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 MMR functional domains pdf.

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).

e As per CMMRD consortium guidelines.

f 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.

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

h Likelihood ratios for segregation can be derived by Bayes factor analysis adapted from the method of Thompson *et al*. 2003. Penetrance estimates for *MLH1* and *MSH2* are from Jenkins *et al.* 2015 and Dowty *et al.* 2013; *MSH6* from Baglietto et al. 2010; *PMS2* from ten Broeke *et al.* 2015

Important Notes

PMS2 NGS results need confirmation by other orthogonal assays as well as functional assessment (e.g. Long-Range or cDNA), if variants are located in the *PMS2CL* pseudogene homologous regions (exons 11-15).

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

Justification for last exon PVS1 boundaries:

Nonsense/frameshift variant introducing Premature Termination Codon (PTC):

1) ≤ codon 753 in MLH1 using location of known pathogenic variant *MLH1* c.2252_2253del
2) ≤ codon 891 in MSH2 using location of known pathogenic variant *MSH2* c.2662del
3) ≤ codon 1341 in MSH6 using location of known pathogenic variant *MSH6* c.3984_3987dup
4) ≤ codon 798 in PMS2 using ≥50 nucleotide NMD-rule.

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.

Derivation of probability values from Odds

0.11 probability corresponds to the odds of 0.48 for Benign Supporting level of benign evidence using 0.2 prior – consistent with ACMG Bayesian model.

0.68 probability corresponds to the odds of 2.08 for Pathogenic Supporting level of evidence using 0.5 prior – consistent with ACMG Bayesian model.

0.81 probability corresponds to the odds of 4.3 for Pathogenic Supporting level of evidence using 0.5 prior – consistent with ACMG Bayesian model.