Modern Pathology (2020) 33:1172–1181
https://doi.org/10.1038/s41379-020-0455-x

ARTICLE

Frequent loss of mutation-specific mismatch repair protein expression in nonneoplastic endometrium of Lynch syndrome patients

Serena Wong[1] · Pei Hui[1] · Natalia Buza[1]

Received: 22 October 2019 / Revised: 12 December 2019 / Accepted: 1 January 2020 / Published online: 13 January 2020

Abstract

Lynch syndrome is most often caused by a germline mutation in one of four DNA mismatch repair (MMR) genes (*MLH1, PMS2, MSH2*, or *MSH6*) or *EPCAM* and is associated with a significantly increased risk of endometrial cancer in affected women. Although universal screening of endometrial cancer for Lynch syndrome is becoming increasingly common by various algorithms using MMR immunohistochemistry and/or microsatellite instability testing by PCR, establishing the diagnosis of Lynch syndrome can be still challenging. MMR-deficient nonneoplastic colonic crypts have been recently described in Lynch syndrome patients with colorectal carcinoma, and have been proposed to be a novel indicator of Lynch syndrome. Presence of MMR-deficient nonneoplastic endometrial glands have not yet been systematically evaluated in Lynch syndrome patients. We performed MMR protein immunohistochemistry in prophylactic hysterectomies and endometrial curettings/biopsies from 27 patients with known Lynch syndrome confirmed by germline mutation analysis. A total of 56 control benign endometrial tissues were also analyzed, and included benign endometrium adjacent to MMR-deficient sporadic (MLH1 promoter hypermethylated) endometrial carcinoma ($n = 9$), adjacent to MMR-intact sporadic endometrial carcinoma ($n = 27$), and normal endometrium from hysterectomies performed for benign disease ($n = 20$). MMR protein deficient nonneoplastic endometrial glands were identified in 70% (19 of 27) of Lynch syndrome patients. In all 19 cases the MMR protein loss was specific for the patients' known germline mutation. None of the control cases showed loss of MMR protein expression in nonneoplastic endometrium. Our findings suggest that MMR-deficient nonneoplastic endometrial glands may be a unique, specific marker of Lynch syndrome, and may provide an important insight into the pathogenesis of Lynch syndrome-associated endometrial cancer. Evaluation of MMR protein expression of benign background endometrium in endometrial cancer patients may be further explored as a possible useful addition to the Lynch syndrome screening algorithm.

Introduction

Lynch syndrome, an autosomal dominant hereditary cancer syndrome, most often results from a germline mutation in one of the four DNA mismatch repair (MMR) genes: *MLH1, PMS2, MSH2*, or *MSH6*. In rare instances, it may also be caused by a germline mutation in the *EPCAM* gene, which leads to inactivation of MSH2 protein [1, 2]. Inheriting any of these mutations is associated with an increased risk of developing certain types of cancer, and usually with an earlier age of onset compared with sporadic tumors [1, 3–6]. The two most common tumor types are colon (53–82% lifetime risk) and endometrial cancer (25–60% lifetime risk), with endometrial cancer presenting as the first malignancy in ~50% of women with Lynch syndrome [1, 3]. Identification of patients with Lynch syndrome is critical as these individuals and their family members may benefit from genetic counseling and appropriate surveillance for cancer prevention or early detection [1, 3–6].

Currently, universal screening for Lynch syndrome has been adopted at many institutions for all patients with endometrial cancer. Testing algorithms may include MMR immunohistochemistry for MLH1, PMS2, MSH2, and

✉ Natalia Buza
natalia.buza@yale.edu

[1] Department of Pathology, Yale University School of Medicine, 310 Cedar Street LH 108, PO Box 208023, New Haven, CT 06520-8023, USA

MSH6 expression, and/or PCR testing for microsatellite instability (MSI) in tumoral tissue, followed by genetic counseling and germline genetic testing of selected patients. However, establishing the diagnosis of Lynch syndrome can be still challenging, as up to half of endometrial or colon cancer cases with MMR protein loss and absence of MLH1 promoter hypermethylation have no detectable pathogenic germline mutation in the MMR genes or *EPCAM*. This group of patients has been termed having "Lynch-like syndrome" and their appropriate clinical management remains problematic [4]. Sequencing of tumor DNA has been recently shown to resolve this uncertainty in up to 70% of such cases by identifying somatic mutations in MMR genes—two pathogenic mutations, or one pathogenic mutation with loss of heterozygosity [7, 8].

Recent studies identified loss of MMR protein expression specific to the known germline mutation in normal nonneoplastic colonic crypts of Lynch syndrome patients [9, 10]. In addition, the same finding was observed in one patient with "Lynch-like syndrome," suggesting that presence of MMR-deficient crypt foci may be a novel indicator of Lynch syndrome [10]. However, this phenomenon has not yet been systematically evaluated in endometrial tissues. We present, to our knowledge, the first comprehensive analysis of MMR protein expression pattern in correlation with the germline MMR gene mutations in nonneoplastic endometrium of Lynch syndrome patients.

Methods

Patients with known Lynch syndrome and available normal endometrial tissues were identified retrospectively in our departmental archives. Clinical information and results of the germline genetic testing were collected from the patients' medical records. Additional cases were identified to serve as controls in three groups: (1) normal endometrial tissue adjacent to sporadic MMR-intact endometrial carcinoma; (2) normal endometrial tissue adjacent to sporadic MMR-deficient endometrial carcinoma with MLH1 promoter hypermethylation (confirmed by methylation specific multiplex PCR); and (3) normal endometrial tissue from patients undergoing hysterectomy for benign indications without clinical suspicion of Lynch syndrome. Hematoxylin–eosin stained slides of the endometrial specimens were retrieved from the archives and were reviewed to select a block containing the most amount of benign endometrial glandular epithelium.

Four MMR immunostains were performed on every Lynch syndrome and control case using primary monoclonal antibodies against MLH1 (clone M1, Ventana, Tucson, AZ), PMS2 (clone EPR3947, Cell Marque, Rocklin, CA), MSH2 (clone G219-1129, Ventana), and MSH6 (clone 44, Ventana), according to the manufacturers' instructions. The immunostained slides were reviewed by at least two of the authors and assessed for presence of background internal positive control and any loss of nuclear expression in the benign endometrial glands (in all Lynch syndrome and control cases) and MMR expression pattern within the endometrial carcinoma (in one Lynch syndrome case and in control groups 1 and 2). In Lynch syndrome cases with no loss of MMR staining on the initial block, two additional tissue blocks were selected and stained for the MMR protein corresponding to the patient's known germline mutation.

Fisher's exact test and Student's t test were used for statistical analysis and p values of <0.05 were considered statistically significant.

Results

We identified 27 female patients with a known diagnosis of Lynch syndrome who had undergone hysterectomy ($n = 19$) or endometrial curettage/biopsy ($n = 8$). The diagnosis of Lynch syndrome was established by germline mutation testing: most patients harbored a pathogenic mutation in *MSH2* (12 of 27, 44%), followed by *MSH6* (7 of 27, 26%), *PMS2* (6 of 27, 22%), and *MLH1* (2 of 27, 7%). The patients' age at the time of procedure ranged between 31 and 61 years (mean: 45.6 years) (Table 1). Five patients (19%) had a personal history of gastrointestinal cancer, four of them of the colorectum and one of the ampulla. In one patient the hysterectomy was performed for a known diagnosis of endometrioid endometrial adenocarcinoma, while the other 18 hysterectomies were prophylactic, two of which harbored incidental complex atypical endometrial hyperplasia. All endometrial curettings/biopsies were performed as part of a routine surveillance and showed benign endometrial tissue. The patient with known endometrial carcinoma and the two patients with incidental complex atypical hyperplasia all displayed the expected pattern of MMR protein loss in the lesional tissue corresponding to their germline mutations (PMS2 in the patient with endometrial carcinoma, and MSH2 in both patients with atypical hyperplasia).

Loss of MMR protein expression was seen in singles or clusters of morphologically normal, nonneoplastic endometrial glands on the initial tissue sections in 15 of the 27 Lynch syndrome cases (56%) (Table 2, cases #1–15). Of the remaining 12 cases, additional tissue blocks were available in 10 cases (all hysterectomy specimens), in which two additional tissue blocks were selected for each case and stained for only the MMR protein corresponding to the patient's known germline mutation. Staining of additional tissue blocks increased the yield of detection of MMR-

Table 1 Mismatch repair protein expression in nonneoplastic endometrial glands by immunohistochemistry.

		Lynch syndrome	Sporadic MMR-intact endometrial cancer	Sporadic MMR-deficient endometrial cancer with MLH1 promoter hypermethylation	Hysterectomy for benign disease
Total number of cases		$N = 27$	$N = 27$	$N = 9$	$N = 20$
Patient age, range (mean)		31–67 years (45.6)	49–78 years (63.9)	40–85 years (67.2)	30–61 years (44.9)
Specimen type:	EMC/EMB	8	0	0	0
	Hysterectomy	19	27	9	20
MMR IHC in benign endometrial glands	Intact	8 (30%)	27 (100%)	9 (100%)	20 (100%)
	Loss	19 (70%) MLH1 and PMS2 ($n = 2$) PMS2 ($n = 4$) MSH2 and MSH6 ($n = 10$)[a] MSH6 ($n = 3$)	0	0	0

MMR mismatch repair, *IHC* immunohistochemistry, *EMC* endometrial curettage, *EMB* endometrial biopsy

[a]Only MSH2 was performed on additional sections in one case

Table 2 Lynch syndrome patients with loss of mismatch repair protein expression in benign endometrial glands by immunohistochemistry.

Case #	Age (years)	Specimen type	Endometrial phase	Germline pathogenic MMR gene mutation	Specific mutation	Loss of MMR protein expression by IHC	Pattern of loss in benign endometrial glands
1	47	Hysterectomy	Proliferative	*MLH1*	Unknown	MLH1 and PMS2	Cluster of glands
2	39	Hysterectomy	Secretory	*MLH1*	Unknown	MLH1 and PMS2	Single gland
3	61	Hysterectomy	Inactive	*PMS2*	*p.K766*	PMS2	Cluster of glands
4	48	EMB	Secretory	*PMS2*	Unknown	PMS2	Cluster of glands
5	40	Hysterectomy	Proliferative	*MSH2*	*c.1147C > T (p.Arg383Ter)*	MSH2 and MSH6	Cluster of glands
6	36	Hysterectomy	Proliferative	*MSH2*	*c.1147C > T (p.Arg383Ter)*	MSH2 and MSH6	Single gland
7	46	Hysterectomy	Secretory	*MSH2*	Unknown	MSH2 and MSH6	Cluster of glands
8	44	Hysterectomy	CAH, Background proliferative	*MSH2*	*c.942 + 2T > A*	MSH2 and MSH6	Cluster of glands (and in CAH)
9	41	Hysterectomy	CAH, Background proliferative	*MSH2*	*c.1023delT*	MSH2 and MSH6	Cluster of glands (and in CAH)
10	31	EMC	Proliferative	*MSH2*	*c.1023delT*	MSH2 and MSH6	Cluster of glands
11	38	EMC	Secretory	*MSH2*	*c.1042delC*	MSH2 and MSH6	Cluster of glands
12	41	EMC	Interval	*MSH2*	*5′UTR_EX2del*	MSH2 and MSH6	Single gland
13	34	EMB	Secretory	*MSH2*	*c.942 + 2T > A*	MSH2 and MSH6	Cluster of glands
14	54	Hysterectomy	Inactive	*MSH6*	*c.3261dupC*	MSH6	Single gland
15	42	EMB	Proliferative	*MSH6*	Unknown	MSH6	Cluster of glands
16	44	Hysterectomy	Weakly Proliferative	*PMS2*	*c.137G > T (p.Ser46Ile)*	PMS2	Single gland
17	52	Hysterectomy	Endometrial carcinoma	*PMS2*	*c.137G > T (p.Ser46Ile)*	PMS2	Cluster of glands (in LUS)
18	55	Hysterectomy	Atrophic	*MSH2*	Unknown	MSH2[a]	Cluster of glands
19	40	Hysterectomy	Inactive	*MSH6*	3261insC	MSH6	Single gland

Loss of MMR protein expression was only identified on additional sections in cases #16–19

MMR mismatch repair, *IHC* immunohistochemistry, *EMB* endometrial biopsy, *EMC* endometrial curettage, *CAH* complex atypical hyperplasia, *LUS* lower uterine segment

[a]Only MSH2 immunostain was performed on additional sections showing loss of expression

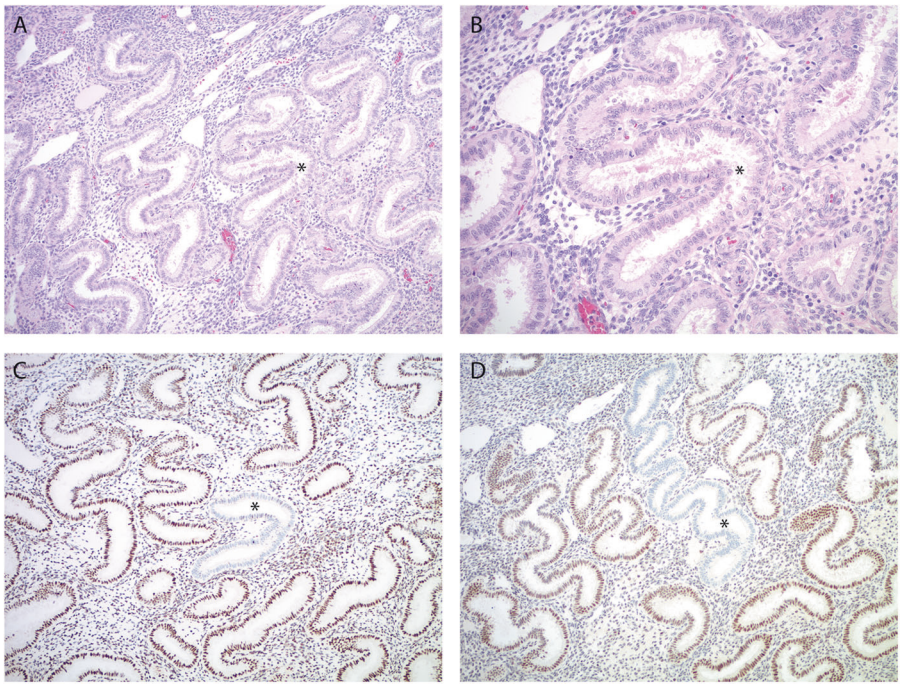

Fig. 1 Lynch syndrome case #2 (Table 2), *MLH1* germline mutation. Prophylactic hysterectomy with morphologically unremarkable secretory endometrium (**a**, **b**). A single gland (marked with * on all panels) shows loss of both MLH1 (**c**) and PMS2 (**d**) expression. (**a**, **c**, **d**: original magnification ×100, **b**: original magnification ×200).

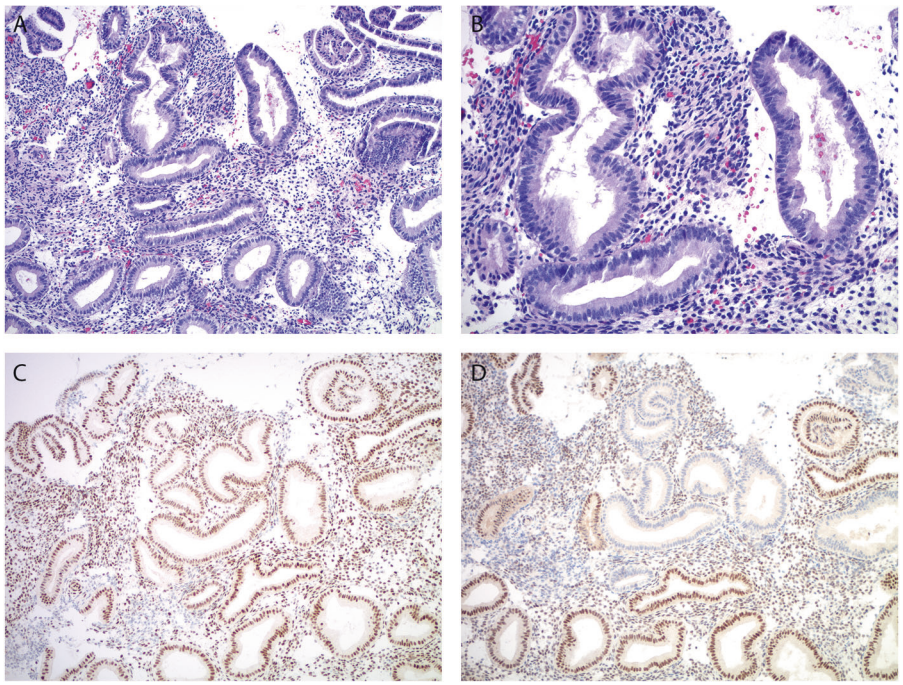

Fig. 2 Lynch syndrome case #4 (Table 2), *PMS2* germline mutation. Routine surveillance endometrial biopsy shows early secretory phase endometrium (**a**, **b**). A cluster of glands is identified with retained MLH1 (**c**) and loss of PMS2 (**d**) expression. (**a**, **c**, **d**: original magnification ×100, **b**: original magnification ×200).

deficient benign endometrial glands to 19 of 27 (70%) Lynch syndrome cases (Tables 1 and 2). Loss of MMR protein expression was specific to the known germline mutation in each patient: two cases of *MLH1* mutation (loss of MLH1 and PMS2 expression), four cases of *PMS2* mutation (loss of PMS2 expression), ten cases of *MSH2* mutation (loss of MSH2 and MSH6 expression), and three cases of *MSH6* mutation (loss of MSH6 expression) (Figs. 1–4). In cases with mutations in *MLH1* or *MSH2*, loss of staining within the same glands were also seen in their paired heterodimers, PMS2 or MSH6, respectively (Figs. 1 and 3). In one case (case #11, Table 2), MMR IHC was performed on both the endometrial curettage and subsequent hysterectomy specimen, and identical loss of MSH2 and MSH6 staining was seen in clusters of benign endometrial glands in both specimens. The two Lynch syndrome cases with atypical hyperplasia (cases #8 and #9 in Table 2) both displayed loss of MMR immunostaining in the area of

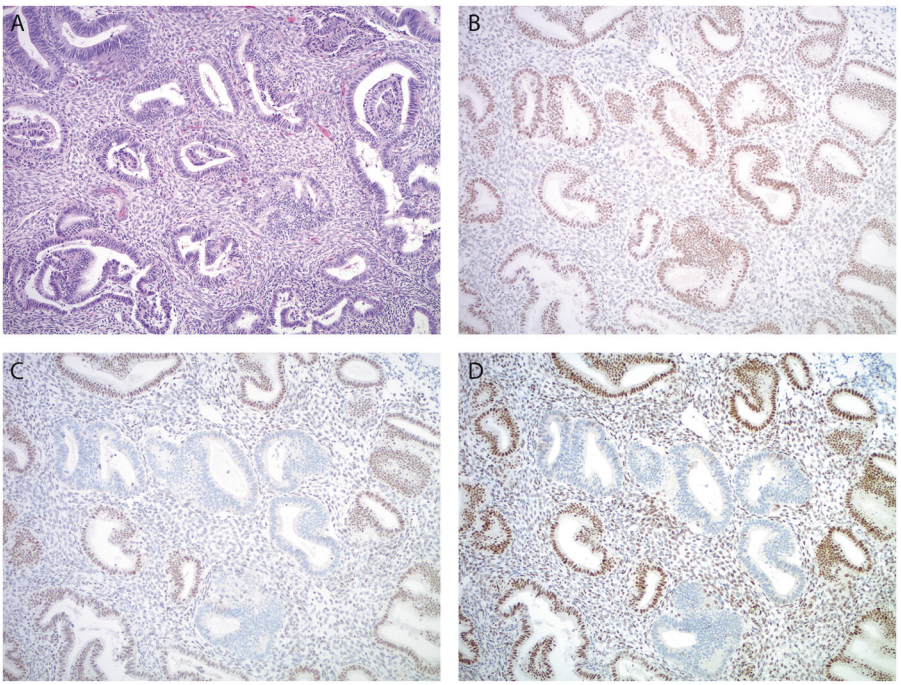

Fig. 3 Lynch syndrome case #11 (Table 2), *MSH2* germline mutation. Routine surveillance endometrial curettage shows secretory phase endometrium (**a**). PMS2 expression is retained (**b**), while paired loss of MSH2 (**c**) and MSH6 expression (**d**) is seen in the same cluster of glands. (All images at original magnification ×100).

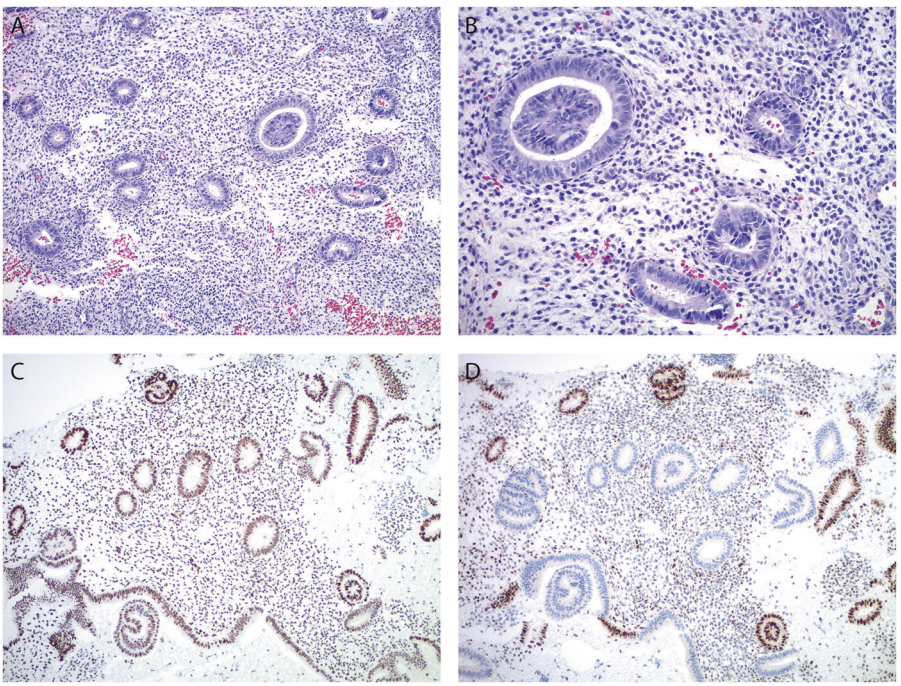

Fig. 4 Lynch syndrome case #15 (Table 2), *MSH6* germline mutation. Routine surveillance endometrial biopsy shows proliferative phase endometrium (**a**, **b**). MSH2 expression is retained (**c**) while loss of MSH6 expression (**d**) is noted in the same cluster of benign glands. (**a**, **c**, **d**: original magnification ×100, **b**: original magnification ×200).

hyperplasia and in clusters of benign endometrial glands immediately adjacent to and also several low magnification fields away from the hyperplastic glands. The only one Lynch syndrome case with endometrial adenocarcinoma (case #17 in Table 2) did not show loss of MMR expression in nonneoplastic glands on the initial sections. However, one of the two additional nonneoplastic sections—from the lower uterine segment, away from the endometrial carcinoma—showed loss of PMS2 immunostaining. The loss of MMR expression involved single glands in six cases and clusters of glands in 13 cases. No statistically significant correlation was observed between the two groups of Lynch syndrome patients (MMR intact versus MMR loss) with regards to the affected germline MMR genes or the patients' age ($p > 0.05$) (Table 3).

A total of 56 cases comprised the control group: 27 sporadic MMR-intact endometrial carcinomas (18 FIGO grade 1 or 2 endometrioid carcinomas, 3 mixed

Table 3 Mismatch repair protein expression pattern in nonneoplastic endometrium in Lynch syndrome cases.

	Lynch syndrome with loss of MMR expression in nonneoplastic endometrium, *n*	Lynch syndrome with intact MMR expression in nonneoplastic endometrium, *n*	*p* value
Number of cases	$n = 19$	$n = 8$	NA
Patient age, range (mean)	31–61 years (43.8)	32–67 years (49.8)	0.1289
Pathogenic germline mutation: total number of cases, $n = 27$			
MLH1, $n = 2$	2	0	0.280
PMS2, $n = 6$	4	2	
MSH2, $n = 12$	10	2	
MSH6, $n = 7$	3	4	

MMR mismatch repair, *NA* not applicable

endometrioid and serous carcinomas, 4 serous carcinomas, 1 clear cell carcinoma, and 1 carcinosarcoma), 9 sporadic MMR-deficient endometrial carcinomas with loss of MLH1 and PMS2 expression and MLH1 promoter hypermethylation (8 FIGO grade 1 or 2 endometrioid carcinoma, 1 dedifferentiated carcinoma), and 20 hysterectomy specimens for benign indications (e.g., leiomyomas or adenomyosis) (Table 1). All cases in the three control groups showed retained (intact) expression of MMR markers in benign endometrial glands.

In addition, we identified two patients harboring germline variants of unknown significance (VUS) in MMR genes *MSH2 c.2293G > A (p.A7657)* and *PMS2 c.2149G > A (p.V717M)*. Neither one of the patients had a personal history of colon cancer. However, both patients had family history of ovarian and breast cancer, and underwent prophylactic hysterectomy due to the perceived uncertainty regarding the risk of endometrial cancer and appropriate clinical management. No histologic evidence of atypical hyperplasia or carcinoma was identified and MMR immunohistochemistry showed no loss of expression in benign endometrial glandular epithelium in either cases.

Discussion

Our study demonstrated loss of germline mutation-specific MMR protein expression by immunohistochemistry in nonneoplastic endometrial glands in 70% of patients with Lynch syndrome. In contrast, all normal endometria in the three control groups—adjacent to MMR-intact, or MMR-deficient MLH1 hypermethylated sporadic endometrial carcinoma, and in benign hysterectomy specimens—showed retained, intact staining patterns with all four MMR markers. These findings may provide important insights into the pathogenesis of Lynch syndrome-associated endometrial cancer, and also raise the possibility that MMR protein expression analysis of preneoplastic or nonneoplastic tissues may be useful in separating true Lynch syndrome from sporadic "Lynch-like" cases.

In the gastrointestinal tract, loss of MMR protein expression has been recently reported in 25–35% of nonneoplastic colonic and small bowel crypts of Lynch syndrome patients, a subset of which also demonstrated MSI by PCR [9–11]. In addition, one of the patients with "Lynch-like syndrome" also showed loss of MSH2 staining in nonneoplastic colonic crypts [10]. The sensitivity of detection was increased by deeper level sectioning of tissue blocks to evaluate additional mucosal surface area [9–11]. MMR deficiency—by either IHC, PCR, or both—has also been identified by other studies in over 70% of colonic adenomas from Lynch syndrome patients [12–14].

The literature is more limited on the prevalence and potential significance of MMR deficiency in precancerous lesions of the endometrium. Identical loss of MMR protein expression by IHC and/or MSI by PCR have been described in areas of endometrial hyperplasia (both with and without atypia) adjacent to endometrial carcinoma in a small number of Lynch syndrome patients [15–17]. Of note, one of these studies found a higher frequency of MSI and an earlier average age of onset of carcinoma in *MSH2* mutation carriers compared with other MMR gene mutation carriers, raising the possibility that *MSH2* mutation may indicate a more rapid rate of tumor progression [15]. In unselected patient populations in two tissue microarray-based studies MMR protein loss was identified in 4.5% [18] and in 20% [19] of atypical endometrial hyperplasia cases, although the case number was much smaller in the latter study. Nieminen et al. analyzed 110 endometrial samples collected over several years of routine cancer surveillance from 54 women with Lynch syndrome, all of whom subsequently developed endometrial hyperplasia or endometrial carcinoma [20]. MMR IHC was performed only on a subset of their Lynch syndrome endometrial samples ($n = 49$) and showed decreased MMR protein expression corresponding to the patients' known germline mutation in 100% of complex hyperplasia without atypia, in 92% of complex hyperplasia

with atypia, in 40% of simple hyperplasia, and in 7% (2 of 29) of normal endometrial samples [20]. The germline mutation affected *MLH1* in both patients in the latter group, although specific details of the decreased MMR staining patterns were not presented. Most recently Lucas et al. studied MMR protein expression in atypical hyperplasia/endometrioid intraepithelial neoplasia from 63 patients with MMR-deficient endometrial carcinoma, including 14 patients with genetically confirmed Lynch syndrome [21]. Background atypical hyperplasia was present in 8 of 14 Lynch syndrome patients, all of which demonstrated the same loss of MMR protein expression as the corresponding carcinoma. Interestingly, normal-appearing background benign endometrium was also present in seven of their cases, but showed no loss of MMR staining [21].

The details of molecular pathogenesis of endometrial cancer in Lynch syndrome are not yet fully understood. In the gastrointestinal tract, Lynch syndrome is not associated with an increased rate of adenoma formation, and Lynch syndrome-associated colorectal cancer is traditionally thought to arise through accelerated progression of pre-formed (MMR-proficient) adenomas [22]. An alternative pathogenetic pathway has also been recently proposed, suggesting that morphologically normal MMR-deficient colonic crypts could give rise to carcinoma, either directly or through an MMR-deficient adenoma phase [12, 23]. Similarly, it is conceivable that MMR-deficient non-neoplastic endometrial glands may represent the initial step in endometrial carcinogenesis in Lynch syndrome patients either leading to the development of atypical hyperplasia or directly progressing to carcinoma. Prior studies demonstrated that Lynch syndrome-associated endometrial carcinoma coexists with complex atypical hyperplasia in ~40% of cases, similar to the rate observed in sporadic endometrial cancer, supporting that there is a continuum of disease progression through complex atypical hyperplasia in Lynch syndrome patients [24, 25]. Interestingly, however, mutations in *PIK3CA, KRAS*, and *CTNNB1* were found to be less frequent in Lynch syndrome-associated atypical hyperplasia and endometrial carcinoma compared with sporadic cases, while *PTEN* mutations and loss of PTEN expression were seen at a similar frequency between the two groups, suggesting that in the presence of MMR deficiency loss of PTEN function may be sufficient for carcinogenesis [24]. PTEN inactivation has also been observed by immunohistochemistry in up to 43% of histologically normal-appearing proliferative endometria from an unselected patient population, and these PTEN-null glands have been hypothesized to represent the initial phase of a multi-step carcinogenesis [26, 27]. Progression would require accumulation of additional genetic abnormalities and the risk is modulated by both hormonal and nonhormonal mechanisms [26, 28]. The significance of the hormonal milieu, i.e., unopposed estrogen effect, in endometrial carcinogenesis is well known and prior studies have shown significant regression of PTEN-null glands following progestin therapy [26, 29, 30]. The possibility of hormonal inactivation of MMR genes have not been extensively studied, but given the specificity of our results—loss of MMR protein expression matched the patients' known germline mutations, and none of the patients in the control groups displayed MMR loss—it is unlikely to be a significant contributing factor. In addition, loss of MMR protein expression was seen in Lynch syndrome patients with a wide age range (31–61 years) and various menstrual cycle phase (inactive, proliferative, interval, and secretory phase; see Table 2).

Other potential mechanisms of MMR deficiency have also been reported in colorectal cancer and in adjacent precursor lesions. For example, CpG island methylation can result in silencing of tumor suppressor genes and *hMLH1* MMR gene and thereby promote tumorigenesis [31–34]. Promoter methylation was also found to be frequent in sporadic endometrial endometrioid carcinomas and in adjacent histologically normal endometria, although *hMLH1* was not included in these studies [35, 36]. We did not observe loss of MLH1 or other MMR protein expression in our control cohort of normal endometrial tissues (including controls adjacent to sporadic MMR-deficient MLH1 hypermethylated tumors), and the only two Lynch syndrome patients with loss of MLH1 expression in benign glands had known germline *MLH1* mutations. In addition, cell differentiation and cell cycle phase are known to affect MMR gene expression, and MMR protein immunohistochemical expression is generally stronger in actively proliferating tissues compared with inactive ones (e.g., proliferative phase versus atrophic endometrium) [37, 38]. Similarly, we observed that the intensity of MMR immunohistochemical staining in inactive/atrophic endometrial tissues was generally weaker (both in the Lynch syndrome and in the control groups) compared with proliferative or secretory phase endometria. However, no complete loss of staining was identified in any of the control cases and the loss of staining among Lynch syndrome cases was specific to the patient's known germline mutation in each case. Several studies have also described aberrant patterns or loss of MMR expression in colorectal cancer and in other tumor types as a result of prior chemo- and/or radiation therapy [39, 40]. The possibility of treatment-related MMR-alteration can be excluded in our Lynch syndrome cohort, as only one of the patients was diagnosed with endometrial carcinoma and she did not receive chemotherapy or radiation prior to the hysterectomy.

Our findings may also have potential implications for Lynch syndrome screening. Many institutions (including ours) recently adopted universal screening of all newly

diagnosed endometrial cancer cases regardless of the patient's age. Most screening algorithms recommend MMR protein immunohistochemistry (for all four—MLH1, PMS2, MSH2, and MSH6—or for at least two—PMS2 and MSH6—markers) as the initial step, followed by MLH1 promoter methylation analysis in cases with combined loss of MLH1/PMS2 [41, 42]. Tumors with MLH1 promoter hypermethylation are likely sporadic and do not require additional genetic workup unless there is a strong clinical suspicion for Lynch syndrome. Patients with loss of both MSH2 and MSH6, or isolated loss of PMS2 or MSH6 expression in their tumors should be referred to genetic counseling and targeted germline testing. MSI testing by PCR has been reported to have a 90% concordance rate with MMR IHC and has been integrated into the testing algorithm either as an initial step, co-testing with IHC to maximize the detection rate, or as a secondary assay for cases with indeterminate/equivocal MMR staining patterns or intact MMR staining and strong clinical suspicion for Lynch syndrome [43, 44]. Universal screening maximizes the detection of Lynch syndrome in endometrial and colorectal cancer patients but it also presents a significant clinical challenge as over 50% of patients with MMR protein loss and absence of MLH1 promoter hypermethylation lack detectable germline MMR gene or *EPCAM* alterations [4, 41, 45]. A proportion of these cases may harbor somatic MMR gene mutations within the tumor, while the remaining "Lynch-like syndrome" patients may have true Lynch syndrome harboring germline MMR gene alterations that are not yet known or are undetectable by currently available methods [46]. In addition, germline testing may identify MMR VUS. Patients harboring germline MMR gene VUS and those with "Lynch-like syndrome" are faced with uncertainty regarding the need or frequency of lifelong cancer surveillance (both colorectal and endometrial) and screening of their asymptomatic family members for Lynch syndrome.

In this study, we report presence of MMR-deficient, morphologically normal, nonneoplastic endometrial glands in 70% of Lynch syndrome cases. While the sensitivity of this phenomenon for Lynch syndrome is only 70% (30% of cases would be false negative if used for screening purposes), the specificity was 100% as it was not observed in any of the control cases. Our detection rate increased significantly (from 56 to 70%) by staining additional tissue blocks, and it is conceivable that further increase in the number of stained blocks and deeper level sections would result in even higher sensitivity. In contrast to the previous observations in the gastrointestinal tract, which only included resection specimens for known colorectal or small bowel carcinoma [9–11], we were able to demonstrate MMR-deficient benign endometrial glands in both hysterectomy and endometrial curettage/biopsy specimens and all but one of our Lynch syndrome cases were prophylactic surgeries or routine surveillance biopsies with no known endometrial cancer. MMR-deficient benign endometrial glands were present in patients as young as 31 years of age, suggesting that they may precede development of atypical hyperplasia or carcinoma by several months or years. The loss of MMR protein expression was specific to the patients' known germline mutations in all cases, and in patients with *MLH1* or *MSH2* germline mutations identical loss of protein expression was also seen in the paired heterodimers, PMS2 and MSH6, respectively. Although we did not observe a statistically significant correlation between the affected germline MMR gene and presence of MMR-deficient benign endometrial glands, it was most frequently identified in *MSH2* (10 of 12, 83%) and *MLH1* (2 of 2, 100%) mutation carriers.

Interpretation of MMR staining results in the Lynch syndrome group was generally straightforward, as the endometrial glands were in proliferative or secretory phase in most cases and both the glandular epithelium and adjacent stroma showed nuclear staining serving as internal controls. Control cases with adjacent carcinoma were typically from older patients showing inactive/atrophic background endometrium, which often showed only weak nuclear MMR expression. However, staining intensity in glandular epithelium was always compared with internal control (surrounding normal stromal tissue and inflammatory cells), and MMR staining was interpreted only in areas with satisfactory internal control staining.

The distribution of germline MMR gene mutations in our study population is different from the known frequency of those previously described among Lynch syndrome patients. Most prior studies reported *MLH1* and *MSH2* mutations to be the most common, accounting for ~60–80% of all Lynch syndrome cases, while the minority of patients (5–10%) have mutations in *PMS2* or *MSH6* [47–49]. In contrast, the most common germline mutation among our Lynch syndrome cases was in *MSH2* (44%), followed by *MSH6* (26%) and *PMS2* (22%), while only 7% of patients harbored germline *MLH1* mutations. A potential explanation may include a selection bias, as most previously reported mutation frequencies were calculated based on Lynch syndrome patient groups with a known diagnosis of colorectal cancer, while only 19% of our patients had a prior diagnosis of colorectal carcinoma. In addition, our relatively small sample size may have also contributed to these differences. Our study also included two cases with germline VUS, involving MMR genes *MSH2* and *PMS2*, both of which showed intact MMR protein expression in benign endometrial glandular epithelium by immunohistochemistry. We did not identify any "Lynch syndrome-like" patients in our study cohorts; however, future studies would be necessary to determine the significance of our findings in those cases.

In summary, we present a novel finding of frequent loss of germline mutation-specific MMR protein expression in benign, morphologically normal endometrial glands in Lynch syndrome patients. We hypothesize, that presence of MMR-deficient nonneoplastic endometrial glands may represent an early detectable marker of Lynch syndrome, and may be further explored as a potentially useful screening tool in endometrial curettings/biopsies from patients with suspected Lynch syndrome. Furthermore, this observation may also have significant pathogenetic implications, raising the possibility—at least in a subset of Lynch syndrome patients—of endometrial carcinogenesis directly from morphologically normal glands without a stepwise progression through a preneoplastic (atypical hyperplasia) phase.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res. 2006;66:7810–7.
2. Lynch HT, Boland CR, Gong G, Shaw TG, Lynch PM, Fodde R, et al. Phenotypic and genotypic heterogeneity in the Lynch syndrome: diagnostic, surveillance and management implications. Eur J Hum Genet. 2006;14:390–402.
3. Buza N, Ziai J, Hui P. Mismatch repair deficiency testing in clinical practice. Expert Rev Mol Diagn. 2016;16:591–604.
4. Mills AM, Sloan EA, Thomas M, Modesitt SC, Stoler MH, Atkins KA, et al. Clinicopathologic comparison of Lynch syndrome-associated and "Lynch-like" endometrial carcinomas identified on universal screening using mismatch repair protein immunohistochemistry. Am J Surg Pathol. 2016;40:155–65.
5. Ryan NAJ, Blake D, Cabrera-Dandy M, Glaire MA, Evans DG, Crosbie EJ. The prevalence of Lynch syndrome in women with endometrial cancer: a systematic review protocol. Syst Rev. 2018;7:121.
6. Stoffel E, Mukherjee B, Raymond VM, Tayob N, Kastrinos F, Sparr J, et al. Calculation of risk of colorectal and endometrial cancer among patients with Lynch syndrome. Gastroenterology. 2009;137:1621–7.
7. Haraldsdottir S, Hampel H, Tomsic J, Frankel WL, Pearlman R, de la Chapelle A, et al. Colon and endometrial cancers with mismatch repair deficiency can arise from somatic, rather than germline, mutations. Gastroenterology. 2014;147:1308–16.e1301
8. Geurts-Giele WR, Leenen CH, Dubbink HJ, Meijssen IC, Post E, Sleddens HF, et al. Somatic aberrations of mismatch repair genes as a cause of microsatellite-unstable cancers. J Pathol. 2014;234:548–59.
9. Kloor M, Huth C, Voigt AY, Benner A, Schirmacher P, von Knebel Doeberitz M, et al. Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study. Lancet Oncol. 2012;13:598–606.
10. Pai RK, Dudley B, Karloski E, Brand RE, O'Callaghan N, Rosty C, et al. DNA mismatch repair protein deficient non-neoplastic colonic crypts: a novel indicator of Lynch syndrome. Mod Pathol. 2018;31:1608–18.
11. Staffa L, Echterdiek F, Nelius N, Benner A, Werft W, Lahrmann B, et al. Mismatch repair-deficient crypt foci in Lynch syndrome–molecular alterations and association with clinical parameters. PLoS ONE. 2015;10:e0121980.
12. Ahadova A, Gallon R, Gebert J, Ballhausen A, Endris V, Kirchner M, et al. Three molecular pathways model colorectal carcinogenesis in Lynch syndrome. Int J Cancer. 2018;143:139–50.
13. Tanaka M, Nakajima T, Sugano K, Yoshida T, Taniguchi H, Kanemitsu Y, et al. Mismatch repair deficiency in Lynch syndrome-associated colorectal adenomas is more prevalent in older patients. Histopathology. 2016;69:322–8.
14. Walsh MD, Buchanan DD, Pearson SA, Clendenning M, Jenkins MA, Win AK, et al. Immunohistochemical testing of conventional adenomas for loss of expression of mismatch repair proteins in Lynch syndrome mutation carriers: a case series from the Australasian site of the colon cancer family registry. Mod Pathol. 2012;25:722–30.
15. de Leeuw WJ, Dierssen J, Vasen HF, Wijnen JT, Kenter GG, Meijers-Heijboer H, et al. Prediction of a mismatch repair gene defect by microsatellite instability and immunohistochemical analysis in endometrial tumours from HNPCC patients. J Pathol. 2000;192:328–35.
16. Ichikawa Y, Tsunoda H, Takano K, Oki A, Yoshikawa H. Microsatellite instability and immunohistochemical analysis of MLH1 and MSH2 in normal endometrium, endometrial hyperplasia and endometrial cancer from a hereditary nonpolyposis colorectal cancer patient. Jpn J Clin Oncol. 2002;32:110–2.
17. Berends MJ, Hollema H, Wu Y, van Der Sluis T, Mensink RG, ten Hoor KA, et al. MLH1 and MSH2 protein expression as a prescreening marker in hereditary and non-hereditary endometrial hyperplasia and cancer. Int J Cancer. 2001;92:398–403.
18. Vierkoetter KR, Kagami LA, Ahn HJ, Shimizu DM, Terada KY. Loss of mismatch repair protein expression in unselected endometrial adenocarcinoma precursor lesions. Int J Gynecol Cancer. 2016;26:228–32.
19. Hardisson D, Moreno-Bueno G, Sanchez L, Sarrio D, Suarez A, Calero F, et al. Tissue microarray immunohistochemical expression analysis of mismatch repair (hMLH1 and hMSH2 genes) in endometrial carcinoma and atypical endometrial hyperplasia: relationship with microsatellite instability. Mod Pathol. 2003;16:1148–58.
20. Nieminen TT, Gylling A, Abdel-Rahman WM, Nuorva K, Aarnio M, Renkonen-Sinisalo L, et al. Molecular analysis of endometrial tumorigenesis: importance of complex hyperplasia regardless of atypia. Clin Cancer Res. 2009;15:5772–83.
21. Lucas E, Chen H, Molberg K, Castrillon DH, Rivera Colon G, Li L, et al. Mismatch repair protein expression in endometrioid intraepithelial neoplasia/atypical hyperplasia: should we screen for Lynch Syndrome in precancerous lesions? Int J Gynecol Pathol. 2019;38:533–42.
22. Lynch HT, Snyder CL, Shaw TG, Heinen CD, Hitchins MP. Milestones of Lynch syndrome: 1895-2015. Nat Rev Cancer. 2015;15:181–94.
23. Shia J, Stadler ZK, Weiser MR, Vakiani E, Mendelsohn R, Markowitz AJ, et al. Mismatch repair deficient-crypts in non-neoplastic colonic mucosa in Lynch syndrome: insights from an illustrative case. Fam Cancer. 2015;14:61–68.

24. Huang M, Djordjevic B, Yates MS, Urbauer D, Sun C, Burzawa J, et al. Molecular pathogenesis of endometrial cancers in patients with Lynch syndrome. Cancer. 2013;119:3027–33.
25. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15:10–17.
26. Lin MC, Burkholder KA, Viswanathan AN, Neuberg D, Mutter GL. Involution of latent endometrial precancers by hormonal and nonhormonal mechanisms. Cancer. 2009;115:2111–8.
27. Mutter GL, Ince TA, Baak JP, Kust GA, Zhou XP, Eng C. Molecular identification of latent precancers in histologically normal endometrium. Cancer Res. 2001;61:4311–4.
28. Mutter GL, Monte NM, Neuberg D, Ferenczy A, Eng C. Emergence, involution, and progression to carcinoma of mutant clones in normal endometrial tissues. Cancer Res. 2014;74:2796–802.
29. Orbo A, Rise CE, Mutter GL. Regression of latent endometrial precancers by progestin infiltrated intrauterine device. Cancer Res. 2006;66:5613–7.
30. Zheng W, Baker HE, Mutter GL. Involution of PTEN-null endometrial glands with progestin therapy. Gynecol Oncol. 2004;92:1008–13.
31. Chan AO, Broaddus RR, Houlihan PS, Issa JP, Hamilton SR, Rashid A. CpG island methylation in aberrant crypt foci of the colorectum. Am J Pathol. 2002;160:1823–30.
32. Ahuja N, Li Q, Mohan AL, Baylin SB, Issa JP. Aging and DNA methylation in colorectal mucosa and cancer. Cancer Res. 1998; 58:5489–94.
33. Issa JP. CpG island methylator phenotype in cancer. Nat Rev Cancer. 2004;4:988–93.
34. Toyota M, Ahuja N, Ohe-Toyota M, Herman JG, Baylin SB, Issa JP. CpG island methylator phenotype in colorectal cancer. Proc Natl Acad Sci USA. 1999;96:8681–6.
35. Arafa M, Kridelka F, Mathias V, Vanbellinghen JF, Renard I, Foidart JM, et al. High frequency of RASSF1A and RARb2 gene promoter methylation in morphologically normal endometrium adjacent to endometrioid adenocarcinoma. Histopathology. 2008; 53:525–32.
36. Zhang QY, Yi DQ, Zhou L, Zhang DH, Zhou TM. Status and significance of CpG island methylator phenotype in endometrial cancer. Gynecol Obstet Investig. 2011;72:183–91.
37. Mjelle R, Hegre SA, Aas PA, Slupphaug G, Drablos F, Saetrom P, et al. Cell cycle regulation of human DNA repair and chromatin remodeling genes. DNA Repair. 2015;30:53–67.
38. Roos WP, Christmann M, Fraser ST, Kaina B. Mouse embryonic stem cells are hypersensitive to apoptosis triggered by the DNA damage O(6)-methylguanine due to high E2F1 regulated mismatch repair. Cell Death Differ. 2007;14:1422–32.
39. Radu OM, Nikiforova MN, Farkas LM, Krasinskas AM. Challenging cases encountered in colorectal cancer screening for Lynch syndrome reveal novel findings: nucleolar MSH6 staining and impact of prior chemoradiation therapy. Hum Pathol. 2011;42:1247–58.
40. Cahill DP, Levine KK, Betensky RA, Codd PJ, Romany CA, Reavie LB, et al. Loss of the mismatch repair protein MSH6 in human glioblastomas is associated with tumor progression during temozolomide treatment. Clin Cancer Res. 2007;13:2038–45.
41. Mills AM, Longacre TA. Lynch syndrome screening in the gynecologic tract: current state of the art. Am J Surg Pathol. 2016;40:e35–44.
42. Moline J, Mahdi H, Yang B, Biscotti C, Roma AA, Heald B, et al. Implementation of tumor testing for lynch syndrome in endometrial cancers at a large academic medical center. Gynecol Oncol. 2013;130:121–6.
43. McConechy MK, Talhouk A, Li-Chang HH, Leung S, Huntsman DG, Gilks CB, et al. Detection of DNA mismatch repair (MMR) deficiencies by immunohistochemistry can effectively diagnose the microsatellite instability (MSI) phenotype in endometrial carcinomas. Gynecol Oncol. 2015;137:306–10.
44. Wu X, Snir O, Rottmann D, Wong S, Buza N, Hui P. Minimal microsatellite shift in microsatellite instability high endometrial cancer: a significant pitfall in diagnostic interpretation. Mod Pathol. 2019;32:650–8.
45. Mas-Moya J, Dudley B, Brand RE, Thull D, Bahary N, Nikiforova MN, et al. Clinicopathological comparison of colorectal and endometrial carcinomas in patients with Lynch-like syndrome versus patients with Lynch syndrome. Hum Pathol. 2015;46:1616–25.
46. Carethers JM. Differentiating Lynch-like from Lynch syndrome. Gastroenterology. 2014;146:602–4.
47. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N. Engl J Med. 2005;352: 1851–60.
48. Yurgelun MB, Kulke MH, Fuchs CS, Allen BA, Uno H, Hornick JL, et al. Cancer susceptibility gene mutations in individuals with colorectal cancer. J Clin Oncol. 2017;35:1086–95.
49. Moreira L, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, et al. Identification of Lynch syndrome among patients with colorectal cancer. JAMA. 2012;308:1555–65.