Concordance between microsatellite instability testing and immunohistochemistry for mismatch repair proteins and efficient screening of mismatch repair deficient gastric cancer

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Abstract. Microsatellite instability (MSI) testing, an established technique that has gained prominence in recent years for its predictive potential regarding the efficacy of immune checkpoint inhibitors, is used to evaluate DNA mismatch repair (MMR) deficiency (dMMR). As with other methods, the immunohistochemistry (IHC) of MMR proteins is also widely adopted. Although both techniques have been validated, their concordance rate remains unknown, particularly regarding non-colorectal cancer. Therefore, the aim of the present study was to explore and elucidate their concordance in the context of gastric cancer (GC). A total of 489 surgically resected primary GC tissues were analyzed to compare the results yielded by the MSI test and those from IHC. Of 488 GC cases, 56 (11.5%) exhibited a loss of MMR proteins, whereas 52 (10.7%) were classified as high-frequency MSI (MSI-H). The concordance rate between these two categories was 99.2%. The microsatellite markers BAT26 and MONO27 demonstrated 100% sensitivity and 99.5% specificity in detecting dMMR GC. In addition, histopathological analysis revealed that MSI-H was more prevalent in GCs exhibiting coexisting Tub2 and Por1 subtypes. However, four discordant cases were observed. All four cases were microsatellite-stable cases but exhibited loss of MLH1 protein expression with hypermethylation of the *MLH1* promoter. The results of the present study highlight that while there is a strong concordance between MSI and IHC testing results for determining dMMR status, IHC testing may offer superior efficacy in detecting dMMR.

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Abbreviations: GC, gastric cancer; MMR, mismatch repair; dMMR, mismatch repair deficiency; MSI, microsatellite instability; IHC, immunohistochemistry; MSI-H, MSI-high; CRC, colorectal cancer; MSI-L, MSI-low; MSS, microsatellite stable; pMMR, proficient MMR

Key words: microsatellite instability, immunohistochemistry, gastric cancer, adenocarcinoma, mismatch repair, immune checkpoint inhibitors

Introduction

Gastric cancer (GC) is the fifth most prevalent cancer globally and the fourth leading cause of all cancer-related deaths; in 2020, GC caused 769,000 deaths worldwide (1). Furthermore, GC has the highest incidence rate in East Asia; however, its age-adjusted occurrence has decreased over the past quarter-century (2). Nevertheless, there is a rise in the number of new cases in Japan, attributed to the aging society (2,3).

Mismatch repair (MMR) deficiency (dMMR) plays an important role in the oncogenic process and in determining the properties of cancer cells in various cancer types (4,5). In addition, the detection of dMMR holds significant diagnostic value for Lynch syndrome (6) and serves as a predictor of the efficacy of immune checkpoint inhibitors (ICI) (7). The microsatellite instability (MSI) test and immunohistochemistry (IHC) for MMR proteins are established methods for determining the dMMR status; a positive outcome is determined by the presence of high-frequency microsatellite instability (MSI-H) or MMR protein loss, respectively (6). The concordance rate between these two tests exceeds 90% (8) in colorectal cancer (CRC). However, some studies have demonstrated discrepancies between them, which might be attributable to the methods used (e.g., microsatellite marker, antibody), the type of cancer, and the genes responsible (9,10). For example, numerous Lynch syndromes identified in patients with uterine cancer are reportedly caused by the *MSH6* gene, a gene prone to yielding false negative results in MSI testing, causing discrepancies in the results obtained from IHC testing (9). Therefore, we posit that procuring datasets detailing the concordance rates between these tests for each cancer type, coupled with an understanding of the causes of discordance, holds the potential to enhance screening accuracy for Lynch syndrome and facilitate the appropriate application of immune checkpoint

inhibitors. GC is classified as one of the cancers associated with Lynch syndrome according to the Revised Bethesda guidelines (11); it is also the third most frequent MSI-H cancer among unresectable/recurrent solid cancers, followed by endometrial cancer and small intestine cancer in Japan (12). This underscores the importance of conducting dMMR testing for GC in clinical settings. However, the comprehensive investigation of the concordance rate between these two testing methods and cases of discordance in GC remains a relatively sparse area of study (13,14). To our knowledge, till date, none of the studies have directly compared the results of IHC with those of MSI testing using the Promega panel, which is used worldwide and serves as a companion diagnostic for ICI in Japan (12). Therefore, we performed MSI testing using the Promega panel and conducted IHC for MMR proteins, which allowed the elucidation of their concordance rate. Furthermore, discordant cases were explored in detail.

Materials and methods

Patients. A total of 489 consecutive patients who had undergone gastrectomy at the Department of Digestive Tract and General Surgery, Saitama Medical Center, Saitama Medical University, between April 2005 and May 2016, were included in the analyses. Clinicopathological data were obtained from the medical records of the patients. The study was approved by the local ethics committee of Saitama Medical Center, Saitama Medical University (approval numbers: 860, 924-VIII, 925, and 926-V), and Saitama Cancer Center (approval number: 1079).

Histological evaluation. All tissue samples were fixed in neutralized 10% formalin after resection and embedded in paraffin using standard procedures. Subsequently, serial sections of 4- and 10-μm thickness were prepared from each specimen. The 4-μm-thick sections were used for hematoxylin-eosin staining and IHC (15), whereas the 10-μm-thick sections were used for DNA extraction. The 489 GC cases were pathologically diagnosed according to the Japanese Classification of Gastric Carcinoma (16). The main histological subtypes were as follows: well-differentiated tubular adenocarcinoma (tub1), moderately differentiated tubular adenocarcinoma (tub2), papillary adenocarcinoma (pap), solid-type, poorly differentiated adenocarcinoma (por1), nonsolid-type, poorly-differentiated adenocarcinoma (por2), signet-ring cell carcinoma (sig), and mucinous carcinoma (muc). Histological subtypes, that are not predominant, characterized by the pathologist as comprising over 10% of the tumor were defined as mixed components. In addition, gastric carcinomas were divided into differentiated and undifferentiated types according to the Nakamura classification (17). The differentiated and undifferentiated types are almost equivalent to the intestinal and diffuse types in Lauren's classification.

Immunohistochemistry (IHC). IHC was performed using the 4-μm-thick GC sections and a DAKO EnVision FLEX system (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol (15). The anti-hMLH1 (clone ES05, DAKO, dilution 1:50), anti-hMSH2 (clone FE11, DAKO, 1:50), anti-hMSH6 (clone EP49, DAKO, 1:50), and anti-hPMS2 (clone EP51, DAKO, 1:40) antibodies were used for detecting MMR proteins.

A case was denoted as MMR-D if a defect was present in one or more MMR proteins in tumor cell nuclei, whereas it was denoted as MMR-P if all MMR proteins were normal in tumor cell nuclei.

DNA extraction. Genomic DNA for MSI testing was extracted from the 10-μm-thick formalin-fixed paraffin-embedded specimens prepared from the resected tumors and corresponding normal gastric tissue using the QIAamp DNA FFPE tissue kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions.

Microsatellite instability test. To assess MSI status, DNA from normal and tumor tissues was evaluated using an MSI test kit (FALCO Biosystems, Kyoto, Japan) as previously reported (12). This kit utilizes the same marker regions as the Promega Panel. MSI testing was performed by a single Clinical Laboratory Improvement Amendments-certified and College of American Pathologists-accredited laboratory (FALCO Biosystems).

In the cases of discordant MSI and IHC findings, in-house MSI analysis was performed using seven microsatellite markers (BAT25, BAT25, NR21, NR24, D2S123, D5S346, and D17S250). Using a fluorescence-based PCR method, amplified products obtained with primers of these marker regions were analyzed using the GenomeLab GeXP Genetic Analysis System (Beckman Coulter Inc., Brea, CA, USA) and CEQ8000 software (Beckman Coulter Inc.) as described previously (18).

The MSI status was classified as MSI-H in the presence of two or more unstable markers, MSI-low (MSI-L) in the presence of only one unstable marker, and microsatellite stable (MSS) in the absence of unstable markers. The markers used in each MSI test are listed in Table SI. Primer information for in-house MSI analysis is shown in Table SII (primer information for the MSI test kit is not available). Reassessment of MSI test results was carried out by KA and OS.

MLH1 promoter methylation analysis. In the case of MSI-H or MMR-D GC, the methylation status of the *MLH1* promoter region was analyzed using the real-time PCR-based method MethyLight. The methylation status of a sample was considered positive at a cut-off percentage of methylated reference volume >10%, following a previous report (19). Primer information for *MLH1* promoter methylation analysis is shown in Table SII. The Methylight method is a semiquantitative analysis of C to T conversion at target sites using bisulfite-treated DNA. Therefore, primers are also used only for sequences after bisulfite treatment.

Statistical analysis. All data were analyzed using SPSS version 22 (SPSS Inc, Chicago, IL, USA). Comparisons among continuous and categorical variables were made using the Mann-Whitney and Fisher's exact tests, respectively. Fisher's exact test was performed separately to determine whether MSI-H GC is more frequently in the elderly (>70), in female, in the lower region, in type 2, and in early stage (stage I and II) compared to MSS. The tests are 2x2 for each category (or categories) and for the other, MSI-H and MSS. P-values <0.01 (two-sided) were considered statistically significant.

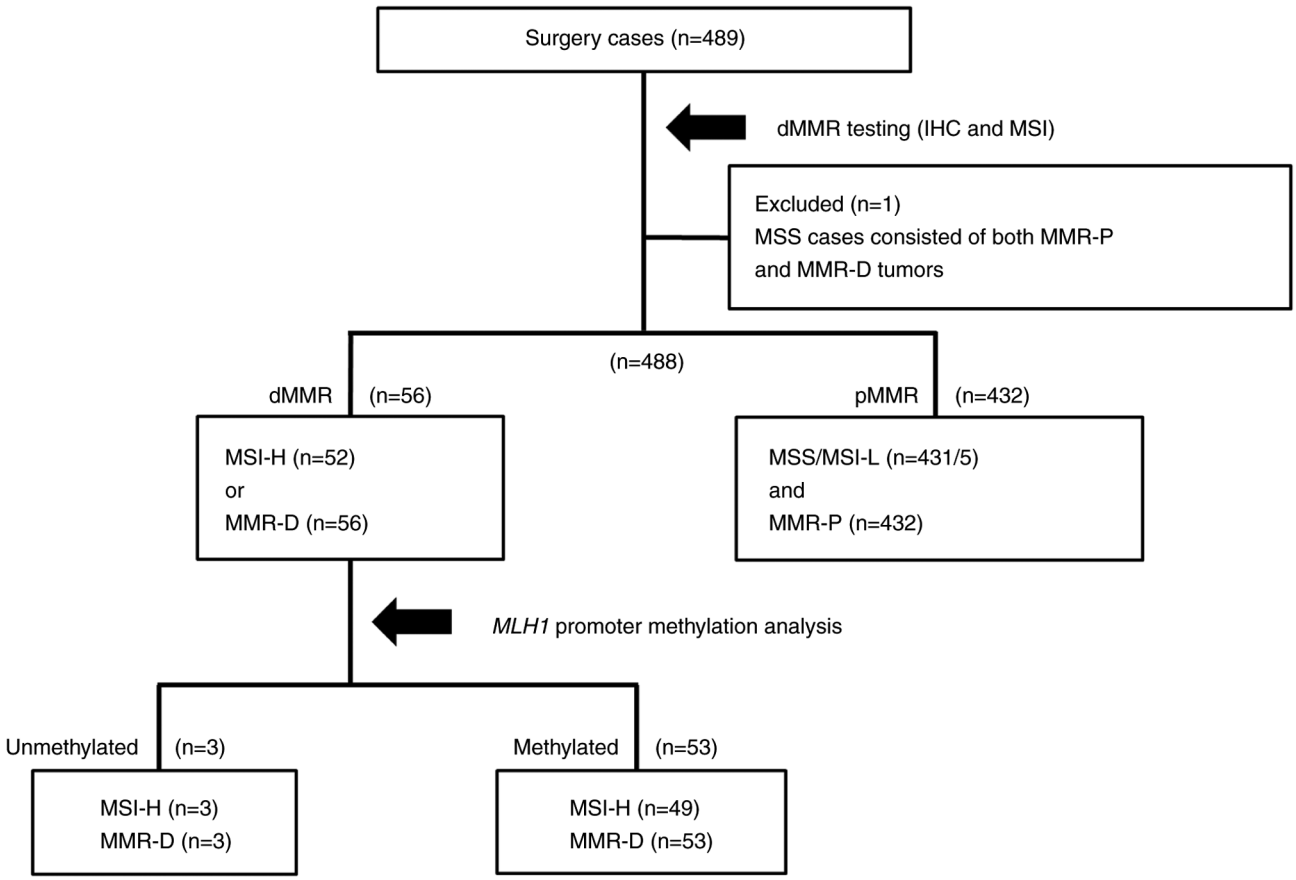

Figure 1. Flowchart of dMMR tests. The numbers indicate the number of cases determined using each test. IHC, immunohistochemistry; MSI, microsatellite instability; dMMR, mismatch repair deficiency; MSS, microsatellite stable; MMR-D, deficiency in ≥1 MMR proteins; pMMR, proficient MMR; MSI-L, MSI-low; MSI-H, MSI-high.

Results

Patient characteristics and MSI status. The flowchart of dMMR testing is shown in Fig. 1. Cases with MMR-D or MSI-H were considered dMMR, whereas those with MMR-P and MSS/MSI-L were considered proficient MMR (pMMR). One case was excluded because it contained biphasic regions of MMR-P and MMR-D within the tumor and could not be evaluated separately for MSI status in the two regions (Fig. S1), leaving a total of 488 cases. Of the 488 GC cases, 52 (10.6%), 5 (1.0%), and 431 (88.3%) were determined to be MSI-H, MSI-L, and MSS, respectively, according to the MSI test; in contrast, 56 (11.5%) and 432 (88.5%) were MMR-D and MMR-P, respectively, according to IHC. In our previous study, germline genetic testing for MMR genes to diagnose Lynch syndrome was performed on three dMMR and Unmethylated cases (Fig. 1). As a result, a pathogenic variant of *MLH1* gene was detected in one case, and this patient was diagnosed with Lynch syndrome (15).

The clinicopathological characteristics of MSI status are shown in Table I. Similar to MMR-D (15), MSI-H GC occurred more frequently at >70 vs. ≤70 years and Lower region, and was often Type 2 vs. the other regions and other types, respectively, and was often Stage I and II vs. stages III-IV, compared with the MSS/MSI-L group (Table I); however, there was no association with histological classifications. Stage IV cases encompass instances determined to be Stage IV through postoperative pathology, cases of palliative surgery, and cases of debulking surgery performed considerably earlier. None of the patients underwent neoadjuvant therapy.

Comparison of MSI and IHC tests for MMR proteins. A comparative analysis of the results of MSI testing and IHC revealed the presence of four discordant cases. MSI and IHC results demonstrated concordance in 484 of the 488 cases (99.2%). The sensitivity and specificity of the MSI test in relation to IHC were 92.9 and 100%, respectively (Table II). The positive rates for BAT25, BAT26, NR21, NR24, and Mono27 in MSI-H GCs were 94.2% (49/52), 100% (52/52), 92.3% (48/52), 92.3% (48/52), and 100% (52/52), respectively (Table III). Thus, most MSI-H cases showed positivity for all five markers (45/52: 86.5%, Table SIII); however, the combination of BAT26 and MONO27 enabled the identification of MSI-H GCs with 100% sensitivity and 99.5% specificity.

Histological subtypes and MSI status. According to classification by predominant histological subtype, 80.8% of MSI-H GCs were classified as tub2 or por1 (Table IV). 451 cases contained some kind of mixed components in addition to the predominant histological subtype (Table SIV). Within tub2 and por1 predominant subtypes, upon analyzing mixed components, MSI-H GCs exhibited the presence of mainly both tub2 and por1 (regardless of which was predominant, Table SV).

Table I. Clinicopathological findings.

Parameter	MSS/MSI-L (n=436; 89.3%)	MSI-H (n=52; 10.7%)	P-value
Age, years			
<41	9	0	
41-50	20	0	
51-60	63	1	
61-70	153	15	
71-80	148	26	
81-90	37	10	
>90	6	0	
Age, median (range)	69 (22-99)	76 (56-87)	<0.01[a]
Sex, n (%)			<0.01[b]
Female	99 (22.7)	29 (55.8)	
Male	337 (77.3)	23 (44.2)	
Tumor location, n (%)			<0.01[b]
Upper	145 (33.3)	5 (9.6)	
Middle	132 (30.1)	8 (15.4)	
Lower	159 (36.5)	39 (75)	
Macroscopic type, n (%)			<0.01[b]
Type 0	33 (7.6)	2 (3.8)	
Type 1	30 (6.9)	1 (1.9)	
Type 2	120 (27.5)	31 (59.6)	
Type 3	170 (39.0)	16 (30.8)	
Type 4	63 (14.4)	0 (0)	
Type 5	20 (4.6)	2 (3.8)	
Histological classification, n (%)			>0.99[b]
Differentiated type	221 (50.7)	28 (53.8)	
Undifferentiated type	215 (49.3)	24 (46.2)	
Immunohistochemistry, n (%)			<0.01[b]
MMR-P	432 (99.1)	0 (0)	
MMR-D	4 (9)	52 (100)	
TNM stage, n (%)			<0.01[b]
Stage I	34 (7.8)	12 (23.1)	
Stage II	118 (27.1)	19 (36.5)	
Stage III	176 (40.4)	19 (36.5)	
Stage IV	108 (24.8)	2 (3.8)	

[a]Mann-Whitney U-test; [b]Fisher's exact test. GC, gastric cancer; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, MSI-low; MSI-H, MSI-high; MMR-P, presence of all mismatch repair (MMR) proteins; MMR-D, deficiency in ≥1 MMR proteins; TNM, tumor node metastasis.

Cases with discordant results between MSI testing and IHC. Four GC cases showed discrepancies between MSI testing (Promega panel) and IHC results. For all cases, the MSI waveform was visually reassessed by two genetics-specialized doctors (KA and OS) proficient in observing such patterns; two cases may be considered MSI-H. The MSI test waveforms and IHC results for these cases are shown in Fig. 2. In Case 1, MLH1 was sparsely negative, PMS2 was negative, *MLH1* promoter region methylation was positive, and the histological subtype was por1. Initially determined to be MSI-L owing to the presence of instability solely in BAT26, a reassessment prompted suspicion of MSI-H because of the instability also observed in MONO27. In Case 2, MLH1 and PMS2 were negative, *MLH1* promoter region methylation was positive, and the histological subtype was sig. Initially determined to be MSS, a subsequent reassessment based on visual inspection indicated instability of BAT25 and MONO27. Thus, these two cases may be classified as MSI-H.

Other cases remained as MSS even after visual inspection (Fig. 3). Next, an in-house MSI test involving three dinucleotide repeat markers (D2S123, D5S346, and D17S250) was performed for these two cases. In Case 3, MLH1 and PMS2 were negative, *MLH1* promoter region methylation was positive, and the histological subtype was sig. Case 4 was similar to Case 3; however,

Table II. Performance of MSI test versus IHC test as reference test.

Group	MMR-D	MMR-P	Total
MSI-H	52	0	52
MSS/MSI-L	4	432	436
Total	56	432	488

Sensitivity, 92.9%; specificity, 100%. PPV, 100%. NPV, 99.1%. PPV, positive predictive value; NPV, negative predictive value; MSI, microsatellite instability; MSI-L, MSI-low; MSI-H, MSI-high; MSS, microsatellite stable; IHC, immunohistochemistry; MMR-P, presence of all mismatch repair (MMR) proteins; MMR-D, deficiency in ≥1 MMR proteins.

Table III. Number of unstable cases per *loci* in MSI-H and MSI-L.

Group	BAT25	BAT26	NR21	NR24	MONO27
MSI-H	49/52	52/52	48/52	48/52	52/52
MSI-L	3/5	1/5	0/5	0/5	1/5

MSI, microsatellite instability; MSI-h, MSI-high; MSI-L, MSI-low.

Table IV. Correlation between histological subtypes and MSI status.

Histological subtypes	MSS/MSI-L, n (%)	MSI-H, n (%)	Ratio of MSI-H, %
Pap	12 (2.8)	0 (0)	0.0
Tub1	54 (12.4)	6 (11.5)	10.0
Tub2	151 (34.6)	22 (42.3)	12.7
Por1	100 (22.9)	20 (38.5)	16.7
Por2	86 (19.7)	2 (3.8)	2.3
Sig	22 (5.0)	1 (1.9)	4.3
Muc	11 (2.5)	1 (1.9)	8.3

MSI, microsatellite instability; MSI-h, MSI-high; MSI-L, MSI-low; MSS, microsatellite stable.

the histological type was tub2 (Fig. 3). In Case 3, the in-house MSI test detected changes in the dinucleotide repeats (D5S346 and D2S123). Conversely, in Case 4, the evaluation of NR24 was revised as 'unstable', whereas D2S123 was changed. Thus, both might be possibly classified as MSI-H (Fig. 4). Several MSS cases were analyzed using the in-house MSI tests; however, no dinucleotide repeats changes were observed (Fig. S2).

Discussion

Determining dMMR status holds immense significance for immunotherapy effectiveness and Lynch syndrome diagnosis. The two major methods for making this assessment are the MSI test and IHC for MMR proteins. However, the concordance rate between the results of these methods in GC remains unclear. In the present study, we investigated this aspect in consecutive series of 488 GC cases, constituting the largest series for a comparative study involving MSI and IHC. Our findings revealed a concordance rate of 99.4%, with sensitivity and specificity of 92.9 and 100%, respectively. This rate surpasses the rates reported in previous studies (13,14), which may be attributable to the different antibodies and microsatellite markers used in each study, thereby affecting the results. The Bethesda panel (11), comprising two mononucleotide markers and three dinucleotide markers, has been a common choice in previous studies. However, subsequent research has demonstrated that five mononucleotide markers (such as in the Promega panel) exhibit more sensitivity and are preferable (12). In our study, all five markers were positive in 86.5% (45/52) of MSI-H cases; particularly, BAT26 and MONO27 showed 100% sensitivity. Therefore, all MSI-H GCs can be identified using only these two markers, streamlining the MSI test in GC. In this regard, incorporating MONO27 may have increased the sensitivity of the MSI test in this study and contributed to the high concordance rate observed with the IHC test, as MONO27 was not included in previous studies (13,14). In contrast, the frequency of MSI-L was 1.0% (5/489), consistent with the results of previous studies that indicated lower frequencies of MSI-L in MSI tests comprising mononucleotide markers (20-22), as opposed to those encompassing dinucleotide markers (23,24).

In this study, the frequency of MSI-H was 10.7% among all GC cases. According to investigations conducted in East Asian countries using universal tumor screening of consecutive patients, the frequency of MSI-H GC ranged from 8.2% to 17.7% (14,25,26). The frequency of MSI-H GC in our study was slightly lower than the frequencies reported in previous studies in Japan (25,26), which may be attributed to the male-female ratio and age distribution, in addition to the different markers used for identifying MSI-H GC, as discussed previously. Previous reports have indicated that MSI-H GC is more common in elderly women and in the lower stomach (25), consistent with the trends observed in this study.

Regarding histopathological findings, the Por1 subtype exhibited the highest frequency in MSI-H GCs (20/120, 16.7%) among histological subtypes; however, within MSI-H GC, the tub2 subtype was most common (22/52, 42.3%). MSI-H cancers are enriched in the por1 subtype in GC and CRCs (25,27,28). Regardless of their individual predominance, it is plausible that MSI-H GCs possess an inherent tendency to contain components of both tub2 and por1 (28). MSI-H GCs have been reported to be frequently found in the pap subtype (25); however, our results depict a scarcity of cases of the pap subtype and no MSI-H GCs in this subtype. This discrepancy might be related to the fact that GC of the papillary type is often resected endoscopically, owing to its propensity for early-stage presentation (29). The relationship between histological subtypes and dMMR status may need to be analyzed in a larger number of cases and not limited to surgical specimens. Recent studies using Artificial intelligence to predict MSI from histopathological images may potentially compensate for errors in MSI and IHC tests (30).

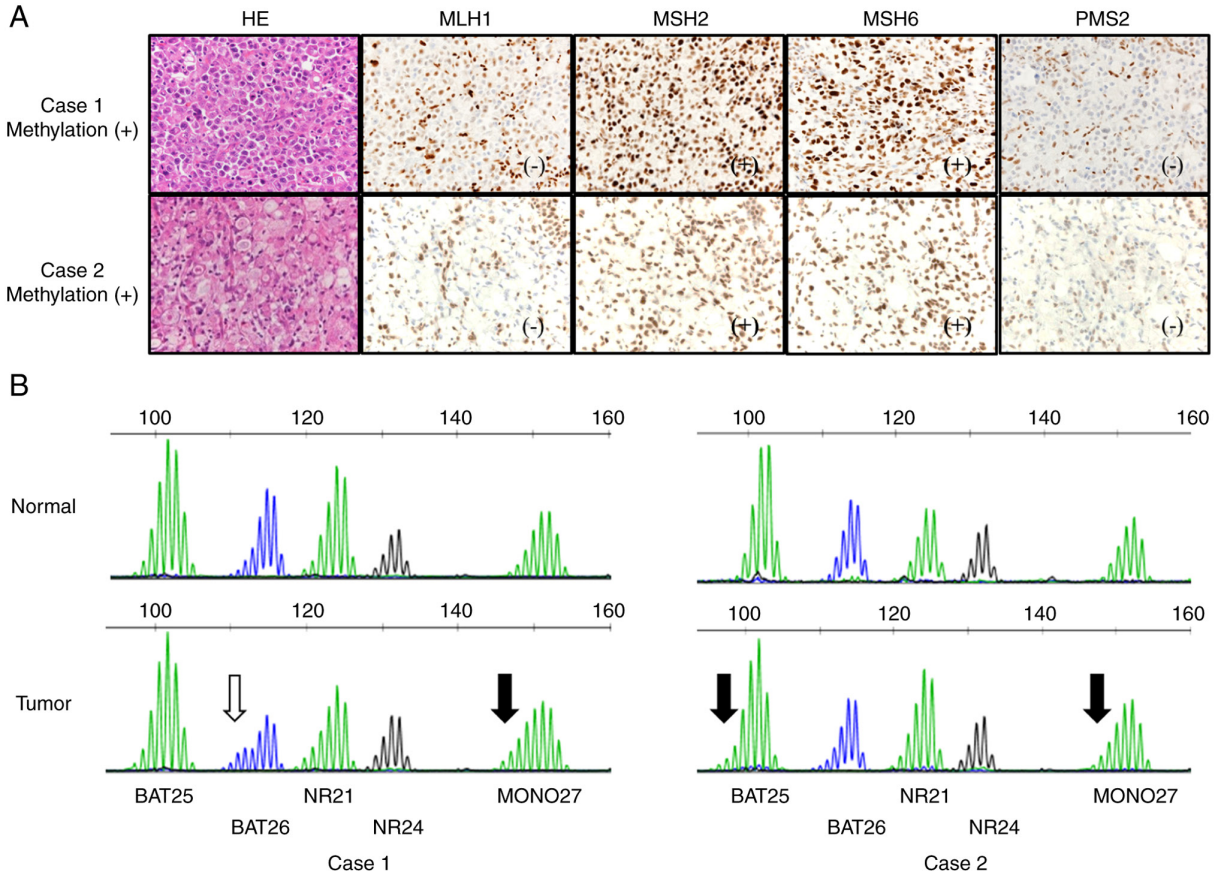

Figure 2. Cases of difficulty in determining MSI. (A) Immunohistochemical findings of cases 1 and 2. The lower right corner of the inset shows each immunostaining result (magnification, x200). (B) Waveform of the MSI test kit. Black arrows indicate marker regions determined to be MSI based on reassessment. The BAT26 of case 1 (white arrow) was initially assessed as unstable. HE, hematoxylin-eosin staining; MSI, microsatellite instability.

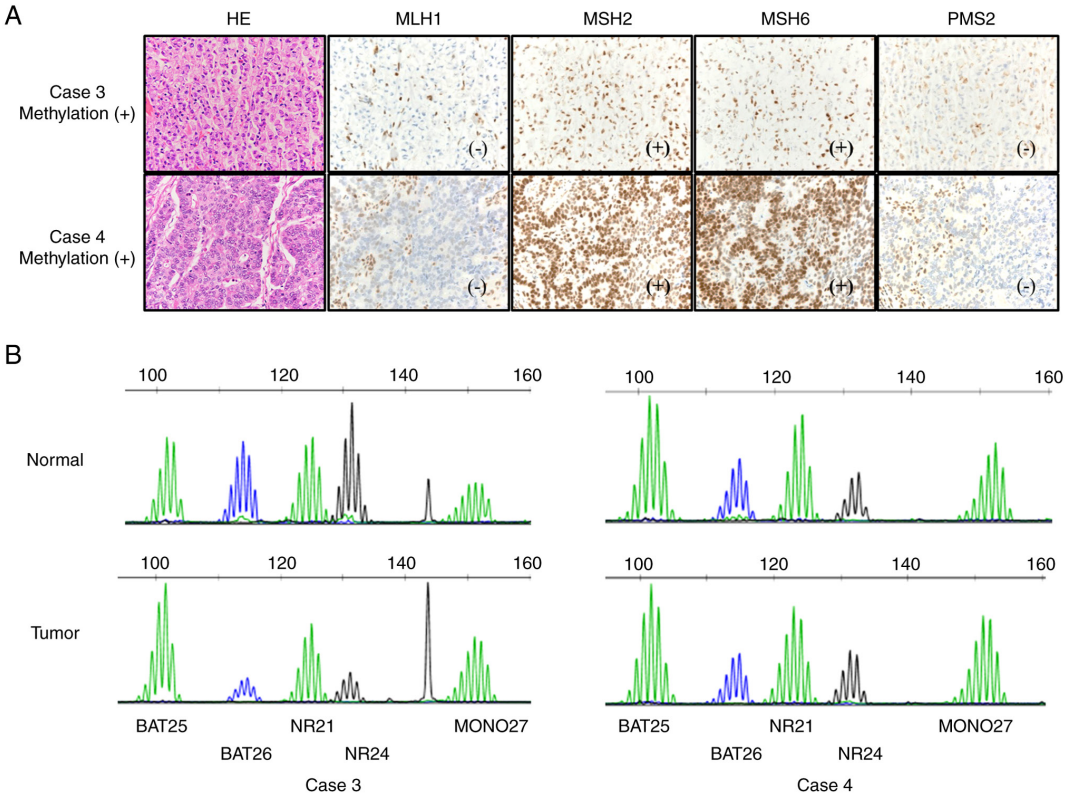

Figure 3. Cases with discordant results between MSI and IHC tests. (A) IHC findings of cases 3 and 4. The lower right corner of the inset shows each immunostaining result (magnification, x200). (B) Waveform of the MSI test kit. HE, hematoxylin-eosin staining; MSI, microsatellite instability; IHC, immunohistochemistry.

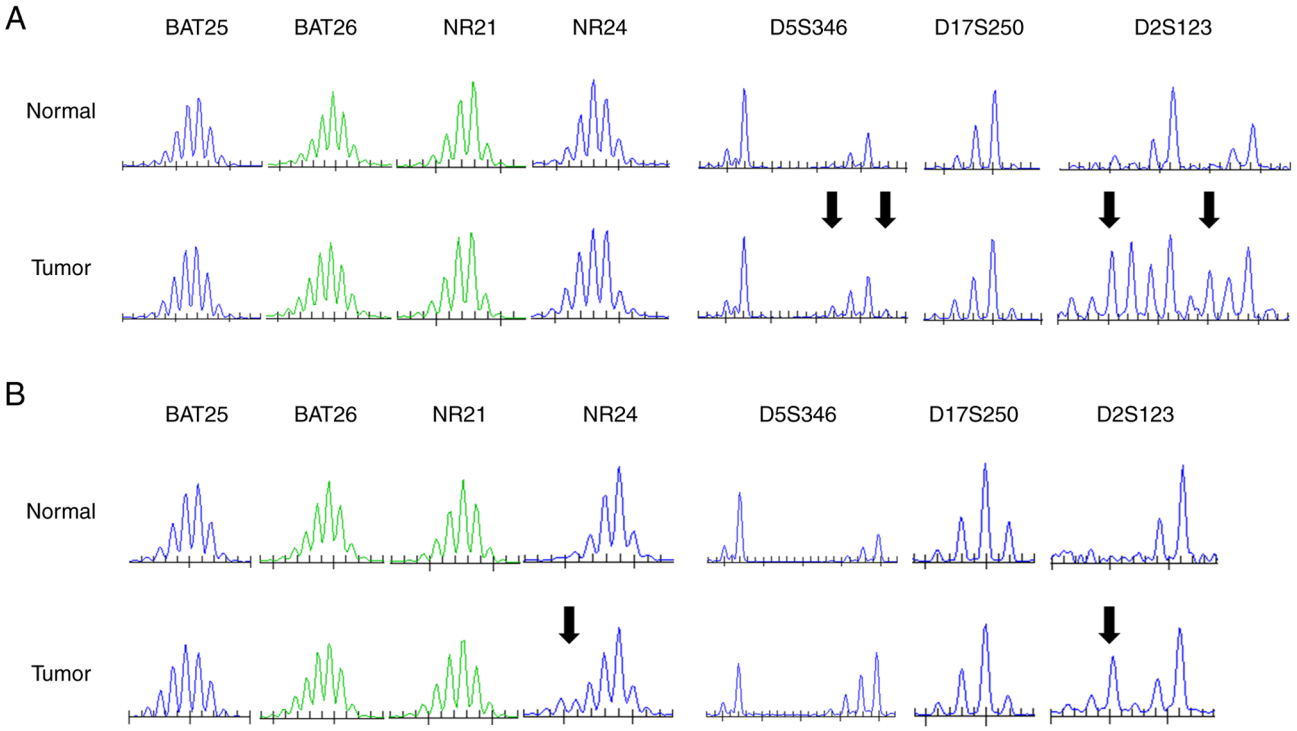

Figure 4. Validation results of MSI test using the in-house method. (A) Waveform of case 3. (B) Waveform of Case 4. Black arrows indicate marker regions determined to be MSI according to the in-house method. MSI, microsatellite instability.

There were four cases with discordant results between the MSI and IHC tests. All of these cases exhibited MSS or MSI-L/MMR-D patterns with a loss of MLH1/PMS2 and *MLH1* promoter hyper-methylation. The high incidence of MSS and loss of MLH1/PMS2 expression in these discordant cases were similar to those reported in previous studies (31). The MSI test is prone to false negatives depending on the amount and proportion of tumor in the specimen and the type of causative MMR gene (8,9,32). Among these discordant cases, three were of poorly differentiated GCs, whereas one was classified as well-differentiated. Additionally, none of the cases showed a single loss of MSH6 or PMS2, which are the proteins prone to false negatives in MSI tests (8). To explore the underlying causes of these discordant results, we conducted a close reevaluation of the waveforms of the MSI test. As a result, two of the cases were potentially regarded as MSI-H. However, the other two cases remained as MSS. Additional MSI tests that included three dinucleotide markers were conducted to further confirm the results. As a result, instability was detected in the dinucleotide repeat region (mainly D2S123). Mononucleotide markers are generally more sensitive in the West (21,22). However, compared with data from Western countries, several reports from Asia suggest that the dinucleotide region is often unstable in GCs and that D2S123 is particularly sensitive (33,34). Although the involvement of *Helicobacter pylori* is suspected as a hallmark of GC in East Asia, its status as a causative factor remains uncertain because some reports have shown no relationship between *H. pylori* and MSI (35). Furthermore, the changes observed during the evaluation of NR24 in in-house MSI tests may be due to the reagents and equipment used. Specifically, in the MSI test (Promega panel), all regions were amplified using multiplex PCR and measured together. Conversely, the in-house MSI test was conducted using PCR with only NR21 and NR24, which may have led to the increased sensitivity observed.

The high intratumor heterogeneity of GC may also be related to this discrepancy (36). In uterine cancer, a heterogeneous *MLH1* promoter methylation state is exhibited within tumor tissues (37) and there are cases of MSS even with the loss of MLH1/PMS2 and *MLH1* promoter hypermethylation (38). Cases of *MLH1* promoter hypermethylation with small changes in mononucleotide markers and large changes in dinucleotide markers have also been reported (39). These trends are similar to those observed in the discordant cases in the present study and are presumed to be possible phenomena in cases of *MLH1* promoter hypermethylation. The concordance rate observed in this study indicates that the Promega panel can yield results similar to those achieved through the IHC test. However, the appropriate selection of dinucleotide markers may improve the determination of dMMR status in Japanese GC.

The current study had some limitations. First, limited number of cases with sufficient DNA for MSI testing may have caused selection bias. Second, the sample size of dMMR cases was small (52 MSI-H and 56 MMR-D GCs). Nevertheless, the paucity of reports detailing the concordance rate between the two tests for ICI application underscores the significance of our comparative findings for clinical practice utilization.

In recent years, the combined positive score derived from IHC utilizing PD-L1 antibody has emerged as a frequent biomarker for guiding the application of ICI in GCs (40). Moreover, research findings have indicated that the efficacy of ICI monotherapy is comparable to that of ICI plus chemotherapy in MSI-H GCs (41,42), and the combination

of nivolumab and ipilimumab may be as highly effective as in MSI-H CRC (43,44). Therefore, the importance of dMMR decision tests for GC is poised to escalate in the future.

Our data show a high number of false positive results for the MSI test. However, even with IHC testing, false positives and false negatives can occur due to factors such as specific mutations, treatment, sample condition, and proficiency of the pathologist (8-10). Prior IHC testing, but if dMMR is suspected based on other clinicopathological information, such as older age, female, mixed tub2 and por1 subtypes, etc., the MSI test should still be performed. In conclusion, the concordance rate between the IHC and MSI tests was very high in the context of dMMR determination. However, the IHC test may have a higher ability to detect than the Promega panel. The underlying difference could stem from genetic or geographical differences, heterogeneity in the MLH1 promoter methylation status or the type of marker used in the MSI test. If MSI testing is used, it is advisable to consider the utilization of a combination of dinucleotide markers, including D2S123, and mononucleotide markers.

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Availability of data and materials

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

Authors' contributions

GY, HI and KA conceived and designed the study. TI, OS and HI collected the gastric cancer cases. TI, OS and NK collected the clinical information. GY, MK, AT, KI and KA performed a dMMR test. TA performed the histological examination. GY, TI, OS and NK analyzed the data. GY, MK, AT, KI and KA drafted the paper. NK, TA, HI and KA reviewed all the data and revised the final paper. GY, TI, OS, AT, HI and KA confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The study was approved by the local ethics committee of Saitama Medical Center, Saitama Medical University (approval nos. 860, 924-VIII, 925 and 926-V), and Saitama Cancer Center (approval no. 1079). Informed consent to be included in the study was obtained from all patients by written form (860, 925 and 926-V) and opt-out (924-VIII and 1079). All procedures followed were in accordance the Helsinki Declaration of 1964 and later versions.

Patient consent for publication

Informed consent to be included in the study was obtained from all patients by written form and opt-out.

Competing interests

The authors declare that they have no competing interests.

Authors' information

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