

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	<div>_VSTR</div> <div>_STR</div> <div>_MOD</div> <div>_SUP</div>	<p>PHE case control data can be used for case-control analysis:</p> <ul style="list-style-type: none"> 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 PHE case series) and series denominator As this is an enriched series, OR\geq10 is required Current data/denominator counts for base substitutions are available at CanVar-UK For non-base-substitutions i.e. deletions/duplications/insertions, PHE counts can be accessed from CanVIG-UK For details of variant frequencies in non-white ethnicities, please contact CanVIG-UK <p>If there are insufficient data to perform case-control analyses, PS4_sup can be applied:</p> <ul style="list-style-type: none"> if there are observations of the variant in \geq5 different families 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)
	<div>_MOD</div> <div>_SUP</div>	<p>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</p>

PVS1: Predicted null variant (in a gene where LOF is a known mechanism of disease)	_VSTR	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 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.																			
	_STR																				
	_MOD																				
	_SUP																				
		<table><tr><th>Gene</th><th>Region</th><th>Bases</th></tr><tr><td rowspan="13">BRCA1</td><td>intron 5 (exon 5 donor)</td><td>c.301+1 c.301+2</td></tr><tr><td>intron6 (exon 7 acceptor)</td><td>c.442-1 c.442-2</td></tr><tr><td rowspan="8">introns 8,9</td><td>c.548-1 c.548-2 c.593+1 c.593+2 c.594-1 c.594-2 c.670+1 c.670+2</td></tr><tr><td>intron 10 (exon 10 donor)</td><td>c.4096+1 c.4096+2</td></tr><tr><td>intron 11 (exon 12 acceptor)</td><td>c.4186-1 c.4186-2</td></tr><tr><td>intron12 (exon 13 acceptor)</td><td>c.4358-1 c.4358-2</td></tr><tr><td rowspan="4">BRCA2</td><td rowspan="4">intron12</td><td>c.6842-1 c.6842-2 c.6937+1 c.6937+2</td></tr></table> Adapted from Spurdle et al, 2017 ¹	Gene	Region	Bases	BRCA1	intron 5 (exon 5 donor)	c.301+1 c.301+2	intron6 (exon 7 acceptor)	c.442-1 c.442-2	introns 8,9	c.548-1 c.548-2 c.593+1 c.593+2 c.594-1 c.594-2 c.670+1 c.670+2	intron 10 (exon 10 donor)	c.4096+1 c.4096+2	intron 11 (exon 12 acceptor)	c.4186-1 c.4186-2	intron12 (exon 13 acceptor)	c.4358-1 c.4358-2	BRCA2	intron12	c.6842-1 c.6842-2 c.6937+1 c.6937+2
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		BRCA2	intron12	c.6842-1 c.6842-2 c.6937+1 c.6937+2																	
				PS1: Same amino acid change as an established variant	_STR		Within forthcoming ENIGMA 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: <ul style="list-style-type: none">Use of PM1_sup/PM4_sup for any variant within BRCA1 RING (aa 1-101), BRCT (aa1650-1863) COILED-COIL DOMAIN (aa 1391-1424) and BRCA2 DNA-binding domain (aa 2481-3186)Use of PM1_mod/PM4_mod for missense at specific residues¹: RING: 22, 37, 39, 41, 44, 61 BRCT: 1685,1688, 1699, 1706, 1708, 1715, 1738, 1764, 1766, 1775, 1787, 1788,1838PM1 cannot be used where functional data are being used for PS3, as per main CanVIG-UK guidancePP2 should not be used for BRCA1/BRCA2Use of PM5, PS1, PP3 otherwise as per CanVIG-UK Consensus Specification														
				PM4: Protein-length-changing variant	_MOD																
	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	<div> <div>_VSTR</div> <div>_STR</div> <div>_MOD</div> <div>_SUP</div> </div>	BRCA1: Findlay et al, 2018 ³ : Strong Bouwman et al, 2020 ⁴ : Strong Fernandes et al, 2019 ⁵ : Supporting Petitalot et al, 2019 ⁶ : Supporting BRCA2: Guidugli et al, 2018 ⁷ /Hart et al, 2019 ⁸ /Richardson et al, 2021 ⁹ : Strong 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	<div> <div>_VSTR</div> <div>_STR</div> <div>_MOD</div> <div>_SUP</div> </div>	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	<div> <div>_STR</div> <div>_MOD</div> <div>_SUP</div> </div>	
PM3: in trans with a pathogenic variant (recessive disorders)	<div> <div>_STR</div> <div>_MOD</div> <div>_SUP</div> </div>	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 <i>In addition</i> , 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: <ul style="list-style-type: none"> • 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	<div> <div>_VSTR</div> <div>_STR</div> <div>_MOD</div> <div>_SUP</div> </div>	Published multifactorial analysis data providing likelihood ratios (LR) or log likelihood ratios (LLR) may be used as data sources encompassing: <ul style="list-style-type: none"> • Segregation (PP1/BS4)

to perform an independent evaluation

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

Where individual likelihood ratios for a particular evidence type do not line up with evidence (exponent) points required for a specific evidence strength, the **combined LLR/LR** encompassing multiple evidence types can be used instead to represent the totality of evidence and applied within PP5

Suitable analyses:

- Easton et al, 2007¹⁰
- Lindor et al, 2011¹¹
- Parsons et al, 2020¹²

If evidence is supplied as LLR (log likelihood ratio, eg Easton et al, 2007), this equates directly to the Evidence (Exponent) Points

If evidence is supplied as LR (likelihood ratio, e.g. Parsons et al, 2020) this should be converted to Evidence (Exponent) points

Likelihood Ratio	Evidence (Exponent) Points	Evidence Strength
2.1	1	SUP
4.3	2	MOD
9	3	
18.7	4	STR
38.9	5	
81	6	
168.4	7	
350.4	8	VSTR

OR

PP5 can be applied at supporting level on the basis of any classification of LP/P after 2018 using ACMG classification from:

- ≥2 accredited North American commercial diagnostic laboratories OR
- ≥1 North American commercial diagnostic laboratory where there is explicit citation of utilisation of otherwise unavailable evidence from their data series OR
- approved ClinGen Expert Group (3 star on ClinVar), ie ENIGMA

This is an **exceptional** application, as per UK-ACGS specification. For conflicts with ENIGMA classifications, contact ENIGMA

PP4: Phenotypic specificity
(Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)

_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 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	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)
	_STR	
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 (αα 1-101), BRCT (αα1650-1683) COILED-COIL DOMAIN (αα 1391-1424) and BRCA2 DNA-binding domain (αα 2481-3186)
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 <i>in vitro</i> or <i>in vivo</i> functional studies show no damaging effect on protein function or splicing	_STR	
	_MOD	
	_SUP	
BS4: Non segregation with disease	_STR	*see PP1
	_SUP	
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis	_STR	*see PM3
	_SUP	
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	_STR	*see PP5
	_SUP	

		<table> <tr> <th>Likelihood Ratio</th><th>Evidence (Exponent) Points</th><th>Evidence Strength</th></tr> <tr> <td>0.48</td><td>-1</td><td>SUP</td></tr> <tr> <td>0.23</td><td>-2</td><td></td></tr> <tr> <td>0.11</td><td>-3</td><td></td></tr> <tr> <td>0.05</td><td>-4</td><td>STR</td></tr> </table>	Likelihood Ratio	Evidence (Exponent) Points	Evidence Strength	0.48	-1	SUP	0.23	-2		0.11	-3		0.05	-4	STR
Likelihood Ratio	Evidence (Exponent) Points	Evidence Strength															
0.48	-1	SUP															
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0.05	-4	STR															
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, 2018³ and Bouwman et al, 2020⁴ 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 \pm 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.

References

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