MMR: CanVIG-UK Gene-Specific Guidance

Date: 25/05/2022 Version: 1.5

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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 MLH1, MSH2, MSH6, PMS2
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. For MLH1 and MSH2, all non-cancer NFE gnomAD controls (both male and female) can be used, but for **MSH6** and **PMS2** only, female non-cancer NFE gnomAD controls should be used. • 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 • For details of variant frequencies in non-white ethnicities, please contact CanVIG-UK • A variant observation cannot be included within the case count used for PS4 case-control analyses if the same family has been used for family history scoring within PP4
PP4: Phenotypic specificity/case counting (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)	_VSTR _STR _MOD _SUP	**Tumour scoring:** **Evidence Points** / **Cellular/molecular phenotype** 0.5: For MLH1 variant with MLH1 promoter methylation status unknown • MSI high **AND/OR** • Loss on immunohistochemistry (IHC) of MLH1+PMS2 **AND/OR** • Loss of MLH1 on IHC (PMS2 IHC status unknown) 1: Informative LOH at chromosomal locus of tumour-suppressor gene For MSH2, MSH6 variant • MSI high **AND/OR** • Loss on IHC of same single protein as variant **AND/OR**

	• Loss on IHC of relevant paired mismatch repair proteins e.g. for MSH2 variant, loss of MSH2+MSH6 For MLH1 variant where MLH1 proven normal MLH1 promoter methylation status • MSI high **AND/OR** • Loss of MLH1+PMS2 on IHC **AND/OR** • Loss of MLH1 on IHC (PMS2 IHC status unknown) For PMS2 variant • MSI high **AND/OR** • Loss of PMS2 alone on IHC **AND/OR** • Loss of MLH1+PMS2 on IHC

- Points can be counted for MSI or IHC, not both
- Points can be counted for both LOH AND MSI/IHC
- Only individuals proven to carry the germline variant can contribute tumour data
- Multiple independent primary tumours can be counted from a single individual

Family History Scoring:

	Isolated single primary or first cancer in proband/family (≥50, 40-49, <40)	Additional family members* or cancers in proband; for each cancer (≥50, 40-49, <40):	Evidence Points
Colon (CRC), Endometrium (EC), TCC (renal pelvis/ureter only), small bowel	(2, 4, 6)	(4, 6, 8)	Divide the sum of family history scores across available families by 7 to get the evidence points • 7= 1EP= sup • 14= 2EP= mod
Rectum, ovary, gastric, hepatobiliary, pancreas, TCC (bladder)	(1, 2, 3)	(2, 3, 4)	

- These scores have been derived from odds ratios of detection of MMR variants in Manchester data series (courtesy of Evans, Woodward)
- *For a multiplex family cluster of ≤3 cases, relatives should be FDRs of each other. In a family cluster of ≥4 cases, one unaffected intervening relative is allowed within the cluster
- The proband is the youngest case in the family with CRC/EC
- A family can only be included for family history scoring when there is concordant tumour data available supporting mismatch repair deficiency (i.e. MSI/IHC)
- A family cannot be used for family history scoring for PP4 if the same family has already been included within the case counts for case-control analyses within PS4 (but can be used for tumour scoring)
- The variant must be present in ≤2 individuals from the Non-Finnish European non-cancer population from gnomAD v2 and ≤1 individuals from each other ethnic group within the non-cancer populations of gnomAD v2
- Where family history score influences final classification (e.g. at VUS/likely pathogenic boundary), cancer family history should be confirmed through cancer registry

- The tumour scoring and family history scoring should be used in combination
- A single family can contribute no more than 2 evidence points
- The same individual can contribute to both tumour and family history scoring
- A maximum of 2 evidence points can be awarded for a single publication
- Cases used for PM3 (biallelic)/PP1(segregation) cannot be used additionally for tumour/family history scoring within PP4

PM2: Absent from controls (or at extremely low frequency if recessive) in ESP, 1000GP, or ExAC	_MOD _SUP	For **MSH6 and PMS2** only female non-cancer gnomAD controls should be used.
PVS1: **Predicted null variant** (in a gene where LOF is a known mechanism of disease)	_VSTR _STR _MOD _SUP	Based on InSiGHT recommendations for initiation codon variants, the following PVS1 strengths apply to truncating variants identified in the first 100 bp of the MMR genes: MLH1 – very strong MSH2 – do not use MSH6 – strong PMS2 – strong
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 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	
PP1: Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease	_VSTR _STR _MOD _SUP	Cases already used for tumour/family history scoring in PP4 cannot additionally be used for PP1

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	A constitutional mismatch repair deficiency (CMMRD) phenotype can be used for PM3 application Cases already used for tumour/family history scoring in PP4 cannot additionally be used for PM3
PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation	_VSTR _STR _MOD _SUP	

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 **controls** should be used
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	
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	
BP2: Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis	_STR _SUP	
BP6: Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation	_STR _SUP	
BP5: Alternate molecular basis for disease	_SUP	

Version History/Amendments

Revised version	Date	Section	Update	Amended by	Approved by
1.4	***03/11/2021***	PP4/PS4	Tumour and family history scoring information combined together in PP4, tumour scoring system updated. Ordering of evidence criteria amended.	Turnbull	CStAG
1.4	***02/12/2021***	PS4	Addition of guidance on using NHSD data for case-control analyses	Garrett	CStAG
1.4	***17/12/2021***	PVS1	Addition of recommendations for truncating variants within first 100bp	Callaway	CStAG
1.4	***17/12/2021***	PS4/PM2	Addition of recommendation for non-cancer female controls to be used for PMS6 and PMS2	Turnbull	CStAG
1.4	***05/01/2022***	PM3/PP1/ PP4	Clarification that a case cannot be used for PP4 if has already been used for PM3/PP1 and vice versa	Garrett	Turnbull
1.5	***25/05/2022***	PM2	Removal of requirement for gnomAD controls to be NFE	Garrett	CStAG

References

1. Thompson BA, Spurdle AB, Plazzer J-P, et al. Application of a five-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants lodged on the InSiGHT locus-specific database. *Nature Genetics* 2014;46(2):107-115. doi:10.1038/ng.2854 [published Online First: 2013/12/22]