MMR: 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 CancerSusceptibility 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. (i.e. both male and female UKBiobank controls can 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 • 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	
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 initiation codon variants and 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 MTAF (maximum tolerated allele frequency) has been calculated via cardiodb using the calculate AF function: prevalence 1 in 15 (*MLH1*), 1 in 15 (*MSH2*), 1 in 36 (*MSH6*), 1 in 36 (*PMS2*); genetic heterogeneity 0.01; allelic heterogeneity 1.0 (BA1) 0.1 (BS1: *MLH1*, *MSH2*, *PMS2*) 0.15 (*MSH6*); penetrance 0.45 (*MLH1*), 0.43 (*MSH2*), 0.41 (*MSH6*), 0.12 (*PMS2*). See training resources from Miranda Durkie for further details. 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	_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
1.6	***30/06/2023***	PS4	Update on case-counting approach where variant seen in multiple cases but also observed in control datasets.	Allen/Garrett	CStAG
1.6	***04/07/2023***	PVS1	Clarification that guidance applies to initiation codon variants	Allen	CStAG
1.6	***04/07/2023***	PS4/PM2	Update of wording to match consensus specification, and removal of sex-matching as requirement for MSH6 and PMS2	Allen/Garrett	CStAG
1.6	***23/10/2023***	BA1/BS1	Clarification of MTAF usage and filtering allele frequency. Addition and clarification of data used in calculation of MTAF for each gene.	Callaway	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]