2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 7</td><td>c.631+1</td><td>N/A</td></tr>
<tr><td>c.631+2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 9</td><td>c.794-1G>C, G>T</td><td>N/A</td></tr>
<tr><td>c.794-2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 10</td><td>c.1909+1</td><td>N/A</td></tr>
<tr><td>c.1909+2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 11</td><td>c.6842-1</td><td>N/A</td></tr>
<tr><td>c.6842-2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 12</td><td>c.6937+1</td><td>N/A</td></tr>
<tr><td>c.6937+2</td><td>N/A</td></tr>
<tr><td rowspan="2">intron 19</td><td>c.8488-1</td><td>Sup</td></tr>
<tr><td>c.8488-2</td><td>Sup</td></tr>
<tr><td>intron 23</td><td>c.9118-1G>A</td><td>Sup</td></tr>
<tr><td rowspan="2">intron 24</td><td>c.9257-1G>C, G>T</td><td>Sup</td></tr>
<tr><td>c.9257-2</td><td>Sup</td></tr>
</table>
Adapted from ENIGMA, 2017[1] and the ENIGMA VCEP Guidelines v1.0.0</td></tr>
<tr><td>PS1: Same amino acid change as an established variant</td><td>_STR</td><td>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:</td></tr>
<tr><td></td><td>_MOD</td><td></td></tr>
</table>

PM4: Protein-length-changing variant	_SUP	• 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 or PM4_mod for a variant 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
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 mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation	_STR _MOD _SUP	
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 from multifactorial analysis data is incorporated within the combined scores described in the PP4/BP5 recommendations. 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	_STR _MOD _SUP	Frequency data regarding co-occurrence in trans is incorporated within the combined scores described in the PP4/BP5 recommendations. ***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.
PP4: Phenotypic specificity (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)	_VSTR _STR _MOD _SUP	Published multifactorial analysis data providing likelihood ratios (LR) or log likelihood ratios (LLR) encompassing multiple evidence types can be applied under PP4/BP5. The **combined score** should be used, representing 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; conversion of an LLR to an LR can be done using the =EXP(LLR) function within Excel if a natural log has been used or the =10^LLR function in Excel if log to base 10 has been used. 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/, where 'ACMG LLR' is equivalent to Evidence (Exponent) points. **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

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

		weaker strength of the two categories it lies between (eg MOD for 3 points)

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 MTAF (maximum tolerated allele frequency) has been calculated using cardiodb using the calculate AF function: prevalence 0.125; genetic heterogeneity 0.01; allelic heterogeneity 1 (BA1) 0.1 (BS1); penetrance 0.72 (BRCA1), 0.69 (BRCA2). See training resources from Miranda Durkie for further details. Cancer-free female controls should be used when determining the maximum allele count / filtering allele frequency See consensus guidelines for further details on the use of cardiodb for calculating the maximum allele count / filtering allele frequency.
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	_STR _SUP	*see PM3

dominant gene/disorder or observed in cis		
BP5: Alternate molecular basis for disease	_VSTR _STR _MOD _SUP	*see PP4 for explanation

Likelihood Ratio	Evidence (Exponent) Points	Evidence Strength towards benignity
0.48 – 0.23	-1	SUP
0.22 – 0.05	-2	MOD
0.049 – 0.00285	-4	STR
<0.00284	-8	VSTR

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

Note: For v1.19 of this guidance, there are two copies (dated 28/09/2023 and 19/10/2023). This version dated 19/10/2023 is the current version, which contains no updates to the guidance, however the below grid has been updated to add more details on the changes made.

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 that CanVIG-UK provide non-white ethnicity counts under PS4	Garrett	CStAG
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
1.19	***26/05/2023***	PS4/PM2/ BA1/BS1	Update of databases to be used in-line with consensus specification.	Garrett	CStAG
1.19	***27/05/2023***	PS4	Update on case-counting approach where variant seen in multiple cases but also observed in control datasets.	Garrett	CStAG
1.19	***15/09/2023***	BA1/BS1	Clarification of MTAF usage and use of the filtering allele frequency.	Callaway	CStAG
1.19	***28/09/2023***	PP4/BP5	Moved multifactorial evidence from PP5/BP6 to PP4/BP5 in alignment with ENIGMA. PP5/BP6 evidence code removed.	CStAG	CStAG
1.19	***29/09/2023***	PM4	Added application at 'Moderate' for in-frame in/dels at specific residues.	Allen	CStAG
1.19	***29/09/2023***	PP1/PM3/ PP4/BP5	Recommendation to use the combined multifactorial score under PP4/BP5, rather than individual subcomponent scores	Garrett	CStAG
1.19	***29/09/2023***	PS4	Change of OR threshold from >10 to >8 for enriched case series where dataset-specific enrichment factors are not available (eg variant count releases from NHSD/NDRS)	CStAG	CStAG
1.19	***29/09/2023***	PVS1	Update of NMD boundary as per ENIGMA VCEP BRCA1 and BRCA2 guidelines	CStAG	CStAG

References

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