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 Cancer Susceptibility Genes.

Evidence towards Pathogenicity

<table>
<tr><th colspan="2">Evidence element and evidence strengths allowed</th><th>Thresholds/data-sources/applications specifically relevant to MLH1, MSH2, MSH6, PMS2</th></tr>
<tr><td>PS4: Case-control: The prevalence of the variant in affected individuals is significantly increased compared with the prevalence in controls</td><td>_VSTR
_STR
_MOD
_SUP</td><td>For cases identified, use the below table to generate a total score using cancer type identified in the proband/family and number of cancers/family members applicable:

<table>
<tr><th></th><th>Isolated single primary or first cancer in proband/family (≥50, 40-49, <40)</th><th>Additional family members* or cancers in proband; for each cancer (≥50, 40-49, <40):</th></tr>
<tr><td>Colon, Endometrium, TCC (renal pelvis/ureter only), small bowel</td><td>(2,4, 6)</td><td>(4,6,8)</td></tr>
<tr><td>Rectum, ovary, gastric, hepatobiliary, pancreas, TCC (bladder)</td><td>(1,2,3)</td><td>(2,3,4)</td></tr>
</table>
*For a multiplex family cluster of ≤3 cases, relatives should be FDRs of each other; in family cluster of ≥4 cases, one unaffected intervening relative is allowed within the cluster. These scores have been derived from odds ratios of detection of MMR variants in Manchester data series (courtesy of Evans, Woodward)

The total score from this table provides a guideline for evidence strength where the variant is present in ≤ 1 in 63,399 NFE in GNOMAD:
• 7 points: PS4_sup (≥1 family)
• 14 points: PS4_mod (≥1 family)
• 28 points: PS4_str (≥2 families)
• 56 points: PS4_vstr (≥3 families)</td></tr>
</table>

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	
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	
PS2/PM6: De novo (maternity and paternity confirmed/unconfirmed) in a patient with the disease and no family history	_STR _MOD _SUP	

<table>
<tr><td>PM3: in trans with a pathogenic variant (recessive disorders)</td><td>_STR
_MOD
_SUP</td><td>A constitutional mismatch repair deficiency (CMMRD) phenotype can be used for PM3 application</td></tr>
<tr><td>PP5: Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation</td><td>_VSTR
_STR
_MOD
_SUP</td><td></td></tr>
<tr><td>PP4: Phenotypic specificity (Patient's phenotype or family history is highly specific for a disease with a single genetic aetiology)</td><td>_STR
_MOD
_SUP</td><td>Points for tumour phenotype can be applied using the table below. Up to two orthogonal tumour phenotype assays can be included per case (i.e. can use one of LOH AND one of MSI/IHC/meth). Up to two members per family can be included; only individuals proven to carry the germline variant can contribute tumour data.
<table>
<tr><th>Tumour (evidence) Points</th><th>Level</th><th>Cellular/molecular phenotype</th></tr>
<tr><td rowspan="2">0.5</td><td rowspan="2">-</td><td>Microsatellite instability (MSI)</td></tr>
<tr><td>Loss of immunohistochemistry of MLH1+PMS2</td></tr>
<tr><td rowspan="2">1</td><td rowspan="2">Sup</td><td>Informative LOH at chromosomal locus of tumour-suppressor gene</td></tr>
<tr><td>Loss on immunohistochemistry of same single protein as variant e.g. MSH6 or PMS2</td></tr>
<tr><td rowspan="2">2</td><td rowspan="2">Mod</td><td>Loss on immunohistochemistry of relevant paired mismatch repair proteins e.g. for MSH2 variant loss or loss of MSH2+MSH6</td></tr>
<tr><td>For MLH1 variant, loss of MLH1+PMS2 on immunohistochemistry and normal MLH1 promoter methylation (for MLH1-related mismatch repair deficiency)</td></tr>
</table>
Each feature is assigned evidence points. Evidence points are summed across contributing families, The sum of the evidence points determines evidence strength:
• 1 evidence point from tumour features: PP4_sup
• 2 evidence point from tumour features: PP4_mod
• 4 evidence point from tumour features: PP4_str</td></tr>
</table>

Evidence towards Benignity

<table>
<tr><td>BA1/BS1: Allele frequency is "too high" in ExAC or gnomAD for disorder</td><td>_SA
_STR</td><td>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</td></tr>
<tr><td>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</td><td>_STR
_SUP</td><td></td></tr>
</table>

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	

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]