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ARTICLE

Importance of gastric cancer for the diagnosis and surveillance of Japanese Lynch syndrome patients

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Abstract

Lynch syndrome (LS) is an autosomal dominantly inherited disease predisposed to not only colorectal cancer but also other LS-related tumors. Although the clinical and genetic characteristics of LS in Western countries have been well characterized, the information of Japanese LS is limited. As a collaborative study of Japanese Society for Cancer of the Colon and Rectum (JSCCR), we registered colorectal cancer (CRC) patients who fulfilled the modified Amsterdam II criteria including gastric cancer as an LS-related tumor. Among 4030 CRC patients initially registered in this project, 85 patients (2.1%) fulfilled the modified criteria. An additional 26 patients who met the same criteria were enrolled in the analysis. We analyzed three major responsible genes, *MLH1*, *MSH2*, and *MSH6* by direct sequencing, and further performed multiplex ligation-dependent probe amplification for *MLH1* and *MSH2*. Consequently, we identified pathogenic variants in 64 of the 111 patients comprising of 34 patients in *MLH1*, 28 in *MSH2*, and 2 in *MSH6*. It is of note that large structural alterations were found in 17 patients. Among the 64 patients, 11 patients would not have been enrolled in the analysis if gastric cancer were not included in the modified criteria. In addition, 10 of the 64 variant carriers (15.6%) had medical history of gastric cancer. Furthermore, the standardized incidence ratio of gastric cancer in the LS patients to the Japanese population is estimated to be as high as 20.2. These data underscore the importance of gastric cancer in the diagnosis and healthcare of Japanese LS patients.

Introduction

Lynch syndrome (LS) or hereditary nonpolyposis colorectal cancer syndrome (HNPCC) is an autosomal dominantly inherited disease. Affected individuals with LS are predisposed to colorectal cancer (CRC) and other LS-related tumors including cancer in the endometrium, small intestine, ureter, renal pelvis, ovary, stomach, pancreas, hepatobiliary tract, brain, and skin [1]. LS is caused by germ line variants in mismatch repair (MMR) genes including *human mutL homolog 1* (*MLH1*), *human mutS homolog 2* (*MSH2*), *human mutS homolog 6* (*MSH6*), and *postmeiotic segregation increased 2* (*PMS2*), or *epithelial cellular adhesion molecule* (*EPCAM*), a gene located in the upstream of *MSH2* [2, 3]. Impaired MMR system results in the enhanced accumulation of somatic mutations, and thus most of the LS-related CRCs demonstrate microsatellite instability (MSI). It is estimated that 2–4% of CRC are attributed to LS [4–7]. To identify at-risk families with LS, an international

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collaboration led to the development of the revised Amsterdam criteria (or the Amsterdam criteria II); (1) three or more family members (one of whom is a first-degree relative of the other two) with LS-associated cancers, (2) two successive affected generations, (3) one or more of the LS-related cancers diagnosed before age 50 years, and (4) exclusion of familial adenomatous polyposis [8]. Under the new criteria, LS-associated cancers include CRC, or cancer of the endometrium, small intestine, ureter, or renal pelvis. The Amsterdam criteria II have been widely used in clinics to find at-risk families basically by their family history. To increase the sensitivity of identification, the revised Bethesda guidelines were developed and applied for patients with CRC [9]. These criteria have assisted the identification of affected patients based primarily on their medical history and histological information. Positive patients for at least one of the revised criteria were recommended to undergo additional analysis such as MSI test and/or immunohistochemistry. Although these guidelines improved the sensitivity of detection, there remain patients who could not be uncovered by the criteria or guidelines [5, 6]. Therefore, several groups recommended universal screening programs for the identification of affected individuals in patients with CRC [6, 10, 11].

Affected individuals have increased risk of CRC and other LS-related tumors. It is reported that the lifetime risk for developing CRC by 70 years of age is between 52 and 82% in the individuals with a pathogenic variant in *MLH1* or *MSH2*, and that the lifetime risk for endometrial cancer is between 25 and 60% in women [12]. These risks are extremely high compared with the general population in which the risks are estimated to be 4.7% and 2.7% for CRC and endometrial cancer, respectively [12]. Previously reported risk for gastric cancer, one of the LS-related tumors, range from 0.2 to 10.9% in the Western individuals with a pathogenic variant in *MLH1* or *MSH2* [13–15]. It is of note that the incidence of gastric cancer is high in Japan and Korea compared with Western countries, and that gastric cancer is the third leading cause of cancer death in Japan. However, the risk estimation for gastric cancer in Japanese patients with LS have not been performed.

To clarify the clinical and genetic characteristics of LS in Japanese, we carried out a collaborative study of LS in CRC patients in Japanese Society for Cancer of the Colon and Rectum (JSCCR). In this study, we performed genetic analyses of three major MMR genes (*MLH1*, *MSH2*, and *MSH6*), and consequently identified pathogenic variants in 64 of the 111 patients. We uncovered that structural alterations are frequent (17/64 cases), and that consideration of gastric cancer in the clinical selection for molecular diagnosis is important for the identification of LS patients in Japanese.

Materials and methods

Patients and samples

This project was approved by the institutional review board of the Institute of medical Science, the University of Tokyo, and the review board of National Cancer Center Japan and the other collaborative hospitals that participated in this project. Written informed consent was obtained from all CRC patients participating in this study who fulfilled the modified Amsterdam criteria II including gastric cancer as an LS-related tumor. A total of 4030 patients with CRC were initially enrolled in this study from September, 2002 to October, 2004. Genetic analyses were carried out for 111 patients, comprising of 85 patients out of the 4030 and an additional 26 patients who fulfilled the same modified criteria. Clinical information was obtained from their medical records.

Genetic analysis

Peripheral blood samples were taken from the patients for genetic analyses. Genomic DNA and RNA were extracted from the blood samples according to the standard phenol extraction/purification procedure and guanidine thiocyanate method, respectively. The coding exons in *MLH1*, *MSH2*, and *MSH6*, were amplified by PCR using the KOD-Plus kit (TOYOBO, Osaka, Japan) with M13-tailed target-specific primers, and Sanger sequencing was performed using the BigDye Direct Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA) on the ABI 3700 automated sequencer (Applied Biosystems). The primer sequences used for the amplification and sequencing are shown in Supplementary Table 1. *PMS2* was not analyzed, because it has a set of pseudogenes and the evaluation of pathogenic variants was difficult. The pathogenicity of germline variations was assessed using the LOVD (Leiden Open Variation Database) databases in The International Society for Gastrointestinal Hereditary Tumours, HGMD (Human Gene Mutation Database, Qiagen), and ClinVar, a database in the National Center for Biotechnology Information. For cases with variants in splice donor or acceptor sites, we performed RT-PCR analysis using RNA from the blood samples to confirm impaired splicing. For the detection of large deletions and duplications, we performed the multiplex ligation-dependent probe amplification (MLPA) assay using the SALSA MLPA P003 kit (MRC-Holland, Amsterdam, The Netherlands) according to the manufacturer's protocol.

Statistical analyses

The data for population-based gastric cancer incidence in the Japanese are available from the Cancer Registry and

Statistics, Cancer Information Service, National Cancer Center, Japan [16]. The incidence of gastric cancer in the patients with pathogenic variants was compared with that in the general population using the Cancer Registry and Statistics data of 2003. Cumulative risk of developing gastric cancer was analyzed by the Kaplan–Meier method using the data of age at gastric cancer onset in the patients with pathogenic variants, and the age-stratified incidence rate in the Japanese population. Observation time ended at the age of CRC diagnosis. In addition, an expected gastric cancer incidence rate (person-years method) of the subjects was calculated based on incidence rate of the Japanese population. The standardized incidence ratio was then calculated by observed/expected incidence rate. Confidence intervals were calculated based on Greenwood's formula for Kaplan–Meier analysis, and Poisson distribution for person-years incidence rate: incidence rate $\pm 1.645 \times$ (incidence/person-years^2)$^{0.5}$. Continuous variables and categorical variables were compared between groups by *t*-test and Fisher's exact test as appropriate. We considered $P < 0.05$ as statistically significant. All analyses were performed using SPSS Statistics 25 (IBM Corp, Chicago, IL).

Results

The frequency and clinical features of CRC patients suspected of LS in Japan

Among 4030 CRC patients who initially visited the collaborative hospitals between September, 2002 and October, 2004, 85 patients (2.1%) fulfilled with the modified Amsterdam II criteria including gastric cancer as an LS-related tumor. In addition, we identified 26 patients who met the same criteria were in this study between October 2004 and August 2007. Among the 111 patients, 77 patients fulfilled the conventional Amsterdam criteria II, in which gastric cancer was not included as an LS-related tumor. The remaining 34 fulfilled the modified criteria that include gastric cancer as an LS-related tumor.

Clinicopathological information of the 111 patients is shown in Table 1. The average age of the 111 patients consisting of 51 males and 60 females was 49.7 years old. Notably, a total of 164 colorectal tumors were found in the 111 patients, and 38 of the 111 patients had synchronous or metachronous colorectal tumors; 12 patients had synchronous multiple colorectal tumors, and 26 patients had the history of metachronous colorectal tumors. Among the 164 colorectal tumors, 77 tumors developed in the right colon (cecum, ascending and transverse colon), and the remaining 87 in the left colon and rectum (descending and sigmoid colon, rectum). Of the 164 tumors, histological information of 141 tumors was obtained from their clinical record, and 22 of the 141 (15.6%) were either poorly differentiated or mucinous adenocarcinomas. Among the 111 patients, medical history of gastric cancer was observed in 13 patients (11.7%), and that of endometrial cancer, renal or ureteral cancer, and cancer in the small intestine was found in 10 (16.7% in women patients), 4 (3.6%), and 4 (3.6%) patients, respectively.

Tumors in first-degree relatives

To clarify the significance of LS-related tumors in the relatives, we analyzed tumors in the first-degree relatives (parents, brothers, sisters, and children) according to the family history information obtained from clinical records. As shown in Table 1, a total of 188 colorectal tumors (0.27 tumor/relative) and 70 gastric cancers (0.10 tumor/relative) were found in 691 first-degree relatives of the 111 patients. The numbers of endometrial cancer, renal or ureteral cancer, and cancer in the small intestine in the first-degree relatives were 19, 5, and 1, respectively. In 484 first-degree relatives of 77 patients who fulfilled the conventional Amsterdam II criteria, we found a total of 158 colorectal tumors (0.33 tumor/relative) and 32 gastric cancers (0.07 tumor/relative). On the other hand, in 207 first-degree relatives of the 34 patients who met the modified criteria by the inclusion of gastric cancer, we detected 30 colorectal tumors (0.15 tumor/relative) and 38 gastric cancers (0.18 tumor/relative).

Identification of pathogenic variants

In this study, we investigated variants in the three major genes associated with LS, namely *MLH1*, *MSH2*, and *MSH6*, by PCR-direct sequencing using the Sanger's method. To investigate large deletions and duplications, MLPA was performed for subjects without pathogenic variants. As a result, 64 (57.7%) of the 111 subjects carried 57 types of pathogenic variants (Supplementary table 2). Twenty-one of the 57 have not reported in LOVD, HGMD, or ClinVar databases, and were novel variants. The 21 variants included c.209_211delAAG, c.319_320delAT, c.464dupT, c.472delA, c.523delA, c.545+3delAC, c.1672_1673insAACT, c.2198_2199insTT, c.1-94968_453+696del109180, c.381-431_453+717del1221, c.1039-4215_1409+2347del6933ins101 in *MLH1*, c.274_276delCTT, c.2300_2303delCAGAinsATATATAT, c.2309delT, c.2455A>T, c.1-7550_211+2019del9780, c.1-19631_1076+10113del42982, c.793-455_1076+5894dup8510, c.943-596_1276+12033del26275, c.1077-10584_1276+207dup10991 in *MSH2*, and c.3403dupC in *MSH6*. In addition, a subject (JLS054) carried a variant, *MSH6* c.3656C>T (p.Thr1219Ile), which was judged as a variant of uncertain significance (VUS) because it was categorized as a VUS in the LOVD, and as uncertain significance or

Table 1 Clinicopathological information of the probands and their relatives

	Modified Amsterdam Criteria II	Conventional Amsterdam Criteria II	Cases included by gastric cancer
Probands			
No. of probands	111	77	34
Sex: male/female	51/60	38/39	13/21
Age	49.7	49.6	52.6
Cases with multiple CRCs (synchronous)	12	11	1
Cases with multiple CRCs (metachronous)	26	21	5
Total no. of CRCs (average)[a]	164 (1.5)	119 (1.6)	45 (1.3)
Location: Rt side-/Lt side-colon	77/87	62/57	15/30
Histology: por or mucinous/total tumors (%)[b]	22/141 (15.6)	20/98 (20.4)	2/43 (4.7)
Past history of gastric cancer (%)	13 (11.7)	10 (13.0)	3 (8.8)
Past history of endometrial cancer (%)[c]	10 (16.7)	6 (15.4)	4 (19.0)
Past history of renal pelvic or ureteral cancer (%)	4 (3.6)	3 (3.9)	1 (2.9)
Past history of cancer in small intestine (%)	4 (3.6)	2 (2.6)	2 (5.9)
Family history of first-degree relatives[d]			
No. of relatives (average)	691 (6.23)	484 (6.29)	207 (6.09)
Total no. of CRCs (average)	188 (27.2)	158 (32.6)	30 (14.5)
Total no. of gastric cancers (average)	70 (10.1)	32 (6.6)	38 (18.4)
Total no. of endometrial cancers (average)	19 (2.7)	14 (2.9)	5 (2.4)
Total no. of renal pelvic and ureteral cancers (average)	5 (0.7)	3 (6.2)	2 (1.0)
Total no. of cancers in small intestine (average)	1 (0.1)	1 (2.1)	0

[a]Total number of tumors including synchronous and metachronous CRCs

[b]Tumors with information of histological classification

[c]Endometrial cancers were selected from tumors of uterus

[d]Parents, brothers and sisters, and children were included

likely pathogenic variant in ClinVar. Among the 64 variant carriers, 34 had a variant in *MLH1*, 28 in *MSH2*, and 2 in *MSH6*. Regarding the method of detection, 46 of the 57 types of variants were identified by PCR-direct sequence in 47 subjects, and 11 were identified by MLPA in 17 subjects, suggesting that 17 out of 64 (26.6%) variant carriers with large structural alterations would have been overlooked if the additional analysis of MLPA was not performed.

It is of note that three types of recurrent variants were identified in this study; *MLH1*, c.199G>A observed in two patients (JLS051 and JLS109), *MLH1*, c.381-431_453 +717del1221 encompassing exon 5 in six patients (JLS039, JLS055, JLS058, JLS070, JLS095, and JLS101), and *MSH2*, c.1-755_211+2019del9780 encompassing exon 1 in two patients (JLS023 and JLS114). It remains to be established whether the three changes are founder variants in Japanese LS.

When CRC patients who fulfilled the conventional Amsterdam criteria II were recruited for the study, pathogenic variants were found in 53 of 77 (68.8%) CRC patients. In other words, pathogenic variants were identified in 11 of 34 (32.3%) patients who were included by the history of gastric cancer. Although this variant frequency is much lower than that observed by conventional Amsterdam criteria II, it will be worthwhile to include gastric cancer in the criteria as far as Japanese CRC patients are concerned.

In addition, of the 64 patients detected with pathogenic variants, 10 (15.6%) had a history of gastric cancer. These data underscore the importance of gastric cancer in the diagnosis and healthcare of Japanese LS patients.

Clinical features of patients with a pathogenic variant

As described above, most of the pathogenic alterations detected in this study (55 of 57 variants) were in *MLH1* or *MSH2*. Therefore, we compared the difference in clinical features between the 34 patients with *MLH1* variants (or *MLH1* carriers) and the 28 patients with *MSH2* variants (or *MSH2* carriers) (Table 2). The average age of *MLH1* and

Table 2 Comparison of clinical features by the mutated genes

Gene	*MLH1*	*MSH2*	*MSH6*	total
Cases	34	28	2	64
Male/female	18/16	13/15	0/2	31/33
Average age	46.8	46.4	64.0	47.2
No. of CRC (average)	58 (1.7)	42 (1.5)	3 (1.5)	103 (1.6)
Location (Rt/Lt)	33/25	17/25	1/2	51/52
Histology of por or muc[a]	5/48	10/37	0/2	15/87
Gastric	6	4	0	10
Endometrial	3	5	1	9
Urinary tract	1	3	0	4
Small intestine	0	3	0	3
Other cancers	2	3	0	5

[a]Poorly differentiated or mucinous adenocarcinomas/CRCs with information of histological classification

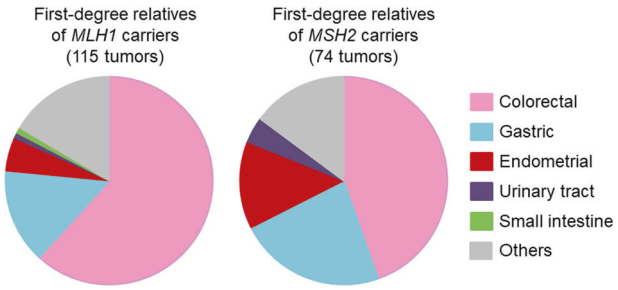

Fig. 1 Tumors in first-degree relatives of the *MLH1* and *MSH2* carriers. A total of 115 tumors developed in 216 first-degree relatives of the *MLH1* carriers, and 74 tumors in 159 first-degree relatives of the *MSH2* carriers

MSH2 carriers was ~46.8 and ~46.4 years old, respectively. The *MLH1* carriers included 18 males and 16 females, and the *MSH2* carriers included 13 males and 15 females. These results suggested that there were no significant differences in age and gender between the two groups. The average age of the two female patients with a pathogenic *MSH6* variant (or *MSH6* carriers) was 64.0, higher than the two groups.

In the 34 *MLH1* carriers, a total of 58 colorectal tumors were found, and 33 of the 58 tumors (56.9%) were located in the right colon. In the 28 *MSH2* carriers, 42 colorectal tumors were detected, and 17 of the 42 tumors (40.5%) were in the right colon. Although the frequency of tumors in the right colon is higher in the *MLH1* carriers than that in the *MSH2* carriers, statistical significance was not observed. Regarding the history of other LS-related cancers, there were six cases of gastric cancer, three endometrial cancers, and one renal or ureteral cancer in the 34 *MLH1* carriers, and there were four cases of gastric cancer, five endometrial cancers, three renal or ureteral cancer, and three cancers in the small intestine in the 28 *MSH2* carriers. In addition, there was one case each of breast cancer and ovarian cancer in the *MLH1* carriers, and there was one case each of prostate cancer, ovarian cancer, and liposarcoma in the thigh in the *MSH2* carriers. In total, 12 extracolonic tumors were found in the 34 *MLH1* carriers, and 18 tumors in the 28 *MSH2* carriers, suggesting that *MSH2* carriers may have higher risks of extracolonic tumors compared with *MLH1* carriers ($p < 0.05$).

Family history of the *MLH1*- or *MSH2*-variant carriers

Since the probands enrolled in this study were patients with CRC, the risk of CRC cannot be evaluated by the probands. Therefore, we analyzed the tumors in the first-degree relatives of the *MLH1* and *MSH2* carriers. For this analysis, we incorporated 216 and 159 first-degree relatives of the *MLH1* and *MSH2* carriers, respectively. Although the relatives were not tested for their genetic status, we can assume that approximately half of the relatives should carry the same germ line variants as the probands. The relatives of *MLH1* carriers had a total of 115 tumors (Fig. 1), which included 71 CRC (61.7%). On the other hand, the relatives of *MSH2* carriers had 74 tumors, which included 33 CRC (44.6%). These data indicated that the relatives of *MLH1* carriers may have higher risks of CRC than the relatives of *MSH2* carriers ($p < 0.05$). Importantly, the frequency of CRC in the relatives of *MLH1* carriers is 0.33 tumor/relative, and that in the relatives of *MSH2* carriers is 0.21 tumor/relative, suggesting that the risk of CRC is much higher in both groups compared with the general population. In terms of gastric cancer, 17 cancers were found in the relatives of *MLH1* carriers, and 17 in the relatives of *MSH2* carriers. It is worth noting that the frequency of gastric cancer in the relatives of *MLH1* carriers is 0.08 tumor/relative, and that in the relatives of *MSH2* carriers is 0.11 tumor/relative. Therefore ~10% of relatives of variant carriers are predisposed to gastric cancer regardless of their genetic status.

Risk of gastric cancer in the variant carriers

According to the medical records in the variant carriers, ten of the 64 variant carriers had a history of gastric cancer, and one of the ten had a history of recurrent gastric cancer. The gender of the ten patients included six males and four females. The age of gastric cancer diagnosis ranged from 48 to 66 years, and the average and standard deviation were 55.3 and 6.1 years, respectively. The ten patients included six *MLH1* carriers and four *MSH2* carriers, but none carried a variant in *MSH6*. According to the information of gastric cancer risks in the Japanese population, it is estimated that cumulative risks of gastric cancer by 70 years of age are

4.4% for males, 1.7% for females, and the overall risk is 3.0% (Supplementary table 3). The frequency of gastric cancer (ten patients in 64 variant carriers) is obviously higher than the general population. The cumulative risks of gastric cancer by 70 years of age are estimated to be 49.7% (90% CI: 24.2–75.2%) for males, 25.1% (90% CI: 6.5–43.7%) for females, and 38.7% (90% CI: 17.7–59.7%) overall (Supplementary table 3). Kaplan–Meier curves for these populations are demonstrated in Fig. 2. Cumulative risk ratio tends to decline by age after 60-year old (Supplementary table 3), and there is a peak at age 55 years for males (35.0, 90% CI: 10.6–59.4) and 60 years for females (31.3, 90% CI 8.1–54.5). The standardized incidence ratio based on person-years method is estimated to be as high as 20.2 (90% CI: 9.7–30.7) (Table 3).

Discussion

In this study, we clarified the clinical and genetic features of Japanese LS. We unveiled that ~2.1% of Japanese CRC patients fulfilled the modified Amsterdam criteria including gastric cancer as an LS-related tumor. In addition, genetic analysis of *MLH1*, *MSH2*, and *MSH6* identified pathogenic variants in 57.7% of cases who fulfilled the criteria. Since two other responsible genes, *PMS2* or *EPCAM*, were not analyzed, a small number of LS cases may have been overlooked. Furthermore, LS patients who did not meet the clinical criteria may be missed because we did not perform a universal screening in this study. It is reported that genetic analysis of *MLH1*, *MSH2*, and *MSH6* genes in 1066 unselected patients with CRC identified pathogenic variants in 23 (2.2%) individuals [6]. Notably, they showed that five (22%) of the 23 cases did not meet neither the revised Amsterdam criteria nor the Bethesda's guideline for MSI test. Another group found pathogenic variants in the three genes in 38 of 870 (4.4%) CRC patients under 55 years of age [7]. In Australasian Colorectal Cancer Family Registry, analysis of five MMR genes (*MLH1*, *MSH2*, *MSH6, PMS2*, and *EPCAM*) identified variants in 42 (5.2%) of 813 CRC patients younger than 60 years of age [17]. Although further studies are needed to clarify the precise frequency of LS, this study has at least shown the approximate frequency of LS patients in Japanese CRC patients.

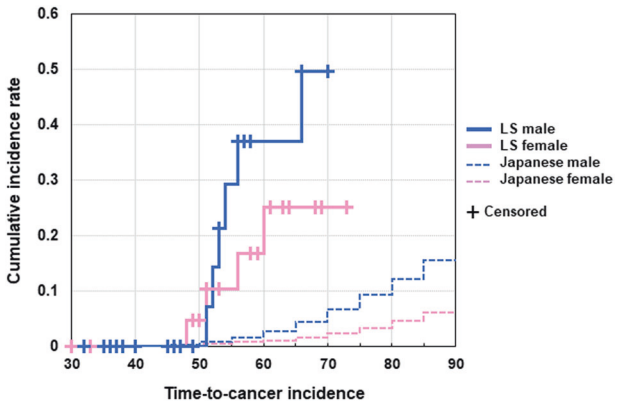

Fig. 2 Cumulative incidence rate of gastric cancer in the LS patients and the Japanese population. The incidence of gastric cancer in the general population using the Cancer Registry and Statistics data of 2003. Cumulative risk of developing gastric cancer was analyzed using the Kaplan–Meier method as described in the "Materials and methods" section

Regarding the genetic features, pathogenic variants of *MSH6* were relatively rare in Japanese LS with CRC although MLPA for *MSH6* was not performed in this study. In addition, we found that the frequency of large structural alterations including deletions and duplications in *MLH1* or *MSH2* was as high as 26.6% (17 of 64) in variant carriers. This result highlighted the importance of MLPA or detection of structural alterations in the genetic diagnosis of LS in the Japanese. In addition, three types of variants were recurrently identified in Japanese LS. Among the three, a deletion of *MLH1* exon 5 was found in six patients. Although future studies are essential to clarify the importance of this variant, the deletion may be a founder variant in the Japanese.

Regarding the risk of gastric cancer, we here clarified that the risk is ~40% by 70 years of age (49.7% for males and 25.1% for females) in Japanese LS patients. In addition,

Table 3 Incidence rate of gastric cancer based on person-years method

			90% CI	
			Lower	Upper
Observed gastric cancer incidence rate (per 1000 person-years)	3.28	Observed incidence/person-years 10/3053	1.57	4.98
Expected gastric cancer incidence rate based on all Japanese population (2003) (per 1000 person-years)	0.16	Expected incidence/person-years 0.495/3050		
Standardized incidence ratio (observed/expected)		20.2	9.7	30.7

Calculated as incidence rate ± 1.645 × (incidence/person-years^2)$^{0.5}$

the standardized incidence ratio of gastric cancer in the LS patients to the Japanese population is estimated to be ~20. These data underscore the importance of gastric cancer in LS patients, although the risk of gastric cancer in the Japanese population is higher than Western countries. On the other hand, retrospective studies reported that the cumulative risk of gastric cancer by the age of 70 years is ~5% in Western LS patients [15]. A study in Netherland showed that the lifetime risk of gastric cancer was 4.8 and 9% for *MLH1* and *MSH2* carriers, respectively [14]. Another prospective study disclosed that the cumulative risk of gastric cancer by the age of 75 is 7.1% (95% CI, 3.5–10.8), 7.7% (95% CI, 1.9–13.6), and 5.3% (95% CI, 0.0–13.1) in variant carriers of *MLH1*, *MSH2*, and *MSH6*, respectively [18]. These data underscore the fact that Japanese LS patients are more susceptible to gastric cancer probably by environmental factor(s), because the incidence of sporadic gastric cancer is also high in the Japanese. In Korea, the calculated risk of gastric cancer in LS patients was 2.1-fold greater than in the general population [19]. From the clinical point of view, the high incidence of gastric cancer should be considered into the assessment of diagnosis and the surveillance of LS patients and variant carriers. The peaks of cumulative risk ratio at age 55 years for males and 60 years for females, and the decline of risk ratio after 60 years may suggest the early onset of gastric cancer. In fact, the age of gastric cancer in the variant carriers ranged from age 48 to 66 years (average of 55.3 years old), and the earliest age of gastric cancer in the first-degree relatives was 35 years. Although further studies on the incidence and onset of gastric cancer are needed, Japanese variant carriers may need to start endoscopy of upper gastrointestinal tract between 30 and 35 years of age, which is consistent with the previous recommendations [15]. Since we did not collect the information of *Helicobacter pylori* infection in this study, we cannot compare the risk of gastric cancer between LS patients with and without infection. Future studies are of necessity to clarify the efficacies of endoscopic surveillance and eradication of *H. pylori* on the development of gastric cancer in variant carriers.

Since this study was carried out using the information of selected CRC patients by the modified Amsterdam criteria, and only the three major responsible genes were analyzed, the results just provide a rough estimation for the prevalence and clinicopathological features of LS in CRC patients. Nevertheless, it has elucidated the high incidence of gastric cancer in LS patients and recapitulated the importance gastric cancer in clinical practice. Future studies with a larger Japanese population will contribute to the clarification of Japanese LS.

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Compliance with ethical standards

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

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CORRECTION

Correction: Importance of gastric cancer for the diagnosis and surveillance of Japanese Lynch syndrome patients

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Since the publication of the above article, the authors of the above paper have noticed errors in the description of variants, and misclassifications of the pathogenicity of two variants in the text and Supplementary Table 2 after its publication. The errors were corrected according to the recommendations of sequence variant nomenclature of Human Genome Variation Society (HGVS) in the text as described below. The misclassifications of two variants, c.2250C>G (p.Tyr750Ter) and c.279_281del (p.Leu94del), have been deleted, because they are categorized as "uncertain significance" and "likely benign," respectively, according to the recent version of ClinVar database. These changes are included in the new version of Supplementary Table 2.

In the "Result" section: Identification of pathogenic variants, we would change the descriptions and the number of pathogenic variants written in italic.

In this study, we investigated variants in the three major genes associated with LS, namely *MLH1*, *MSH2*, and *MSH6*, by PCR-direct sequencing using the Sanger's method. To investigate large deletions and duplications, MLPA was performed for subjects without pathogenic variants. As a result, 64 (57.7%) of the 111 subjects carried 55 types of pathogenic variants (Supplementary Table 2). Twenty one of the 55 have not reported in LOVD, HGMD, or ClinVar databases, and were novel variants. The 21 variants included *c.213_215delAGA*, *c.320_321delTA*, *c.469dupT*, *c.473delA*, *c.526delA*, *c.545+4_545+5delCA*, *c.1673_1676dupAACT*, *c.2200_2201dupTT*, *c.1-94948_453+716del*, *c.381-415_453+733del*, *c.1038+960_1410-429delins(101)* in *MLH1*, *c.2300_2303delinsATATATAT*, *c.2310delT*, c.2455A>T, *c.1-7545_211+2024del*, *c.1-19631_1077-3200del*, *c.793-453_1076+5896dup*, *c.943-584_1277-3562del*, *c.1077-10584_1276+207dup* in *MSH2*, and *c.3404dupC* in *MSH6*. In addition, a subject (JLS054) carried a variant, *MSH6* c.3656C>T (p.Thr1219Ile), which was judged as a variant of uncertain significance (VUS) because it was categorized as a VUS in the LOVD, and as uncertain significance or likely pathogenic variant in ClinVar. Moreover, another two subjects (JSL066 and JSL128), the former carried a variant, *MLH1 c.2250C>G* (*p.Tyr550Ter*), and the latter carried a variant, *MSH2 c.279_281del* (*p.Leu94del*), were judged as uncertain significance and likely benign, respectively, in ClinVar database. Among the 64 variant carriers, 34 had a variant in *MLH1*, 28 in *MSH2*, and 2 in *MSH6*. Regarding the method of detection, 46 of the 55 types of variants were identified by PCR-direct sequence in 47 subjects, and 11 were identified by MLPA in 17 subjects, suggesting that 17 out of 64 (26.6%) variant carriers with large structural alterations would have been overlooked if additional analysis of MLPA were not performed.

It is of note that three types of recurrent variants were identified in this study; *MLH1*, c.199G>A observed in two patients (JLS051 and JLS109), *MLH1*, *c.381-415_453+733del* encompassing exon 5 in six patients (JLS039, JLS055, JLS058, JLS070, JLS095, and JLS101), and *MSH2*, *c.1-7545_211+2024del* encompassing exon 1 in two patients (JLS023 and JLS114). It remains to be established whether the three changes are founder variants in Japanese LS.

These corrections do not alter the conclusion and discussions of the paper. The authors would like to apologize for the errors and misclassifications.