Published in final edited form as:
Fam Cancer. 2014 June ; 13(2): 219–225. doi:10.1007/s10689-013-9688-x.

Inversion of exons 1–7 of the *MSH2* gene is a frequent cause of unexplained Lynch syndrome in one local population

Jennifer Rhees,
Gastrointestinal Cancer Research Laboratory, Division of Gastroenterology, Department of Internal Medicine, Baylor University Medical Center, Dallas, TX, USA. Baylor Research Institute and Sammons Cancer Center, Baylor University Medical Center, 250 Hoblitzelle, 3500 Gaston Ave, Dallas, TX 75246, USA

Mildred Arnold, and
Gastrointestinal Cancer Research Laboratory, Division of Gastroenterology, Department of Internal Medicine, Baylor University Medical Center, Dallas, TX, USA. Baylor Research Institute and Sammons Cancer Center, Baylor University Medical Center, 250 Hoblitzelle, 3500 Gaston Ave, Dallas, TX 75246, USA

C. Richard Boland
Gastrointestinal Cancer Research Laboratory, Division of Gastroenterology, Department of Internal Medicine, Baylor University Medical Center, Dallas, TX, USA. Baylor Research Institute and Sammons Cancer Center, Baylor University Medical Center, 250 Hoblitzelle, 3500 Gaston Ave, Dallas, TX 75246, USA

Jennifer Rhees: jennifrh@baylorhealth.edu; C. Richard Boland: rickbo@baylorhealth.edu

Abstract

Germline mutations in DNA mismatch repair (MMR) genes, such as *MSH2*, cause Lynch syndrome, an autosomal dominant predisposition to colorectal as well as other cancers. Our research clinic focuses on hereditary colorectal cancer, and over the past 9 years we have identified germline mutations in DNA MMR genes in 101 patients using commercial genetic reference laboratories. We also collected samples from twelve patients with absent MSH2 protein expression and microsatellite instability in tumor tissue, with a family history suggestive of Lynch syndrome, but negative germline test results. The most likely explanation for this set of results is that the germline testing did not detect true germline mutations in these patients. Two of our patients with failed commercial testing were later found to have deletions in the 3′ region of *EPCAM*, the gene just upstream of *MSH2*, but no explanation could be found for inactivation of *MSH2* in the other ten patients. We used allelic dropout in long PCR to look for potential regions of rearrangement in the *MSH2* gene. This method detected a potential rearrangement breakpoint in the same region of *MSH2* where one breakpoint of a 10 Mb inversion was reported previously. We tested these ten patients for this inversion. Six of 10 patients had the inversion, indicating the importance of including testing for this inversion in patients suspected of having MSH2-type

Correspondence to: Jennifer Rhees, jennifrh@baylorhealth.edu.

Conflict of interest The authors declare no competing financial interests.

Lynch syndrome in our population. Additionally, this method could be further developed to look for inversions in other genes where current methods of testing fail to find a causative mutation.

Keywords

Lynch syndrome; Inversion; MSH2; Colon cancer

Introduction

Lynch syndrome is the familial cancer syndrome caused by a germline mutation in one of the DNA mismatch repair (MMR) genes [1]. Cancers in this syndrome characteristically have microsatellite instability (MSI) and abnormal immunohistochemistry (IHC), in which the MMR gene with the germline mutation is not expressed in the tumor [2, 3]. *MSH2* is one of the most important genes causing Lynch syndrome, but traditional exon-by-exon sequencing will not always detect the germline mutation. *MSH2* incurs frequent Alu-mediated large deletions, which cannot be detected by exon sequencing [4]. Additionally, silencing of *MSH2* can occur due to deletion of the polyadenylation signal of the *EPCAM* gene located 5′ to *MSH2*. Deletions of the 3′ end of the *EPCAM* gene abolish transcription termination, which results in transcription read-through into the MSH2 gene, and subsequent methylation-induced silencing of the *MSH2* gene in tissues that express *EPCAM* [5]. The presence of MSI with the absence of MSH2 expression in a colorectal cancer (CRC) is highly suggestive of Lynch syndrome-MSH2 type, but in some instances, no germline mutation can be found in the *MSH2* gene, even when testing for large deletions in *MSH2* or *EPCAM*.

Our referral clinic focuses on hereditary CRC, and over the past 9 years we have identified germline mutations in DNA MMR genes in 101 patients using commercial genetic reference laboratories. We have also collected samples from twelve patients who had tumors with absent MSH2 protein expression, MSI, a family history suggestive of Lynch syndrome, but negative germline test results. The most likely explanation for this combination is that the germline testing was unable to detect true germline mutations in these patients. Even the best commercial testing methods do not detect mutations deep within introns or copy number-neutral inversions whose breakpoints are located in noncoding regions of the gene. Two of our patients with failed commercial testing were later found to have deletions in the 3′ region of *EPCAM*, but no explanation could be found for inactivation of *MSH2* in the other ten [5].

One important type of mutation not examined by current testing methods is the presence of large inversions which result in rearrangement of the order of the exons of the gene. We sought to identify locations of potential inversion breakpoints in *MSH2* by looking for allelic drop-out of single nucleotide polymorphisms (SNPs) in a series of long overlapping (~10 kb) PCR products. Starting with one patient with suspected *MSH2*-type Lynch syndrome, we found a long PCR product which was completely homozygous at all SNPs sequenced. This product encompassed exon 8 and part of intron 7 of *MSH2*. Upon review of the literature, we found two publications which had previously described a 10 Mb inversion in

the *MSH2* gene, in which the 3′ breakpoint was located in the same region as our long PCR amplicon [6, 7]. Thus, the objective of our study was to determine if this inversion of the *MSH2* gene previously described was present in a group of patients with suspected *MSH2*-type Lynch syndrome of unknown origin.

Methods

Patients

Patients were selected from our familial CRC registry based upon suspicion of having *MSH2*-type Lynch syndrome. Patients previously had tested negative for *MSH2* germline mutations by commercial genetic testing services. All patients had exhibited loss of *MSH2* expression by IHC. Germline testing, IHC, and MSI results listed in the table were provided by CLIA certified labs. Family members of patients who tested positive for the inversion were subsequently enrolled in our study and tested for the inversion when possible. All patients provided written informed consent, and the study was approved by the Baylor Research Institute institutional Review Board.

Control Patients

Five control patients with no known family history of CRC were tested using the primers designed by Wagner et al. [6] for the 5′ inversion breakpoint. 22 controls with no known history of familial CRC were tested using the primers designed to amplify across the 3′ breakpoint.

Inversion PCR

Patients and controls were tested for the 5′ inversion breakpoint using primers F3 and R3 published by Wagner et al. [6]. Primers F4MV and B3MV were designed to amplify across the 3′ inversion breakpoint using MacVector (Cary, NC, USA). The forward primer sequence was 5′-GGGAGGGGAAAATGACTTACAAAG-3′. The reverse primer sequence was 5′-GCAAAAGGAACAGTCAGCAG AAGG-3′. PCR was performed using HotStar Taq (Qiagen, Valencia, CA, USA). Both inversion primer pairs only amplify a product in carriers of the inversion. Inversion PCR products were sequenced on an ABI 3100-Avant sequencer (Applied Biosystems, Foster City, CA, USA). An additional 1.6 kb PCR that amplifies exons 12–13 of MSH2 was included on all patient samples to exclude the possibility of false negative results due to poor DNA integrity (Fig. 1).

SNP genotyping

Patients were genotyped at multiple SNPs in *MSH2* by PCR and DNA sequencing and/or denaturing high performance liquid chromatography (dHPLC). Primers and dHPLC conditions are available upon request.

Allelic drop out PCR

Two PCRs were designed to look for allelic drop out in a long PCR product from inversion carriers. A short PCR product was designed to amplify and genotype SNP rs7607076, which is located in *MSH2* intron 7 downstream of the 3′ inversion breakpoint. A second set of

primers anneal to each side of the 3′ inversion breakpoint and only amplify the wild type allele. This results in allelic drop out in the long PCR product in carriers of the inversion who are heterozygous at SNP rs7607076. PCR products from both the short and long PCR products were sequenced and genotyped at rs7607076 (Fig. 2).

Results and discussion

Starting with one patient with suspected *MSH2*-type Lynch syndrome, we found a long PCR product which was completely homozygous at all SNPs sequenced. Sequencing of short PCR products containing multiple SNPs confirmed allelic drop out in the long PCR product. This product encompassed exon 8 and part of intron 7 of *MSH2*. Upon review of the literature, we found a previously described 10 Mb inversion in the *MSH2* gene, in which the 3′ breakpoint was located in the same region as our long PCR amplicon. PCR and sequencing using inversion-specific primers described by Wagner revealed that this patient carried the inversion previously reported [6].

We next performed PCR of the 5′ inversion breakpoint on our remaining suspected *MSH2*-type Lynch syndrome patients, and six patients were positive for the inversion (Table 1; Fig. 3). We designed additional primers to amplify the 3′ inversion breakpoint using the sequence data provided by Chen [7]. PCR products from both the 5′ and 3′ breakpoints were sequenced for confirmation (Fig. 1). No amplification of the 3′ inversion PCR product was seen in 22 controls. A 1.6 kb PCR amplification was included in all samples to exclude the possibility of false negative results due to problems with DNA integrity.

After identifying the six initial patients, we tested additional family members where possible, and 21 family members from five families revealed an additional seven inversion carriers (Fig. 3). To confirm our initial results we designed an additional PCR amplification to test for allelic drop-out in carriers of the 10 Mb inversion. Similar to the original long PCR, this PCR amplification relies upon a common SNP located near one of the inversion breakpoints. Primers were designed which amplified a region of the *MSH2* gene encompassing both the common SNP and the neighboring region where the 3′ breakpoint is located. This strategy amplifies only the wild type allele of *MSH2*, and therefore patients who are heterozygous for the internal SNP are homozygous in the PCR product if they also carry the inversion in *MSH2*. Only carriers of the inversion displayed allelic drop out in the long PCR, and no inversion carriers had amplification of both alleles (Fig. 2).

Initial screening for mutations in the *MSH2* gene had indicated that all carriers of the inversion also carried the C allele at SNP rs2303428. Genotyping of several additional SNPs within the *MSH2* locus revealed that all inversion carriers may have had a common haplotype, suggesting the possibility that the inversion derived from a common ancestor (Table 2). Upon additional in-depth analysis of the pedigrees of the five affected families, we discovered that two of the carriers were first cousins. Based upon information in the pedigrees, we believe a third family may be related to these two families; however an exact connection could not be determined. No additional connections could be made among these three families and the other two families. None of these families was previously known to be related to the others.

Based upon our current information we are unable to determine if the mutation we have found represents a founder effect in our local population, or if it applies to a wider population of suspected Lynch syndrome cases. In order to address this issue, we are currently in collaboration with multiple groups to collect DNA samples from mutation negative Lynch syndrome patients throughout the United States.

Our results resolved a considerable proportion of previously unexplained *MSH2*-type Lynch syndrome families in our registry. Of 10 patients tested, six carried this specific inversion. Of the remaining four unexplained cases, two cases (213,474) had non-synonymous alterations in the coding region of the *MSH2* gene which were categorized as "variants of uncertain significance" by the reference laboratory, which may have accounted for Lynch syndrome in these two patients, as both are predicted to be deleterious by SIFT analysis and "probably damaging" by PolyPhen analysis. Of the remaining two cases, one case (328) was negative for standard diagnostic criteria for Lynch syndrome (Amsterdam II criteria, Bethesda guidelines, and a low PREMM 1,2,6 score [8], but was included in the study because of loss of *MSH2* expression in a Lynch syndrome-associated cancer and a family history of multiple cancers. Although it is generally assumed that loss of *MSH2* expression occurs only in the setting of Lynch syndrome, it remains possible that in some cases the loss is due to somatic mutation, especially in cases with weak or no family history of CRC [9]. Our study did not include exhaustive testing for other inversions in *MSH2* in the remaining patients. It is possible that other inversions may explain additional cases of Lynch syndrome in which genetic testing fails to find a causative mutation. Current genetic testing methodology uses convenient approaches and only looks for mutations which can be identified by direct exon sequencing and copy number variation. Even more labor intense methods such as Southern blots and sequencing of full length RT-PCR transcripts may fail to find more obscure mutations such as inversions. This limitation is exemplified in our study. Although Wagner initially discovered the 10 Mb inversion using Southern blotting, a commercial testing service performed Sothern blotting on one of our patients and failed to find this *MSH2* gene inversion. New techniques need to be developed to capture all mutations causing Lynch syndrome, and other diseases in which the culpable mutation cannot be identified by current methods. In our analysis, we used allelic drop out in long PCR analysis as a method to find regions of possible inversions. This method could be further developed to look for other inversion in the *MSH2* gene, as well as in other genes where current methods of testing fail to find a causative mutation. In the meantime, clinical testing services should add an analysis for the 10 Mb inversion to their clinical testing repertoire. This study also demonstrates the importance of enrolling patients with suspected Lynch syndrome, but negative germline testing results, into research registries where their DNA can be tested until an answer is found.

Acknowledgments

This work supported by the National Institutes of Health grant CA72851 and funds from the Baylor Research Institute, Baylor University Medical Center at Dallas, and the Charles A. Sammons Cancer Center. We also thank Linda Robinson, MS, CGC, Assistant Director, Clinical Cancer Genetics, Simmons Comprehensive Cancer Center, UT Southwestern Medical Center at Dallas, Texas, USA for patient referrals, Margaret M. Hinshelwood, Ph.D., manager, Office of Scientific Publications at the Sammons Cancer Center for her suggestions and the preparation of the manuscript, and Thomas Jascur, Ph.D., for helpful discussion on PCR primer design.

References

1. Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer. 2005; 4(3):211–218.10.1007/s10689-004-4489-x [PubMed: 16136380]
2. Boland CR, Thibodeau SN, Hamilton SR, 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 Res. 1998; 58(22):5248–5257. [PubMed: 9823339]
3. Hampel H, Frankel WL, Martin E, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med. 2005; 352(18):1851–1860.10.1056/NEJMoa043146 [PubMed: 15872200]
4. Gylling A, Ridanpaa M, Vierimaa O, et al. Large genomic rearrangements and germline epimutations in Lynch syndrome. Int J Cancer. 2009; 124(10):2333–2340.10.1002/ijc.24230 [PubMed: 19173287]
5. Lynch HT, Riegert-Johnson DL, Snyder C, et al. Lynch syndrome-associated extracolonic tumors are rare in two extended families with the same EPCAM deletion. Am J Gastroenterol. 2011; 106(10):1829–1836.10.1038/ajg.2011.203 [PubMed: 21769135]
6. Wagner A, van der Klift H, Franken P, et al. A 10-Mb paracentric inversion of chromosome arm 2p inactivates MSH2 and is responsible for hereditary nonpolyposis colorectal cancer in a North-American kindred. Genes Chromosomes Cancer. 2002; 35(1):49–57.10.1002/gcc.10094 [PubMed: 12203789]
7. Chen JM. The 10-Mb paracentric inversion of chromosome arm 2p in activating MSH2 and causing hereditary nonpolyposis colorectal cancer: re-annotation and mutational mechanisms. Genes Chromosomes Cancer. 2008; 47(6):543–545. doi:10.1002/gcc.20556. [PubMed: 18335504]
8. Kastrinos F, Steyerberg EW, Mercado R, et al. The PREMM(1, 2, 6) model predicts risk of MLH1, MSH2, and MSH6 germline mutations based on cancer history. Gastroenterology. 2011; 140(1):73–81.10.1053/j.gastro.2010.08.021 [PubMed: 20727894]
9. Rodriguez-Soler M, Perez-Carbonell L, Guarinos C, et al. Risk of cancer in cases of suspected Lynch syndrome without germline mutation. Gastroenterology. 2013; 144(5):926 e1–932 e1. (quiz e13–e14). 10.1053/j.gastro.2013.01.044 [PubMed: 23354017]

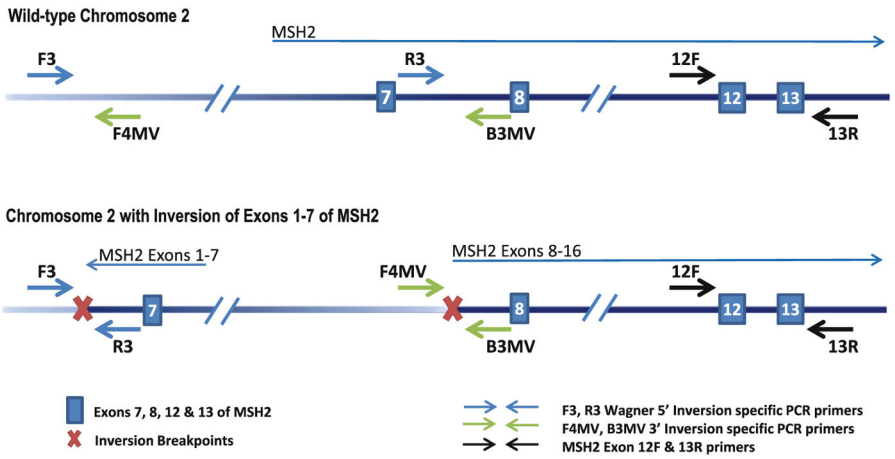

Fig. 1.

Inversion-specific PCR. Representation of PCR assays used to detect the *MSH2* inversion. Primers F3 and R3 were described by Wagner et al. [6] and are used to amplify the 5′ inversion breakpoint. Primers F4MV and B3MV were designed in our lab to amplify the 3′ inversion breakpoint. Amplification is achieved in carriers of the inversion due to re-orientation of the primer directionality. No amplification occurs in patients with wild-type *MSH2*. Amplification of exons 12–13 of *MSH2* using 12F and 13R was used to verify DNA integrity

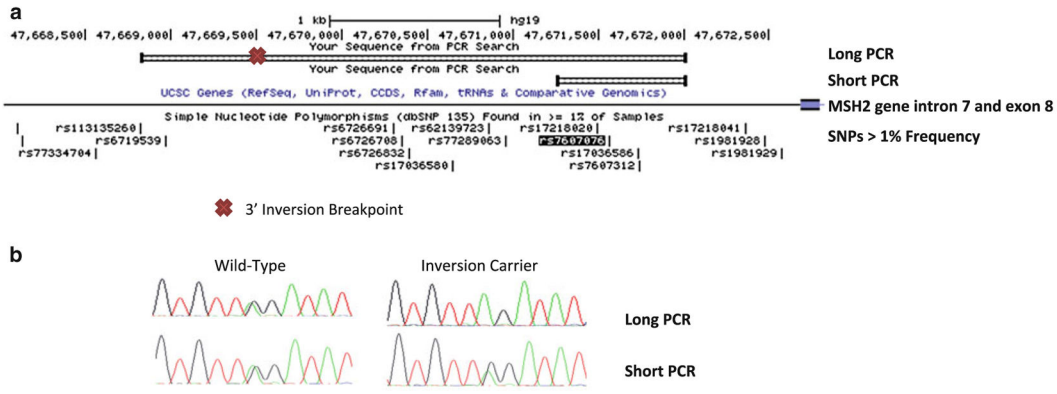

Fig. 2.

Design of PCR analyses used to detect allelic drop out in carriers of the *MSH2* inversion. **a** Long and short PCR amplicons and their positions on chromosome 2 relative to the 3′ inversion breakpoint, *MSH2* intron 8, and SNP rs7607076 are depicted. **b** Sequencing results of rs7607076 in the long and short amplicons for patients tested for the *MSH-2* inversion by use of inversion-specific PCR. Only one allele is amplified in the long PCR of inversion carriers which results in the SNP appearing to be homozygous

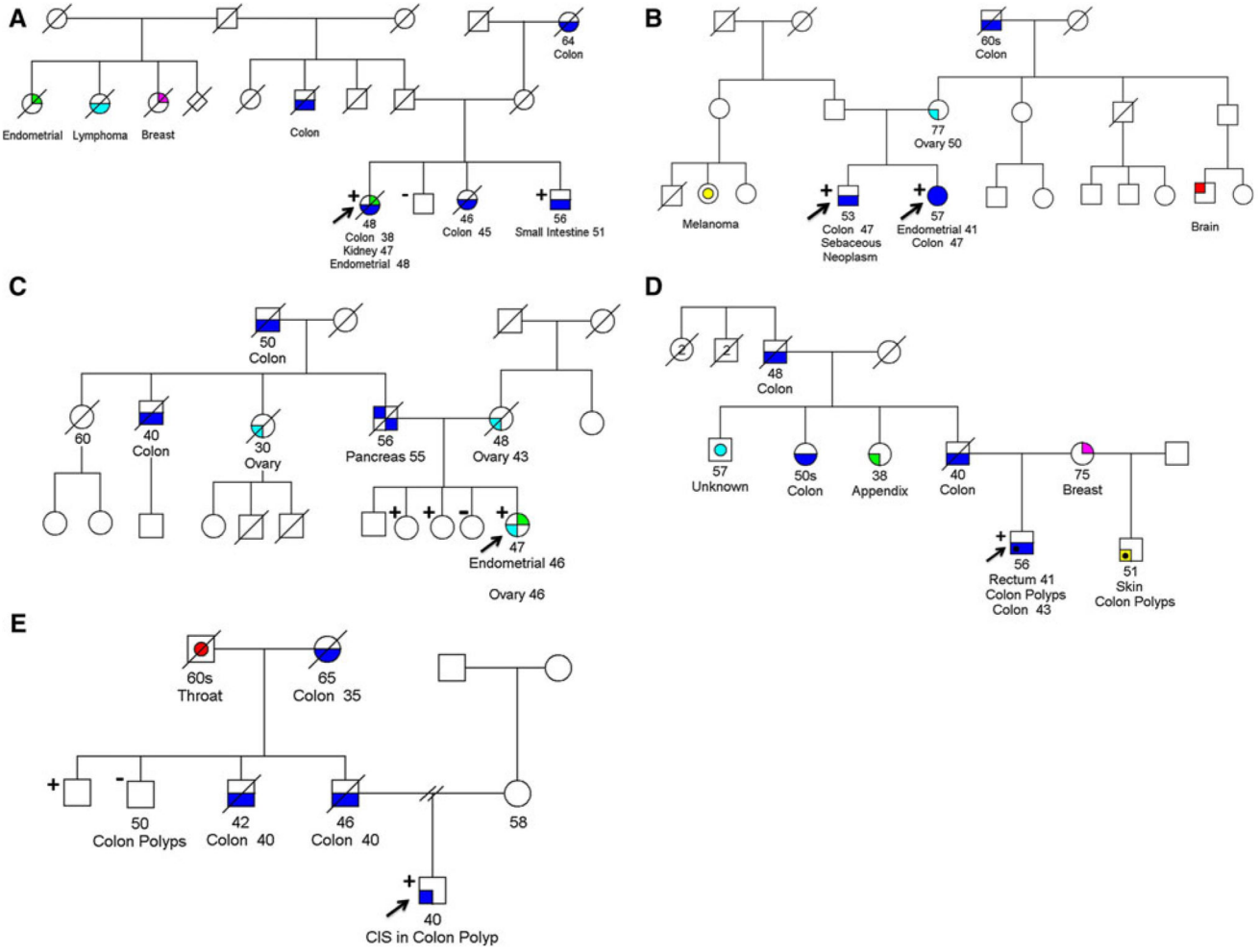

Fig. 3.

Pedigrees of patients who are carriers of the *MSH2* gene inversion. Cancer history is illustrated as reported by the probands. Only Lynch syndrome-related cancers are shown. Inconsistencies in cancer incidence and ages of onset were found when comparing related kindreds, emphasizing the necessity for caution when interpreting family histories provided by patients. *CIS* carcinoma-in situ. *Plus sign* indicates the individual is a carrier of the inversion. *Minus sign* indicates the individual tested does not carry the inversion

Table 1

Clinical characteristics of unexplained MSH2-type Lynch syndrome patients

Diagnosis		Age at diagnosis	MSH2/MSH6 IHC	MSI testing	Germline testing	Amsterdam I criteria	Amsterdam II criteria	Bethesda criteria	PREMM 1,2,6	Inversion (%)
213	Endometrial adenocarcinoma	55	Absent	ND	**MSH2-VUS L800P**	Negative	Positive	Positive	27	No
314	Synchronous endometrial & ovarian adenocarcinoma	46	Absent	MSI-H	NMD	Negative	Positive	Negative	38	**Yes**
328	Endometrial adenocarcinoma	44	Absent	MSI-H	NMD	**Negative**	**Negative**	**Negative**	**<5**	No
370	Endometrial adenocarcinoma Mucinous cecal adenocarcinoma	41 47	Absent	MSI-H	NMD	Positive	Positive	Positive	36.90	**Yes**
372	Muir-Torre syndrome Stage I cecal poorly differentiated adenocarcinoma	41 47	Absent	MSI-H	NMD	Positive	Positive	Positive	65.80	**Yes**
381	Colon adenocarcinoma Endometrial adenocarcinoma Squamous cell kidney cancer	41 47 48	Absent	MSI-H	NMD	Positive	Positive	Positive	94	**Yes**
431	Stage II mucinous hepatic flexure adenocarcinoma	57	Absent	ND	NMD	Negative	Positive	Positive	29	No
474	Stage III cecal adenocarcinoma	40	Absent	ND	**MSH2-VUS V163D**	Positive	Positive	Positive	62	No
538	Rectal adenocarcinoma Terminal ileum adenocarcinoma	41 43	Absent	MSI-H	NMD	Positive	Positive	Positive	89.80	**Yes**
572	CIS in a large colonic polyp	40	Absent	ND	ND	Positive	Positive	Positive	25.20	**Yes**

For six of the patients, the positive inversion test results are highlighted. For two of the remaining four patients who are negative for the inversion, the VUS identified in their germline testing results is indicatd in bold. Of the remaining two unsolved cases, the negative Amsterdam and Bethesda criteria as well as low PREMM score are indicated in bold. Only one unexplained case remains

CIS Carcinoma in situ (high grade dysplasia), *MTS* Muir-Torre syndrome, *ND* not done, *NMD* no mutation detected, *VUS* variant of uncertain significance

Table 2

Patient genotypes at multiple SNPs within the *MSH2* locus

PatinetID#	rs10191478	rs2347794	rs17224360	rs6726691	rs6726832	rs7607076	rs7607312	rs1981928	rs1981929	rs7602094	rs6711675	rs3732182	rs12998837	rs3732183	rs2303428	rs2042649
314	GT	AG	TT	CG	AG	AG	AC	AT	AG	CT	AG	GT	AA	AG	CT	CT
370	GT	AG	TT	CG	AG	AG	AC	AT	AG		AG		AA	AG	CT	
372				CG	AG	AG	AC	AT	AG	CT		GT	AA	AG	CT	CT
372 Tumor														A	C	
381	TT	AG	TT	CG	AG	AG	AC	AT	AA	CT	AG	GT	AA	AG	CT	CT
538	TT	AA	TT	CG	AA	AG	CC	AT	AA	CT	GG	GT	AA	AG	CT	CT
572	n	AA	TT	CC	AA	GG	CC	AA	AA	CC	GG	TT	AA	AA	CC	CC
Inferred Inversion Carrier Haplotype	T	A	T	C	A	G	C	A	A	C	G	T	A	A	C	C

It is possible to assign one common haplotype to all carriers. The retained SNP genotype in the tumor tissue of 372 is assumed to represent LOH of the wild-type allele and retention of the allele carrying the inversion of *MSH2*. The inferred haplotype of the *MSH2* allele carrying the inversion is shown at the bottom of the table