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Clinical and Pathological Characterization of Lynch-Like Syndrome

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This article has an accompanying continuing medical education activity, also eligible for MOC credit, on page e22. Learning Objective–Upon completion of this activity, successful learners will be able to identify Lynch-like syndrome, distinguish several pathogenic mechanisms associated with Lynch-like syndrome, and describe the management of patients with Lynch-like syndrome.

BACKGROUND & AIMS: **Lynch syndrome is characterized by DNA mismatch repair (MMR) deficiency. Some patients with suspected Lynch syndrome have DNA MMR deficiencies but no detectable mutations in genes that encode MMR proteins—this is called Lynch-like syndrome (LLS). There is no consensus on management of patients with LLS. We collected data from a large series of patients with LLS to identify clinical and pathology features.**

METHODS: **We collected data from a nationwide-registry of patients with colorectal cancer (CRC) in Spain. We identified patients whose colorectal tumors had loss of MSH2, MSH6, PMS2, or MLH1 (based on immunohistochemistry), without the mutation encoding V600E in *BRAF* (detected by real-time PCR), and/or no methylation at *MLH1* (determined by methylation-specific multiplex ligation-dependent probe amplification), and no pathogenic mutations in MMR genes, *BRAF*, or *EPCAM* (determined by DNA sequencing). These patients were considered to have LLS. We collected data on demographic, clinical, and pathology features and family history of neoplasms. The χ^2 test was used to analyze the association between qualitative variables, followed by the Fisher exact test and the Student t test or the Mann-Whitney test for quantitative variables.**

Abbreviations used in this paper: CRC, colorectal cancer; IHC, immunohistochemistry; LLS, Lynch-like syndrome; LS, Lynch syndrome; MMR, mismatch repair; MSI, microsatellite instability; PCR, polymerase chain reaction; SD, standard deviation.

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RESULTS: **We identified 160 patients with LLS; their mean age at diagnosis of CRC was 55 years and 66 patients were female (41%). The Amsterdam I and II criteria for Lynch syndrome were fulfilled by 11% of cases and the revised Bethesda guideline criteria by 65% of cases. Of the patients with LLS, 24% were identified in universal screening. There were no proportional differences in sex, indication for colonoscopy, immunohistochemistry, pathology findings, or personal history of CRC or other Lynch syndrome-related tumors between patients who met the Amsterdam and/or Bethesda criteria for Lynch syndrome and patients identified in universal screening for Lynch syndrome, without a family history of CRC.**

CONCLUSIONS: **Patients with LLS have homogeneous clinical, demographic, and pathology characteristics, regardless of family history of CRC.**

Keywords: Familial; Colon Tumor; Risk; Genetic; Polyp.

See editorial on page 294.

Lynch syndrome (LS) is the most frequent cause of hereditary colorectal cancer (CRC). It is mainly characterized by a high risk of developing CRC and endometrial cancer as well as other neoplasms, namely of the ovaries, urinary tract, stomach, small intestine, pancreas, biliary tract, skin, and brain.[1–3] LS is caused by germline mutations in one of the DNA mismatch repair (MMR) genes.[4] The inactivation of these genes increases the rate of mutations during DNA synthesis, with an increase in structural anomalies that tend to appear in repetitive DNA sequences. This characteristic is called microsatellite instability (MSI) and is observed in more than 95% of tumors in patients with CRC or other tumors associated with LS.[5] The presence of MSI suggests a defect in the MMR genes; however, its specificity is low because it also occurs in approximately 15% of sporadic CRC cases, usually because of hypermethylation of the promoter region of the *MLH1* gene in the tumor tissue.[6] Immunohistochemistry (IHC) with antibodies against MMR proteins can be useful to identify MMR if there is loss of expression of these proteins.[7]

However, in an increasing number of cases, the presence of MSI or loss of immunochemical expression of MMR genes is found, but the presence of germline pathogenic mutations in these genes could not be found. These patients are considered to have "probably non-sporadic" MMR-defective CRC or Lynch-like syndrome (LLS), which represents approximately 30% of all patients with unstable tumors.[8] A previous study from our group showed that these cases and their first-degree relatives show a risk of CRC that is between that found in relatives of LS patients and sporadic cases. This result suggests that these LLS patients are probably a heterogeneous group that includes patients with an unidentified hereditary syndrome, as well as sporadic cases. Testing for somatic mutations in MMR genes has been proposed for differential diagnosis between hereditary and sporadic cases; however, this testing is not widely performed, and there is no consensus about management of LLS cases or follow-up of patients and their relatives.[9,10]

The aim of this study was to describe the clinical and pathologic features of a large nationwide series of LLS patients and to analyze whether patients with a suspected hereditary or sporadic origin show any different clinical or pathologic characteristics.

Methods

Patient Data

Data were extracted from a descriptive, observational, multicenter, nationwide registry (EPICOLON-III) on familial CRC that involved 25 Spanish hospitals. Patients with CRC were included when their tumors showed immunohistochemical loss of MSH2, MSH6, PMS2, or loss of MLH1 with BRAF–wild-type and/or no *MLH1* methylation, and in whom germline mutations could not be found in these genes or in *EPCAM*. Immunohistochemical study of the tumors was performed because of fulfillment of Amsterdam criteria and/or revised Bethesda guidelines[11] or because of universal molecular screening for LS.[12] In these cases, the fulfillment of Amsterdam criteria and/or revised Bethesda guidelines was also reviewed. Patients were investigated according to common protocols,[13] and in all cases the family history was collected through the realization of pedigrees that included at least one generation backward and forward to the index case.

These patients were included in the national registry EPICOLON-III (www.epicolon.es); demographic, clinical, and pathologic variables were registered, as well as family history of neoplasms.

Microsatellite Instability, Immunohistochemistry Staining, and Detection of Germline Mutations

MSI and/or IHC analysis was performed in all patients. Although IHC was not performed for 6 patients, we confirmed their inclusion because of the presence of high MSI. MSI status was analyzed by using multiplex polymerase chain reaction (PCR) patterns at the following monomorphic repetitive markers: BAT26, BAT25, NR21, NR24m, and NR27.[14,15] Amplicon

detection and analysis were performed by using an ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA) and Genotyper software (Life Technologies, Carlsbad, CA), respectively. A diagnosis of MSI was considered positive when 2 or more markers showed an altered pattern. IHC analysis of MLH1, MSH2, MSH6, and PMS2 was performed in formalin-fixed, paraffin-embedded tumor tissue, as previously described.[16]

In patients with a loss of MLH1, methylation of *MLH1* and/or somatic *BRAF* mutation status was analyzed. *MLH1* methylation analysis was performed by using methylation-specific multiplex ligation-dependent probe amplification according to the manufacturer's protocol using the SALSA MS-MLPA Kit ME011 Mismatch Repair Genes (MRC-Holland, Amsterdam, The Netherlands).[17] The V600E *BRAF* mutation was detected by using specific TaqMan probes in real-time PCR (ABI Prism 7500; Applied Biosystems) and allelic discrimination software as described previously.[18]

Germline mutation analysis was performed in accordance with the results of IHC analysis as described previously.[8] Patients with loss of MSH2 expression with no detected mutation were analyzed for *EPCAM* rearrangements by using multiplex ligation-dependent probe amplification according to the manufacturer's recommended protocol. DNA sequencing was performed to characterize the deletion breakpoints.[19] Large rearrangements (deletions and insertions) were tested by using multiplex ligation-dependent probe amplification according to the manufacturer's protocol. The results of genetic analysis were interpreted on the basis of the American College of Medical Genetics Recommendations for Standards for Interpretation of Sequence Variations (2000) and the InSIGHT database.[20]

Statistical Analysis

The statistical analysis was carried out by using the SPSS program (SPSS 19.0; Chicago, IL). Regarding the descriptive analysis, the qualitative variables are presented as percentages. Continuous quantitative variables are described as the mean and standard deviation (SD) or the median and interquartile range, depending on whether they follow a normal distribution. The χ^2 test was used to analyze the association between qualitative variables, followed by Fisher exact test and the Student *t* test or the Mann-Whitney test for quantitative variables, according to whether the variables followed a normal distribution. A *P* value $<.05$ was considered statistically significant.

Results

We included 160 patients diagnosed with CRC who met the diagnostic criteria for LLS. The characteristics of patients with LLS are shown in Table 1. Mean age at diagnosis of CRC was 54.9 years (SD, 14.2), and 53 patients (33%) were younger than 50 years at diagnosis; 41.2% of patients were female. The majority of cases were diagnosed because of symptoms (87.4%). The most frequent IHC finding was lack of MLH1/PMS2

What You Need to Know

Background

Lynch syndrome is characterized by DNA mismatch repair (MMR) deficiency. Some patients with suspected Lynch syndrome have DNA MMR deficiencies but no detectable mutations in genes that encode MMR proteins; this is called Lynch-like syndrome (LLS).

Findings

In analysis of a database of patients with colorectal cancer in Spain, we identified 160 patients with LLS. These patients had homogeneous clinical, demographic, and pathology features, regardless of family history of CRC.

Implications for patient care

Family history of CRC does not identify all patients with LLS.

Table 1. Characteristics of Patients With Lynch-like Syndrome

Lynch-like syndrome	n = 160
Female sex, n (%)	66 (41.3)
Mean age, *y* (SD)	63.5 (14.4)
Mean age at CRC diagnosis, *y* (SD)	54.9 (14.2)
Indication for colonoscopy, n (%)	
Symptoms	118 (87.4)
CRC screening	17 (12.6)
Immunohistochemistry, n (%)	
Loss of MLH1 and PMS2	77 (48.1)
Loss of MSH2 and MSH6	43 (26.9)
Isolated loss of MSH6	20 (12.5)
Isolated loss of PMS2	14 (8.8)
IHC non-available; MSI-H	6 (3.7)
Reason for IHC, n (%)	
Amsterdam I and II criteria	18 (11.2)
Revised Bethesda guidelines	103 (64.4)
Universal screening	39 (24.4)
Location, n (%)	
Right colon	89 (61.4)
Left colon and rectum	56 (38.6)
Median tumor size (range), *cm*	5 (0.6–30)
Histology, n (%)	
Poor differentiation	33 (20.6)
Lymphocytic infiltration	37 (23.1)
Mucinous tumor	46 (28.7)
Vascular invasion	18 (11.3)
Metachronous CRC, n (%)	5 (3.1)
Personal history of non-CRC tumors, n (%)	27 (16.8)
Personal history of non-CRC LS-associated tumors, n (%)	5 (3.1)
Family history of CRC, n (%)	80 (50)

CRC, colorectal cancer; IHC, immunohistochemistry; LS, Lynch syndrome; MSI, microsatellite instability; SD, standard deviation.

expression in 50% of cases, followed by lack of MSH2/MSH6 expression (27.9%). Isolated loss of MSH6 (13%) or PMS2 (9.1%) was less frequent. Regarding family history, 64.4% of cases fulfilled revised Bethesda guidelines and 11.2% fulfilled Amsterdam criteria for LS diagnosis. Fifty percent of patients reported any family history of CRC, and 38.7% reported family history of another LS-related cancer. In 24.4% of cases, IHC of MMR proteins was performed in the context of universal LS screening. Five patients (3.1%) developed a second CRC within a median of 7 years (SD, 3.9 years), 16.8% had a history of non-CRC tumors, and 3.1% had a history of other non-CRC LS-related neoplasms.

With the aim of identifying whether there was any difference between LLS patients with suspected hereditary origin and those with probable sporadic origin, an analysis was performed comparing LLS patients who met the Amsterdam and/or Bethesda criteria with those who did not meet these criteria and in whom the diagnosis was made because of the realization of universal screening for LS diagnosis. The only differences we found were related to the definition of cases, with a mean age at CRC diagnosis of 65.5 years (SD, 10.1) in patients diagnosed by universal screening versus 51.6 years (SD, 13.7) in patients who fulfilled Amsterdam and/or Bethesda criteria ($P = .02$). In addition, 57% of patients who met Amsterdam and/or Bethesda criteria reported a family history of CRC versus 28% of patients identified through universal LS screening ($P < .001$). No statistical differences were observed between the 2 groups with respect to sex, indication for colonoscopy, IHC findings, tumor characteristics (location, size, TNM stage, pathology), personal history of CRC or other LS-associated cancer, or family history of non-colorectal cancer associated with LS (Table 2).

A second analysis compared patient characteristics based on their age at CRC diagnosis (<50 years of age) and/or the presence of a family history of tumors associated with LS versus those patients with diagnosis of CRC at ≥50 years of age and lack of family history of LS-associated tumors. This analysis also did not reveal any significant differences between the 2 groups regarding sex, vital status, indication for colonoscopy, IHC, tumor characteristics (location, size, TNM stage, histology), or personal history of CRC or other LS-associated cancer (Table 3). Also here the only differences found were related to the selection criteria.

Table 2. Patient Characteristics Based on Reason for Lynch-like Syndrome Diagnosis

	Amsterdam or Bethesda guidelines, n = 121	Universal screening of LS, n = 39
Median age at CRC diagnosis (SD), *y*	51.6 (13.7)	65.5[a] (10.1)
Female sex, n (%)	47 (38.8)	19 (48.7)
Indication for colonoscopy, n (%)		
Symptomatic	89 (87.3)	29 (87.9)
CRC screening	13 (12.7)	4 (12.1)
Immunohistochemistry, n (%)		
MLH1 and PMS2	57 (49.1)	20 (52.6)
MSH2 and MSH6	30 (25.9)	13 (34.2)
MSH6	16 (13.8)	4 (10.5)
PMS2	13 (11.2)	1 (2.6)
Location, n (%)		
Right colon	66 (60.6)	23 (63.9)
Rectum and left colon	43 (39.4)	13 (36.1)
Median tumor size (range), *cm*	5.88 (4.9–6.8)	4.5 (3.7–5.2)
Histology, n (%)		
Poor differentiation	25 (20.7)	8 (20.5)
Lymphocytic infiltration	30 (24.8)	7 (17.9)
Mucinous	36 (29.8)	10 (25.6)
Vascular infiltration	17 (14)	1 (2.6)
Personal history, n (%)		
CRC or other LS-associated cancer	11 (9.1)	1 (2.6)
Metachronous CRC	4 (3.3)	1 (2.6)
Synchronous CRC	2 (1.7)	0 (0)
Non-CRC LS tumor	5 (4.1)	0 (0)
Family history of CRC, n (%)	69 (57)	11 (28.2)[a]

CRC, colorectal cancer; LS, Lynch syndrome; SD, standard deviation.
[a]$P < .05$.

Discussion

In this study, which includes the largest published cohort of patients with LLS, we describe clinical and pathologic characteristics of these patients. We found that cases with suspected hereditary origin that was due to family history, young age at diagnosis, and/or fulfillment of Amsterdam or Bethesda criteria are similar to cases with suspected sporadic origin with respect to clinical, molecular, and pathologic characteristics. These results support that in the absence of a molecular marker able to differentiate both groups, these patients should be managed homogeneously.

The implementation of universal LS screening has led to an increase in the percentage of tumors that exhibit MSI or loss of expression of the MMR proteins but lack any germline pathogenic mutation or other cause of MMR deficiency.[8] This situation, called LLS or MMR tumors of unknown origin, is associated with uncertainty regarding preventive management of patients and their relatives, because there is no consensus about whether it should be considered a likely hereditary or sporadic condition. There are different mechanisms that may cause this phenotype (Figure 1). The first potential cause is the presence of atypical germline alterations in MMR genes (regulatory regions, inversions, or translocations) or cryptic mutations (not detected with current methods) that could provoke somatic alteration of the remaining MMR allele. This group of patients actually have unidentified LS. Another possible cause is the presence of germline alterations in other genes

Table 3. Patient Characteristics Based on Age at CRC Diagnosis and Family History of LS-Associated Neoplasms

	CRC diagnosed <50 y old and/or family history of LS-related cancer, n =128	CRC diagnosed ≥50 y old and no family history of LS-related cancer, n = 32
Median age at CRC diagnosis (SD), *y*	52.05 (14)	65.71[a] (9)
Female sex, n (%)	52 (40.6)	14 (43.7)
Indication for colonoscopy, n (%)		
Symptomatic	100 (92.6)	18 (66.7)
CRC screening	8 (7.4)	9 (33.3)
Immunohistochemistry, n (%)		
MLH1 and PMS2	58 (47.5)	19 (59.3)
MSH2 and MSH6	34 (27.9)	9 (28.1)
MSH6	18 (14.8)	2 (6.3)
PMS2	12 (9.8)	2 (6.3)
Location, n (%)		
Right colon	71 (61.7)	18 (60)
Rectum and left colon	44 (38.3)	12 (40)
Median tumor size (range), *cm*	5.97 (5–6.9)	3.98 (3.1–4.8)
Histology, n (%)		
Poor differentiation	27 (21.1)	6 (18.7)
Lymphocytic infiltration	27 (21.1)	10 (31.3)
Mucinous	33 (25.8)	13 (40.6)
Vascular infiltration	15 (11.7)	3 (9.4)

CRC, colorectal cancer; LS, Lynch syndrome; SD, standard deviation.
[a]$P < .05$.

(eg, *MUTYH, POLD1, POLE*) that could also alter the MMR system. Finally, we can also observe sporadic tumors with biallelic MMR alterations as a result of somatic alterations in cancer genes (tumor suppressor genes, oncogenes, repair genes), somatic biallelic alterations in MMR genes, or a combination of both findings.

Clinically, patients with LLS are probably represented by at least 2 different subsets. The first group includes cases in which the clinical characteristics strongly suggest a hereditary origin, but in which the genetic defect has not yet been identified through routine protocols. These patients probably have an undiagnosed hereditary condition with high risk of CRC for them and their first-degree relatives. The second subset includes a significant proportion of families with LLS who do not have a history of cancer and for whom the only element leading to suspicion of LS is the presence of MSI or the loss of expression of some of the MMR proteins. In this latter group, a double somatic mutation in MMR genes is probably the underlying cause of the MSI phenotype. This second group of patients has sporadic tumors, and specific preventive measures are not necessary for them or their relatives.

It has been proposed that current LS diagnostic strategy should be complemented with algorithms that integrate other molecular data from the tumors, allowing differential diagnosis between LLS cases of sporadic versus hereditary origin.[21] In that sense, different authors have proposed the investigation of somatic mutations in MMR genes and other genes that might explain

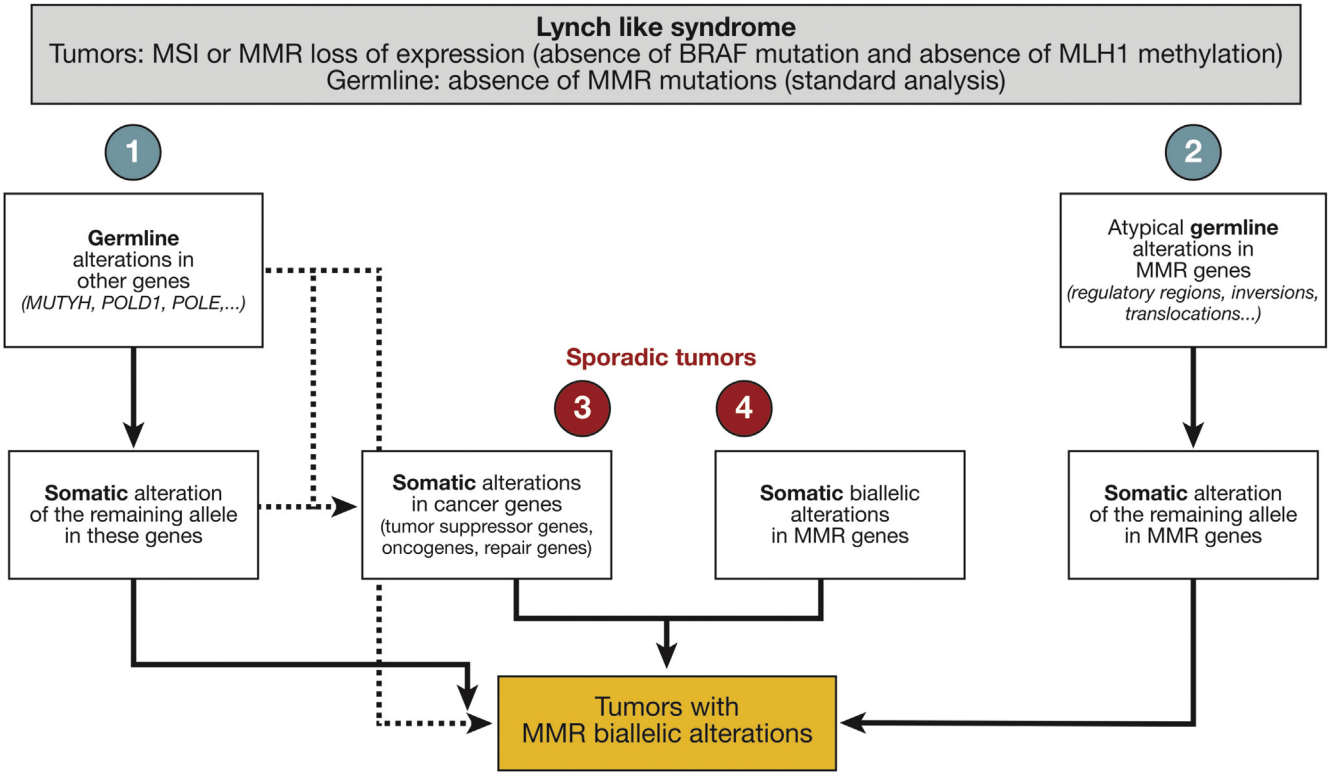

Figure 1. Potential mechanisms for Lynch-like syndrome. MMR, mismatch repair; MSI, microsatellite instability.

sporadic CRC cases with LLS. Preliminary studies found a frequency of somatic mutations in LLS patients that ranges between 22% and 69%.[22–24]

Studies that used these somatic mutations to classify LLS patients as hereditary or sporadic also did not find any clinical or pathologic characteristics that were able to differentiate between the 2 populations. In a recently published study by Hemminger et al,[25] the presence of double somatic mutation in the MMR was observed in 69% of patients with unexplained MMR deficiency who lacked MLH1 methylation and germline mutation. They analyzed whether histomorphology could distinguish patients with double somatic mutations from those with LS, but no significant differences in histologic features were found between tumors in LS patients and tumors with double somatic mutations. This similar tumor histology might be a result of a similar underlying oncogenesis involving defective MMR function, leading to a hypermutated phenotype. Also, in a previous study, Mas-Moya et al[26] compared clinicopathologic differences in CRCs between patients with LS and a group of 21 patients with LLS. Curiously, they found a higher percentage of CRC in the right colon in LLS patients than in LS patients (93% versus 45%; $P < .002$); however, there were no significant differences related to tumor stage, tumor grade, tumor size, tumor-infiltrating lymphocytes, Crohn-like lymphocytic reaction, mucinous differentiation, signet ring cell differentiation, or medullary differentiation. Finally, Hampel et al[21] proposed that current LS diagnostic strategy should be improved with universal up-front tumor sequencing, obtaining better performance in the detection of LS cases and differentiation between hereditary and sporadic cases when no germline mutation is found.

However, the use of multigene panel testing or other tools for diagnosis has not yet been routinely implemented in the majority of centers because of discrepancies about the appropriate somatic gene analysis, with no specific methodology uniformly recommended. Also, the high cost of this approach, the need of next-generation sequencing technology, and the difficulties of applying this technology in paraffin samples are barriers to the implementation of this diagnostic tool for the adequate classification of LLS patients as sporadic or likely hereditary cases. Moreover, the addition of somatic mutations to the diagnostic algorithm of LS has not yet been validated in research studies. Finally, a relationship between these somatic mutations and germline inactivation of still unknown genes related to MMR deficiency has not yet been fully ruled out (Figure 1), and only a germline exome approach or a clinical follow-up validation could finally confirm the sporadic behavior of these LLS tumors with somatic mutations. For these reasons, the majority of LLS cases remain unclassified, and patients and their relatives are followed heterogeneously. If we consider LLS patients as a group, the risk of CRC in patients and their first-degree relatives lies between the risk found in LS syndrome and the risk of sporadic CRC; the incidence of CRC is significantly lower in families of patients with LLS than in families with confirmed LS but is higher than in families with sporadic CRC,[10] and because of that, some preventive measures should be guaranteed in this population.

The main limitation of this study is precisely the lack of molecular information about somatic mutations in the LLS cases; however, we would like to perform a clinical description of these cases pointing out the difficulties of classifying them only on the basis of clinical characteristics. Our study underlines the lack of value of clinical criteria for classification of this heterogeneous group of patients. Moreover, as previously noted, there is not a validated methodology for detecting true somatic mutations, and some consensus between experts is needed to adequately classify these cases as truly sporadic or probably hereditary. In contrast, the main strength of our study is the large number of patients included in a nationwide registry of hereditary CRC cases, which allows adequate and uniform classification of cases and the possibility of establishing cohort studies that will provide more information about this subset of patients.

In summary, we found that there are no clinical or pathologic features differentiating tumors with a suspected hereditary or sporadic origin. These data support that in the absence of any molecular or genetic tool to assist in the classification of this group of patients, we should consider them a homogeneous group, applying preventive measures with periodic colonoscopies for patients and their relatives. Because as a group, the risk of CRC in first-degree relatives of patients with LLS is between that found in LS and in sporadic cases,[10] we can recommend screening for first-degree relatives, at least in the limit of that recommended for LS, with colonoscopy every 3 years. Moreover, validation studies should be approved that aim to determine whether family history or age at CRC diagnosis might be helpful for identifying cases needing a more or less intensive surveillance protocol. Our findings also support the need to increase the study of CRC pathogenesis in these patients, as well as the appropriate way to identify cases as truly hereditary or sporadic.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Clinical Gastroenterology and Hepatology* at www.cghjournal.org, and at https://doi.org/10.1016/j.cgh.2019.06.012.

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See Appendix 1 for list of study participants.

Conflicts of interest
The authors disclose no conflicts.

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Appendix 1

Study Participants

Hospital General Universitario de Elche: Ana Beatriz Sánchez Heras, Victor M. Barberá, Isabel Castillejo; Hospital General Universitario de Alicante: Cristina Mira, Alejandro Martínez-Roca, Carolina Mangas-Sanjuan, Lucía Medina, Sandra Baile, Artemio Payá; Hospital de la Santa Creu i Sant Pau, Barcelona: Consol López, Eva Jiménez, Nuria Calvo; Hospital Universitari Parc Taulí, Sabadell; Consorci Sanitari de Terrassa, Barcelona: Eva Martínez Bauer, Cristina Romero Mascarell; Hospital Universitario de Móstoles: Jorge López-Vicente; Hospital Universitario Virgen del Rocío, Sevilla: Ángeles Pizarro; Hospital del Mar, Barcelona: Faust Riu Pons, Laura Carot Bastard, Agustín Seoane, Xavier Bessa; Hospital Universitario Vall d'Hebron, Barcelona: Judith Balmaña, Neus Gadea, Estela Carrasco, Sara Torres-Esquius; Hospital Universitario de Canarias: Beatriz Alonso Alvarez.