RAD51C/RAD51D: CanVIG-UK Gene-Specific Guidance

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CanVIG-UK review of *RAD51C/RAD51D* May 2023: Consensus to use relevant recommendations from the ClinGen *ATM* VCEP guidance (attached and also available at: https://clinicalgenome.org/affiliation/50039/) for *RAD51C/RAD51D* variants reported under indication R207 and/or R208 of the UK Genomic Test Directory. This scope of this test indication currently includes truncating variants (defined as: nonsense, frameshift and canonical splice site (+/- 1/2) variants). Additional points of specification are given below. Evidence items in grey are not relevant to truncating variants.

This guidance is intended for use in classification of truncating variants only as per current UK Test Directory.

Evidence towards Pathogenicity

Evidence element and evidence strengths allowed		Thresholds/data-sources/applications specifically relevant to *RAD51C* and *RAD51D*
PS4: Case-control: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls	_STR	As per *ATM* VCEP guidance.
PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC	_SUP	As per *ATM* VCEP guidance.
PVS1: **Predicted null variant** (in a gene where LOF is a known mechanism of disease)	_VSTR _STR _MOD _SUP	For *RAD51C*, as per draft *RAD51C* VCEP guidance: • PVS1 is not applicable for the initiation codon or for any nonsense variants 5' of p.M10 • PVS1_vstr may be applied for exon deletions affecting the Walker-A region (p.125-132) • PVS1_vstr may be applied for exon deletions removing >10% of the protein (<113nt, <38 aa) • For the following 1,2 splice sites, apply PVS1 at the following evidence strengths:

Position	Strength	Position	Strength
c.145+1	VSTR	c.145+2	VSTR
c.146-1	VSTR	c.146-2	VSTR
c.404+1	VSTR	c.404+2	VSTR
c.405-1	VSTR	c.405-2	VSTR
c.571+1	VSTR	c.571+2	VSTR
c.572-1	VSTR	c.572-2	VSTR
c.705+1	VSTR	c.705+2	VSTR
c.706-1	VSTR	c.706-2A>C, A>T	VSTR
		c.706-2A>G	STR
c.837+1	STR	c.837+2	STR
c.838-1	VSTR	c.838-2	VSTR
c.904+1	VSTR	c.904+2	VSTR
c.905-1	STR	c.905-2	STR
c.965+1	STR	c.965+2	STR
c.966-1	SUP	c.966-2	SUP
c.1026+1	STR	c.1026+2	STR
c.1027-1	N/A	c.1027-2	N/A

- Otherwise follow *ATM* VCEP guidance.

For *RAD51D* follow *ATM* VCEP guidance.

PS1: Same amino acid change as an established variant	_STR _MOD	As per *ATM* VCEP guidance.
PM4: Protein-length-changing variant	_MOD	As per *ATM* VCEP guidance.
PM5: Novel missense change at an amino acid residue where a different missense change determined to be pathogenic seen before	_SUP	Per *ATM* VCEP guidance, PM5 may be applied at supporting level for truncating variants if nonsense mediated decay (NMD) is predicted. For *RAD51C* and *RAD51D* specific boundaries for NMD, please see below: • *RAD51C*: NMD predicted if the variant is 5' of p.Leu326 • *RAD51D*: gene-specific NMD boundary not yet established; assume NMD unless in the final exon or the last 50 base pairs of the penultimate exon.
PP3: In silico: Multiple lines of computational evidence support a deleterious effect on the gene or gene product	_SUP	As per *ATM* VCEP guidance.
PM1, PP2: Enrichment/constraint:		PM1/PP2 N/A for truncating variants.

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		
PS3: Functional: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product	_MOD _SUP	No functional assays in *RAD51C/RAD51D* assessed by CanVIG-UK.
PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease		N/A as per *ATM* VCEP guidance
PS2/PM6: De novo (maternity and paternity confirmed/unconfirmed) in a patient with the disease and no family history		N/A as per *ATM* VCEP guidance
PM3: in trans with a pathogenic variant (**recessive disorders)**	_VSTR _STR _MOD _SUP	For *RAD51C*, please see the CanVIG-UK *BRCA2* guidance relating to biallelic Fanconi anaemia.
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation		N/A as per *ATM* VCEP guidance
PP4: Phenotypic specificity (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)		N/A as per *ATM* VCEP guidance

Evidence towards Benignity

BA1/BS1: Allele frequency is "too high" in ExAC or gnomAD for disorder	_SA _STR	*RAD51C* draft VCEP guidance states MTAF of: BA1: 0.000583 (0.0583%); BS1: 0.0000583 (0.00583%) Use these MTAFs for *RAD51C* and *RAD51D*. The MTAF (maximum tolerated allele frequency) has been calculated using cardiodb using the calculate AF function:

		prevalence 1 in 78; genetic heterogeneity 0.01; allelic heterogeneity 1 (BA1) 0.1 (BS1); penetrance 0.11 . Cancer-free controls should be used when determining the maximum allele count / filtering allele frequency; therefore it is permissible to use the cancer-free PopMAX FAF on gnomAD against the MTAF cutoffs for BA1/BS1. See consensus guidelines for further details on PopMAX FAF, and 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		N/A as per *ATM* VCEP guidance
BP4: In silico: Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)	_SUP	As per *ATM* VCEP guidance
BP1: Missense variant in a gene for which primarily truncating variants are known to cause disease		N/A as per *ATM* VCEP guidance
BP7: Synonymous (silent) variant for which splicing prediction algorithms predict no impact to the splice consensus sequence	_STR _MOD _SUP	As per *ATM* VCEP guidance (BP7_O)
BP3: In-frame deletions/insertions in a repetitive region		N/A as per *ATM* VCEP guidance
BS3: Well-established *in vitro* or *in vivo* functional studies show no damaging effect on protein function or splicing	_MOD _SUP	No functional studies assessed by CanVIG-UK
BS4: Non segregation with disease		N/A as per *ATM* VCEP guidance
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis	_STR _SUP _SUP	As per *ATM* VCEP guidance
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation		N/A as per *ATM* VCEP guidance
BP5: Alternate molecular basis for disease		N/A as per *ATM* VCEP guidance

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.0	28/09/2023	--	Initial Version	--	CStAG