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Research Article

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

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Abstract

Colorectal cancers associated with Lynch syndrome 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 Lynch syndrome–associated polyps. Sixty-two colorectal polyps—37 adenomatous polyps, 23 hyperplastic polyps, and 2 sessile serrated polyps (SSP)—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. High-level MSI (MSI-H) was seen in 15 of 37 (41%) adenomatous polyps, one of 23 (4%) hyperplastic polyps, and one of two (50%) SSPs. Loss of MMR protein expression was seen in 18 of 36 (50%) adenomatous polyps, zero of 21 hyperplastic polyps, and zero of two SSPs. Adenomatous polyps 8 mm or larger in size were significantly more likely to show MSI-H [OR, 9.98; 95% confidence interval (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 less than 8 mm in size. All (six of six) adenomatous polyps 10 mm or larger in size showed both MSI-H and loss of MMR protein expression by immunohistochemistry. Our finding that the prevalence of MMR deficiency increases with the size of adenomatous polyps suggests that loss of MMR function is a late event in Lynch syndrome–associated colorectal neoplasia. Although testing large adenomatous polyps may be of value in the diagnostic evaluation of patients with suspected Lynch syndrome, the absence of an MMR-deficient phenotype in an adenoma cannot be considered as a strong evidence against Lynch syndrome, as it is with colorectal carcinomas. *Cancer Prev Res; 5(4); 574–82. ©2012 AACR.*

Introduction

Lynch syndrome, formerly known as hereditary nonpolyposis colorectal cancer (HNPCC), is the most commonly inherited colorectal cancer (CRC) syndrome (1). CRC is the most common Lynch syndrome–associated malignancy (2) and approximately 2% to 3% of all CRC diagnoses can be attributed to Lynch syndrome (2, 3). In the absence of risk-reducing interventions, individuals with Lynch syndrome have an estimated 70% lifetime risk of CRC (3, 4), with many of these tumors developing at early ages and showing 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; refs. 12, 13). High-level MSI (MSI-H) is seen in nearly 90% of Lynch syndrome–associated CRCs, compared with 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 Lynch syndrome (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

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Lynch syndrome (20, 21). Therefore, establishing the diagnosis can help ensure that individuals at risk undergo colonoscopies at 1- to 2-year intervals, as recommended by evidence-based expert guidelines (22). Given the importance of identifying patients at risk for Lynch syndrome 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 immunohistochemistry 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 3 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 examinations (23), and the remaining subjects had colorectal polyps removed during colonoscopies conducted 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, hyperplastic polyp, 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. Hornick) 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.

MSI analysis

DNA was microdissected from paraffin-embedded tissue blocks for MSI testing. For each sample, a pentaplex PCR was carried out targeting 5 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 Lynch syndrome–associated CRCs compared with the National Cancer Institute (NCI)-endorsed panel, which uses 2 mononucleotide markers (BAT-25 and BAT-26) and 3 dinucleotide markers (D2S123, D17S250, and D5S346; refs. 18, 25). Unlike the NCI-endorsed panel, this pentaplex PCR can be carried out as a single reaction and does not require simultaneous analysis of corresponding germline DNA from subjects (24). Polyps were considered to have MSI-H if 40% or more of the markers that gave results were unstable. When 1% to 39% of the markers that gave results were unstable, polyps were considered to have low-level MSI (MSI-L); polyps were considered microsatellite stable (MSS) when no instability was detected in any of the markers.

DNA MMR immunohistochemical analysis

Immunohistochemistry was conducted following pressure cooker heat–induced epitope retrieval (0.01 mol/L 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), mouse anti-MSH2 monoclonal antibody (1:150 dilution; clone FE11; Calbiochem), mouse anti-PMS2 monoclonal antibody (1:50 dilution; clone MRQ-28; Cell Marque), and mouse anti-MSH6 monoclonal antibody (1:400 dilution; clone PU29; Novocastra), and the Envision Plus detection System (Dako). Expression of MLH1, MSH2, PMS2, and MSH6 was assessed in a blinded fashion by one of the authors (J.L. Hornick) 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" immunohistochemistry. Nonneoplastic 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 immunohistochemistry were available, concordance between the 2 tests was assessed. Polyps were classified as having concordant results if they had intact immunohistochemistry and were MSS/MSI-L or if they had abnormal immunohistochemistry 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

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

MSI analysis was conducted on all 37 adenomatous polyps. Fifteen (41%) showed MSI-H, 3 (8%) were MSI-L, and the remaining 19 (51%) were MSS. MSI-H was seen in 6 of 6 (100%) adenomatous polyps ≥10 mm, 2 of 7 (29%) adenomatous polyps 5–9 mm, and 7 of 22 (32%) adenomatous polyps <5 mm (Table 2). The logistic regression model with the dichotomous MSI status as outcome (MSI-H vs. MSI-L/MSS) showed a significant association between MSI and adenomatous polyp size, with larger (≥8 mm) adenomatous polyps having a significantly higher likelihood of showing MSI-H than smaller (<8 mm) ones [OR, 9.98; 95% confidence interval (CI), 1.52–65.65; $P = 0.02$] (Table 3). There was also a significant association between MSI-H and subject age, with adenomatous polyps 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 adenomatous polyps from *MSH6* mutation carriers was MSI-H, compared with 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 adenomatous polyps. Thirty (83%) had sufficient tissue to give conclusive results for all 4 MMR proteins, 5 (14%) had results on 3 of the 4 MMR proteins, and 1 (3%) adenomatous polyp from an *MSH2* mutation carrier had results for MLH1 and MSH2 staining only. In total, 18 (50%) of the 36 adenomatous polyps were classified as having abnormal IHC results. Abnormal immunohistochemistry was observed in 6 of 6 (100%) adenomatous polyps ≥10 mm in size, 4 of 7 (57%) adenomatous polyps 5–9 mm, and 8 of 21 (38%) adenomatous polyps <5 mm (Table 2). In the logistic regression model, the prevalence of abnormal MMR immunohistochemistry was significantly associated with adenomatous polyp size, with larger (≥8 mm) adenomatous polyps having a significantly higher likelihood of showing abnormal immunohistochemistry (OR, 3.17; 95% CI, 1.20–8.37; $P = 0.02$) compared with smaller (<8 mm) adenomatous polyps. Size as a continuous predictor lost significance but only marginally (OR per mm, 1.08; 95% CI, 0.996–1.16; $P = 0.06$) (Table 3).

Among the 12 adenomatous polyps from *MLH1* carriers, 8 (67%) had absent MLH1 staining (Fig. 1A), 7 of which also showed absence of PMS2 staining. Of the 20 adenomatous polyps from *MSH2* mutation carriers, 10 (50%) had absent MSH2 staining (Fig. 1B), 9 of which also showed absence of MSH6 staining (the remaining one had very weak MSH6 staining and absent MSH2 staining). None of the 4 adenomatous polyps from *MSH6* mutation carriers had abnormal MMR immunohistochemistry, compared with 67% and 50% of adenomatous polyps 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 immunohistochemistry with increasing age (Table 3).

Thirty-six of the 37 adenomatous polyps had results for both MSI and MMR immunohistochemistry (one MSI-H adenomatous polyp had insufficient tissue for IHC analysis). Thirteen of the 14 adenomatous polyps with MSI-H also had deficient MMR protein expression by immunohistochemistry. In comparison, only 13 of 18 adenomatous polyps with deficient MMR protein expression by immunohistochemistry also showed MSI-H. Thus, 6 (17%) of the 36 adenomatous polyps had MSI and IHC results that were discordant. All 6 of the adenomatous

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Table 1. Clinical and pathological demographics of Lynch syndrome–associated polyps

Subject	Sex	Age, y	Polyp size, mm	Polyp location	Germline mutation	MSI status	MMR IHC results
Adenomatous polyps							
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[a]	Abnormal[a]
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[a]	Abnormal[a]
		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[a]	Abnormal[a]
		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
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[a]	Abnormal[a]
		35	3	Proximal		MSS[a]	Abnormal[a]
1,016	F	46	2	Proximal	*MSH6*	MSI-H[a]	Intact[a]
		46	2	Proximal		MSS	Intact
		46	2	Distal		MSS	Intact
1,024	M	23	7	Proximal	*MSH2*	MSS	Intact
Hyperplastic polyps							
4	F	48	2	Distal	*MSH2*	MSS	Intact
		61	3	Distal		MSS	Intact
5	F	67	3	Distal	*MLH1*	MSI-H[a]	Intact[a]
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

(Continued on the following page)

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Table 1. Clinical and pathological demographics of Lynch syndrome–associated polyps (Cont'd)

Subject	Sex	Age, y	Polyp size, mm	Polyp location	Germline mutation	MSI status	MMR IHC results
Hyperplastic polyps							
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
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
1,024	M	23	1	Distal	*MSH2*	MSS	Intact
1,032	F	26	2	Distal	*MSH2*	MSS	Intact
Sessile Serrated Polyps							
8	F	45	5	Proximal	*MLH1*	MSS	Intact
16	F	38	10	Proximal	*MSH2*	MSI-H[a]	Intact[a]

[a]Discordance between MSI and IHC results. n/a = not available.

polyps with discordant results were from subjects younger than 50 years and ranged from 2 to 8 mm in size. Only one adenomatous polyp was MSI-H with intact MMR protein expression, and this was a proximally located 2-mm adenoma from an *MSH6* mutation carrier.

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

Table 2. Rates of MSI and abnormal DNA MMR 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, y				
0–49 (*N* = 18)	4 (22)	14 (78)	8 (44)	10 (56)
≥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], mm				
<5 (*N* = 22)	7 (32)	15 (68)	8 (38)	13 (62)
5–9 (*N* = 7)	2 (29)	5 (71)	4 (57)	3 (43)
≥10 (*N* = 6)	6 (100)	0 (0)	6 (100)	0 (0)

NOTE: All values are expressed as *N* (%).

[a]All *N* values refer to number of adenomas.

[b]Size data were unknown for 2 MSS adenomas, both with intact immunohistochemistry.

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Table 3. Factors predicting for MSI-H and deficient MMR protein expression in Lynch syndrome adenomas

Characteristic	MSI status[a] OR (95% CI)	MMR IHC[b] OR (95% CI)
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 studied as a dichotomous variable.

Hyperplastic and sessile serrated polyps

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

MSI analysis was conducted on all 23 hyperplastic polyps. MSI-H was seen in 1 of 23 (4%) and 3 of 23 (13%) were MSI-L; the remaining 19 of 24 (83%) hyperplastic polyps were MSS. There was no significant association between MSI status and subject gender, age, mutated MMR gene, hyperplastic polyp location, or hyperplastic polyp size. DNA MMR IHC results were available on 21 hyperplastic polyps; 100% had intact MMR protein expression by immunohistochemistry.

Two polyps were sessile serrated polyps 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 4 MMR proteins (Table 1).

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 Lynch syndrome–associated CRCs (14–16). The likelihood of having an MMR-deficient phenotype is significantly associated with increasing adenoma size, and 6 of 6 large (≥10 mm) adenomas exhibited both MSI-H and abnormal immunohistochemistry. Neither subject gender, younger subject age nor proximal colon location showed a significant association with either MSI status or IHC results for Lynch

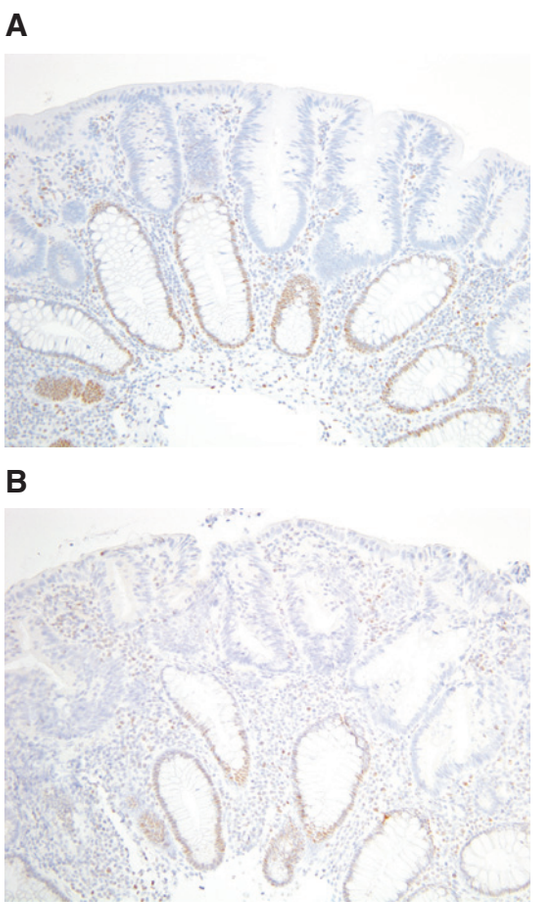

Figure 1. A, an adenoma from a subject with a germline mutation in *MLH1* shows loss of MLH1 protein expression in the adenomatous epithelium by immunohistochemistry. Note the intact nuclear staining in non-neoplastic epithelial cells of the lower crypts (original magnification, 200×). B, an adenoma from a subject with a germline mutation in *MSH2* shows loss of MSH2 protein expression in the adenomatous epithelium (original magnification, 200×).

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syndrome–associated adenomatous polyps. Of note, none of the adenomatous polyps from *MSH6* mutation carriers had abnormal MMR protein expression by immunohistochemistry. Of the Lynch syndrome–associated hyperplastic polyps, only 1 of 23 was MSI-H and none showed abnormal MMR protein expression.

Adenomatous polyps are believed to be the precursor lesions for Lynch syndrome–associated CRCs, 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 Lynch syndrome–associated adenomatous polyps have used various criteria for defining Lynch syndrome and have reported rates of MSI-H and abnormal immunohistochemistry ranging from 33% to 89% and 25% to 82%, respectively (27–34).

Our findings add to the existing literature by showing a significant association between the likelihood of an MMR-deficient phenotype and increasing polyp size in Lynch syndrome–associated adenomatous polyps. Although the overall prevalence of both MSI-H and deficient MMR protein expression by immunohistochemistry in our study was lower than in prior reports (27, 34), the comparatively small size of the adenomatous polyps (mean size, 5.0 mm) removed from subjects undergoing intensive colonoscopic surveillance may account for this difference. Our observation that adenomatous polyps 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 showed 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 2 tests. Furthermore, ours is the first study of its kind to include an extensive analysis of hyperplastic polyps from subjects with genetically confirmed Lynch syndrome.

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 adenomatous polyps 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 Lynch syndrome–associated neoplasia. Our study only included subjects with confirmed Lynch syndrome and thus does not provide any information about the use of MSI and MMR IHC testing of unselected colorectal polyps, although prior data suggest that the rate of MMR deficiency in sporadic adenomatous polyps is extremely low (<2%; refs. 37–39). Because we did not include polyps from non–Lynch syndrome 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 Lynch syndrome.

Our findings have diagnostic implications in that they suggest that MMR IHC testing could provide useful information in the evaluation of patients with suspected Lynch syndrome 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 Lynch syndrome 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 Lynch syndrome.

We believe that our findings also provide important insight into the biology of Lynch syndrome–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 Lynch syndrome–associated adenomatous polyps in our study showed an MMR-deficient phenotype—a phenomenon that requires biallelic inactivation of MMR gene function. We would thus hypothesize that the early steps in Lynch syndrome–associated colorectal neoplasia are likely similar to those in sporadic adenomatous polyp 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 adenomatous polyps, along with the well-described high rates of MMR deficiency in Lynch syndrome–associated CRCs (1, 2, 15) would suggest that the "second hit" somatic loss of MMR activity is a relatively late event in Lynch syndrome–associated neoplasia.

The observation that MSI analysis is even less sensitive than DNA MMR immunohistochemistry in small (<8 mm) Lynch syndrome–associated adenomatous polyps 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*; refs. 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) Lynch syndrome–associated adenomatous polyps show a phenotype of deficient DNA MMR function at a frequency comparable with what is seen

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

Disclosure of Potential Conflicts of Interest

S. Syngal reports having served as a consultant for Archimedes, Inc. No potential conflicts of interests were disclosed by other authors.

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