Criteria Specification

ClinGen InSiGHT Hereditary Colorectal Cancer/Polyposis Expert Panel Specifications to the ACMG/AMP Variant Interpretation Guidelines for MSH6 Version 1.0.0

Affiliation: InSiGHT Hereditary Colorectal Cancer/Polyposis VCEP
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Initial release

Rules for MSH6

Gene: MSH6 (HGNC:7329)
Transcripts:
NM_000179.3

HGNC Name: mutS homolog 6
Disease:
Lynch syndrome (MONDO:0005835) **Mode of Inheritance:** Autosomal dominant inheritance
mismatch repair cancer syndrome 1 (MONDO:0010159) **Mode of Inheritance:** Autosomal recessive inheritance

Criteria & Strength Specifications

PVS1

Original ACMG Summary

Null variant (nonsense, frameshift, canonical +/−1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF) is a known mechanism of disease.
Caveats:
- Beware of genes where LOF is not a known disease mechanism (e.g. GFAP, MYH7).
- Use caution interpreting LOF variants at the extreme 3’ end of a gene.
- Use caution with splice variants that are predicted to lead to exon skipping but leave the remainder of the protein intact.
- Use caution in the presence of multiple transcripts.

Very Strong

Nonsense/frameshift variant introducing Premature Termination Codon (PTC)[a] ≤ codon

1341 in *MSH6*. Refer to Appendix for details.

OR

Large genomic alterations[a] of single or multi-exon size.

OR

Variants at IVS±1 or IVS±2[a,c] where exon skipping or use of a cryptic splice site disrupts reading frame and is predicted to undergo NMD. Not to be combined with PP3 and not to be used for a confirmed splice defect (see PVS1 for variants where patient mRNA assays indicate splicing aberration). If exon skipping or use of a cryptic splice site preserves reading frame and the altered region is critical to protein function[b] then use PVS1_Strong. If exon skipping or use of a cryptic splice site disrupts reading frame and is NOT predicted to undergo NMD then use PVS1_Moderate.

OR

Variants where mRNA assays using RNA derived from patient constitutional biological samples indicate that the variant allele results in a splicing aberration (with evidence that the variant allele produces no full-length/reference transcript) leading to premature stop codon or in-frame deletion disrupting a functional domain[b] or protein conformation. Splicing aberration must be confirmed in a minigene assay or an additional RNA assay from an independent laboratory if it is a non-canonical splice site variant.

Modification Type: General recommendation

Strong

Variants in the initiation codon of *MSH6*.

OR

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 that involve the UTR (i.e. exon 1 or last exon) and whole gene duplications.

OR

G>non-G at last base of exon if first 6 bases of the intron are not GTRRGT. If confirmed to cause a splice defect, then PVS1 should be used instead.

OR

Variants at IVS±1 or IVS±2[a,c] where exon skipping or use of a cryptic splice site preserves reading frame and the altered region is critical to protein function[b]. Not to be combined with PP3 and not to be used for a confirmed splice defect (see PVS1 for variants where patient mRNA assays indicate splicing aberration).

Modification Type: General recommendation

Moderate

Nonsense/frameshift variant introducing premature termination codon between codons 1342 & 1360 in MSH6. Refer to Appendix for details.

Modification Type: Gene-specific

PS1

Original ACMG Summary

Same amino acid change as a previously established pathogenic variant regardless of nucleotide change.
Example: Val->Leu caused by either G>C or G>T in the same codon.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Strong

A predicted missense substitution that encodes the same amino acid change with a different underlying nucleotide change previously established by this VCEP as Pathogenic (not a predicted or confirmed splice defect).

OR

Variants affecting the same non-canonical splice nucleotide as a confirmed pathogenic splice variant with similar or worse splicing in silico prediction using SpliceAI.

Modification Type: General recommendation

Moderate

A predicted missense substitution that encodes the same amino acid change with a different underlying nucleotide change as a previously established Likely Pathogenic missense variant with normal RNA result*, and PM2_supporting is met.

*Otherwise, if the previously established Likely pathogenic missense variant truly is a splice defect, the new missense variant also has to be investigated on a functional level for RNA splicing.

Modification Type: General recommendation

Instructions: SpliceAI masked score option should be checked on.

PS2

Original ACMG Summary

De novo (both maternity and paternity confirmed) in a patient with the disease and no

family history.
Note: Confirmation of paternity only is insufficient. Egg donation, surrogate motherhood, errors in embryo transfer, etc. can contribute to non-maternity.

Very Strong

≥ 4 *de novo* points

Modification Type: Disease-specific

Strong

2 or 3 *de novo* points

Modification Type: Disease-specific

Moderate

1 *de novo* point

Modification Type: Disease-specific

Supporting

0.5 *de novo* points

Modification Type: Disease-specific

Instructions: Proband with a *de novo* variant with both maternity and paternity confirmed in a case with MMR deficient LS spectrum tumor* (i.e. MSI/IHC consistent with affected gene, with no MLH1 methylation in tumor tissue, with the exception of MLH1 constitutional promoter methylation. If there is no tumor data, see PS2_Moderate). Refer to Appendix for protein expression consistent with variant location. **2 points per proband**

OR

Proband with a *de novo* variant with both maternity and paternity confirmed in a case with LS spectrum tumor* (with no tumor data for MSI/IHC/methylation, otherwise see PS2). **1 point per proband**

OR

Proband with assumed *de novo* variant and maternity and/or paternity unconfirmed with LS spectrum tumor* (No tumor data for MSI/IHC/methylation). **0.5 points per proband**

*Lynch Syndrome (LS) tumors include: colorectal/colon/rectal, endometrial, ovarian, small bowel/small intestine, renal pelvis, ureter, and

stomach/gastric carcinomas, sebaceous skin tumors (adenomas and carcinomas), gliomas.

PS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product.
Note: Functional studies that have been validated and shown to be reproducible and robust in a clinical diagnostic laboratory setting are considered the most well-established.

Strong

Calibrated functional assays with functional odds for Pathogenicity > 18.7

Modification Type: General recommendation

Moderate

Calibrated functional assays with functional odds for pathogenicity >4.3 and <= 18.7

OR

MMR function defect following functional assay flowchart*

OR

Variants with monoallelic expression: complete loss of expression (<10% of wild-type in cDNA without puromycin) of the variant allele. Full-length transcript should be analysed with and without NMD block.

Modification Type: General recommendation

Supporting

Calibrated functional odds for Pathogenicity >2.08 and <= 4.3

Modification Type: General recommendation

Instructions: Refer to file ‘Functional assay SVI documentation (MMR genes)’ for calibrated functional assays.

*The functional assay flowchart is a general framework for evaluating functional assays that were already performed, or from historic publications, not for prospective studies on variants. The information describing these assays are generic. The VCEP recommends use of the calibrated assays for prospective testing.

PS4

Original ACMG Summary

The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls.
Note 1: Relative risk (RR) or odds ratio (OR), as obtained from case-control studies, is >5.0 and the confidence interval around the estimate of RR or OR does not include 1.0. See manuscript for detailed guidance.
Note 2: In instances of very rare variants where case-control studies may not reach statistical significance, the prior observation of the variant in multiple unrelated patients with the same phenotype, and its absence in controls, may be used as moderate level of evidence.

Not Applicable

Comments: Due to the availability of tumor IHC data for variant classification (see PP4), PS4 has not been utilized for MMR variant classification using proband counting.

PM1

Original ACMG Summary

Located in a mutational hot spot and/or critical and well-established functional domain (e.g. active site of an enzyme) without benign variation.

Not Applicable

Comments: There are no recognized mutational hot spots that could be used for classification purposes. While there are functional domains in the MMR genes, the distribution of pathogenic variants is generalized over all the domains (unpublished data).

PM2

Original ACMG Summary

Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.
Caveat: Population data for indels may be poorly called by next generation sequencing.

Supporting

Absent/extremely rare (<1 in 50,000 alleles) in gnomAD v4 dataset

Modification Type: General recommendation

PM3

Original ACMG

Summary

For recessive disorders, detected in trans with a pathogenic variant
Note: This requires testing of parents (or offspring) to determine phase.

Very Strong

≥ 4 points

Modification Type: Disease-specific

Strong

≥ 2 and < 4 points

Modification Type: Disease-specific

Moderate

≥1 and < 2 points

Modification Type: Disease-specific

Supporting

= 0.5 points

Modification Type: Disease-specific

Instructions: Co-occurrence with a known pathogenic/likely pathogenic sequence variant in the same gene in a patient with clinical features consistent with CMMRD as per Aronson et al 2022 - Refer to “Table for CMMRD diagnosis.pdf”. For MLH1 variants - the variant has to meet PM2_Supporting criteria.

Classification/zygosity of other variant:

Pathogenic/Likely Pathogenic *in trans*: 1.0 point;

Pathogenic/Likely Pathogenic - phase unknown: 0.5 points

Sum all cases with the above evidence to determine the PM3 strength.

PM4

Original ACMG

Summary

Protein length changes due to in-frame deletions/insertions in a non-repeat region or stop-loss variants.

Not Applicable

Comments: Protein length change from an in-frame variant is not used due to lack of evidence.

PM5

Original ACMG

Summary

Novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before.
Example: Arg156His is pathogenic; now you observe Arg156Cys.
Caveat: Beware of changes that impact splicing rather than at the amino acid/protein level.

Moderate

Missense change at an amino acid residue where a different missense change was classified by this VCEP as Pathogenic on the protein level and not due to aberrant splicing. Only use PM5 if PP3 is supporting for the missense change. Use PM5_Supporting if other variant is Likely Pathogenic due to a missense alteration.

Modification Type: General recommendation

Supporting

Missense change at an amino acid residue where a different missense change was classified as Likely Pathogenic on the protein level and not due to aberrant splicing. Only use PM5_Supporting if PP3 is supporting for the missense change. Use PM5 if other variant is Pathogenic due to a missense alteration.

Modification Type: General recommendation

PM6

Original ACMG

Summary

Assumed de novo, but without confirmation of paternity and maternity.

Not Applicable

Comments: Please see PS2

PP1

Original ACMG Summary

Co-segregation with disease in multiple affected family members in a gene definitively known to cause the disease.
Note: May be used as stronger evidence with increasing segregation data.

Strong

Co-segregation with disease in pedigree(s) with a combined* Bayes Likelihood Ratio[h] >18.7 in ≥2 families.

Modification Type: General recommendation

Moderate

Co-segregation with disease in pedigree(s) with a combined* Bayes Likelihood Ratio[h] >4.3 & ≤18.7.

Modification Type: General recommendation

Supporting

Co-segregation with disease in pedigree(s) with a combined* Bayes Likelihood Ratio[h] >2.08 & ≤4.3.

Modification Type: General recommendation

Instructions: *For multiple pedigrees, results are combined by multiplying together.

Recommended segregation analysis tool: COOL(COsegregation OnLine) v3 https://fenglab.chpc.utah.edu/cool3/manual.html

Copy the example pedigree format and complete the fields to build the pedigree in text format. Refer to online manual for cancer types to enter into pedigree. Click on the 'Analysis' tab to view the webform for pedigree file upload and enter appropriate parameters for population and allele frequency. Penetrance file and relative risk file are not required for MMR genes. Use the 'overall Bayes Factor' to determine evidence strength.

PP2

Original ACMG Summary

Missense variant in a gene that has a low rate of benign missense variation and where missense variants are a common mechanism of disease.

Not Applicable

Comments: Missing variant in a gene with low rate of benign missense changes does not apply.

PP3

Original ACMG Summary

Multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.).
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm should not be counted as an independent criterion. PP3 can be used only once in any evaluation of a variant.

Moderate

Missense variant with HCI prior probability for pathogenicity >0.81 as per https://hci-priors.hci.utah.edu/PRIORS

Modification Type: General recommendation

Supporting

Missense variant with HCI prior probability for pathogenicity >0.68 & ≤0.81 as per https://hci-priors.hci.utah.edu/PRIORS

OR

Predicted splice defect for non-canonical splicing nucleotides using SpliceAI with delta score >= 0.2 as per Walker et al 2023.

Modification Type: General recommendation

Instructions: SpliceAI masked score option should be checked on. For HCI-PRIORS, ensure correct gene is selected from the tabs, and enter the nucleotide number in either HGVS position or HG38 genomic co-ordinate and click ‘view’. The output shows 3 substitutions at the nucleotide location, with probability based on splicing and protein predictions. Ensure the ‘applicable prior’ is used that corresponds to the variant under review.

PP4

Original ACMG Summary

Patient’s phenotype or family history is highly specific for a disease with a single genetic etiology.

Strong

≥3 independent CRC/Endometrial MSI-H tumors in ≥2 families using a standard panel of 5-10 markers[g] or tumor genome **and/or** loss of MMR protein expression consistent with the variant location. MSI-H tumor with inconsistent protein expression does not meet PP4_Strong. Independent tumors can be from the same patient/family.

Modification Type: Disease-specific

Moderate

2 independent CRC/Endometrial MSI-H tumors using a standard panel of 5-10 markers[g] or tumor genome **and/or** loss of MMR protein expression consistent with the variant location. MSI-H tumor with inconsistent protein expression does not meet PP4_Moderate.

Modification Type: Disease-specific

Supporting

1 CRC/Endometrial MSI-H tumor using a standard panel of 5-10 markers[g] or tumor genome **and/or** loss of MMR protein expression consistent with the variant location. MSI-H tumor with inconsistent protein expression does not meet PP4.

Modification Type: Disease-specific

Instructions: [g]Standard MSI markers panel: BAT25, BAT26, BAT40, BAT34, D5S346, D17S250, ACTC, D18S55, D10S197, MYCL; D2S123, D18S69; NR21, NR24, NR27

Protein Expression and consistency with variant location:

IHC evidence should be consistent with the variant gene and the protein that is tested and take into account the MutSα and MutLα heterodimers: MLH1 and PMS2 loss is consistent with an MLH1 pathogenic variant, MSH2 and MSH6 loss is consistent with an MSH2 pathogenic variant, MSH6 loss is consistent with an MSH6 pathogenic variant, and PMS2 loss is consistent with a PMS2 pathogenic variant.

PP5

Original ACMG Summary

Reputable source recently reports variant as pathogenic, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant

Interpretation VCEP Review Committee. PubMed : 29543229

BA1

Original ACMG Summary

Allele frequency is above 5% in Exome Sequencing Project, 1000 Genomes or Exome Aggregation Consortium.

Stand Alone

GnomAD v4 Grpmax filtering allele frequency ≥ 0.0022 (0.22%) and variant is excluded as founder pathogenic variant.

Modification Type: Gene-specific

BS1

Original ACMG Summary

Allele frequency is greater than expected for disorder.

Strong

GnomAD v4 Grpmax filtering allele frequency ≥ 0.00022 and < 0.0022 (0.022-0.22%) and variant is excluded as founder pathogenic variant.

Modification Type: Gene-specific

BS2

Original ACMG Summary

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.

Strong

Co-occurrence *in trans* with a known pathogenic sequence variant in the same gene in a patient with colorectal cancer after age 45 (or other LS cancer above the median age of onset for that cancer in LS[f]), and who has no previous or current evidence of clinical manifestations of CMMRD as per Aronson et al 2022 (Refer to 'Table for CMMRD diagnosis.pdf'). Confirmation of phase requires testing of parents or offspring.

Modification Type: Disease-specific

BS3

Original ACMG Summary

Well-established in vitro or in vivo functional studies show no damaging effect on protein function or splicing.

Strong

Calibrated functional assays with functional odds for Pathogenicity ≤ 0.05

OR

Synonymous substitutions and intronic variants with no associated mRNA aberration (either splicing or allelic imbalance) as determined by laboratory assays conducted with nonsense-mediated decay inhibition. Whenever abnormal transcripts are identified at similar levels in controls they will be considered naturally occurring isoforms and not mRNA aberrations.

Modification Type: General recommendation

Supporting

Calibrated functional assays with functional odds for Pathogenicity >0.05 & ≤0.48

OR

Variant-specific proficient function in protein and mRNA-based lab assays as per MMR functional assay flowchart.

Modification Type: General recommendation

Instructions: Refer to file ‘Functional assay SVI documentation (MMR genes)’ for calibrated functional assays.

*The functional assay flowchart is a general framework for evaluating functional assays that were already performed, or from historic publications, not for prospective studies on variants. The information describing these assays are generic. The VCEP recommends use of the calibrated assays for prospective testing.

BS4

Original ACMG Summary

Lack of segregation in affected members of a family.
Caveat: The presence of phenocopies for common phenotypes (i.e. cancer, epilepsy) can mimic lack of segregation among affected individuals. Also, families may have more than

one pathogenic variant contributing to an autosomal dominant disorder, further confounding an apparent lack of segregation.

Strong

Lack of co-segregation with disease in pedigree(s) with a combined* Bayes Likelihood Ratio[h] <0.05.

Modification Type: General recommendation

Supporting

Lack of co-segregation with disease in pedigree(s) with a combined* Bayes Likelihood Ratio[h] >0.05 & ≤0.48.

Modification Type: General recommendation

Instructions: *For multiple pedigrees, results are combined by multiplying together.

Recommended segregation analysis tool: COOL(COsegregation OnLine) v3 https://fenglab.chpc.utah.edu/cool3/manual.html

Copy the example pedigree format and complete the fields to build the pedigree in text format. Refer to online manual for cancer types to enter into pedigree. Click on the 'Analysis' tab to view the webform for pedigree file upload and enter appropriate parameters for population and allele frequency. Penetrance file and relative risk file are not required for MMR genes. Use the 'overall Bayes Factor' to determine evidence strength.

BP1

Original ACMG Summary

Missense variant in a gene for which primarily truncating variants are known to cause disease.

Not Applicable

Comments: Missense variant in a gene where only loss of function causes disease is not applicable.

BP2

Original ACMG Summary

Observed in trans with a pathogenic variant for a fully penetrant dominant gene/disorder or observed in cis with a pathogenic variant in any inheritance pattern.

Not Applicable

Comments: BS2 is used instead.

BP3

Original ACMG Summary

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

Not Applicable

Comments: In-frame deletions/insertions in a repetitive region without a known function is not used.

BP4

Original ACMG Summary

Multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc)
Caveat: As many in silico algorithms use the same or very similar input for their predictions, each algorithm cannot be counted as an independent criterion. BP4 can be used only once in any evaluation of a variant.

Supporting

Missense variant with HCI-prior probability of pathogenicity <0.11 as per https://hci-priors.hci.utah.edu/PRIORS

OR

For intronic and synonymous variants: SpliceAI predicts no splicing impact with delta score <= 0.1 as per Walker et al 2023.

Modification Type: General recommendation

Instructions: SpliceAI masked score option should be checked on. For HCI-PRIORS, ensure correct gene is selected from the tabs, and enter the nucleotide number in either HGVS position or HG38 genomic co-ordinate and click ‘view’. The output shows 3 substitutions at the nucleotide location, with probability based on splicing and protein predictions. Ensure the ‘applicable prior’ is used that corresponds to the variant under review.

BP5

Original ACMG Summary

Variant found in a case with an alternate molecular basis for disease.

Strong

≥ 4 tumors: CRC/Endometrial tumors with MSS and/or no loss of MMR protein expression and/or LS spectrum tumors[f] with loss of MMR protein(s) that is inconsistent with the gene demonstrating genetic variation

OR

≥2 BRAF V600E (CRC only)/*MLH1* methylation (in LS spectrum tumor only) with MSI-H/*MLH1* loss.

Modification Type: Disease-specific

Supporting

2 or 3 tumors: CRC/Endometrial tumors with MSS and/or no loss of MMR protein expression and/or LS spectrum tumors[f] with loss of MMR protein(s) that is inconsistent with the gene demonstrating genetic variation.

OR

1 BRAF V600E (Colon only)/*MLH1* methylation (in LS spectrum tumor only) with MSI-H/*MLH1* loss.

Modification Type: Disease-specific

BP6

Original ACMG Summary

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Not Applicable

This criterion is not for use as recommended by the ClinGen Sequence Variant Interpretation VCEP Review Committee. PubMed : 29543229

BP7

Original ACMG Summary

A synonymous variant for which splicing prediction algorithms predict no impact to the splice consensus sequence nor the creation of a new splice site AND the nucleotide is not highly conserved.

Supporting

A synonymous (silent) or intronic variant at or beyond -21/+7 (5′/3′ exonic). Variants may satisfy both BP7 and BP4.

Modification Type: General recommendation

Rules for Combining Criteria

Pathogenic

1 Very Strong *(PVS1)* **AND ≥ 1 Strong** *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)*

1 Very Strong *(PVS1)* **AND ≥ 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 2 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)* **AND ≥ 3 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Very Strong *(PVS1)* **AND ≥ 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)*

Likely Pathogenic

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)* **AND 1 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

≥ 3 Moderate *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)*

2 Moderate *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)* **AND ≥ 2 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Moderate *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)* **AND ≥ 4 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

1 Strong *(PVS1_Strong, PS1, PS2, PS3, PP1_Strong, PP4_Strong)* **AND 2 Moderate** *(PVS1_Moderate, PS1_Moderate, PS2_Moderate, PS3_Moderate, PM3, PM5, PM6, PP1_Moderate, PP3_Moderate, PP4_Moderate)*

1 Very Strong *(PVS1)* **AND 1 Supporting** *(PS3_Supporting, PM2_Supporting, PM5_Supporting, PP1, PP3, PP4)*

Benign

≥ 2 Strong *(BS1, BS2, BS3, BS4, BP5_Strong)*

1 Stand Alone *(BA1)*

Likely Benign

1 Strong *(BS1, BS2, BS3, BS4, BP5_Strong)* **AND 1 Supporting** *(BS3_Supporting, BS4_Supporting, BP4, BP5, BP7)*

≥ 2 Supporting *(BS3_Supporting, BS4_Supporting, BP4, BP5, BP7)*

Files & Images

MMR PVS1 Decision Tree: PVS1 Decision Tree for MMR genes

MMR functional domains: MMR functional domains

Appendix: Appendix

MMR Functional assay flowchart: Flowchart demonstrating the interpretation of functional assay data

VCEP pilot variants - MMR: VCEP pilot variants - MMR

Table for CMMRD diagnosis: Scoring system for aiding CMMRD diagnosis

Functional assay SVI documentation: Functional assay SVI documentation (MMR genes)

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