Application of a five-tiered scheme for standardized classification of 2,360 unique mismatch repair gene variants lodged on the InSiGHT locus-specific database

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Supplementary Information

Supplementary Note

Mismatch Repair Gene Variant Classification Criteria

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Rules for Variant Classification:

Rules describing the 5 class system for classification of MMR gene variants were devised and documented by Amanda Spurdle and Bryony Thompson in September 2009 for standardised classification of variants in the Colon Cancer Family Registry database, and revised by Amanda Spurdle, Bryony Thompson, Sean Tavtigian and Marc Greenblatt during April 2011, in collaboration with the InSiGHT Variant Interpretation Committee (VIC), modulated by input from committee members during ongoing VIC meetings. They are based on the following:

- The 5 class system described for quantitative assessment of variant pathogenicity in Plon et al.[1], using a multifactorial likelihood model[2-5] as applied to MMR gene variants[6,7];
- The 5 class system for interpretation of splicing variants and aberrations by Spurdle et al.[8];
- The classification of sequence changes according to standard clinical practice – that is, description of variants generally considered pathogenic (clinically relevant in a genetic counselling setting such that germline variant status is used to inform patient and family management) or non-pathogenic (significant evidence against being a dominant high-risk pathogenic mutation); and
- The documentation of non-quantitative methods that have been used to classify variants in the literature.

For a given class, a variant is required to satisfy all the criteria listed for at least one bullet-point that falls within that class. The symbol "✓" represents an "AND" statement. The footnote # describes the rationale for suggested sample numbers, and the rationale for use of indicative MSI or IHC information. The interpretation of functional assays is assisted by a flowchart developed for this purpose (See Fig. 1b, main text).

These criteria provide a baseline for standardized clinical classification of MMR gene sequence variation that may be linked to patient and family management in the genetic counseling arena according to published guidelines [1]. Use of the InSiGHT database, and associated interpretation relating to pathogenicity, is subject to User discretion and responsibility. Whereas InSiGHT has developed processes to assign pathogenicity utilizing high-level expertise available through its membership, such assignments are subject to change with the availability of new information and interpretation processes. Information submitted to the InSiGHT database is available for individual enquiry for clinical use, but any collective use of the data for research or other purposes transgresses agreements InSiGHT has with the valuable community of researchers, clinicians and diagnostic laboratories that generously support the database through their submissions. All users of the database are encouraged to submit their own variants to support the InSiGHT international collaboration to share gene variant data relating to gastrointestinal tumors. Submissions should be directed to the InSiGHT curator John-Paul Plazzer (johnpaul@variome.org).

Class 5 – Pathogenic

- Variants with probability of pathogenicity >0.99 using a multifactorial likelihood model
- Coding sequence variation resulting in a stop codon i.e. nonsense or frameshift pathogenic alteration that is predicted to result in interruption of known functional protein domains or highly conserved exonic sequences
- Variants where mRNA assays in **patient RNA** indicate that the variant allele results in a splicing aberration (with evidence that the variant allele produces no wild-type transcript) leading to premature stop codon or in-frame deletion disrupting a functional domain or protein conformation
- Large genomic deletions
- Large genomic duplications shown by laboratory studies to result in a frameshift before the last splice junction
- Variants demonstrating all of the following characteristics with no conflicting results (combined evidence achieves estimated LR >100:1, posterior >0.99 with prior 0.5):
  - ✓ Variant-specific abrogated function in protein or mRNA based lab assays (see MMR functional assay supplementary material)
    - OR - for *MLH1* and *MSH2*, co-occurrence *in trans* with a known pathogenic sequence variant in the same gene in a patient with clinical features consistent with Constitutional Mismatch Repair Deficiency (CMMRD) and reported LS cancer in the parent of origin for the variant to be classified. If parental genotype is unknown, then both parents should have early-onset LS cancer
    - OR - presence of the variant on different haplotypes across families indicating that reported LS clinical features are not due to an undiscovered sequence change *in cis* with the variant
  - ✓ Evidence for co-segregation with disease where pedigree information provided allows calculation of likelihood ratio (LR) of ≥10:1*****
    - OR - at least one revised Amsterdam criteria [9] family with ≥4 affected carriers
    - OR - across ≥2 revised Amsterdam criteria families reported to show segregation with disease (no further information provided)
    - OR - ≥2 families with ≥3 affected non-proband carriers
  - ✓ ≥2 independent tumors with MSI using a standard panel of 5-10 markers**** and/or** loss of MMR protein expression consistent with the variant location (may include tumor information from proband)
  - ✓ evidence that variant is not an undescribed polymorphism at allele frequency >1% (minor allele frequency [MAF] >0.01) in healthy controls from an appropriate population (i.e. 1000 genomes)

Class 4 – Likely pathogenic

- Variants with probability of pathogenicity between 0.95-0.99 using a multifactorial likelihood model
- Variants at IVS±1 or IVS±2, or G>non-G at last base of exon if first 6 bases of the intron are <u>not</u> GTRRGT, that are untested for splicing aberrations *in vitro* - *irrespective of bioinformatic predictions*
- Variants demonstrating (combined evidence achieves LR >20:1, posterior 0.95-0.99 with prior 0.5):
  - ✓ variant-specific abrogated function in protein or mRNA based lab assays (see MMR functional assay supplementary material)
    - OR - for *MLH1* and *MSH2*, co-occurrence *in trans* with a known pathogenic sequence variant in the same gene in a patient with clinical features consistent with CMMRD and reported LS cancer in the parent of origin for the variant to be classified. If parental genotype is unknown, then both parents should have early-onset LS cancer
    - OR - presence of the variant on different haplotypes across families indicating that reported LS clinical features are not due to an undiscovered sequence change *in cis* with the variant
  - ✓ **plus one of the following:**
    - co-segregation with disease assessed by available pedigree information allows calculation of LR of >5:1*****
    - OR - at least one revised Amsterdam criteria family with ≥3 affected carriers
    - OR - ≥2 families with ≥2 affected non-proband carriers
    - OR - ≥2 independent tumours with MSI using a standard panel of 5-10 markers**** and/or** loss of MMR protein expression consistent with the variant location (may include tumour information from proband)

Class 3 – Uncertain

- Variants with probability of pathogenicity between 0.05-0.949 using a multifactorial likelihood model
- Variants that have insufficient evidence (molecular or otherwise) to classify, which may include large genomic duplications not yet shown by laboratory studies to result in a frameshift before the last splice junction, missense alterations, small in-frame insertions/deletions, silent variants, intronic variants, promoter and regulatory region variants

Class 2 – Likely not pathogenic/little clinical significance

- Variants with probability of pathogenicity between 0.001-0.049 using a multifactorial likelihood model
- 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 to be naturally occurring isoforms and not mRNA aberrations
- Variants reported to occur in a specific ethnic group at allele frequency ≥1%, (MAF ≥ 0.01, tested in ≥ 160 individuals) and that have not yet been excluded as known founder pathogenic sequence variants ("founder mutations")
- Variants demonstrating:
  - ✓ variant-specific proficient function in protein and mRNA based lab assays (see MMR functional assay supplementary material)
    - OR - 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***), and who has no previous or current evidence of clinical manifestations of CMMRD
  - ✓ **plus one of the following:**
    - present in control reference groups at allele frequency 0.01-1% (MAF 0.0001-0.01, tested in ≥ 160 individuals)
    - OR - lack of co-segregation with disease consistent with a dominant high-risk pathogenic sequence variant in pedigrees (LR≤ 0.01)
    - OR - estimated risk with upper bound 95% confidence limit <4, as determined by large well-designed case-control studies that consider size, geography/ethnicity and quality control measures
    - OR - ≥3 colorectal tumors with MSS **and/or** no loss of MMR protein expression **and/or** loss of MMR protein(s) in LS spectrum tumors, that is inconsistent with the gene demonstrating genetic variation
- Variants demonstrating any **three** of the following:
  - present in control reference groups at allele frequency 0.01-1% (MAF 0.0001-0.01, tested in ≥ 160 individuals)
  - **OR -** lack of co-segregation with disease consistent with a dominant high-risk pathogenic sequence variant in pedigrees (LR≤ 0.01)
  - OR - estimated risk with upper bound 95% confidence limit <4, as determined by large well-designed case-control studies that consider size, geography/ethnicity and quality control measures
  - OR - ≥3 colorectal tumors with MSS **and/or** no loss of MMR protein expression **and/or** loss of MMR protein(s) in LS spectrum tumors, that is inconsistent with the gene demonstrating genetic variation

Class1 – Not pathogenic/no clinical significance

- Variants with probability of pathogenicity <0.001 using a multifactorial likelihood model
- Variants reported to occur in control reference groups at allele frequency ≥1% (MAF ≥ 0.01, tested in ≥ 160 individuals) and excluded as founder pathogenic sequence variants
- Variants demonstrating:
  - ✓ Variant-specific proficient function in protein and mRNA based lab assays (see MMR functional assay supplementary material)
    - OR - 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***), and who has no previous or current evidence of clinical manifestations of CMMRD
  - ✓ **Plus any two of the following:**
    - present in control reference groups at allele frequency 0.01-1% (MAF 0.0001-0.01, tested in ≥ 160 individuals)
    - OR - lack of co-segregation with disease consistent with a dominant high-risk pathogenic sequence variant in pedigrees (LR≤ 0.01)
    - OR - estimated risk with upper bound 95% confidence limit <4, as determined by large well-designed case-control studies that consider size, geography/ethnicity and quality control measures
    - OR - ≥3 colorectal tumors with MSS **and/or** no loss of MMR protein expression **and/or** loss of MMR protein(s) in LS spectrum tumors, that is inconsistent with the gene demonstrating genetic variation
- Variants demonstrating all of the following:
  Present in control reference groups at allele frequency 0.01-1% (MAF 0.0001-0.01, tested in ≥ 160 individuals)
  - ✓ Lack of co-segregation with disease consistent with a dominant high-risk pathogenic sequence variant in pedigrees (LR≤ 0.01)
  - ✓ Estimated risk with upper bound 95% confidence limit <4, as determined by large well-designed case-control studies that consider size, geography/ethnicity and quality control measures
  - ✓ ≥3 colorectal tumors with MSS **and/or** no loss of MMR protein expression **and/or** loss of MMR protein(s) in LS spectrum tumors, that is inconsistent with the gene demonstrating genetic variation

Footnotes to Mismatch Repair Gene Variant Classification Guidelines:

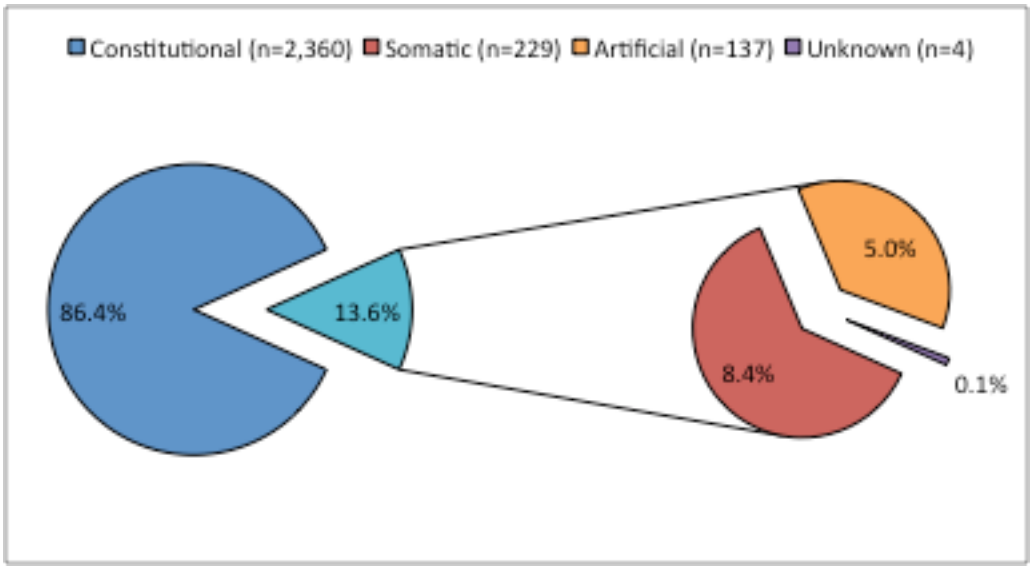
The upper 95% confidence limit of frequency for an allele observed once in 160 individuals (320 chromosomes) is <0.01, the allele frequency considered sufficient for classification as class 1 (not pathogenic/low clinical significance). Characteristics of a pathogenic variant were selected to achieve a combined likelihood ratio of >100:1 in conjunction with a prior of 0.5 (estimated prior irrespective of *in silico* predictions). Namely, the co-segregation and family history descriptions were selected to estimate segregation odds of 10:1, results from functional assays were assumed to have likelihood ratio 5:1 and MSI/IHC data was conservatively assumed to carry a likelihood ratio of 5:1. Tumor data was based on two tumors for MSI and/**or** IHC to allow for the possibility that pathogenic missense alterations may not all demonstrate loss of MMR protein expression. Where both MSI and IHC information are available, MSI-H results will take precedence over normal immunohistochemical results, since MSI is more specific than MMR immunohistochemistry in colorectal cancers[10,11]. For variants that demonstrate tumor MSS and loss of MMR protein expression, technical or other explanations[10,12-14] for the discrepancy should be investigated. The need for multiple results was implemented to minimize the chance of a "sporadic" MSI-H or negative immunohistochemical result for *MLH1* methylated tumors (0.15x0.15 = 0.0225). Independent tumors can include multiple primary tumors from a single individual.

***** Likelihood ratios for segregation can be derived by Bayes factor analysis adapted from the method of Thompson et al[15], as described previously[6]. Penetrance estimates for *MLH1* and *MSH2* variants to be derived from Quehenberger et al[16] and those for *MSH6* and *PMS2* variants to be derived from Baglietto et al[17] and Senter et al[18].

****** Standard MSI markers panel: BAT25, BAT26, BAT40, BAT34, D5S346, D17S250, ACTC, D18S55, D10S197, MYCL [19]; D2S123, D18S69 [20]; NR21, NR24, NR27 [21]

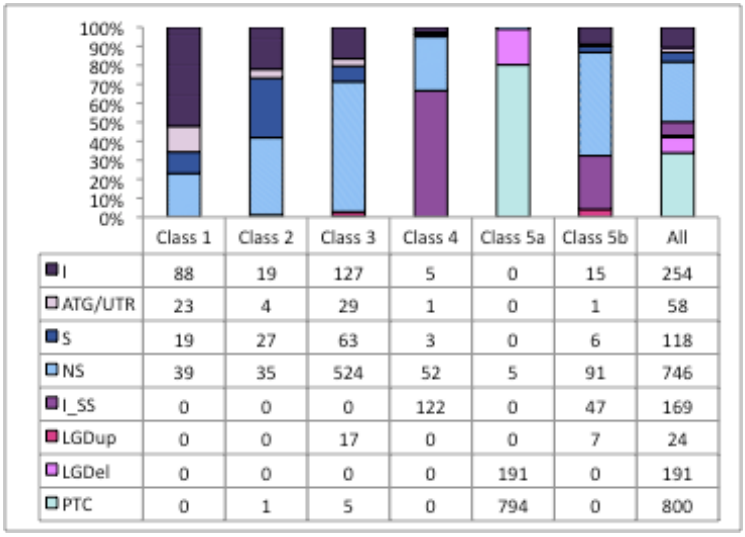
******* Lynch syndrome tumors include: colorectal/colon/rectal, endometrial, ovarian, small bowel/small intestine, renal pelvis, ureter, and stomach/gastric carcinomas, sebaceous skin tumors (adenomas and carcinomas), gliomas.

Supplementary Figure 1 Distribution of unique MMR gene variants (constitutional and non-constitutional) on the InSiGHT database at study initiation. There were 2,370 unique variants in the database after 3,458 nomenclature alterations due to formatting to standardized Human Genome Variation Society nomenclature[22] and systematic review of the literature. There were 448 somatic (tumor or cell line derived) entries in the InSiGHT database, 229 of which have not been reported as constitutional variants. There were 3,322 artificial (synthesized for *in vitro* or *in vivo* assays or subjected to *in silico* analyses) entries in the database, 137 of which have not been reported as constitutional variants. In addition, four variants were termed "unknown" since the source could not be established (n=1) or the nomenclature provided in the reference was inconsistent with sequence trace data provided in the source publication (n=3). Details of the non-constitutional variants are shown in Supplementary Table 1.



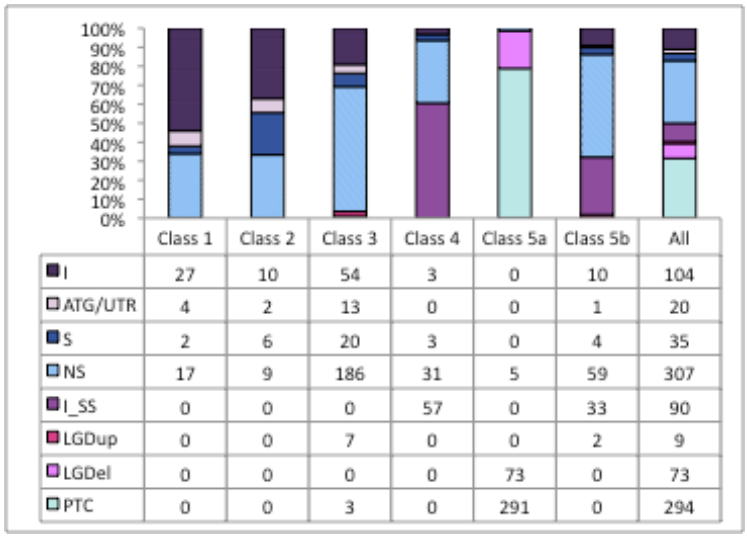
Supplementary Figure 2 Breakdown by MMR gene, of the proportion of different variant types in the 5-tiered classifications. Class 5a is a subset of Class 5 containing the assumed pathogenic mutations caused by nonsense mutations, small frameshift indels, and large deletions. Class 5b includes not-obviously truncating variants considered to be pathogenic on the basis of combined evidence (see Supplementary Note). The different variant types are: PTC – variants that introduce premature terminating codons, i.e. nonsense mutations and small frameshift indels; NS – not obviously truncating non-synonymous variants; I – intronic variants outside the canonical splicing dinucleotides; LGDel – large genomic deletions or disrupting inversions; LGDup – large genomic duplications; SS – variants in the canonical splice site dinucleotides; S – synonymous variants; ATG/UTR – variants in the initiation codon (n=9), and the 5' or 3' untranslated regions (n=49).

A) All genes (n=2360)



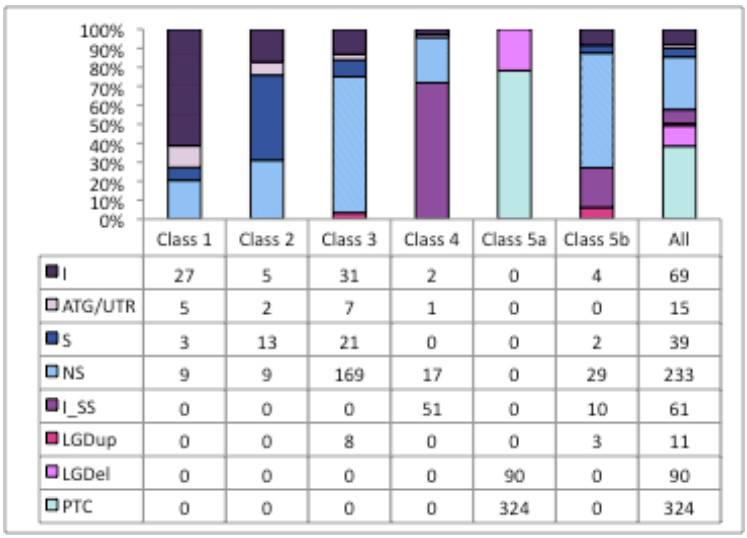
	Class 1	Class 2	Class 3	Class 4	Class 5a	Class 5b	All
I	88	19	127	5	0	15	254
ATG/UTR	23	4	29	1	0	1	58
S	19	27	63	3	0	6	118
NS	39	35	524	52	5	91	746
I_SS	0	0	0	122	0	47	169
LGDup	0	0	17	0	0	7	24
LGDel	0	0	0	0	191	0	191
PTC	0	1	5	0	794	0	800

B) *MLH1* (n=932)



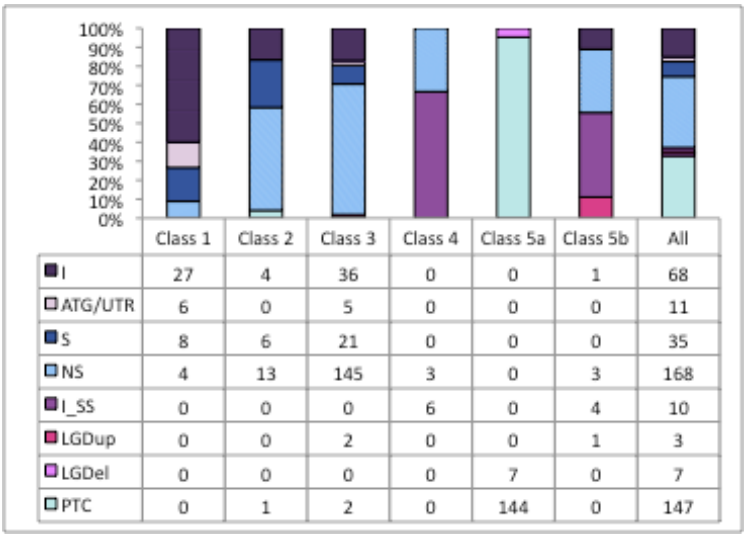
	Class 1	Class 2	Class 3	Class 4	Class 5a	Class 5b	All
I	27	10	54	3	0	10	104
ATG/UTR	4	2	13	0	0	1	20
S	2	6	20	3	0	4	35
NS	17	9	186	31	5	59	307
I_SS	0	0	0	57	0	33	90
LGDup	0	0	7	0	0	2	9
LGDel	0	0	0	0	73	0	73
PTC	0	0	3	0	291	0	294

C) *MSH2* (n=842)



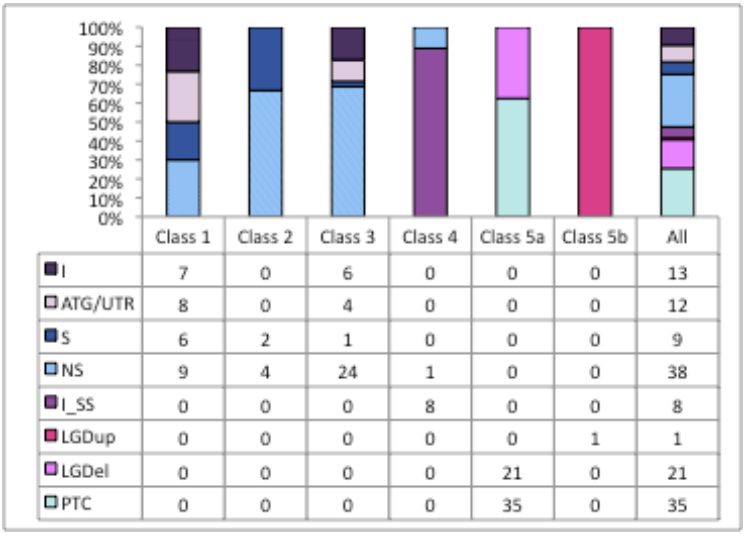
	Class 1	Class 2	Class 3	Class 4	Class 5a	Class 5b	All
I	27	5	31	2	0	4	69
ATG/UTR	5	2	7	1	0	0	15
S	3	13	21	0	0	2	39
NS	9	9	169	17	0	29	233
I_SS	0	0	0	51	0	10	61
LGDup	0	0	8	0	0	3	11
LGDel	0	0	0	0	90	0	90
PTC	0	0	0	0	324	0	324

D) *MSH6* (n=449)



	Class 1	Class 2	Class 3	Class 4	Class 5a	Class 5b	All
I	27	4	36	0	0	1	68
ATG/UTR	6	0	5	0	0	0	11
S	8	6	21	0	0	0	35
NS	4	13	145	3	0	3	168
I_SS	0	0	0	6	0	4	10
LGDup	0	0	2	0	0	1	3
LGDel	0	0	0	0	7	0	7
PTC	0	1	2	0	144	0	147

E) *PMS2* (n=137)



	Class 1	Class 2	Class 3	Class 4	Class 5a	Class 5b	All
I	7	0	6	0	0	0	13
ATG/UTR	8	0	4	0	0	0	12
S	6	2	1	0	0	0	9
NS	9	4	24	1	0	0	38
I_SS	0	0	0	8	0	0	8
LGDup	0	0	0	0	0	1	1
LGDel	0	0	0	0	21	0	21
PTC	0	0	0	0	35	0	35

Supplementary Table 2 Database information used to select discordantly classified variants for detailed examination by the Variant Interpretation Committee: *MLH1*:c.731G>A p.(Gly244Asp) as an example.
The current database contains data from three merged public databases: the original InSiGHT database[23,24] (contributions from individual submitters); MMR Gene Unclassified Variants Database[25] (functional assay results); and the Mismatch Repair Genes Variant Database[26] publication database. Additionally, Human Variome Project country/regional LOVD sub-databases also contribute to the central database, e.g. the Chinese LOVD MMR database[27]. Classifications were commonly assigned to variants by submitters based on a single point of evidence. Variants with different submitter-assigned classifications were prioritized for initial review.

Test Method	Test Type	Result	Classification	Reference
dominant negative effect	reporter assays in yeast	DNE in 0 out of 3 tests	PATHOGENIC	Takahashi et al., 2007
expression level of mutant allele	in vivo assay in human cell line	comparable to WT	NEUTRAL	Blasi et al., 2006
mutation rate at HPRT gene	in vivo assay in human cell line	increased mutation rate	PATHOGENIC	Blasi et al., 2006
tolerance to methylating agents	in vivo assay in human cell line	tolerance as in WT	NEUTRAL	Blasi et al., 2006
MMR activity assay	in vitro assay	19,4% compared to 0% in MLH1 deficient cell line	VUS	Takahashi et al., 2007
MLH1 expression	protein abundancy	>75% of WT level	NEUTRAL	Takahashi et al., 2007
comparison of mutation rate between haploid yeast	functional assays using yeast	mutation rate in a lys+ reporter gene comparable to a MLH1 deficient haploid yeast	PATHOGENIC	Shcherbakova et al, 1999
pSPL3 minigene	splicing assay	no change in exon inclusion	NEUTRAL	Lastella et al., 2006
human cell extract+in vitro MMR assay	cell based in vitro MMR functional assay using a human expression system	reduced repair efficiency compared to the WT	PATHOGENIC	Trojan et al., 2002

Supplementary Table 3 The InSiGHT Variant Interpretation Committee classification process.

InSiGHT Variant Interpretation Committee: 40 members												
Purpose of VIC Meetings	Classification Criteria testing: TCs or face-to-face meeting (June 2012) included variant classification, criteria amendments and clarifications. Average duration of TCs was 2 ½ hours.						Functional assay interpretation TC	TC for splicing variant classification	Variant classification via email discussion	Two TCs to reassess functional flowchart	Re-assessment of classification for variants with functional assay data via email.	Finalization of variant classifications
Meeting date	Oct 2011	Dec 2011	Jan 2012	May 2012	Jun 2012	Oct 2012	Dec 2012	Jan 2013	Feb 2013	Jun 2013	Jul 2013	Aug 2013
Participants (continents covered)	14 (3)	11 (3)	15 (4)	10 (3)	39 (5)	14 (4)	8 (3)	10 (2)	11 (2)	9 & 6 (3)	6 (3)	20 (3)
Variants assessed	12	20	20	20	10	35	23	47	177	NA	36	128
Reviews per variant	4-6	2-5	2-6	2-4	4	1-4	NA	2-4	3-7	NA	4	3-7

TC – teleconference; VIC - Variant Interpretation committee; NA – not applicable.

Note: A set of discordantly classified variants remaining after curation for nomenclature (n=117, see “curation” results section for further detail) were used to test the robustness of the guidelines in first set of teleconferences and a face-to-face meeting (June 2012). Due to apparent discordances in results from functional assays, several teleconferences were devoted solely to functional assay interpretation. Amendments and clarifications to classification criteria were incorporated through all these meetings. Documentation of evidence and preliminary classification of the remaining mismatch repair variants was performed by author BAT, and a subset of variants with available splicing data were discussed and classified in a teleconference in January 2013. Some variants were discussed on multiple teleconferences. Of the remaining mismatch repair variants, those with sufficient data to warrant examination against the classification criteria were assessed independently by at least 3 committee members and consensus classification reached through electronic discussion, or face-to-face meeting (August 2013). The Variant Interpretation committee covers the following continents: Australia, Europe, Asia, Africa, and North America.

Supplementary Table 4 Rationale for InSiGHT classification criteria

Class	Criteria	Rationale
Class 5: pathogenic	Posterior probability of pathogenicity >0.99	IARC recommendation for Class 5[1]
	Variant resulting in premature termination codon (PTC -nonsense/frameshift predicted to interrupt functional domains or conserved sequences)	Treated clinically as pathogenic
	Full expression of aberrant splice transcripts by variant allele in lab assays, that leads to PTC/disruption of a domain	Treated clinically as pathogenic
	Large genomic deletion (assumed PTC or domain deletion)	Treated clinically as pathogenic
	Large genomic duplication resulting in frameshift (lab-tested)	Treated clinically as pathogenic
	Variant-specific abrogated function: functional assays	Excludes undetected causal mutation *in cis*; assumed LR 5:1[28]
	CMMRD (MIM 276300) phenotype for co-occurrence with known pathogenic mutation *in trans*	Clinical phenotype for "*in vivo*" functional abrogation
	Presence of variant on different haplotypes across families	Excludes undetected causal mutation *in cis*
	Evidence that variant is not an undescribed polymorphism	High-risk variants are not common in the general population
	Evidence for co-segregation with disease (segregation score, reported segregation in AM2 families, or reported variant status of multiple affected family members)	Provides evidence that the variant is associated with the clinical phenotype. Total estimated segregation odds ≥10:1.
	Evidence that variant is associated with MMR deficiency in vivo (≥2 tumours with MSI and/or appropriate IHC loss)	Provides evidence that the variant is associated with the clinical phenotype. Assumed conservative LR ≥5:1 for tumor data.
Class 4: likely pathogenic	Posterior probability of pathogenicity 0.95-0.99	IARC recommendation for Class 4[1]
	Variants untested for splicing aberrations at IVS±1 or IVS±2 or G>non-G at last base of exon when adjacent intronic sequence is not GTRRGT	Disruption of conserved bases at acceptor and donor splice sites results in splicing aberrations.
	Variant-specific abrogated function: functional assays	Excludes undetected causal mutation *in cis*; assumed LR 5:1[28]
	CMMRD (MIM 276300) phenotype for co-occurrence with known pathogenic mutation *in trans*	Clinical phenotype for "*in vivo*" functional abrogation
	Presence of variant on different haplotypes across families	Excludes undetected causal mutation *in cis*
	Evidence for co-segregation with disease (segregation score, or reported variant status of multiple affected family members – less stringent than class 5)	Provides evidence that the variant is associated with the clinical phenotype. Total estimated segregation odds ≥5:1
	Evidence that variant is associated with MMR deficiency in vivo (≥2 tumors with MSI and/or appropriate IHC loss)	Provides evidence that the variant is associated with the clinical phenotype. Assumed LR >≥5:1 for tumor data
Class 3: uncertain	Posterior probability of pathogenicity 0.05-0.949	IARC recommendation for Class 3[1]
	Insufficient evidence to classify variant	Does not fit prescribed criteria for other classes

Class 2: likely not pathogenic or of little clinical significance	Posterior probability of pathogenicity 0.001-0.0049	IARC recommendation for Class 2[1]
	Intronic and synonymous variants with no associated variant-specific mRNA aberration in lab assays (excludes naturally occurring isoforms at similar levels to controls)	In the absence of mRNA aberrations, function is unlikely to be deficient for intronic and synonymous nucleotide substitutions
	Variant reported at ≥1% allele frequency, but in only one specific population and has not been excluded as "founder mutation"	High-risk variants are not common in the general population, but may be increased in individuals from specific geographical regions or in specific ethinc groups. The prevalence of Lynch syndrome is 1-3% in colorectal and endometrial cancer patients, which corresponds to an incidence between 1:660 and 1:2000 when extrapolated to the general population[29-31].
	Proficient protein function in protein and mRNA based lab assays	*In vitro* evidence for proficient function. Assumed LR 0.2[28]
	Co-occurrence *in trans* with a known pathogenic mutation with no unusual clinical features	*In vivo* evidence for proficient function
	Variant with allele frequency of 0.01-1%	Present in the general population/healthy controls, but not at frequency >1% consistent with no severe effect on function or risk. The prevalence of Lynch syndrome is 1-3% in colorectal and endometrial cancer patients, which corresponds to an incidence between 1:660 and 1:2000 when extrapolated to the general population[29-31].
	≥3 MSS and/or no IHC loss and/or IHC loss inconsistent with gene involved, in colorectal tumors	Provides evidence that the variant is not associated with the clinical phenotype. Estimated LR 0.1 for MSS[32]
	Lack of co-segregation with disease (LR≤0.01)	Sufficient to derive posterior probability of 0.01
	Odds Ratio with upper 95% CI <4 in case-control study	Cut-off is indicative of a low/moderate risk variant[33]. Odds ratio below this level of risk would not lead to implementation of clinical management recommendations for Lynch syndrome.
Class 1: not pathogenic or of no clinical significance	Posterior probability of pathogenicity <0.001	IARC recommendation for Class 1[1]
	Variant with reported frequency ≥1% in the general population, and no evidence that it is a founder mutation	High-risk variants are not common in the general population. The prevalence of Lynch syndrome is 1-3% in colorectal and endometrial cancer patients, which is an incidence between 1:660 and 1:2000 when extrapolated to the general population[29-31].
	Proficient protein function in protein and mRNA based lab assays	*In vitro* evidence for proficient function. Assumed LR 0.2[28]
	Co-occurrence *in trans* with a known pathogenic mutation with no unusual clinical features	*In vivo* evidence for proficient function
	Variant with allele frequency of 0.01-1%	Present in the general population/healthy controls, but not at frequency >1% consistent with no severe effect on function or risk. The prevalence of Lynch Syndrome is 1-3% in colorectal and endometrial cancer patients, which is an incidence between 1:660 and 1:2000 when extrapolated to the general population[29-31].
	≥3 MSS and/or no IHC loss and/or IHC loss inconsistent with gene involved in colorectal tumors	Provides evidence that the variant is not associated with the clinical phenotype. Estimated LR 0.1 for MSS[32]
	Lack of co-segregation with disease (LR≤0.01)	Sufficient to derive posterior probability of 0.01
	Odds Ratio with upper 95% CI <4 in case-control study	Cut-off is indicative of a low/moderate risk variant[33]. Odds ratio below this level of risk would not lead to implementation of clinical management recommendations for Lynch syndrome.

Supplementary Table 5 Description of assays used in the interpretation of mismatch repair gene variants

Subclassification	Functional Assays	Assay details	Considerations for Assay interpretation	Required Controls	MMR publications using assays
Assays assessing impact on gene transcripts					
mRNA expression level and splicing analysis	Reverse-transcriptase PCR (RT-PCR)	PCR amplication of cDNA fragments. Gel electrophoresis used to analyze transcript size and level of mRNA expression. Cloning can be used to separate out alternate transcripts for sequencing.	Cells used in assay may not be tissue of interest. Naturally occurring alternate splicing isoforms may complicate interpretation. Semi-quantitative measure of expression. NMD inhibitors can be used to stabilise transcripts that contain premature stop codons. Different PCR designs can lead to different results.	Wild type. Recommend minimum of 5 controls, in order to capture naturally occuring isoforms.	6,34-79
Splicing analysis	Minigene constructs	Exonic and intronic gDNA inserted into a minigene construct, which is transfected into a cell line. RNA extracted from cells and subjected to RT-PCR analysis	*Ex vivo* system: different levels of exon inclusion between constructs and cell lines. Representative of genomic context but still a heterologous system	Wild type.	45,56,65,70,71,80-83
	Diploid to haploid conversion analysis	Hybrid cell lines containing separate alleles, generated by fusing lymphoblastoid cell line with a mouse embryonic cell line. RNA extracted from cells and subjected to RT-PCR analysis.	Specialty laboratory: STR genotyping or cell treatment with negative selection reagents required to confirm cell line fusion. Interference associated with the use of a heterologous system.	Wild type; unfused LCL and mouse embryonic cell line	84-88
	Protein truncation test (PTT) or *in vitro* synthesized protein assay (IVSP)	*In vitro* transcription and translation (IVTT) of cDNA transcripts	*In vitro* system: small differences in protein sizes are difficult to visualize.	Wild type.	45,89-109

Allelic expression	Allelic expression ratios	Heterozygous coding SNP or variant used as marker of allelic expression in single nucleotide extension assays, such as MassArray MALDI-ToF mass spectometry, Snapshot, Snupe, Pyrosequencing.	Require the sequence information from samples to identify coding SNPs	gDNA	65,72,79,110-112
Promoter or 3'UTR activity	Luciferase assay	gDNA sequence of interest is inserted into a plasmid upstream of the luciferase gene. Construct transiently transfected into a cell line. Relative activity measured by the level of fluorescence from luciferase expression	*In vitro* system: ensure full-characterized promoter sequence is tested for 5'UTR variants.	Wild type, positive & empty vector.	113-115 116 Empty vector & positive control were not used
Assays assessing MMR protein repair capacity as a complete process					
Functional assays using cell-free systems	In vitro MMR complementation assays	An *in vitro* test of the repair of mismatched DNA by protein extracts. Baculovirus infected insect cell extracts are used to complement MMR-deficient cell extract; MMR genes transfected into MMR-deficient cell line; or IVTT of PCR fragments complement MMR-deficient cell extracts. DNA repair substrates: mismatch within restriction site or LacZ domain.	False negative results possible for variants that are pathogenic due to poor expression or protein stability. Variants defective in nuclear import may yield false-positive results. Subtle defects will not be detected if amount of protein is saturating.	Wild type; known defective (untransfected MMR deficient cell line or pathogenic control). Transfection efficiency for assays involving transient expression in cell lines.	49,79,112,117-134 135-137 Transfection efficiency not measured 66 Level of MMR activity not quantified

Functional assays using mammalian cell-based systems	Cellular-based MMR functional assay using a human/mouse expression system	Monitor the repair capacity as a whole through expression of mutant human MMR gene constructs in human/mouse cell lines. MMR status measured using: cellular response to methylating agents (MMR-deficient cells have acquired tolerance to these agents), spontaneous mutation rate at the endogenous *HPRT* gene, repair of an exogenously added mismatch-containing GFP plasmid, or measuring microsatellite instability.	Best to use cell lines that lack endogenous expression of the MMR protein. Level of protein expression is critical: poor expression can produce false-negative results; variant MMR gene expression is unregulated and may be toxic to cells. "Knock-in" of the variant allele through oligonucleotide gene targeting avoids unregulated expression.	Wild type; known defective.	85,133,138-141
Assays assessing MMR protein expression					
Mammalian assay	Transient or stable expression of MMR genes	Transient or stable expression of MMR genes in relevant MMR-deficient cell line: MLH1 deficient - HCT116 (human CRC) or HEK293T (human embryonic kidney fibroblast); MSH2 deficient - LoVo (human CRC), Hec59 (human endometrial cancer); MSH6 deficient - HCT15 (human CRC); PMS2 deficient - HEC1-A (human endometrial cancer); knockout murine embryonic fibroblasts. Immunoblot of MMR proteins.	Level of protein expression is unregulated and may be toxic to cells. C-terminal fluorescent labelling can affect MutLa expression.	Wild type; known defective; internal loading. Transfection efficiency for assays involving transient expression in cell lines.	72,76,79,85,112,125,134,138-143 14,49,121,122,135-137,144-147 Transfection efficiency not measured 123,124 & known defective not included 144,148,149 Internal loading control not shown

Protein stability assays	Pulse-chase assay/cycloheximide treatment	Measure half-life of protein: radiolabelled for pulse-chase assay or inhibiting *de novo* protein expression with cycloheximide		Wild type; known defective; tranfection efficiency	72[142]Known defected not included
Assays assessing a specific biological or biochemical function of a MMR protein					
MMR protein subcellular localization	Localization experiments	Expression of fluorescent MMR proteins in mammalian/yeast cells or immunostaining to localize the distribution of these proteins in the cell.	Overexpression of MMR proteins might interfere with proper localization. Overexpression of MMR proteins might be toxic, can be assessed by investigating cell survival/apoptosis. C-terminal fluorescent labelling can affect MutLa expression. Mammalian assay would be superior.	Wild type.	79,112,122,128,143,144,147,149-152[153]Localization in yeast

Note: Yeast-based assays[76,125,154-169] were not used in variant classification, because of concerns about using non-mammalian systems for diagnostic purposes[170,171]. These concerns were corroborated by detailed examination of results from yeast-based assays for variants considered Class 1 (not pathogenic) based on multifactorial analysis posterior probability or allele frequency >1% in the population: abrogated/intermediate function or discordant results were reported for 8/19 (42%) of variants assayed. This compares to only 1/18 (5.5%) for results from mammalian assays. Assays measuring specific functions in MMR, such as subunit interaction and ATPase assays were not used to assist variant interpretation, because these functions are tested in the MMR activity assays as a whole.

Supplementary Table 6 MMR activity and protein expression values for pathogenic and neutral controls, measured in different laboratories

	Variant or cell line control	IARC Class†	MMR activity (% normalized to WT)[125]	Protein expression (% relative to WT)[125]	MMR activity (% relative to WT)[79,112]	Protein expression (% relative to WT)[72,79,112]	MMR activity (% relative to WT)[172]	Protein expression (% relative to WT)[172]	MMR activity (% normalized to WT)[135-137]	Protein expression (% relative to WT)[137]	MMR activity (% normalized to WT)[129,130,133]	MMR activity (% normalized to WT)[49,117-124,126-128,131,173]	Protein expression (% relative to WT)[76]	MMR activity (% normalized to WT)[139]
Variants included in studies as pathogenic controls	HCT116	-	**0%**	**1%**					**30%**		**10%**	**0-12%**		
	HEK293T	-			**0%**	**0%**	**15%**	**0%**	**0-30%**	**0%**			**0%**	
	LoVo	-									**5%**	**0-13%**		
	Msh2 -/- ESC	-									**13%**			
	HCT15	-										**0%**		
	Hec259	-												**9.7%**
	MLH1 p.S44F	5	29%	<25%							***20%***			
	MLH1 p.G67R	5	7%	13.5%	**5%**	20%					***5%***	2.4%		
	MLH1 p.T117M	5	**44%**	<25%			5%	45%	6%		***15%***			
	MLH1 p.L622H	5	87%	50%		25-48%	80%	**42%**						
	MLH1 p.E633_E663del	-										**2.3%**		
	MLH1 p.P654L	5	61%	51%			67%	**25%**		31%	10%	111%	8%	
	MLH1 p.R659P	-	31%	<25%			4%	12%		<25%	***15%***	**26%**	42%	
	MLH1 p.A681T	5	88%	>75%			99%	**51%**				115%	41%	
	MLH1 p.W714*	-	0%	47%										
	MLH1 p.L749P	-			**10%**	50%	15%	85%	33%	>75%				
	MSH2 p.P622L	5									**7%**			9.7%
	MSH2 p.A636P	5									***20%***	16%		
	MSH6 p.G1139S	-									***5%***			
	MSH6 p.T1219I	-									***10%***			
	PMS2 p.D70N	-			***10%***	90%								
Class 5: Pathogenic variants, classified using	MLH1 p.M35R	5	29%	51%										
	MLH1 p.N38K	5									15%			
	MLH1 p.N38H	5					15%	15%			15%			
	MLH1 p.C77Y	5	14%	46%										
	MLH1 p.F80V	5	30%	>75%								14%	96%	
	MLH1 p.T82I	5	34%	>75%			5%	41%						

mutlifactorial likelihood analysis	MLH1 p.A128P	5	31%	66%					0%					
	MLH1 p.V185G	5	11%	63%					5.8%			19%		
	MLH1 p.G244D	5	24%	>75%					23%					
	MLH1 p.K618del	5	49%	22%								86%		
	MLH1 p.P648L	5	49%	25-75%			92%	22%		28%	85%	102%	64%	
	MLH1 p.R659L	5					84%	28%		36%				
	MLH1 p.R687W	5	72%	47%								123%		
	MSH2 p.G162R	5										12%		
	MSH2 p.G164R	5										14%		
	MSH2 p.L187P	5										13%		
	MSH2 p.C697F	5									0%	18%		
Variants included in studies as neutral controls	MLH1 p.I219V	1	**76%**	**45%**	**95%**	**130%**	**96%**	**94%**	100%		**70%**	88%		
	MLH1 p.S406N	1	92%	>75%							**85%**			
	MLH1 p.E578G	1	64%	34%			**90%**	**70%**			70%			
	MLH1 p.K618A	1	104%	35%			**92%**	**78%**	92%		80%	104%		
	MLH1 p.V716M	1	94%	52%			**86%**	**74%**			**65%**	110%		
	MLH1 p.H718Y	1	106%	54%			**110%**	**72%**						
	MSH2 p.G322D	1									**85%**	53%-123%		
	MSH2 p.A834T	2									**100%**	>100%		
	MSH6 p.S144I	1									**55%**	97%		
	MSH6 p.P1087R	-									**95%**	106%		
	MSH6 p.P1087T	-									**80%**	100%		
	PMS2 p.R20Q	1			**90%**	**85%**								
Class 1: not pathogenic variants, classified using multifactorial likelihood analysis or MAF >1% in general population	MLH1 p.D132H	1	79%	50%					120%					
	MLH1 p.I219L	1	107%	>75%										
	MLH1 p.V213M	1	107%	>75%								93%		
	MLH1 p.E268G	1	99%	25-75%										
	MLH1 p.V326A	1	**34%**	68%					80%					
	MLH1 p.V384D	1	81%	>75%										
	MLH1 p.L390F	1									90%			
	MLH1 p.K618T	1	61%	66%										
	MLH1 p.Q689R	1	85%	56%									**7%**	
	MSH2 p.N127S	1										107%-116%		
	MSH6 p.L396V	1									85%			

	MSH6 p.V878A	1									85%	84%		
	PMS2 p.P470S	1			90%	95%								
Additional variants with relevant functional data from at least two independent studies	MLH1 p.K84E	-	28%	>75%			8%	85%				2%	36%	
	MLH1 p.H329P	-	32%	>75%								71%		
	MLH1 p.L550P	-									30%	96%	22%	
	MLH1 p.A586P	-	35%	71%			21%	50%	24%	50%			37%	
	MLH1 p.A589D	-									25%	99%	10%	
	MLH1 p.T662P	-	80%	70%			93%	22%	89%	25%				
	MLH1 p.R755W	-					10%	72%	7%	68%				
	MSH2 p.R524P	-												9.7%‡
	MSH2 p.E749K	-									4%	18%		

†IARC class as defined by multifactorial likelihood analysis conducted as part of this study. ‡Also found to be deficient in Boyer et al[174].

Note: Red or blue highlighted cells with black text are the pathogenic or neutral controls (respectively) used in the studies tabulated. Colored font indicates interpretation of values by members of the Functional Assay Group within the Variant Interpretation Committee. Namely, protein expression within the conservative protein expression cut-offs set in Figure 1b ie <25% (red text) and >75% (blue text), and abrogated function (red text) or normal function (blue text) relative to the pathogenic and assay controls for MMR activity. For the study by Takahashi et al[125], which reported results for only a single cell line control, assay interpretation was modulated by also considering results from at least two other studies that had assayed the same variant. This indicated 35% activity as an appropriate upper % to indicate abrogated function, 64% activity as an upper % to indicate normal function, but also highlighted two variants with outlying results (purple text): MLH1 p.V326A variant classified as low clinical significance (and thus assumed to have normal MMR activity) with 34% activity; and MLH1 p.T117M with 44% activity, but evidence for abrogated function from three independent assays. Another clear outlier result was the low protein expression level for MLH1 p.Q689R (7%), classified as not pathogenic by multifactorial analysis. These discrepant findings highlight the need for caution when interpreting existing functional data that has been generated using assays not yet assessed for sensitivity and specificity. Therefore, concordant results from independent assays from 2 different laboratories are required to assign the functional effect of variants (See Fig 1b).

Supplementary Table 7 Validation set of 170 "assumed pathogenic" truncating/large deletion variants. Sufficient tumor data included the presence of microsatellite instability and/or loss of protein expression in two or more tumors. Sufficient segregation data included fulfilling the segregation requirements of Class 5 "pathogenic" (see supplementary note). In most cases the truncating variants that were in Class 3 and Class 4 had one or two affected carriers, or did not include informative meioses. None of the variants were found in the 1000 genomes data. NA – not available.

InSiGHT Class	Gene	Variant	Protein	Evidence Variant Selected On	Tumor Characteristics	Family History and co-segregation with disease
5a	*MLH1*	c.(?_-198)_1558+?del	p.?	Variant allele not expressed (diploid-haploid conversion analysis [pmid:12658575 Wagner:2003])	2 MSI-H tumours: 1 [pmid:19690142 Mueller:2009]; 1 [USC CCFR]	Sufficient segregation data: 2 ACI families (4 affected carriers & 3 affected carriers [USC CCFR])
4a	*MLH1*	c.-54519_1731+2263del	p.?	Exon 1-15 genomic deletion confirmed (LR-PCR & breakpoint analysis [pmid:16837128 Pistorius:2007]; diploid-haploid conversion analysis [pmid:18330910 Staaf:2008])	2 MSI-H: 1 [pmid:15849733 Mangold:2005], [pmid:16837128 Pistorius:2007]; 1 [lovd:Lagerstedt Robinson; 00078]	Summary family history only: ACI [lovd:Lagerstedt Robinson; 00078]. Bethesda [pmid:15849733 Mangold:2005], [pmid:16837128 Pistorius:2007].
4a	*MLH1*	c.(?_-198)_207+?del c.-381_207+606del	p.?	Aberrant transcript (allelic imbalance [pmid:19173287 Gylling:2009]; PTT [pmid:19459153 Chong:2009])	MSI-H: 1 [pmid:16423994 Zhang:2006], [pmid:19173287 Gylling:2009]. MLH1 absent: 1 [pmid:19459153 Chong:2009]	Summary family history only: Bethesda [pmid:16143124 Baudhuin:2005]. 2 ACI families ([pmid:16423994 Zhang:2006], [pmid:19173287 Gylling:2009], [pmid:19459153 Chong:2009])
3a	*MLH1*	c.104_105insAA	p.(Met35Ilefs*7)	Allelic imbalance [pmid:20860725 Santibez Koref:2010]	MSS: 1 [pmid:11720433 Coleman:2001]	Summary family history only: ACI [pmid:11720433 Coleman:2001]
4a	*MLH1*	c.1011dup	p.Asn338Glnfs*24	Aberrant transcript (IVSP [pmid:9833759 Hutter:1998]; PTT [pmid:19459153 Chong:2009])	MSI-H: 1 [pmid:9833759 Hutter:1998]. MLH1 absent: 1 [pmid:19459153 Chong:2009]	Summary family history only: 2 ACI families ([pmid:9833759 Hutter:1998]; [pmid:19459153 Chong:2009])
3a	*MLH1*	c.1039-?_(*193_?)del	p.?	Truncated polypeptide (IVSP, nucleotide not identified [pmid:09718327 Farrington:1998])	MSI-H: 1 [pmid:09718327 Farrington:1998]	Summary family history only
5a	*MLH1*	c.1039-?_1409+?del	p.Thr347Lysfs*8	Truncated polypeptide (IVSP & RT-PCR of patient RNA [pmid:8128251 Papadopoulos:1994]; [pmid:08574961 Liu:1996]; RT-PCR of patient RNA [pmid:8776590 Nystrom-Lahti:1996])	5 MSI-H: 1 [pmid:08574961 Liu:1996]; 2 [pmid:19690142 Mueller:2009]; 1 [Aus CCFR]; 1 [lovd:Elke Holinski-Feder and Moni Morak; 00057]	Sufficient segregation data: Linkage to MLH1 in 2 families [pmid:8776590 Nystrom-Lahti:1996].

3a	*MLH1*	c.117-691_306+1011del	p.Cys39Trpfs*6	Confirmed genomic deletion (LR-PCR, non-homologous recombination [pmid:15942939 van der Klift:2005])	MSI-H: 1 [pmid:15849733 Mangold:2005]	Summary family history only
4a	*MLH1*	c.117-?_545+?del c.117-707_545+1338delinsTCCCGGGTTCAAGCGATTCT	p.Cys39_Arg182delinsTrp	Truncated polypedtide (PTT [Desiree du Sart])	MSI-H: 1 [pmid:16142001 Becouarn:2005]. MLH1 absent: 1 [Desiree du Sart].	Summary family history only
3a	*MLH1*	c.1217_1223dup	p.Gln409Serfs*10	Frameshift aberration (PTT [pmid:10190329 Bapat:1999])	MSI-H: 1 [pmid:10190329 Bapat:1999]	Summary family history only
5a	*MLH1*	c.1410-?_1558+?del c.1409+1156_1558+1385del c.1409+1127_1558+4255del	p.Arg470Serfs*8	Truncated polypeptide (~3kb deletion identified by IVSP [pmid:09718327 Farrington:1998], [pmid:19459153 Chong:2009])	2 MSI-H: 2 [orcid:Tops; 0000-0002-6769-7290]	Sufficient segregation data: 2 families, 4 affected carriers & 3 affected carriers [Mayo CCFR]; 3 affected carriers [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794]; 2 affected carriers [orcid:Tops; 0000-0002-6769-7290]
3a	*MLH1*	c.1410-?_1731+?del	p.Lys471Serfs*13	Aberrant transcript (RT-PCR of patient RNA [pmid:8971183 Mauillon:1996])	NA	Summary family history only
5a	*MLH1*	c.1411_1414del	p.Lys471Aspfs*19	Truncated polypeptide (IVSP [pmid:08574961 Liu:1996])	3 MSI-H: 1 [pmid:08574961 Liu:1996]; 1 [pmid:15926618 Wolf:2005]; 1 [pmid:18389388 Goldberg:2008]. MSS: 1 [pmid:15926618 Wolf:2005]	Sufficient segregation data: segregation LR 12:1 [Mayo CCFR]
5a	*MLH1*	c.1449del	p.Asp484Metfs*7	Truncated polypeptide (IVTT & RT-PCR of patient RNA [pmid:8880570 Froggatt:1996])	>2 MSI-H: number not specified [pmid:8880570 Froggatt:1996]	Sufficient segregation data: LOD 1.38, 6 affected [pmid:8880570 Froggatt:1996]

5a	*MLH1*	c.1459C>T	p.Arg487*	Truncated polypeptide (PTT [pmid:10713887 Fidalgo:2000]; no effect on splicing in diploid-haploid conversion analysis [pmid:15713769 Casey:2005])	15 MSI-H: 1 [pmid:15849733 Mangold:2005], [pmid:16216036 Mangold:2005]; 1 [pmid:18726168 Yap:2009]; 1 [pmid:20924129 Giraldez:2010]; 4 [orcid:Soto; 0000-0003-0234-9188]; 4 CRC & 1 adenoma [orcid:Capella; 0000-0002-4669-7320]; 1 [orcid: Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 2 [orcid:Yuen; 0000-0002-1768-4184]; 1 [lovd:Lagerstedt Robinson; 00078]	Sufficient segregation data: 4 affected carriers [orcid:Tops; 0000-0002-6769-7290]; 16 affected carriers over 4 families [orcid:Capella; 0000-0002-4669-7320]
4a	*MLH1*	c.1462A>T	p.Lys488*	Truncated polypeptide (PTT [Desiree du Sart])	2 MLH1 absent: 2 [1003053][1003098]	Summary family history only
4a	*MLH1*	c.1489del	p.Arg497Glyfs*11	Truncated polypeptide (PTT [Desiree du Sart])	5 MSI-H: 1 [Desiree du Sart]; 1 [pmid:15872200 Hampel:2005]; 1 gastric, 1 renal & 1 CRC [orcid:Varesco; 0000-0003-4871-6668]	Insufficient segregation data: revised Bethesda, 2 affected carriers [orcid:Varesco; 0000-0003-4871-6668]
5a	*MLH1*	c.1489dup	p.Arg497Profs*6	Frameshift aberration (PTT [pmid:10323887 Lamberti:1999]; [Desiree du Sart])	MSI-H: 3 [Mayo CCFR]	Sufficient segregation data: 3 families, segregation LR 56:1 [Mayo CCFR]; 11 affected carriers over 4 ACI families [Moni Morak].
4a	*MLH1*	c.1491del	p.(Arg498Glufs*10)	Defective PMS2 interaction (GST-fusion assay [pmid:12810663 Kondo:2003])	MSI-H: 2 [pmid:9500462 Yamamoto:1998]	Summary family history only
5a	*MLH1*	c.1554dup	p.(Glu519*)	Allelic imbalance [pmid:20860725 Santibez Koref:2010]	MSI-H: 2 [Aus CCFR]	Sufficient segregation data: 4 affected carriers [Aus CCFR]
3a	*MLH1*	c.1559-1322_1668-391del	p.Leu521Lysfs*34	Truncated polypeptide (PTT [pmid:10495924 Wahlberg:1999])	MSI-H: 1 [pmid:17312306 Lagerstedt Robinson:2007];	Summary family history only
3a	*MLH1*	c.1622del	p.(Ala541Aspfs*50)	Defective PMS2 interaction (GST-fusion assay [pmid:12810663 Kondo:2003])	MSI-H: 1 [pmid:15849733 Mangold:2005], [pmid:16216036 Mangold:2005]; 1 adenoma [Moni Morak]	Summary family history only

4a	*MLH1*	c.1669G>T	p.Glu557*	Truncated polypeptide (PTT [Desiree du Sart])	MSI-H: 1 [pmid:15655560 Apessos:2005]	Sufficient segregation data: 3 affected carriers & 3 obligate carriers [pmid:15655560 Apessos:2005]
4a	*MLH1*	c.1672G>T	p.Glu558*	Frameshift aberration (PTT [pmid:10480359 Wang:1999])	2 MSI-H: 1 [pmid:16216036 Mangold:2005]; 1 [Moni Morak]	Summary family history only
5a	*MLH1*	c.1732-?_1896+?del c.1731+270_1896+73del c.1732-2243_1896+404del	p.Pro579_Glu633del	Aberrant transcript (3.5kb genomic deletion, Finnish Founder mutation, PTT [pmid:10200055 Holmberg:1998])	6 MSI-H tumours (6 [pmid:17312306 Lagerstedt Robinson:2007])	Sufficient segregation data: Finnish/Swedish founder mutation (>30 kindreds [pmid:8776590 Nystrom-Lahti:1996])
4a	*MLH1*	c.1758dup	p.(Met587Hisfs*6)	Defective PMS2 interaction (GST-fusion assay [pmid:12810663 Kondo:2003])	Absent: 1 EC [pmid:17973265 Yoon:2008]	Sufficient segregation data: 3 affected carriers [pmid:7757073 Han:1995]. Korean founder mutation (segregates in 11 families [pmid:15365995 Shin:2004]).
4a	*MLH1*	c.1764del	p.Ala589Profs*2	Truncated polypeptide (PTT [pmid:19459153 Chong:2009])	MSI-H: 1 [pmid:09831355 Yuan:1998]. MLH1 absent: 1 [pmid:19459153 Chong:2009]	Insufficient segregation data: ACI (French Canadian segregation LR 7.8:1 [pmid:09831355 Yuan:1998])
4a	*MLH1*	c.1772_1775del	p.(Asp591Valfs*24)	Defective PMS2 interaction (GST-fusion assay [pmid:12810663 Kondo:2003])	3 MSI-H: 1 [pmid:17312306 Lagerstedt Robinson:2007], [orcid:Nordling; 0000-0002-4047-4994]; 2 [pmid:8872463 Moslein:1996]	Insufficient segregation data: Bethesda family with segregation LR 1.9:1 [lovd:Lagerstedt Robinson; 00078], [orcid:Nordling; 0000-0002-4047-4994]
5a	*MLH1*	c.1783_1784del	p.(Ser595Trpfs*14)	Defective PMS2 interaction (GST-fusion assay [pmid:12810663 Kondo:2003])	4 MSI-H: 1 [pmid:16216036 Mangold:2005]; 1 [pmid:10601588 Fornasarig:2000]; 2 [0000-0002-1768-4184]	Sufficient segregation data: 5 affected carriers [pmid:8571956 Wijnen:1996]. 4 affected carriers [0000-0002-1768-4184]
5a	*MLH1*	c.1831_1832del	p.Ile611Cysfs*2	Truncated polypeptide (PTT [pmid:15253764 Thiffault:2004])	MSI-H: 1 gastric [pmid:16237216 Bacani:2005]. 2 MLH1 absent: 2 [pmid:15253764 Thiffault:2004]	Sufficient segregation data: 2 families, 4 affected carriers each [pmid:15253764 Thiffault:2004]
5a	*MLH1*	c.184C>T	p.Gln62*	Truncated polypeptide (2 cases: PTT [pmid:10323887 Lamberti:1999])	MSI-H: 1 [lovd:Elke Holinski-Feder, Moni Morak; 00057]. 7 MLH1 absent: 3 CRC & 1 EC [pmid:16034045 Stormorken:2005], [pmid:20587412 Sjursen:2010], [Pal Moller]; 3 [Pal Moller]	Sufficient data: 7 affected carriers over 2 families, 4 informative meioses [pmid:20587412 Sjursen:2010], [Pal Moller]

3a	*MLH1*	c.1852A>T	p.Lys618*	Truncated polypeptide (IVSP [pmid:9833759 Hutter:1998])	MSI-H: 1 BrCa [pmid:22034109 Buerki:2012]	Summary family history only: ACI (Swiss proband CRC age 34 [pmid:9833759 Hutter:1998])
4a	*MLH1*	c.1897-?_1989+?del	p.(Glu633_Glu663del)	Deficient MMR activity [pmid:16083711 Raevaara:2005]	2 MSI-H: 1 [pmid:10732761 Dieumegard:2000]; 1 [pmid:16837128 Pistorius:2007]	Summary family history only
5a	*MLH1*	c.1975C>T	p.[Glu633_Glu663del, Arg659*]	r.[1897_1989del, 1975c>u] (Truncated polypeptide and partial splicing aberration, IVSP [pmid:09718327 Farrington:1998]; PTT [pmid:10200055 Holmberg:1998]; exon 17 skipping: RT-PCR of patient RNA [pmid:10534773 Nystrom-Lahti:1999], [pmid:15235038 Renkonen:2004]; diploid-haploid conversion[pmid:15713769 Casey:2005])	5 MSI-H: 1 [Ontario CCFR]; 2 [Aus CCFR]; 1 [Moni Morak]; 1 [orcid:Ian Frayling @ Dundee; 0000-0002-3420-0794]	Sufficient segregation data: 6 affected carriers [Ontario CCFR]; 3 affected carriers [Aus CCFR]
4a	*MLH1*	c.1975_1976del	p.(Arg659Thrfs*4)	r.1897_1989del (exon 17 skipping, RT-PCR of patient RNA [pmid:08808596 Kohonen-Corish:1996])	NA	Sufficient segregation data: 3 affected carriers & 6 asymptomatic carriers [Aus CCFR]
4a	*MLH1*	c.1986_1989+1delinsC	p.Glu633_Glu663del	r.1897_1989del (Exon 17 skipping, no full-length expressed by variant allele in RT-PCR of patient RNA [pmid:16341550 Pagenstecher:2006])	MSI-H (1 CRC [pmid:16341550 Pagenstecher:2006])	Sufficient segregation data: ACI (German family, at least 4 affected carriers [pmid:16341550 Pagenstecher:2006])
5a	*MLH1*	c.208-?_306+?del	p.Lys70_Glu102del	Truncated polypeptide (PTT [Desiree du Sart])	4 MLH1 absent: 1 EC [Desiree]; 2 [Aus CCFR], [Rodney Scott]; 1 [orcid:Capella; 0000-0002-4669-7320]	Sufficient segregation data: 3 affected carriers & 2 obligate carriers [Aus CCFR]
4a	*MLH1*	c.208-?_453+?del	p.Lys70_Thr151del	Truncated polypeptide (RT-PCR of patient RNA [pmid:9593786 Aaltonen:1998]; PTT & RT-PCR [pmid:10200055 Holmberg:1998])	2 MSI-H: 1 [pmid:16423994 Zhang:2006]; 1 [pmid:19173287 Gylling:2009]	Insufficient segregation data: Segregation with disease reported [pmid:19173287 Gylling:2009]
5a	*MLH1*	c.207+1560_546-871del	p.Lys70Valfs*9	Truncated polypeptide (PTT [Desiree du Sart])	2 MSI-H: 2 [Desiree du Sart]	Sufficient segregation data: 2 familes with 3 affected carriers each [Aus CCFR]

4a	*MLH1*	c.210_213del	p.[Glu71Ilefs*20, Lys70_Glu102del]	Truncated polypeptide (partial exon 3 skipping in RT-PCR of patient RNA [pmid:15235038 Renkonen:2004]; PTT [Desiree du Sart])	2 MSI-H: 1 [orcid:Genuardi; 0000-0002-7410-8351]; 1 [Maria Grazia Tibiletti]	Insufficient segregation data: Segregation with disease reported [pmid:15235038 Renkonen:2004]
4a	*MLH1*	c.2135G>A	p.Trp712*	Truncate polypeptide (IVSP [pmid:08574961 Liu:1996])	2 MSI-H: 1 [pmid:08574961 Liu:1996]; 1 [pmid:11720433 Coleman:2001]	Summary family history only
5a	*MLH1*	c.2141G>A	p.Trp714*	Truncated polypeptide (IVSP [pmid:8863153 Hutter:1996], [pmid:9833759 Hutter:1998])	2 MSI-H: 1 [pmid:8880570 Froggatt:1996]; 1 [pmid:17054581 Sheng:2006]	Sufficient segregation data: ACI (Swiss family, 7 affected carriers [pmid:8863153 Hutter:1996])
3a	*MLH1*	c.2163T>A	p.Tyr721*	Truncated polypeptide (PTT [pmid:10713887 Fidalgo:2000])	NA	Summary family history only
5a	*MLH1*	c.2195_2198dup	p.His733Glnfs*14	Truncated polypeptide (PTT [pmid:10190329 Bapat:1999])	5 MSI-H: 1 BrCa [pmid:8646682 Risinger:1996]; 1 [pmid:15217520 Kunstmann:2004]; 3 CRC [pmid:10190329 Bapat:1999]	Sufficient segregation data: ACI (North American family, segregation LR 52:1 [pmid:8646682 Risinger:1996])
5a	*MLH1*	c.298C>T	p.Arg100*	Aberrant transcript & truncated polypeptide (PTT [pmid:10480359 Wang:1999]; SnuPe [pmid:14512394 Renkonen:2003])	4 MSI-H: 1 [pmid:15872200 Hampel:2005]; 1 [orcid:Soto; 0000-0003-0234-9188]; 1 [Ontario CCFR]; 1 [orcid:Tops; 0000-0002-6769-7290]	Sufficient segregation data: Finnish ACI family, 9 affected carriers [pmid:14512394 Renkonen:2003]. Dutch ACI family, 3 affected carriers [orcid:Tops; 0000-0002-6769-7290].
4a	*MLH1*	c.307-820_380+896del c.307-1420_380+624del	p.Ala103Serfs*13	Exon 4 deletion confirmed (LR-PCR & breakpoint analysis, Alu-Alu [pmid:12494471 Wang:2003])	2 MSI-H: 1 [pmid:15849733 Mangold:2005]; 1 [orcid:Capella; 0000-0002-4669-7320]	Summary family history only
4a	*MLH1*	c.307-245_454-365del	p.Ala103_Thr151del	Truncated polypeptide (PTT [Desiree du Sart])	2 MSI-H: 1 [Desiree du Sart]; 1 [Aus CCFR]	Insufficient segregation data: Bethesda proband segregation LR 1.9:1 [Aus CCFR], [Rodney Scott]
4a	*MLH1*	c.307-?_545+?del	p.Ala103Valfs*9	Exon 4-6 deletion confirmed as contiguous exons in PCR [pmid:19173287 Gylling:2009].	2 MSI-H: 1 OvCa [pmid:16360201 Malander:2006]; 1 [pmid:19173287 Gylling:2009]	Insufficient segregation data: segregation reported in ACI family [pmid:19173287 Gylling:2009].
5a	*MLH1*	c.307-797_677+1061del	p.Ala103Argfs*8	Truncated polypeptide (PTT [pmid:10495924 Wahlberg:1999]; RT-PCR of patient RNA & genotyping intragenic SNPs [pmid:11260232 Liu:2001])	2 MSI-H tumours [pmid:10564582 Liu:2000]	Insufficient segregation data: Bethesda (Swedish family, 2 affected carriers [pmid:11260232 Liu:2001])

3a	*MLH1*	c.378del	p.Tyr126*	Frameshift aberration (PTT [pmid:10480359 Wang:1999])	NA	Summary family history only
4a	*MLH1*	c.381-415_453+733del	p.Ala128Trpfs*8	Exon 5 deletion confirmed (RT-PCR of patient RNA [pmid:12624159 Sumitsuji:2003])	2 MLH1 absent: 2 [Aus CCFR]	Insufficient segregation data: ACI fam with seg LR 1.9:1 [Aus CCFR]; Bethesda fam seg LR 1.4:1 [Hawaii CCFR]: total seg LR 2.7:1
5a	*MLH1*	c.454-?_545+?del c.454-505_546-1102del c.454-665_545+49del c.454-432_546-1030del c.454-466_546-1062del	p.Glu153Phefs*8	Truncated polypeptide & exon 6 deletion confirmed (LR-PCR [pmid:14635101 Taylor:2003], [pmid:12402334 Viel:2002]; PTT [Desiree du Sart]; breakpoint analysis [pmid:18330910 Staaf:2008], [pmid:15942939 van der Klift:2005])	8 MSI-H: 1 [pmid:18625694 Ramsoekh:2008]; 2 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 1 duodenal & 1 CRC [orcid:Tops; 0000-0002-6769-7290]; 2 [Robert Hofstra]; 1 [pmid:21387278 Bozzao:2011]	Sufficient segregation data: segregation LR 4.2:1 [Ian Frayling - Belfast]; Bethesda family segregation LR 1.9 [Robert Hofstra]; Italian family, 2 affected carriers [pmid:21387278 Bozzao:2011].
3a	*MLH1*	c.[453+625_545+921delinsTG;545+1271_677+737delinsCA]	p.Val152Argfs*8	Complex deletion of exons 6-7 confirmed (PTT, g.37024184_37026321delinsTG + g.37026671_37029331delinsCA [pmid:16098012 McVety:2005])	NA	Summary family history only
4a	*MLH1*	c.546-?_790+?del	p.Tyr183Serfs*42	Exon 7-9 deletion confirmed (RT-PCR of patient RNA [pmid:16423994 Zhang:2006])	3 MSI-H: 1 OvCa, 2 CRC [pmid:16423994 Zhang:2006]	Summary family history only: ACI (Swiss proband CRC age 61 [pmid:16423994 Zhang:2006])
3a	*MLH1*	c.546-361_885-811del	p.Arg182_Leu294del	Exon 7-10 deletion confirmed (8kb deletion identified by LR-PCR and breakpoint analysis [pmid:12494471 Wang:2003])	MSI-H: 1 [pmid:16216036 Mangold:2005]	Insufficient segregation data: ACI family with 2 affected carriers [pmid:12494471 Wang:2003]
3a	*MLH1*	c.586A>T	p.Lys196*	Truncated polypeptide (PTT [pmid:10480359 Wang:1999])	NA	Summary family history only
3a	*MLH1*	c.588del	p.Lys196Asnfs*6	Truncated polypeptide (PTT [Desiree du Sart])	MLH1 absent: 1 [Aus CCFR]	Summary family history only
3a	*MLH1*	c.673_676del	p.Ser225Glufs*3	Truncated polypeptide (PTT [pmid:10480359 Wang:1999])	NA	Summary family history only

5a	*MLH1*	c.676C>T	p.(Arg226*)	Homozygote with CMMRD ([pmid:9927033 Ricciardone:1999], [pmid:17889038 Alotaibi:2008])	7 MSI-H: 1 [pmid:18307539 Yan:2008]; 1 [pmid:19690142 Mueller:2009]; 1 [pmid:20045164 Chang:2010]; 1 [pmid:20924129 Giraldez:2010]; 1 [orcid:Soto; 0000-0003-0234-9188]; 1 [orcid:Genuardi; 0000-0002-7410-8351]; 1 [Moni Morak]	Sufficient segregation data: 4 affected non-proband carriers [pmid:9927033 Ricciardone:1999], [pmid:17889038 Alotaibi:2008]. 4 affected non-proband carriers [pmid:21247423 Castillejo:2011], [pmid:21868491 Perez-Carbonell:2012]. 3 affected non-proband carriers [pmid:15655560 Apessos:2005]. Segregation LR 3.8:1 [Mayo CCFR]. 2 affected carriers [orcid:Genuardi; 0000-0002-7410-8351]. German ACII family with segregation LR 3.5:1 [Nils Rahner]: total segregation LR 13.3:1
3a	*MLH1*	c.678-?_884+?del	p.Glu227_Ser295del	Truncated polypeptide (PTT [Desiree du Sart])	MLH1 absent: 1 [Desiree du Sart]	Summary family history only
3a	*MLH1*	c.791-?_884+?del	p.His264Leufs*2	Exon 10 deletion confirmed (1kb deletion identified by LR-PCR & breakpoint analysis [pmid:16451135 Kurzawski:2006])	NA	Summary family history only
5a	*MLH1*	c.806C>G	p.(Ser269*)	CMMR-D family [pmid:15571801 Rey:2004].	2 MSI-H: 1 [pmid:12547705 Hendriks:2003]; 1 [orcid:Tops; 0000-0002-6769-7290]	Sufficient segregation data: ACI family with 7 affected carriers & ACI fam with 4 affected carriers [orcid:Tops; 0000-0002-6769-7290].
3a	*MLH1*	c.884_884+3del	p.(His264Leufs*2)	r.791_884del (Exon 10 skipping: RT-PCR of patient RNA [pmid:12362047 Kurzawski:2002], [pmid:16451135 Kurzawski:2006])	NA	Summary family history only
4a	*MLH1*	c.885-?_1038+?del c.885-493_1039-372del c.885-594_1038+1123del	p.Ser295Argfs*21	Exon 11 deletion confirmed (LR-PCR, homologous recombination [pmid:15942939 van der Klift:2005], [pmid:11420710 Chan:2001])	MSI-H: 1 CRC [pmid:11420710 Chan:2001]. MLH1 absent: 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]	Insufficient segregation data: 2 affected carriers [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]

4a	*MLH1*	c.885-206_997del	p.?	Large deletion confirmed by break-point analysis [pmid:18330910 Staaf:2008]	2 MSI-H: 1 [pmid:17312306 Lagerstedt Robinson:2007]; 1 [lovd:Lagerstedt Robinson; 00078]	Summary family history only
3a	*MLH1*	c.889G>T	p.Glu297*	Truncated polypeptide (IVSP of patient RNA [pmid:09399661 Wang:1997], [pmid:10480359 Wang:1999])	NA	Insufficient segregation data: Bethesda (French proband CRC age 35 [pmid:09399661 Wang:1997]). German ACI family with segregation LR 4.3:1 [Nils Rahner].
5a	*MLH1*	c.901C>T	p.Gln301*	Aberrant transcript (Allelic imbalance [pmid:20860725 Santibez Koref:2010])	7 MSI-H: 1 [pmid:14871975 de Jong:2004]; 2 [pmid:10448273 Capozzi:1999]; 1 [pmid:18307539 Yan:2008]; 1 [orcid:Tops; 0000-0002-6769-7290]; 2 [orcid:Viel; 0000-0003-2804-0840]	Sufficient segregation data: AC1 fam with segregation LR 1.9:1 [pmid:08993976 Viel:1997], [orcid:Viel; 0000-0003-2804-0840]; Bethesda family with segregation LR 7.2:1 [orcid:Tops; 0000-0002-6769-7290]: total seg LR 13.7:1
4a	*MLH1*	c.954del	p.His318Glnfs*49	Truncated polypeptide (PTT [pmid:10660333 Panariello:1998])	MSI-H/ACII (1 [pmid:15849733 Mangold:2005])	Sufficient segregation data: 4 affected carriers & 3 affected carriers in 2 families [pmid:10660333 Panariello:1998].
5a	*MSH2*	c.(?_-68)_(*272_?)del	p.?	Whole gene deletion (confirmed as contiguous exons [pmid:19173287 Gylling:2009])	2 MSI-H: 1 [pmid:16216036 Mangold:2005]; 1 [orcid:Genuardi; 0000-0002-7410-8351]	Sufficient segregation data: segregation with disease reported [pmid:19173287 Gylling:2009]. 6 affected carriers [Aus CCFR]. 2 families with 2 affected carriers each [orcid:Genuardi; 0000-0002-7410-8351].
5a	*MSH2*	c.(?_-68)_1076+?del c.-11844_1077-6021delins155 c.-823_1076+5984del c.-956_1077-5607del	p.?	Truncated polypeptide (IVSP [pmid:07704024 Liu:1995]; conversion analysis [pmid:10693791 Yan:2000])	7 MSI-H: 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 1 [Moni Morak]; 2 [pmid:12938096 Nakagawa:2003]; 3 [pmid:18809606 Hampel:2008]	Sufficient segregation data: 7 affected carriers [orcid:Ian Frayling @ Cardiff; 0000-0002-3420-0794]; North American founder mutation, segregates in 9 families [pmid:14871915 Lynch:2004].
5a	*MSH2*	c.(?_-68)_1276+?del c.-47156_1277-4980del	p.?	Exon 1-7 deletion confirmed (conversion analysis [pmid:10693791 Yan:2000]; Southern Blot [pmid:15942939 van der Klift:2005]; aCGH	4 MSI-H tumours: 2 [pmid:16251890 Pastrello:2006]; 1 [orcid:Tops; 0000-0002-6769-7290]; 1 [pmid:12938096 Nakagawa:2003].	Segregation with sufficient data: segregation with disease in ACI family reported [pmid:15949572 Zhu:2005]. ACI family with 4 affected carriers [orcid:Tops; 0000-

				[pmid:18330910 Staaf:2008]; break-point analysis [pmid:12938096 Nakagawa:2003])		0002-6769-7290].
5a	*MSH2*	c.(?_-68)_1386+?del	p.?	Confirmed as contiguous exons [pmid:19173287 Gylling:2009]	5 MSI-H tumours: 1 [pmid:16251890 Pastrello:2006]; 2 [orcid:Ian Frayling @ Cardiff; 0000-0002-3420-0794]; 1 [Moni Morak]; 1 [lovd:Lagerstedt Robinson; 00078]. MSS: 1 [lovd:Lagerstedt Robinson; 00078].	Sufficient segregation data: segregation with disease in ACII family reported [pmid:19173287 Gylling:2009]. 3 affected carriers [Aus CCFR]. 4 affected carriers [Mayo CCFR]. 2 affected carriers [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794]. 2 affected carriers [Moni Morak].
4a	*MSH2*	c.(?_-68)_1759+?del c.-75398_1759+1708del	p.?	Exon 1-11 deletion confirmed [pmid:16885385 Hampel:2006]	2 MSI-H tumours: 1 CRC [pmid:15872200 Hampel:2005]; 1 EC [pmid:16885385 Hampel:2006].	Summary family history only
4a	*MSH2*	c.(?_-68)_211+?del	p.?	Exon 1 deletion confirmed 2.1kb deletion identified by Southern blot [pmid:16826164 Wijnen:1998]. Allele not transcribed [pmid:15870828 Wehner:2005].	7 MSI-H tumours: 1 EC [orcid:Soto; 0000-0003-0234-9188]; 1 [pmid:16142001 Becouarn:2005]; 1 sebaceous epithelioma [pmid:16826164 Ponti:2006]; 2 CRC & 1 EC [pmid:17453009 Overbeek:2007]; 1 [Moni Morak]	Insufficient segregation data: 2 families: segregation LR 1.7 [Aus CCFR]
5a	*MSH2*	c.(?_-68)_366+?del c.-4729_367-353del	p.?	Exon 1-2 deletion confirmed by diploid-haploid conversion analysis [pmid:12938096 Nakagawa:2003]	4 MSI-H tumours: 1 [orcid:Soto; 0000-0003-0234-9188]; 1 CRC [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 1 EC [orcid:Capella; 0000-0002-4669-7320]; 1 [pmid:12938096 Nakagawa:2003]	Sufficient segregation data: 4 affected carriers [Aus CCFR]. 2 affected carriers [orcid:Capella; 0000-0002-4669-7320].
3a	*MSH2*	c.(?_-68)_792+?del	p.?	Exon 1-4 deletion confirmed by LR-PCR [pmid:11830542 Charbonnier:2002]	MSI-H: 1 [pmid:18307539 Yan:2008]; 1 adenoma [orcid:Viel; 0000-0003-2804-0840]	Insufficient segregation data: Bethesda fam with 2 affected carriers [orcid:Viel; 0000-0003-2804-0840]
4a	*MSH2*	c.1034G>A	p.Trp345*	Loss of expression of affected allele due to NMD (diploid-haploid conversion analysis [pmid:15713769 Casey:2005])	2 MSI-H tumours: 1 [pmid:21387278 Bozzao:2011], [Monica Pedroni]; 1 [pmid:16142001 Becouarn:2005]	Insufficient segregation data: segregation LR 4.9 [Mayo CCFR]
5a	*MSH2*	c.1077-?_1276+?del	p.Leu360Lysfs*16	Truncated polypeptide (PTT on	3 MSI-H tumours: 1	Sufficient segregation data:

		c.1077-220_1276+6245del		patient RNA [Desiree du Sart])	[pmid:19690142 Mueller:2009], 1 [pmid:20924129 Giraldez:2010]; 1 [pmid:21778331 Perez-Cabornero:2011]. MSS [pmid:19690142 Mueller:2009].	Spanish founder mutation, 3 families: 4 affected carriers, 2 affected carriers [pmid:21778331 Perez-Cabornero:2011]
4a	*MSH2*	c.1077-?_1386+?del	p.Leu360Trpfs*8	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	7 MSI-H tumours: 1 [pmid:16423994 Zhang:2006]; 1 [pmid:16251890 Pastrello:2006], [Maria Grazia Tibiletti]; 1 [pmid:16736289 Spaepen:2006]; 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 1 [orcid:Tops; 0000-0002-6769-7290]; 1 [Robert Hofstra]; 1 [Paola Sala]	Insufficient segregation data: segregation LR 1.94 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000].
4a	*MSH2*	c.1077-?_1661+?del	p.Arg359_Asn553del	Exon 7-10 deletion confirmed (PTT & RT-PCR of patient RNA [pmid:10495924 Wahlberg:1999])	3 MSI-H tumours: 1 [pmid:19173287 Gylling:2009]; 1 [Moni Morak]; 1 [orcid:Nordling; 0000-0002-4047-4994].	Insufficient segregation data: segregation reported with disease [pmid:19173287 Gylling:2009].
3a	*MSH2*	c.1077_1078ins173	p.?	Truncated polypeptide (IVSP of patient RNA [pmid:08574961 Liu:1996])	NA	Summary family history only
5a	*MSH2*	c.1147C>T	p.Arg383*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	5 MSI-H tumours: 1 [pmid:19731080 Jasperson:2010]; 1 BrCa [pmid:22034109 Buerki:2012]; 1 [0000-0002-1768-4184]; 1 [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794]; 1 Sebaceous adenoma [orcid:Capella; 0000-0002-4669-7320]	Sufficient segregation data: 3 ACI families (Combined segregation LR 83.5, 1 ACI family [Aus CCFR] & 2 ACI families [Mayo CCFR]). 2 affected carriers [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794]. German Bethesda family with segregation LR 1.8:1 [Nils Rahner]. Total segregation LR 150.3:1

5a	*MSH2*	c.1165C>T	p.Arg389*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart], [pmid:19459153 Chong:2009])	7 MSI-H tumours: 1 [pmid:21868491 Perez-Carbonell:2012]; 1 carcinosarcoma [pmid:19130300 Nilbert:2009]; 1 [orcid:Genuardi; 0000-0002-7410-8351]; 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 2 [Moni Morak]; 1 [orcid:Ian Frayling@Liverpool; 0000-0002-3420-0794].	Sufficient segregation data: 2 affected carriers [orcid:Genuardi; 0000-0002-7410-8351]. 4 affected carriers [Ontario CCFR]. 2 affected carriers [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000].
5a	*MSH2*	c.1189C>T	p.Gln397*	Truncated polypeptide (IVSP on patient RNA [pmid:8880570 Froggatt:1996])	4 MSI-H tumours: 1 [pmid:15235030 Mangold:2004]; 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 2 [Moni Morak]	Sufficient segregation data: 5 affected carriers [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000].
3a	*MSH2*	c.1196_1197dup	p.Asn400Glnfs*13	Frameshift aberration (PTT on patient RNA [pmid:10882759 Montera:2000])	MSI-H: 1 [pmid:10882759 Montera:2000]	Summary family history only
5a	*MSH2*	c.1216C>T	p.Arg406*	Truncated polypeptide (IVSP of patient RNA [pmid:08062247 Liu:1994]; PTT of patient RNA [pmid:19459153 Chong:2009]; allele not expressed in diploid-haploid conversion analysis [pmid:15713769 Casey:2005])	10 MSI-H tumours: 1 [pmid:21590452 Perea:2011]; 8 [pmid:10564582 Liu:2000]; 1 [orcid:Genuardi; 0000-0002-7410-8351]	Sufficient segregation data: 2 affected carriers [pmid:8261515 Leach:1993]. 3 affected carriers [pmid:15952990 Sarroca:2005]. 8 tumours from carriers in ACI family [pmid:10564582 Liu:2000]. 2 affected carriers [orcid:Genuardi; 0000-0002-7410-8351]. 3 families with 3 affected carriers each [orcid:Capella; 0000-0002-4669-7320].

5a	*MSH2*	c.1226_1227del	p.Gln409Argfs*7	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	7 MSI-H tumours: 1 [pmid:21778331 Perez-Cabornero:2011]; 1 [Mayo CCFR]; 2 [pmid:15955785 Mueller-Koch:2005], [Moni Morak]; 1 [pmid:17312306 Lagerstedt Robinson:2007]; 1 [orcid:Tops; 0000-0002-6769-7290]; 1 [Moni Morak]	Sufficient segregation data: 2 families with segregation LR 2.0:1 [Aus CCFR], [Mayo CCFR]. Dutch family with segregation LR 3.7:1 [orcid:Tops; 0000-0002-6769-7290]. 5 affected carriers over 2 ACI families [Moni Morak]. German ACI family with segregation LR 0.9:1 [Nils Rahner]. Combined segregation LR 6.7:1
4a	*MSH2*	c.1255C>T	p.Gln419*	Truncated polypeptide (IVSP on patient RNA [pmid:10080150 Lin:1999])	2 MSI-H tumours: 1 [orcid:Soto; 0000-0003-0234-9188]; 1 [pmid:10404063 Bai:1999]	Summary family history only
4a	*MSH2*	c.1277-?_(*272_?)inv	p.?	Inversion of exons 8-16 confirmed (LR-PCR of patient RNA [pmid:15942939 van der Klift:2005]; diploid-haploid conversion analysis [pmid:12658575 Wagner:2003])	NA	Sufficient segregation data: ACI (North American, 5 affected carriers [pmid:12203789 Wagner:2002])
5a	*MSH2*	c.1277-?_1386+?del c.1277-572_1386+2326del c.1276+232_1386+3798del	p.Lys427Glyfs*4	Exon 8 deletion confirmed (2 cases: PTT & RT-PCR of patient RNA [pmid:10190329 Bapat:1999]; RT-PCR of patient RNA [pmid:16451135 Kurzawski:2006]; PTT on patient RNA [Desiree du Sart])	9 MSI-H: 1 [pmid:19173287 Gylling:2009]; 1 [pmid:21590452 Perea:2011]; 4 [pmid:20682701 Woods:2010]; 1 [pmid:21636617 Win:2011]; 1 [Moni Morak]; 1 CRC [pmid:12938096 Nakagawa:2003]	Sufficient segregation data: Sardinian founder mutation, 13 families: 3, 9 & 4 affected non-proband carriers [pmid:22781090 Borelli:2012]
5a	*MSH2*	c.1277-?_2634+?del	p.Gly426Alafs*3	Truncated polypeptide (IVSP & RT-PCR on patient RNA [pmid:08062247 Liu:1994])	2 MSH2 absent: 2 CRC [USC CCFR]	Sufficient segregation data: 4 affected carriers [USC CCFR].
4a	*MSH2*	c.1373T>G	p.Leu458*	Truncated polypeptide (IVSP on patient RNA [pmid:08062247 Liu:1994])	5 MSI-H tumours: 2 EC & 1 OvCa [pmid:10432927 Ichikawa:1999]; 1 [pmid:11304573 Salahshor:2001], [pmid:17312306 Lagerstedt Robinson:2007]; 1 [pmid:16216036 Mangold:2005]	Insufficient segregation data: German ACI family with segregation LR 6.7 [Nils Rahner]
5a	*MSH2*	c.1387-?_1510+?del	p.Val463Alafs*22	Exon 9 deletion confirmed (2 families: RT-PCR of patient RNA [pmid:16451135 Kurzawski:2006])	7 MSI-H tumours: 1 [pmid:18931482 Sheng:2008]; 1 EC [pmid:17453009 Overbeek:2007]; 4 CRC & 1 EC [orcid:Tops; 0000-0002-6769-7290]	Sufficient segregation data: ACI fam with 10 affected carriers, Bethesda fam with 2 affected carriers, ACI fam with 3 affected carriers [orcid:Tops; 0000-0002-6769-7290]

5a	*MSH2*	c.1387-?_1661+?del	p.Val463Glnfs*7	Exon 9-10 deletion confirmed (RT-PCR of patient RNA [pmid:19173287 Gylling:2009]; PTT on patient RNA [pmid:19459153 Chong:2009])	6 MSI-H tumours: 1 [pmid:19173287 Gylling:2009]; 1 [pmid:16251890 Pastrello:2006]; 1 [pmid:15235030 Mangold:2004]; 3 [orcid:Capella; 0000-0002-4669-7320]	Sufficient segregation data: segregation with disease reported [pmid:19173287 Gylling:2009]. 13 affected carriers [Aus CCFR]. 4 affected carriers [orcid:Capella; 0000-0002-4669-7320].
5a	*MSH2*	c.1457_1460del	p.Asn486Thrfs*10	Frameshift aberration (2 cases: IVSP on patient RNA [pmid:10413423 Chan:1999])	11 MSI-H tumours: 2 [pmid:10413423 Chan:1999]; 9 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]	Sufficient segregation data: Chinese founder mutation, segregates in 10 families [pmid:15042510 Chan:2004] & segregates in 8 families [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000].
5a	*MSH2*	c.1552_1553del	p.Gln518Valfs*10	Frameshift aberration (PTT on patient RNA [pmid:10200055 Holmberg:1998])	MSI-H in 4 tumours: 1 [pmid:18307539 Yan:2008]; 3 [pmid:10564582 Liu:2000]	Sufficient segregation data: 2 ACI families (Finnish families, 11 affected carriers over 2 kindreds [pmid:10200055 Holmberg:1998], [pmid:14574010 Peltomaki:2001]
3a	*MSH2*	c.1552C>T	p.Gln518*	Truncated polypeptide (PTT on patient RNA [pmid:10713887 Fidalgo:2000])	MSH2 absent: 1 CRC [Kate Green]	Summary family history only
5a	*MSH2*	c.1566C>G	p.Tyr522*	Low expression of affected allele (diploid-haploid conversion analysis [pmid:15713769 Casey:2005])	MSI-H: 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]. 2 MSH2 absent: 1 [pmid:15713769 Casey:2005]; 1 [Aus CCFR]	Sufficient segregation data: 2 families, segregation LR 36.5 [Aus CCFR], [Mayo CCFR], [pmid:15713769 Casey:2005]. 2 affected carriers [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000].
4a	*MSH2*	c.1578del	p.Cys527Valfs*16	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 [pmid:15235030 Mangold:2004]. MSH2 absent: 1 [Desiree du Sart].	Insufficient segregation data: German MTS family [pmid:15235030 Mangold:2004]. ACII fam with seg LR 1.9:1 [Kate Green].
4a	*MSH2*	c.1662-?_2458+?del c.1662-374_2458+467del	p.Ser554Argfs*4	Exon 11-14 deletion confirmed (8.4kb deletion identified by LR-PCR & breakpoint analysis, Alu-	5 MSI-H tumours: 1 [pmid:14645426 Berends:2003], [pmid:16636019 Niessen:2006];	Summary family history only

				Alu [pmid:12494471 Wang:2003])	2 [orcid:Tops; 0000-0002-6769-7290]; 2 [pmid:16216036 Mangold:2005].	
5a	*MSH2*	c.1738G>T	p.Glu580*	Truncated polypeptide (IVSP on patient RNA [pmid:10413423 Chan:1999]; low expression of affected allele in conversion analysis [pmid:15713769 Casey:2005]; PTT on patient RNA [Desiree du Sart])	4 MSI-H tumours: 3 CRC [pmid:15849733 Mangold:2005], [pmid:16216036 Mangold:2005]; 1 [pmid:19731080 Jasperson:2010]	Sufficient segregation data: 8 affected carriers [pmid:15713769 Casey:2005], [Aus CCFR].
3a	*MSH2*	c.1760-?_2005+?del c.1759+305_2006-34del	p.Tyr588_Gly669del	Exon 12 deletion confirmed (1.9kb deletion identified by LR-PCR [pmid:18307539 Yan:2008])	MSI-H: 1 [pmid:18307539 Yan:2008]	Summary family history only
4a	*MSH2*	c.1760-361_2634+838del	p.Gly587Alafs*3	Exon 12-15 deletion confirmed (7kb deletion identified by LR-PCR & breakpoint analysis, Alu-Alu [pmid:12494471 Wang:2003])	MSI-H/ACII (1 CRC [pmid:15849733 Mangold:2005])	Sufficient segregation data: 4 affected carriers In ACI family [pmid:12494471 Wang:2003]
4a	*MSH2*	c.1760del	p.Gly587Alafs*3	Frameshift aberration (IVSP on patient RNA [pmid:10413423 Chan:1999])	2 MSI-H tumours: 1 glioma [pmid:9777949 Leung:1998]; 1 CRC [pmid:12386821 Yuen:2002]	Summary family history only
4a	*MSH2*	c.1801C>T	p.Gln601*	Truncated polypeptide (IVSP on patient RNA [pmid:07585065 Liu:1995], [pmid:09718327 Farrington:1998])	MSI-H: 1 [pmid:07704024 Liu:1995]	Sufficient segregation data: 6 affected carriers, LOD 3.08 [pmid:7713503 Kolodner:1994].
3a	*MSH2*	c.1885C>T	p.Gln629*	Truncated polypeptide (PTT on patient RNA [pmid:15991306 Kohonen-Corish:2005])	NA	Summary family history only
5a	*MSH2*	c.1889_1892del	p.Gly630Glufs*4	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSH2 absent in 3 tumours: 3 [Aus CCFR]	Sufficient segregation data: 4 families, segregation LR 18.6:1 [Aus CCFR]
3a	*MSH2*	c.1996_1997del	p.Ile666Hisfs*9	Truncated polypeptide (PTT on patient RNA [pmid:10080150 Lin:1999])	NA	Summary family history only
5a	*MSH2*	c.2006-?_2210+?del	p.Pro670Leufs*7	Truncated polypeptide (IVSP & RT-PCR of patient RNA [pmid:08062247 Liu:1994])	2 MSI-H tumours: 1 [Aus CCFR]; 1 [pmid:11407044 Soravia:2001]	Sufficient segregation data: 4 affected carriers [pmid:15951966 Akrami:2005]

5a	*MSH2*	c.2038C>T	p.Arg680*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	7 MSI-H tumours: 1 CRC [pmid:18809606 Hampel:2008]; 1 CRC [pmid:20305446 Alvarez:2010]; 1 malignant fibrous histiocytoma [pmid:21598002 Brieger:2011]; 2 [orcid:Genuardi; 0000-0002-7410-8351]; 2 [Moni Morak]	Sufficient segregation data: 4 affected carriers [Aus CCFR]. 2 affected carriers [orcid:Genuardi; 0000-0002-7410-8351]. 5 affected carriers [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794]. 4 affected carriers [Moni Morak]. 5 informative meisoses [Pal Moller].
3a	*MSH2*	c.2074_2081del	p.Gly692Cysfs*4	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 CRC [pmid:16116158 Southey:2005], [Desiree du Sart]	Summary family history only
4a	*MSH2*	c.212-?_1276+?del c.211+1566_1277-3954del	p.Ala72_Gly426del	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	2 MSH2 absent: 1 OvCa [pmid:16034045 Stormorken:2005]; 1 [Desiree du Sart]	Summary family history only
4a	*MSH2*	c.212-?_1386+?del	p.Ala72Glyfs*4	Exon 2-8 deletion confirmed (PTT & RT-PCR of patient RNA [pmid:10190329 Bapat:1999])	MSI-H: 1 [pmid:11074494 Davoodi-Semiromi:2000]	Sufficient segregation data: 3 affected carriers in ACI family [pmid:10190329 Bapat:1999]
5a	*MSH2*	c.212-?_366+?del	p.Ala72Phefs*9	Exon 2 deletion confirmed (5.4kb deletion identified by southern blot [pmid:16826164 Wijnen:1998], [pmid:15942939 van der Klift:2005]; RT-PCR of patient RNA [Moni Morak])	8 MSI-H tumours: 1 [pmid:12373605 Gille:2002]; 1 EC [pmid:14645426 Berends:2003]; 1 [pmid:15289847 Caldes:2004]; 1 [pmid:17312306 Lagerstedt Robinson:2007], [lovd:Lagerstedt Robinson; 00078]; 3 [pmid:17453009 Overbeek:2007]; 1 [orcid:Tops; 0000-0002-6769-7290].	Sufficient segregation data: ACI family with 7 affected carriers, 2x ACI families with 2 affected carriers, ACI fam with 3 affected carriers [orcid:Tops; 0000-0002-6769-7290].
5a	*MSH2*	c.2131C>T	p.Arg711*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	7 MSI-H tumours: 1 [pmid:21387278 Bozzao:2011]; 1 [pmid:21868491 Perez-Carbonell:2012]; 1 [orcid:Soto; 0000-0003-0234-9188]; 1 EC & 2 CRC [orcid:Genuardi; 0000-0002-7410-8351]; 1 [lovd:Lagerstedt Robinson; 00078].	Sufficient segregation data: 4 affected carriers [Aus CCFR].
5a	*MSH2*	c.226C>T	p.(Gln76*)	Homozygote (CMMRD in 2 families [pmid:17483304 Barwell:2007]; [pmid:17601929 Scott:2007])	MSI-H: 1 CRC [pmid:22480969 Bonnet:2012]. MSH2 absent: 1 CRC [pmid:16034045 Stormorken:2005], [Pal Moller]	Sufficient segregation data: ACII (2 Kuwaiti families, 15 affected carriers [orcid:Marafie; 0000-0003-0853-2039])

4a	*MSH2*	c.2294del	p.Ala765Valfs*47	Truncated polypeptide (IVSP on patient RNA [pmid:9218993 Pensotti:1997])	NA	Sufficient segregation data: 4 affected carriers [pmid:9218993 Pensotti:1997].
5a	*MSH2*	c.2347del	p.(His783Ilefs*29)	Loss of MSH2 expression in transfected LoVo cells [pmid:12377806 Brieger:2002]	MSI-H: 1 [pmid:11601928 Raedle:2001]. MSH2 absent: 1 EC [pmid:11291077 Berends:2001]	Sufficient segregation data: 3 affected carriers & 3 obligate carriers [pmid:7726159 Wijnen:1995]
5a	*MSH2*	c.2459-?_(*272_?)del	p.?	Complete loss of one copy of MSH2 gene (FISH on patient DNA [pmid:15943554 Grabowski:2005])	3 MSI-H tumours: 3 [pmid:15943554 Grabowski:2005], [Moni Morak]	Sufficient segregation data: 4 affected carriers [Moni Morak].
4a	*MSH2*	c.2466_2467del	p.Cys822*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	2 MSI-H tumours: 1 [Desiree du Sart]; 1 [pmid:15365996 Kruger:2004]	Summary family history only
3a	*MSH2*	c.2485del	p.His829Metfs*12	Frameshift aberration (PTT on patient RNA [pmid:15991306 Kohonen-Corish:2005])	MSH2 absent: 1 [Desiree du Sart]	Summary family history only
4a	*MSH2*	c.2502_2508del	p.Asn835Leufs*4	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 [pmid:11720433 Coleman:2001]. 3 MSH2 absent: 2 [Aus CCFR]; 1 [Diane Cairns]	Insufficient segregation data: 3 families, segregation LR 3.7:1 [Aus CCFR]
3a	*MSH2*	c.2581C>T	p.Gln861*	Frameshift aberration (PTT on patient RNA [pmid:10480359 Wang:1999])	NA	Summary family history only
5a	*MSH2*	c.2633_2634del	p.Glu878Alafs*3	Frameshift aberration (PTT on patient RNA [pmid:10190329 Bapat:1999])	11 MSI-H tumours: 4 [orcid:Soto; 0000-0003-0234-9188]; 1 [Aus CCFR]; 1 [pmid:8690195 Konishi:1996]; 1 [pmid:11208710 Terdiman:2001]; 1 [Moni Morak]; 3 [Robert Hofstra]	Sufficient segregation data: Bethesda family, with 2 affected carriers [Robert Hofstra]. ACI (Canadian family, segregation LR 15:1 [pmid:10196371 Millar:1999], [pmid:15845562 Durno:2005].
4a	*MSH2*	c.269_290dup	p.Tyr98Argfs*9	Truncated polypeptide (PTT on patient RNA [pmid:10323887 Lamberti:1999])	2 MSI-H tumours: 1 sebaceous epithelioma & 1 sebaceous adenoma [pmid:08931714 Kruse:1996]	Insufficient segregation data for Class 5: German ACI family with segregation LR 5.5:1 [Nils Rahner & Brigitte Royer-Pakora]
4a	*MSH2*	c.367-?_1386+?del	p.Ala123_Gln462del	Exon 3-8 deletion confirmed by LR-PCR, homologous recombination [pmid:15942939 van der Klift:2005]	2 MSI-H: 1 CRC & 1 sebaceous carcinoma [Maria Grazia Tibiletti]	Summary family history only: ACI (North American proband CRC age <50 [pmid:12658575 Wagner:2003])
5a	*MSH2*	c.367-?_645+?del c.367-480_645+644del c.367-681_646-956del c.367-371_646-513del	p.Ala123_Gln215del	Exon 3 deletion comfirmed (PTT & RT-PCR on patient RNA [pmid:10190329 Bapat:1999]; LR-PCR [pmid:12494471 Wang:2003]; PTT on patient	9 MSI-H tumours: 1 CRC [orcid:Mensenkamp & Ligtenberg; 0000-0003-3805-877X]; 1 [pmid:12547705 Hendriks:2003]; 1	Sufficient segregation data: 2 families: 1 affected non-proband carrier & 7 affected carriers [orcid:Genuardi; 0000-0002-7410-8351]. Segregates with disease in

				RNA [Desiree du Sart], [pmid:19459153 Chong:2009]; Southern blot [pmid:16826164 Wijnen:1998])	[pmid:10190329 Bapat:1999]; 2 [pmid:12373605 Gille:2002]; 1 [pmid:16216036 Mangold:2005]; 1 [orcid:Genuardi; 0000-0002-7410-8351]; 1 [pmid:12547705 Hendriks:2003]; 1 [pmid:12373605 Gille:2002].	3 families [orcid:Tops; 0000-0002-6769-7290]; 3 affected carriers [pmid:15655560 Apessos:2005]. Segregates with disease in 2 families [orcid:Tops; 0000-0002-6769-7290].
4a	*MSH2*	c.367-?_942+?del	p.Ala123_Gln314del	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 [pmid:15943554 Grabowski:2005]. MSH2 absent: 1 [Desiree du Sart]	Insufficient segregation data: segregation LR 2.67:1 [orcid:Capella; 0000-0002-4669-7320].
5a	*MSH2*	c.388_389del	p.(Gln130Valfs*2)	15 Portuguese families: different haplotypes [pmid:23170986 Pinheiro:2012]	MSI-H: 1 [pmid:22480969 Bonnet:2012]. 10 MSH2 absent: 1 [pmid:22480969 Bonnet:2012]; 9 [pmid:23170986 Pinheiro:2012]	Sufficient segregation data: 55 affected carriers in 15 Portuguese families: different haplotypes [pmid:23170986 Pinheiro:2012]
4a	*MSH2*	c.508C>T	p.Gln170*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 [Aus CCFR]. MSH2 absent: 1 BrCa [Aus CCFR]	Sufficient segregation data for Class 4: ACI family with segregation LR 4.3:1 [Aus CCFR]. German ACI family with segregation LR 1.6:1 [Nils Rahner]. Total segregation LR 6.9.
3a	*MSH2*	c.513del	p.Lys172Asnfs*2	Frameshift aberration (IVSP on patient RNA [pmid:10413423 Chan:1999])	MSI-H: 1 [pmid:10413423 Chan:1999]	Insufficient segregation data: segregation with disease reported [pmid:10413423 Chan:1999].
3a	*MSH2*	c.547C>T	p.Gln183*	Truncated polypeptide (PTT on patient RNA [pmid:10713887 Fidalgo:2000])	MSI-H: 1 [pmid:12200596 Ward:2002]	Summary family history only
3a	*MSH2*	c.643C>T	p.Gln215*	Truncated polypeptide (PTT [pmid:10190329 Bapat:1999])	MSI-H: 1 [pmid:10190329 Bapat:1999]	Summary family history only
4a	*MSH2*	c.645+791_1076+4894del c.645+967_1076+5075del c.646-?_1076+?del c.645+539_1077-3451del	p.Ile217Glufs*28	Exon 4-6 deletion (Alu-Alu recombination identified by aCGH & breakpoint analysis - [pmid:18330910 Staaf:2008])	NA	Sufficient segregation data for Class 4: ACI (3 affected carriers [pmid:19760518 Martinez-Bouzas:2009])
5a	*MSH2*	c.646-?_1386+?del	p.Ile216_Gln462del	Truncated polypeptide (IVSP on cell line [pmid:07704024 Liu:1995])	3 MSI-H tumours: 2 [pmid:21778331 Perez-Cabornero:2011]; 1 [orcid:Capella; 0000-0002-4669-7320].	Sufficient segregation data: Spanish founder mutation, segregates in 4 families; 2 affected carriers, 3 affected carriers, 4 affected carriers [pmid:21778331 Perez-Cabornero:2011].

4a	*MSH2*	c.646-?_792+?del	p.Ile216_Gln264del	Truncated polypeptide (PTT on patient RNA [pmid:10480359 Wang:1999])	2 MSI-H: 1 [Robert Hofstra]; 1 [Mayo CCFR]	Summary family history only
4a	*MSH2*	c.687del	p.Ala230Leufs*16	Frameshift aberration (PTT on patient RNA [pmid:10480359 Wang:1999])	3 MSI-H tumours: 1 [pmid:10404063 Bai:1999]; 1 [pmid:15926618 Wolf:2005]; 1 [Moni Morak]	Summary family history only
4a	*MSH2*	c.754C>T	p.Gln252*	Truncated polypeptide (IVSP on patient RNA [pmid:07585065 Liu:1995], [pmid:09718327 Farrington:1998])	3 MSI-H tumours: 1 [pmid:07585065 Liu:1995]; 2 [pmid:16807412 Barnetson:2006]	Sufficient segragation data for Class 4: 2 affected carriers [pmid:16807412 Barnetson:2006]. Segregation LR 3.4:1 [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794].
3a	*MSH2*	c.793-?_1386+?del	p.Ala266_Val463del	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	NA	Summary family history only
5a	*MSH2*	c.793-?_942+?del c.793-6_942+450del c.792+8_943-450del	p.Val265_Gln314del	Exon 5 deletion confirmed (RT-PCR [pmid:15063132 Miyaki:2004]; PTT on patient RNA [Desiree du Sart]; breakpoint analysis [pmid:10850409 Charbonnier:2000], [pmid:15942939 van der Klift:2005])	2 MSH2 absent: 1 [Aus CCFR]; 1 [pmid:15942939 van der Klift:2005]	Sufficient segregation data: 3 families: 4 affected carriers, 3 affected carriers & 2 affected carriers [Aus CCFR]
4a	*MSH2*	c.811_814del	p.Ser271Argfs*2	Frameshift aberration (IVSP on patient RNA [pmid:07585065 Liu:1995], [pmid:09718327 Farrington:1998])	3 MSI-H tumours: 1 [pmid:09718327 Farrington:1998]; 1 transitional cell Roupret 2004; 1 [pmid:17312306 Lagerstedt Robinson:2007], [lovd:Lagerstedt Robinson; 00078]	Summary family history only
5a	*MSH2*	c.943-?_1076+?del c.943-926_1077-1449del	p.Gly315Ilefs*29	Exon 6 deletion (Southern blot & breakpoint analysis [pmid:16826164 Wijnen:1998], [pmid:15942939 van der Klift:2005])	3 MSI-H tumours: 1 EC [pmid:17453009 Overbeek:2007]; 1 [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 1 [Moni Morak]	Sufficient segregation data: 3 affected carriers [orcid:Leung et al; 0000-0001-8614-4619; 0000-0002-1768-4184; 0000-0002-8420-6633; 0000-0002-8390-2442; 0000-0002-4716-5000]; 7 affected carriers & 4 affected carriers [orcid:Tops; 0000-0002-6769-7290].
4a	*MSH2*	c.970C>T	p.Gln324*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	3 MSI-H tumours: 2 [pmid:16142001 Becouarn:2005]; 1 [Moni Morak]	Summary family history only

4a	*MSH6*	c.(?_-152)_457+?del c.-3097_457+2010del	p.?	Exon 1-2 deletion (LR-PCR [pmid:12920072 Plaschke:2003])	3 MSI-H tumour: 1 uroethial cell tumour [pmid:17453009 Overbeek:2007]; 1 [Daniela Barana]; 1 [pmid:15483016 Plaschke:2004].	Summary family history only
3a	*MSH6*	c.762_763del	p.Ser256*	Truncated polypeptide (PTT on patient RNA [pmid:19459153 Chong:2009])	MSH6 absent 1 in tumour [pmid:19459153 Chong:2009]	Summary family history only: ACI [pmid:19459153 Chong:2009]
4a	*MSH6*	c.1139_1143del	p.Asp380Alafs*6	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSH6 absent in 2 tumours: 1 [Desiree du Sart]; 1 OvCa [orcid:Tops; 0000-0002-6769-7290].	Insufficient segregation data for Class 5: ACII fam with seg LR 7.4:1 [orcid:Tops; 0000-0002-6769-7290].
4a	*MSH6*	c.1628_1629del	p.Lys543Argfs*19	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 OvCa [orcid:Tops; 0000-0002-6769-7290]. MSS: 1 [Aus CCFR]. MSI-L: 1 [Desiree du Sart]. MSH6 absent: 1 [Aus CCFR].	Insufficient segregation LR: Bethesda proband segregation LR 1.8:1 [orcid:Tops; 0000-0002-6769-7290].
4a	*MSH6*	c.2150_2153del	p.Val717Alafs*18	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	5 MSI-H tumours: 1 OvCa [pmid:22006311 Walsh:2011]; 1 [Robert Hofstra]; 1 CRC, 1 EC, 1 sebaceous [orcid:Viel; 0000-0003-2804-0840].	Insufficient segregation data: Bethesda MTS family with segregation LR 3.7:1 [orcid:Viel; 0000-0003-2804-0840].
4a	*MSH6*	c.2765del	p.Arg922Glnfs*23	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSH6 absent in 2 tumours [Desiree du Sart]. MSS: 1 [Desiree du Sart]	Summary family history only
3a	*MSH6*	c.3053_3054del	p.Leu1018Hisfs*4	Truncated polypeptide (PTT on patient RNA [pmid:11245474 Huang:2001])	MSS: 1 [pmid:17312306 Lagerstedt Robinson:2007]	Insufficient segregation data: ACI (Swedish family, segregatio LR 2.1:1 [pmid:11245474 Huang:2001])
4a	*MSH6*	c.3103C>T	p.Arg1035*	Truncated polypeptide (PTT on patient RNA [pmid:10471527 Planck:1999])	2 MSI-H tumours: 1 [pmid:10471527 Planck:1999]; 1 OvCa [pmid:22006311 Walsh:2011]	Summary family history only
3a	*MSH6*	c.3173-433_3556+228del	p.Asp1058_Ser1185del	Exon 5-6 deletion confirmed (breakpoint analysis [pmid:16885385 Hampel:2006])	MSH6 absent: 1 EC [pmid:16885385 Hampel:2006]	Summary family history only

5a	*MSH6*	c.3202C>T	p.Arg1068*	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	MSI-H: 1 [orcid:Tops; 0000-0002-6769-7290]. MSH6 absent in 7 tumours: 2 [pmid:18301448 Steinke:2008]; 2 [Desiree du Sart]; 1 EC & 1 CRC [Moni Morak]; 1 [orcid:Capella; 0000-0002-4669-7320]	Sufficient segregation data: Total segregation LR 10.2:1. Segregation LR ~3.5:1 in revised Bethesda family [orcid:Capella; 0000-0002-4669-7320]. Segregation LR 0.79:1 [orcid:Ian Frayling @ Cardiff; 0000-0002-3420-0794]; segregation LR 3.7:1 [Aus CCFR].
5a	*MSH6*	c.3261del	p.Phe1088Serfs*2	Truncated polypeptide (PTT on patient RNA [Desiree du Sart])	3 MSI-H tumours: 1 [pmid:22480969 Bonnet:2012]; 1 EC [orcid:Mensenkamp & Ligtenberg; 0000-0003-3805-877X]; 1 [orcid:Capella; 0000-0002-4669-7320]	Sufficient segregation data: 4 affected carriers [pmid:10508506 Wijnen:1999]. 2 affected carriers [Rodney Scott]. Revised Bethesda with 2 affected carriers & ACI family with 3 affected carriers [orcid:Capella; 0000-0002-4669-7320].
4a	*MSH6*	c.3939_3957dup	p.Ala1320Serfs*5	Truncated polypeptide (PTT on patient RNA [pmid:19459153 Chong:2009], [Desiree du Sart])	3 MSI-H tumours: 1 [pmid:18809606 Hampel:2008]; 1 [pmid:16807412 Barnetson:2006]; 1 [Aus CCFR]. MSS: 1 rectal [pmid:16807412 Barnetson:2006], [orcid:Ian Frayling@Edinburgh; 0000-0002-3420-0794].	Summary family history only
4a	*MSH6*	c.3991C>T	p.Ala1268Glyfs*6	r.3802_4001del (exon 9 skipping causing frameshift, no full-length expressed by mutant allele: RT-PCR of patient RNA [pmid:16418736 Plaschke:2006])	MSI-H: 1 [pmid:18301448 Steinke:2008]. MSH6 absent in 2 tumours: 2 EC [pmid:16034045 Stormorken:2005], [pmid:20587412 Sjursen:2010].	Insufficient segregation data: Bethesda (German proband rectal cancer age 19 & EC age 24 [pmid:18301448 Steinke:2008]). 5 carriers & 10 carriers in 2 revised Behtesda families, affected status unknown [pmid:20587412 Sjursen:2010].
4a	*PMS2*	c.1145-1350_ *20545del	p.?	Exon 11-15 deletion (36kb deletion identified by Southern blot [pmid:16472587 Hendriks:2006])	PMS2 absent in 2 tumours [pmid:16472587 Hendriks:2006]	Insufficient segregation data: segregation LR 2.7:1 [pmid:16472587 Hendriks:2006], [orcid:Tops; 0000-0002-6769-7290]
4a	*PMS2*	c.164-518_803+252delinsCG	p.?	Exon 3-7 skipping frameshift (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	PMS2 absent in 2 tumours [orcid:Tops; 0000-0002-6769-7290]	Insufficient segregation data: 2 families: segregation LR 4.23:1 [orcid:Tops; 0000-0002-6769-7290]

4a	*PMS2*	c.1882C>T	p.Arg628*	RNA subject to NMD (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	2 MSI-H tumours: 1 CRC [orcid:Mensenkamp & Ligtenberg; 0000-0003-3805-877X]; 1 [orcid:Tops; 0000-0002-6769-7290]	Insufficient segregation data for Class 5: 2 families: segregation LR 5.0:1 (1) [pmid:16472587 Hendriks:2006], [orcid:Tops; 0000-0002-6769-7290]
4a	*PMS2*	c.219_220dup	p.Gly74Valfs*3	RNA subject to NMD (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	MSI-H: 1 uterine [orcid:Tops; 0000-0002-6769-7290], [Robert Hofstra]. MSI-L: 1 [orcid:Tops; 0000-0002-6769-7290]. PMS2 absent in 3 tumours: 1 lymphoma homozygote & 1 CRC [orcid:Tops; 0000-0002-6769-7290], [pmid:20186688 van der Klift:2010]; 1 [Robert Hofstra].	Insufficient segregation data: segregation LR 2.73:1 [orcid:Tops; 0000-0002-6769-7290]
4a	*PMS2*	c.2192_2196del	p.Leu731Cysfs*3	RNA subject to NMD (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	PMS2 absent in 4 tumours: 1 Nagakawa 2004; 1 [pmid:20186688 van der Klift:2010]; 2 [orcid:Tops; 0000-0002-6769-7290]	Insufficient segregation data: Bethesda family segregation LR 1.7:1 [orcid:Tops; 0000-0002-6769-7290].
4a	*PMS2*	c.2276-113_2445+1596del	p.Ala759Glyfs*8	Exon 14 skipping frameshift (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	PMS2 absent in 2 tumours: 2 [pmid:20186688 van der Klift:2010].	Summary family history only
4a	*PMS2*	c.2404C>T	p.(Arg802*)	Deficient (in vivo function Hec-1A EC cell line [pmid:07629132 Risinger:1995])	PMS2 absent in 2 tumours: 1 [pmid:18602922 Senter:2008]; 1 [Aus CCFR]	Summary family history only
5a	*PMS2*	c.24-12_107delinsAAAT	p.Ser8Argfs*5	r.24_163del (Exon 2 skipping causing frameshift: RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	PMS2 absent in 3 tumours: 2 [pmid:20186688 van der Klift:2010]; 1 [Kate Green]. PMS2 present: 1 [Kate Green]. MSS: 1 [orcid:Tops; 0000-0002-6769-7290]	Sufficient segregation data: segregation LR 2.1:1 [orcid:Tops; 0000-0002-6769-7290]. ACI family with segregation LR 5.5:1 [Kate Green]. Combined segregation LR 11.6:1.
4a	*PMS2*	c.400C>T	p.Arg134*	Frameshift aberration (PTT [pmid:7661930 Hamilton:1995])	PMS2 absent in 2 tumours: 1 [pmid:18602922 Senter:2008], [Mayo CCFR]; 1 [Moni Morak]	Summary family history only
3a	*PMS2*	c.697C>T	p.Gln233*	RNA subject to NMD (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	PMS2 absent: 1 EC [orcid:Tops; 0000-0002-6769-7290]	Summary family history only

5a	*PMS2*	c.736_741delinsTGTGTGTGAAG	p.Pro246Cysfs*3	RNA subject to NMD (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	MSI-H: 1 [pmid:22120844 Schofield:2011]. PMS2 absent in 15 tumours: 1 EC & 1 CRC [pmid:20205264 Vaughn:2010]; 1 CRC [pmid:20682701 Woods:2010]; 2 [Aus CCFR]; 4 CRC, 2 duodenal, 2 EC [orcid:Tops; 0000-0002-6769-7290]; 1 [Pal Moller]; 3 [lovd:Lagerstedt Robinson; 00078]. Present: 1 [Aus CCFR].	Sufficient segregation data: 3 Australian probands, segregation LR 1.9:1 [Aus CCFR]. CMMRD fam seg LR 3.7, Bethesda fam with seg LR 2.4:1 [orcid:Tops; 0000-0002-6769-7290]. Combined segregation LR 16.9:1
3a	*PMS2*	c.804-?_2006+?del	p.Ile269_Ser669del	Exon 8-11 deletion in frame (IVSP & RT-PCR of patient RNA [pmid:8072530 Nicolaides:1994])	MSI-H (1 [pmid:8072530 Nicolaides:1994])	Summary family history only: ACI ([pmid:8072530 Nicolaides:1994])
4a	*PMS2*	c.861_864del	p.Arg287Serfs*19	RNA subject NMD (RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	2 MSI-H tumours: 1 EC & 1 CRC [pmid:16472587 Hendriks:2006], [orcid:Tops; 0000-0002-6769-7290]	Insufficient segregation data: segregation LR 1.9:1 [pmid:16472587 Hendriks:2006], [orcid:Tops; 0000-0002-6769-7290]
4a	*PMS2*	c.989-296_1144+706del	p.Glu330_Glu381del	PMS2 exon 10 deletion (Southern blot analysis [pmid:16472587 Hendriks:2006]; RT-PCR of patient RNA [pmid:20186688 van der Klift:2010])	2 MSI-H tumours: 1 [Aus CCFR]; 1 Trichoepithelioma/trichoblastoma [pmid:17453009 Overbeek:2007]	Insufficient segregation data: 3 Australian probands with common haplotype, segregation LR 1.53:1 [pmid:22577899 Tomsic:2012], [Aus CCFR]

Supplementary Table 9 Qualifications and expertise covered by the 40 members of the InSiGHT Variant Interpretation Committee (VIC)

VIC member	Affiliation	Academic Qualifications and Expertise
Dr Kiwamu Akagi	Div. Molecular Diagnosis and Cancer Prevention, Saitama Cancer Center, Saitama, Japan	MD, PhD: Molecular Pathology
Prof Fahd Al-Mulla	Department of Pathology, Faculty of Medicine, Health Sciences Center, Kuwait University, Safat, Kuwait	BSc, M.B.,Ch.,B., PhD, FRCPE: Molecular Pathology
Prof Bharati Bapat	Department of Lab Medicine and Pathobiology, University of Toronto, Canada	BSc, MSc, PhD: Cancer Genetics, Molecular Pathology
Dr Inge Bernstein	Surgical Gastroenterology Department, Aalborg University Hospital, Aalborg, Denmark; Danish HNPCC Registry, Copenhagen, Denmark	MD, PhD, MHM: Gastrointestinal Surgery
Dr Gabriel Capella	Hereditary Cancer Program. Catalan Institute of Oncology-IDIBELL, Barcelona, Spain	MD PhD: Molecular Pathology and Genetic Oncology Research
Dr Desirée du Sart	Molecular Genetics Lab, Victorian Clinical Genetics Services, Murdoch Childrens Research Institute, Melbourne, Australia	PhD: Diagnostic molecular genetics
Aurelie Fabre	INSERM UMR S910, Department of Medical Genetics and Functional Genomics, Marseille, France	Cancer Genetics Research
Michael Farrell	Department of Cancer Genetics, Mater Private Hospital, Dublin, Ireland	R.G.N., H. Dip. Applied Science, B.Sc. in Computing, Grad. Dip. in Oncology Nursing, M. Sc. in Molecular Medicine: Genetic counseling
Dr Susan M Farrington	Colon Cancer Genetics Group, Institute of Genetics and Molecular Medicine, University of Edinburgh, Scotland	BSc, PhD: Cancer and Molecular Genetics Research
Dr Ian Frayling	Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK	MA (Cambridge; Medical Sciences with Honours in Biochemistry), MB BChir (Cambridge), PhD (Manchester: DNA repair), FRCPath (Clinical Molecular and Cytogenetics), FEBLM (Founder Fellow European Board of Laboratory Medicine): Molecular Pathology
Prof Thierry Frebourg	Inserm U614, Faculty of Medicine, Institute for Biomedical Research, University of Rouen, France	PhD: Molecular genetics diagnostic and research
Prof Maurizio Genuardi (committee chair)	1: Department of Biomedical, Experimental and Clinical Sciences, University of Florence, Italy; 2: Fiorgen Foundation for Pharmacogenomics, Sesto Fiorentino, Italy	MD: Clinical genetics
Dr David Goldgar	Department of Dermatology; University of Utah Medical School; Salt Lake City, Utah, U.S.	PhD: Genetic epidemiology
Dr Marc Greenblatt	University of Vermont; Burlington, Vermont, U.S.	MD: Medical Oncology

Dr Christopher D. Heinen	Neag Comprehensive Cancer Center & Center for Molecular Medicine, UConn Health Center, Farmington, CT, U.S.	PhD: Cancer Genetics Research (functional assays)
Prof Elke Holinski-Feder	Klinikum der Universität München, Campus Innenstadt, Medizinische Klinik und Poliklinik IV, Munich, Germany; MGZ – Medizinisch Genetisches Zentrum, Munich, Germany	MD, PhD: Diagnostic molecular genetics
A/Prof Maija Kohonen-Corish	Garvan Institute of Medical Research, Kinghorn Cancer Centre & St Vincent's Clinical School, University of NSW & School of Medicine, University of Western Sydney, Sydney, Australia	BSc, MSc, PhD, MHGSA: Cancer genetics research
Dr Kristina Lagerstedt-Robinson	Department of Molecular Medicine and Surgery, Karolinska Institutet, Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden	PhD: Diagnostic molecular genetics
Prof Suet Yi Leung	Hereditary Gastrointestinal Cancer Genetic Diagnosis Laboratory, Department of Pathology, The University of Hong Kong, Queen Mary Hospital, Pokfulam, Hong Kong	MBBS, MD, FRCPath(UK), FRCPA, FHKAM (Pathology), FHKCPath: Diagnostic molecular genetics; Cancer genetics and genomics; Molecular pathology
Prof Finlay Macrae	Department of Colorectal Medicine and Genetics, Royal Melbourne Hospital, Australia	MBBS (Hons1 Monash), MD (Melb), FRACP, FRCP (UK), AGAF: Gastroenterology
Dr Alexandra Martins	Inserm U1079, University of Rouen, Institute for Research and Innovation in Biomedicine, Rouen, France	PhD: Diagnostic molecular genetics, Cancer genetics and RNA biology research, RNA splicing assays
Senior scientist, Senior consultant Pal Moller	Research Group on Inherited Cancer, Department of Medical Genetics; Oslo University Hospital, The Norwegian Radium Hospital Oslo, Norway	MD, PhD: Clinical genetics, Cancer genetics, Genetic epidemiology
Dr Monika Morak	Klinikum der Universität München, Campus Innenstadt, Medizinische Klinik und Poliklinik IV, Munich, Germany; MGZ – Medizinisch Genetisches Zentrum, Munich, Germany	PhD: Cancer genetics research, splicing and cDNA assays.
Prof Minna Nyström	Department of Biosciences, Genetics; University of Helsinki, Finland	PhD Cancer genetics research (functional assays)
Prof Paivi Peltomaki	Department of Medical Genetics, Haartman Institute; University of Helsinki, Finland	MD, PhD: Cancer genetics research and InsiGHT database curator
Dr Marta Pineda	Hereditary Cancer Program. Catalan Institute of Oncology-IDIBELL, Barcelona, Spain	PhD: Diagnostic molecular genetics
John-Paul Plazzer	Department of Colorectal Medicine and Genetics, Royal Melbourne Hospital, Australia	BEng (Comp), GradDip (Bioinf): Database curator

Prof Ming Qi	Center for Genetic & Genomic Medicine; The First Affiliated Hospital of Zhejiang University School of Medicine; James Watson Institute of Genomic Sciences, Beijing Genome Institute, China; University of Rochester Medical Center, NY, USA	PhD, FACMG: Diagnostic moelcular genetics
Prof Rajkumar Ramesar	MRC Human Genetics Research Unit, Division of Human Genetics, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, South Africa	PhD: Diagnostic molecular genetics
Managing Director, Prof Lene Juel Rasmussen	Center for Healthy Aging, University of Copenhagen, Denmark	PhD: Cancer genetics research (functional assays)
Prof Brigitte Royer-Pokora	Institute of Human Genetics, University of Düsseldorf, Germany	PhD: Diagnostic molecular genetics and functional assays
Prof Rodney Scott	Discipline of Medical Genetics, Faculty of Health, University of Newcastle, The Hunter Medical Research Institute, NSW, Australia & The Division of Molecular Medicine, Hunter Area Pathgology Service, John Hunter Hospital, Newcastle, NSW, Australia	PhD, DSc, FRCPath, FHGSA, FFSc(RCPA): Cancer genetics, diagnositic and research
Prof Rolf Sijmons	Dept of Genetics, University of Groningen, University Medical Center Groningen, Groningen, the Netherlands	MD PhD: Clinical genetics
A/Prof Amanda Spurdle	Queensland Institute for Medical Research, Brisbane, Australia	PhD: molecular cancer epidemiology research
A/Prof Sean Tavtigian	Huntsman Cancer Institute, Salt Lake City, UT, U.S.	PhD: Cancer genetic research - bioinformatics
Bryony Thompson	Queensland Institute for Medical Research, Brisbane, Australia; School of Medicine, University of Queensland, Brisbane, Australia	BSc (Hons), PhD scholar: cancer genetics research
Dr Carli Tops	Center of Human and Clinical Genetics, Leiden University Medical Centre, The Netherlands	PhD: Diagnostic molecular Genetics
Prof Thomas Weber	State University of New York at Downstate, Brooklyn, NY, U.S.	MD: Surgery
A/Prof Juul Wijnen	Center of Human and Clinical Genetics, Leiden University Medical Centre, The Netherlands	PhD: Diagnostic molecular Genetics
A/Prof Michael Woods	Discipline of Genetics, Faculty of Medicine, Memorial University of Newfoundland; St. John's, NL, Canada	PhD: Diagnostic molecular genetics and database curator

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