**ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1**

This version is specified for the following genes:
- MLH1
- MSH2
- MSH6
- PMS2

**DRAFT VERSION**

These guidelines are under review and not yet approved.

**Related publication(s):**
- Visit [https://www.clinicalgenome.org/docs/assertion/50099](https://www.clinicalgenome.org/docs/assertion/50099)

**Date Approved:**
- Visit [https://www.clinicalgenome.org/docs/assertion/50099](https://www.clinicalgenome.org/docs/assertion/50099)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Disease (MONDO ID)</th>
<th>Transcript</th>
<th>Gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>Lynch syndrome I (MONDO: 0007356)</td>
<td>NM_000025.5</td>
<td>MLH1</td>
</tr>
<tr>
<td>MSH2</td>
<td>Lynch syndrome I (MONDO: 0007356)</td>
<td>NM_000027.2</td>
<td>MSH2</td>
</tr>
<tr>
<td>MSH6</td>
<td>Lynch syndrome I (MONDO: 0007356)</td>
<td>NM_000027.2</td>
<td>MSH6</td>
</tr>
<tr>
<td>PMS2</td>
<td>Lynch syndrome I (MONDO: 0007356)</td>
<td>NM_000024.9</td>
<td>PMS2</td>
</tr>
</tbody>
</table>

### PATHOGENIC CRITERIA

<table>
<thead>
<tr>
<th>Criteria Description</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsense/Terminal variant introducing Premature Termination Codon</td>
<td>Reference to Appendix for details.</td>
</tr>
</tbody>
</table>

### VERY STRONG CRITERIA

<table>
<thead>
<tr>
<th>Criteria Description</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large genomic deletions of single or multi-exon size, specifically:</td>
<td>Refer to Appendix for details.</td>
</tr>
<tr>
<td>Very Strong Criteria</td>
<td>Ref. PVS1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gene-Specific</th>
<th>General</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVS1</td>
<td>Refer to Appendix for details.</td>
</tr>
<tr>
<td></td>
<td>1) ≤ codon 753 in MLH1</td>
</tr>
<tr>
<td></td>
<td>2) ≤ codon 891 in MSH2</td>
</tr>
<tr>
<td></td>
<td>3) ≤ codon 1341 in MSH6</td>
</tr>
<tr>
<td></td>
<td>4) ≤ codon 798 in PMS2</td>
</tr>
</tbody>
</table>

**Expert Panel Page:** [https://www.clinicalgenome.org/affiliation/50099](https://www.clinicalgenome.org/affiliation/50099)
<table>
<thead>
<tr>
<th>Recommendation</th>
<th>PVS1-Strong</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOT predicted to undergo NMD then use PVS1-Moderate.</td>
<td></td>
</tr>
<tr>
<td>If exon skipping or use of a cryptic splice site disrupts reading frame and the altered region is critical to protein function then use PVS1-Strong.</td>
<td></td>
</tr>
<tr>
<td>If exon skipping or use of a cryptic splice site preserves reading frame and the altered region is not critical to protein function then use PVS1-Moderate.</td>
<td></td>
</tr>
</tbody>
</table>

**PVS1**

Variants at IVS±1 or IVS±2 where exon skipping or use of a cryptic splice site disrupts reading frame and is predicted to undergo NMD.

- If exon skipping or use of a cryptic splice site is NOT predicted to undergo NMD then use PVS1-Moderate.
- If exon skipping or use of a cryptic splice site disrupts reading frame and is NOT predicted to undergo NMD then use PVS1-Moderate.

**General recommendation**

- Large genomic duplications shown by laboratory studies (which define the breakpoints of the duplication) to result in an in-frame insertion OR deletion genes: duplications shown by laboratory studies (which define the breakpoints of the duplication) to result in a frameshift before the last splice junction.
- Large genomic duplications shown by laboratory studies (which define the breakpoints of the duplication) to result in an in-frame deletion genes: duplications shown by laboratory studies (which define the breakpoints of the duplication) to result in a frameshift before the last splice junction.
<table>
<thead>
<tr>
<th>Strength</th>
<th>General Recommendation</th>
<th>Gene-Specific Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVS1-Strong</td>
<td>Variants where mRNA assays using RNA derived from patient tissue are consistent with a predicted splice site mutation.</td>
<td>No a predicted splice site mutation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mRNA assay from independent laboratory is his own.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Combined with evidence from functional domain or protein expression.</td>
</tr>
</tbody>
</table>

**STRONG CRITERIA**

- **PVS1-VeryStrong**: Presumed by default in tandem duplication of ≥1 exon resulting in a frameshift before the last splice junction. This rule does not apply for variants involving the UTR (i.e., exon 1 or last exon) and whose gene expression that involve the UTR (i.e., exon 1 or last exon) and whose gene expression is critical for the onset of disease. This rule does not apply for variants involving the UTR (i.e., exon 1 or last exon) and whose gene expression is critical for the onset of disease.

**Gene-Specific Strengths**

- **PVS1-Strong**: Splicing aberration must be confirmed in a minigene assay or an additional RNA assay from an independent laboratory if is not a predicted splice site mutation.
- **PVS1-Moderate**: Presumed by default in tandem duplication of ≥1 exon resulting in a frameshift before the last splice junction. This rule does not apply for variants involving the UTR (i.e., exon 1 or last exon) and whose gene expression is critical for the onset of disease.

**General Recommendation**

- Variants in the initiation codon of MLH1 and MSH2 for MSH2 further ATGs exist in exon 2, so this criterion is not applicable at any evidence weight.
- Variants in the initiation codon of MSH6 and PMS2 for MSH2 further ATGs exist in exon 2, so this criterion is not applicable at any evidence weight.

**Variants where mRNA assays using RNA derived from patient tissue are consistent with a predicted splice site mutation.**

- mRNA assay from independent laboratory is his own.
- Combined with evidence from functional domain or protein expression.

**Variants involving the UTR (i.e., exon 1 or last exon) and whose gene expression is critical for the onset of disease.**

- This rule does not apply for variants involving the UTR (i.e., exon 1 or last exon) and whose gene expression is critical for the onset of disease.

**Splicing aberration must be confirmed in a minigene assay or an additional RNA assay from an independent laboratory if is not a predicted splice site mutation.**

- Combined with evidence from functional domain or protein expression.
- mRNA assay from independent laboratory is his own.
### Disease-Specific

<table>
<thead>
<tr>
<th>General Recommendation</th>
<th>Disease-Specific Recommendation</th>
<th>PVS1</th>
<th>PSS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n/a predicted splice site detected</td>
<td>n/a established class 5 pathogenic missense variant (RNA not tested and not a predicted missense substitution that encodes the same amino acid substitution)</td>
<td>OR MMRF functional Odds for Pathogenicity &gt; 18.72</td>
<td>OR CIMRA functional Odds for Pathogenicity &gt; 18.72</td>
</tr>
<tr>
<td>PS2</td>
<td>PS2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PS2</td>
<td>PS2</td>
<td></td>
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<tr>
<td>PS2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>PS2</td>
<td>PS2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Strength

- **PVS1-Strong**: if confirmed to cause a splice defect, then PVS1 should be used instead.
- **PS2-Strong**: non-G at least base 6 of exon if first 6 bases of the intron are not GTRRGT.
- **PSS-Strong**: confirmed to cause a splice defect, then PSS should be used instead.

**Related Publication(s):**

Visit https://www.clinicalgenome.org/affiliation/50099

**Date Approved:**

Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion-criteria for the most recent version.

---

**ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1**

This version specified for the following genes:

- MLH1
- MSH2
- MSH6
- PMS2

These guidelines are under review and not yet approved.
<table>
<thead>
<tr>
<th>Genes-Specific, Gene-Specific</th>
<th>Strength</th>
<th>PP1_Strong</th>
<th>PP2_Strong</th>
<th>PP4_Strong</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>N/A</td>
<td>PP1_Strong</td>
<td>PP2_Strong</td>
<td>PP4_Strong</td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>N/A</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PP1_Strong**
- ≥3 independent CRC/EMT/H tumors in ≥2 families
- Standard panel of 5-10 markers
- Consistent protein expression consistent with variant location
- For multiple pedigrees, results are combined.
- MLH1 variants, MLH1 promoter methylation is to be excluded in the tumors.
- MLH1, MSH2, MSH6, PMS2
- Between codons 754, 755, 756 in MLH1; between codons 892, 894 in MSH2; between codons 1342, 1360 in MSH6; between codons 799, 801 in PMS2.
- Refer to Appendix for details.

**PP2_Strong**
- Increased compared with the prevalence in controls.
- For multiple pedigrees, results are combined.
- Consistent protein expression consistent with variant location.
- For multiple pedigrees, results are combined.
- MLH1 variants, MLH1 promoter methylation is to be excluded in the tumors.
- MLH1, MSH2, MSH6, PMS2
- Between codons 754, 755, 756 in MLH1; between codons 892, 894 in MSH2; between codons 1342, 1360 in MSH6; between codons 799, 801 in PMS2.
- Refer to Appendix for details.

**PP4_Strong**
- ≥3 independent CRC/EMT/H tumors in ≥2 families
- Standard panel of 5-10 markers
- Consistent protein expression consistent with variant location
- For multiple pedigrees, results are combined.
- MLH1 variants, MLH1 promoter methylation is to be excluded in the tumors.
- MLH1, MSH2, MSH6, PMS2
- Between codons 754, 755, 756 in MLH1; between codons 892, 894 in MSH2; between codons 1342, 1360 in MSH6; between codons 799, 801 in PMS2.
- Refer to Appendix for details.
<table>
<thead>
<tr>
<th>Disease-Specific</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1, MSH2, MSH6, PMS2</td>
<td>To use PM3 criteria, the variant has to meet PM2-Supporting criteria.</td>
</tr>
</tbody>
</table>

**PM3**
- Use CMMRD “indication criteria” from Table 2. If ≥2 points then it is consistent with CMMRD.
- Use CMMRD “indication criteria” from Table 3 and Table 4. Documented MMR deficiency in normal cells (Table 3 and Table 4) or patient with clinical features consistent with CMMRD and/or pathogenic/likely pathogenic sequence variant in the same gene in a co-occurrence (in trans/phased unknown) with a known pathogenic/likely pathogenic sequence variant.

**Strength**

<table>
<thead>
<tr>
<th>N/A</th>
<th>PM1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Functional domain located in a mutational hot spot or critical and well-established</td>
</tr>
</tbody>
</table>

**PM2**
- Point per proband – can be combined with PS2/PM6 points to increase evidence strength as per Table 1.RODUCTION.

**PS2**
- De novo variants with both maternal and paternal confirmed (Table 3). If four variant alleles, then use PS2-Very Strong.

**PM3**
- If the previously classified variant is Class 5, then use PS2-Very Strong.
- Missense variant at a functional level for RNA splicing.
- Missense variant that is splice donor.
- Missense variant that is splice acceptor.
- Missense variant that is stop gain or frameshift.
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- Missense variant that is stop gain or frameshi

<table>
<thead>
<tr>
<th>Disease-Specific</th>
<th>PM6</th>
<th>General Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>case with 15 section tumour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>or no data for</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pathogenic point.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**PM4**

**Protein length changes due to in-frame deletions/insertions in a non-coding region of stop-loss variants.**

- Sum all cases with the above evidence to determine the PM3 strength as points.
- Heterozygous occurrence (max points from homoygotes = 1.0): 0.5
- Pathogenic/Likely pathogenic in trans: 1.0 point, Pathogenic/Likely pathogenic in cis: 0.5 point

**PM5**

- Missense changes at an amino acid residue where a different missense change was classified as Class 5 pathogenic on the protein level and not only due to aberrant splicing.
- Use PM5 if PM3 is supporting for the missense change.

**PM5_Supporting**

- Variants affecting the same splice site as a confirmed splice variant with.

**PM6**

- Assumed de novo with maternity and/or paternity unconfirmed in a.
- Pathogenic from http://predictkinetik phenome/bioinfo/PBuilPs)
- Pathogenic, missense, splice, or frameshift on the protein level and other variants affecting the same splice site as a confirmed splice variant with.

**PM7**

- Variants affecting the same splice site as a confirmed splice variant with.

**PM8**

- Variants affecting the same splice site as a confirmed splice variant with.
### Draft version

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# InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes:

- MLH1
- MSH2
- MSH6
- PMS2

## DRAFT VERSION

### These guidelines are under review and not yet approved.

### URL

Visit [https://www.clinicalgenome.org/affiliation/50099](https://www.clinicalgenome.org/affiliation/50099) for the most recent version.

### Date Approved

Refer to [https://www.clinicalgenome.org/affiliation/50099/docs/assertion-criteria](https://www.clinicalgenome.org/affiliation/50099/docs/assertion-criteria) for the most recent version.

---

### Supporting Criteria

#### PP1

**Strength**

Co-segregation with disease in pedigree(s) with a combined Bayes likelihood ratio $>2.08$ and $\leq 4.3$

**PP2**

Supporting evidence for pathogenicity $\geq 18.7$. Can be combined with PS2/PM6 points to increase evidence strength as per Table 1.

#### PP3

**Strength**

CIMRA Functional Odds for Pathogenicity $>4.3$ and $\leq 18.7$.

#### PP4

**Strength**

2 independent CRC/Endometrial MSI-H tumours using a standard panel of 5-10 markers and/or loss of MMR protein expression consistent with the variant location. MSI-H tumour with inconsistent protein expression does not meet PP4_Moderate.

For MLH1 variants, MLH1 promoter methylation is to be excluded in the tumours.

Refer to Appendix for protein expression consistent with variant location.

#### PS2

**Strength**

- Absent/extremely rare (<1 in 50,000 alleles) in gnomAD using the non-cancer dataset.

#### PM2

**Strength**

- Absent/extremely rare (<1 in 50,000 alleles) in gnomAD using the non-cancer dataset.

#### PM3

**Strength**

Co-segregation with disease in pedigree(s) with a combined Bayes likelihood ratio $>4.3$ and $\leq 18.7$.

**Strength**

See [http://priors.hci.utah.edu/PRIORS](http://priors.hci.utah.edu/PRIORS) for functional odds for pathogenicity $>4.3$ and $\leq 18.7$.

**Strength**


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### Related Publication(s)

Visit [https://www.clinicalgenome.org/affiliation/50099](https://www.clinicalgenome.org/affiliation/50099) for the most recent version.
### PP3: Support

- Refer to Appendix for protein expression consistent with variant.
- For MLH1 variants, MLH1 promoter methylation is to be excluded in the tumour.
- Does not meet PP4.

#### Disease-Specific

- Predicted splice defect using recommended splicing algorithms.
  - OR
  - Predicted splice defect using HPPRNS: [link](http://priors.hci.utah.edu/PRIORS)
  - OR
  - See HPPRNS: [link](http://priors.hci.utah.edu/PRIORS)

#### PP4

- CRC/Endometrial MSI-H tumour using a standard panel of 5-10 markers.
- N/B: MSI screen negative.
- Does not meet PP4.

### PP2

- OR

<table>
<thead>
<tr>
<th>PP2</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

---

**Recommended segregation tool:** [COOL](http://fengbj-laboratory.org/cool2/manual.html)

**PP2**

- OR

- OR

- OR

**PP3**

- OR

**PP4**

- OR

**PP5**

- OR

---

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Expert Panel Page: [https://www.clinicalgenome.org/affiliation/50099/docs](https://www.clinicalgenome.org/affiliation/50099/docs)

- Recommended publications:
  - N/A

---

**PP2**

- OR

- OR

- OR

**PP3**

- OR

**PP4**

- OR

**PP5**

- OR

---

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- Recommended publications:
  - N/A
### Strong Criteria

<table>
<thead>
<tr>
<th>Gene-Specific</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM5</td>
<td>Supporting Missense change at an amino acid residue where a different missense change was classified as Class 5 due to absence of evidence.</td>
</tr>
<tr>
<td>PM5</td>
<td>Supporting if PP3 is supporting for the missense change.</td>
</tr>
</tbody>
</table>

### Stand Alone Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>BA1</td>
<td>Variant reported to occur in control reference groups above Maximum Credible Allele Frequency (MCAF) cutoffs and excluded as founder sequence variants (use continental-scale population databases).</td>
</tr>
</tbody>
</table>

### Benign Criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP5</td>
<td>Reputable source recently reports variant as pathogenic but the evidence is not available to the laboratory to perform an independent evaluation.</td>
</tr>
</tbody>
</table>

**Note:**
- SS: Source Score
- MCAF: Maximum Credible Allele Frequency
- BA1: Base Allele Frequency
- PM5: Supporting Missense change at an amino acid residue where a different missense change was classified as Class 5 due to absence of evidence.
Disease-Specific

- Refer to Table 3 for clinical features of CMMRD.
- No previous or current evidence of clinical manifestations of CMMRD cancer above the median age of onset for that cancer in I(5), and who
- share genes in a patient with colorectal cancer after age 45 (or other LS
- gene).

<table>
<thead>
<tr>
<th>Gene-Specific</th>
<th>BS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNS: 0.0028 (0.28%)</td>
<td></td>
</tr>
<tr>
<td>MSH6: 0.0022 (0.22%)</td>
<td></td>
</tr>
<tr>
<td>MSH2: 0.001 (0.1%)</td>
<td></td>
</tr>
<tr>
<td>MLH1: 0.001 (0.1%)</td>
<td></td>
</tr>
</tbody>
</table>

MCAF Cutoffs:

- Variants reported to occur in control reference groups above Maximum Credible Allele Frequency (MCAF) and not yet excluded as
candidate Allele Frequency (MAF)<(MAF) due to the filtering Allele Frequency from the non-cancer dataset.

Gene-Specific

- Refer to Table 3 for clinical features of CMMRD.
- No previous or current evidence of clinical manifestations of CMMRD cancer above the median age of onset for that cancer in I(5), and who
- share genes in a patient with colorectal cancer after age 45 (or other LS
- gene).

<table>
<thead>
<tr>
<th>Gene-Specific</th>
<th>BS2</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNS: 0.0028 (0.28%)</td>
<td></td>
</tr>
<tr>
<td>MSH6: 0.0022 (0.22%)</td>
<td></td>
</tr>
<tr>
<td>MSH2: 0.001 (0.1%)</td>
<td></td>
</tr>
<tr>
<td>MLH1: 0.001 (0.1%)</td>
<td></td>
</tr>
</tbody>
</table>

MCAF Cutoffs:

- Variants present in control reference groups above Maximum Credible Allele Frequency (MCAF) within the Maximum
- Credible Allele Frequency (MCAF) cutoffs and not yet excluded as
candidate Allele Frequency (MAF)<(MAF) due to the filtering Allele Frequency from the non-cancer dataset.

<table>
<thead>
<tr>
<th>Gene-Specific</th>
<th>BS1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MNS: 0.0028 (0.28%)</td>
<td></td>
</tr>
<tr>
<td>MSH6: 0.0022 (0.22%)</td>
<td></td>
</tr>
<tr>
<td>MSH2: 0.001 (0.1%)</td>
<td></td>
</tr>
<tr>
<td>MLH1: 0.001 (0.1%)</td>
<td></td>
</tr>
</tbody>
</table>

MCAF Cutoffs:
## Expert Panel Page:

https://www.clinicalgenome.org/affiliation/50099/docs/assertion

### Related publication(s):

Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion for the most recent version.

---

### BS3

**Variant-specific proficient function in protein and mRNA-based assays**

- OR
  - Synonymous substitutions and intronic variants with no associated mRNA aberrations (either splicing or allelic imbalance) as determined by laboratory assays conducted with nonsense-mediated decay inhibition.
  - Whenever biallelic truncating variants are identified at similar levels in controls and carriers, association studies conducted with nonsense-mediated decay inhibition may be performed (either splicing or allelic imbalance) as determined by synchronous substitutions and intronic variants with no associated mRNA aberrations as per MMR functional assay overview in Figure I.

**Disease-Specific**

- **BP5**
  - Strong
    - BS5
      - For multiple pedigrees, results are combined.
    - BS4
      - Lack of co-segregation with disease in pedigrees with a combined Bayes’ Likelihood Ratio (B/LR) < 0.05.
    - BS3
      - CIMRA: Functional Odds for Pathogenicity ≥ 0.05.

**Strength**

- OR
  - Functional associations they will be considered naturally occurring isoforms and not MMR portion (that is inconsistent with the gene demonstrating genetic phenomenon expression and/or loss of MMR protein expression and/or loss of MMR protein expression and/or loss of MMR protein expression) with MSS and/or loss of MMR protein expression and/or loss of MMR protein expression) with MSS and/or loss of MMR protein expression and/or loss of MMR protein expression.

**DRAFT VERSION**

These guidelines are under review and not yet approved.
## Disease-Specific

<table>
<thead>
<tr>
<th>Supporting Criteria</th>
<th>Bp1</th>
<th>Bp2</th>
<th>Bp3</th>
<th>Bp4</th>
</tr>
</thead>
<tbody>
<tr>
<td>bp1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bp2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bp3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bp4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### BP1
- Missense variant predicted to be pathogenic by PolyPhen-2 and P-LoD 0.01 or lower.

### BP2
- Missense variant in a gene where only loss of function causes disease.

### BP3
- In-frame deletions/insertions in a repetitive region without a known function.

### BP4
- Missense variant with MAPP+PolyPhen-2 prior probability for pathogenicity > 0.01.

### Additional Criteria
- BRAF V600E (CRC only)/MLH1 methylation (in tumour only) with MSI-H drug sensitivity data.

---

**Note:** These guidelines are under review and not yet approved.
<table>
<thead>
<tr>
<th><strong>Strength</strong></th>
<th><strong>Recommended Curation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>General</strong></td>
<td>1. <strong>Recommended splicing algorithms (MaxEntScan, NNSplice, SpliceAI)</strong> suggest no impact on gene or gene product.</td>
</tr>
<tr>
<td></td>
<td>2. Evidence of loss of expression or protein truncation.</td>
</tr>
<tr>
<td></td>
<td>3. Lack of cosegregation with disease in pedigrees with a combined risk of colorectal cancer/Polyposis or endometrial cancer with MSS and/or no loss of MMR protein expression and/or LS spectrum tumors with loss of MMR protein(s) that is inconsistent with the gene demonstrating genetic variation.</td>
</tr>
<tr>
<td></td>
<td>4. 1 BRAF V600E (Colon only)/MLH1 methylation (in tumor only) with MSI.</td>
</tr>
<tr>
<td></td>
<td>5. H+MTH1 loss.</td>
</tr>
<tr>
<td>N/A</td>
<td>6. Reputable source recently reports variant as benign but the evidence is not available to the laboratory to perform an independent evaluation.</td>
</tr>
</tbody>
</table>

**Disease-Specific**

- **BP6**: CIMRA A functional odds for polygenic risk >0.05 & ≤0.48
- **BP7**: Variants may satisfy both BP7 and BP4. A synonymous (silent) or intronic variant at or beyond +7/-21 (5′/3′ exonic) variants may satisfy both BP7 and BP4.
- **BP5**: Recommended segregating populations (exons/5′, NSsplice, Nspliceal).

**BS3** Supporting

- **BS4** Supporting

**BS2** Supporting

- **BS3** Supporting

**BS4** Supporting

- **BS5** Supporting

**BS6** Supporting

- **BS7** Supporting

- **BS8** Supporting

**BS9** Supporting

- **BS10** Supporting
InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

Key:

Disease-Specific: Disease-specific modifications are based on what is known about Lynch Syndrome; Gene-Specific: Gene-specific modifications are based on decreasing strength of criteria based on the amount of evidence; N/A: not applicable for Lynch Syndrome.

Updated guidance for Lynch Syndrome; Strength: Increasing or decreasing strength of criteria; General Recommendation: Criteria is applicable for the original ACMG/AMP guidelines with general notes from the VCEP and/or updates from ClinGen.

Rules for Combining Pathogenic Criteria:

Pathogenic

1. Very Strong AND 1 (a) ≥1 Very Strong OR (b) ≥2 Moderate OR (c) 1 Moderate and 1 Supporting OR (d) ≥2 Supporting

2. ≥2 Strong

3. 1 Strong

4. 2 Strong

5. 1 Strong AND 1 Supporting OR 2 Supporting

6. 1 Moderate AND 2 Supporting

Likely Pathogenic

1. Very Strong AND 1 Moderate

2. Strong AND 1 Moderate

3. Moderate AND 1 Supporting OR 2 Supporting

4. Moderate AND 2 Supporting

5. ≥2 Moderate

6. 1 Moderate AND 2 Supporting OR 2 Supporting

7. 1 Moderate

8. Moderate AND 1 Supporting OR 2 Supporting

9. 1 Moderate and 1 Supporting OR 2 Supporting

N/A: Changes made to existing criteria definitions.

None: No changes made to existing criteria definitions.
RULES FOR COMBINING BENIGN CRITERIA

Benign

1. 1 Strong Alone (BA1)

2. ≥2 Strong and 1 Supporting (BS1-BS4)

Likely Benign

1. 1 Strong and 1 Supporting

2. 2 Supporting
This version specified for the following genes: MLH1, MSH2, MSH6, PMS2

ClinGen InSiGHT Colorectal Cancer/Polyps ACMG Specifications v1

Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion-criteria for the most recent version.

Related publication(s):

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyps Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

DRAFT VERSION

These guidelines are under review and not yet approved.

Date Approved:

Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion-criteria for the most recent version.
Figure 1. Flowchart used to assist in interpretation of functional assay data. Adapted from Thompson et al. 2020.
**ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1.0**

These guidelines are under review and not yet approved.

**Table 1:** The combined point value of all de novo occurrences is used to determine the applicable evidence strength level.

<table>
<thead>
<tr>
<th></th>
<th>4</th>
<th>2</th>
<th>1</th>
<th>0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM6</td>
<td>Very Strong</td>
<td>Strong</td>
<td>Moderate</td>
<td>Supporting</td>
</tr>
<tr>
<td>PM2</td>
<td>Strong</td>
<td>Moderate</td>
<td>Supporting</td>
<td>Very Strong</td>
</tr>
<tr>
<td>PM2</td>
<td>Moderate</td>
<td>Supporting</td>
<td>Strong</td>
<td>Very Strong</td>
</tr>
</tbody>
</table>

**SV1 Recommendation for De Novo Criteria (PM2 & PM6) - Version 2.0**

This version specified for the following genes: MLH1, MSH2, MSH6, PMS2

**SVI Recommendation for In Trans Criterion (PM3) - Version 1.0**

- Supporting
- Moderate
- Strong
- Very Strong

**Figure 2:** Linear schematic of mismatch repair gene functional domains according to amino acid position, adapted from Borras et al., 2018

Related publication(s): https://www.clinicalgenome.org/site/assets/files/3461/svi_proposal_for_de_novo_criteria_v1_0.pdf

Supporting (PM3_Supporting)
Moderate (PM3_Moderate)
Strong (PM3_Strong)
Very Strong (PM3_VeryStrong)
Table 2: Recommendation for determining the appropriate ACMG/AMP evidence strength level for trans-occurrence(s)

<table>
<thead>
<tr>
<th>Scoring</th>
<th>Evidence Strength Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Strong Evidence</td>
</tr>
<tr>
<td>2</td>
<td>Moderate Evidence</td>
</tr>
<tr>
<td>1</td>
<td>Limited Evidence</td>
</tr>
<tr>
<td>0.5</td>
<td>No Evidence</td>
</tr>
</tbody>
</table>

---

These guidelines are under review and not yet approved.

---

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

This version specified for the following genes: MLH1, MSH2, MSH6, PMS2

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Date Approved: [Date]

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DRAFT VERSION

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Related publication(s): [Publication Links]

---

Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion for the most recent version.
The table below provides diagnostic criteria for constitutional mismatch repair deficiency syndrome (CMMRD) as suggested by the European consortium 'Care for CMMRD' (C4CMMRD) and includes test information for comparison.

### Table 3: Diagnostic criteria for constitutional mismatch repair deficiency syndrome

<table>
<thead>
<tr>
<th>Test</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunohistochemistry (IHC) of the 4 MMR genes</td>
<td>High sensitivity and specificity. New normal tissue can be assessed.</td>
<td>Access to normal tissue may not be available.</td>
</tr>
<tr>
<td>Germline Microsatellite Instability (MSI)</td>
<td>Specific for CMMRD.</td>
<td>Uninterpretable results in ~16% of patients.</td>
</tr>
<tr>
<td>Ex vivo MSI + Methylation Tolerance</td>
<td>High sensitivity and specificity. Concordant results with the two tests strengthen interpretation.</td>
<td>Discordant results between tests may require additional ancillary testing.</td>
</tr>
<tr>
<td>Germline or Tissue from a Normal Tissue Tissue biopsy (e.g., skin, normal colon)</td>
<td>Rapid result.</td>
<td>Time to develop lymphoblastic cell line.</td>
</tr>
<tr>
<td>MSI + Methylation Tolerance</td>
<td>Rapid result.</td>
<td>Not widely available commercially.</td>
</tr>
<tr>
<td>MSI + Methylation Tolerance</td>
<td>Rapid result.</td>
<td>Not widely available commercially outside Europe.</td>
</tr>
</tbody>
</table>

**Test**

- MSI: Microsatellite instability
- MMR: Mismatch repair
- CMMRD: Constitutional mismatch repair deficiency
- C4CMMRD: Consortium for Care of CMMRD

**Pros**

- High sensitivity and specificity
- Concordant results with other tests strengthen interpretation

**Cons**

- Uninterpretable results in ~16% of patients
- May require additional ancillary testing
- Not widely available commercially outside Europe

**Test**

- MSI: Microsatellite instability
- MMR: Mismatch repair
- CMMRD: Constitutional mismatch repair deficiency
- C4CMMRD: Consortium for Care of CMMRD

**Pros**

- High sensitivity and specificity
- Concordant results with other tests strengthen interpretation

**Cons**

- Uninterpretable results in ~16% of patients
- May require additional ancillary testing
- Not widely available commercially outside Europe

---

Table 3: Diagnostic criteria for constitutional mismatch repair deficiency syndrome: Suggestions of the European Consortium Care for CMMRD (C4CMMRD)
In vitro repair assay

► High sensitivity and specificity

NGS detection, low-level MSI in tissue

Table 4: Examples of ancillary tests available to assist in CMMRD diagnosis, adapted from Aronson et al. 2009.

<table>
<thead>
<tr>
<th>Test Available</th>
<th>Cost-effective and scalable</th>
<th>NGS detection, low-level MSI in tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widely available</td>
<td>High sensitivity and specificity</td>
<td>High sensitivity and specificity</td>
</tr>
</tbody>
</table>

Footnotes

a PVS1 criteria is adapted from Tayoun et al. 2018.

b A known functional protein domain is reported to harbor sequence variants that introduce deleterious changes to protein function (via missense alteration, protein sequence deletion, or protein truncation in the last exon) AND are associated with high risk of cancer. Physical boundaries for functional domains are shown in Figure 2.

c IVS±1 and IVS±2 are the least invariant nucleotides in a splice site.

d Outbred control reference groups currently used for this purpose: Genome Aggregation Database non-cancer dataset (gnomad.broadinstitute.org).

f As per CMMRD consortium guidelines 5,6.

g Lynch Syndrome (LS) tumours include: colorectal/colon/rectal, endometrial, ovarian, small bowel/small intestine, renal pelvis, ureter, and stomach/gastric carcinomas.

h Penetrance estimates for MLH1 and MSH2 are from Jenkins et al. 2015 and Dowty et al. 2013; MSH6 from Ponsioen et al. 2010; PMS2 from ten Broeke et al. 2015.

These guidelines are under review and not yet approved.

DRIFT VERSION


Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion for the most recent version.

DRAFT VERSION

These guidelines are under review and not yet approved.

Visit https://www.clinicalgenome.org/affiliation/50099/docs/assertion for the most recent version.
Physician's overview of the ACMG/AMP DRAFT V1.0

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Variant Curation Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines Version 1

Visit https://www.clinicalgenome.org/services/50099/docs/assertion to determine whether these guidelines have been approved.

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Date Approved: Visit https://www.clinicalgenome.org/services/50099/docs/assertion for the most recent version.

Important Notes

Gene-specific penetrance estimates are available at https://lscarisk.org/

PMS2, PMS2CL pseudogene homologous regions (exons 11-15)

PMS2 CL variants need confirmation by other orthogonal assays as well as functional assessment (e.g. long-range or CDNA) if variants are located in the last exon.

APPENDIX

Important Notes

Protein expression and consistency with variant location

1. Codon 798 in PMS2 using 50 bp 3' of the penultimate exon.

2. Codon 753 in MLH1 using location of known pathogenic variant MLH1:c.2252_2253del

3. Codon 841 in MSH6 using location of known pathogenic variant MSH6:c.2662delC

4. Codon 789 in PMS2 using 50 bp 3' of the penultimate exon.

Justification for last exon PVS1 boundaries:

1. Nonsense frameshift variant introducing Premature Termination Codon (PTC):

   a) ≤ codon 753 in PMS2 using location of known pathogenic variant PMS2:c.2252_2253del

   b) ≤ codon 798 in PMS2 using 50 bp 3' of the penultimate exon.

Do not combine PP3 criteria with any PVS1 criteria.

Gene-specific penetrance estimates are available at https://lscarisk.org/

PMS2 CL variants need confirmation by other orthogonal assays as well as functional assessment (e.g. long-range or CDNA) if variants are located in the last exon.

APPENDIX
In general, for pathogenic variants: an MLH1 variant is consistent with MLH1 and PMS2 loss, an MSH2 variant is consistent with MSH2 and MSH6 loss, an MSH6 variant is consistent with MSH6 loss and a PMS2 variant is consistent with PMS2 loss.

The decision-making process involves considering both ACMG/Bayesian model and evidence from family data. The probabilities derived from the ACMG/Bayesian model are used to weigh the evidence from other sources. The final decision is made considering all available evidence, including cosegregation and tumor characteristics.

References:
