Research Square

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Comparison of Molecular, Clinicopathological, and Pedigree Differences Between Lynch-like and Lynch Syndromes

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Abstract

Background: In this study, we compared the molecular, clinical, and pathological characteristics, as well as pedigrees, between patients with Lynch-like syndrome (LLS) and confirmed Lynch syndrome (LS) to develop appropriate management strategies for patients with LLS and their affected family members.

Methods: Between June 2008 and September 2018, 81 patients with LLS and 47 patients with LS who developed colorectal cancer (CRC) were enrolled in this study. Multigene panel testing included 139 genes and was performed for all patients. The variants identified in each group were described, and clinicopathological characteristics and pedigrees were compared between the two groups.

Results: In the LLS group, a total of 52 variants were detected in 44 (54.3%) patients. Among the 52 variants, 17 were variants of unknown significance in mismatch repair genes, and the other most frequently mutated genes were *MUYTH*, *POLE*, *BRCA2*, and *GJB2.* The proportion of early-onset patients was significantly higher among the LS probands than among the LLS probands (74.5% and 53.1%, respectively; $\chi^2 = 5.712$, P = 0.017). On the other hand, the proportion of primary CRC developed in the rectum was higher in the LLS group than in the LS group (25.9% and 10.6%, respectively; $\chi^2 = 2.358$, P = 0.046). There were no significant differences in the occurrence of metachronous CRC (P = 0.632) and extra-CRC (P = 0.145) between the two groups. However, analysis of pedigrees showed that more patients developed CRC in the LS families (P = 0.013), whereas more patients with extra-CRC were observed in the LLS families (P = 0.045). A higher prevalence of male patients was observed in the LLS families (P = 0.036).

Conclusions: LLS should be classified as a mixed entity, containing cases of true LS, other hereditary cancer syndromes, and sporadic CRC. The high risks of CRC and extra-CRC, found in our study, suggest that aggressive surgical management should be performed for LLS probands. In addition, we recommend a stringent surveillance protocol that should involve regular follow-ups of the intestine and other organs for probands and affected family members.

Background

Lynch syndrome (LS) is an autosomal dominantly inherited predisposition to colorectal cancer (CRC), accounting for approximately 2–5% of all CRC cases [1, 2]. The consequent tumors present the phenotypes of mismatch repair (*MMR*) protein deficiency and microsatellite instability (MSI). However, there is a lack of information on pathogenic variants (PVs) in *MMR* genes for up to 50–70% of patients with *MMR*-deficient CRC tumors who were identified in population-based studies [1, 3]. The majority of cases in this subset are characterized by hypermethylation of the *MLH1* promoter, which is also observed in approximately 15% of sporadic CRC cases [4, 5]. Variants in the *BRAF* oncogene are able to distinguish LS from sporadic *MMR*-deficient CRC; this has been demonstrated to be a powerful method for screening patients with LS [6, 7]. A subset of patients with CRC, who manifest the *MMR* deficiency but have no identified germline variant in either *MMR* genes or the *BRAF* gene (absence of MLH1 methylation), have been defined as having Lynch-like syndrome (LLS) [1, 3, 8]. It has been reported that LLS may account for up to 70% of clinically suspected LS cases with a high MSI and an *MMR*-deficient profile [9].

Molecular etiology of LLS still remains unknown, although previous findings have revealed that some groups of patients with LLS may be a mixture of true LS cases, with non-detected germline variants, and sporadic CRC cases [8, 10]. Some researchers found that the risk of CRC was lower in families with LLS than in those with genetically confirmed LS [10, 11], while the age of CRC onset was similar for both diseases [12, 13]. Nevertheless, the inability to determine the etiology of LLS hampers the development of effective screening and management policies for patients with LLS and the implementation of surveillance recommendations for these individuals and their affected relatives.

In the last decade, a wider application of multigene panel tests has provided more accurate molecular evidence for the diagnosis of LS; in the meantime, a considerable number of patients with LLS were identified at our center. Even though the genetic etiology of LLS is not defined, analyses of molecular, clinical, and pathological characteristics, as well as pedigrees of patients, may help guide decision making regarding surgical management, surveillance, and other interventions to reduce the future risks of cancer. This study was undertaken to compare the features of LS and LLS at the largest hereditary CRC research center of China, which could provide more information for the comprehensive understanding of LLS and guide management decisions for LS and LLS patients.

Methods

Patients

Between June 2008 and September 2018, a total of 139 patients with suspected LS and *MMR*-deficient profiles underwent curative surgeries, depending on the location of tumors, at the Fudan University Shanghai Cancer Centre. Multigene panel testing that included 139 genes was performed for all patients and some of their affected relatives. Informed consent for genetic analyses was obtained from all the patients. For patients with *MMR* deficiency variants in the *MLH1* or *MLH1* and *PMS2* genes, detection of *BRAF V600* variants was performed to exclude sporadic CRC.

The inclusion criteria of our study were as follows: (a) CRC confirmed by post-operative pathology; (b) *MMR* deficiency confirmed by immunohistochemistry; (c) the wild-type *BRAF V600* variant confirmed in patients without PVs in *MMR* genes. A total of 128 patients who met the inclusion criteria were enrolled in this study. Among these, 47 (36.7%) patients who were found to carry PVs in *MMR* genes were classified into the LS group, and 81 (63.3%) patients without PVs in *MMR* genes and without *BRAF V600* variants were classified into the LLS group. Carriers of variants of unknown significance (VUS) in *MMR* genes were also classified into the LLS group. Eleven patients without PVs in *MMR* genes but carrying a *BRAF* variant were excluded.

Data Collection and Follow-up

For the 128 enrolled patients, the demographic information, pathological results, and tumor histories were retrospectively collected. The pedigrees of their families were obtained through interviews of patients and their first- and second-degree relatives, including children, siblings, parents, grandparents, aunts, and uncles. The patient and each relative were asked to report whether the relative had ever been diagnosed with cancer.

For each relative, the sex of the patient, type of cancer, and age at diagnosis were recorded. Pathology documentation of cancers among relatives was systematically collected, if available.

Follow-ups were conducted for all recruited patients every 2–3 months. During the follow-up evaluation, the occurrence of metachronous CRC, distant metastases, and extra-CRC was recorded. Treatment options for these events were formulated based on the recommendations of our multidisciplinary team. Meanwhile, new cases of tumors in their families were noted, and next-generation sequencing (NGS) was recommended for these patients. This study was censored on May 30, 2020.

Next-generation sequencing

Peripheral blood (10 mL) was collected, stored in ethylenediaminetetraacetic acid tubes, and allowed to stand at 25°C for 2 h. The supernatant was transferred to a 15-mL centrifuge tube and then centrifuged for 10 min at 2200 *g* at 4°C. Thereafter, the intermediate white blood cells were transferred to a 1.5-ml centrifuge tube. The DNA was recovered using the MagPure FFPE DNA LQ Kit (Magen). NGS was conducted on the germline DNA as a standard genetic testing for germline analysis.

DNA quantification was performed using the Qubit 2.0 Fluorimeter with the dsDNA HS assay kits (Life Technologies, Carlsbad, CA). A minimum of 50 ng of DNA was required for NGS library construction. DNA shearing was performed using Covaris M220, followed by end repair, phosphorylation, and adaptor ligation. Fragments measuring 200-400 bp were selected using AMPure beads (Agencourt AMPure XP Kit), followed by hybridization with capture probes baits, hybrid selection with magnetic beads, and PCR amplification. The quality and size range of amplified fragments were then assessed by performing bioanalyzer high-sensitivity DNA assay. Paired-end sequencing of the indexed samples was performed on a NextSeq 500 sequencer (Illumina, Inc., USA).

Sequence data were mapped to the reference human genome (hg19) using BWA aligner 0.7.10. Local alignment optimization was performed using GATK 3.2. Germline SNVs were identified using Varscan with default parameters. Germline indels were identified using Varscan and GATK. Pathogenic variants were determined by a clinical molecular geneticist according to the guidelines of the American College of Medical Genetics. ClinVar and Enigma were used during manual curation for final confirmation of the results. The InSIGHT database was used for the pathogenicity classification of the *MMR* genes.

Prediction of Pathogenicity

The pathogenicity was predicted for all detected variants using two commonly used tools, SIFT and PolyPhen2.

BRAF Variant Analysis

In all cases, surgical cancer tissues were used for the *BRAF* variant analysis. BRAF exon 15 was bidirectionally sequenced using an ABI 3730XL instrument and the BigDye Terminator v. 3.1 cycle sequencing kit (Applied Biosystems, Carlsbad, CA, United States). Three independent experiments were performed to confirm positive samples. DNA from patients was tested using the AmoyDx *BRAF* variant

detection kit (Amoy Diagnostics, Xiamen, China) based on the principles of the amplification-refractory variant system. All results were confirmed according to the criteria suggested by the manufacturer.

Statistical analysis

Continuous variables were reported as mean ± standard deviation. Differences in categorical variables and continuous variables between these two groups were analyzed with the Chi square test or Fisher's exact test and with Student's t test, respectively, using the SPSS version 21.0 software (SPSS, Chicago, IL, USA). Two-tailed P values less than 0.05 were considered statistically significant.

Results

Molecular Characteristics

In the LS group, PVs of *MLH1* were identified in 17 (36.2%) probands, and those of *MSH2*, *MSH6*, and *PMS2* were identified in 18 (38.3%), 10 (21.3%), and 2 (4.2%) probands, respectively.

In the LLS group, a total of 52 variants were detected in 44 (54.3%) individuals, of which eight patients carried multiple variants. Among the 52 variants, 17 were VUS in *MMR* genes, including 8 in *MLH1*, 5 in *MSH2*, 3 in *MSH6*, and 1 in *PMS2*. Of the 17 VUS in the *MMR* genes, 8 were predicted to be possibly/probably damaging using PolyPhen2, and 4 were predicted to be deleterious using SITF. Other than *MMR* genes, the most frequently mutated genes were *MUYTH*, *POLE*, *BRCA2*, and *GJB2*. There were three biallelic missense variants in *MUYTH* (*p.Arg19Ter*, *p.Gly272Glu*, and *p.Gln267Ter*), two frameshift variants in *GJB2* (*p.His100fs* and *p.Leu79Cysfs*), and one frameshift variant in *RAD50* (*p.Glu995fs*), which were defined as pathogenic. One case of a missense variant in *BUB1B* (*p.Arg550Gln*) was defined as likely pathogenic. Using PolyPhen2, 22 variants were predicted to be possibly/probably damaging, among which most were mutations in the *MLH1* and *POLE* genes. Using SIFT, 20 variants were predicted to be deleterious, among which most were mutations in the *POLE*, *MUTYH*, and *GJB2* genes. All the variants and prediction of their deleteriousness in the 44 patients from the LLS group are summarized in Table 1.

Table 1
Variants and prediction of deleteriousness in 44 patients of LLS group.

Gene	Variants (HGVS)	Clinvar	Polyphen2#	SIFT*
MLH1	*NC_000003.12:g.37050541T > G (p.Val720Gly)*	VUS	Possibly damaging (0.892)	Deleterious (-5.056)
APC	*NC_000005.10:g.112843926G > T (p.Ala2778Ser)*	VUS	Probably damaging (1.000)	Neutral (-0.824)
MLH1	*NC_000003.11:g.37035002A > G (5 Prime UTR Variant)*	VUS	unpredictable	unpredictable
MLH1	*NC_000003.11:g.37035004C > T(5 Prime UTR Variant)*	VUS	unpredictable	unpredictable
MLH1	*NC_000003.11:g.37035057G > T(p.Val7Phe)*	VUS	Possibly damaging (0.485)	Neutral (-1.603)
MLH1	*NC_000003.11:g.37035051G > C(p.Ala5Pro)*	VUS	Possibly damaging (0.859)	Neutral (-1.578)
MLH1	*NC_000003.11:g.37061844A > G (p.Thr310Ala)*	VUS	Probably damaging (1.000)	Deleterious (-4.886)
MLH1	*NC_000003.12:g.37012077A > G (p.Ile219Val)*	VUS	Benign (0.018)	Neutral (-0.460)
MLH1	*NC_000003.11:g.37092140G > A (p.Arg687Gln)*	VUS	Possibly damaging (0.819)	Neutral (-1.040)
MSH2	*NC_000001.10:g.236912509G > T(p.Arg534Leu)*	VUS	Probably damaging (1.000)	Deleterious (-6.708)
MSH2	*LRG_218:g.4955G > A (5Prime UTR Variant)*	VUS	unpredictable	unpredictable
MSH2	*NC_000002.11:g.47630246G > A(5 Prime UTR Variant)*	VUS	unpredictable	unpredictable
MSH2	*NC_000002.11:g.47703650G > A (p.Ser717Asn)*	VUS	Benign (0.263)	Neutral (-2.425)
MSH2	*NC_000002.11:g.47703539G > C(p.Arg680Pro)*	VUS	Probably damaging (1.000)	Deleterious (-6.534)
MSH6	*NC_000002.12:g.47783304C > T(p.Ser24Leu)*	VUS	Benign (0.007)	Neutral (-0.535)

These data are presented as prediction (predicted score); *these data are presented as prediction (PROVEAN score).

Gene	Variants (HGVS)	Clinvar	Polyphen2#	SIFT*
MSH6	*NC_000002.11:g.48033801_48033825del (Splice Donor Variant)*	VUS	unpredictable	unpredictable
RAD50	*NC_000005.10:g.132609343_132609346del (p.Glu995fs)*	Pathogenic	unpredictable	Deleterious (-6.228)
MSH6	*NC_000002.12:g.47795968C > T(p.Arg178Cys)*	VUS	Probably damaging (0.974)	Neutral (-1.577)
NSD1	*NC_000005.10:g.177211614G > A (p.Arg1072Gln)*	VUS	Benign (0.009)	Deleterious (0.041)
PMS2	*NC_000007.14:g.5987462G > A (p.His435Tyr)*	VUS	Benign (0.017)	Neutral (-0.795)
MUTYH	*NC_000001.10:g.45800123G > A (p.Arg19Ter)*	Pathogenic	unpredictable	Deleterious (-2.625)
MUTYH	*NC_000001.10:g.45797914C > T (p.Gly272Glu)*	Pathogenic	Probably damaging (1.000)	Deleterious (-7.553)
MUTYH	*NC_000001.10:g.45797972G > A(p.Gln267Ter)*	Pathogenic	unpredictable	Deleterious (-9.745)
MUTYH	*NC_000001.11:g.45334474C > T(p.Pro18Leu)*	VUS	Benign (0.006)	Neutral (-1.436)
MUTYH	*NC_000001.10:g.45797760T > C(5 Prime UTR Variant)*	VUS	unpredictable	unpredictable
TP53	*NC_000017.10:g.7579705C > T (p.Val31Ile)*	VUS	Benign (0.001)	Neutral (-0.142)
GJB2	*NC_000013.10:g.20763421_20763422del (p.His100fs)*	Pathogenic	unpredictable	Neutral (-2.065)
GJB2	*NC_000013.10:g.20763488del (p.Leu79fs)*	Pathogenic	unpredictable	Deleterious (-13.990)
GJB2	*NC_000013.10.20763486G>-(p.Leu79Cysfs)*	Pathogenic	unpredictable	Deleterious (-13.990)
GJB2	*NC_000013.10:g.20763150A > G (p.Phe191Leu)*	VUS	Probably damaging (1.000)	Deleterious (-5.719)
ATM	*NC_000011.10:g.108250816C > T(p.Arg451Cys)*	VUS	Probably damaging (1.000)	Neutral − 2.171

These data are presented as prediction (predicted score); *these data are presented as prediction (PROVEAN score).

Gene	Variants (HGVS)	Clinvar	Polyphen2#	SIFT*
POLE	*NC_000012.11:g.133215747C > T(p.Arg1839His)*	VUS	Probably damaging (0.993)	Deleterious (-4.652)
POLE	*NC_000012.11:g.133225520G > A(p.Arg1382Cys)*	VUS	Probably damaging (0.994)	Deleterious (-5.698)
POLE	*NC_000012.11:g.133201290C > T(p.Gly2285Asp)*	VUS	Benign (0.000)	Neutral (-0.437)
POLE	*NC_000012.11:g.133250198G > A(p.Pro441Leu)*	VUS	Probably damaging (1.000)	Deleterious (-8.776)
POLE	*NC_000003.11:g.37090494C > G(p.Pro697Ala)*	VUS	Benign (0.000)	Deleterious (-3.425)
POLE	*NC_000012.12:g.132676107T > C(p.Asn336Ser)*	VUS	Probably damaging (1.000)	Deleterious (-4.472)
POLD1	*NC_000023.10:g.152034432G > A (p.Gly205Ser)*	VUS	Benign (0.150)	Neutral (-0.030)
BRCA1	*NC_000017.11:g.43106514G > A(p.Leu52Phe)*	VUS	Probably damaging (1.000)	Neutral (-0.587)
BRCA2	*NC_000013.10:g.32913723G > T(p.Ser1744Ile)*	VUS	Benign (0.048)	Neutral (-2.182)
BRCA2	*NC_000013.10:g.32906967G > A(p.Ser451Asn)*	VUS	Benign (0.009)	Neutral (-0.431)
ATR	*NC_000003.11:g.142172064G > C(p.Thr2556Ser)*	VUS	Possibly damaging (0.830)	Neutral (-1.301)
EPCAM	*NC_000002.11:g.47600631G > A (p.Val36Ile)*	VUS	Benign (0.002)	Neutral (-0.350)
MSH3	NC_000005.10:g.80813659T > G(p.Leu911Val)	VUS	Benign (0.174)	Deleterious (-2.721)
PMS1	NC_000002.11:g.190649220G > A(5 Prime UTR Variant)	VUS	unpredictable	unpredictable
APC	NC_000005.10:g.112843926G > T(p.Ala2778Ser)	VUS	Probably damaging (1.000)	Neutral (-0.824)
SMAD4	NC_000018.10:g.51059908A > G (p.Asn316Ser)	VUS	Benign (0.002)	Neutral (-0.829)

These data are presented as prediction (predicted score); *these data are presented as prediction (PROVEAN score).

Gene	Variants (HGVS)	Clinvar	Polyphen2[#]	SIFT*
BUB1B	NC_000015.10:g.40202609G > A(p.Arg550Gln)	Likely benign	Benign (0.001)	Neutral (0.332)
CDH1	NC_000016.10:g.68811716G > A (p.Ala289Thr)	VUS	Probably damaging (0.932)	Neutral (-2.459
CDH1	NC_000016.9:g.68856080C > G(p.Leu630Val)	VUS	Probably damaging (0.998)	Deleterious (-2.726)
CHEK2	NC_000022.10:g.29083956G > A (p.Arg521Trp)	VUS	Probably damaging (1.000)	Deleterious (-4.126)
DICER1	NC_000014.8:g.95590896T > G(p.Glu338Ala)	VUS	Benign (0.310)	Neutral (-0.642)

[#] These data are presented as prediction (predicted score); *these data are presented as prediction (PROVEAN score).

The distribution of *MMR* deficiencies in the two groups was compared, and the results are summarized in Table 2. A total of 19.1% (9/46) of the patients in the LS group manifested deficiency in *MSH6* by immunohistochemistry, which was significantly higher than that (7.4%, 6/81) in the LLS group ($\chi^2 = 3.963$, $P = 0.046$). No significant differences were observed in case of other *MMR* deficiencies.

Table 2
Distribution of *MMR* deficiency in the two groups.

MMR deficiency	LS group (N = 47)	LLS group (N = 81)	χ^2 value	p value
MLH1/PMS2			0.482	0.487
Presence	14(29.8%)	29(35.8%)		
MSH2/MSH6			0.182	0.670
Presence	12(25.5%)	18(22.2%)		
Isolated *MLH1*			0.504	0.478
Presence	2(4.3%)	6(7.4%)		
Isolated *MSH2*			0.019	0.891
Presence	5(10.6%)	8(9.9%)		
Isolated *MSH6*			3.963	0.046
Presence	9(19.1%)	6(7.4%)		
Isolated *PMS2*			2.044	0.153
Presence	3(6.4%)	12(14.8%)		
Other			0.313	0.576
Presence	2(4.3%)	2(2.5%)		
LS: Lynch syndrome, LLS: Lynch-like syndrome.				

Demographic And Clinical Characteristics

The demographic and clinical characteristics of the 128 enrolled patients were compared between the LS and LLS groups and are summarized in Table 3. There were significant differences in the proportion of patients with the earliest onset age of CRC and in the primary CRC location between the two groups. In the LS group, 74.5% (35/47) of the patients were characterized by early-onset (< 50 years old) CRC, which was significantly higher than the proportion (53.1%, 43/81) found in the LLS group (χ^2 = 5.712, P = 0.017). In the LLS group, 25.9% (21/81) of the patients developed primary CRC in the rectum, which was remarkably higher than the proportion (10.6%, 5/47) found in the LS group (χ^2 = 2.358, P = 0.046).

Table 3
Demographic and clinical characteristics of 128 patients with colorectal cancer in the two groups.

Characteristic	LS group (N = 47)	LLS group (N = 81)	χ^2 / t value	P value
Gender			0.985	0.321
Male	26(55.3%)	52(64.2%)		
Female	21(44.7%)	29(35.8%)		
Age(years)[a]	44.36 ± 11.26	48.12 ± 13.09	-1.715	0.089
< 50	35(74.5%)	43(53.1%)	5.712	0.017
≥ 50	12(2.5.5%)	38(46.9%)		
Diagnostic criteria			4.297	0.117
Amsterdam I	11(23.4%)	9(11.1%)		
Amsterdam II	23(43.9%)	39(48.1%)		
Bethesda	13(27.7%)	33(40.8)		
CEA (ng/ml)			0.145	0.714
< 5.2	7(14.9%)	15(18.5%)		
≥ 5.2	40(85.1%)	66(81.5%)		
Location of colorectal cancer			7.994	0.046
Right colon	18(38.3%)	32(39.5%)		
Left colon	20(42.6%)	19(23.5%)		
Rectal	5(10.6%)	21(25.9%)		
Multiple	4(8.5%)	9(11.1%)		
Multiple tumors			2.358	0.125
Occurrence	12(25.5%)	12(14.8%)		
Absence	35(74.5%)	69(85.2%)		
Tumor size [a](cm)	5.17 ± 2.61	5.11 ± 2.49	0.127	0.899
Pathological classification			5.156	0.076

[a] These data are presented as mean ± standard deviation; other values are presented as number of patients followed by percentage in parentheses.

LS: Lynch syndrome, LLS: Lynch-like syndrome.

Characteristic	LS group (N = 47)	LLS group (N = 81)	χ^2 / t value	P value
Adenocarcinoma	34(72.3%)	66(81.5%)		
Adenocarcinoma with partial mucinous adenocarcinoma	5(10.7%)	11(13.6%)		
Mucinous adenocarcinoma	8(17.0%)	4(4.9%)		
Differentiation			0.365	0.833
Well differentiated	1(2.1%)	3(3.7%)		
Moderately differentiated	28(59.6%)	45(55.6%)		
Poorly differentiated	18(38.3%)	33(40.7%)		
Cancerous node			1.780	0.182
Occurrence	2(4.3%)	9(11.1%)		
Absence	45(95.7%)	72(88.9%)		
Vascular invasion			0.045	0.832
Occurrence	8(17.0%)	15(18.5%)		
Absence	39(83.0%)	66(81.5%)		
Perineural invasion			0.079	0.779
Occurrence	6(12.8%)	9(11.1%)		
Absence	41(87.2%)	72(88.9%)		
T stage			0.804	0.669
T1	7(14.9%)	8(9.9%)		
T2	8(17.0%)	13(16.0%)		
T3	32(68.1%)	60(74.1%)		
N stage			0.911	0.634
N0	34(72.3%)	53(65.4%)		
N1	9(19.1%)	17(21.0%)		
N2	4(8.6%)	11(13.6%)		

[a] These data are presented as mean ± standard deviation; other values are presented as number of patients followed by percentage in parentheses.

LS: Lynch syndrome, LLS: Lynch-like syndrome.

Characteristic	LS group (N = 47)	LLS group (N = 81)	χ^2 / t value	P value
Metastasis			0.313	0.576
Occurrence	2(4.3%)	3(3.7%)		
Absence	45(95.7%)	78(96.3%)		
TNM stage			1.152	0.764
I	13(27.7%)	16(19.8%)		
II	17(36.2%)	32(39.5%)		
III	15(31.8%)	30(37.0%)		
IV	2(4.3%)	3(3.7%)		
[a] These data are presented as mean ± standard deviation; other values are presented as number of patients followed by percentage in parentheses.				
LS: Lynch syndrome, LLS: Lynch-like syndrome.				

Pathological Characteristics

Comparison of the pathological results showed no significant differences in the pathological TNM stage (χ^2 = 1.152, P = 0.764) and differentiation of the CRC tumors (χ^2 = 0.365, P = 0.833) between the two groups. The proportion of patients with mucinous CRC was 17.0% (8/47) in the LS group, which was higher than that (4.9%, 4/81) in the LLS group, whereas the proportions of patients with adenocarcinoma and partial mucinous CRC were similar between the two groups. Thus, no significant differences were observed in pathological classification (χ^2 = 5.516, P = 0.076). The pathological characteristics of the CRC tumors in the two groups are summarized in Table 3.

Primary And Metachronous Crc In Probands

During the follow-up period, 34.0% (16/47) of the patients in the LS group and 38.3% (31/81) in the LLS group developed metachronous CRC, with no significant difference observed between the groups (χ^2 = 0.229, P = 0.632). The period between the occurrence of primary and metachronous CRC was 28.78 ± 29.14 months in the LS group and 38.58 ± 24.89 months in the LLS group, with no significant difference being observed between the groups (t = − 1.033, P = 0.108).

The mean age of cancer onset was 43.40 ± 11.17 years in the LS group and significantly higher (47.56 ± 12.99 years) in the LLS group (t = − 2.008, P = 0.049). The locations of the metachronous CRC tumors were similar to those of primary CRC. In the LLS group, 38.3% (31/81) of the patients developed rectal cancer,

which was markedly higher than the proportion (17.0%, 8/47) found in the LS group (χ^2 = 6.340, P = 0.012). The tumor histories in the probands from the two groups are summarized in Table 4.

Table 4
Comparison of patients' tumor histories between LS group and LLS group.

Characteristic	LS group (N = 47)	LLS group (N = 81)	χ^2 / t value	p value
Earliest onset age of CRC (years)[a]	44.36 ± 11.26	48.12 ± 13.09	-1.175	0.089
Total number of CRCs[a]	1.55 ± 0.75	1.51 ± 0.59	0.392	0.696
Metachronous CRC			0.229	0.632
Occurrence	16(34.0%)	31(38.3%)		
Right colon cancer			0.001	0.972
Occurrence	28(59.6%)	48(59.3%)		
Left colon cancer			2.505	0.113
Occurrence	30(63.8%)	40(49.4%)		
Rectal cancer			6.340	0.012
Occurrence	8(17.0%)	31(38.3%)		
Synchronous or metachronous CRC			0.118	0.874
Occurrence	20(42.6%)	37(45.7%)		
Earliest onset age of extra-colorectal cancer (years)[a]*	48.45 ± 12.68	49.79 ± 10.28	-0.345	0.732
Synchronous or metachronous extra-colorectal cancer			2.128	0.145
Occurrence	11(23.4%)	29(35.8%)		
Earliest onset age of cancer (years) [a]	43.40 ± 11.17	47.56 ± 12.99	-2.008	0.049
Total number of cancers [a]	1.89 ± 1.03	1.96 ± 0.94	-0.389	0.698

[a] These data are presented as mean ± standard deviation; other values are presented as number of patients followed by percentage in parentheses. * These data are limited to patients who developed extra-colorectal cancer. LS: Lynch syndrome, LLS: Lynch-like syndrome, CRC: colorectal cancer.

Extra-crc In Probands

In the LS group, 11 patients developed 15 cases of primary extra-CRC, including 5 cases of endometrial cancer, 5 cases of gastric cancer, 2 cases of small intestinal cancer, and 1 case each of ovarian, breast, and cutaneous cancer. In the LLS group, 29 patients developed 29 cases of extra-CRCs, including 8 cases of gastric cancer, 6 cases of endometrial cancer, 4 cases each of small intestinal and breast cancer, 2 cases each of prostate and ovarian cancer, and 1 case each of ureteral carcinoma, renal cancer, and pancreatic cancer. The proportions of synchronous or metachronous extra-CRC were 23.4% (11/47) in the LS group and 35.8% (29/81) in the LLS group, with no significant difference observed between the groups ($\chi^2 = 2.128$, $P = 0.145$).

Family Pedigrees

A total of 142 first- and second-degree relatives who developed LS-associated cancer in the LS families and 210 of those in the LLS families were enrolled in the pedigree analysis.

In the LS families, the mean number of patients who developed CRC was 3.26 ± 2.08, which was significantly higher than that (2.42 ± 1.65) in the LLS families ($t = 2.506$, $P = 0.013$). The mean earliest age of CRC onset was 37.53 ± 8.63 years in the LS families, which was significantly lower than that (44.51 ± 13.64 years) in the LLS families ($t = -3.156$, $P = 0.002$). In terms of the tumor distribution, left colon cancer was observed in 91.5% (43/47) of the LS families, which was significantly more frequent than that (70.4%, 57/81) in the LLS families ($\chi^2 = 7.762$, $P = 0.005$).

In addition to CRC, the mean number of patients who developed extra-CRC was 1.59 ± 1.38 in the LLS families, which was significantly higher than that (1.09 ± 1.37) in the LS families ($t = -2.017$, $P = 0.045$). The pedigrees of the LS and LLS families were compared, and the results are summarized in Table 5.

Table 5
Comparison of pedigrees between the LS group and LLS group.

Variable	LS group (N = 47)	LLS group (N = 46)	t/χ^2 value	p value
Patients with cancer (cases) [a]	4.02 ± 2.48	3.59 ± 1.99	1.073	0.285
Male patients(cases) [a]	2.28 ± 1.72	2.04 ± 1.63	0.786	0.433
Female patients (cases) [a]	1.74 ± 1.42	1.54 ± 1.32	0.808	0.421
First degree relatives (cases)[a]	1.98 ± 1.69	1.79 ± 1.58	0.635	0.526
Second degree relatives (cases)[a]	1.04 ± 1.55	0.80 ± 1.23	0.968	0.335
Cases of cancer[a]	5.13 ± 3.10	4.83 ± 2.84	0.558	0.578
Patients with CRC (cases) [a]	3.26 ± 2.08	2.42 ± 1.65	2.506	0.013
Cases of CRC[a]	3.91 ± 2.54	3.12 ± 2.40	2.109	0.047
Patients with right colon cancer (cases) [a]	1.45 ± 1.02	1.27 ± 1.13	0.877	0.382
Cases of right colon cancer[a]	1.49 ± 1.06	1.32 ± 1.31	0.749	0.455
Right colon cancer			1.356	0.244
Occurrence	38(80.9%)	58(71.6%)		
Patients with left colon cancer (cases) [a]	1.72 ± 1.19	0.98 ± 0.87	3.764	< 0.001
Cases of left colon cancer[a]	1.94 ± 1.54	1.04 ± 1.01	3.588	0.001
Left colon cancer			7.762	0.005
Occurrence	43(91.5%)	57(70.4%)		
Patients with rectal cancer (cases) [a]	0.49 ± 0.69	0.77 ± 0.99	-1.688	0.094
Cases of rectal cancer[a]	0.49 ± 0.69	0.77 ± 0.99	-1.688	0.094
Rectal cancer			2.608	0.106
Occurrence	18(38.3%)	43(53.1%)		
Patients with extra-colorectal cancer (cases) [a]	1.09 ± 1.37	1.59 ± 1.38	-2.017	0.046

[a] These data are presented as mean ± standard deviation; other values are presented as number of patients followed by percentage in parentheses. ***** These data are limited to families that developed extra-colorectal cancer.

LS: Lynch syndrome, LLS: Lynch-like syndrome, CRC: colorectal cancer.

Variable	LS group (N = 47)	LLS group (N = 46)	t/χ^2 value	p value
Cases of extra-colorectal cancers[a]	1.21 ± 1.49	1.70 ± 1.52	-2.005	0.045
Extra-colorectal cancer			1.140	0.286
Occurrence	30(63.8%)	59(72.8%)		
Synchronous or metachronous CRC			0.060	0.807
Occurrence	21(44.7%)	38(46.9%)		
Synchronous or metachronous extra-colorectal cancer			1.948	0.163
Occurrence	15(31.9%)	36(44.4%)		
Earliest onset age of cancer (years) [a]	36.66 ± 8.75	41.60 ± 11.91	-2.690	0.008
Earliest onset age of CRC (years) [a]	37.53 ± 8.63	44.51 ± 13.64	-3.156	0.002
Earliest onset age of extra-colorectal cancer (years)*	45.00 ± 10.27	47.97 ± 10.09	-1.303	0.196
[a] These data are presented as mean ± standard deviation; other values are presented as number of patients followed by percentage in parentheses. * These data are limited to families that developed extra-colorectal cancer.				
LS: Lynch syndrome, LLS: Lynch-like syndrome, CRC: colorectal cancer.				

Analysis of the sex distribution showed that the mean number of the male patients in the LLS families was 2.04 ± 1.63, which was significantly higher than that (1.54 ± 1.32) of the female patients (t = 2.116, P = 0.036). In the LS families, the mean numbers of the male and female patients were 2.28 ± 1.72 and 1.74 ± 1.42, respectively, with no significant difference being observed (t = 1.637, P = 0.105).

Discussion

With respect to oncologic outcomes, *MMR*-deficient CRC is associated with a better prognosis and therapeutic responses because the *MMR* pathway is involved in triggering cell death after chemotherapy-induced DNA damage [14]. The prognosis in patients with *MMR*-deficient CRC tends to be better, with regard to stage-for-stage comparison, than in those with *MMR*-proficient cancer [14]. Patients with early-stage *MMR*-deficient CRC do not appear to benefit from adjuvant 5-Fluorouracil monotherapy [15]; however, in some patients with metastatic *MMR*-deficient CRC, treatment with immune checkpoint inhibitors has been associated with an excellent response [16].

However, a considerable number of *MMR*-deficient CRC tumors have an unknown etiology, other than confirmed LS and methylation of MLH1. In our study, a high proportion of patients with *MMR*-deficient CRC

were diagnosed as having LLS, which was consistent with the data of a previous study [9]. Therefore, multigene panel testing should be recommended for all *MMR*-deficient patients to distinguish LS and LLS.

While management of LS has been well described, the inability to define the molecular basis of the LLS entity not only hampers the appropriate clinical management of probands, but also the cancer screening recommendations for affected families. Comparison of clinical and molecular characteristics of patients with LLS and features of their CRC tumors with those of confirmed patients with LS can contribute to the development of appropriate management recommendations for patients with LLS and their affected family members.

The genetic causes of LLS are still unknown, although advanced NGS approaches have facilitated the discovery of novel genetic events that may allow the definition of clinical and molecular phenotypes of LLS. In our study, variants were unidentified in nearly half of the LLS cohort. Current techniques of analysis may be missing complex or cryptic variants in *MMR* genes, and some deep intronic variants may be overlooked [17, 18]. Furthermore, there may be some unidentified variants in the regulatory regions of *MMR* genes, which are hardly screened [19]. Thus, we suggest that this subset may have been a mixture of patients with LS, whose germline variants were not detected, and those with sporadic CRC. Future advances in NGS techniques may allow obtaining more accurate genetic information for discriminating between patients with LS and LLS.

Among the variants identified in this study, the largest category was VUS in *MMR* genes. The classification of these patients is still uncertain, and they were grouped as patients with LLS in the current study. Some of the patients carrying VUS in *MMR* genes may have been true patients with LS, which was supported by a high frequency of metachronous CRC. The pathogenicity of these VUS should be confirmed in functional experiments. The high frequency of metachronous CRC observed in our study suggests that patients with LLS should be considered high-risk cases, and strategies for cancer prevention must be implemented for this group of patients and their relatives. In clinical practice, aggressive surgical protocols, such as extended colectomy and subtotal colectomy, should be recommended. Platinum-based chemotherapy should be routinely applied, and treatment with immune checkpoint inhibitors may provide a considerable benefit to patients with metastatic *MMR*-deficient CRC. Meanwhile, stringent colonoscopy surveillance should be performed in patients with LLS, who carry both PVs and VUS in *MMR* genes.

In addition to *MMR* genes, most of the other PVs and likely PVs were detected in the *MUTYH* and *GJB2* genes. Biallelic *MUTYH* variants have been detected in 1.8–3.1% of patients with LLS [20, 21]. *MUTYH*-associated polyposis is extremely variable, ranging from severe polyposis coli to attenuated forms with a late age of onset or few adenomas, or CRC, which creates a phenotypic overlap with LS [20, 22]. *GJB2* encodes a gap junction protein, also known as connexin 26. Variants in this gene are responsible for as much as 50% of prelingual, recessive deafness [23]. The cytoplasmic Cx26 protein has been associated with the tumor progression and a poor prognosis in patients with breast cancer and esophageal squamous cell carcinoma [24, 25]. To the best of our knowledge, this is the first study to demonstrate the involvement of *GJB2*, as a novel candidate gene, in LLS-linked CRC. The pathogenicity of the frameshift variant in *GJB2* is being evaluated by functional analysis, and the results will be reported separately. Variants in the

exonuclease domain of the polymerase proofreading genes *POLE* and *POLD1* cause polymerase proofreading-associated polyposis, which is a dominant-inheritance and high-penetrance hereditary syndrome conferring a predisposition to attenuated colorectal polyposis and early-onset CRC [26]. The association between variants of polymerase proofreading genes and *MMR* deficiency has been reported previously [27]. In our study, VUS in the *POLE* and *POLD1* genes were predicted to be deleterious and were among the most frequently detected variants. Some other variants were identified in *BRCA1*, *BRCA2*, and *RAD50*, which are involved in the homologous recombination pathway. Defects in the *BRCA* genes are known to be pathogenic causes of hereditary breast and ovarian cancers [28], in addition to conferring a high risk of developing CRC [29].

Therefore, it is possible that some cases of LLS can be due to the pleiotropism of certain gene variants, manifesting as genetic overlaps with other hereditary cancer syndromes. Because of the mixture, a higher prevalence of extra-CRCs and a lower prevalence of CRCs were revealed in the LLS families. The high risk of extra-CRCs found in our study suggests that stringent surveillance of other organs should be recommended for probands and their affected family members. The surveillance regimen can be based on the gene variants and family history. For example, gastroduodenoscopy should be regularly performed in patients carrying *MUTYH* variants, while gynecological and breast examinations would be recommended for patients carrying *BRCA* variants. Furthermore, functional analysis of the undefined variants found in patients with LLS should be performed to elucidate the underlying molecular etiology of LLS.

The difference in the age at onset of CRC between patients with LS and LLS remains controversial; some studies demonstrated similar proportions of early-onset patients in the LS and LLS groups [13], whereas one report showed that the population of patients with LLS was older [30]. Our results supported the latter findings, with age differences being manifested in both probands and related family members. Variants in genes such as *POLE* and *BRCA*, which were found in patients with LLS, may confer a higher risk of CRC; however, these variants show moderate penetrance [31]. Because sporadic CRC is combined with moderate penetrance of other variants, a delayed onset of CRC was demonstrated in probands with LLS. It is noteworthy that more than half of the patients in the LLS group were early-onset cases, which is significantly higher than the reported rate of sporadic CRC [32]. Therefore, MSI and multigene panel testing should be recommended for the early-onset subset, and screening colonoscopy at an early age should be performed in affected family members.

In terms of the CRC localization, our study showed a striking clustering of tumors in the rectum of probands with LLS, indicating that the rectum as the preferred organ can be described as a clinical feature of LLS-associated CRC. A higher frequency of left colon cancer was consistent with the findings of our previous study, which investigated clinical features of LS in an Asian population [33]. While LS-associated CRC is characterized by mucinous differentiation [34], a reasonably lower proportion of mucinous tumors was observed in the LLS cohort in this study.

Another interesting finding was a larger number of male patients in LLS families. A higher prevalence of male patients in LS families was reported in a previous review [35], but has not been previously described in

LLS families. This discovery of the sex-dependent tendency of disease in LLS families indicates that more attention should be paid to the screening and surveillance of male members.

There are some limitations of our study. First, because this was a retrospective study, the potential bias in the selection of subjects could not be eliminated. Second, MSI testing was not performed, which may have resulted in an incorrect interpretation of the molecular evidence. Lastly, the sample size needs to be increased, and a long-term follow-up is required.

Conclusions

In conclusion, LLS should be classified as a mixed entity, containing cases of true LS, other hereditary cancer syndromes, and sporadic CRC. The high risks of CRC and extra-CRCs, which were found in this study, suggest that aggressive surgical management should be performed for probands with LLS, and a stringent surveillance protocol, which involves regular follow-ups of the intestine and other organs, should be recommended for probands and affected family members. The surveillance regimen can be based on gene variants and the family history. The preference for CRC development in the rectum should be described as a clinical feature of LLS. A higher prevalence of male patients was discovered, for the first time, in LLS families; this indicates that more attention should be paid to the screening and surveillance of male members.

Abbreviations

LS
Lynch syndrome; CRC:colorectal cancer; MMR:mismatch repair; MSI:microsatellite instability; PV:pathogenic variants; LLS:Lynch-like syndrome; VUS:variants of unknown significance; NGS:Next-generation sequencing

Declarations

Ethics approval and consent to participate

All examinations and treatments were conducted at the Fudan University Shanghai Cancer Center (Shanghai, China) and were in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of the Fudan University Shanghai Cancer Center. Written informed consent was obtained from all patients included in this study

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

YX, YX and FQL conceived and designed the study. YX, CL, YQZ, and TAG collected and analyzed the datas. YX provided statistical expertise and were involved in data analysis and interpretation of results. YX wrote the paper. CL, YX and FQL reviewed the paper. All authors have read and approved the manuscript.

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