

Pathogenic germline variants in mismatch repair genes in patients with microsatellite instability-high gastric cancer

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Abstract

Objective: Lynch syndrome (LS) increases the risk of various cancers, including colorectal cancer, endometrial cancer and gastric cancer (GC). The incidence of LS among microsatellite instability-high (MSI-H) GC and their association in South Korea remains underexplored. This study investigates LS-associated pathogenic germline variants in MSI-H GC patients using whole-exome sequencing (WES) on normal tissues.

Methods: This retrospective study included patients who underwent gastrectomy for GC at Soonchunhyang University Bucheon and Cheonan Hospitals from January 2011 to October 2023. Among 1,537 patients screened for MSI status, 127 (8.3%) were identified as MSI-H. WES was performed on normal tissues from 123 patients. Pathogenic/likely pathogenic (P/LP) variants in mismatch repair (MMR) genes were identified using *in silico* models and protein loss assessments in corresponding tumor tissues.

Results: Of the 127 MSI-H GC cases, characteristics aligned with typical MSI-H GC. The average age was 70.02 years, with 98 (77.2%) located in the lower body and 81 (63.8%) of the intestinal type. All five MSI markers were positive in 46.5% of cases, whereas four markers were positive in 27.6%. Of the MSI-H GCs, 10 LS candidates were identified. Three patients had known P/LP variants [*MLH1* (c.1758dup), *MSH6* (c.3261dup), *MSH2* (c.1241T>C)]. Seven patients had variants of unknown significance (VUS) in MMR genes. Six (4.9%) patients were identified as having LS or possible LS, including one patient with the *MLH1* (c.1153C>T) variant previously classified as VUS but now considered LS-associated.

Conclusions: This large-scale screening for LS in MSI-H GC patients using retrospective samples confirmed the lower incidence of LS than those of colorectal or endometrial cancer and GC patients in Western countries, emphasizing the need for clinical consideration in managing MSI-H GC patients.

Keywords: Lynch syndrome; microsatellite instability; gastric cancer; germline mutation; genetic screening

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Introduction

Lynch syndrome (LS) is a representative hereditary cancer syndrome caused by germline mutations in mismatch repair (MMR) genes that lead to tumors with microsatellite instability-high (MSI-H) characteristics (1). Historically, LS has been most closely associated with hereditary non-polyposis colorectal cancer (HNPCC), with previous research focusing primarily on its role in colorectal cancer (2). However, advances in genomics during the era of precision medicine have expanded our understanding of hereditary cancers, revealing that the spectrum of cancers associated with LS is broader than previously thought (3).

Gastric cancer (GC) is a key type of cancer that exhibits MSI-H characteristics (4,5). The association between this subtype and LS suggests that a subset of GC may also be associated with this hereditary condition. Nonetheless, research exploring this connection has been predominantly conducted in Western populations, where GC is relatively rare (3). Consequently, there is a lack of comprehensive data on the relationship between LS and GC, particularly in regions where GC is more prevalent, such as East Asia.

In East Asia, specifically South Korea, GC remains a major health concern, with significant advances in its diagnosis and treatment having been made in recent decades (6-9). Despite these developments, research on hereditary forms of GC, including LS, has been limited, in contrast to the extensive studies published on LS-associated colorectal and endometrial cancers focusing on MSI-H tumors (10-12). Notably, the prevalence of hereditary diffuse GC caused by *CDH1* mutations is high among Hispanic populations (13), but considerably lower among Koreans (14). This disparity highlights the fact that the prevalence of hereditary cancer syndromes can vary significantly among different ethnic groups. Consequently, there is a critical need for more comprehensive research to understand the genetic and epidemiological aspects of LS in GC within Asian populations, where such data are currently lacking. This study aimed to address this knowledge gap by examining the prevalence and characteristics of LS in patients with MSI-H GC in South Korea.

Materials and methods

Population

This retrospective study included patients who underwent

gastrectomy for GC between January 2011 and October 2023 at the Soonchunhyang University Bucheon and Cheonan Hospitals. Among these patients, those with a confirmed MSI status were selected for analysis. The inclusion criteria were as follows: 1) confirmation of MSI-H through polymerase chain reaction (PCR) testing; and 2) availability of normal formalin-fixed paraffin-embedded (FFPE) tissue suitable for germline testing. Normal tissue obtained from the resection margin was histologically verified as free of cancer cells. Institutional Review Board (IRB) approval was obtained from both Soonchunhyang University Bucheon Hospital (No. 2023-12-018-001) and Cheonan Hospital (No. 2023-12-065-001) to ensure compliance with ethical standards. Given the retrospective nature of the study, the IRBs waived the requirement for informed patient consent.

Clinicopathological variables

This study gathered clinicopathological data of patients, including variables such as sex, age, number of lesions, tumor size and location, number of retrieved and metastatic lymph nodes, extent of resection, Lauren classification, histology according to the World Health Organization (WHO) classification, depth of tumor invasion, and staging based on the American Joint Committee on Cancer (AJCC) 8th edition.

Definition of MSI-H

MSI-H status was determined using a PCR approach with a panel of markers. This included two mononucleotide repeat markers (BAT25 and BAT26) and three dinucleotide repeat markers (D5S346, D2S123, and D17S250) (15) or, alternatively, three quasi-monomorphic dinucleotide markers (NR21, NR24, and NR27) (16). MSI-H was defined as instability in ≥ 2 loci, microsatellite instability-low (MSI-L) as instability in 1 locus, and microsatellite instability-stable (MSS) as no instability, following the National Cancer Institute (NCI) guidelines.

Immunohistochemistry (IHC) of MMR genes in tumors

IHC was performed to assess the protein expression of MMR genes in tumors from patients carrying germline pathogenic (P) or likely pathogenic (LP) variants of these genes. The following antibodies were used: MLH1 (ready-to-use, clone M1; Roche, Indianapolis, IN, USA), MSH2 (ready-to-use, clone G219-1129; Roche), MSH6 (dilution 1:100, clone 44; Cell Marque, Rocklin, CA, USA), and

PMS2 (dilution 1:40, clone MRQ28; Cell Marque).

Whole-exome sequencing (WES)

WES was performed on histologically confirmed normal tissue samples from patients with MSI-H GC. Genomic DNA was extracted from FFPE tissues. SureSelect sequencing libraries were prepared according to the manufacturer's protocol using the Agilent SureSelect All Exon V6 kit (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed using an Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA) with read lengths of 2×100 base pairs (bp).

Sequencing reads were aligned to the human reference genome (hg38) using Burrows-Wheeler Aligner software (Version 0.7.17; Heng Li, Broad Institute, Cambridge, MA, USA). Post-alignment processing was performed using the bcbio-nextgen pipeline (Version 1.2.3; bcbio project) (17), which included duplicate read removal using the Picard's Mark Duplicates tool. Variant calling was performed using the Genome Analysis Toolkit (GATK) HaplotypeCaller (v4.2). High-confidence germline variants were identified following GATK Best Practices (18). This involved a series of filtering steps to ensure the accurate identification of germline variants (*Supplementary Figure S1*). The analysis focused on cancer-related genes by cross-referencing the identified variants with established cancer driver consensus gene lists (19). To refine the analysis to high-confidence heterozygous germline variants, an additional selection criterion based on the sequencing depth and variant allele frequency (VAF) was applied. Variants with a VAF between 0.4 and 0.6 were selected to ensure the identification of heterozygous variants. All identified variants were annotated using the ensembl variant effect predictor (VEP; Version 109, Ensembl, EMBL-EBI, Hinxton, Cambridge, UK) (20) with the AlphaMissense plugin (AlphaMissense, DeepMind, London, UK) (21), combined annotation dependent depletion (CADD, v1.6) (22), and rare exome variant ensemble learner (REVEL, v1.3) (23). These tools provide comprehensive functional annotations and predict whether the variants are deleterious. Finally, we focused on MMR genes (*MLH1*, *PMS2*, *MSH2*, *MSH6*, and *EPCAM*) to identify LS. The minor allele frequencies (MAF) for the Eastern Asian (EAS) population and overall populations were obtained from the gnomAD database (v4.1.0) (24). Rare variants were defined as those with an MAF of <0.001.

Germline variants were classified as P, LP, variant of uncertain significance (VUS), likely benign, or benign

according to the American College of Medical Genetics and Genomics (25). The ClinVar database was used to identify the known P/LP variants (26).

Statistical analysis

All statistical analyses and graphical representations were performed using the R software (Version 4.1.2; R Foundation for Statistical Computing, Vienna, Austria). Descriptive statistics were used for categorical and continuous variables. Categorical variables were summarized as frequencies and percentages, while continuous variables were expressed as the $\bar{x} \pm s$. Graphical representations were generated using the ggplot2 package.

Results

Baseline characteristics of MSI-H GC patients

Of the 1,537 patients included in the study, 127 (8.3%) had MSI-H GC. The baseline characteristics of the patients are summarized in *Supplementary Table S1*. Consistent with the known characteristics of MSI-H GC (27,28), our cohort exhibited similar patterns. The mean age of the MSI-H patients was 70.02 years, and most patients (97.6%) had a single tumor with a mean tumor size of 4.26 cm. Most tumors (77.2%) were located in the lower body of the stomach; thus, most patients (n=116, 91.3%) underwent distal gastrectomy. According to the Lauren classification, the predominant type was the intestinal type, which was observed in 81 (63.8%) patients, and differentiated histology, defined as tubular adenocarcinoma including well-differentiated (WD) and moderately differentiated (MD) types, was present in 83 (65.4%) patients. The most common depth of tumor invasion was T1, which was observed in 53 (41.7%) patients. The N0 stage was the most frequent, occurring in 88 (69.3%) patients. Overall, stage I was the most prevalent, with 77 (60.6%) patients classified at this stage.

MSI marker analysis

Among the five markers used to define MSI, the most common finding was instability in all five markers, observed in 46.5% of the cases (*Figure 1A*). Instability of the four markers was noted in 27.6% of cases. Specifically, the mononucleotide markers BAT26 and BAT25 exhibited the highest rates of instability, with 92.1% and 81.9% cases (*Figure 1B*), respectively. In contrast, the dinucleotide

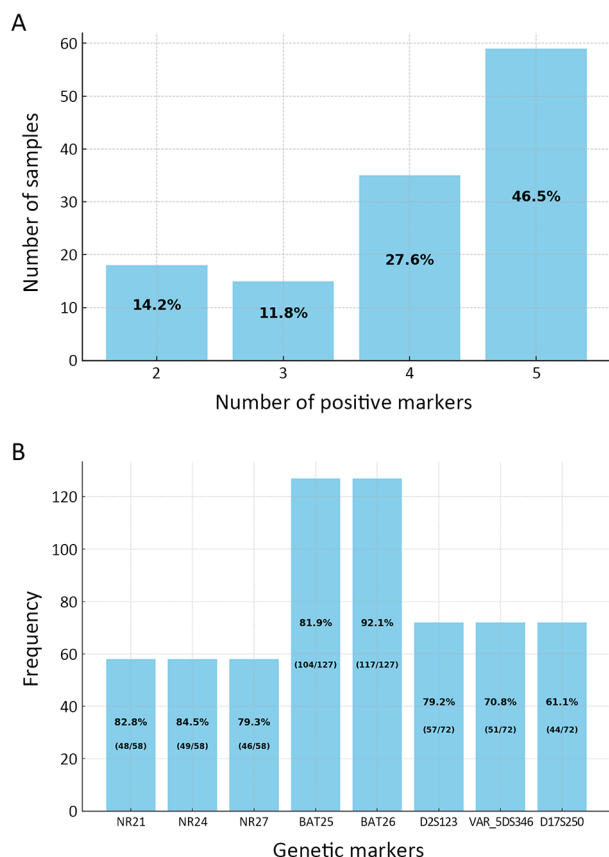


Figure 1 Distribution of positive markers and frequency of genetic markers. (A) Distribution of number of positive markers in the sample population; (B) Frequency of specific genetic markers across the sample population.

marker D17S250 showed the lowest frequency of instability, being unstable in only 61.1% of the cases.

Germline WES and variant identification

Germline WES was successfully conducted in 123 MSI-H patients, whereas sequencing failed in four samples (*Supplementary Figure S1*). A total of 6,068,902 high-quality variants were identified in these patients. Among these, 29,756 heterozygous variants were identified in consensus cancer driver genes. Our analysis focused specifically on MMR genes to identify LS-associated P/LP variants in patients with MSI-H GC, resulting in the identification of 10 candidate variants.

The characteristics of the identified MMR variants are summarized in *Table 1*. Ten variants were identified in 10 patients: two were frameshift insertions and the remaining were missense variants. All these were rare variants (MAF<0.001). Three known P/LP variants were found in

MLH1 (c.1758dup), *MSH6* (c.3261dup), and *MSH2* (c.1241T>C) in three patients. Additionally, two recurrent variants were identified in two patients, *MLH1* (c.1153C>T) and *MSH6* (c.3529C>G), both of which were classified as VUS. *In silico* protein damage prediction tools indicated that *MLH1* (c.1153C>T) was deleterious, whereas *MSH6* (c.3529C>G) showed inconsistent predictions of potential damage. Another possible deleterious variant was *MSH2* (c.2064G>A).

Loss of heterozygosity (LOH) and LS classification

To identify LOH, as suggested by Knudson (29), the effects of the identified variants in MMR genes on GC were evaluated by analyzing the corresponding tumors (*Table 2*). Specifically, if the protein loss of the corresponding MMR gene was observed in the tumor of a patient with a germline P/LP variant of the gene, it was considered indicative of LS.

In two patients (MSI_085 and MSI_093) with known germline P/LP variants, protein loss in the corresponding MMR gene was identified in their tumors, confirming these cases as LS. Two additional patients (MSI_033 and MSI_095) harboring rare and recurrent deleterious variants in *MLH1* (c.1153C>T) also showed loss of *MLH1* protein in their tumors. However, since *MLH1* promoter hypermethylation is the most frequent cause of MSI-H tumors, and tumor sequencing was not performed in this study, we could not definitively rule out sporadic MSI-H in these cases. Therefore, we classified them as possible LS.

Atypical and ambiguous cases

There were some ambiguous cases. One patient (MSI_088) with a known LP germline variant in *MSH6* (c.3261dup) did not exhibit any protein loss in the four MMR genes despite the tumor being confirmed as MSI-H. Another patient (MSI_126) carrying a deleterious variant of *MSH2* (c.2064G>A) showed no protein loss for *MSH2* in the tumor. Instead, the tumor exhibited a loss of *MLH1* and *PMS2* proteins. Because of these atypical findings, both patients (MSI_088 and MSI_126) were classified as having possible LS based on the presence of deleterious germline variants.

Age distribution in LS and possible LS patients

The age distribution of patients with LS or possible LS among those with MSI-H GC was analyzed (*Figure 2*). The

Table 1 Germline variants at MMR genes in patients with MSI-H gastric cancer

Patients	Symbol	Chr	Pos	Ref	Alt	DP (reads)	VAF	HGVSc	HGVSp	MAF_EAS	MAF_Overall	Consequence	AlphaMissense	CADD	REVEL	Class_AM	Germline classification*
MSL_033	MLH1	Chr3	37025751	C	T	89	0.55	c.1153C>T	p.Arg385Cys	<0.001	<0.001	Missense	0.946	28.500	0.840	Likely pathogenic	VUS
MSL_040	MSH6	Chr2	47805000	C	G	100	0.53	c.3529C>G	p.Leu1177Val	<0.001	<0.001	Missense	NA	24.900	0.660	NA	VUS
MSL_055	PM52	Chr7	5987366	C	T	132	0.57	c.1399G>A	p.Val467Ile	<0.001	<0.001	Missense	0.065	0.020	0.120	Likely benign	VUS
MSL_059	MSH6	Chr2	47783346	C	T	85	0.44	c.113C>T	p.Pro38Leu	<0.001	<0.001	Missense	0.124	16.000	0.230	Likely benign	VUS
MSL_085	MLH1	Chr3	37047543	G	GC	43	0.51	c.1758dup	p.Met587fs	NA	NA	Frameshift insertion	NA	NA	NA	NA	Pathogenic
MSL_088	MSH6	Chr2	47803500	A	AC	112	0.59	c.3261dup	p.Phe1086fs	<0.001	<0.001	Frameshift insertion	NA	NA	NA	NA	Pathogenic
MSL_093	MSH2	Chr2	47429906	T	C	31	0.45	c.124T>C	p.Leu414Pro	NA	NA	Missense	0.986	28.600	0.900	Likely pathogenic	Likely pathogenic
MSL_095	MLH1	Chr3	37025751	C	T	111	0.50	c.1153C>T	p.Arg385Cys	<0.001	<0.001	Missense	0.946	28.500	0.840	Likely pathogenic	VUS
MSL_096	MSH6	Chr2	47805000	C	G	91	0.54	c.3529C>G	p.Leu1177Val	<0.001	<0.001	Missense	NA	24.900	0.660	NA	VUS
MSL_126	MSH2	Chr2	47476425	G	A	76	0.47	c.2064G>A	p.Met688Ile	0.001	<0.001	Missense	0.938	28.700	0.820	Likely pathogenic	VUS

MMR, mismatch repair; MSI-H, microsatellite instability-high; DP, depth (number of reads); VAF, variant allele frequency; HGVS, human genome variation society; MAF, minor allele frequency by gnomAD; EAS, eastern Asian; CADD, combined annotation dependent depletion; REVEL, rare exome variant ensemble learner; Class_AM, classification of missense variant by AlphaMissense; NA, not applicable; VUS, variant of uncertain significance; VUS >0.564 for AlphaMissense; >20.000 for CADD; and >0.7 for REVEL. * Germline classification was based on ClinVar. AlphaMissense predictions (e.g., “Likely pathogenic”) were used as supplementary *in silico* annotations and are not the basis for final clinical classification.

patients’ ages were evenly distributed and showed no specific trends or patterns.

Discussion

In the present study, 2%–5% (two of LS and four of possible LS) of 123 MSI-H GC cases were associated with LS, a proportion that seemed to be lower than previous findings. One of the largest cohort studies, the MSKCC-IMPACT trial, which analyzed over 15,000 pan-cancer patients (326 of whom had MSI-H solid tumors), found that 16.3% of the MSI-H cases were associated with LS (3). However, the study population was predominantly non-Hispanic white (>75%), with less than 10% being Asian. Moreover, LS is primarily observed in colorectal and endometrial MSI-H cancers, with GC as a minor component. In contrast, a study focusing on colorectal cancer patients in South Korea identified LS in 7.7% of MSI-H patients (25 of 326) (12). This suggests that the lower prevalence of LS (<5%) among patients with MSI-H GC in our study could be influenced by ethnic and cancer type differences.

Several challenging cases emerged in defining LS in this study. Two patients (MSI_033 and MSI_095) harbored deleterious mutations in MLH1, which are rare variants in the general population (MAF in EAS <0.001). The recurrent identification of these variants in our highly selected cohort (MSI-H GC; considering GC incidence of 55 per 100,000 in Korea (30), with MSI-H comprising less than 10% of cases) strongly suggests their association with LS. Additionally, MLH1 protein loss was confirmed in their corresponding tumors. However, given that over 90% of MSI-H cases are sporadic GC caused by *MLH1* promoter CpG hypermethylation (31), we could not definitively classify these cases as LS without additional tumor analyses (e.g., WES or methylation profiling).

Furthermore, case MSI_088 presented another diagnostic challenge. Despite harboring a known LP variant in *MSH6* and exhibiting MSI-H status, IHC showed intact expression of all four MMR proteins, including MSH6. Similar cases have been reported in colorectal cancer, where patients with germline frameshift mutations and exon 5 skipping mutations in *MSH6* developed MSI-H tumors while maintaining intact MMR protein expression by IHC (32). This discrepancy likely reflects the lower sensitivity of IHC-based dMMR detection compared to PCR-based methods (33). Additionally, the antibodies used in IHC may fail to recognize specific epitopes affected by

Table 2 Characteristics of corresponding gastric cancer in patients with variants at MMR genes

Patients	Age (year)	Sex	Lauren	pT	pN	TNM	Tumor location	Symbol	Corresponding GC MMR IHC				Family cancer history	Other malignancy	Estimated pathogenicity*	Decision
									MLH1	PMS2	MSH2	MSH6				
MSI_033	75	F	Mixed	T3	2	IIIA	MB	MLH1	Loss	Loss	Intact	Intact	None	None	Likely pathogenic	LS
MSI_040	74	F	Intestinal	T4	3	IIIC	LB	MSH6	Loss	Loss	Intact	Intact	None	None	VUS	Non-LS
MSI_055	60	M	Intestinal	T1	0	IA	LB	PMS2	Loss	Loss	Intact	Intact	None	None	Likely benign	Non-LS
MSI_059	77	F	Intestinal	T3	1	IIB	LB	MSH6	Loss	Loss	Intact	Intact	None	None	Likely benign	Non-LS
MSI_085	82	F	Intestinal	T1	0	IA	LB	MLH1	Loss	Loss	Intact	Intact	None	Endometrial & colon cancer	Pathogenic	LS
MSI_088	57	M	Intestinal	T2	0	IB	LB	MSH6	Intact	Intact	Intact	Intact	None	None	Pathogenic	Possible LS
MSI_093	52	F	Mixed	T3	1	IIB	MB	MSH2	Intact	Intact	Loss	Loss	None	Endometrial cancer	Likely pathogenic	LS
MSI_095	59	M	Intestinal	T3	0	IIA	MB	MLH1	Loss	Loss	Intact	Intact	None	Kidney cancer	Likely pathogenic	LS
MSI_096	80	M	Mixed	T3	0	IIA	LB	MSH6	Loss	Loss	Intact	Intact	None	None	VUS	Non-LS
MSI_126	71	M	Mixed	T2	0	IB	LB	MSH2	Loss	Loss	Intact	Intact	None	None	Likely pathogenic	Possible LS

MMR, mismatch repair; MSI, microsatellite instability; GC, gastric cancer; IHC, immunohistochemistry; F, female; M, male; MB, mid body; LB, lower body; VUS, variant of unknown significance; LS, Lynch syndrome. *, Estimated based on the ClinVar classification and results of *in silico* protein prediction tools (Table 1).

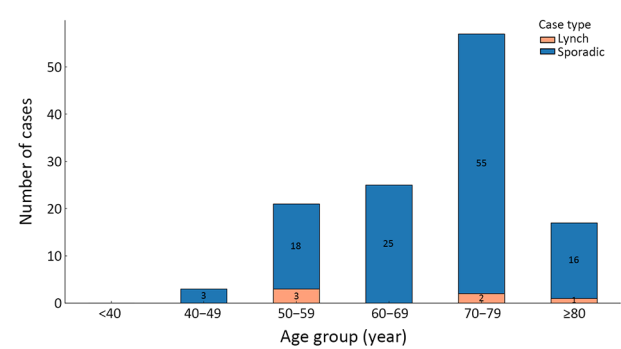


Figure 2 Distribution of sporadic and Lynch syndrome cases by age group. Comparison of sporadic and Lynch syndrome cases across different age groups.

these variants, potentially leading to false-negative results.

While only approximately 5% of MSI-H GC patients are associated with LS, there are several important reasons for conducting genetic testing in this subset of patients, despite the seemingly low yield. First, identifying LS allows patients to implement preventive actions and engage in early detection for other LS-associated cancers as subsequent primary cancers (34), thereby reducing cancer morbidity and mortality. Secondly, genetic testing enables early detection and prevention strategies for family members who may also be at risk, facilitating a holistic family wise approach to cancer prevention, screening, and treatment. Third, the ongoing identification of LS advances our genetic understanding of both LS and GC and contributes to precision medicine by providing insights

that enhance patient care and treatment strategies. Therefore, germline evaluation should be considered in patients with MSI-H GC.

Our study showed that age distribution in patients with LS among MSI-H GC cases was even, with no discernible trend. This finding is comparable to those of previous studies in the West and colorectal and multiple cancers in Korea (12,35), and challenges the traditional view that hereditary cancer syndromes, such as LS, predominantly affect younger individuals (36). This suggests that MSI-H GC, possibly linked to LS, can be present at any age, necessitating the vigilance of LS-associated genetic testing across all age groups. This insight is crucial for clinical guidelines that typically recommend genetic testing based on age (37) and could lead to updates in screening protocols to include a broader age range for LS evaluation.

Recently, a large-scale study in Japanese patients with GC (38) reported that *BRCA1/2* germline P/LP variants were the most common but did not significantly increase GC risk in the absence of *Helicobacter pylori* (*H. pylori*) infection. This suggests that preventing a second hit, such as LOH induced by *H. pylori*, may mitigate the risk of GC, even in individuals with hereditary risk factors. Although similar findings have not been established for LS-associated GC, it can be hypothesized that the eradication of *H. pylori* may reduce the risk of developing GC in patients with LS by preventing a second hit in the context of germline MMR gene variants. However, further research is required to explore this preventive strategy.

Although the present study provides valuable insights into LS in GC and identifies a variant possibly associated with LS [*MLH1* (c.1153C>T)], several limitations need to be addressed. Owing to its retrospective design, some important clinical information was unavailable, such as family history of cancer, history of other cancers and the status of *H. pylori*, which is a major risk factor and potential cause of the second hit in GC. The cohort consisted exclusively of Korean patients, which may represent the Eastern Asian population, but has limited generalizability to other ethnicities. Despite we evaluated MMR protein loss in the corresponding tumors, we did not assess *MLH1* promoter methylation to exclude sporadic MSI-H cases. Additionally, due to the absence of tumor sequencing, we could not determine the specific second hit mechanisms (e.g. deleterious mutation in opposite allele or biallelic imbalance) in tumors from patients with confirmed or possible LS. While the sample size is relatively modest, this must be considered in the context of the low prevalence of MSI-H GC (approximately 10%) (27) and the even lower frequency of LS-associated cases. Despite this limitation, our study represents one of the largest MSI-H GC cohorts to date. Given the rarity of LS-associated MSI-H GC, further data are needed to achieve a more comprehensive understanding of LS in GC.

Conclusions

This study provides foundational data on LS in MSI-H GC and may serve as a cornerstone for future research. The results highlight the need for large-scale studies that consider both patients and their families and explore the preventive effects of *H. pylori* eradication. Therefore, clinicians should consider the possibility of LS in patients with MSI-H-type GC.

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Author contributions

Conceptualization: YY Choi and MS Lee; Data curation: All authors; Writing – original draft: JH Yun, GJ Song and YY Choi; Writing – review & editing: YY Choi and MS

Lee; Methodology: YY Choi, MS Lee and JH Yun; Visualization: JH Yun, GJ Song and YY Choi; Investigation: All authors; Formal analysis: JH Yun, GJ Song and YY Choi; Funding acquisition: YY Choi and JH Yun; Resources: MW Son, SC Yun, I Cho and SH Kim; Supervision: YY Choi and MS Lee. All authors read and approved the final manuscript.

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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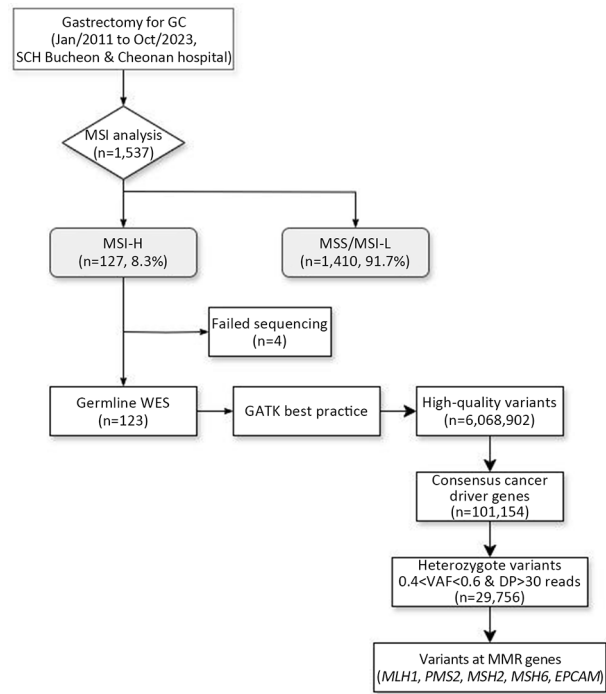


Figure S1 Study workflow. Detailed steps and analysis flow of MSI in gastric cancer patients from SCH Bucheon and Cheonan hospitals. MSI, microsatellite instability; SCH, Soonchunhyang; GC, gastric cancer; MSS, microsatellite instability-stable; WES, whole-exome sequencing; GATK, Genome Analysis Toolkit; VAF, variant allele frequency; DP, depth.

Table S1 Clinicopathological characteristics

Variables	n (%)		Total (N=127)
	Hereditary MSI-H*	Sporadic MSI-H	
Sex			
Male	3 (50.0)	79 (65.2)	82 (64.6)
Female	3 (50.0)	42 (34.7)	45 (35.4)
Age (year) ($\bar{x}\pm s$)	67.83 $\pm$ 13.41	70.13 $\pm$ 9.56	70.02 $\pm$ 9.71
No. of lesions ($\bar{x}\pm s$)	1.17 $\pm$ 0.41	1.02 $\pm$ 0.13	1.02 $\pm$ 0.15
Tumor size (cm) ($\bar{x}\pm s$)	5.87 $\pm$ 3.14	4.18 $\pm$ 2.87	4.26 $\pm$ 2.89
No. of retrieved lymph nodes ($\bar{x}\pm s$)	33.00 $\pm$ 16.28	36.09 $\pm$ 15.80	35.94 $\pm$ 15.77
No. of metastatic lymph nodes ($\bar{x}\pm s$)	1.33 $\pm$ 1.97	2.03 $\pm$ 4.82	2.00 $\pm$ 4.72
Extent of resection			
Distal gastrectomy	5 (83.3)	111 (91.7)	116 (91.3)
Total gastrectomy	1 (16.7)	9 (7.4)	10 (7.9)
Completion gastrectomy	0 (0)	1 (0.8)	1 (0.8)
Tumor location			
Lower body	3 (50.0)	95 (78.5)	98 (77.2)
Mid body	3 (50.0)	22 (18.2)	25 (19.7)
Upper body	0 (0)	4 (3.3)	4 (3.2)
Lauren classification			
Intestinal	4 (66.7)	77 (63.6)	81 (63.8)
Diffuse	0 (0)	21 (17.4)	21 (16.5)
Mixed	2 (33.3)	23 (19.0)	25 (19.7)
WHO classification			
Tubular adenocarcinoma, WD	0 (0)	10 (8.3)	10 (7.9)
Tubular adenocarcinoma, MD	3 (50.0)	70 (57.9)	73 (57.5)
Tubular adenocarcinoma, PD	2 (33.3)	17 (14.0)	19 (15.0)
Signet-ring cell carcinoma	0 (0)	9 (7.4)	9 (7.1)
Mucinous adenocarcinoma	0 (0)	4 (3.3)	4 (3.1)
Others	1 (16.7)	11 (9.1)	12 (9.4)
T classification			
T1	1 (16.7)	52 (43.0)	53 (41.7)
T2	1 (16.7)	29 (24.0)	30 (23.6)
T3	4 (66.7)	24 (19.8)	28 (22.0)
T4	0 (0)	16 (13.2)	16 (12.6)
N classification			
N0	3 (50.0)	85 (70.2)	88 (69.3)
N1	2 (33.3)	14 (11.6)	16 (12.6)
N2	1 (16.7)	8 (6.6)	9 (7.1)
N3	0 (0)	14 (11.6)	14 (11.0)
AJCC stage			
I	2 (33.3)	75 (62.0)	77 (60.6)
II	3 (50.0)	24 (19.8)	27 (21.3)
III	1 (16.7)	22 (18.2)	23 (18.1)

WHO, World Health Organization; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; AJCC, American Joint Committee on Cancer; MSI-H, microsatellite instability-high; LS, Lynch syndrome. *, The hereditary MSI-H group included patients classified as LS and possible LS (see *Table 2*). No statistically significant differences were observed between hereditary and sporadic cases across all variables ($P<0.05$ as the significance threshold). However, given the small sample size of the hereditary group, caution is required when interpreting these results.