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Risk of Cancer in Cases of Suspected Lynch Syndrome Without Germline Mutation

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This article has an accompanying continuing medical education activity on page e13. Learning Objective: Upon completion of this CME activity, successful learners will be able to assess the different diagnostic tests to establish a diagnosis of Lynch syndrome.

See editorial on page 868.

BACKGROUND & AIMS: Colorectal cancers (CRCs) with microsatellite instability (MSI) and a mismatch repair (MMR) immunohistochemical deficit without hypermethylation of the *MLH1* promoter are likely to be caused by Lynch syndrome. Some patients with these cancers have not been found to have pathogenic germline mutations and are considered to have Lynch-like syndrome (LLS). The aim of this study was to determine the risk of cancer in families of patients with LLS. **METHODS:** We studied a population-based cohort of 1705 consecutive patients, performing MSI tests and immunohistochemical analyses of MMR proteins. Patients were diagnosed with Lynch syndrome when they were found to have pathogenic germline mutations. Patients with MSI and loss of MSH2 and/or MSH6 expression, isolated loss of PMS2 or loss of MLH1 without *MLH1* promoter hypermethylation, and no pathogenic mutation were considered to have LLS. The clinical characteristics of patients and the age- and sex-adjusted standardized incidence ratios (SIRs) of cancer in families were compared between groups. **RESULTS:** The incidence of CRC was significantly lower in families of patients with LLS than in families with confirmed cases of Lynch syndrome (SIR for Lynch syndrome, 6.04; 95% confidence interval [CI], 3.58–9.54; SIR for LLS, 2.12; 95% CI, 1.16–3.56; $P < .001$). However, the incidence of CRC was higher in families of patients with LLS than in families with sporadic CRC (SIR for sporadic CRC, 0.48; 95% CI, 0.27–0.79; $P < .001$). **CONCLUSIONS: The risk of cancer in families with LLS is lower that of families with Lynch syndrome but higher than that of families with sporadic CRC. These results confirm the need for special screening and surveillance strategies for these patients and their relatives.**

Keywords: Inherited Colon Cancer; Cancer Risk; Genetic Testing; Immunohistochemistry.

Lynch syndrome (LS) is the most common inherited colon cancer susceptibility syndrome and is caused by germline mutations in one of several DNA mismatch repair (MMR) genes, mainly *MLH1* and *MSH2* but also *MSH6* and *PMS2*.[1–3] Patients with LS have an increased risk of colorectal cancer (CRC), endometrial cancer, and several other cancers, including ovarian, upper urinary tract, gastric, small bowel, biliary/pancreatic, skin, and brain cancers. The molecular signature of LS is microsatellite instability (MSI), which is found in more than 95% of LS-associated CRCs.[4] However, MSI is also present in up to 15% of sporadic CRCs due to hypermethylation of the promoter region of *MLH1* in tumor cells. Immunohistochemical (IHC) studies of MMR proteins have been shown to be equivalent to MSI in detecting MMR-defective CRC.[5] CRC with MSI and a lack of staining of MSH2, MSH6, or MLH1 without promoter hypermethylation is a strong indicator of *MSH2*, *MSH6*, or *MLH1* germline mu-

Abbreviations used in this paper: CI, confidence interval; CRC, colorectal cancer; IHC, immunohistochemical; LLS, Lynch-like syndrome; LS, Lynch syndrome; LSRC, Lynch syndrome–related cancer; MMR, mismatch repair; MSI, microsatellite instability; SIR, standardized incidence ratio.

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tations.[6] However, some of these cases of CRC do not have pathogenic mutations in MMR genes. These cases are suspected to be nonsporadic because no mechanism of inactivation is known for these genes other than germline mutations in the context of LS. These patients are considered to have "probably nonsporadic" CRC or Lynch-like syndrome (LLS), and decisions about their management are not simple because of unconfirmed suspicions of hereditary cancer. These cases must be distinguished from familial CRC type X, in which tumors do not show MMR deficiency. No studies have characterized these patients with CRC, and the risk of cancer in this group of families is not known. Therefore, the surveillance strategy for these patients and their relatives is unclear.

We analyzed the clinical and familial characteristics of patients diagnosed with LLS, LS, or sporadic CRC. The main aim of this study was to determine the risk of cancer in families of patients with LLS.

Patients and Methods

Patients and Data Collection

This population-based, observational, cohort study included 1705 patients with CRC from 2 nationwide multicenter studies: EPICOLON I and EPICOLON II. EPICOLON I included consecutive patients with a new diagnosis of CRC between November 2000 and October 2001 with the main goal of estimating the incidence of LS in Spain.[7] EPICOLON II also included consecutive patients with newly diagnosed CRC between March 2006 and December 2007 with the aim of investigating different aspects related to the diagnosis of hereditary CRC.[8] All of the patients provided written informed consent. Both studies were approved by the institutional review boards of the participating hospitals.

Patients were divided into 3 groups based on genetic data: (1) the LS group, in which patients had a confirmed pathogenic mutation in *MLH1*, *MSH2*, *MSH6*, or *PMS2*; (2) the LLS group, in which patients had MSI and loss of MSH2/MSH6 expression, isolated loss of PMS2, or loss of expression of MLH1 without *MLH1* promoter hypermethylation in which no germline mutation was found; and (3) the sporadic group, in which patients with CRC and microsatellite stable tumors had normal expression of MMR genes or a loss of MLH1 expression with *MLH1* promoter hypermethylation.

Demographic, clinical, and pathologic data were collected at the time of diagnosis. Cancer pedigrees were built at diagnosis for cases of CRC in the EPICOLON I and II studies. The pedigrees were traced backward and laterally as far as possible. This information was verified by reviewing medical records when available. Standardized incidence ratios (SIRs) for cancer were calculated as the ratio of the observed to expected number of cases diagnosed in the families at the time of inclusion in the EPICOLON I or II cohorts. To avoid recall bias, only cases of cancer in first-degree relatives were included in the calculation of SIR. We considered tumors in the endometrium, ovaries, upper urinary tract, stomach, small intestine, and hepatobiliary system as noncolorectal LS-related cancers (LSRCs). The index case was excluded for the analysis of family history at the time of diagnosis. Calculation of the SIR was only possible in families with complete pedigrees and information about the ages of all family members, including relatives without cancer.

In 2011, the pedigrees were updated by asking patients and/or relatives about new cases of cancer after diagnosis of the index case. We include the index case for this analysis, and the appearance of metachronous CRC or a new case of noncolorectal LSRC in the index case was considered a new case in the family.

MSI, Immunohistochemical Staining, and Detection of Germline Mutations

MSI analysis was performed in all patients. We ascertained MSI status using BAT26 and NR24 quasi-monomorphic markers as described previously.[9] MSI was present when one of the 2 markers was unstable. IHC analysis of the 4 MMR proteins MLH1, MSH2, MSH6, and PMS2 in tumor tissue was performed in all patients using tissue microarrays as described previously.[10] In patients with a loss of MLH1, methylation of *MLH1* and *BRAF* mutation status was analyzed. *MLH1* methylation analysis was performed using methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) according to the manufacturer's protocol using SALSA MS-MLPA Kit ME011 Mismatch Repair Genes (MRC-Holland, Amsterdam, The Netherlands).[11] The V600E *BRAF* mutation was detected using specific TaqMan probes in real-time polymerase chain reaction (ABI Prism 7500; Applied Biosystems, Foster City, CA) and allelic discrimination software as described previously.[12]

Germline mutation analysis was performed in accordance with the results of IHC analysis as described previously.[10] Patients with loss of MSH2 expression with no detected mutation were analyzed for *EPCAM* rearrangements using MLPA (MRC-Holland) according to the manufacturer's recommended protocol. DNA sequencing was performed to characterize the deletion breakpoints.[13] Large rearrangements (deletions and insertions) were tested using MLPA according to the manufacturer's protocol. The results of genetic analysis were interpreted based on the ACMG Recommendations for Standards for Interpretation of Sequence Variations (2000) and the InSIGHT database.[14]

Statistical Analysis

Continuous variables are reported as mean ± standard deviation or median and 25th and 75th percentiles for non-normally distributed data. Categorical variables are reported as frequencies or percentages. Significant differences between groups were analyzed using the χ^2 test for categorical data and the non-parametric Mann–Whitney *U* test for quantitative data.

The SIR of each cancer was calculated as the ratio of the observed to expected number of cases among relatives. Person-years were calculated from 20 years of age to the earliest cancer diagnosis or death. The expected number of cases was calculated as the sum of the products of the number of person-years for each 5-year age/sex group and the corresponding age/sex-specific incidence rates in Spanish regional registers.[15] The confidence limits were based on Byar's approximation of the exact Poisson distribution, which is accurate even with small numbers.[16] All reported *P* values are 2 sided, and $P < .05$ was considered significant. All calculations were performed using SPSS 19.0 software (Chicago, IL).

Results

A total of 1705 patients with CRC were included in the study. The median age was 71 years (range, 27–101 years), and 59% of patients were male. Sixteen patients were excluded because of discrepancies between the IHC and MSI analyses; no mutation was found in these pa-

Table 1. Comparison of Demographic, Clinical, and Pathologic Data from the LLS, LS, and Sporadic CRC Groups

	LS (n = 16)	LLS (n = 43)	Sporadic CRC (n = 1630)
Female sex	62.5 (10)	55.8 (24)[a]	40.1 (654)
Median age, *y* (interquartile range)	69 (51–75)	66 (55–73)[a]	71 (64–78)
Fulfilled revised Bethesda Guidelines	81.3 (13)	51.2 (22)[ab]	22.0 (358)
Location, right colon	56.3 (9)	55.8 (24)[a]	26.9 (438)
TNM stage II	50.0 (8)	59.0 (25)[a]	39.6 (645)
Histology			
Poor differentiation	25.0 (4)	4.9 (2)[b]	8.3 (135)
Lymphocytic infiltration	25.0 (4)	28.6 (12)	29.2 (475)
Mucinous	20.0 (3)	36.6 (15)[a]	13.0 (212)
Metachronous CRC	12.5 (2)	0 (0)	1.2 (20)
Personal history of noncolorectal LS cancer	43.8 (7)	11.6 (5)[ab]	3.3 (54)
Synchronous CRC	12.5 (2)	9.3 (4)	5.6 (91)

NOTE. All values are expressed as percent (n) unless otherwise noted.
[a]$P < .05$, patients with LLS versus patients with sporadic CRC.
[b]$P < .05$, patients with LS versus patients with LLS.

tients. Therefore, data from 1689 patients were analyzed. Tumors from all patients were analyzed. A total of 135 patients (8%) exhibited in their tumors MSI and loss of expression of any of the MMR proteins. In 104 patients (6.1%), loss of MLH1 expression was found on IHC analysis. Of these patients, 25 (1.4%) did not exhibit hypermethylation of the promoter region. We also used *BRAF* mutation as a sporadic CRC marker in these 25 cases, but no case of *BRAF* mutation indicating sporadic origin was found. Loss of MSH2 expression was seen in the IHC analysis of 22 patients (1.3%). Three patients (0.2%) had an isolated loss of MSH2 expression, and 19 (1.1%) had a combined loss of MSH2 and MSH6. An isolated loss of MSH6 was found in 6 patients (0.3%), and an isolated loss of PMS2 was found in 3 patients (0.2%).

A germline pathogenic mutation in any of the MMR genes was found in 16 patients (0.9%), who were considered to have LS. Three of these patients exhibited MSI with nonvaluable expression of MMR proteins on IHC analysis. Four of the patients with LS were found to have a pathogenic mutation in *MLH1*, 8 in *MSH2*, 3 in *MSH6*, and one in *PMS2*. All of these patients exhibited MSI. No case was found with deletions in *EPCAM*. Variants of uncertain significance were found in 5 patients (Supplementary Table 1). Forty-three patients (2.5%) exhibited MSI and loss of MSH2/MSH6, PMS2, or MLH1 expression without promoter hypermethylation, but no pathogenic germline mutation was found. These patients were considered to have LLS. Among the patients with LLS, 21 were found to have loss of MLH1 protein expression and 22 loss of MSH2, MSH6, or PMS2 expression (14 with loss of MSH2 and MSH6, 6 with isolated loss of MSH6, and 2 with isolated loss of PMS2). Finally, 1630 patients (96%) were considered to have sporadic CRC.

Demographic, Clinical, and Pathologic Characteristics of Patients With LLS

The characteristics of the LLS group (n = 43) were compared with those of the LS (n = 16) and sporadic CRC (n = 1630) groups (Table 1). Fewer patients with LLS fulfilled the revised Bethesda guidelines than patients with LS, and patients with LLS less often had a personal history of non-colorectal LSRC compared with patients with LS. There were no differences in the presence of metachronous CRC, median age at diagnosis of CRC, sex, and tumor characteristics, such as location or TNM classification. Patients with LLS were younger at diagnosis, predominantly female, and more frequently fulfilled the revised Bethesda guidelines compared with patients with sporadic CRC. A personal history of noncolorectal LSRC was more frequent in patients with LLS without differences in the presence of metachronous or synchronous CRC (Table 1).

Risk of Familial Cancer

A total of 13 families with LS and 25 families with LLS had complete pedigrees, including the ages of relatives without cancer. A random sample of 115 families with sporadic CRC was used for comparison. A total of 1102 first-degree relatives were included: 80 from families with LS, 177 from families with LLS, and 845 from families with sporadic CRC. The mean number of first-degree relatives was 6.1 for families with LS, 7.0 for families with LLS, and 7.3 for families with sporadic CRC.

In families with LS, we identified 18 cases of CRC and 6 cases of noncolorectal LSRC. There were no cases of cancer other than the index case in only 4 families (30.7%). In families with LLS, we found 14 cases of CRC and 8 cases of noncolorectal LSRC. In 12 of 25 families (48%), no case of cancer was found other than the index case. The characteristics of patients and distribution of cases of cancer in families with LLS are provided in Table 2. Finally, in families with sporadic CRC, 15 first-degree relatives had CRC and 27 had noncolorectal LSRC. No cases of cancer other than the index case were identified in 85 families (79.9%).

The SIRs of CRC and noncolorectal LSRC are shown in Table 3. The incidence of CRC was significantly lower in families with LLS compared with confirmed families with LS (SIR in LS, 6.04; 95% confidence interval [CI], 3.58–

Table 2. Characteristics and Family History of Patients With LLS

Patient	Protein lost	Age of index case (*y*)	No. of relatives with CRC[a] (n = 14)	No. of relatives with noncolorectal LSRC[b] (n = 8)	Total no. of first-degree relatives (n = 177)	Cases of CRC (%)[c]	Cases of noncolorectal LSRC (%)[d]
1	PMS2	71	0	1 (pancreas)	10	0	10
2	MLH1/PMS2	81	0	0	9	0	0
3	MLH1/PMS2	73	0	0	7	0	0
4	MLH1/PMS2	75	1	1 (stomach)	7	14.2	14.2
5	MLH1/PMS2	45	3	1 (ovary)	10	30	10
6	MSH2/MSH6	38	0	0	5	0	0
7	MSH2/MSH6	74	0	0	6	0	0
8	MSH2/MSH6	32	2	0	3	66.6	0
9	MLH1/PMS2	72	2	3 (uterus)	11	18.1	27.2
10	MLH1/PMS2	51	0	0	4	0	0
11	MLH1/PMS2	46	1	1 (pancreas)	3	33.3	33.3
12	MSH2/MSH6	69	1	0	9	11.1	0
13	MSH6	55	0	0	6	0	0
14	MSH6	56	0	0	4	0	0
15	MLH1/PMS2	72	1	0	7	14.2	0
16	MSH2/MSH6	69	0	0	5	0	0
17	MSH2/MSH6	66	0	1 (stomach)	6	0	16.6
18	MSH2/MSH6	66	0	0	9	0	0
19	MLH1/PMS2	63	1	0	8	12.5	0
20	MLH1/PMS2	46	0	0	11	0	0
21	MLH1/PMS2	71	0	0	4	0	0
22	MLH1/PMS2	69	1	0	12	8.3	0
23	MLH1/PMS2	59	0	0	4	0	0
24	MSH6	79	1	0	8	12.5	0
25	MSH6	54	0	0	9	0	0

[a]Number of first-degree relatives diagnosed with CRC at any time until diagnosis of the index case.
[b]Number of first-degree relatives diagnosed with noncolorectal LSRC at any time until diagnosis of the index case.
[c]Percentage of family members diagnosed with CRC at any time until diagnosis of the index case.
[d]Percentage of family members diagnosed with noncolorectal LSRC at any time until diagnosis of the index case.

9.54; SIR in LLS, 2.12; 95% CI, 1.16–3.56; $P < .001$). However, the incidence of CRC was significantly greater in the families with LLS than in the families with sporadic CRC (SIR in sporadic CRC, 0.48; 95% CI, 0.27–0.79; $P < .001$). The SIR for noncolorectal LSRC was not significantly higher in families with LS (SIR, 2.81; 95% CI, 1.03–6.12) compared with families with LLS (SIR, 1.69; 95% CI, 0.73–3.34; $P = .09$). There were no differences in the SIR for noncolorectal LSRC between families with LLS and families with sporadic CRC (SIR, 1.20; 95% CI, 0.79–1.74; $P = .5$). Taken together, the results indicate that, for CRC and noncolorectal LSRC, the highest risk is for families with LS (SIR, 4.69; 95% CI, 3.00–6.98), followed by families with LLS (SIR, 1.94; 95% CI, 1.22–2.94; $P < .001$). The risk in families with LLS was significantly higher than the risk in relatives of patients with sporadic CRC (SIR, 0.78; 95% CI, 0.56–1.05; $P < .001$).

Figure 1 shows the cumulative age-of-onset curves for CRC among all relatives in the LS, LLS, and sporadic CRC groups. The relatives of patients with LLS developed CRC at an earlier mean age (53.71 ± 16.8 years) than those with sporadic CRC (68.8 ± 9 years; $P = .004$) but at a mean age similar to that in patients with LS (48.5 ± 14.13; $P = .23$).

After a median of 8.3 years of prospective follow-up, cancer pedigrees were updated in 93 families: 10 in the LS group, 16 in the LLS group, and 67 in the sporadic CRC group. A total of 533 first-degree relatives were included (including the index case): 41 from families with LS, 89 from families with LLS, and 403 from families with sporadic CRC. During this period, 7 (17.1%) new cases of CRC or non-colorectal LSRC appeared in families with LS, 4 (4.5%) new cases in families with LLS, and 4 (0.9%) new cases in families with sporadic CRC (Table 4).

Table 3. SIRs Between Families With LLS and Families With LS/Sporadic CRC

	LS (n = 80)		*P*	LLS (n = 177)		*P*	Sporadic CRC (n = 845)	
	No. of tumors	SIR (95% CI)	value[a]	No. of tumors	SIR (95% CI)	value[b]	No. of tumors	SIR (95% CI)
CRC	18	6.04 (3.58–9.54)	<.001	14	2.12 (1.16–3.56)	<.001	15	0.48 (0.27–0.79)
Non-CRC LSRC	6	2.81 (1.03–6.12)	.09	8	1.69 (0.73–3.34)	.5	27	1.20 (0.79–1.74)
Total	24	4.69 (3.00–6.98)	<.001	22	1.94 (1.22–2.94)	<.001	42	0.78 (0.56–1.05)

[a]Comparing the SIR of the LS and LLS groups.
[b]Comparing the SIR of the LLS and sporadic CRC groups.

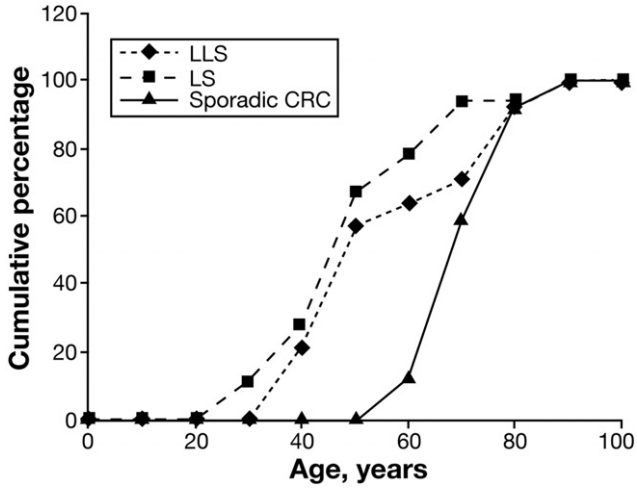

Figure 1. Cumulative age of onset of CRC in first-degree relatives of patients with LS, LLS, and sporadic CRC.

Discussion

The main finding of our study was that the risk of CRC is lower in families with LLS than among patients with genetically confirmed LS but significantly higher than in cases of sporadic CRC. The results confirm the need for a special surveillance strategy for these patients and their relatives. In addition, the age of onset for CRC in families with LLS was similar to that for families with LS. Differences between families with LLS and families with sporadic CRC were more prominent in regard to risk of CRC than for risk of other noncolorectal LSRCs.

Recent studies have shown that MSI testing and IHC analysis of MMR genes in all patients with CRC improve the detection of patients with LS.[10,17,18] Because of the generalization of this universal strategy following the Jerusalem guidelines,[19] an increasing number of patients exhibit a loss of MMR protein expression with no pathogenic mutation. In cases of CRC with a loss of MSH2, MSH6, PMS2, or MLH1 without hypermethylation, no cause of MMR gene inactivation is known other than germline mutation. In these cases, when a germline mutation is not found, a high suspicion of LS persists, and these patients and their relatives should be followed up appropriately. The clinical characteristics of some of these patients suggest that they are clear hereditary cases, even though we were not able to find a genetic defect. Some of the pedigrees of patients with LLS showed a significant history of CRC with metachronous and synchronous tumors and fulfillment of the Amsterdam criteria. In these cases, the genetic defect was not found, probably because it was located in a still unknown part of the gene or simply because our technical capacity is not yet able to detect the pathogenic mutation. Some of these cases have been explained in the literature by alterations in other genes, such as in cases of *EPCAM* deletions or MLH1 constitutional epimutations. Other mechanisms, including inversions and duplications, could also explain some of these cases.[20–26] However, some cases do not show any specific characteristics that suggest a hereditary origin. A notable proportion of families with LLS do not have a history of other cancers, and the only reason to suspect LS is the presence of MSI and loss of MMR protein expression. In these cases, determining the appropriate counseling for patients and their relatives is difficult. It is possible that some of these patients with LLS could be cases with CRC who may have false-positive results on IHC analysis and/or MSI or sporadic MMR-deficient CRC, and patients with LLS would be a mixture of patients with true LS with nondetected germline mutation and cases of sporadic CRC. However, the high risk of CRC found in our study suggests that, in its entirety, patients with LLS should be considered high-risk cases and strategies for prevention of cancer must be implemented in this group of patients and their relatives. The SIR of CRC for families with LLS was similar to that described in a group of families with familial colorectal cancer syndrome type X, but in this syndrome no molecular alteration has been found.[27] Even though LLS is a completely different entity because of the presence of MSI, the similar risk of CRC should guarantee at least a similar surveillance program, even in cases without a previous family history. Our results can contribute some rationale for designing follow-up strategies and, together with family history, can help clinicians appropriately schedule surveillance for these patients and their relatives. In our study, the age at onset of CRC was similar to that of LS, and therefore surveillance strategies should start at the same age as recommended for cases of LS. On the other hand, the frequency of CRC screening should be individualized. Given that the risk of CRC is lower in families with LLS than in families with LS, longer surveillance intervals for patients with LLS and relatives without a prominent family history of CRC may be recommended. We have not found a higher risk of noncolorectal LSRC in families with LLS compared with families with sporadic CRC. However, that can be attributable to the small num-

Table 4. Differences in the Prospective Appearance of New Cases of Cancer Between Families With LLS and Families With LS/Sporadic CRC During Follow-up

	LS (n = 41)	*P* value[a]	LLS (n = 89)	*P* value[b]	Sporadic CRC (n = 403)
CRC (%)	3 (7.3)	.16	2 (2.2)	.2	3 (0.7)
Non-CRC LSRC (%)	4 (9.8)	.05	2 (2.2)	.02	1 (0.2)
Total cancers (%)	7 (17.1)	.01	4 (4.5)	.01	4 (0.9)

[a]Comparing the percentage in the LS and LLS groups.
[b]Comparing the percentage in the LLS and sporadic CRC groups.

ber of cases detected in our series. For this reason, specific recommendations for endometrial and other noncolorectal LSRC cannot be appropriately supported by our data.

The limitations of our study are the possibility of underreporting or misreporting cancers because our information was not always confirmed with objective clinical and pathologic data. However, we believe this limitation is minor because it would affect the LLS group to the same extent as the other groups. Another limitation is the relatively small number of families, especially in the prospectively followed up cases, which precludes finding clear differences between groups. Moreover, the follow-up time for these cases could be considered too short.

The main strength of our study is its population-based approach, with cases ascertained from general clinics and not from specialized high-risk clinics. This approach provides robustness to our data in terms of potential applicability to general practice. The risk of cancer can be overestimated in studies from select populations in genetic high-risk clinics. Studies based on recruitment through cancer genetics clinics do not usually correct for the selection bias caused by the overrepresentation of families with multiple cases in the data set.[28,29] Our results attempt to provide a rationale for follow-up and surveillance of this growing group of patients who will mostly be seen in general clinics and not in high-risk clinics. New research is necessary to refine the classification of these patients to distinguish between sporadic and true hereditary cases.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi:10.1053/j.gastro.2013.01.044.

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Conflicts of interest

The authors disclose no conflicts.

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Supplementary Table 1. Variants of Uncertain Significance (VUS)

Patient	Sex	Age (*y*)	MSI	MMR protein loss	Gene	VUS (DNA change)	Protein change	Reported insight (times)	Mainly reported as
1	Female	80	No	MLH1/PMS2	MLH1	c.1013 A>G	p.Asn338Ser	14	-? (10/14)
2	Female	80	Yes	MLH1/PMS2	MLH1	c.1959 G>T	p.Leu653Leu	20	? (11/20)
3	Female	72	Yes	MLH1/PMS2	MLH1	c.1331 A>G	p.Asn444Ser	Not reported	
					MLH1	c.2401 G>A	p.Ala681Thr	71	?‘ (43/71)
4	Female	83	Yes	MSH2/MSH6	MSH2	c.1021 C>G	p.Leu341Val	Not reported	
5	Male	66	Yes	MSH2/MSH6	MSH2	c.366 +6 T>C		Not reported	

?, variant of uncertain clinical significance; -?, likely not pathogenic.