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Heritable somatic methylation and inactivation of *MSH2* in families with Lynch syndrome due to deletion of the 3' exons of *TACSTD1*

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Supplementary information page

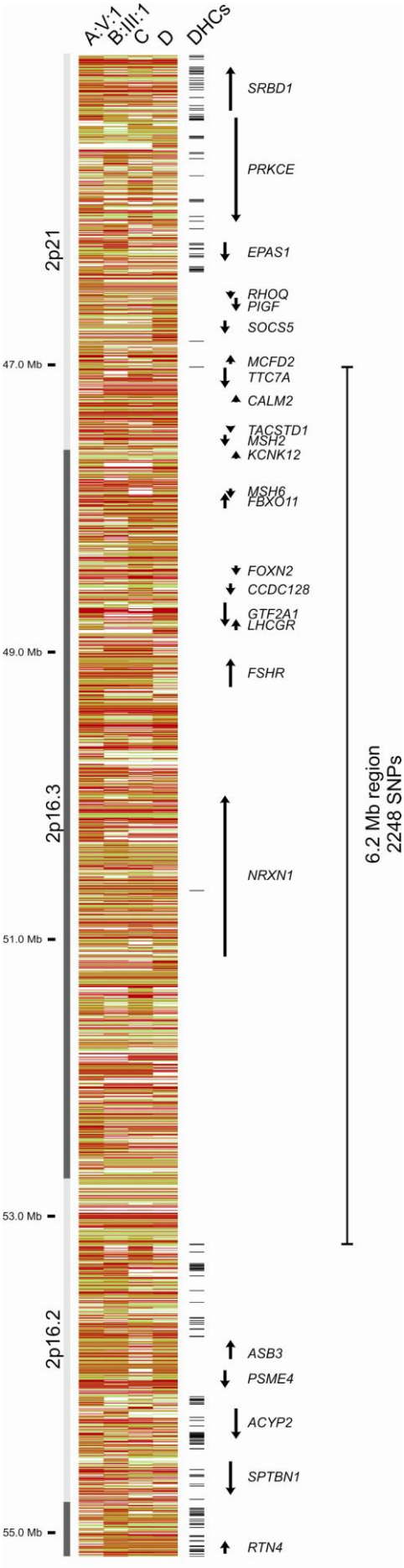
Recruitment criteria of the subjects

Dutch subjects were recruited from the population of subjects visiting the clinical genetics department of the Radboud University Nijmegen Medical Centre in between 2002 and 2007. Subjects were eligible for diagnostic analysis of germline mutations in *MSH6* after written informed consent if they were known with a tumor with microsatellite instability or were diagnosed with colorectal or endometrial cancer below age 50. Microsatellite instability testing was offered to subjects who fulfilled the Amsterdam criteria[1] or the Bethesda guidelines[2,3].
Affected family members of the index subjects with the *TACSTD1* deletion were informed about the presence of a putative pathogenic mutation in their family and invited to participate after written consent.
Hong Kong subjects were recruited for microsatellite instability testing by the Hereditary Gastrointestinal Cancer Genetic Diagnosis Laboratory based at Queen Mary Hospital, The University of Hong Kong, if they fulfilled the Amsterdam criteria[1] or the Bethesda guidelines[2,3].

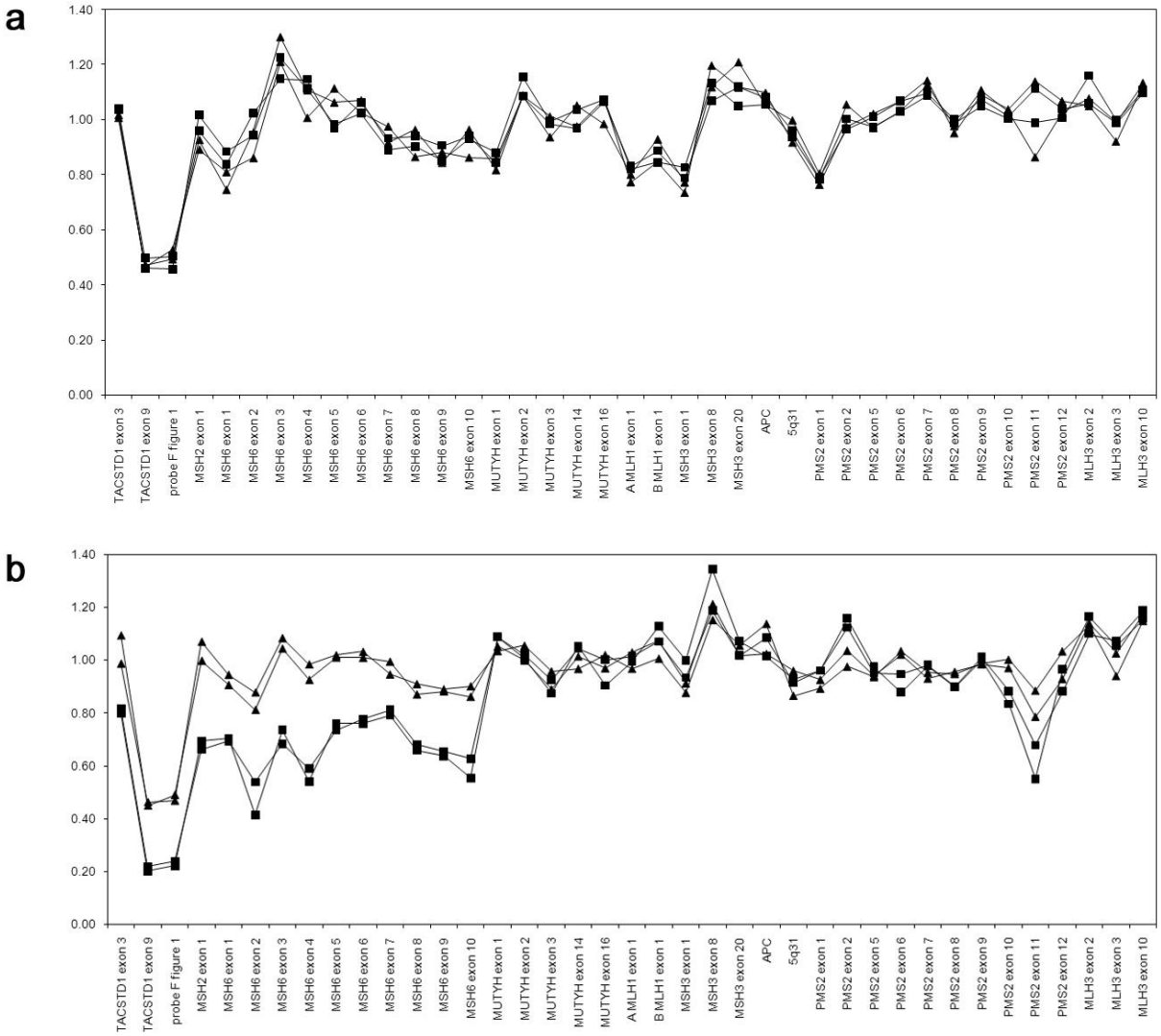
1. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 116, 1453-1456 (1999).

2. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW et al. A national cancer institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Research 58, 5248-5257 (1998).

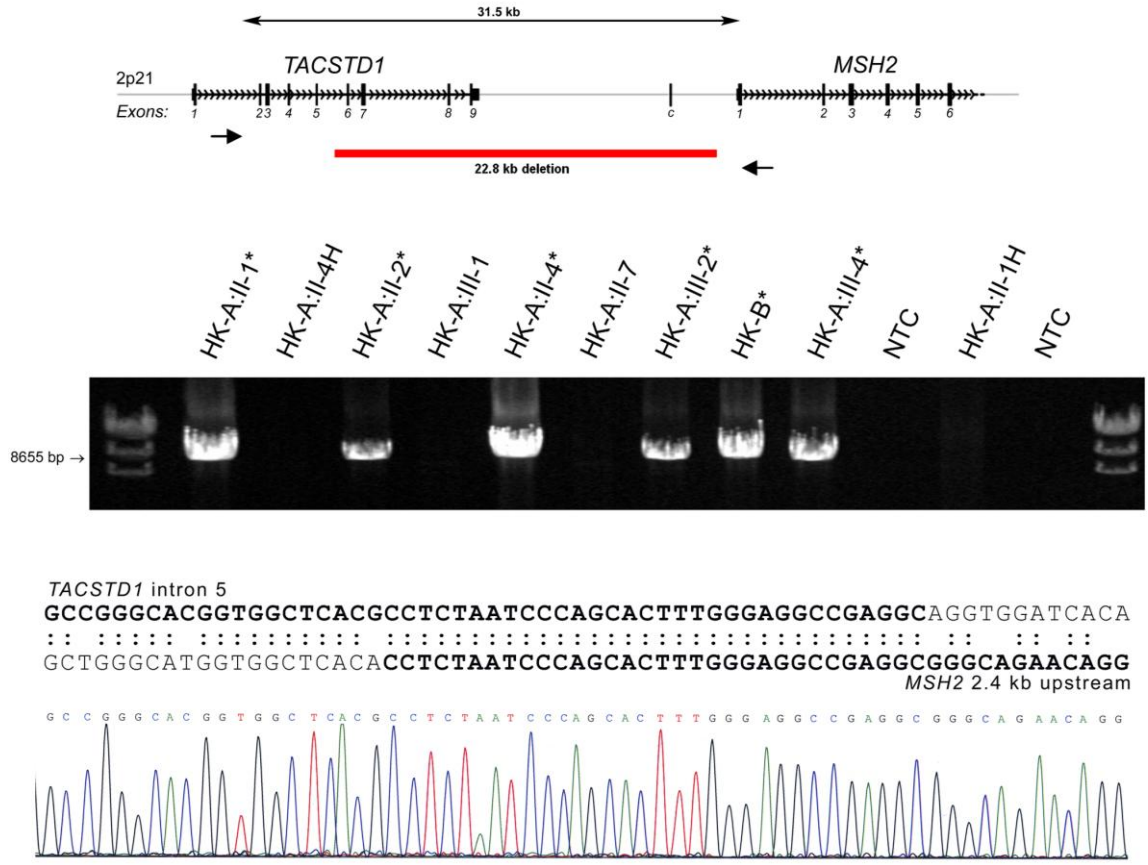
3. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Rüschoff J, Fishel R, Lindor NM, Burgart LJ, Hamelin R, Hamilton SR, Hiatt RA, Jass J, Lindblom A, Lynch HT, Peltomaki P, Ramsey SD, Rodriguez-Bigas MA, Vasen HF, Hawk ET, Barrett JC, Freedman AN, Srivastava S. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst. 96, 261-268 (2004).



Supplementary Fig. 1. Representation of Affymetrix SNP6.0 array data of the genomic region around *TACSTD1*/*MSH2* in one subject from each of the four Dutch families with a *TACSTD1* 3' end deletion (A-D) showing a shared haplotype block. Haplotypes of chromosome 2 were derived from homozygous calls of the 75,933 SNPs on this chromosome, and positions of discordant homozygous calls (DHCs) between the four subjects (i.e. AA in at least one of the four subjects and BB in at least one of the others) were marked. Indicated are the locations of homozygous AA (green) or BB (red) and heterozygous AB (white) calls in a region of 3850 SNPs around *TACSTD1* and *MSH2* (lanes 1-4), as well as the positions of DHCs. Within this region, two large stretches of 1341 and 906 SNPs, respectively, without DHCs in either one of the four subjects were identified, separated by a single DHC, strongly suggesting the presence of a haplotype block shared by all families. *TACSTD1* and *MSH2* are located within the largest of these two stretches encompassing 3.8 Mb of genomic sequence. No other such stretches were identified on chromosome 2. The total length of the two stretches is 6.2 Mb (2248 SNPs).

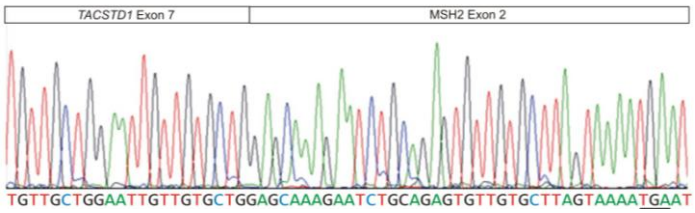


Supplementary Fig 2. Loss of Heterozygosity analysis using SALSA MLPA P008 *MSH6*/*PMS2* probe mix (MRC-Holland, Amsterdam, The Netherlands) with addition of synthetic probe F (for location see Figure 1). MLPA was performed using 400 ng DNA isolated from formalin fixed paraffin embedded material. Data were normalized such that the mean of the probes that are not located on chromosome 2 (i.e. the reference probes) is 1. (a) Duplicate analysis of both DNA from normal caecal mucosa (triangles) and caecal carcinoma containing 80% tumor cells (rectangles) of subject A:IV:3 shows the constitutional deletion of the 3' end of *TACSTD1* in both tissues but no loss of the wild type allele in the tumor. (b) Duplicate analysis of DNA from normal caecal mucosa (triangles) and ileocaecal carcinoma containing 60% tumor cells (rectangles) of subject C shows the constitutional deletion of the 3' end of *TACSTD1* as well as loss of the wild type allele in the tumor. In the carcinoma, the relative amounts of the probes on chromosome 2 outside the constitutional deletion (i.e. *TACSTD1* exon 3, *MSH2* exon 1 and *MSH6* exons 1-10) decrease to a mean of 0.68, which is in line with the somatic loss of one allele covering these genes in the tumor cells. The relative amounts of the probes within the constitutional deletion (i.e. *TACSTD1* exon 9 probe and probe F) drop to 0.22 indicating that it is the wild type allele that is lost in the tumor. The amounts of these latter probes do not decrease to zero due to the admixture of 40% normal cells in the tumor tissue. This tumour shows a corresponding loss of Ep-cam protein expression.

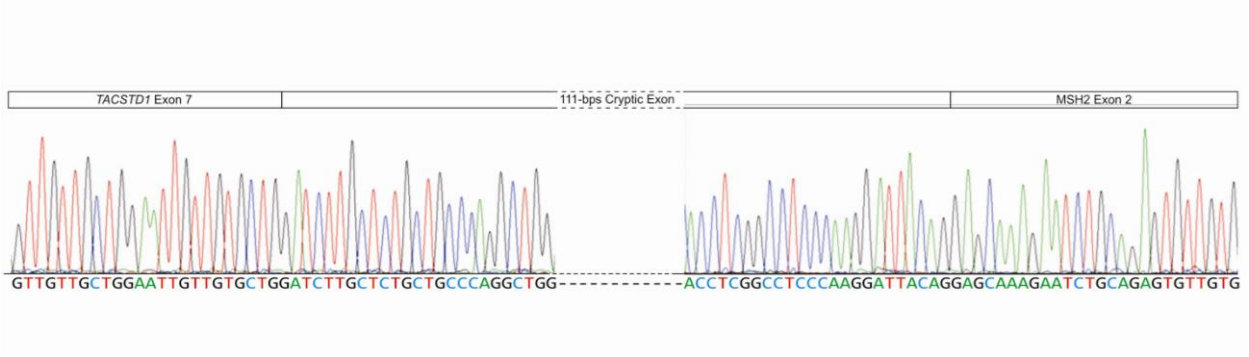


Supplementary Fig. 3. Long range PCR amplification spanning 31.5 kb of chromosomal region showed an aberrant amplicon of 8655 bp in individuals with *MSH2* promoter methylation (denoted by *), but not in the family members without methylation. Direct sequencing showed a 22.8 kb deletion involving alu repeats with the breakpoint residing in a 32 bp region with 100% homology. NTC; no template control.

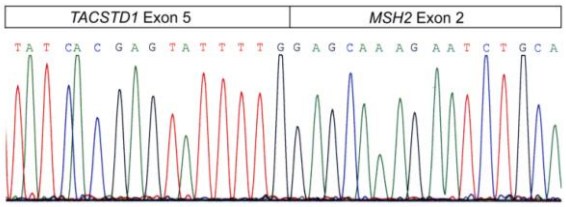
a



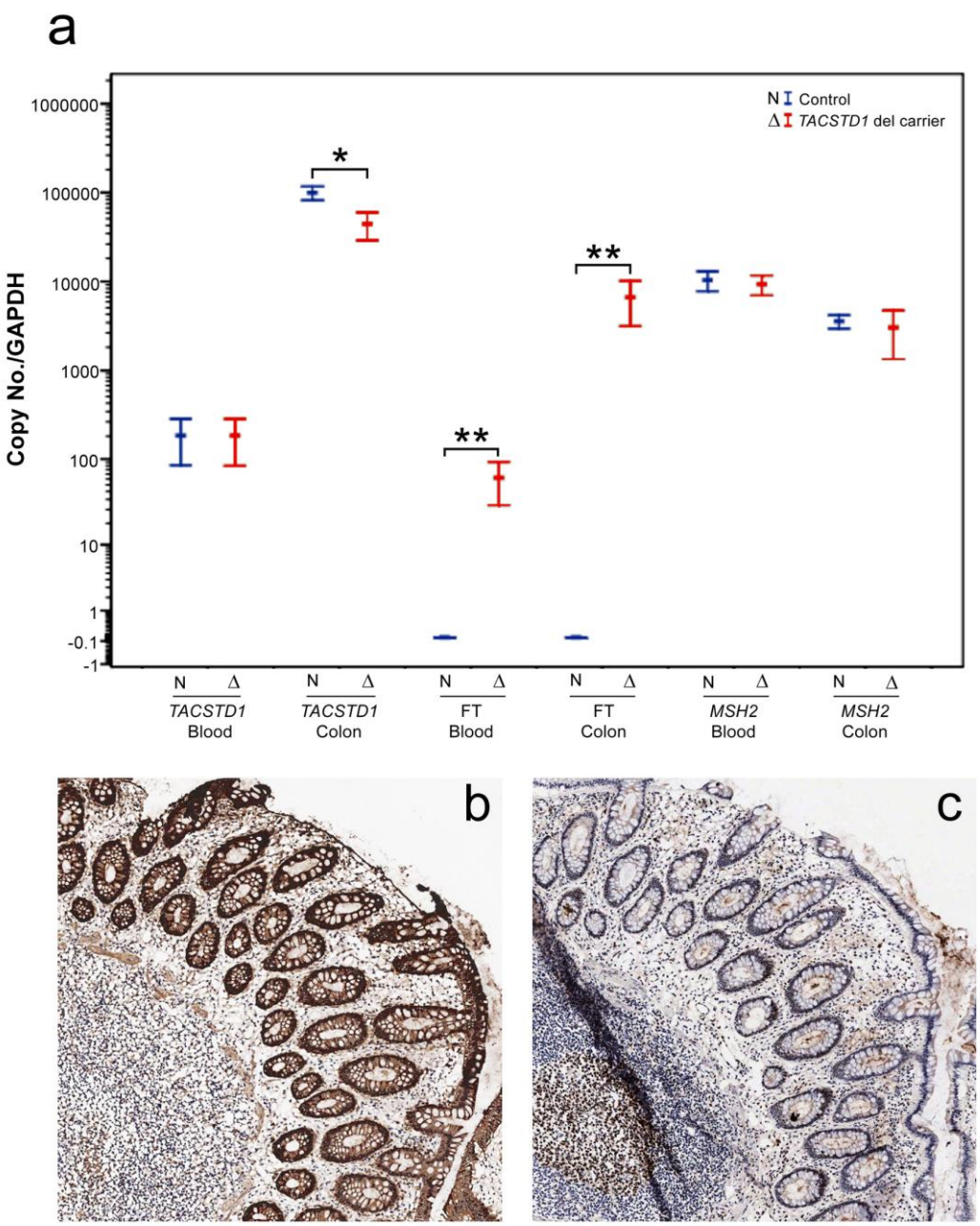
b



c



Supplementary Fig. 4. Sequence chromatograms of the *TACSTD1-MSH2* fusion transcripts in the Dutch and Hong Kong families. In the Dutch family A two fusion transcripts were detected. **a.** In transcript 1 *TACSTD1* exon 7 is fused to *MSH2* exon 2. The open reading frame of transcript 1 encounters a premature stop 12 codons downstream of *TACSTD1* exon 7 in *MSH2* exon 2 (underlined). **b.** In transcript 2 a cryptic exon located at position -4256 to -4146 relative to the translational start site of *MSH2* is spliced in between *TACSTD1* exon 7 and *MSH2* exon 2. The open reading frame of transcript 2 includes 37 codons of the cryptic exon and encounters the same premature stop codon in *MSH2* exon 2. **c.** In HK-family A a fusion transcript was detected between *TACSTD1* exon 5 and *MSH2* exon 2, again leading to a premature stop at the same position.



Supplementary Fig. 5. Relative levels of wild type *TACSTD1*, fusion and *MSH2* transcripts in blood leukocytes and colon or rectal mucosa. **a.** Real-time quantitative RT-PCR shows high level of wild type *TACSTD1* transcripts in colon or rectal mucosa and a significant reduction in the deletion carriers. In the deletion carriers the amount of fusion transcipt is lower than that of residual wild type *TACSTD1* transcript, which might be explained by nonsense mediated decay of the fusion transcript. The expression level of *MSH2* is not significantly reduced in the colon or rectal mucosa of the deletion carriers, which can be attributed to the high level of *MSH2* expression in lymphoid and stromal cells residing in the rectal mucosa. *p<0.01; **p<0.001 by Mann-Whitney U test. **b&c.** Immunohistochemical staining of Ep-CAM (b) and MSH2 (c) in frozen rectal mucosa tissue of a *TACSTD1* deletion carrier demonstrates that only a subset of MSH2-positive cells expresses *TACSTD1*. Expression of Ep-CAM is limited to the epithelial cells, whereas expression of *MSH2* is strong in germinal centers of lymphoid follicles and weaker in basal proliferative crypt epithelial cells and stromal cells in the lamina propria. The expression of *MSH2* will only be blocked in the subset of cells expressing *TACSTD1*, which only partially contributes to the total *MSH2* transcript level.

Supplementary Table 1. Genotyping of SNPs in the promoter region of *MSH2* in Dutch subjects that are heterozygous for the *TACSTD1* deletion.

subject ID	*TACSTD1* deletion	SNP rs1863332 (-433)	SNP rs2303425 (-118)
family A			
A:III:3	p	T T	T T
A:III:4	p	T T	T T
A:IV:1	p	T T	T T
A:IV:3	p	T T	T T
A:V:1	p	T G	T T
allele cosegregating with deletion		T	T
allele methylated		T	T
family B			
B:III:1	p	T T	T C
B:III:4	p	T T	T T
allele cosegregating with deletion		T	T
allele methylated		T	T
family C			
C	p	T T	T T
family D			
D	p	T T	T T

Supplementary Table 2. Primers used in this study

primer	application	Sequence
A: TACSTD1_ex_03_120	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**TACAGGTGTGTGAACACTGCTGGGGTCAGAA**-3'** 5'-GAACAGACAAGGACACTGAAATAACCTGCTCTGAGCGAGTCATGCAG**TCTAGATTGGATCTTGCTGGCAC-3'**
B: TACSTD1_ex_05_88	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGTGCACTTCAGAAGGAGATCA**-3'** 5'-CAACGCGTTATCAACTGGATCCA**TCTAGATTGGATCTTGCTGGCAC-3'**
C: TACSTD1_intr_06_104	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GTTTTACACAGATCAACCAAATGGTTCGCTG**-3'** 5'-CTGCCGTTAATTTTGTCCTCCCTCAGGAAAC**TCTAGATTGGATCTTGCTGGCAC-3'**
D: TACSTD1_ex_07_92	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GTGGTTGTGGTGATAGCAGTTGTTG**-3'** 5'-CTGGAATTGTTGTGCTGGTGAGTAC**TCTAGATTGGATCTTGCTGGCAC-3'**
E: TACSTD1_ex_09_116	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GAAGAAGGGAAATAGCAAATGGACACAA**-3'** 5'-ATTACAAATGTGTGTGCGTGGGACGAAGACATCTTTGAAGGTCATG**TCTAGATTGGATCTTGCTGGCAC-3'**
F: MSH2_PROM_10_100	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GATGCTGGGGGAAAGCTATTCCTAGGTAG**-3'** 5'-GTGTCTCAGCAGACATGGAAAGCAGCCTA**TCTAGATTGGATCTTGCTGGCAC-3'**
H: MSH2_PROM_08_124	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CTAGAATGGGGATGGTGCAGGAGGTACCCTCCGAAGTTAGC**-3'** 5'-TGCGTCTGCAGTCTCTGACACTCAGTAGCTTCTCTGCTCGC**TCTAGATTGGATCTTGCTGGCAC-3'**
J: MSH2_PROM_03_128	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GCACACAGACGGTTAAGCATACAGAATCTGGAAGAAGACTGGA**-3'** 5'-TTCTAGATCTATCACTTACTAGCTCTGTGATCTGGCGCAAGGC**TCTAGATTGGATCTTGCTGGCAC-3'**
M: MSH2_EX1C_108	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGTAGCTAAAGTCACCAGCGTGCGCGGGAAGC**-3'** 5'-TGGGCCGCGTCTGCTTATGATTGGTTGCCGCGG**TCTAGATTGGATCTTGCTGGCAC-3'**
Ctrl probe_1_CAB45_e2_80	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGGAGGCCATGGAGGAGA**-3'** 5'-GCAAGACACACTTCCGCGC**TCTAGATTGGATCTTGCTGGCAC-3'**
Ctrl probe_2_VIPR2_e12_96	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CGCGCCCAGTCCTTCCTGCAAACGGAG**-3'** 5'-ACCTCGGTCATCTAGCCCCACCCCTGC **TCTAGATTGGATCTTGCTGGCAC-3'**
Ctrl probe_4_MRPL41_e1_132	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GACCCTGACAACCTGGAAAAGTACGGCTTCGAGCCCACACAGGAG**-3'** 5'-GGAAAGCTCTTCCAGCTCTACCCCAGGAACTTCCTGCGCTAGCTG**TCTAGATTGGATCTTGCTGGCAC-3'**
Ctrl probe_5_KIAA0056_e8_136	**MLPA primers (probemixA)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGCAATTATGCCAGCCTGACCTACCTTCAGATGGCTTGAAATGGTT**-3'** 5'-TACTACAGTCTGCATCACTATGTCTGAGACCCTTGTGTTCTCCATCC**TCTAGATTGGATCTTGCTGGCAC-3'**
F: MSH2_PROM_10_100	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GATGCTGGGGGAAAGCTATTCCTAGGTAG**-3'** 5'-GTGTCTCAGCAGACATGGAAAGCAGCCTA**TCTAGATTGGATCTTGCTGGCAC-3'**
G: MSH2_PROM_09_120	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GGGAAGAATTGTCTGCATGTTTTGTAAGAGCCAGGTCTC**-3'** 5'-TACCTCCCTCTTTAGAAGTTTAAGGGCAGAGATCCTCTC**TCTAGATTGGATCTTGCTGGCAC-3'**
H: MSH2_PROM_08_124	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CTAGAATGGGGATGGTGCAGGAGGTACCCTCCGAAGTTAGC**-3'** 5'-TGCGTCTGCAGTCTCTGACACTCAGTAGCTTCTCTGCTCGC**TCTAGATTGGATCTTGCTGGCAC-3'**
I: MSH2_PROM_06_112	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CATTTGGTGTCAAGAGCCTGGACACTGGCTGGGGT**-3'** 5'-CAAAGTCCCACTGGAATCGTGGGACCTCCCCTAGT**TCTAGATTGGATCTTGCTGGCAC-3'**
J: MSH2_PROM_03_128	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GCACACAGACGGTTAAGCATACAGAATCTGGAAGAAGACTGGA**-3'** 5'-TTCTAGATCTATCACTTACTAGCTCTGTGATCTGGCGCAAGGC**TCTAGATTGGATCTTGCTGGCAC-3'**
K: MSH2_PROM_02_88	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGGGTGATTCTGCTGCTGAATT**-3'** 5'-AGGTTTTGGAACCACTTCCATGG**TCTAGATTGGATCTTGCTGGCAC-3'**
L: MSH2_PROM_01_104	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CTCAGCCCTGCTAATATCTGGGATCACAGAC**-3'** 5'-GTGGGTTTTACCATGTTGCCCAGGATGGTGT**TCTAGATTGGATCTTGCTGGCAC-3'**
M; MSH2_EX1C_108	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGTAGCTAAAGTCACCAGCGTGCGCGGGAAGC**-3'** 5'-TGGGCCGCGTCTGCTTATGATTGGTTGCCGCGG**TCTAGATTGGATCTTGCTGGCAC-3'**

N: MSH2_EX1B_92	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CGGCTTCGTGCGCTTCTTTCAGGGC-**3'** 5'-ATGCCGGAGAAGCCGACCACCACAG**TCTAGATTGGATCTTGCTGGCAC-3'**
Control probe_1_CAB45_e2_80	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGGAGGCCATGGAGGAGA-**3'** 5'-GCAAGACACACTTCCGCGC**TCTAGATTGGATCTTGCTGGCAC-3'**
Control probe_2_VIPR2_e12_96	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA** CGCGCCCAGTCCTTCCTGCAAACGGAG-**3'** 5'-ACCTCGGTCATCTAGCCCCACCCCTGC **TCTAGATTGGATCTTGCTGGCAC-3'**
Control probe_3_VIPR2_e2_116	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GCCTGCGGTGAACACTGTGAACGTGCAGATCGCCTTC-**3'** 5'-TCCGGTTTGACAGAGGCATGCTGGGCCATCATGCTCC**TCTAGATTGGATCTTGCTGGCAC-3'**
Control probe_4_MRPL41_e1_132	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GACCCTGACAACCTGGAAAAGTACGGCTTCGAGCCCACACAGGAG-**3'** 5'-GGAAAGCTCTTCCAGCTCTACCCCAGGAACTTCCTGCGCTAGCTG**TCTAGATTGGATCTTGCTGGCAC-3'**
Control probe_5_KIAA0056_e8_136	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**CAGCAATTATGCCAGCCTGACCTACCTTCAGATGGCTTGAAATGGTT-**3'** 5'-TACTACAGTCTGCATCACTATGTCTGAGACCCTTGTGTTCTCCATCC**TCTAGATTGGATCTTGCTGGCAC-3'**
Control probe_6_SPG7_e1_84	**MLPA primers (probemixB)** [1)]	**5'-GGGTTCCCTAAGGGTTGGA**GATCACGCAGGCGCGGCTTTC-**3'** 5'-AGGCCAACATGGCCGTGCTGC**TCTAGATTGGATCTTGCTGGCAC-3'**
TACSTD1 intron7F	**LR-PCR deletion forward primer (Dutch)**	5'-GGAAAAGGAGGATGTGGAAGAATGC-**3'**
TACSTD1 5KdelR1	**LR-PCR deletion reverse primer (Dutch)**	5'-CAGTGTTGACCTTAGCGAAAGTAGC-**3'**
TACSTD1 5KdelR2	**LR-PCR deletion internal rev primer (Dutch)**	5'-TAGCCAAGTATAGTGGTGGGTG-**3'**
TACSTD1 intron5F	**Deletion primer HK**	5'-TGAATAGTGTGACGGAACTCTTTAA **-3'**
TACSTD1 intron5R	**Deletion primer HK**	5'-CATTCGGCTATTTTGTATGTTTTTAG **-3'**
23K delR	**Deletion primer HK**	5'-ATCTCTTGACCTCCTGTTCTGCC**-3'**
11081: TACSTD1 exon 6 F	**RT-PCR primer fusion transcript (Dutch)** [2)]	5'-TGTAAAACGACGGCCAGTACTCAGAATGATGTGGAC-**3'**
11082: TACSTD1 exon 7 F	**RT-PCR primer fusion transcript (Dutch)** [2)]	5'-TGTAAAACGACGGCCAGTGAATTCTCAATGCAGGGTC-**3'**
3847: MSH2 exon 2 R	**RT-PCR primer fusion transcript (Dutch)** [2)]	5'-ATGCCAAATACCAATCATTC-**3'**
P461: MSH2 exon 2 R	**RT-PCR primer fusion transcript (Dutch)** [2)]	5'-ACTGACGAACCAGAAGAAG-**3'**
TACSTD1 exon 4 F	**RT-PCR primer fusion transcript (HK)** [2)]	5'-ACAAAGCAAGAGAAAAACCTTATG-**3'**
MSH2 exon 5 R	**RT-PCR primer fusion transcript (HK)** [2)]	5'-AGTTGGAATCATCTGATAAGAGTTCT-**3'**
TACSTD1 exon 5 F	**Cloning of WT *TACSTD1* plasmid**	5'-CGCGTTATCAACTGGATCC-**3'**
TACSTD1 exon 7 R		5'-TTTTAGACCCTGCATTGAGAA-**3'**
MSH2 exon 1 F	**Cloning of WT *MSH2* plasmid**	5'-TTCTATACGGCGCACGGCGA-**3'**
MSH2 exon 3 R		5'-TTAACACCCACAACACCAATG-**3'**
TACSTD1 exon 4 F	**Cloning of HK-fusion plasmid**	5'-ACAAAGCAAGAGAAAAACCTTATG-**3'**
MSH2 exon 5 R		5'-AGTTGGAATCATCTGATAAGAGTTCT-**3'**

TACSTD1 exon 5 F	**Q-PCR for WT *TACSTD1***	5'-CGCGTTATCAACTGGATCC**-3'**
TACSTD1 exon 7 R		5'-TTTTAGACCCTGCATTGAGAA**-3'**
MSH2 exon 1 F	**Q-PCR for WT *MSH2***	5'-TTCTATACGGCGCACGGCGA**-3'**
MSH2 exon 3 R		5'-TTAACACCCACAACACCAATG**-3'**
TACSTD1 exon 4 F	**Q-PCR for HK-fusion transcript**	5'-ACAAAGCAAGAGAAAAACCTTATG**-3'**
MSH2 exon 3 R		5'-TTAACACCCACAACACCAATG**-3'**
MSP1U-F	**methylation analysis** [3)]	5'-GGTTGTTGTGGTTGGATGTTGTTT**-3'**
MSP1U-R	**methylation analysis** [3)]	5'-CAACTACAACATCTCCTTCAACTACACCA**-3'**
MSP1M-F	**methylation analysis** [3)]	5'-TCGTGGTCGGACGTCGTTC**-3'**
MSP1M-R	**methylation analysis** [3)]	5'-CAACGTCTCCTTCGACTACACCG**-3'**
MSP3U-F	**methylation analysis** [3)]	5'-AGTGTTTTTTTTGGTTGTATTGTTATGTTG**-3'**
MSP3U-R	**methylation analysis** [3)]	5'-CCACATCTACTTATAATTAATTACCACAAC**-3'**
MSP3M-F	**methylation analysis** [3)]	5'-CGTTTTTTTCGGTTGTATCGTTATGTC**-3'**
MSP3M-R	**methylation analysis** [3)]	5'-CGTCTACTTATAATTAATTACCGCGACA**-3'**
MSP5U-F	**methylation analysis** [3)]	**5'-TGTAAAACGACGGCCAGT**GGTGGGAGATTTTTGGTAGGTATTGAGTTT**-3'**
MSP5U-R	**methylation analysis** [3)]	**5'-CAGGAAACAGCTATGACC**AAAACTATATAAAATAAACCCATAATCCCA**-3'**
MSP5M-F	**methylation analysis** [3)]	**5'-TGTAAAACGACGGCCAGT**GGTGGGAGATTTTCGGTAGGTATTGAGTTC**-3'**
MSP5M-R	**methylation analysis** [3)]	**5'-CAGGAAACAGCTATGACC**AAAACTATATAAAATAAACCCGTAATCCCG**-3'**
NP2-F	**methylation analysis** [3)]	5'-GGTGTAGTAAGGGTAGGTTGTTATT**-3'**
NP2-R	**methylation analysis** [3)]	5'-AAACACACRTTTTAACAAAATACTAA**-3'**
NP2-IR	**methylation analysis** [3)]	5'-TATTACTCCCATACTTC**-3'**

1) List of probes included in two synthetic probe mixes. M13 tags used in the amplification are in bold. Probes F, H, J, and M are included in both mixes and allow integration of the data
2) RT-PCR was performed using neat concentration of cDNA in HK families because of the low abundance of the fusion transcript in blood leukocytes.
3) The MSP1, MSP3 and NP2 primer sets were described previously[3,13]. The MSP1 primers amplify the forward strand, whereas for both MSP3, MSP5 and NP2 the reverse strand is amplified. Primers are specific for unmethylated (U) and methylated (M) DNA as indicated in the primer name. M13 tags used in the amplification are in bold.