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Analysis of Families with Lynch Syndrome Complicated by Advanced Serrated Neoplasia: The Importance of Pathology Review and Pedigree Analysis

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Abstract

The identification of Lynch syndrome has been greatly assisted by the advent of tumour immunohistochemistry (IHC) for mismatch repair (MMR) proteins, and by the recognition of the role of acquired somatic *BRAF* mutation in sporadic MMR-deficient colorectal cancer (CRC). However, somatic *BRAF* mutation may also be present in the tumours in families with a predisposition to develop serrated polyps in the colorectum. In a subgroup of affected members in these families, CRCs emerge which demonstrate clear evidence of MMR deficiency with absent MLH1 staining and high-level microsatellite instability (MSI). This may result in these families being erroneously classified as Lynch syndrome or, conversely, an individual is considered “sporadic” due to the presence of a somatic *BRAF* mutation in a tumour. In this report, we describe two Lynch syndrome families who demonstrated several such inconsistencies. In one family, IHC deficiency of both MSH2 and MLH1 was demonstrated in tumours from different affected family members, presenting a confusing diagnostic picture. In the second family, MLH1 loss was observed in the lesions of both *MLH1* mutation carriers and those who showed normal *MLH1* germline sequence. Both families had Lynch syndrome complicated by an independently segregating serrated neoplasia phenotype, suggesting that in families such as these, tumour and germline studies of several key members, rather than of a single proband, are indicated to clarify the spectrum of risk.

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Introduction

Colorectal cancer (CRC) risk is increased in individuals with a family history of the condition¹. Inherited predispositions account for approximately 10-15% of cases of CRC¹ and the two most well-characterised clinical syndromes, familial adenomatous polyposis (FAP) and hereditary non-polyposis colon cancer (HNPCC or Lynch syndrome), comprise up to one-third of these. In most instances, a striking phenotype of early-onset multiple colonic adenomas leads to the clinical diagnosis of FAP, which is associated with germline mutations in the *APC* gene in the majority of patients.

In contrast to FAP, patients with Lynch syndrome present with very few adenomas and a phenotype of early-onset colorectal or endometrial cancer. Lynch syndrome tumours show distinctive features at the molecular pathology level, resulting from autosomal dominantly-inherited germline defects in genes responsible for DNA mismatch repair, primarily either *MLH1* or *MSH2*, with a minor subset of families (approximately 20% of Lynch syndrome) showing mutation in *MSH6* or *PMS2*². As a consequence, tumours in Lynch syndrome families are characterised by a high level of microsatellite instability (MSI). The diagnosis of Lynch syndrome has been facilitated by the advent of immunohistochemical (IHC) testing of tumour material from an affected family member, where absence of staining for one or more MMR proteins identifies which of the four major MMR genes is likely to be mutated in a given family.

Although tumours with absence of *MSH2*, *MSH6*, or *PMS2* on IHC (though with retained *MLH1*) suggest a diagnosis of Lynch syndrome with a high degree of certainty, this is not the case with tumours which lack expression of *MLH1*. Tumours with absent *MLH1* staining on IHC are present in 10-15% of all CRC in the population, and only a minor subset of these are due to Lynch syndrome. The remainder frequently develop from somatic epigenetic inactivation of *MLH1* by promoter hypermethylation³, or less frequently through other undetermined mechanisms. Importantly, *MLH1* methylation can also be present as a second hit in a small proportion of CRC arising in Lynch syndrome⁴. Several studies have sought to define differences between the two major *MLH1*-absent tumour subsets which have diagnostic utility⁵. In this regard, age of onset of less than 55 years increases the likelihood of Lynch syndrome, however, patients of an intermediate age (55-60), and without extensive knowledge of their family history, continue to present a diagnostic problem⁶.

Significant progress towards a resolution of this diagnostic challenge has resulted from molecular pathology findings, which reflect the non-overlapping developmental stages underlying these two subsets of *MLH1*-absent tumours⁶. There are two common epithelial polyp lineages in the colorectum; traditional adenomas and serrated polyps. Lynch syndrome CRC evolves from pre-malignant, traditional adenomas whilst sporadic MSI-H CRC has its origins in the serrated neoplasia pathway, characterised by advanced serrated polyps as precursor lesions⁷. Although there are histological differences between these CRC subsets⁶, including nuclear structure, the presence of eosinophilic cytoplasm, and differential detection of membranous beta-catenin, a clinically useful difference has been the identification of somatic *BRAF* activating mutations (p.V600E) in the subset of colorectal tumours which arises in serrated polyps⁸. Detection of the somatic *BRAF* mutation in an *MLH1*-absent tumour therefore generally excludes a diagnosis of Lynch syndrome as it unequivocally indicates serrated pathway commitment in a tumour^{8,9}, and testing has been incorporated into clinical use in some centres¹⁰. A descriptive classification of colorectal polyps is given in Table 1¹¹ 12.

Recently, several families have been described where advanced serrated polyps are common, and in whom the presence of the *BRAF* mutation as a somatic alteration in tumours excludes a diagnosis of Lynch syndrome despite their fulfilling Amsterdam criteria¹³. Families in which

the proband has a somatic *BRAF* mutation have also been reported by others^{14,15}, and in a subset of these probands, MSI-H tumours arose where MLH1 was absent by IHC, resulting in the probability that the family may be erroneously diagnosed as having Lynch syndrome¹³. Conversely, the presence of a somatic *BRAF* mutation in a tumour from an individual family member could result in their CRC being designated “sporadic”, even if young-onset. Lynch syndrome families have been observed where not all the CRC can be accounted for by the germline MMR mutation known to segregate in the family. To highlight this diagnostic dilemma we report on two families where, despite recent advances in diagnostic knowledge, detailed pedigree analysis, pathology review, somatic *BRAF* mutation detection in tumours and germline MMR mutation testing were required to fully understand the aetiology of their CRC.

Patients and Methods

Non-FAP CRC families were registered with the Colon Collaborative Family Registry (Colon CFR), an NCI-sponsored database of epidemiology data and biospecimens, and gave informed consent in writing to participation in research¹⁶. During triage for Lynch syndrome, which included MSI testing and IHC for MMR proteins, two families were identified where indications of both Lynch syndrome and somatic *BRAF* mutation were present. Both families fulfilled the Amsterdam I criteria for identification of Lynch syndrome¹⁷. Pathology review was undertaken by a single pathologist (JRJ). Polyps were designated advanced serrated polyps if their morphology was consistent with sessile serrated adenoma, serrated adenoma or mixed polyp (Table 1.)

Tissue screen for DNA MMR deficiency

Immunohistochemistry staining was carried out as previously described¹⁸ for the four MMR proteins MLH1, MSH2, MSH6, and PMS2. Microsatellite instability was analysed from formalin fixed paraffin embedded normal and tumour paired tissue DNA using a 10 marker panel also as previously described¹⁹.

BRAF p.V600E Allele Specific PCR Assay

The somatic c.1799T>A mutation resulting in the p.V600E missense variant in the *BRAF* gene was determined using a fluorescent allele specific PCR assay. Briefly, 20-50ng of DNA, extracted from formalin-fixed paraffin embedded tumour tissue, was amplified in a 25µl reaction containing 100nM each of allele specific primers tagged with differing fluorophores and a common reverse primer, together with 2.5units of Taq polymerase (Eppendorf), 1× buffer and 1mM of dNTPs. Primers for the reaction were as follows; Forward Mutant Primer (F1) 6-Fam-5'-**CAGT**GATTTTGGTCTAGCTtCAGA-3', Forward Wildtype Primer (F2) NED - 5'-**TGATTTTGGTCTAGCTaCAGT**-3', and Common Reverse Primer 5'-CTCAATTCTTACCATCCACAAAATG-3'. The cycling conditions consisted of an initial denaturation of 95°C for 2mins followed by 35 cycles of 94°C for 30sec, 59°C for 30sec and 65°C for 30sec then a final extension of 65°C for 10mins. After amplification, 1µl of the PCR product was added to an 8.7µl mix of HiDi formamide and ROX Genescan 500 size marker (Applied Biosystems, Foster City, CA). The mutant allele (c.1799A) primer generated a PCR product of 97bp, 3bp larger than the wildtype PCR product after separation on an ABI 3100 genetic analyser. GeneMarker (SoftGenetics) software was used to identify the different size and fluorescent allele PCR products. Positive controls were run in each experiment and 10% of samples were replicated with 100% concordance. Example results are given in Figure 1.

MMR Mutation Testing

Genomic DNA isolated from blood lymphocytes using a standard ethanol-salt purification method was used as the template for amplification of MLH1 and MSH2. Briefly, each exon

specific amplicon contained 50ng of gDNA, 20pmol of sequence specific primers (primer sequences available on request), 1.25 units Hotmaster Taq polymerase and 1× Hotmaster Taq buffer (Eppendorf, Hamberg, Germany) and 200μM dNTPs in a 25μl reaction. Universal cycling conditions utilised a touch-down PCR approach and included an initial denaturation at 95°C for 2mins then 12 cycles of 94°C for 20sec, 64°C for 10sec (drop by 0.5°C/cycle) and 65°C for 40sec followed by 30 cycles of 94°C for 20sec, 50°C for 10sec and 65°C for 40sec. After amplification, 1μl of PCR product was used for a 1/8-strength sequencing reaction with the BigDye Terminator v3.1 reagents and protocol (Applied Biosystems) in a 12μl reaction, with 2pmol of the appropriate primer. Reactions were performed in both forward and reverse directions. Sequencing product was cleaned with the DyeEx 96 Kit (Qiagen, Hilden, Germany) using the standard protocol. The product was dried, resuspended in HiDi Formamide (Applied Biosystems) and analysed on an ABI PRISM 3100 (Applied Biosystems). Results were compared to reference sequence NC_000003.10 (genomic) and NM_000249.2 (cDNA) for MLH1, and NC_000002.10 (genomic) and NM_000251.1 (cDNA) for MSH2. Families were also screened for the presence of exon rearrangements in MLH1 and MSH2 using the Salsa MLPA kit P003 in accordance with the manufacturer's protocol (MRC-Holland, Holland).

Case Reports

Family 1

A 58-year old female (Figure 2, individual 7) initially referred herself to her local familial cancer clinic in 1998 as she had been informed by mail from a distant clinic that her sister had been identified as having a mismatch repair gene mutation (specifically *MSH2* mutation in exon 5: c.854delA p.N285fs). Prior to being seen, she had been undergoing regular colonic surveillance due to a personal history of rectal bleeding and diarrhoea. In 1994 two polyps were identified. One was a sessile adenoma of undefined sub-type with severe dysplasia in the caecum which was removed by snare polypectomy, and the other a recto-sigmoid adenoma treated with sigmoid colectomy since it was considered unsuitable for endoscopic resection. Predictive gene testing showed that the proband carried the family *MSH2* mutation.

On further investigation a substantial family history of various cancers was ascertained (Figure 2). This included four individuals with isolated colorectal cancers (individuals 16, 19, 20, 22 ranging in age of onset from 29 to 56 years), one with both uterine and pancreatic cancer (individual 17), 2 with both breast and colorectal cancer (individuals 8 and 14), one with isolated breast cancer (individual 10) and one with a melanoma (individual 12). Interestingly, one of the individuals with both breast and colon cancer (patient 14) had already tested negative for the family *MSH2* mutation. However due to a previous finding of “multiple adenomas” she continued colonic surveillance. The patient had at least 30 polyps, and examination of her excised colorectal lesions revealed multiple polyps of both serrated and adenomatous sub-types, with serrated polyps predominating. This picture is typical of hyperplastic polyposis syndrome (HPS), a condition with increased risk for development of CRC^{20,21}. Several of the more advanced polyps demonstrated immunohistochemical loss of MLH1, high-level microsatellite instability and somatic *BRAF* mutation. The patient then went on to develop a CRC at age 64, several years after her negative test for the family germline mutation. The tumour proved to be MLH1-deficient.

All other CRC-affected individuals who underwent testing were shown to carry the family *MSH2* mutation. A later age of onset in this family for CRC is seen in individual 7 who at 58 had no evidence of colorectal malignancy, and individual 8 whose CRC was detected at age 59. A summary of the investigations undertaken is shown in Table 2. A representative CRC showing *MSH2* loss is illustrated in Figure 3. Figure 3 also demonstrates the contrasting loss of MLH1 in individual 14 who is a non-mutation carrier, but has a predisposition to develop serrated polyps.

Family 2

In 1996, following her gastric cancer diagnosis, Individual 23 (Figure 4) discussed her extensive CRC family history with her gastroenterologist. Several family members were diagnosed with CRC prior to 1996 including the proband's father, sister, niece, five paternal aunts and uncles, five paternal cousins, and her paternal grandfather. Following collaboration between the proband, her gastroenterologist and a geneticist, several affected family members were approached and genetic testing was initiated, resulting in the identification of an *MLH1* mutation in exon 4: c.350C->T p.T117M. Though this is a missense mutation, functional assays have confirmed its deleterious nature^{22,23}. Subsequently, forty-six family members took up the offer of genetic testing and recommended surveillance, resulting in the identification of 19 *MLH1* mutation carriers. Interestingly, the proband did not carry the family mutation, and nor did her sister (individual 24, presenting with 5 polyps at the age of 51), despite the observation of *MLH1* loss in her polyp (Figure 5). Between 1996 and 2008, an additional three family members were diagnosed with CRC, and 16 individuals had polyps removed at time of screening, four of whom tested negative for the family *MLH1* mutation. Of the 12 *MLH1* mutation carriers who underwent subsequent polypectomy, five also had serrated polyps removed. Four mutation carriers and two non-mutation carriers with serrated polyps underwent pathology review and IHC testing for this report. Importantly, a young-onset CRC patient who carried the family mutation demonstrated an *MLH1*-deficient CRC with a *BRAF* mutation (Individual 50, aged 39 years). In several key relatives, including two siblings and her son (individuals 46, 47 and 57), who all carried the family mutation, examination of histological samples revealed the presence of serrated polyps. In her siblings, aged 43 and 44 respectively, and who each had at least 5 polyps, multiple large serrated adenomas and sessile serrated adenomas were observed. Advanced serrated polyps (serrated adenomas and mixed polyps) were also reported in three female relatives in her maternal generation, including two in whom the germline mutation was not detected (individuals 24 and 26). A later age of onset for CRC is also seen in this family with individual 21 being diagnosed with her first CRC at 68 years. A summary of the investigations undertaken is shown in Table 3, and representative colorectal neoplasms showing *MLH1* loss are illustrated in Figure 5.

Discussion

The recognition of familial predisposition to CRC is an important factor in the prevention of morbidity and mortality in both the proband and their relatives. MSI and IHC analysis in a single tumour from the proband, followed by mutation testing in the proband and subsequently in at-risk relatives, is usually adequate to identify those individuals in Lynch syndrome families who carry deleterious mutations in an MMR gene. However, in a small number of Lynch syndrome families the CRC risk is likely due to two independent germline factors. This is particularly important when one susceptibility is for advanced serrated polyps which can produce both somatic *BRAF* mutation-bearing CRC, which may be MSI-H due to *MLH1* deficiency, and mixed serrated-adenomatous polyps, which may also demonstrate IHC loss of *MLH1*²⁴.

Families with a mixed lineage disorder involving Lynch syndrome have been reported previously²⁵. Skoglund et al described a family where both Lynch syndrome, due to a deleterious mutation in *MSH2*, as well as a second predisposition linked to a region on 9q concurred^{26,27}. A further family report detailed the counterintuitive findings of adenomatous polyposis in the presence of an *MSH6* germline mutation also suggesting two co-occurring predispositions²⁸. Mixed lineage families have also been described where *MUTYH* and *MSH6* mutations co-occur²⁹. Given the recent findings regarding individuals with advanced serrated polyps presenting with biallelic mutations in *MUTYH*³⁰, both families were screened for the two common variants of *MUTYH* found in northern Europeans (data not shown)³¹, and

all sixty-five individuals who underwent testing were wild-type for both G382D and Y165C. Our findings regarding *MUTYH* are not unexpected as the lesions in both families demonstrated somatic *BRAF* rather than *KRAS* mutation³⁰. In this case report, we describe another factor which may complicate the management of a Lynch syndrome family, namely a co-existing susceptibility to develop advanced serrated polyps.

It is important to be aware of these mixed lineage families because of the risk of confounding both diagnosis and management. The identification of an MSI-H CRC demonstrating IHC absence of MLH1 in an individual at a relatively young age and with a family history of CRC is likely to result in a diagnosis of Lynch syndrome. The individual in whom the tumour is found will be designated as the family proband, and mutation testing will proceed in their germline tissue. In the majority of cases, this will result in diagnostic confirmation through the ascertainment of a pathogenic germline MMR gene mutation. However, should this tumour be the result of a serrated neoplasia predisposition, a search for a germline mutation in *MLH1* may prove fruitless if this individual is in fact a Lynch syndrome phenocopy.

In this report we describe two families where advanced serrated polyps coincided with Lynch syndrome. In Family 1, an individual with hyperplastic polyposis syndrome (HPS) developed several neoplasms where MLH1 was absent. Given her family history, including multiple CRCs and endometrial cancer, it would be reasonable to suspect that this person also, had Lynch syndrome. Though the multiple CRC in this family indeed resulted from Lynch syndrome, specifically due to a germline defect in *MSH2*, the individual with HPS did not carry this mutation. Instead, she was at increased risk for CRC due to her predisposition to develop multiple serrated polyps. In Family 2, a CRC demonstrated a somatic *BRAF* mutation in a young-onset female mutation carrier. This was an unexpected finding, however, serrated polyps, including multiple sessile serrated adenomas and traditional serrated adenomas were detected in several of her first-degree relatives, and advanced serrated polyps, including traditional serrated adenomas and mixed polyps were observed in three female relatives in her maternal generation. In two of these relatives, the family germline mutation was not detected, suggesting independent segregation. Importantly, in one of these relatives, the serrated polyp demonstrated IHC loss of MLH1 in the absence of a germline mutation.

Though small serrated polyps, especially those occurring in the distal colon, are relatively common in the population, available evidence suggests that serrated polyps which are large, proximal, and of atypical histological appearance (advanced serrated polyps), especially if multiple, may be associated with a genetic predisposition³². Such predispositions are thought to be relatively rare, however recent evidence suggests that they may be prevalent in some populations³³, specifically more common in Europeans²¹, and in Anglo-Celtic populations in particular³⁴. The mode of inheritance in families with serrated neoplasia predisposition ranges from isolated individuals with numerous serrated polyps reminiscent of a recessive condition, exemplified by family 1, to families with multiple affected members where polyp numbers are relatively low but where advanced serrated polyps are frequent as seen in family 2. There is currently no robust clinical test which can discriminate serrated polyps with malignant potential from innocuous serrated lesions, nor is there any robust germline genetic test available which can definitively identify individuals at risk for high-risk serrated polyps. Histological examination remains the best available technique. This report does not conflict with the conventional wisdom that diminutive serrated polyps in the distal colon are unlikely to be of clinical concern. In both families, the serrated polyps are multiple and advanced, and therefore are likely to be associated with an increased risk for colorectal cancer, though precise risk estimates are not able to be derived from currently available data³⁵.

CRC in Lynch syndrome develops from traditional adenomas, and studies of Lynch syndrome families have observed that IHC absence of an MMR protein is not observed in serrated or

hyperplastic polyps in MMR mutation carriers³⁶, in contrast to this alteration being demonstrated in 70% of traditional adenomas³⁷. The occurrence of the somatic *BRAF* mutation in tumours in Lynch syndrome families is rare^{8,9,38-42} and, consistent with this finding, advanced serrated polyps are also rare in Lynch syndrome⁴³. Confirmed Lynch syndrome families in which somatic *BRAF* mutation is detected in a tumour therefore warrant closer investigation, especially when detected in a young-onset individual, as this may indicate that other family members are at risk for CRC independent of their carrier status for MMR mutations. The clinical team managing these mixed lineage families, in whom advanced serrated polyps are observed, need to build a model for dealing with the co-existing genetic predispositions, and update the risk status of the family when each new result becomes available. This management model depends on the study of more than one affected individual in the diagnostic strategy²⁸ and examination of the pathology of pre-malignant lesions developing in the colon. The recognition of a serrated neoplasia predisposition originally came about in the family cancer clinic attended by the authors because multiple tumours from each family were routinely characterised during Lynch syndrome triage, allowing for detection of a pattern of observations delineating serrated neoplasia families from those with Lynch syndrome¹³.

In summary, we have reported two mixed lineage families in whom tumours have arisen from both Lynch syndrome and serrated neoplasia predispositions, overlapping in some but not all affected individuals. Such families will likely come to light as tumour *BRAF* mutation testing becomes more widespread as a screening test to exclude Lynch syndrome. The finding of incongruous occurrences of young-onset CRC in a Lynch syndrome family should suggest consideration of mixed lineage and prompt further investigations, including detailed pedigree analysis, namely segregation of phenotypes, somatic *BRAF* mutation testing, and pathology review, especially in families with European ancestry.

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Figure 1. BRAF V600E Allele Specific PCR Detection

Allele specific amplification plots for detection of the *BRAF* p.V600E mutation with A) displaying only the amplification of a 94bp product corresponding to the BRAF p.V600E wildtype allele and B) a sample positive for the p.V600E mutation displaying a mutant allele-specific amplification product at 97bp as well as the wildtype allele (94bp).

Figure 2.
Pedigree for Family 1. Dotted symbols=hyperplastic or serrated polyps, C=mutation carrier, NC=non-mutation carrier.

Figure 3.

Two tumours from Family 1. Panel A illustrates haematoxylin and eosin-stained sections, panel B MLH1 stain, and panel C MSH2 stain. Row 1 shows serial sections from a CRC derived from an MSH2 mutation carrier (Individual 19). Row 2 shows serial sections from the CRC arising in a non-mutation carrier (Individual 14).

Figure 4.
Pedigree for Family 2. Dotted symbols=serrated polyps, C=mutation carrier, NC=non-mutation carrier.

Figure 5.

Three tumours from Family 2. Panel A illustrates haematoxylin and eosin-stained sections, panel B MLH1 immunostaining, and panel C PMS2 immunostaining. Row 1 shows serial sections from a serrated polyp derived from a non-mutation carrier but showing MLH1 loss (Individual 24). Row 2 shows serial sections from a traditional serrated adenoma arising in a mutation carrier (Individual 47). Row 3 shows serial sections from a severely dysplastic lesion derived from a mutation carrier but which demonstrates somatic *BRAF* mutation (Individual 50).

Table 1

Classification of Common Colorectal Polyps (adapted from Jass, 2005⁹ and O'Brien *et al*, 2006¹⁰). Advanced serrated polyp sub-types are shown in bold. Note the difference in frequency of somatic *BRAF* mutation between the adenoma (<1%) and serrated polyps (up to 83%).

Polyp Name	Alternative Terminology	Key features
Adenoma	Adenomatous polyp	Neoplastic polyp with malignant potential.Somatic <i>BRAF</i> mutation in < 1%
Serrated polyp		A term that encompasses all polyps listed below
Hyperplastic polyp	Metaplastic polyp	General term for common lesion with glandular serration.Somatic <i>BRAF</i> mutation in up to 50%
Serrated adenoma	Mixed hyperplastic adenomatous polyp Atypical hyperplastic polyp Traditional serrated adenoma	Neoplastic polyp having a serrated architecture reminiscent of hyperplastic polyp but with traditional adenomatous dysplasia Somatic <i>BRAF</i> mutation in 62%
Mixed polyp	Admixed serrated polyp	Polyp that includes two separate morphologic components; usually dysplastic and non-dysplastic
Sessile serrated adenoma	Sessile serrated polyp Serrated polyp with atypical proliferation.	Type of serrated polyp with abnormalities of architecture and proliferation but lacking the classical features of epithelial dysplasia (intraepithelial neoplasia)Somatic <i>BRAF</i> mutation in 83%

Table 2

Individual	Age	Sex	Tumours	MLH1 IHC	MSH2 IHC	MSH6 IHC	PMS2 IHC	MSI	BRAF p.V600E	MSH2 Mutation
8	59	female	CRC	Normal	Absent	Absent	Normal	MSI-H	Not detected	Detected
	59		Breast cancer	Normal	Normal	Normal	Normal	MSS		
14	58	female	Hyperplastic polyp 1	Absent	Normal	Normal	Absent	MSI-H	Detected	Not Detected
	62		Hyperplastic polyp 2	Normal	Normal	Normal	Normal	MSS	Detected	
	62		Traditional adenoma 1	Normal	Normal	Normal	Normal	MSI-L	Not detected	
	62		Traditional adenoma 2	Normal	Normal	Normal	Normal	MSS	Detected	
	62		Traditional adenoma 3	Normal	Normal	Normal	Normal	MSS	Not detected	
	62		Hyperplastic polyp 3	Absent	Normal	Normal	Absent	MSS	Detected	
	62		Hyperplastic polyp 4	Normal	Normal	Normal	Normal	MSS	Detected	
	62		Hyperplastic polyp 5	Normal	Normal	Normal	Normal	MSS	Detected	
	62		Hyperplastic polyp 6	Normal	Normal	Normal	Normal	MSS	Not Tested	
	62		Hyperplastic polyp 7	Normal	Normal	Normal	Normal	MSS	Detected	
			Breast cancer	Normal	Normal	Normal	Normal	MSS	Not Tested	
	64		CRC	Absent	Normal	Normal	Absent	MSI-H	Detected	
19	39	male	CRC	Normal	Absent	Absent	Normal	MSI-H	Not Detected	Detected

Legend: CRC=colorectal cancer; MSS=microsatellite stable; MSI-H=high-level microsatellite instability; N/A result not available

Table 3

Individual	Age	Sex	Tumours	MLH1 IHC	MSH2 IHC	MSH6 IHC	PMS2 IHC	MSI	BRAF p.V600E	MLH1 Mutation
17	27	female	CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	Detected
	53		CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
	62		Endometrial cancer	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
39	42	female	CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	Detected
24	51	female	Hyperplastic polyp	Absent	Normal	Normal	Absent	N/A	N/A	Not Detected
	51		Adenomatous polyp	Normal	Normal	Normal	Normal	N/A	N/A	N/A
27	36	male	CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	N/A
39	50	female	Traditional serrated adenoma	Absent	Normal	Normal	Absent	N/A	Not Detected	Detected
	51		CRC	Absent	Normal	Normal	Absent	N/A	Not Detected	
	51		Traditional serrated adenoma	Absent	Normal	Normal	N/A	MSS	Not Detected	
21	68	female	CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	Detected
	71		CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
	71		Adenomatous polyp 1	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
	71		Adenomatous polyp 2	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
26	69	female	Mixed polyp	Normal	Normal	Normal	Normal	MSS	Not Detected	Not Detected
46	44	female	Endometrial cancer	Absent	Normal	Normal	Absent	MSI-H	Not Detected	Detected
	44		Sessile serrated adenoma	Normal	Normal	Normal	Normal	MSS	Detected	
	44		Hyperplastic polyp	Normal	Normal	Normal	Normal	MSS	Not Detected	
	44		Hyperplastic polyp	Normal	Normal	Normal	Normal	MSS	Detected	
47	43	male	Traditional serrated adenoma	Absent	Normal	Normal	Absent	MSI-H	Not Detected	Detected
	43		Adenomatous polyp	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
	43		Traditional serrated adenoma	Absent	Normal	Normal	Absent	MSI-H	Not Detected	
50	39	female	CRC	Absent	Normal	Normal	Absent	MSI-H	Detected	Detected
	39		CRC	Absent	Normal	Normal	Absent	MSI-H	Not Detected	

Legend: CRC=colorectal cancer; MSS=microsatellite stable; MSI-H=high-level microsatellite instability; N/A result not available