Clinicopathologic Implications of Genetic Instability in Intestinal-Type Gastric Cancer and Intestinal Metaplasia as a Precancerous Lesion

Proof of Field Cancerization in the Stomach

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Abstract

To clarify field cancerization *in the stomach by genetic alterations, we studied 83 cases of intestinal-type gastric cancer (GC) and paired intestinal metaplasia (IM) distant from GC and 39 cases of chronic gastritis with IM (CG-IM) for genetic instability (GIN). Microsatellite instability (MSI) and loss of heterozygosity (LOH) were evaluated at 5 microsatellite loci.*

The incidence of GIN was 21% (8/39) in CG-IM, 48% (40/83) in GC-IM, and 65% (54/83) in GC and showed a significant difference among these 3 categories. By tumor location, MSI showed the highest incidence in GC and GC-IM with the tumor located in the upper third of the stomach. GIN in GC and GC-IM significantly increased with the progression of tumor invasion from mucosal to advanced cancer. GIN, especially LOH, was more frequently detected in cases with vs without lymphatic or vascular invasion and lymph node involvement in GC and GC-IM.

The GIN of GC and GC-IM was significantly similar in relation to clinicopathologic features. Biologic detection of GIN in IM may be a surrogate marker for GC risk and for clinical evaluation of malignant potential. The condition is consistent with the hypothesis of field cancerization in the stomach.

Gastric cancer (GC) is the second leading cause of cancer death and the fourth most common malignant tumor in the world.[1,2] GC is histologically divided into 2 types, intestinal and diffuse.[3] The former is thought to arise from intestinal metaplasia (IM) and may be associated with *Helicobacter pylori* infection.[4] In general, IM is believed to be a precancerous lesion of the stomach, which increases the risk of gastric adenocarcinoma, especially the intestinal type.[5,6]

GC develops through the accumulation of genetic and epigenetic alterations.[7,8] Current knowledge on the molecular mechanisms underlying gastric carcinogenesis indicates that 2 major genetic instability (GIN) pathways are involved in the pathogenesis of GC, microsatellite instability (MSI) and chromosome instability, including loss of heterozygosity (LOH).[9] MSI, defined as the presence of replication errors in simple repetitive microsatellite sequences, is responsible for a well-defined subset of GC and has been recognized as one of the earliest changes in GC carcinogenesis.[9-11] LOH, characterized by gross chromosomal alterations, qualitative or quantitative, is an early event in tumor formation that increases with tumor progression.[10] Although the MSI and chromosomal instability phenotypes can be distinguished from one another, evidence suggests some overlap.[10,11] To date, there are several studies on the occurrence of GIN in GC. It has been reported that intestinal-type GC more commonly involves MSI and LOH than does the diffuse type.[12-14] However, data for GIN in intestinal-type cancer are conflicting.[15,16]

Previous studies found that MSI, but not LOH, as a genetic alteration in IM possibly had a role in the early events leading to gastric carcinogenesis.[17-19] This finding indicates that MSI may be a useful marker in identifying high-risk IM that may develop into intestinal-type GC. To date, the relationship

between IM with GC and GIN, eg, MSI or LOH, has been demonstrated extensively, with divergent results.[17,18,20,21] However, no studies have described the relationship between clinical features and GIN in IM with regard to the background mucosa of intestinal-type cancer.

To make a plausible prediction on the outcome of IM as a premalignant gastric mucosal lesion, a comprehensive genetic indicator is needed in addition to identification of the specific genetic change contributing to the determination of tumor progression and enhancement of the molecular basis underlying the malignant transformation. This type of study may lead to the identification of new diagnostic and prognostic molecular markers.

Multiple GCs, which constitute 4% to 10% of all GCs, are associated with more extensive IM. With regard to the genesis of multiple GCs, multicentricity (independent origin) rather than multifocality (local or lateral spread of one cancer) has been the favored theory. Conventional morphologic study, however, has not provided convincing evidence in support of multicentricity.[22]

The aims of the present study were to clarify GIN in intestinal-type GC and IM as its background mucosa in relation to clinicopathologic features of cancer and to verify the concept of *field cancerization* in the stomach from the analysis of genetic alterations.

Materials and Methods

Cases

We randomly selected 83 cases of intestinal-type GC from the histopathology files of Asahikawa Medical College, Asahikawa, Japan, between January 1997 and March 2006. The patients had undergone surgical operation or endoscopic mucosal resection. All GC cases studied showed extensive IM (GC-IM) in the stomach. None of the patients had received preoperative adjuvant therapy or *H pylori* eradication. None of the patients had a family history suggestive of hereditary nonpolyposis colorectal cancer. Furthermore, 39 cases of chronic gastritis with IM (CG-IM) were also analyzed as the control samples for this study.

Histologic types were determined according to the Lauren classification.[3] Tumor location and invasion were classified based on the classification of the Japanese Gastric Cancer Association.[23] Tumor location was subdivided into 3 groups: upper third, middle third, and lower third of the stomach. Tumor invasion was classified into 2 stages: early (involvement of the mucosa or submucosa) and advanced (involvement of the muscularis propria or deeper). The samples in the study included 59 early GCs, including 32 mucosal and 27 submucosal cancers, and 24 advanced cancers. All GC-IM and CG-IM cases were of the incomplete type lacking Paneth cells. In all cases, 4 biopsy specimens were obtained to assess *H pylori* infection, 2 from the greater curvature of the antrum and the others from the greater curvature of the corpus. *H pylori* status was determined to be present by a positive result for Warthin-Starry staining, *H pylori* culture, or both.

Written informed consent was obtained from patients before they were interviewed for this study. The ethics committee of Asahikawa Medical College approved the study.

Genomic DNA Extraction

Specimens were fixed in 10% formalin and embedded in paraffin wax, and 4-μm consecutive sections were used for histologic examination by H&E staining. From the paraffin-embedded blocks, two 7-μm tissue sections were cut. DNA was extracted from the cancerous area, from IM (GC-IM) **Image 1** distant from the cancerous area, from normal mucosa without metaplasia, and from CG-IM cases as the control group. For DNA from the IM, biopsy or surgically resected samples obtained from the antrum distant from the tumor, but not from the surrounding mucosa of cancer area, were used. For the DNA extraction procedure, tissue was precisely microdissected under microscopic visualization using a P.A.L.M. MG III Laser Capture Microdissection System (MEIWAFOSIS, Osaka, Japan) to avoid DNA contamination of inflammatory or stromal cell nuclei, based on previous reports.[19,24]

Analysis of MSI and LOH by High-Resolution Fluorescent Microsatellite Analysis

As reported previously,[19] we examined 5 microsatellite loci on chromosomes for MSI and LOH based on the Bethesda panel[25] as follows: 2p (BAT26), 4q (BAT25), 2p (D2S123), 5q (D5S346), and 17p (D17S250). One primer for each primer pair was fluorescence labeled at the 5′ end. Polymerase chain reaction (PCR) amplification was carried out in a reaction volume of 10 μL, which contained 100 ng of genomic DNA, 1× PCR buffer (Perkin Elmer Applied Biosystems Division, Foster City, CA), 200 μmol/L of each deoxynucleoside triphosphate, 600 μmol/L of each primer, and 1.5 U of AmpliTaq Gold polymerase (Perkin Elmer). The magnesium chloride concentration was 1.5 mmol/L. The following PCR cycle conditions were used for amplification: 95°C for 10 minutes, 30 cycles of 95°C for 45 seconds, 55°C for 1 minute, and 72°C for 30 seconds.

PCR products were evaluated for MSI and LOH by capillary electrophoresis using an ABI Prism 310 genetic analyzer (Perkin Elmer) and automatic sizing of the alleles using a Gene Scan (Applied Biosystems). MSI was defined as positive when unequivocal extra peak bands in tumor or IM DNA different by multiples of 2 base pairs in dinucleotide

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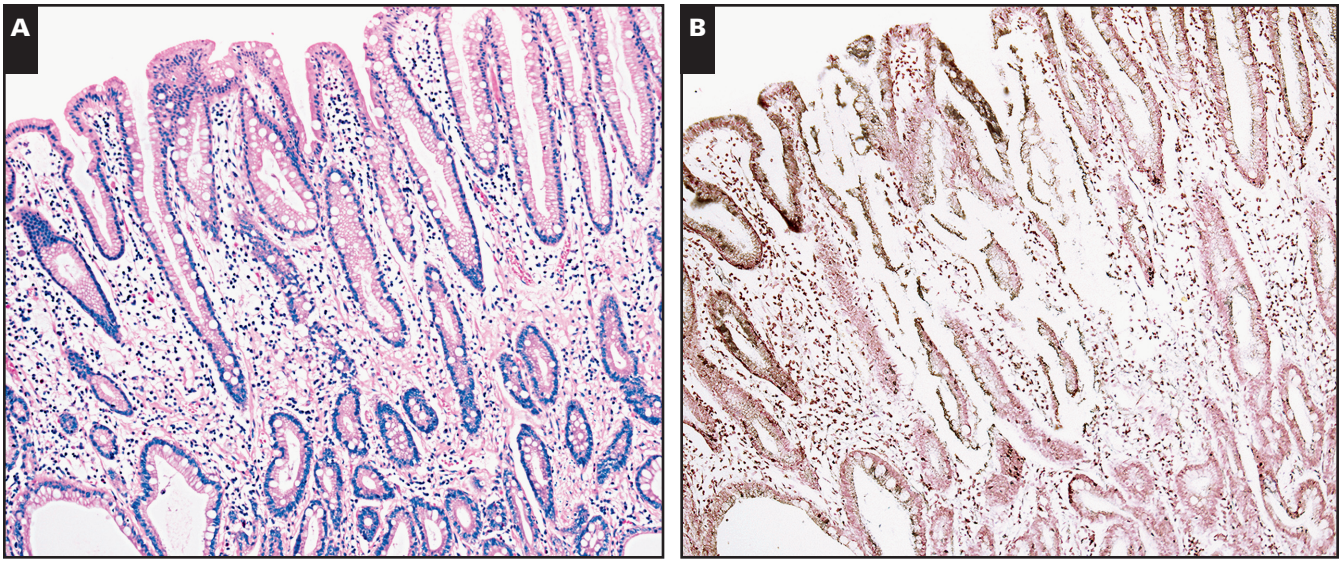

Image 1 **A**, The glands of intestinal metaplasia were isolated by laser capture microdissection (H&E, ×100). **B**, The same section after removal of metaplastic glands (hematoxylin, ×100).

markers or 1 base pair in mononucleotide markers from DNA in normal mucosa were observed and was also characterized by the appearance of drastic additional alleles in the tumor or IM DNA. The former type of MSI was judged as the minor pattern (extra peak bands) **Figure 1A** and the latter type as the major pattern (additional alleles) **Figure 1B**, as reported previously.[19,21,24,26]

Tumors or IMs were defined as having high MSI (MSI-H) when unstable loci were observed in 2or more of 5 microsatellite markers and as having low MSI (MSI-L) when unstable loci were observed in only 1 of 5 markers studied.[26] The tumor or IM was considered microsatellite stable (MSS) if no unstable loci were found. Generally, most of the clinical and molecular features in MSI-L cancers are considered similar to those of MSS cancers and different from those of MSI-H cancers.[27-29] In the literature, the MSI phenotype is categorized into 2 groups: MSI-H and MSI-L/MSS, and a sample is defined as MSI only when MSI-H is observed.

LOH is determined to be positive when an allelic ratio (AR = T1:T2/N1:N2) is less than 0.7, as used by Kobayashi et al[21] in a GC study. Briefly, T1 and N1 represent the highest respective peak areas of the shorter allele in cancerous and normal mucosa samples and T2 and N2 the highest respective peak areas of the longer allele. For cases in which the AR was more than 1.0, the ratio was inverted (1/AR) to obtain results in the range of 0.0 to 1.0 **Figure 1C** and **Figure 1D**.

Statistical Analysis

Statistical differences were assessed by using the Mann-Whitney *U* test between independent groups and by using the χ^2 test or Fisher exact test between 2 proportions. Statistical significance was defined as a *P* value of less than .05.

Results

The data for age, sex, and *H pylori* infection rate from 83 patients with GC are summarized in **Table 1**. All patients with CG were positive for *H pylori* infection. No association between clinical data and GIN was found.

In cancer cases, GIN was seen in 54 (65%) of 83 cases in the cancer area and in 40 (48%) of 83 GC-IM cases, showing a significant difference between GC-IM and the cancer area ($P < .05$) **Table 2**. In CG-IM, 8 (21%) of 39 cases were positive for GIN. The frequency of GIN was significantly lower in CG-IM than in the cancer area and in GC-IM ($P < .0001$ and $P < .005$, respectively). The incidence of cases with both MSI and LOH at different loci was 14% (12/83) in the cancer area, 8% (7/83) in GC-IM, and 11% (1/9) in CG-IM.

Correlation Between GIN and Tumor Location

GIN in each location is shown in **Table 3** and **Table 4**. GIN in the cancer area tended to be higher in the upper region when compared with that of the middle region ($P < .1$). MSI was significantly higher in the upper region than in the middle region ($P < .05$); however, there was no significant difference in LOH (Table 3).

GIN in GC-IM showed a significantly higher incidence in the upper region than in the middle and lower regions ($P < .001$ and $P < .005$, respectively). Similarly, MSI was significantly higher in the upper region than in the middle and lower regions ($P < .0005$ and $P < .05$, respectively). LOH in the upper region showed a high tendency toward significance compared with that in the lower region ($P < .1$) (Table 4).

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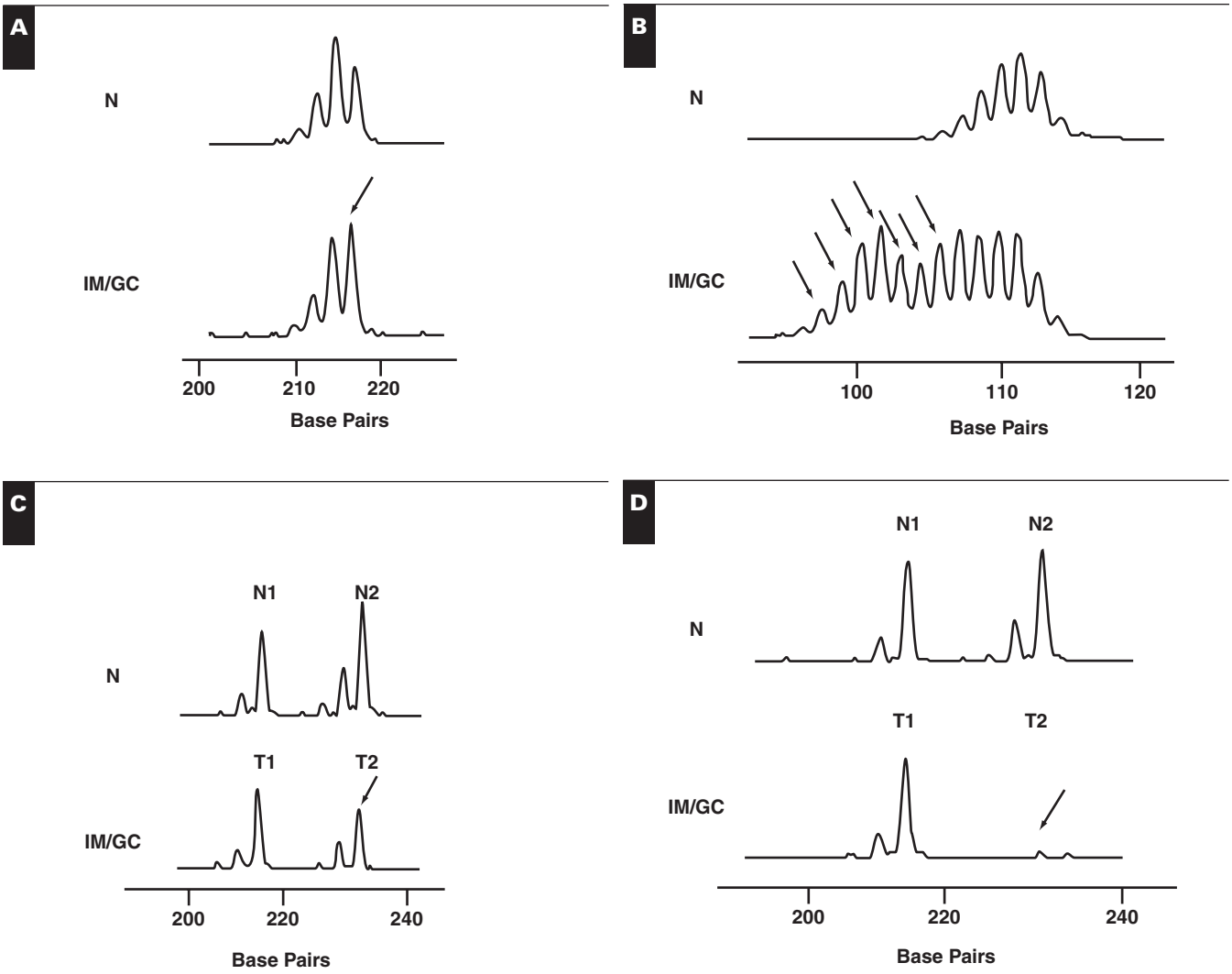

Figure 1 Examples of microsatellite instability (MSI) and loss of heterozygosity (LOH) were detected in intestinal metaplasia (IM) and gastric cancer (GC) by high-resolution fluorescent microsatellite analysis. DNA was isolated from IM and GC and matching normal mucosa without IM (N). **A**, Representative case of a minor pattern of MSI on D2S123. MSI is seen as an unequivocal extra peak shift (arrow) compared with normal mucosa. **B**, Representative case of a major pattern of MSI on BAT26. MSI is characterized by the appearance of multiple drastic additional alleles (arrows). **C**, T1 and N1 represent the highest respective peak areas of the shorter allele in cancerous or intestinal metaplastic and normal mucosa samples and T2 and N2 the highest respective peak areas of the longer allele. LOH on D17S250 (longer allele, T2) of IM/GC DNA is seen (arrow). **D**, Representative case of LOH on D17S250. The longer allele (T2) is not detected in IM/GC (arrow).

Table 1
Characteristics of Patients With Gastric Cancer

		GIN+	
	GIN– (n = 29)	MSI (n = 31)	LOH (n = 35)
Mean ± SD age (y)	65.6 ± 9.8	69.3 ± 9.6	65.6 ± 11.1
Sex			
Male	25	23	30
Female	4	8	5
No. (%) with *Helicobacter pylori* infection	25 (86)	25 (81)	29 (83)

GIN, genetic instability; LOH, loss of heterozygosity; MSI, microsatellite instability.

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Table 2
Incidence of Genetic Instability in Cancer Area and IM in Patients With Gastritis and Cancer

		GIN+		
	GIN–	No. of Cases/Total (%)	MSI	LOH
IM				
Gastritis	31	8/39 (21)*†	5	4
Cancer	43	40/83 (48)†‡	21	25
Cancer area	29	54/83 (65)*‡	31	35

GIN, genetic instability; IM, intestinal metaplasia; LOH, loss of heterozygosity; MSI, microsatellite instability.
* $P < .0001$.
† $P < .005$.
‡ $P < .05$.

Table 3
Clinicopathologic Characteristics and Genetic Instabilities in Gastric Cancer*

		GIN+		
	GIN–	Total	MSI	LOH
Tumor location				
Upper third (n = 27)	6 (22)	21 (78)†	14 (52)‡	13 (48)
Middle third (n = 34)	15 (44)	19 (56)†	8 (24)‡	15 (44)
Lower third (n = 22)	8 (36)	14 (64)	9 (41)	7 (32)
Tumor invasion				
Early (n = 59)	24 (41)	35 (59)	21 (36)	22 (37)
Mucosal (n = 32)	15 (47)	17 (53)‡	10 (31)	10 (31)
Submucosal (n = 27)	9 (33)	18 (67)	11 (41)	12 (44)
Advanced (n = 24)	5 (21)	19 (79)‡	10 (42)	13 (54)
Lymphatic/vascular invasion				
Positive (n = 36)	8 (22)	28 (78)‡	17 (47)	19 (53)
Negative (n = 47)	21 (45)	26 (55)‡	14 (30)	16 (34)
Lymph node metastasis				
Positive (n = 22)	3 (14)	19 (86)	9 (41)	14 (64)†
Negative (n = 24)	8 (33)	16 (67)	13 (54)	9 (38)†

GIN, genetic instability; LOH, loss of heterozygosity; MSI, microsatellite instability.
* Data are given as number (percentage). Advanced cancer involves the muscularis propria or deeper.
† $P < .1$.
‡ $P < .05$.

Table 4
Clinicopathologic Characteristics and Genetic Instabilities in Intestinal Metaplasia of Patients With Cancer*

		GIN+		
	GIN–	Total	MSI	LOH
Tumor location				
Upper third (n = 27)	6 (22)	21 (78)†‡	14 (52)‡§	12 (44)‖
Middle third (n = 34)	22 (65)	12 (35)†	3 (9)‡	9 (26)
Lower third (n = 22)	15 (68)	7 (32)‡	4 (18)§	4 (18)‖
Tumor invasion				
Early (n = 59)	34 (58)	25 (42)‖	16 (27)	14 (24)§
Mucosal (n = 32)	19 (59)	13 (41)	8 (25)	7 (22)
Submucosal (n = 27)	15 (56)	12 (44)	8 (30)	7 (26)
Advanced (n = 24)	9 (38)	15 (63)‖	5 (21)	11 (46)§
Lymphatic/vascular invasion				
Positive (n = 36)	13 (36)	23 (64)§	10 (28)	17 (47)‡
Negative (n = 47)	30 (64)	17 (36)§	11 (23)	8 (17)‡
Lymph node metastasis				
Positive (n = 22)	8 (36)	14 (64)	3 (14)§	11 (50)
Negative (n = 24)	10 (42)	14 (58)	11 (46)§	8 (33)

GIN, genetic instability; LOH, loss of heterozygosity; MSI, microsatellite instability.
* Data are given as number (percentage). Advanced cancer involves the muscularis propria or deeper.
† $P < .001$.
‡ $P < .005$.
§ $P < .05$.
‖ $P < .1$.

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Correlation Between GIN and Depth of Tumor Invasion

The total incidence of GIN in the cancer area was significantly higher in advanced than in mucosal cancer ($P < .05$), although no significant differences were seen in MSI or LOH irrespective of tumor invasion (Table 3). In GC-IM, the incidence of GIN tended to be higher in the advanced compared with the early stage ($P < .1$). LOH increased significantly with the progression of tumor invasion ($P < .05$) (Table 4).

Correlation Between GIN and Vascular or Lymphatic Invasion

In the cancer area, the overall incidence of GIN increased significantly with lymphatic or vascular invasion ($P < .05$), whereas there was no significant difference in MSI or LOH in cases with or without lymphatic or vascular invasion (Table 3). Similarly, GIN, including LOH in GC-IM, was significantly higher in cases with lymphatic or vascular invasion than in cases without it ($P < .05$). No significant difference was found in MSI irrespective of lymphatic and vascular invasion.

Correlation Between GIN and Lymph Node Metastasis

LOH in the cancer area tended to be higher in node-positive cases compared with node-negative cases ($P < .1$). However, there was no significant difference in MSI between either type of case for lymph node metastasis (Table 3).

The incidence of LOH in GC-IM was higher in node-positive cases than in node-negative cases but did not reach statistical significance. In contrast, the incidence of MSI in GC-IM was significantly higher in cases without lymph node metastasis than in cases with it ($P < .05$) (Table 4).

Correlation of GIN Between Cancer and IM

It is interesting that cancer tissues positive for MSI and LOH showed a tendency to be accompanied by positive GC-IM. This finding was statistically significant ($P < .0001$ for MSI and LOH) **Table 5** and **Table 6**.

The corresponding rates in GIN, eg, MSI and LOH, between the cancer area and GC-IM were 81% (67/83) and 80% (66/83), respectively. The frequency of GIN at different loci is summarized in **Figure 2**. For each marker analyzed, GIN in the cancer area showed a higher frequency than that in IM. BAT25 and D2S123 showed the most frequent incidence among the 5 microsatellite markers. In contrast, the frequency of GIN in BAT26 was the lowest among the markers studied.

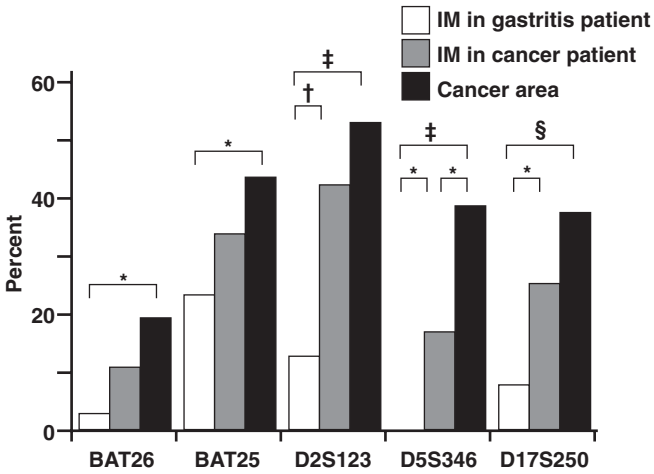

Figure 2 The frequency of genetic instability at different loci in intestinal metaplasia (IM) and the cancer area. * $P < .05$. † $P < .005$. ‡ $P < .0001$. § $P < .001$.

The overlap rate between MSI and LOH in the cancer area and IM is summarized in **Table 7**. The overlap between MSI and LOH was 14% (12/83) in the cancer area and 8% (7/83) in GC-IM. It is interesting that we identified 29 GCs (35%) that were negative for MSI and LOH.

Table 7
Overlap Rate Between MSI and LOH in 83 Paired Gastric Cancers and Intestinal Metaplasia*

	MSI+	MSI–
Intestinal metaplasia		
LOH+	7 (8)	18 (22)
LOH–	14 (17)	44 (53)
Cancer		
LOH+	12 (14)	23 (28)
LOH–	19 (23)	29 (35)

LOH, loss of heterozygosity; MSI, microsatellite instability.
* Data are given as number (percentage).

Table 5
MSI in Paired Gastric Cancers and Intestinal Metaplasia

	Intestinal Metaplasia	
Cancer	MSI+	MSI–
MSI+*	18	13
MSI–*	3	49

MSI, microsatellite instability.
* $P < .0001$.

Table 6
LOH in Paired Gastric Cancers and Intestinal Metaplasia

	Intestinal Metaplasia	
Cancer	LOH+	LOH–
LOH+*	22	14
LOH–*	3	44

LOH, loss of heterozygosity.
* $P < .0001$.

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Discussion

This is the first study that demonstrates the clinicopathologic implications of GIN in IM as a precancerous lesion in GC and compares GIN in GC and IM in relation to clinical features. In the present study, we clearly found the following: (1) GIN including MSI or LOH is frequently detected and tends to be coincident in cancerous lesions and IM. (2) GIN is frequently observed in increasing order in CG-IM, GC-IM, and the cancer area. (3) Alterations in the paired samples of GC and GC-IM in the background mucosa have similar molecular features of GIN in relation to clinicopathologic features. Thus, our findings support the argument that IM may be a precancerous lesion for intestinal-type GC, which occurs with sequential accumulation of genetic alterations in histologic progression from IM to cancer.

Our data showed that the incidence of MSI in GC was the highest in the proximal location compared with other regions. Several investigators have reported that MSI is associated with a distal location of GC,[11,12,20,30] but others did not detect this association.[31] There are some explanations for this discrepancy. As mentioned previously, it can be explained by the method of DNA extraction. The laser capture microdissection used in this study allows procurement of relatively pure tumor cell populations from complex heterogeneous cell mixtures.[32] Therefore, the specificity of genetic alterations in DNA extracted selectively from the area is higher than hand-microdissected samples.[33] Second, in assessing tumors for the presence of MSI or LOH, many studies differ in terms of which and how many loci should be analyzed. Third, the method of analysis for MSI or LOH affects the outcome. When conventional methods are used, the electrophoretic profiles of PCR products may not always be reproducible.[34,35] Moreover, assessment of MSI using an autoradiograph is difficult.[34,35] The high-resolution fluorescent microsatellite analysis assay used in the present study allows more accurate assessment compared with the conventional method.[34] It is interesting that the incidence of GIN in GC-IM showed similar tendencies, as well as in the cancer area.

GIN in GC showed a significantly higher incidence in cases with lymphatic or vascular invasion, although this difference was not significant in MSI or LOH. Furthermore, GIN, including LOH in GC-IM, occurred significantly more often in cases with vs without lymphatic or vascular invasion. Similarly, LOH tended to be higher in cases with lymph node metastasis in comparison with cases without metastasis in the cancer area and in GC-IM, although the difference was not significant. These results suggest that a similar molecular event exists in GC and the precancerous lesion, GC-IM. Our data agree with the report that these two lesions may be chronologically connected[21] and the observation that chromosomal instability is an early event in tumor formation that increases with tumor progression.[10]

In the present study, MSI was observed in 21 (25%) of 83 GC-IM cases and 31 (37%) of 83 GC cases, showing a higher incidence than that reported by Kobayashi et al.[21] In contrast, Leung et al[20] reported a higher incidence in the level of MSI-H than in the present study. Generally, most of the clinical and molecular features of MSI-L cancers are considered similar to those of MSS cancers and different from those of MSI-H cancers. In the literature, therefore, the MSI phenotype is categorized into 2 groups: MSI-H and MSI-L/MSS, and a sample should be defined as MSI only when MSI-H is observed.[27-29]

Previous reports showed that BAT26 is considered highly sensitive and specific in identifying MSI.[36,37] In our study, BAT26 showed the lowest incidence, whereas BAT25 and D2S123 showed the highest levels of MSI among the 5 markers. Bacani et al[38] reported, similar to findings in the present study, that BAT26 alone is not an adequate marker for checking the level of MSI. The difference in MSI rates may be associated with the number of markers used and the method of assessment of MSI. There is an interesting report, however, that states that because the sensitive markers identifying MSI have different results in different populations, the selection of markers should be carefully considered when analyses are performed in people with different genetic backgrounds or from different geographic regions.[39] Thus, further studies will be required with a larger sample to clarify this issue in the same manner.

Some investigators have attempted to study the relationship between clinicopathologic features or outcome and LOH status in GC.[14,40] LOH has been demonstrated to be a valuable prognostic factor and tumor stage indicator in GC, and survival is reduced in patients with GC with a high LOH level,[14,41] suggesting that tumor progression correlates with accumulation of GIN.[10,14,39] In the present study, the incidence of GIN in cancer areas significantly increased with the degree of tumor invasion, and LOH in GC-IM also showed a significant increase with tumor invasion. The aforementioned results are the first to document that similar genetic alterations occur coincidentally in the cancer area and in GC-IM in background mucosa.

Multiple GCs are commonly observed in approximately 10% of patients with GC.[42] Esaki et al[43] reported that multiple GCs often showed similarities in macroscopic and microscopic morphologic features, which may suggest similar pathways of tumor development. It has also been reported that secondary cancers occur more frequently in patients with multiple GCs than in patients with a single GC.[44] These results suggest that patients with multiple GCs may be more prone to developing additional cancers than patients with a single GC. Consequently, it is possible

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that these patients may have a genetic predisposition to the development of cancer.

Previous studies showed that MSI might have an important role in the development of synchronous and metachronous GCs and that it may be used clinically as a molecular marker for the prediction of multiple GCs.[45,46] In previous studies of the relationship between MSI and IM in patients with GC, corresponding samples of IM were acquired from areas adjacent to the GC. However, in the present study, GC-IM was obtained from the antral portion distant from the tumor but not from the adjacent IM of GC. Thus, our findings indicate that the entire stomach with GC-IM harboring GIN is considered to be in a precancerous state. In this study, the similar molecular backgrounds of IM may explain the pathogenesis of synchronous and metachronous GCs even after resection of tumors due to persistent uncorrected accumulated errors of DNA mismatch repair in IM. A subset of IM with GIN has the possibility for the development of GC after a certain time. Therefore, genetic markers and possible cutoffs that predict development of GC should be determined to identify the cancer risk.

The development of GC and IM is closely associated with *H pylori* infection.[4] Some investigators have reported a significant association between *H pylori* infection and GC with MSI,[20,30] but others have not.[47,48] In the present series, the *H pylori* infection rate was more than 80%, and no association was found between infection and GIN. It has been reported by some Japanese physicians that *H pylori* treatment reduced the incidence of metachronous GC in patients who underwent endoscopic mucosal resection of early-stage GC.[49,50] However, the molecular changes associated with gastric carcinogenesis due to *H pylori* infection were not well defined. We previously found a significant association between *H pylori* infection and GIN in GC-IM in patients with GC, and the accumulation of genetic alterations is carried on from IM to GC. As demonstrated herein and consistent with our previous report,[19] it is possible that the eradication of *H pylori* will change the molecular behavior of IM as evident by reversion of GIN and may decrease the risk of gastric carcinogenesis.

The results of our study show for the first time that when IM coexists with GC, similar genetic alterations to GIN are detectable in both lesions. Moreover, the biologic detection of GIN in precancerous gastric lesions, such as IM, may be a surrogate marker of risk for GC and for the clinical evaluation of malignant potential. Our findings support the argument that IM with GIN is a precancerous lesion for intestinal-type GC, and the condition is consistent with the hypothesis that field cancerization in the stomach is mucosal intestinalization.[51] Future studies are required with a larger series of samples to determine the possible role of GIN in IM as a precancerous lesion. Hereafter, we shall attempt to investigate not only genetic but also epigenetic alterations by using other markers to clarify the mechanisms involved in gastric carcinogenesis.

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