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Differences in genetic instability and cellular phenotype among Barrett's, cardiac, and gastric intestinal metaplasia in a Japanese population with *Helicobacter pylori*

J Watari, K Moriichi¹, H Tanabe¹, R Sato, M Fujiya¹, K M Das², and Y Kohgo¹

Department of Gastroenterology, Kushiro Medical Association Hospital, Kushiro, Japan

¹Division of Gastroenterology and Haematology/Oncology, Department of Medicine, Asahikawa Medical College, Asahikawa, Japan

²Crohn's and Colitis Center of New Jersey, Division of Gastroenterology and Hepatology, Department of Medicine and Pathology, UMDNJ-Robert Wood Johnson Medical School, New Brunswick, NJ, USA

Abstract

Aims—Intestinal metaplasia is considered to be a precursor lesion in both Barrett's and intestinal-type gastric cancer. The aim was to clarify the differences in molecular pathology between specialized intestinal metaplasia (SIM) in Barrett's oesophagus (BO), cardiac (CIM) and gastric intestinal metaplasia (GIM).

Methods and results—Eighty-eight SIM cases with BO, 30 CIM cases and 52 GIM cases in patients with or without *Helicobacter pylori* infection were analysed for genetic instability and Das-1. Microsatellite instability and a loss of heterozygosity were evaluated at five microsatellite loci. The incidence of genetic instability was 55.7% in SIM, 40.0% in CIM and 23.1% in GIM, revealing a significant difference between SIM and GIM ($P < 0.0005$). For each microsatellite marker analysed, there were obvious differences in frequency among the three conditions. Das-1 reactivity was significantly higher in SIM than in CIM or GIM ($P < 0.0001$, both). Interestingly, both genetic instability and Das-1 reactivity in SIM showed a significantly higher incidence in patients with *H. pylori* infection than in those without ($P < 0.005$ and $P < 0.01$, respectively).

Conclusions—SIM is distinct from CIM and GIM, and the pathogenesis of SIM, like that of GIM, is associated to some degree with *H. pylori* infection in a Japanese Population

Keywords

Barrett's oesophagus; cardiac intestinal metaplasia; Das-1; gastric intestinal metaplasia; genetic instability; *Helicobacter pylori*; specialized intestinal metaplasia

Introduction

Gastric cancer is the second most frequent kind of malignant tumour and contributes significantly to cancer mortality, particularly in Asia.¹ It is histologically divided into two types: intestinal and diffuse.² The former is thought to arise from gastric intestinal metaplasia (GIM) and may be associated with *Helicobacter pylori* infection.^{3,4} Barrett's oesophagus (BO) is also a premalignant condition and the most important known risk factor for oesophageal adenocarcinoma (OAC),⁵ whose incidence has been rapidly rising over the past few decades in Western countries,^{6,7} although not in Asia.⁸ To diagnose BO, the presence of specialized intestinal metaplasia (SIM) in a macroscopically identifiable segment of columnar mucosa is required, and the metaplasia–dysplasia–adenocarcinoma sequence is a well-known marker of BO carcinogenesis.⁹ Additionally, cardiac intestinal metaplasia (CIM) is also a precancerous condition of adenocarcinoma at the oesophagogastric junction.¹⁰ However, BO and CIM probably differ in their oncogenic potential carcinogenesis and in their roles in the development of adenocarcinoma at the cardiac region, including OAC.¹¹ Unlike the case with GIM and some cases of CIM, however, an inverse statistically significant relationship is commonly assumed between *H. pylori* infection and BO, including both SIM and OAC, thus suggesting that these entities protect against *H. pylori* infection.^{12,13}

As regards the definition of endoscopically suspected BO, there is a serious problem in patients with *H. pylori* infection. Commonly, the oral end of the gastric fold of the stomach is defined as the oesophagogastric junction. However, in patients with severe atrophic gastritis that spreads through the cardia from the antrum, especially in Japanese, who have high rates of *H. pylori* infection, atrophic gastritis makes it difficult for the oral end of a gastric fold to be used as a marker of the oesophagogastric junction. It is thus important to determine whether the metaplastic epithelium originates from the oesophagus or from the gastric mucosa, i.e. CIM or GIM.

Numerical and structural genomic changes occur during the development of BO and gastric carcinogenesis, indicating that a great number of gene variations may be involved in oesophageal adenocarcinoma and gastric cancer.^{14–17} Genomic instability describes conditions involving the widespread loss of DNA integrity. The development of genetic instability is an important event in the multistep progression of human carcinogenesis. It has been proposed that genetic or genomic instability in human cancers can be divided into two types: chromosomal instability or loss of heterozygosity (LOH), which can result from errors in chromosome partitioning, and microsatellite instability (MSI), which is usually equated with DNA polymerase errors^{18–20} We have previously proposed that genetic instability may play an important role in BO²¹ and gastric^{22,23} carcinogenesis.

Das *et al.* have developed a monoclonal antibody (mAb), Das-1 (formerly known as 7E₁₂H₁₂, IgM isotype), that specifically reacts with colonic epithelium (both goblet and non-goblet absorptive cells), but with neither enterocytes (including goblet cells) from the jejunum or ileum nor normal epithelium from the stomach and oesophagus.^{24,25} However, mAb Das-1 reacts sensitively (95%) and specifically (100%) to BO and oesophageal adenocarcinoma.^{26,27} These data suggest that BO is a colonic type of intestinal metaplasia.²⁶

We have also reported recently that colonic phenotype GIM, detected by mAb Das-1, is strongly associated with gastric cancer.^{28,29}

As described above, intestinal metaplasia has been widely recognized as an associated risk factor and a probable precursor of both oesophageal adenocarcinoma and intestinal-type gastric cancer,^{4,10,30–33} but little is known about its pathogenesis. In the present study, we examined the differences in genetic alterations and cellular phenotypes, as identified by mAb Das-1, between intestinal metaplasia in the oesophagus and that in the stomach in a Japanese population with or without *H. pylori* infection.

Materials and methods

Patients

We diagnosed endoscopically suspected BO as a columnar-lined epithelium between the lower end of the longitudinal capillary vessels of the lower oesophagus and the squamocolumnar junction (SCJ) based on a report by Goda *et al.*³⁴ Biopsy specimens were obtained to assess the presence of SIM, namely goblet cell metaplasia. It is sometimes difficult to distinguish endoscopically suspected BO from gastric cardia because of the anatomical variations in these regions and hiatal hernia. Therefore, a biopsy for CIM was performed 10–20 mm distal from the lower end of the longitudinal capillary vessels of the lower oesophagus or from the squamocolumnar junction that was present at the same anatomical level as the oesophagogastric junction.³⁵ To assess the presence of GIM, biopsy specimens were taken from the corpus and antral mucosa. All GIM investigated here were also goblet cell metaplasias histologically. Eighty-eight cases of BO, all of which were short-segment BE, and 82 cases of chronic gastritis with CIM ($n = 30$) or GIM ($n = 52$) were randomly selected from the histopathology files of Asahikawa Medical College Hospital between 2002 and 2008.

To determine the presence of *H. pylori* infection, two biopsy specimens from the greater curvature of the antrum and two from the greater curvature of the corpus of the stomach were also taken. The *H. pylori* status of each patient was analysed by two methods: Warthin–Starry staining and *H. pylori* culture. A patient was regarded as having *H. pylori* infection if they showed positive results on at least one of these tests. Of the 88 BE cases with SIM and the 30 individuals with CIM, 49 and 22 were positive for *H. pylori* infection, respectively. In contrast, all chronic gastritis patients with GIM were positive for *H. pylori* infection. Written informed consent was obtained from all patients, and the ethics committee of Asahikawa Medical College approved this study.

The specimens were fixed in 10% formalin and embedded in paraffin wax, after which 4- μ m consecutive sections were used for histological examination by haematoxylin and eosin staining.

DNA extraction

From the paraffin-embedded blocks, two 7- μ m tissue sections were cut. DNA was extracted from SIM, CIM and GIM obtained from biopsy samples. In this DNA extraction procedure, the sample was precisely microdissected under microscopic visualization, using a P.A.L.M.

MG III (Meiwafosis, Osaka, Japan) or a PixCell (Arcturus Engineering, Mountain View, CA, USA) Laser Capture Microdissection System to avoid DNA contamination of inflammatory or stromal cell nuclei based on the methodology described in previous reports.^{22,23,36}

Analysis of MSI and LOH by high-resolution fluorescent microsatellite analysis

As reported previously,^{22,23} we examined five microsatellite loci on chromosomes for MSI and LOH based on the Bethesda panel.³⁷ Briefly, polymerase chain reaction (PCR) amplification was carried out in a reaction volume of 10 μ l, which contained 100 ng of genomic DNA, 1 $\times$ PCR buffer (Perkin Elmer Applied Biosystems, Foster City, CA, USA), 200 μ mol/l of deoxyribonucleotide triphosphate, 600 μ mol/l of each primer, and 1.5 units of AmpliTaq Gold polymerase (Perkin Elmer). The MgCl₂ concentration was 1.5 mmol/l. The following PCR cycle conditions were used for amplification: 95 °C for 10 min, 30 cycles of 95 °C for 45 s, 55 °C for 1 min, and 72 °C for 30 s. The 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). The status of MSI and of LOH were judged according to previous reports.^{22,23,36} Intestinal metaplasia was defined as having high MSI (MSI-H) when unstable loci were observed in two or more of five microsatellite markers and as having low MSI (MSI-L) when unstable loci were observed in only one of the five markers studied based on the criteria established by the International Workshop in 1997.³⁷ The lesion, i.e. SIM, CIM or GIM, was considered microsatellite stable (MSS) if no unstable loci were found. Generally, most of the clinical and molecular features of MSI-L cancers are thought to be similar to those of MSS cancers and different from those of MSI-H cancers.^{38–40} Therefore, the MSI phenotype was categorized into two groups, MSI-H and MSI-L/MSS. In our study, a sample was defined as MSI only when MSI-H was observed.

LOH was defined using the criteria of Kobayashi *et al.*⁴¹ and our previous papers.^{21–23,36} No metaplasias exhibiting MSI at a given locus were evaluated for LOH.

Immunoperoxidase assays with mAb Das-1

Serial sections were stained with mAb Das-1 using sensitive immunoperoxidase assays as described previously.^{26–29} Briefly, after deparaffinization and rehydration, free aldehydes were reduced with 0.05% sodium borohydride for 20 min at 4 °C. They were then sequentially incubated with mAb Das-1 (hybridoma supernatant diluted to 1:5) for 45 min at room temperature, followed by biotinylated rabbit antimouse Ig (Dako, Glostrup, Denmark), 3% hydrogen peroxide and streptavidin peroxidase (Dako). Subsequently, the slides were treated with diaminobenzidine–H₂O₂ solution for 20 min at room temperature. Positive controls for mAb Das-1 consisted of normal colon sections. As negative controls, the jejunum was used. Reactivity to mAb Das-1 was considered positive if cells were stained a crisp golden brown. A substantial number of cells, and more than one gland, had to be reactive to this mAb before a specimen was considered positive. If only an occasional goblet cell was stained, the sample was defined as negative.^{26–29}

Statistical analysis

Statistical analyses were made by the Mann–Whitney *U*-test, the χ^2 test, and Fisher's exact test. Statistical significance was defined as a *P*-value < 0.05.

Results

The data on the mean age and gender of patients in each group are summarized in Table 1. The mean age was significantly higher in patients with SIM, regardless of *H. pylori* infection, than in chronic gastritis patients with GIM ($P < 0.0001$). Likewise, the mean age in CIM patients was higher than that in GIM individuals, though not significantly so ($P = 0.06$). Among CIM patients, no statistical significance was found in mean age between those who were positive for *H. pylori* infection and those who were negative.

Incidence of genetic instability in SIM, CIM and GIM

Genetic instability was found in 55.7% (49/88) of SIM patients, in 40.0% (12/30) of CIM patients, and in 23.1% (12/52) of GIM patients, revealing a significant difference between SIM and GIM ($P < 0.0005$). In contrast, although genetic instability was more frequent in CIM than in GIM, the difference was not statistically significant. Of the three conditions, the highest incidence of *H. pylori* positivity was found in cases of genetic instability in SIM (SIM vs. GIM, $P < 0.0001$; SIM vs. CIM, $P = 0.05$; CIM vs. GIM, $P = 0.05$). Intriguingly, the incidence of genetic instability in SIM was significantly higher in patients with *H. pylori* infection (69.4%) than in patients without (38.5%) ($P < 0.005$), but this was not the case in CIM individuals (Table 2).

Frequency of genetic instability at different loci in SIM, CIM and GIM

In SIM, genetic instability occurred more frequently in BAT25 (54.5%) than in any of the other microsatellite markers; the differences were significant compared with BAT26 and D5S346 ($P < 0.0001$ and $P < 0.005$, respectively) (Figure 1). None of the markers showed any significant difference in frequency of genetic instability between patients with and without *H. pylori* infection (Figure 2). As regards CIM, D2S123 had the highest incidence (46.7%), which was significantly higher than that of BAT26 or D17S250 ($P < 0.05$, both). In GIM, genetic instability showed the most frequent incidence in D2S123 (30.8%) among the five markers, and had a significantly higher incidence than in BAT26, D5S346 or D17S250 ($P < 0.005$, $P < 0.0001$ and $P < 0.01$, respectively).

Next, the incidence of MSI or LOH was analysed (Table 3). The frequency of MSI was grossly lower in GIM than in SIM in all the markers except D2S123. In all three conditions, MSI was most frequently observed at BAT25. MSI at D5S346 was more frequent in CIM than in the other two categories, such as SIM and GIM. In contrast, LOH was not detected in either BAT26 or BAT25, and the frequency of LOH at D2S123 and D17S250 was similar among the three categories. LOH at D5S123 was detected more frequently in SIM than in either CIM or GIM.

Incidence of reactivity to mAb Das-1 in SIM, CIM and GIM

The reactivity of mAb Das-1 was diffusely present in the cytoplasm of SIM, CIM and GIM goblet cells (Figure 3). mAb Das-1 did not react with the non-metaplastic area in the biopsy samples evaluated. Reactivity against SIM was detected in 78.4% (69/88) of cases, significantly higher than in CIM (30.0%) or GIM (38.5%) ($P < 0.0001$, both) (Table 2). Interestingly, mAb Das-1 reactivity in SIM patients was found in 85.7% (42/49) of *H. pylori* positives and in 61.5% (24/36) of *H. pylori* negatives, also indicating a significant difference ($P < 0.01$). However, this was not detected in CIM patients.

Discussion

Many authors have examined the differences between SIM and CIM by using immuno- or mucin-histochemistry.^{35,42–53} It is important to distinguish between these conditions, because the aetiology and risk of developing adenocarcinoma differ between them.^{11,54} The pathogenesis of SIM and that of CIM are thought to be associated with gastro-oesophageal reflux disease and *H. pylori* infection, respectively,⁵⁵ but are still controversial.⁵⁶ Also unclear are the molecular and cellular phenotypic differences among SIM, CIM and GIM. The aim of this study was to clarify the differences in genetic alterations and cellular phenotypes among these three conditions in cases with or without *H. pylori* infection.

A few reports have investigated the genetic alterations comprising MSI and LOH in BO patients, and have indicated that genetic instability occurs at an early stage of SIM development.^{57,58} In our results, genetic instability was frequently observed in SIM. The current and previous data²¹ together suggest that genetic instability in SIM may be a key molecular event in the pathogenesis of BE. Sharma *et al.* have shown that the risk of malignancy is substantially greater in SIM than in CIM.¹¹ Our results also showed that genetic instability was more frequent in SIM than in CIM. Similarly, it is noteworthy that its incidence was significantly higher in SIM than in GIM ($P < 0.0001$). Thus, SIM may have a more malignant potential than CIM and GIM from the standpoint of genetic alteration. It has been demonstrated that genetic alterations in gastrointestinal malignancies are associated with ageing.^{59,60} In fact, SIM patients had the highest mean age among the three conditions. Therefore, ageing may be related to the high frequency of genetic instability in SIM.

Some aetiological studies have suggested that the pathogenesis of BO is related to gastro-oesophageal reflux disease and not to *H. pylori* infection.^{54,61} More recently, a meta-analysis showed that *H. pylori* infection protects against the development of BO.¹² From the perspective of molecular pathology, however, the frequency of genetic instability in SIM was significantly higher in individuals with than in those without *H. pylori* infection. As we reported recently,²¹ this result supports that *H. pylori* infection may affect the genetic alterations in SIM as well as in GIM,²² and may accelerate those molecular alterations associated with the pathogenesis of SIM, at least to some degree, in the Japanese population. For each microsatellite marker analysed, there was an obvious difference in the frequency of genetic instability among SIM, CIM and GIM, as shown in Figure 1. Furthermore, the frequency of genetic instability did not differ significantly among the five different loci in SIM between patients with and those without *H. pylori* infection (Figure 2), suggesting that

SIM is distinct from *H. pylori*-associated GIM, in terms of genetic instability, irrespective of *H. pylori* infection.

Previous reports have shown that BAT26 is highly sensitive to and specific for identifying MSI.^{62,63} However, our study has revealed that BAT25 is a highly sensitive marker for identifying MSI in the three conditions, whereas BAT26 showed little sensitivity in this regard. Bacani *et al.* reported findings similar to those of the present study: that BAT26 alone is not an adequate marker of MSI level.⁶⁴ The reason for this discrepancy in the frequency of MSI between these markers may be associated with the method of assessing MSI. Moreover, there is an interesting report that markers should be selected carefully depending on a patient's genetic background or geographical origin, because MSI markers produce different results in different populations.⁶⁰

We previously reported a highly significant reactivity of mAb Das-1 against GIM adjacent to gastric cancer when compared with GIM from patients without cancer.^{28,29} Recently, we found that *H. pylori* eradication reduced mAb Das-1 reactivity in GIM.²⁹ We have also reported that the reactivity of mAb Das-1 in other precancerous conditions, such as BO, is highly sensitive and specific.^{26,27} In the small intestine, although mAb Das-1 does not react with normal epithelium, mAb Das-1 reactivity is evident in most cases of small intestinal adenoma and adenocarcinoma.⁶⁵ Together, these results suggest that the incomplete or colonic phenotype of metaplasia identified by this mAb is a pre-cancerous condition for the oesophagus, stomach and small intestine, and is a biomarker of carcinogenesis. The incidence of mAb Das-1 reactivity was significantly higher in SIM than in CIM or GIM ($P < 0.0001$, both). This indicates that the cellular phenotype detected by this mAb may be an early event in the pathogenesis of intestinal metaplasia in BO rather than in the stomach. Also, SIM reacted more frequently (78.4%) to mAb Das-1 in Japanese patients as well as in US patients.^{21,26–29,35,42,52} Interestingly, mAb Das-1 reactivity against SIM, as well as GIN, showed a significantly higher incidence in patients with than in those without *H. pylori* infection. Therefore, considering the present results, *H. pylori* infection may be associated with the enhancement of the colonic phenotype identified by the mAb in the pathogenesis of BO with SIM.

In this study, we compared GIN and cellular phenotypes among SIM, CIM and GIM. Piazuolo *et al.* reported that the immunoreactivity of SIM shared phenotypic characteristics with that of GIM, but not with that of CIM, by using various markers of mucin protein types, cytokeratins, inflammation, and proliferation.^{35,42,52} In contrast, some studies have demonstrated that the immunophenotypic features of SIM in both short segment BO and long-segment BO were different from those of GIM, but were similar between GIM and CIM.^{51,54} Considering the results of previous immuno- or mucin-histochemical studies, it seems that SIM has a different nature to that of GIM, and the differences between CIM and GIM also remain controversial. There are a few possible explanations for these discrepancies. First, the evaluation of this anatomical area is complicated by the fact that it is normally very difficult for endoscopists, particularly those with no special interest in this field, to discriminate BO from other regions, such as the distal oesophagus and the proximal stomach at the oesophagogastric junction³⁵ Therefore, in this study we defined endoscopically suspicious BO as a columnar-lined epithelium between the lower end of the

longitudinal capillary vessels of the lower oesophagus and the squamocolumnar junction. Moreover, gastric cardia was defined as the region 10–20 mm distal from the above location.³⁵ Second, there may be dual aetiologies for CIM: gastro-oesophageal reflux disease in some, *H. pylori* infection in others.^{52,66,67} In the current study, 20% of CIM patients were actually negative for *H. pylori* infection. However, all of those cases also had GIM. It is well known that gastric atrophy and intestinal metaplasia progress gradually with age in patients with *H. pylori* infection, but the mucosa with intestinal metaplasia is not a hospitable environment for the bacteria, and indeed is associated with the dramatic reduction or even disappearance of the organism.^{68–70} Our finding that CIM patients tended to be older than GIM patients may suggest that intestinal metaplasia spreads through the cardia from the antrum with long-term *H. pylori* infection, after which *H. pylori* is naturally eliminated in 20% of patients. Hence, it is possible that *H. pylori* is more likely than gastro-oesophageal reflux disease to be the aetiology of CIM in a Japanese population with a high rate of infection.

In conclusion, this is the first study to provide evidence that three conditions, i.e. SIM, CIM and GIM, are different from a molecular pathology standpoint, although the cellular phenotype of CIM, as identified by mAb Das-1, is similar to that of GIM. However, most BO in Japanese individuals is short-segment BO, as found here, and this is quite different from reports from Western countries.⁷¹ Therefore, further investigation is required using a larger series of samples from different countries to clarify the molecular–pathological differences among *H. pylori*-related SIM, CIM and GIM.

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Abbreviations

BO	Barrett's oesophagus
CIM	cardiac intestinal metaplasia
OAC	oesophageal adenocarcinoma
GIM	gastric intestinal metaplasia
LOH	loss of heterozygosity
MSI	microsatellite instability
MSI-H	high microsatellite instability
MSI-L	low microsatellite instability
MSS	microsatellite stable
PCR	polymerase chain reaction
SIM	specialized intestinal metaplasia;

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Figure 1.

The frequency of genetic instability at different loci in specialized intestinal metaplasia (SIM), cardiac (CIM), and gastric intestinal metaplasia (GIM). ^a $P < 0.0001$ compared with BAT25, ^b $P < 0.005$ compared with BAT25, ^{c,d} $P < 0.05$ compared with D2S123, ^e $P < 0.005$ compared with D2S123, ^f $P < 0.0001$ compared with D2S123, ^g $P < 0.001$ compared with D2S123.

Figure 2.

The frequency of genetic instability at different loci in specialized intestinal metaplasia (SIM) in patients with or without *Helicobacter pylori* infection. None of the markers shows any significant differences in the frequency of genetic instability between patients with and those without *H. pylori* infection. NS, not significant.

Figure 3. Representative pictures from specialized intestinal metaplasia (SIM) (a), cardiac (CIM) (b) and gastric intestinal metaplasia (GIM) (c) stained with mAb Das-1. The reactivity of mAb Das-1 is present in goblet cells in three lesions.

Table 1

Characteristics of patients with specialized intestinal metaplasia (SIM), cardiac (CIM) and gastric intestinal metaplasia (GIM)

	No.	Age $\pm$ SD	M:F ratio	<i>H. pylori</i> +:– ratio
SIM	88	69.1 $\pm$ 10.1 ^a	72:16	49:39
<i>H. pylori</i> +ve	49	69.9 $\pm$ 10.0	42:7	–
<i>H. pylori</i> –ve	39	68.6 $\pm$ 10.4	30:9	–
CIM	30	67.2 $\pm$ 12.4 ^b	24:6	22:8
<i>H. pylori</i> +ve	22	65.0 $\pm$ 12.8	18:4	–
<i>H. pylori</i> –ve	8	67.2 $\pm$ 12.4	6:2	–
GIM	52	62.7 $\pm$ 11.2 ^{ab}	37:15	52:0

M, male; F, female.

^a $P < 0.0001$,

^b $P = 0.06$.

Table 2

The frequency of genetic instability (GIN) and mAb Das-1 reactivity in specialized intestinal metaplasia (SIM), cardiac (CIM) and gastric intestinal metaplasia (GIM)

	Genetic instability	mAb Das-1 reactivity
SIM	49/88 (55.7%) ^a	69/88 (78.4%) ^{f,g}
<i>H. pylori</i> +ve	34/49 (69.4%) ^{b,c,d}	42/49 (85.7%) ^h
<i>H. pylori</i> -ve	15/39 (38.5%) ^b	24/39 (61.5%) ^h
CIM	12/30 (40.0%)	9/30 (30.0%) ^f
<i>H. pylori</i> +ve	10/22 (45.5%) ^{d,e}	7/22 (31.8%)
<i>H. pylori</i> -ve	2/8 (25.0%)	2/8 (25.0%)
GIM	12/52 (23.1%) ^{a,c,e}	20/52 (38.5%) ^g

mAb, monoclonal antibody.

^a $P < 0.0005$,

^b $P < 0.005$,

^{d, e} $P = 0.05$,

^{c,f,g} $P < 0.0001$,

^h $P < 0.01$.

Table 3

The frequency of microsatellite instability and loss of heterozygosity at different loci in specialized intestinal metaplasia (SIM), cardiac (CIM) and gastric intestinal metaplasia (GIM)

	MSI				LOH			
	SIM	CIM	GIM	P-value	SIM	CIM	GIM	P-value
BAT26	22/88 (25.0%)	5/30 (16.7%)	3/52 (5.8%)	0.015	0/88 (0%)	0/30 (0%)	0/52 (0%)	–
BAT25	48/88 (54.5%)	13/30 (43.3%)	13/52 (25.0%)	0.003	0/88 (0%)	0/30 (0%)	0/52 (0%)	–
D2S123	32/88 (36.4%)	11/30 (36.7%)	12/52 (23.1%)	0.229	12/88 (13.6%)	3/30 (10.0%)	4/52 (7.7%)	0.545
D5S346	15/88 (17.0%)	9/30 (30.0%)	0/52 (0%)	0.0005	13/88 (14.6%)	0/30 (0%)	1/52 (1.9%)	0.006
D17S250	30/88 (34.1%)	2/30 (6.7%)	3/52 (5.8%)	<0.0001	10/88 (11.4%)	4/30 (13.3%)	2/52 (3.8%)	0.244

MSI, microsatellite instability; LOH, loss of heterozygosity.