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Microsatellite Instability and DNA Mismatch Repair Protein Deficiency in Lynch Syndrome Colorectal Polyps

Matthew B. Yurgelun[1], **Ajay Goel**[2], **Jason L. Hornick**[3], **Ananda Sen**[4], **D. Kim Turgeon**[4,9], **Mack T. Ruffin IV**[4,9], **Norman E. Marcon**[5,9], **John A. Baron**[6,9], **Robert S. Bresalier**[7,9], **Sapna Syngal**[3,8,9], **Dean E. Brenner**[4,9,10], **C. Richard Boland**[2], and **Elena M. Stoffel**[3,4,8,9]

[1]Beth Israel Deaconess Medical Center, Boston, Massachusetts, 02215, USA

[2]Baylor University Medical Center, Baylor Research Institute and Charles A. Sammons Cancer Center, Dallas, Texas, 75246, USA

[3]Brigham and Women's Hospital, Boston, Massachusetts, 02115, USA

[4]University of Michigan Medical Center, Ann Arbor, Michigan, 48109, USA

[5]The Wellesley Site-St. Michael's Hospital, Toronto, Ontario, Canada

[6]University of North Carolina, Chapel Hill, North Carolina, 27599, USA

[7]University of Texas MD Anderson Cancer Center, Houston, Texas, 77030, USA

[8]Dana-Farber Cancer Institute, Boston, Massachusetts, 02115, USA

[9]Great-Lakes New-England Clinical Epidemiology and Validation Center of the Early Detection Research Network (GLNE-EDRN)

[10]VA Medical Center, Ann Arbor, MI 48105

Abstract

Colorectal cancers associated with Lynch syndrome (LS) are characterized by deficient DNA mismatch repair (MMR) function. Our aim was to evaluate the prevalence of microsatellite instability (MSI) and loss of MMR protein expression in LS-associated polyps. Sixty two colorectal polyps – 37 adenomas (APs), 23 hyperplastic polyps (HPs), and 2 sessile serrated polyps (SSPs) – from 34 subjects with germline MMR gene mutations were tested for MSI using a single pentaplex PCR for five mononucleotide repeat microsatellite markers, and also for expression of MLH1, MSH2, MSH6, and PMS2 proteins by immunohistochemistry (IHC). High-level MSI (MSI-H) was seen in 15/37 (41%) APs, 1/23 (4%) HPs, and 1/2 (50%) SSPs. Loss of MMR protein expression was seen in 18/36 (50%) APs, 0/21 HPs, and 0/2 SSPs. APs ≥8 mm were significantly more likely to demonstrate MSI-H (OR = 9.98, 95% CI: 1.52-65.65, p = 0.02) and deficient MMR protein expression (OR = 3.17, 95% CI: 1.20-8.37, p = 0.02) compared with those <8 mm. All (6/6) APs ≥10 mm demonstrated both MSI-H and loss of MMR protein expression by IHC. Our finding that the prevalence of MMR deficiency increases with the size of APs suggests that loss of MMR function is a late event in LS-associated colorectal neoplasia. Although testing large APs may be of value in the diagnostic evaluation of patients with suspected LS, the absence of an MMR deficient phenotype in an adenoma cannot be considered strong evidence against LS, as it is with colorectal carcinomas.

Corresponding Author: Elena Stoffel, MD, MPH University of Michigan Division of Gastroenterology 1500 East Medical Center Drive Taubman Center 3912 Ann Arbor, MI 48109 Phone: 734-936-4785 Fax: 734-936-7392 estoffel@med.umich.edu.

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Keywords

Lynch Syndrome; adenomas; microsatellite instability

Introduction

Lynch syndrome (LS), formerly known as hereditary nonpolyposis colorectal cancer (HNPCC), is the most common inherited colorectal cancer (CRC) syndrome.(1) CRC is the most common LS-associated malignancy(2) and approximately 2-3% of all CRC diagnoses can be attributed to LS.(2, 3) In the absence of risk-reducing interventions, individuals with LS have an estimated 70% lifetime risk of CRC,(3, 4) with many of these tumors developing at early ages and demonstrating accelerated malignant transformation.

Lynch syndrome results from germline mutations in one of the genes involved with DNA mismatch repair (MMR): *MLH1, MSH2*, *MSH6, PMS2,* or *EPCAM/TACSTD1*.(5-11) Functional impairment of the DNA MMR system results in the accumulation of insertion/deletion lesions at loci of DNA repeat sequences termed microsatellites, thereby producing a phenotype known as microsatellite instability (MSI).(12, 13) High-level microsatellite instability (MSI-H) is seen in nearly 90% of LS-associated CRCs, compared to only 15% of sporadic CRCs.(11, 14-17) Analyzing CRCs for MSI and deficient MMR protein expression by immunohistochemical (IHC) staining has become a useful strategy for identifying patients who should undergo genetic evaluation for LS(2, 15, 16, 18) and some have even advocated for the routine testing of all CRCs.(17, 19)

Intensive colonoscopic surveillance is effective in reducing CRC-related morbidity and mortality in patients with LS.(20, 21) Therefore, establishing the diagnosis can help ensure that individuals at risk undergo colonoscopies at 1-2 year intervals, as recommended by evidence-based expert guidelines.(22) Given the importance of identifying patients at risk for LS prior to the diagnosis of CRC, it would be useful to know whether testing for evidence of MMR deficiency is informative in premalignant lesions.

The aim of this study was to determine the prevalence of MSI and loss of MMR protein expression by IHC in colorectal polyps from patients with genetically-confirmed Lynch syndrome.

Materials and Methods

Subjects

Individuals with known pathogenic germline mutations in one of the DNA MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM/TACSTD1*) were identified from registries at three U.S. cancer centers (Dana-Farber Cancer Institute, Boston, MA; University of Michigan Comprehensive Cancer Center, Ann Arbor, MI; MD Anderson Cancer Center, Houston, TX). Subjects who had available colorectal polyp tissue from previous endoscopic or surgical resections were considered for analysis. Approximately half of the MMR mutation carriers with colorectal polyps had participated in a randomized trial comparing colorectal polyp detection using chromoendoscopy and conventional colonoscopy exams (23) and the remaining subjects had colorectal polyps removed during colonoscopies performed as part of their clinical care. Subject gender, age at the time of polyp removal, and MMR gene known to be mutated were recorded.

Polyp Characteristics

Each colorectal polyp was classified as an adenomatous polyp (AP), hyperplastic polyp (HP), or sessile serrated polyp (SSP), based on the original pathology report issued as part of the patients' routine clinical care, and reviewed by a gastrointestinal pathologist (J.L.H.) to confirm the histologic classification. Polyp size in millimeters was obtained from pathology reports. Polyp location within the colon was obtained from endoscopic and pathology reports; polyps located in the cecum through the transverse colon proximal to the splenic flexure were considered to have a proximal location; polyps from the splenic flexure through the rectum were considered to have a distal location.

Microsatellite Instability Analysis

DNA was microdissected from paraffin-embedded tissue blocks for microsatellite instability (MSI) testing. For each sample, a pentaplex polymerase chain reaction (PCR) was performed targeting five mononucleotide repeat microsatellite markers (BAT-25, BAT-26, NR-21, NR-24, and NR-27), as has been previously described.(24) This panel has recently been shown to have superior sensitivity and specificity for detecting LS-associated CRCs compared to the National Cancer Institute (NCI)-endorsed panel, which uses two mononucleotide markers (BAT-25 and BAT-26) and three dinucleotide markers (D2S123, D17S250, and D5S346).(18, 25) Unlike the NCI-endorsed panel, this pentaplex PCR can be performed as a single reaction and does not require simultaneous analysis of corresponding germline DNA from subjects.(24) Polyps were considered to have high-level microsatellite instability (MSI-H) if ≥40% of the markers that gave results were unstable. When 1-39% of the markers that gave results were unstable, polyps were considered to have low-level microsatellite instability (MSI-L); polyps were considered microsatellite stable (MSS) when no instability was detected in any of the markers.

DNA Mismatch Repair Immunohistochemical Analysis

Immunohistochemistry was performed following pressure cooker heat-induced epitope retrieval (0.01 M citrate buffer, pH 6.0) on 4-μm thick formalin-fixed paraffin-embedded tissue sections using mouse anti-MLH1 monoclonal antibody (1:100 dilution; clone ES05; Novocastra, Newcastle Upon Tyne, U.K.), mouse anti-MSH2 monoclonal antibody (1:150 dilution; clone FE11; Calbiochem, Gibbstown, NJ), mouse anti-PMS2 monoclonal antibody (1:50 dilution; clone MRQ-28; Cell Marque, Rocklin, CA), and mouse anti-MSH6 monoclonal antibody (1:400 dilution; clone PU29; Novocastra), and the Envision Plus detection system (Dako, Carpinteria, CA). Expression of MLH1, MSH2, PMS2, and MSH6 was assessed in a blinded fashion by one of the authors (J.L.H.) and was scored as "intact" or "deficient" in lesional cells (epithelial cells in the polyp). The overall IHC status of a polyp was classified as "abnormal" if the polyp had deficient expression of the MMR protein whose gene was known to be mutated in that particular patient. Polyps that had intact IHC staining for the MMR protein known to be mutated in that particular patient (as well as all other MMR proteins that gave interpretable IHC results) were deemed to have "intact" IHC. Non-neoplastic cells (epithelial cells, lymphocytes, and stromal cells) served as an internal positive control in all tissue sections.

MSI and IHC Concordance

For all polyps in which results for both MSI and MMR protein expression by IHC were available, concordance between the two tests was assessed. Polyps were classified as having concordant results if they had intact IHC and were MSS/MSI-L or if they had abnormal IHC and were MSI-H.

Statistical Analyses

Adenoma size was examined as both a continuous and dichotomous variable (<8 mm, and ≥8 mm). Subject age at the time of polypectomy was studied as a continuous variable. Other characteristics – including subject gender, polyp location, and germline MMR gene mutation – were examined as categorical variables. MMR gene mutation was included in the model as a dichotomous variable (*MSH2* mutation vs. other) to overcome model convergence difficulties. A generalized estimating equations approach with an exchangeable working correlation matrix was adopted to account for clustering within subjects. Separate models were fit using polyp size either as a continuous or a dichotomous variable. The validity of using a linear polyp size variable in the regression model was confirmed from a rough linear pattern in an *added variable plot* in which the residuals from the logistic regression model without polyp size as a covariate was plotted against the residuals from a linear model with polyp size as a dependent variable and the same covariates as in the logistic regression model. A similar logistic regression analysis was also carried out to model the likelihood of observing a specific MSI status (MSI-H vs. MSI-L/MSS). The regression analysis was conducted for adenomatous polyps (APs) only. *P*-values <0.05 were considered to be statistically significant.

Results

Subject Characteristics

Sixty two colorectal polyps from 34 subjects with known pathogenic germline mutations in one of the DNA MMR genes (*MLH1*, *MSH2*, *MSH6*, or *PMS2*, or *EPCAM/TACSTD1*) were identified (mean of 1.8 polyps per subject; range 1-6). Fourteen (41%) of the subjects were men; subjects' median age at the time of first polyp removal was 47.5 years (range 23-67). Twenty-one (62%) subjects had germline mutations in *MSH2*, 11 (32%) in *MLH1*, and 2 (6%) in *MSH6*.

Adenomatous Polyps

Thirty-seven of the polyps from 21 subjects were identified as APs. Fifteen (41%) were from males and 18 (49%) were from subjects who were ≥50 years of age at the time of polypectomy. Mean size of APs was 5.0 mm (range 1-15 mm); 22 (59%) were <5, 7 (19%) were between 5-9 mm, and 6 (16%) ≥10 mm. Sixteen (43%) were located in the proximal colon. Further details regarding subject demographics and polyp characteristics and are presented in Table 1A.

MSI analysis was performed on all 37 APs. Fifteen (41%) demonstrated MSI-H, 3 (8%) were MSI-L, and the remaining 19 (51%) were MSS. MSI-H was seen in 6/6 (100%) APs ≥10 mm, 2/7 (29%) APs 5-9 mm, and 7/22 (32%) APs <5 mm. The logistic regression model with the dichotomous MSI status as outcome (MSI-H vs. MSI-L/MSS) showed a significant association between MSI and AP size, with larger (≥8 mm) APs having a significantly higher likelihood of demonstrating MSI-H than smaller (<8 mm) ones (OR=9.98, 95% CI: (1.52, 65.65), p=0.02). There was also a significant association between MSI-H and subject age, with APs diagnosed at older ages having significantly higher odds of being MSI-H (OR=1.18, 95% CI: (1.09, 1.28), p < 0.0001). Polyp size as a continuous variable remained a statistically significant predictor of MSI status (OR =1.19, 95% CI: (1.02, 1.39), p=0.025). The significant association between age and MSI status was also retained in the model using continuous polyp size (OR per mm = 1.17, 95% CI: (1.09, 1.25), p < 0.0001).

Only 1 (25%) of 4 APs from *MSH6* mutation carriers was MSI-H, compared to 33% and 48% from *MLH1* and *MSH2* mutation carriers respectively; this difference, however, was

not statistically significant (p=0.26). There was no significant association between MSI status and subject gender (Table 3).

Conclusive MMR IHC results were available on 36 APs. Thirty (83%) had sufficient tissue to give conclusive results for all four MMR proteins, 5 (14%) had results on three of the four MMR proteins, and 1 (3%) AP from an *MSH2* mutation carrier had results for MLH1 and MSH2 staining only. In total, 18 (50%) of the 36 APs were classified as having abnormal IHC results. Abnormal IHC was observed in 6/6 (100%) APs ≥10 mm in size, 4/7 (57%) APs 5-9 mm, and 8/21 (38%) APs <5 mm. In the logistic regression model, the prevalence of abnormal MMR IHC was significantly associated with AP size, with larger (≥8 mm) APs having a significantly higher likelihood of demonstrating abnormal IHC (OR=3.17, 95% CI: (1.20, 8.37), p=0.02) compared to smaller (<8 mm) APs. Size as a continuous predictor lost significance, but only marginally (OR per mm = 1.08, 95% CI: (0.996, 1.16), p=0.06).

Among the 12 APs from *MLH1* carriers, 8 (67%) had absent MLH1 staining (Figure 1A), 7 of which also demonstrated absence of PMS2 staining. Of the 20 APs from *MSH2* mutation carriers, 10 (50%) had absent MSH2 staining (Figure 1B), 9 of which also demonstrated absence of MSH6 staining (the remaining one had very weak MSH6 staining and absent MSH2 staining). None of the 4 APs from *MSH6* mutation carriers had abnormal MMR IHC, compared with 67% and 50% of APs from *MLH1* and *MSH2* mutation carriers, respectively; this, however, did not result in any statistical significance. There was no significant association between MMR IHC results and subject gender or age, although the data suggested a nonsignificant trend toward higher odds of abnormal IHC with increasing age (Table 3).

Thirty six of the 37 APs had results for both MSI and MMR IHC (one MSI-H AP had insufficient tissue for IHC analysis). Thirteen of the 14 APs with MSI-H also had deficient MMR protein expression by IHC. In comparison, only 13 of 18 APs with deficient MMR protein expression by IHC also demonstrated MSI-H. Thus, 6 (17%) of the 36 APs had MSI and IHC results that were discordant. All six of the APs with discordant results were from subjects under the age of 50 and ranged from 2-8 mm in size. Only one AP was MSI-H with intact MMR protein expression, and this was a proximally-located 2 mm adenoma from an *MSH6* mutation carrier.

Overall, 20/37 (54%) APs demonstrated a phenotype of MMR deficiency with MSI-H, abnormal IHC, or both. All (100%) six of the APs ≥10 mm in size were both MSI-H and had deficient MMR protein expression by IHC.

Hyperplastic and Sessile Serrated Polyps

Twenty-three polyps from 19 subjects were identified as HPs. Six (26%) of the 23 HPs came from male subjects and 8 (35%) were from patients who were ≥50 years old. Size data were available on all 23 HPs, with a mean of 3.0 mm (range 1-7 mm); 21 (91%) were <5 mm, while the remaining 2 (9%) were between 5-9 mm. Four (17%) of the 23 HPs were located in the proximal colon. Further details regarding subject demographics and polyp characteristics are presented in Table 1B.

MSI analysis was performed on all 23 HPs. MSI-H was seen in 1/23 (4%) and 3/23 (13%) were MSI-L; the remaining 19/24 (83%) HPs were MSS. There was no significant association between MSI status and subject gender, age, mutated MMR gene, HP location, or HP size. DNA MMR IHC results were available on 21 HPs; 100% had intact MMR protein expression by IHC. Two polyps were SSPs and came from 2 different subjects. One 10 mm SSP from an *MSH2* mutation carrier was MSI-H and the other 5 mm SSP from an

MLH1 mutation carrier was MSS. Both of the SSPs had intact IHC staining for all four MMR proteins (Table 1C).

Discussion

Overall, we detected an MMR deficient phenotype in approximately half of all adenomas from confirmed MMR mutation carriers, a prevalence that is much lower than what is seen in LS-associated CRC.(14-16) The likelihood of having an MMR deficient phenotype is significantly associated with increasing adenoma size, and 6/6 large (≥10 mm) adenomas exhibited both MSI-H and abnormal IHC Neither subject gender, younger subject age, nor proximal colon location showed a significant association with either MSI status or IHC results for LS-associated APs. Of note, none of the APs from *MSH6* mutation carriers had abnormal MMR protein expression by IHC. Of the LS-associated HPs, only 1 of 23 was MSI-H and none demonstrated abnormal MMR protein expression.

Adenomatous polyps (APs) are believed to be the precursor lesions for LS-associated CRC, making them a potential target for diagnostic testing.(26, 27) Prior studies attempting to define the prevalence of MSI and deficient MMR protein expression in LS-associated APs have used various criteria for defining LS and have reported rates of MSI-H and abnormal IHC ranging from 33-89% and 25-82%, respectively.(27-34)

Our findings add to the existing literature by demonstrating a significant association between the likelihood of an MMR deficient phenotype and increasing polyp size in LS-associated APs. Although the overall prevalence of both MSI-H and deficient MMR protein expression by IHC in our study was lower than in prior reports,(27, 34) the comparatively small size of the APs (mean size 5.0 mm) removed from subjects undergoing intensive colonoscopic surveillance may account for this difference. Our observation that APs from *MSH6* mutation carriers showed universally intact MMR IHC staining reinforces previous reports that colorectal neoplasms in *MSH6* mutation carriers have a relatively mild MMR deficient phenotype.(9, 35) This phenomenon can potentially be explained by prior work, which demonstrated that isolated loss of *MSH6* gene function is not, by itself, sufficient to lose MMR activity due to the partially overlapping function of *MSH3*.(36)

Our study has several strengths. This was a multicenter study that included only subjects who were confirmed to carry a pathogenic germline mutation in one of the DNA MMR genes. We also obtained both MSI and MMR IHC results on nearly all of our polyps, thereby allowing us to describe the concordance between the two tests. Furthermore, ours is the first study of its kind to include an extensive analysis of HPs from subjects with genetically-confirmed LS.

We recognize that our study has several limitations. While the number of MMR mutation carriers is large compared with other published studies, the overall small number of polyps examined limits the statistical power of our analyses. We examined only 4 APs from 2 subjects with *MSH6* mutations and no polyps from *PMS2* or *EPCAM/TACSTD1* mutation carriers, thereby limiting the conclusions that can be made about MSI and MMR IHC testing in these subjects. Likewise, the small number of SSPs in our study precludes us from commenting on their role in LS-associated neoplasia. Our study only included subjects with confirmed LS and thus does not provide any information regarding the utility of MSI and MMR IHC testing of unselected colorectal polyps, although prior data suggest that the rate of MMR deficiency in sporadic APs is extremely low (<2%).(37-39) Since we did not include polyps from non-LS control subjects, we are unable to define the specificity, positive predictive value, or negative predictive value of MSI and MMR IHC for detecting cases of LS.

Our findings have diagnostic implications in that they suggest that MMR IHC testing could provide useful information in the evaluation of patients with suspected LS who have large adenomas, but no cancer tissue available for testing. Absent expression of one or more DNA MMR proteins would provide justification for formal genetic evaluation for LS with the added benefit of identifying which genes should be targeted for germline sequencing. It is important to recognize, however, that the finding of intact MMR function in such adenomas cannot be considered evidence against a diagnosis of LS.

We believe that our findings also provide important insight into the biology of LS-associated colorectal neoplasia. Although all of the subjects in our study were confirmed carriers of a pathogenic germline mutation in a DNA MMR gene and thus had a “first hit” monoallelic loss of DNA MMR gene expression at baseline, only about half of the LS-associated APs in our study demonstrated an MMR deficient phenotype – a phenomenon that requires biallelic inactivation of MMR gene function. We would thus hypothesize that the early steps in LS-associated colorectal neoplasia are likely similar to those in sporadic AP formation with abrogation of WNT signaling via biallelic inactivation of the *APC* gene or through mutations in *β-catenin*.(40) Our observation of high rates of deficient MMR protein expression in large APs, along with the well-described high rates of MMR deficiency in LS-associated CRCs(1, 2, 15) would suggest that the “second hit” somatic loss of MMR activity is a relatively late event in LS-associated neoplasia.

The observation that MSI analysis is even less sensitive than DNA MMR IHC in small (<8 mm) LS-associated APs further supports this hypothesis. The phenotype of MSI is determined by evaluating markers that are highly-sensitive “hotspots” which, when mutated, give an accurate reflection of deficient DNA MMR function.(18, 24, 25, 40) In and of themselves, however, mutations at these particular sequences are probably of minimal biologic relevance and are instead simply indicators of an MMR deficient phenotype. MSI-induced carcinogenesis instead occurs via biallelic mutations in the coding microsatellites of genes that regulate cell proliferation (such as *TGFβR2*) and apoptosis (such as *BAX*).(40, 41) Thus, we would expect that mutations seen in the pentaplex markers are passengers and relatively late phenomena seen in more advanced neoplasms, occurring only after the acquisition of biallelic loss of DNA MMR function.

In summary, large (≥8 mm) LS-associated APs demonstrate a phenotype of deficient DNA MMR function at a frequency comparable to what is seen in LS-associated CRCs, whereas small (<8 mm) APs are significantly less likely to exhibit MSI-H and/or abnormal DNA MMR protein expression. Our findings provide important insight into LS-associated carcinogenesis and imply that loss of DNA MMR function is likely to be a relatively late event in LS-associated neoplasia. Our data suggest that the finding of MMR deficiency in APs can have clinical utility, particularly in the evaluation of patients with suspected LS who have not developed colorectal adenocarcinoma. Although the absence of an MMR deficient phenotype would not be sufficient to exclude a diagnosis of LS, the finding of MSI-H and/or abnormal MMR expression in an AP from a patient with a concerning family history could provide justification for formal genetic evaluation and germline sequencing.

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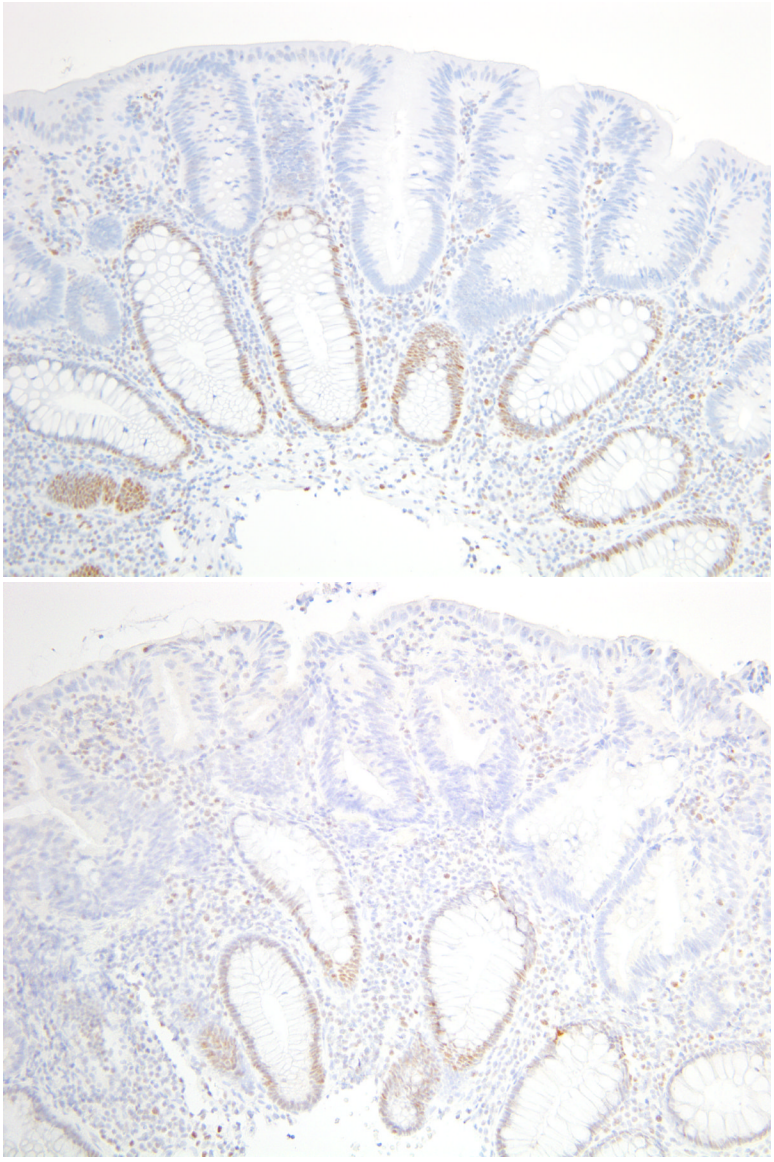

Figure 1.
(A) An adenoma from a subject with a germline mutation in *MLH1* shows loss of MLH1 protein expression in the adenomatous epithelium by immunohistochemistry. Note the intact nuclear staining in non-neoplastic epithelial cells of the lower crypts (original magnification x200). (B) An adenoma from a subject with a germline mutation in *MSH2* shows loss of MSH2 protein expression in the adenomatous epithelium (original magnification x200).

Table 1

Clinical and pathological demographics of Lynch syndrome-associated polyps

A - Adenomatous Polyps							
Subject	**Sex**	**Age (Years)**	**Polyp Size (mm)**	**Polyp Location**	**Germline Mutation**	**MSI Status**	**MMR IHC Results**
1	M	51	n/a	Distal	MSH2	MSS	INTACT
2	M	53	n/a	Distal	MSH2	MSS	INTACT
3	F	58	3	Distal	MSH6	MSS	INTACT
4	F	45	10	Distal	MSH2	MSI-H	ABNORMAL
		51	2	Proximal		MSI-H	ABNORMAL
		58	8	Distal		MSI-H	ABNORMAL
		61	15	Distal		MSI-H	ABNORMAL
6	M	31	3	Distal	MLH1	MSS	INTACT
7	M	44	2	Proximal	MLH1	MSS	INTACT
		46	2	Distal		MSS *	ABNORMAL *
10	M	53	5	Proximal	MLH1	MSI-H	ABNORMAL
		62	10	Proximal		MSI-H	ABNORMAL
		69	4	Distal		MSI-H	ABNORMAL
11	M	38	6	Distal	MSH2	MSS *	ABNORMAL *
		39	4	Distal		MSI-H	ABNORMAL
15	F	51	10	Distal	MSH2	MSI-H	ABNORMAL
		51	15	Distal		MSI-H	ABNORMAL
16	F	40	3	Proximal	MSH2	MSI-L	INTACT
		40	3	Distal		MSI-L	INTACT
233	M	57	3	Proximal	MSH2	MSI-H	ABNORMAL
242	F	40	8	Distal	MLH1	MSS *	ABNORMAL *
		40	8	Distal		MSS	INTACT
279	F	52	2	Proximal	MSH2	MSS	INTACT
		52	2	Distal		MSS	INTACT
		52	4	Distal		MSS	INTACT
		52	5	Proximal		MSS	INTACT
402	M	50	10	Distal	MSH2	MSI-H	ABNORMAL

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A - Adenomatous Polyps

Subject	Sex	Age (Years)	Polyp Size (mm)	Polyp Location	Germline Mutation	MSI Status	MMR IHC Results
439	M	27	4	Proximal	MSH2	MSS	INTACT
787	F	49	2	Proximal	MLH1	MSI-H	ABNORMAL
867	M	59	2	Distal	MSH2	MSI-H	n/a
901	F	59	1	Proximal	MLH1	MSI-L	INTACT
992	F	35	3	Proximal	MLH1	MSS*	ABNORMAL*
		35	3	Proximal		MSS*	ABNORMAL*
1016	F	46	2	Proximal	MSH6	MSI-H*	INTACT*
		46	2	Proximal		MSS	INTACT
		46	2	Distal		MSS	INTACT
1024	M	23	7	Proximal	MSH2	MSS	INTACT

B - Hyperplastic Polyps

Subject	Sex	Age (Years)	Polyp Size (mm)	Polyp Location	Germline Mutation	MSI Status	MMR IHC Results
4	F	48	2	Distal	MSH2	MSS	INTACT
		61	3	Distal		MSS	INTACT
5	F	67	3	Distal	MLH1	MSI-H*	INTACT*
9	F	43	2	Distal	MLH1	MSI-L	INTACT
10	M	57	4	Distal	MLH1	MSS	INTACT
12	F	51	3	Distal	MLH1	MSS	n/a
16	F	38	2	Distal	MSH2	MSI-L	INTACT
		40	3	Distal		MSI-L	INTACT
242	F	40	2	Distal	MLH1	MSS	INTACT
251	F	26	2	Distal	MSH2	MSS	INTACT
		26	7	Distal		MSS	INTACT
386	F	50	2	Distal	MSH2	MSS	INTACT
		50	3	Distal		MSS	INTACT
439	M	27	4	Proximal	MSH2	MSS	INTACT
607	F	25	5	Proximal	MSH2	MSS	INTACT
732	M	49	3	Distal	MSH2	MSS	INTACT

B - Hyperplastic Polyps

Subject	Sex	Age (Years)	Polyp Size (mm)	Polyp Location	Germline Mutation	MSI Status	MMR IHC Results
821	M	67	4	Distal	MSH2	MSS	INTACT
858	F	39	1	Proximal	MSH2	MSS	n/a
929	M	35	4	Proximal	MSH2	MSS	INTACT
947	F	51	3	Distal	MSH2	MSS	INTACT
992	F	35	3	Distal	MLH1	MSS	INTACT
1024	M	23	1	Distal	MSH2	MSS	INTACT
1032	F	26	2	Distal	MSH2	MSS	INTACT

C - Sessile Serrated Polyps

Subject	Sex	Age (Years)	Polyp Size (mm)	Polyp Location	Germline Mutation	MSI Status	MMR IHC Results
8	F	45	5	Proximal	MLH1	MSS*	INTACT*
16	F	38	10	Proximal	MSH2	MSI-H	INTACT

* Discordance between MSI and IHC results

Table 2

Rates of microsatellite instability (MSI) and abnormal DNA mismatch repair protein expression (MMR IHC) in Lynch syndrome-associated adenomas

Adenomas (N=37)[a]		MSI-H (N=15)	MSI-L/MSS (N=22)	Abnormal MMR IHC (N=18)	Intact MMR IHC (N=18)
Subject Sex	Male (N=15)	7 (47%)	8 (53%)	8 (57%)	6 (43%)
	Female (N=22)	8 (36%)	14 (64%)	10 (45%)	12 (55%)
Subject Age (Years)	0-49 (N=18)	4 (22%)	14 (78%)	8 (44%)	10 (56%)
	Age ≥50 (N=19)	11 (58%)	8 (42%)	10 (56%)	8 (44%)
Germline MMR Mutation	MLH1 (N=12)	4 (33%)	8 (67%)	8 (67%)	4 (33%)
	MSH2 (N=21)	10 (48%)	11 (52%)	10 (50%)	10 (50%)
	MSH6 (N=4)	1 (25%)	3 (75%)	0 (0%)	4 (100%)
Adenoma Location	Proximal (N=16)	6 (38%)	10 (63%)	7 (44%)	9 (56%)
	Distal (N=21)	9 (43%)	12 (57%)	11 (55%)	9 (45%)
Adenoma Size[b]	<5 mm (N=22)	7 (32%)	15 (68%)	8 (38%)	13 (62%)
	5-9 mm (N=7)	2 (29%)	5 (71%)	4 (57%)	3 (43%)
	≥10 mm (N=6)	6 (100%)	0 (0%)	6 (100%)	0 (0%)

[a] All N values refer to number of adenomas

[b] Size data were unknown for two MSS adenomas, both with intact IHC

Table 3

Factors predicting for MSI-H and deficient mismatch repair (MMR) protein expression in Lynch syndrome adenomas

Characteristic	MSI Status[a] Odds Ratio (95% Confidence Interval)	MMR IHC[b] Odds Ratio (95% Confidence Interval)
Subject Sex (Reference: Male)	0.14 (0.01, 1.42)	0.33 (0.05, 2.18)
Subject Age[c]	1.18 (1.09, 1.28)	1.07 (0.99, 1.15)
Subject's Germline Mutation (Reference: *MSH2* mutation)	3.53 (0.39, 32.3)	1.39 (0.23, 8.36)
Adenoma Size[c]	1.19 (1.02, 1.39)	1.08 (0.996, 1.16)
Adenoma Size[d] (Reference: <8 mm)	9.98 (1.52, 65.65)	3.17 (1.20, 8.37)

[a] Modeling the odds of observing MSI-H versus MSI-L/MSS.

[b] Modeling the odds of observing abnormal MMR IHC versus intact MMR IHC.

[c] Subject age and adenoma size studied as continuous variables.

[d] Adenoma size as a dichotomous variable.