and specificity in the non-invasive diagnosis of *Helicobacter pylori* (*H. pylori*) infection, making the UBT one of the most valuable non-invasive tests for diagnosing *H. pylori* infection and eradication throughout the world[1]. This test has been recommended in the 2005 revision of the guidelines for *H. pylori* diagnosis and treatment of the European *Helicobacter* study group (Maastricht Ⅲ)[2].

The stool antigen test, using polyclonal and monoclonal antibodies, is another non-invasive method for detecting *H. pylori*[3], with comparable sensitivity and specificity to the UBT. Maastricht Ⅲ has also recommended the stool antigen test as a simple, easy and useful method for detecting the presence or eradication of *H. pylori*[2]. Native catalase was recently identified as an antigen produced by *H. pylori*[4]. Catalase is characterized by its stability, and the stool antigen test using a monoclonal antibody to catalase is both rapid, taking only about 70 min, and considered more specific than methods using polyclonal antibodies[4]. Although the UBT is non-invasive and the laboratory procedure is recognized as simple and easy, it requires a longer period of time and trained medical staff. Moreover, the UBT is difficult for some patients, including children, handicapped individuals with deteriorated activities of daily life (ADL) and elderly individuals. The stool antigen test is a simpler analytical process, does not require as many medical staff members and can be more readily applied for patients with reduced ADL, children, and the elderly.

Proton pump inhibitors (PPIs) have strong antigastric secretion effects, as well as being bacteriostatic against *H. pylori*. Since UBT may yield false-negative results in patients being treated with PPIs[5,6], it has been recommended that PPI treatment be suspended for at least 2 wk prior to UBT for *H. pylori* infection. One of the stool antigen tests available is the Testmate pylori antigen enzyme immunoassay (EIA)[7], which uses native catalase as an antigen. Thus, the precision of this test in diagnosing *H. pylori* infection should remain stable even during PPI treatment. To determine their comparative accuracy in patients being treated with PPIs, we compared this stool antigen test and the UBT in patients before and after PPI administration.

MATERIALS AND METHODS

We assessed 28 patients [16 men and 12 women; mean age, (63.1 ± 5.9) years; range, 25-84 years] who had been referred to the Department of Gastroenterology at Oita University Hospital and diagnosed with peptic ulcer, reflux esophagitis, or other diseases requiring PPI treatment.

The UBT and stool antigen test were performed before and after PPI administration. Patients were treated for > 2 wk with the 3 types of PPI commercially available in Japan: omeprazole (10 mg/d or 20 mg/d); lansoprazole (15 mg/d or 30 mg/d); or rabeprazole (10 mg/d or 20 mg/d).

Breath samples for UBT were collected before and 20 min after each subject took a 100 mg UBIT tablet® (Otsuka Pharmaceutical, Tokyo, Japan). $^{13}CO_2/^{12}CO_2$ ratios were analyzed by INIS (UBiT-IR300; Otsuka Electronics, Tokyo, Japan), with a cut-off point of 2.5‰.

Stool samples were collected from each patient before and after PPI treatment, and stored at -80 ℃ until assayed. The stool antigen test was performed using the Testmate pylori antigen EIA (Wakamoto Pharmaceutical, Tokyo, Japan; Kyowa Medex, Tokyo, Japan). An aliquot of 100 mg stool was diluted into 0.4 mL of diluted buffer, and 50 μL, together with peroxidase-conjugated anti-catalase monoclonal antibody, were added to a well and incubated for 1 h at 25 ℃. Absorbance was measured at wavelengths of 450 nm and 630 nm, with a cut-off value of 0.120.

Table 1 Results of urea breath test and stool antigen tests before proton pump inhibitor administration

	Urea breath test		
	Positive	Negative	Total
Stool antigen test			
Positive	20	2	22
Negative	1	5	6
Total	21	7	28

Sensitivity 95.2% (20/21), specificity 71.4% (5/7), agreement 89.3% (25/28).

Statistical analysis

Statistical comparisons were performed using the χ^2 test, the Wilcoxon signed-rank test, and the paired *t*-test.

RESULTS

Prior to PPI administration, both the UBT and stool antigen tests yielded positive results in 20 of 28 patients and negative results in 5. One patient was positive on the UBT and negative on the stool antigen test, and 2 patients were negative on the UBT and positive on the stool antigen test. Using UBT as the standard, the sensitivity, specificity and agreement of the stool antigen test before PPI treatment were 95.2%, 71.4%, and 89.3%, respectively (Table 1).

Following PPI administration, both the UBT and stool antigen test showed positive results in 16 patients and negative results in 9. Two patients were positive on the UBT and negative on the stool antigen test, and one was showed on the UBT and positive on the stool antigen test. Using UBT as the standard, the sensitivity, specificity and agreement of the stool antigen test after PPI treatment were 88.9%, 90.9%, and 89.3%, respectively (Table 2).

Positivity of the UBT and stool antigen test before and after PPI treatment

The UBT positivity rates were 75.0% (21/28) before and 64.3% (18/28) after PPI treatment (P = 0.55; Table 3). The stool antigen test positivity rates were 78.6% (22/28) before and 60.7% (17/28 cases) after PPI treatment (P = 0.15). No significant differences in the positivity ratios of

Table 2 Results of urea breath test and stool antigen tests after proton pump inhibitor administration

	Urea breath test		
	Positive	Negative	Total
Stool antigen test			
Positive	16	1	17
Negative	2	9	11
Total	18	10	28

Sensitivity 88.9% (16/18), specificity 90.9% (9/10), agreement 89.3% (25/28).

Table 3 Differences in positivity rates from before to after proton pump inhibitor treatment

	Before PPI treatment	After PPI treatment	*P* value
Urea breath test	75.0% (21/28)	64.3% (18/28)	0.55
Stool antigen test	78.6% (22/28)	60.7% (17/28)	0.15

PPI: Proton pump inhibitor.

the two assays were observed before ($P = 0.75$) or after ($P = 0.58$) PPI treatment.

Measured values for UBT ($^{13}CO_2/^{12}CO_2$ ratios) and stool antigen test (A values) before and after PPI treatment

The mean UBT values were 23.98% ± 5.33% before and 16.19% ± 4.75% after PPI treatment ($P = 0.068$), and were 24.53% ± 8.53% and 12.58% ± 4.49%, respectively ($P = 0.048$), in the 15 patients treated with PPIs for ≥ 4 wk (Figure 1).

The mean *A* ratios on the stool antigen test were 1.16 ± 0.20 before and 1.17 ± 0.24 after PPI treatment ($P = 0.989$), and were 1.02 ± 0.26 and 0.69 ± 0.28, respectively ($P = 0.099$), in the 15 patients treated with PPI for ≥ 4 wk (Figure 2).

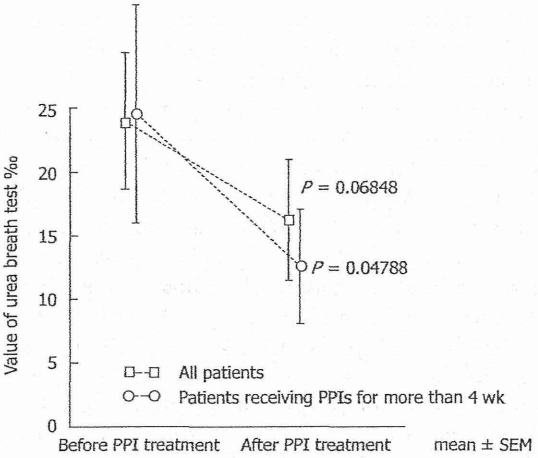

Figure 1 Urea breath test results ($^{13}CO_2/^{12}CO_2$ ratios) before and after proton pump inhibitor treatment. UBT results in all 28 patients were not altered by PPI treatment ($P = 0.068$). However, among patients treated for ≥ 4 wk, UBT results decreased significantly after PPI treatment ($P = 0.048$). UBT: Urea breath test; PPI: Proton pump inhibitor.

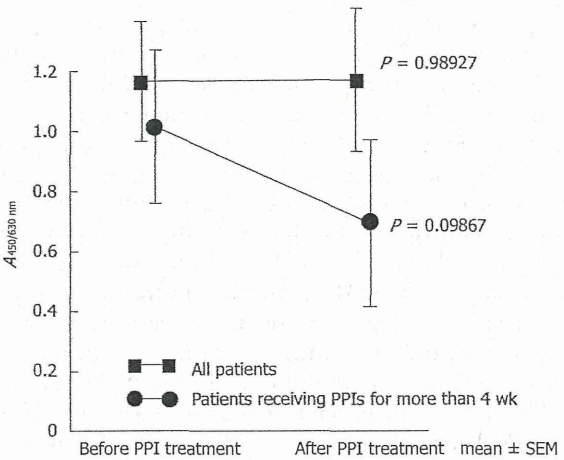

Figure 2 Stool antigen test results (A_{450}/A_{630} ratios) before and after proton pump inhibitor treatment. PPI treatment did not significantly alter stool antigen test results, both in all patients ($P = 0.989$) and in those treated for ≥ 4 wk ($P = 0.099$). PPI: Proton pump inhibitor.

DISCUSSION

The stool antigen test is a non-invasive test for *H. pylori*, similar to the UBT. The test is simple and easy to perform, with good sensitivity and specificity[8]. An analysis of 89 reports found that the mean sensitivity, specificity, positive predictive value and negative predictive value were 91%, 93%, 92%, and 87%, respectively, among 10 858 subjects who were not treated with *H. pylori* eradication therapy[3]. In addition, 8 reports of tests with monoclonal antibody showed significantly better results ($P < 0.001$), with mean sensitivity, specificity, positive predictive value and negative predictive value of 96%, 97%, 96%, and 97%, respectively. The Maastricht Ⅲ guidelines have found that the reliability of the stool antigen test is comparable to that of the UBT[2].

Despite its non-invasive nature, and high sensitivity and specificity, the UBT can lead to false-negative results in patients treated with drugs showing bacteriostatic activity against *H. pylori*, such as PPIs, or inhibiting urease activity[5,6,9-12]. In those studies, the false-negative rates of the UBT in patients treated with omeprazole or lansoprazole for 2 wk or 4 wk were ≥ 50%. We previously observed false-negative UBT results in 1 of 16 patients (6.3%) treated with 30 mg/d lansoprazole for 2 wk[6]. Several studies have also reported that the rate of false-negative results on the stool antigen test also increase in patients treated with PPI[13,14]. A comparison of stool antigen test and UBT results for 9 *H. pylori*-positive patients receiving PPI for 2 wk found a smaller degree of change on the stool antigen test than on the UBT[15].

The authors found that, before PPI treatment, the stool antigen test showed high sensitivity, but lower specificity (71.4%). This was likely due to the small number of patients showing negative results on the UBT ($n = 7$).

Similarly, although many reports have shown high sensitivity and specificity for stool antigen tests, others have reported lower specificity (54%-78%)[3].

The sensitivity of the stool antigen test fell slightly after PPI administration, but its specificity remained high level (90.9%). The concordance rate of UBT and stool antigen test results was high (89.3%) before and after PPI administration. Using the UBT as standard, the stool antigen test showed good sensitivity and specificity both before and after PPI administration. Although stool antigens have generally shown high sensitivity and specificity, divergent sensitivity and specificity between the UBT and stool antigen test have been reported[16]. However, increasing the cut-off for the stool antigen test reduced the percentage of conflicting results[16]. This discrepancy was attributed to the urease-based UBT not detecting the coccoid form of *H. pylori*, as well as the low cut-off index for the stool antigen test. The discrepancies we observed, with positive results on the stool antigen test and negative results on the UBT, were likely due to the same mechanism.

There were no significant differences in positive rates on the UBT and stool antigen test before and after PPI therapy, suggesting that the stool antigen test is a useful and reliable diagnostic test for *H. pylori*, similar to the UBT. The positivity rate for UBT decreased from 75.0% before to 64.3% after PPI administration, and the positivity rate for the stool antigen test decreased from 78.6% to 60.7%. These reductions indicate that the stool antigen test should be performed after limiting PPI administration as much as possible, but that, when PPI treatment cannot be stopped, the stool antigen test shows comparable utility to the UBT. Although both the stool antigen test and UBT results did not change significantly from before to after PPI treatment, the reductions were much less pronounced on the stool antigen test than on the UBT. Moreover, in patients treated for $\geq$ 4 wk, UBT, but not stool antigen test, results decreased significantly after PPI treatment. These findings indicate that, while the results of both assays were influenced by the bacteriostatic actions of PPIs, the stool antigen test was less influenced than the UBT.

Although several studies have reported increased false-negative results for the stool antigen test during PPI treatment[13,14], we found that stool antigen test results were more stable than UBT results in patients being treated with PPIs.

Despite the benefits of the UBT as a non-invasive test for *H. pylori*[17] and the slightly lower accuracy of the stool antigen test, the latter has several advantages, including ease, rapidity and lower cost. Others have also reported that the stool antigen test is highly sensitive and specific, with high utility due to the speed and simplicity of testing[18].

The 2005 Maastricht Ⅲ consensus report recommended that the stool antigen test be used for diagnosis when the UBT is unavailable[2]. The numbers of patients receiving PPI therapy have been increasing around the world, and many patients have difficulty undergoing the UBT, such as elderly patients and patients with reduced ADL. In these patients, the stool antigen test should be considered reliable and useful.

In conclusion, the stool antigen test showed stable results even during PPI treatment as well as sensitivity comparable to the UBT. In patients treated with PPI for $\geq$ 4 wk, the stool antigen test showed more stable results than the UBT. The stool antigen test is therefore a useful and reliable diagnostic method for *H. pylori* infection.

COMMENTS

Background

Although the urea breath test (UBT) is a reliable, non-invasive diagnostic test for *Helicobacter pylori* (*H. pylori*), it has a potential for false-negative results in patients treated with proton pump inhibitor (PPI). The stool antigen test is another useful, easy to perform, non-invasive assay for *H. pylori*, with high sensitivity and specificity. However, the effects of PPI treatment on stool antigen test results are unclear.

Research frontiers

Due to the increase in patients with gastroesophageal reflux disease and those treated with nonsteroidal anti-inflammatory drugs, the numbers treated with PPIs has increased throughout the world. Thus, reliable diagnostic tests for *H. pylori* are needed for patients treated with PPIs. The study describe the utility of a stool antigen test, using the TestMate pylori enzyme immunoassay, in patients treated with PPIs.

Innovations and breakthroughs

Although many studies have reported results of stool antigen tests in patients treated with PPIs, none has assessed the relationship between measured value of stool antigen test and UBT results. To our knowledge, this study is the first to clarify the stability of the stool antigen test, relative to the UBT, in patients treated with PPIs.

Applications

Elderly patients and those with reduced daily activities who are treated with PPIs have increased. The UBT is somewhat difficult to perform in these patients. The authors found that the stool antigen test may be a useful alternative to the UBT in these patients.

Terminology

The stool antigen test is a diagnostic assay for *H. pylori*. which identifies *H. pylori* antigens in feces using antigen-antibody reactions. This non-invasive assay has equal sensitivity and specificity to the UBT, and may be especially useful for children or elderly patients.

Peer review

The manuscript is well written. The methods are adequate. The results justify the conclusions drawn.

REFERENCES

1 **Gisbert JP**, Pajares JM. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. *Aliment Pharmacol Ther* 2004; **20**: 1001-1017

2 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781

3 **Gisbert JP**, Pajares JM. Stool antigen test for the diagnosis of Helicobacter pylori infection: a systematic review. *Helicobacter* 2004; **9**: 347-368

4 **Suzuki N**, Wakasugi M, Nakaya S, Kokubo N, Sato M, Kajiyama H, Takahashi R, Hirata H, Ezure Y, Fukuda Y, Shimoyama T. Catalase, a specific antigen in the feces of human subjects infected with Helicobacter pylori. *Clin Diagn Lab Immunol* 2002; **9**: 784-788

5 **Laine L**, Estrada R, Trujillo M, Knigge K, Fennerty MB. Effect of proton-pump inhibitor therapy on diagnostic testing for Helicobacter pylori. *Ann Intern Med* 1998; **129**: 547-550

6 **Murakami K**, Sato R, Okimoto T, Watanabe K, Nasu M, Fu-

jioka T, Kodama M, Kagawa J. Influence of anti-ulcer drugs used in Japan on the result of (13)C-urea breath test for the diagnosis of Helicobacter pylori infection. *J Gastroenterol* 2003; **38**: 937-941

7 **Suzuki N**, Wakasugi M, Nakaya S, Okada K, Mochida R, Sato M, Kajiyama H, Takahashi R, Hirata H, Ezure Y, Koga Y, Fukuda Y, Shimoyama T. Production and application of new monoclonal antibodies specific for a fecal Helicobacter pylori antigen. *Clin Diagn Lab Immunol* 2002; **9**: 75-78

8 **Malfertheiner P**, Mégraud F, O'Morain C, Hungin AP, Jones R, Axon A, Graham DY, Tytgat G. Current concepts in the management of Helicobacter pylori infection--the Maastricht 2-2000 Consensus Report. *Aliment Pharmacol Ther* 2002; **16**: 167-180

9 **Chey WD**, Woods M, Scheiman JM, Nostrant TT, DelValle J. Lansoprazole and ranitidine affect the accuracy of the 14C-urea breath test by a pH-dependent mechanism. *Am J Gastroenterol* 1997; **92**: 446-450

10 **Connor SJ**, Seow F, Ngu MC, Katelaris PH. The effect of dosing with omeprazole on the accuracy of the 13C-urea breath test in Helicobacter pylori-infected subjects. *Aliment Pharmacol Ther* 1999; **13**: 1287-1293

11 **Savarino V**, Bisso G, Pivari M, Zentilin P, Bilardi C, Dulbecco P, Mele MR, Tracci D, Vigneri S. Effect of gastric acid suppression on 13C-urea breath test: comparison of ranitidine with omeprazole. *Aliment Pharmacol Ther* 2000; **14**: 291-297

12 **Adachi K**, Fujishiro H, Mihara T, Komazawa Y, Kinoshita Y. Influence of lansoprazole, famotidine, roxatidine and rebamipide administration on the urea breath test for the diagnosis of Helicobacter pylori infection. *J Gastroenterol Hepatol* 2003; **18**: 168-171

13 **Manes G**, Balzano A, Iaquinto G, Ricci C, Piccirillo MM, Giardullo N, Todisco A, Lioniello M, Vaira D. Accuracy of the stool antigen test in the diagnosis of Helicobacter pylori infection before treatment and in patients on omeprazole therapy. *Aliment Pharmacol Ther* 2001; **15**: 73-79

14 **Bravo LE**, Realpe JL, Campo C, Mera R, Correa P. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for Helicobacter pylori infection. *Am J Gastroenterol* 1999; **94**: 2380-2383

15 **Fukuda Y**, Tomita T, Kosaka T, Hori K. [Evaluation of diagnostic methods for Helicobacter pylori infection]. *Nihon Rinsho* 2004; **62**: 459-463

16 **Masoero G**, Lombardo L, Della Monica P, Vicari S, Crocillà C, Duglio A, Pera A. Discrepancy between Helicobacter pylori stool antigen assay and urea breath test in the detection of Helicobacter pylori infection. *Dig Liver Dis* 2000; **32**: 285-290

17 **Manes G**, Zanetti MV, Piccirillo MM, Lombardi G, Balzano A, Pieramico O. Accuracy of a new monoclonal stool antigen test in post-eradication assessment of Helicobacter pylori infection: comparison with the polyclonal stool antigen test and urea breath test. *Dig Liver Dis* 2005; **37**: 751-755

18 **Kato S**, Ozawa K, Okuda M, Nakayama Y, Yoshimura N, Konno M, Minoura T, Iinuma K. Multicenter comparison of rapid lateral flow stool antigen immunoassay and stool antigen enzyme immunoassay for the diagnosis of Helicobacter pylori infection in children. *Helicobacter* 2004; **9**: 669-673

S- Editor Zhang SJ **L- Editor** O'Neill M **E- Editor** Zhang DN

UICC
global cancer control

IJC
International Journal of Cancer

Elevated risk of colorectal adenoma with *Helicobacter pylori*-related chronic gastritis: a population-based case-control study

Izumi Inoue[1], Chizu Mukoubayashi[1], Noriko Yoshimura[2], Tohru Niwa[1], Hisanobu Deguchi[1], Mika Watanabe[1], Shotaro Enomoto[1], Takao Maekita[1], Kazuki Ueda[1], Mikitaka Iguchi[1], Kimihiko Yanaoka[1], Hideyuki Tamai[1], Kenji Arii[1], Masashi Oka[1], Mitsuhiro Fujishiro[3], Tatsuya Takeshita[4], Masataka Iwane[5], Osamu Mohara[5] and Masao Ichinose[1]

[1] Department of Gastroenterology, School of Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Wakayama, Japan
[2] Department of Joint Disease Research, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan
[3] Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan
[4] Department of Public Health, School of Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Wakayama, Japan
[5] Wakayama Wellness Foundation, 1850 Minato, Wakayama City, Wakayama, Japan

This study investigated correlations between *Helicobacter pylori* infection or chronic atrophic gastritis (CAG) and risk of colorectal adenoma in a population-based case-control study. Subjects comprised asymptomatic, middle-aged, male Japanese factory workers who participated in an annual health check-up program, including cancer screening with colonoscopy. We selected 239 colorectal adenoma cases based on histological evaluation and 239 age-matched adenoma-free controls, and evaluated colorectal adenoma risk according to stage of *H. pylori*-related chronic gastritis as determined by serum tests for *H. pylori* antibody titer and pepsinogen. Subjects with colorectal adenoma were more likely to be smokers and have hypercholesterolemia. *H. pylori* infection was a risk factor for adenoma as a whole (crude odds ratio [OR]: 2.26, 95% confidence interval [CI]: 1.44–3.55). Analysis of distal adenoma cases showed that adenoma risk was significantly increased in the presence of *H. pylori* infection, but there was no further increase in risk with CAG. In contrast, proximal adenoma risk increased stepwise with the presence and progression of *H. pylori*-related chronic gastritis and showed a maximal and significant increase with CAG (crude OR: 4.51, 95% CI: 1.43–14.2). Subjects with more extensive and severe gastritis showed still higher risk not only for proximal but also for distal adenoma. *H. pylori*-related chronic gastritis is likely to be involved in the development of colorectal neoplasms, and its progression appears to increase the risk, particularly for proximal adenomas. Knowing the *H. pylori*-related chronic gastritis stage will probably be useful for evaluation of risk for colorectal neoplasia.

Helicobacter pylori infection induces chronic inflammation in the stomach mucosa of both humans and animals, and *H. pylori*-related chronic gastritis is deeply involved in the development of gastric neoplasms, such as adenoma or cancer.[1] *H. pylori* infection is now widely accepted as a major driving force in the progression of a series of carcinogenic cascades representing the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[2] Our previous seroepidemiological study clearly demonstrated a positive correlation between progression of chronic gastritis and risk of gastric cancer. Subjects with extensive chronic atrophic gastritis (CAG), as determined by serum pepsinogen (PG) levels, showed an annual cancer incidence rate of 0.24%.[3]

Recently, promotion of tumor development by *H. pylori* infection in extragastric target organs has been reported.[4] In addition, some clinical and epidemiological studies have revealed close correlations between incidence rates of gastric and colorectal mucosal neoplasms.[5,6] Furthermore, previous studies have suggested that the most common second primary site of synchronous and metachronous cancer in cases of gastric cancer is the colorectum.[7,8] In Japan, areas with high age-adjusted mortality rates from stomach cancer among the 47 municipal districts, such as Wakayama Prefecture (51.1/100,000 person-years), have also reported high rates of colorectal cancer mortality (38.4/100,000 person-years).[9] Progressive chronic gastritis induced by persistent *H. pylori* infection leads to extensive glandular atrophy and reduced acid secretion, which induces hypergastrinemia, a putative trophic factor for large bowel mucosa,[10] and it alters the gastrointestinal microenvironment composed of bacterial flora,[11] and thus may contribute to colorectal

Key words: colorectal adenoma, pepsinogen, chronic atrophic gastritis, case-control study, cancer risk
Abbreviations: BMI: body mass index; CAG: chronic atrophic gastritis; CI: confidence interval; CIMP+: CpG island methylator phenotype; CIN: chromosomal instability; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; MSI+: microsatellite instability; OR: odds ratio; PG: pepsinogen; SD: standard deviation; TC: total cholesterol; TG: triglyceride
Grant sponsor: Cancer Research from the Ministry of Health, Labor and Welfare of Japan
DOI: 10.1002/ijc.25931
History: Received 21 Jul 2010; Accepted 22 Dec 2010; Online 10 Jan 2011
Correspondence to: Izumi Inoue, Second Department of Internal Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Wakayama 641-0012, Japan, Tel.: +81-734-47-2300, Fax: +81-734-45-3616, E-mail: izumiino@wakayama-med.ac.jp

carcinogenesis. On the basis of these findings, we investigated the possibility that gastric cancer/adenoma and colorectal cancer/adenoma have common risk factors of *H. pylori* infection and/or its end result, CAG. The aim of our study was to examine etiological links among precancerous colorectal lesions, adenomas and *H. pylori* infection or CAG.

Material and Methods

Subjects

Between April 1996 and March 2004, a total of 4,655 middle-aged male factory workers (mean age ± standard deviation [SD]: 49.5 ± 4.6 years; range: 40–59 years) underwent annual health check-ups at a workplace in Wakayama City, located in the southwestern part of the main island of Japan. All patients were inhabitants from the Wakayama area. This type of screening program is common in various workplaces throughout Japan to detect incident diseases in early stages. Thus, subjects were symptom-free and workers with symptoms requiring prompt medical care were excluded from the study. As a result, all subjects in this study could be considered to represent healthy Japanese individuals.

As part of the screening program, study subjects underwent a series of screening tests and procedures: an interview to ascertain general state of health, physical examination, chest radiography, electrocardiography, blood laboratory testing, urinalysis and barium X-ray. In addition, subjects could select to undergo screening for colorectal cancer using their preferred method among fecal occult blood testing (FOBT), barium enema or colonoscopy. During the study period, a total of 1,605 middle-aged men underwent colonoscopy for cancer screening. Subjects with a previous history of colorectal neoplasia or inflammatory bowel disease were excluded from the study. In addition, subjects who underwent colonoscopy because of positive FOBT or a positive finding on barium enema were also excluded. As a result, a total of 1,019 subjects were analyzed for the study. When each case of colorectal adenoma was identified, an age-matched control (within 3 years) was randomly selected from among the participants of the health check-up program who were confirmed to be colorectal neoplasm-free.

Questionnaire

Information about baseline characteristics (age, height, weight, sociodemographic characteristics, personal medical history, family history, smoking and alcohol consumption) was obtained from the questionnaire completed at the time of the aforementioned interview.

Evaluation of CAG and *H. pylori* infection

Aliquots of separated sera from blood samples collected as routine laboratory tests for the general health check-up were stored below −20°C until measurement of serum levels of *H. pylori* immunoglobulin (Ig) G antibody titer and serum PG. *H. pylori* IgG antibody titers were measured using an enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan). Antibody titers >50 U/ml were classified as indicating *H. pylori* infection. The sensitivity and specificity of the ELISA used in our study were 93.5% and 92.5%, respectively.[12] Serum PG levels were measured using PGI/PGII RIA-Bead Kits (Dainabbot, Tokyo, Japan), which use a modified radioimmunoassay method that we previously established.[13] Subjects with extensive CAG were diagnosed on the basis of the previously described PG test-positive criteria (PG I $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0).[14,15] These criteria have 70.5% sensitivity and 97% specificity.[14] Subjects who had been prescribed medications before examination that might affect gastrointestinal function, such as proton pump inhibitors, H2 blockers or nonsteroidal anti-inflammatory drugs, as well as subjects who had a previous history of gastric resection, *H. pylori* eradication therapy or renal failure, were excluded from analysis of PG test results.

In our study, all but four CAG cases diagnosed by the above PG-test positive criteria were *H. pylori*-antibody positive. Endoscopic examination of these four *H. pylori*-negative CAG cases (one control and three adenoma cases) revealed extensive metaplastic gastritis involving both antrum and corpus. Thus, the negative result for *H. pylori* antibody is considered to reflect a spontaneous eradication of the bacteria, an end result of the progression of *H. pylori*-related CAG. Furthermore, the prevalence of autoimmune gastritis is extremely low in Japan; the incidence rate is reported to be 0.6/100,000 person-years.[16] Thus, the possibility of autoimmune gastritis in the analyzed CAG cases including these four *H. pylori*-negative cases is considered to be negligible.

Screening for colorectal neoplasia

Subjects who selected colonoscopy for colorectal cancer screening underwent a full colonoscopic examination with adequate bowel preparation. A colonoscope (CF 240 I, Olympus, Tokyo, Japan) was inserted to the cecum, except in cases with advanced adenocarcinoma. The adenoma cases were classified into three groups according to the location of the detected polypoid lesion: proximal (cecum, ascending colon, hepatic flexure and transverse colon), distal (splenic flexure, descending colon, sigmoid colon and rectum) and bilateral (lesions located in both sides). All polypoid lesions found during colonoscopy were biopsied, immediately fixed in 10% formalin and embedded in paraffin. Tissue sections were stained with hematoxylin-eosin and examined under light microscopy. Routine histological evaluation was performed by staff pathologists.

The retrospective analysis of the clinical data in our study was approved by the ethics committee of Wakayama Medical University. Informed consent for the use of the clinical data from the health check-up was obtained from the screened subjects at the time of their first screening.

Table 1. Baseline characteristics of study subjects

	Control $n = 239$	Case $n = 239$	p^1	Proximal $n = 38$	p^1	Bilateral $n = 78$	p^1	Distal $n = 123$	p^1
Age (years)									
Mean (SD)	49.4 (4.3)	49.9 (3.9)	0.21	49.7 (3.9)	0.71	49.6 (3.8)	0.65	50.1 (4.0)	0.14
BMI (kg/m^2)									
Mean (SD)	23.4 (2.9)	23.7 (2.8)	0.16	23.7 (2.5)	0.53	24.0 (2.7)	0.08	23.6 (3.0)	0.50
Current smoker (−)/(+)	103/136	83/156	0.08	18/20	0.75	18/60	0.002	47/76	0.43
Alcohol use (−)/(+)[2]	73/166	62/177	0.31	9/29	0.5	18/60	0.26	35/88	0.77
TC (mg/dl)									
Mean (SD)	204.5 (31.3)	210.0 (34.2)	0.07	205.1 (39.3)	0.92	207.6 (32.8)	0.44	212.9 (33.3)	0.02
TG (mg/dl)									
Mean (SD)	167.7 (130.3)	183.1 (137.6)	0.21	175.0 (94.4)	0.74	205.3 (155.4)	0.06	171.5 (136.0)	0.79
***H. pylori* IgG (U/ml)**									
Mean (SD)	288.0 (479.7)	352.0 (462.6)	0.14	354.7 (463.4)	0.42	316.4 (438.8)	0.63	373.8 (479.1)	0.11
PG I (ng/ml)									
Mean (SD)	58.7 (29.5)	58.1 (25.9)	0.81	54.5 (23.3)	0.4	59.2 (23.6)	0.89	58.6 (28.0)	0.96
PG II (ng/ml)									
Mean (SD)	15.8 (10.0)	18.5 (11.0)	0.006	18.1 (8.7)	0.18	18.0 (10.7)	0.10	18.9 (11.8)	0.02
PG I/II									
Mean (SD)	4.4 (2.2)	3.7 (1.8)	<0.001	3.3 (1.7)	0.003	3.9 (1.9)	0.03	3.6 (1.8)	<0.001
Clinicopathological features									
Size									
The proportion of the cases with adenoma <10 mm (%)				177/239 (74.1)		32/38 (85)		49/78 (62.7)	96/123 (77.9)
The proportion of the cases with adenoma ≥10 mm (%)				62/239 (25.9)		6/38 (15)		29/78 (37.3)[3]	27/123 (22.1)
Number									
The proportion of the cases with a single adenoma (%)				120/239 (50.2)		31/38 (81.6)		0/78 (0)	89/123 (72.3)
The proportion of the cases with two or more adenoma (%)				119/239 (49.8)		7/38 (18.4)		78/78 (100)	34/123 (27.7)
Histopathology									
The proportion of the cases with tubular adenoma (%)				228/239 (95.4)		36/38 (95)		76/78 (98)	116/123 (94.1)
The proportion of the cases with tubulovillous, villous adenoma (%)				11/239 (4.6)		2/38 (5)		2/78 (2)	7/123 (5.9)
The grade of dysplasia									
The proportion of the cases with mild or moderate adenoma (%)				226/239 (94.6)		35/38 (92.1)		73/78 (93.6)	118/123 (95.9)
The proportion of the cases with sevear adenoma (%)				13/239 (5.4)		3/38 (7.9)		5/78 (6.4)	5/123 (4.1)

[1]Two-sided *p*-values for the difference between cases and controls were based on the χ^2 test and *t* test. [2]Drinking alcohol at least once a week for the past 5 years.[3]$p < 0.05$: vs proximal based on χ^2 test.
Abbreviations: BMI, body mass index; TC, total cholesterol; TG, triglycerides; PG, pepsinogen; CAG, chronic atrophic gastritis; SD, standard deviation.

Statistical analysis

Data were analyzed using SPSS version 11.0 (Chicago, IL) and STATA (College Station, TX). Data for continuous variables were expressed as mean ± SD, and the differences were tested for significance using *t* tests for comparison of two groups and analysis of variance (ANOVA) for comparison among multiple groups. Categorical variables were compared using the chi-squared test. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to describe associations. ORs with corresponding 95% CIs were obtained by conditional logistic regression analysis. Trend tests were assessed using an ordinal score for each categorical variable. All two-sided *p*-values less than 5% were considered statistically significant.

Results

In our study, 239 middle-aged men with colorectal adenoma were identified. Although four cases of adenocarcinoma were also detected during the study period, they were excluded from the study because of the small number. The same number of age-matched adenoma-free controls was randomly selected from among asymptomatic middle-aged factory workers. Baseline characteristics of colorectal adenoma cases

Table 2. Association between *Helicobacter pylori* infection or chronic atrophic gastritis and colorectal adenoma

	Controls (n = 239)	Total adenoma cases (n = 239)	Proximal (n = 38)	Bilateral (n = 78)	Distal (n = 123)
***H. pylori* infection**					
(–)	72	38	5	16	17
(+)	167	201	33	62	106
Crude OR (95% CI)	1	2.26 (1.44–3.55)	2.85 (1.07–7.58)	1.67 (0.90–3.10)	2.69 (1.50–4.81)
Adjusted OR[1] (95% CI)	1	2.52 (1.57–4.05)	2.84 (1.07–7.58)	1.72 (0.92–3.22)	2.88 (1.60–5.21)
CAG					
(–)	176	162	22	56	84
(+)	63	77	16	22	39
Crude OR (95% CI)	1	1.31 (0.89–1.93)	2.03 (1.004–4.11)	1.10 (0.62–1.94)	1.30 (0.81–2.09)
Adjusted OR[1] (95% CI)	1	1.45 (0.97–2.17)	2.03 (0.99–4.14)	1.17 (0.65–2.10)	1.38 (0.85–2.24)

[1]Adjusted for current smoking status and total cholesterol by conditional logistic regression analysis.
Abbreviations: OR, odds ratio; CI, confidence interval; CAG, chronic atrophic gastritis.

and controls are summarized in Table 1. No significant differences in mean age, body mass index (BMI), alcohol consumption or serum triglyceride levels (TG) were apparent between cases and controls. Smoking and hypercholesterolemia tended to be more frequent among cases ($p < 0.1$), reflecting those of bilateral and distal adenoma cases, respectively. Therefore, smoking habits and total serum cholesterol (TC) levels were included in the model to control for confounding effects in the following analyses. The serum PG II level was significantly higher in cases than in controls, especially among distal adenoma cases, while the PG I/II ratio was significantly lower among adenoma cases irrespective of their location. The clinicopathologic features of the adenoma cases are also shown in Table 1. The proportion of cases with a lesion $\geq$10 mm in size and the proportion of cases with two or more lesions were larger in bilateral, distal and proximal adenoma cases, in that order. A significant difference was observed between bilateral and proximal cases. There was no significant difference in the histopathology or the grade of dysplasia according to the location of the polyps.

Since PG II and the PG I/II ratio are believed to reflect the activity of inflammation and extent of atrophy, respectively, during the course of *H. pylori*-related chronic gastritis,[17–19] further analyses were performed with special reference to the infection. Table 2 shows that *H. pylori* infection was significantly more prevalent among cases (84.1%) than among controls (69.9%), with a crude OR of 2.26 (95% CI: 1.44–3.55) (Table 2). The risk of adenoma was significantly elevated by *H. pylori* infection regardless of its location, both in the proximal and distal colon, although there was no increase in risk in bilateral adenoma cases. The percentage of subjects with CAG, as determined by serum PG levels using the criteria of PG $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0, was 32.2% in cases and 26.4% in controls, indicating that the presence of CAG did not lead to a significant increase in the risk of colorectal adenoma (crude OR: 1.31; 95% CI: 0.89–1.93) (Table 2). Although adenoma risk was marginally significantly elevated by the presence of CAG in a subgroup of subjects with proximal adenoma, the adjusted OR showed no significantly increased risk.

H. pylori-related chronic gastritis can be classified into three stages based on the results of the two serologic tests: *H. pylori* antibody titer and PG (3). The classification reflects each stage of a serial change in stomach mucosa induced by chronic *H. pylori* infection. The three groups were: Group A: *H. pylori*-negative and PG test-negative; Group B: *H. pylori*-positive and PG test-negative; and Group C: PG test-positive. Group A corresponds to an *H. pylori*-free healthy stomach, Group B corresponds to *H. pylori*-related nonatrophic gastritis and Group C corresponds to the presence of extensive CAG. Table 3 shows the correlations between these three stages of *H. pylori*-related chronic gastritis and risk of colorectal adenoma. The presence of *H. pylori*-related chronic gastritis significantly increased the risk for colorectal adenoma as a whole (Group B: crude OR: 2.61, 95% CI: 1.54–4.41), but the progression of chronic gastritis and resulting CAG development did not show any further increase in the risk of adenoma (Group C: crude OR: 2.30, 95% CI: 1.38–3.83). There was no significant difference in risk between Groups B and C (crude OR: 1.01, 95% CI: 0.66–1.54). Cases were further stratified into three groups based on location of the tumor (proximal, distal or bilateral). The risk of distal adenoma, a major subgroup of colorectal adenoma, was significantly increased with *H. pylori* infection (Group B: crude OR: 2.87, 95% CI: 1.54–5.35), but there was no further increase in risk with the presence of CAG. In contrast, analysis of proximal adenoma cases showed that the adenoma risk increased in a stepwise manner with the presence and progression of *H. pylori*-related chronic gastritis, and it showed a maximal and significant increase in the presence of *H. pylori*-related CAG (Group C: crude OR: 4.51, 95% CI: 1.43–14.2). Bilateral adenoma cases showed no significant risk elevation in the presence of either *H. pylori* infection or CAG.

Stricter criteria for positive PG I ($\leq$30 ng/ml) and PG I/II ratio ($\leq$2.0) are used to detect subjects with more extensive and

Table 3. Association between development of colorectal adenoma and stage of *Helicobacter pylori–related* chronic gastritis

	H. pylori	CAG	Controls (n = 239)	Total adenoma cases (n = 239)	Proximal (n = 38)	Bilateral (n = 78)	Distal (n = 123)
Group A	(–)	(–)	71	35	4	15	16
Group B	(+)	(–)	105	127	18	41	68
Group C		(+)	63	77	16	22	39
Crude OR							
A:B (95% CI)			1	2.61 (1.54–4.41)	3.04 (0.99–9.37)	1.85 (0.95–3.59)	2.87 (1.54–5.35)
A:C (95% CI)			1	2.30 (1.38–3.83)	4.51 (1.43–14.2)	1.65 (0.79–3.46)	2.75 (1.40–5.39)
B:C (95% CI)			1	1.01 (0.66–1.54)	1.48 (0.71–3.11)	0.89 (0.49–1.64)	0.96 (0.58–1.58)
Adjusted OR[1]							
A:B (95% CI)			1	2.81 (1.64–4.81)	3.06 (0.99–9.42)	1.85 (0.94–3.62)	3.05 (1.62–5.73)
A:C (95% CI)			1	2.70 (1.58–4.62)	4.51 (1.43–14.2)	1.76 (0.83–3.74)	3.05 (1.54–6.07)
B:C (95% CI)			1	1.04 (0.68–1.60)	1.54 (0.73–3.27)	0.94 (0.51–1.75)	1.00 (0.60–1.66)
Trend (p value)				0.002	0.009	0.188	0.007

[1]Adjusted for current smoking status and total cholesterol by conditional logistic regression analysis.
Abbreviations: CAG, chronic atrophic gastritis; OR, odds ratio; CI, confidence interval.

Table 4. Association between development of colorectal adenoma and stage of *Helicobacter pylori*-related chronic gastritis (PG test-positive criteria : PGI $\leq$ 30 ng/ml, PG I/II $\leq$ 2)

	H. pylori	CAG	Controls (n = 239)	Total adenoma cases (n = 239)	Proximal (n = 38)	Bilateral (n = 78)	Distal (n = 123)
Group A	(–)	(–)	71	35	4	15	16
Group B	(+)	(–)	155	180	29	56	95
Group C		(+)	13	24	5	7	12
Crude OR							
A:B (95% CI)			1	2.36 (1.49–3.73)	3.32 (1.12–9.80)	1.71 (0.91–3.23)	2.72 (1.49–4.95)
A:C (95% CI)			1	3.75 (1.70–8.23)	6.83 (1.61–28.9)	2.55 (0.87–7.46)	4.10 (1.58–10.6)
B:C (95% CI)			1	1.59 (0.78–3.23)	2.06 (0.68–6.21)	1.49 (0.57–3.93)	1.51 (0.66–3.44)
Adjusted OR[1]							
A:B (95% CI)			1	2.45 (1.54–3.90)	3.33 (1.13–9.83)	1.75 (0.92–3.33)	2.91 (1.58–5.36)
A:C (95% CI)			1	4.20 (1.88–9.40)	7.00 (1.64–29.9)	2.67 (0.88–8.12)	5.16 (1.92–13.9)
B:C (95% CI)			1	1.73 (0.84–3.58)	2.31 (0.75–7.11)	1.48 (0.55–4.04)	1.76 (0.75–4.12)
Trend (p value)				<0.001	0.005	0.049	<0.001

[1]Adjusted for current smoking status and total cholesterol by logistic regression analysis.
Abbreviations: PG, pepsinogen; CAG, chronic atrophic gastritis; OR, odds ratio; CI, confidence interval.

severe CAG.[15] Using these criteria in the study subjects, 31.2% (24/77) who were diagnosed as CAG positive by the less strict criteria (PG I $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0) were considered to be in a more advanced stage of CAG. Table 4 shows that these advanced-stage CAG subjects were at even higher risk for proximal adenoma (crude OR: 6.83, 95% CI: 1.61–28.9), and they were also at higher risk for distal adenoma (crude OR: 4.10, 95% CI: 1.58–10.6) compared to CAG-positive subjects diagnosed by the less strict criteria (PG I $\leq$ 70 ng/ml and PG I/II $\leq$ 3.0). In contrast, the adenoma risk of *H. pylori*-infected CAG-free subjects detected by the stricter criteria was at a comparable level to the subjects diagnosed by the less strict criteria.

Discussion

Our study investigated correlations between *H. pylori* infection and risk of colorectal adenoma. Once established in the stomach mucosa, *H. pylori*-related chronic gastritis is generally believed to trigger a series of events involved in stomach carcinogenesis, as the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[2] We therefore stratified study subjects based on the stage of *H. pylori*-related chronic gastritis as determined by two serum tests (*H. pylori* antibody titer and PG) and then evaluated colorectal adenoma risk in each stage. As a result, our study clearly indicated that *H. pylori* infection was a risk for colorectal adenoma, which is consistent with

the results from a limited number of previous hospital-based case-control studies[20,21] and comparative studies[22–26] that reported an increased risk of colorectal neoplasia with *H. pylori* infection. However, most of these studies were confounded by uncontrolled factors, so the relationship between colorectal cancer/adenoma and *H. pylori* infection remained unclear. The present population-based case-control study of middle-aged male factory workers was adjusted by potentially confounding factors and clearly demonstrated an increase in the risk of colorectal adenoma in the presence of *H. pylori* infection, although there remains a possibility of uncontrolled confounding factors remaining.

As for the correlation between colorectal neoplasia and CAG, to the best of our knowledge, a single report by Machida *et al.* showed an insignificant increase in the prevalence of CAG among colorectal cancer patients in a hospital-based case-control study.[27] Likewise, in our study, the presence of CAG, as determined by serum PG level, did not contribute to an increase in colorectal adenoma risk as a whole. Accumulating evidence suggests that the risk of colorectal neoplasia associated with various environmental and genetic factors differs for proximal and distal neoplasms,[28] probably reflecting two recently proposed and different tumorigenic pathways based on the molecular features of CpG Island methylator phenotype (CIMP+) and microsatellite instability (MSI+) predominantly occurring in the proximal colon, and chromosomal instability (CIN) occurring in the distal colon.[28] Adenoma cases were thus stratified into proximal and distal groups, and the adenoma risk of each group was analyzed. As a result, a subgroup of subjects with proximal adenoma showed a stepwise increase in adenoma risk with the presence and progression of *H. pylori*-related chronic gastritis, and it reached a maximal and significantly high risk level with the development of CAG, whereas the adenoma risk of the major subgroup with distal adenoma showed no further increase with the development of CAG after *H. pylori* infection. Furthermore, the adenoma risk for both proximal and distal cases appeared to be still higher in about a third of the subjects with CAG, who were in a more advanced stage. Given all these findings, *H. pylori* infection is likely to be involved in the development of colorectal adenoma, and the resultant CAG and its progression appears to further increase the risk, particularly for proximal adenoma.

Various interpretations have been suggested for the mechanism by which *H. pylori* is involved in an increased risk of colorectal neoplasia. First, *H. pylori* infection increases gastrin secretion, which could contribute to colorectal carcinogenesis by inducing mucosal cell proliferation in the colon.[10] As for the correlation between colorectal neoplasia and gastrin, a limited number of epidemiological studies have been done with inconsistent results, some indicating positive correlations[29,30] and others, including a recent large nested case-control study, finding no correlation.[23,31] The differences in these results might be attributable to gastrin precursors such as progastrin or glycine-extended gastrin acting as more important promoters of colorectal carcinogenesis than the fully amidated form of the hormone measured by most commercially available assays.[10,32] Second, *H. pylori* infection might also affect the normal gastrointestinal flora, which contributes to colorectal carcinogenesis,[33–35] as a result of the reduced gastric acid secretion caused by *H. pylori*-related chronic gastritis.

Previous studies have indicated that the presence of an enteric infection and bacterial overgrowth, including intestinal bacteria, are considered to be directly related to a reduction in gastric acid secretion.[36–38] Indeed, our previous study revealed that CAG-positive asymptomatic middle-aged subjects, as determined by serum PG levels of PG I $\leq$ 70 ng/ml and PG I/II ratio of $\leq$ 3.0, were found to have more colonic microflora than CAG-negative subjects.[11] Bacterial overgrowth is reported to lead to an increase in unabsorbed nutrients in the lower intestine due to impaired gastric protein digestion,[39] so some metabolites derived from bacterial fermentation of malabsorbed proteins probably play a role in the etiopathogenesis of colonic disorders, including epithelial neoplasia.[40,41]

In the present results, the association between CAG and adenoma appeared to be particularly high in the proximal colon, but the reason for this is currently unclear. As described above, altered DNA methylation is proposed to be involved in the carcinogenic process of the proximal colon, and it is also known that chronic inflammation induces aberrant DNA methylation in normal tissues.[42] From this viewpoint, it is interesting that interleukin-6, a pro-inflammatory cytokine, whose polymorphisms are involved in the susceptibility to various cancers, is reported to induce expression and activity of DNA methyltransferase.[43] Thus, it is possible that CAG-induced colonic bacterial overgrowth can generate methylation changes to which the proximal colon is more susceptible. In addition, colonic bacterial overgrowth is also known to lead to an enhanced production of secondary bile acids, which are reported to increase the risk for proximal colon cancer.[44] Also, bile acids are presumed to cause DNA damage and activation of the carcinogenic pathway involving DNA methylation particularly in proximal colonic mucosa, and finally lead to the development of cancer.[45,46] Third, *H. pylori* urease could turn gastric juice urea into ammonia and carbon dioxide,[47] which might also affect the normal gastrointestinal flora and contribute to colorectal carcinogenesis. Some studies have correlated high concentrations of luminal ammonia with colon carcinogenesis.[48] Fourth, subjects with *H. pylori* infection might have lifestyles that increase susceptibility to carcinogenesis of the stomach and the rest of the gastrointestinal tract.

This study had some limitations. First, the subjects were asymptomatic men who were susceptible to colon cancer because of their age and who were self-referred for colonoscopy. As such, these subjects may have had a different overall prevalence of colorectal adenoma and risk profile for colorectal cancer compared to the general working population. It is also possible that the subjects were more health-conscious or had undisclosed reasons for suspecting they had colorectal

disease. Although we do not claim a complete absence of selection bias, the prevalence of colorectal adenoma (23.5%) in our study is in a range similar to the recently reported value of 26.5%, based on colonoscopy, of asymptomatic subjects in Japan.[49] Second, patients with hypergastrinemia and hyperchlorhydria secondary to Zollinger-Ellison syndrome show increased proliferation of rectal mucosa,[50] and Machida *et al.* reported that atrophic gastritis with gastric acid reduction (presence of CAG) might increase the risk of rectal cancer.[27] However, we failed to detect a significant association with rectal adenoma, as we did not have a sufficient sample size for tumors located only in the rectum. Third, with respect to the misclassification of exposures, the diagnosis of *H. pylori* infection and atrophic gastritis were based on serological tests. However, misclassification was likely to have occurred equally among cases and controls, and the risk of developing adenomas following infection might have been underestimated.

In conclusion, it is probable that *H. pylori* infection is involved in an increased risk of colorectal adenoma, and the risk of adenoma, particularly in the proximal colon, appears to be further enhanced by the presence and progression of CAG. The stage of *H. pylori*-related chronic gastritis, as determined by the two serologic markers *H. pylori* antibody and PG, will probably be useful for the evaluation of risk of colorectal neoplasia, and may contribute to the selection of high-risk individuals who warrant surveillance by colonoscopy. Further investigation into the role of *H. pylori* infection in the carcinogenesis of the colorectum is necessary. In addition, whether eradication therapy for *H. pylori*-infected subjects reduces the risk of colorectal neoplasia is a problem for future study.

Acknowledgements

The authors would like to express their deepest thanks to Ms. Kazu Konishi for her excellent secretarial assistance.

References

1. Whiting JL, Sigurdsson A, Rowlands DC, Hallissey MT, Fielding JWL. The long term results of endoscopic surveillance of premalignant gastric lesions. *Gut* 2002;50: 378–81.
2. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—first American cancer society award lecture on cancer epidemiology and prevention. *Cancer Res* 1992;52:6735–40.
3. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, Yoshikawa A, Yanaoka K, Arii K, Tamai H, Shimizu Y, Ichinose M, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. *Int J Cancer* 2004;109:138–43.
4. Konturek SJ, Konturek PC, Pieniążek P, Bielański W. Role of *Helicobacter pylori* infection in extragastroduodenal disorders: introductory remarks. *J Physiol Pharmacol* 1999;50:683–94.
5. Shemesh E, Czerniak A, Pines A, Bat L. Is there an association between gastric polyps and colonic neoplasms? *Digestion* 1989;42: 212–6.
6. Shimizu Y, Kakei N, Wada T, Yahagi N, Kido M, Ishihama S, Tsukada S, Matsushima M, Ichinose M, Miki K, Kurokawa K, Takahashi K. Two cases of early colorectal cancer associated with gastric adenoma detected by serum pepsinogen screening method. *Adv Exp Med Biol* 1995;362:149–54.
7. Eom BW, Lee H-J, Yoo M-W, Cho JJ, Kim WH, Yang H-K, Lee KU. Synchronous and metachronous cancers in patients with gastric cancer. *J Surg Oncol* 2008;98: 106–10.
8. Lee JH, Bae JS, Ryu KW, Lee JS, Park SR, Kim CG, Kook MC, Choi IJ, Kim YW, Park JG, Bae J-M. Gastric cancer patients at high-risk of having synchronous cancer. *World J Gastroenterol* 2006;12:2588–92.
9. Statistics and Information Department, Minister's Secretariat, Ministry of Health, Labor and Welfare. Age-adjusted death rates by prefecture. Special report on vital statistics of Japan, 2008. Tokyo: Health and Welfare Statistics Association.
10. Ciccotosto GD, McLeish A, Hardy KJ, Shulkes A. Expression, processing, and secretion of gastrin in patients with colorectal carcinoma. *Gastroenterology* 1995;109:1142–53.
11. Kanno T, Matsuki T, Oka M, Utsunomiya H, Inada K, Magari H, Inoue I, Maekita T, Ueda K, Enomoto S, Iguchi M, Ichinose M, et al. Gastric acid reduction leads to an alteration in lower intestinal microflora. *Biochem Biophys Res Commun* 2009;381: 666–70.
12. Chen TS, Chang FY, Lee SD. Serodiagnosis of *Helicobacter pylori* infection: comparison and correlation between enzyme-linked immunosorbent assay and rapid serological test results. *J Clin Microbiol* 1997;35:184–6.
13. Ichinose M, Miki K, Furihata C, Kageyama T, Hayashi R, Niwa H, Oka H, Matsushima T, Takahashi T. Radioimmunoassay of serum group I and group II pepsinogens in normal controls and patients with various disorders. *Clinica Chimica Acta* 1982;126:183–91.
14. Ichinose M, Yahagi N, Oka M, Ikeda H, Miki K, Omata M. Screening for gastric cancer in Japan. In: Wu GY, Aziz K, eds. Cancer screening. Totowa, NJ: Humana Press, 2001. 255–68.
15. Yanaoka K, Oka M, Yoshimura N, Mukoubayashi C, Enomato S, Iguchi M, Magari H, Utsunomiya H, Tamai H, Arii K, Mohara O, Ichinose M, et al. Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer Epidemiol Biomarkers Prev* 2008;17:838–45.
16. Komine M. Megaloblastic anemia. *Int J Hematol* 2000;71 (Suppl. 1):8.
17. Plebani M, Basso D, Cassaro M, Brigato L, Scrigner M, Toma A, Mario FD, Rugge M. *Helicobacter pylori* serology in patients with chronic gastritis. *Am J Gastroenterol* 1996;91:954–8.
18. Mårdh E, Mårdh S, Mårdh B, Borch K. Diagnosis of gastritis by means of a combination of serological analyses. *Clin Chim Acta* 2002;320:17–27.
19. Miki K, Ichinose M, Shimizu A, Huang SC, Oka H, Furihata C, Matsushima T, Takahashi K. Serum pepsinogens as a screening test of extensive chronic gastritis. *Gastroenterol Jpn* 1987;22:133–41.
20. Meucci G, Tatarella M, Vecchi M, Ranzi ML, Biguzzi E, Beccari G, Clerici E, de Franchis R. High prevalence of *Helicobacter pylori* infection in patients with colonic adenomas and carcinomas. *J Clin Gastroenterol* 1997;25:605–7.
21. Breuer-Katschinski B, Nemes K, Marr A, Rump B, Leiendecker B, Breuer N, Goebell H; Colorectal Adenoma Study Group. *Helicobacter pylori* and the risk of colonic adenomas. *Digestion* 1999;60:210–5.
22. Mizuno S, Morita Y, Inui T, Asakawa A, Ueno N, Ando T, Kato H, Uchida M, Yoshikawa T, Inui A. *Helicobacter pylori* infection is associated with colon adenomatous polyps detected by high-

resolution colonoscopy. *Int J Cancer* 2005; 117:1058–9.
23. Fireman Z, Trost L, Kopelman Y, Segal A, Sternberg A. *Helicobacter pylori*: seroprevalence and colorectal cancer. *IMAJ* 2000;2:6–9.
24. Shmuely H, Passaro D, Figer A, Niv Y, Pitlik S, Samra Z, Koren R, Yahav J. Relationship between *Helicobacter pylori* CagA status and colorectal cancer. *Am J Gastroenterol* 2001;96:3406–10.
25. Fujimori S, Kishida T, Kobayashi T, Sekita Y, Seo T, Nagata K, Tatsuguchi A, Gudis K, Yokoi K, Tanaka N, Yamashita K, Sakamoto C, et al. *Helicobacter pylori* infection increases the risk of colorectal adenoma and adenocarcinoma, especially in women. *J Gastroenterol* 2005;40: 887–93.
26. Jones M, Helliwell P, Pritchard C, Tharakan J, Mathew J. *Helicobacter pylori* in colorectal neoplasms: is there an aetiological relationship? *World J Surg Oncol* 2007;5:51.
27. Machida-Montani A, Sasazuki S, Inoue M, Natsukawa S, Shaura K, Koizumi Y, Kasuga Y, Hanaoka T, Tsugane S. Atrophic gastritis, *Helicobacter pylori*, and colorectal cancer risk: a case-control study. *Helicobacter* 2007;12:328–32.
28. Iacopetta B. Are there two sides to colorectal cancer? *Int J Cancer* 2002;101: 403–8.
29. Thorburn CM, Friedman GD, Dickinson CJ, Vogelman JH, Orentreich N, Parsonnet J. Gastrin and colorectal cancer: a prospective study. *Gastroenterology* 1998; 115:275–80.
30. Georgopoulos SD, Polymeros D, Triantafyllou K, Spiliadi C, Mentis A, Karamanolis DG, Ladas SD. Hypergastrinemia is associated with increased risk of distal colon adenomas. *Digestion* 2006;74:42–6.
31. Penman ID, El-Omar E, Ardill JES, McGregor JR, Galloway DJ, O'Dwyer PJ, McColl KEL. Plasma gastrin concentrations are normal in patients with colorectal neoplasia and unaltered following tumor resection. *Gastroenterology* 1994;106: 1263–70.
32. Aly A, Shulkes A, Baldwin GS. Gastrins, cholecystokinins and gastrointestinal cancer. *Biochim Biophys Acta* 2004;1704: 1–10.
33. Moore WEC, Moore LH. Intestinal floras of populations that have a high risk of colon cancer. *Appl Environ Microbiol* 1995; 61:3203–7.
34. Kado S, Uchida K, Funabashi H, Iwata S, Nagata Y, Ando M, Onoue M, Matsuoka Y, Ohwaki M, Morotomi M. Intestinal microflora are necessary for development of spontaneous adenocarcinoma of the large intestine in T-cell receptor β chain and p53 double-knockout mice. *Cancer Res* 2001;61:2395–8.
35. Horie H, Kanazawa K, Okada M, Narushima S, Itoh K, Terada A. Effects of intestinal bacteria on the development of colonic neoplasm: an experimental study. *Eur J Cancer Prev* 1999;8:237–45.
36. Howden CW, Hunt RH. Relationship between gastric secretion and infection. *Gut* 1987;28:96–107.
37. Drasar BS, Shiner M, McLeod GM. Studies on the intestinal flora: I. The bacterial flora of the gastrointestinal tract in healthy and achlorhydric persons. *Gastroenterology* 1969;56:71–9.
38. Thorens J, Froehlich F, Schwizer W, Saraga E, Bille J, Gyr K, Duroux P, Nicolet M, Pignatelli B, Blum AL, Gonvers JJ, Fried M. Bacterial overgrowth during treatment with omeprazole compared with cimetidine: a prospective randomized double blind study. *Gut* 1996;39:54–59.
39. Evenepoel P, Claus D, Geypens B, Maes B, Hiele M, Rutgeerts P, Ghoos Y. Evidence for impaired assimilation and increased colonic fermentation of protein, related to gastric acid suppression therapy. *Aliment Pharmacol Ther* 1998;12:1011–9.
40. Aarbakke J, Schjönsby H. Value of urinary simple phenol and indican determinations in the diagnosis of the stagnant loop syndrome. *Scand J Gastroenterol* 1976;11: 409–14.
41. Visek WJ. Diet and cell growth modulation by ammonia. *Am J Clin Nutr* 1978;31: 216–20.
42. Nakajima T, Enomoto S, Ushijima T. DNA methylation: a marker for carcinogen exposure and cancer risk. *Environ Health Prev Med* 2008;13:8–15.
43. Hodge DR, Xiao W, Clausen PA, Heidecker G, Szyf M, Farrar WL. Interleukin-6 regulation of the human DNA methyltransferase (HDNMT) gene in human erythroleukemia cells. *J Biol Chem* 2001;276:39508–11.
44. Giovannucci E, Colditz GA, Stampfer MJ. A meta-analysis of cholecystectomy and risk of colorectal cancer. *Gastroenterology* 1993;105:130–41.
45. Pereira MA, Wang W, Kramer PM, Tao L. DNA hypomethylation induced by non-genotoxic carcinogens in mouse and rat colons. *Cancer Lett* 2004;212: 145–51.
46. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. *Mutat Res* 2005;589:47–65.
47. Le Veen HH, Le Veen EG, Le Veen RF. Awakenings to the pathogenicity of urease and the requirement for continuous long-term therapy. *Biomed Pharmacother* 1994; 48:157–66.
48. Clinton SK, Bostwick DG, Olson LM, Mangian HJ, Visek WJ. Effects of ammonium acetate and sodium cholate on N-methyl-N′-nitro-N-nitrosoguanidine-induced colon carcinogenesis of rats. *Cancer Res* 1988;48:3035–9.
49. Sawada S, Shinchi K, Imanishi K. Prevalence of colorectal adenomas in a healthy Japanese middle-aged male population. *Scand J Gastroenterol* 1998;33: 783–84.
50. Renga M, Brandi G, Paganelli GM, Calabrese C, Papa S, Tosti A, Tomassetti P, Miglioli M, Biasco G. Rectal cell proliferation and colon cancer risk in patients with hypergastrinaemia. *Gut* 1997; 41:330–2.

UICC
global cancer control

IJC
International Journal of Cancer

Alu and Satα hypomethylation in *Helicobacter pylori*-infected gastric mucosae

Takeichi Yoshida[1,2], Satoshi Yamashita[1], Takeji Takamura-Enya[3], Tohru Niwa[1], Takayuki Ando[1], Shotaro Enomoto[2], Takao Maekita[2], Kazuyuki Nakazawa[2], Masae Tatematsu[4], Masao Ichinose[2] and Toshikazu Ushijima[1]

[1] Carcinogenesis Division, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan
[2] Second Department of Internal Medicine, Wakayama Medical University, Wakayama City, Wakayama, Japan
[3] Department of Applied Chemistry, Kanagawa Institute of Technology, Atsugi City, Kanagawa, Japan
[4] Division of Oncological Pathology, Aichi Cancer Center Research Institute, Nagoya City, Aichi, Japan

Global hypomethylation and regional hypermethylation are supposed to be hallmarks of cancer cells. During gastric carcinogenesis, in which *Helicobacter pylori* infection is causally involved, aberrant hypermethylation is already present in *H. pylori*-infected gastric mucosae. In contrast, little is known about global hypomethylation, which can be caused by hypomethylation of individual repetitive elements and other sequences. We, therefore, investigated hypomethylation of individual repetitive elements and the global 5-methylcytosine content in four groups of gastric mucosal samples that represented the time course of *H. pylori* infection and gastric carcinogenesis [gastric mucosae of *H. pylori*-negative healthy volunteers (G1, n = 34), *H. pylori*-positive healthy volunteers (G2, n = 42), *H. pylori*-positive gastric cancer patients (G3, n = 34) and *H. pylori*-negative gastric cancer patients (G4, n = 20)] and 52 primary gastric cancers. Major variants of Alu, LINE1 and Satα were identified, and their methylation levels were quantified by bisulfite pyrosequencing. Compared with G1, the Alu methylation level was decreased in G2, G3, G4 and cancers (89.2–97.1% of that in G1, $p < 0.05$). The Satα methylation level was decreased in G2 (91.6%, $p < 0.05$) and G3 (94.3%, $p = 0.08$) but not in G4 and cancers. The LINE1 methylation level was decreased only in cancers. The 5-methylcytosine content was at similar levels in G2, G3 and G4 and highly variable in cancers. These results showed that Alu and Satα hypomethylation is induced in gastric mucosae by *H. pylori* infection during gastric carcinogenesis, possibly in different target cells, and that global hypomethylation is not always present in human gastric cancers.

Global hypomethylation and regional hypermethylation are supposed to be hallmarks of cancer cells.[1] Global hypomethylation, defined as the content of 5-methylcytosine in the genome,[2] is considered to be due to hypomethylation of repetitive elements, which are normally heavily methylated,[3] and other sequences. Global hypomethylation is known to cause chromatin decondensation that results in chromosomal instability and cancer development.[4–7] In addition, hypomethylation of repetitive elements is associated with its elevated transcription,[8] and that of normally methylated promoter CpG islands can lead to elevated expression of tumor antigens and possible oncogenes.[8–10] On the other hand, hypermethylation is observed in normally unmethylated promoter CpG islands and silences downstream genes, including tumor suppressor and other passenger genes.[11]

Hypermethylation of CpG islands can be present not only in cancers but also in noncancerous tissues.[12] Especially in gastric mucosae, aberrant DNA hypermethylation is induced by *Helicobacter pylori* infection, a major cause of gastric cancers.[13,14] The methylation levels of CpG islands are very low in gastric mucosae of *H. pylori*-negative healthy individuals (G1; incidence of gastric cancers = 0.03% per year or less[15]). They are very high in gastric mucosae of *H. pylori*-positive healthy individuals (G2; incidence = 0.14%[16]) and in noncancerous gastric mucosae of *H. pylori*-positive gastric cancer patients (G3; incidence of secondary gastric cancer = 4.1%[17]). They are high but lower than in G2 and G3 in noncancerous gastric mucosae of *H. pylori*-negative gastric cancer patients (G4; incidence = 6.2%[18]). *H. pylori* infection is known to disappear when severe gastric atrophy is induced as a result of chronic *H. pylori* infection,[19–22] and the four groups, G1–G4, are considered to represent the natural history of *H. pylori* infection. Methylation levels correlate with gastric cancer risk only in *H. pylori*-negative individuals,[13,14] suggesting that methylation levels in these individuals reflect the degree of epigenetic damage in stem cells.[23,24]

In contrast, global hypomethylation during gastric carcinogenesis remains unclear, not only when but also where in the genome it takes place. Major normally methylated repetitive elements consist of Alu, LINE1 and Satα, which

Key words: hypomethylation, repeat sequence, gastric cancer, *Helicobacter pylori*, carcinogenesis
Additional Supporting Information may be found in the online version of this article.
DOI: 10.1002/ijc.25534
History: Received 16 Mar 2010; Accepted 14 Jun 2010; Online 2 Jul 2010
Correspondence to: Toshikazu Ushijima, Carcinogenesis Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan, Tel.: [+81 3 3547 5240], Fax: +[81 3 5565 1753], E-mail: tushijim@ncc.go.jp

constitute 10, 17 and 4% of the genome, respectively,[25–27] and collectively cover over 30% of the total CpG sites in the genome.[28,29] Alu and LINE1 belong to interspersed elements,[25] and Satα is a tandem repeat element[30,31] confined to the centromeres.[32] Hypomethylation of Satα is known to be induced by loss-of-function mutations of DNA methyltransferase 3B.[33] As a fundamental basis to understand gastric carcinogenesis, we have to clarify whether or not hypomethylation is present in *H. pylori*-infected gastric mucosae and, if present, which repetitive elements or global 5-methylcytosine content are mainly affected.

In this study, we aimed to clarify these issues. To this end, we first identified major variants of Alu, LINE1 and Satα, and then measured their methylation levels by bisulfite pyrosequencing of DNA from gastric mucosal samples of G1, G2, G3 and G4 and gastric cancer tissues.

Material and Methods

Tissue samples

Gastric mucosae were collected by endoscopic biopsy of the antral region in 34 *H. pylori*-negative (G1: 16 male and 18 female; average age = 51 years, range = 25–91 years) and 42 *H. pylori*-positive healthy volunteers (G2: 21 male and 21 female; average age = 57 years, range = 23–86 years; 19 with gastric atrophy and 23 without; nine with gastric ulcers, eight with duodenal ulcers and three with hyperplastic polyps) and noncancerous gastric mucosae from 34 *H. pylori*-positive (G3: 26 male and 8 female; average age = 68 years, range = 39–87 years; 23 early cancers and 11 advanced cancers) and 20 *H. pylori*-negative gastric cancer patients (G4: 15 male and 5 female; average age = 69 years, range = 38–84 years; 17 early cancers and three advanced cancers). Gastric cancer tissues were obtained from 52 gastric cancer patients (cancers: 52 male; average age = 60 years, range = 29–84 years) who underwent gastrectomy. Informed consents were obtained from all the individuals. Gastric mucosae, noncancerous gastric mucosae and cancer tissues were frozen in liquid nitrogen immediately after biopsy or resection and stored at −80°C until extraction of genomic DNA.

All cancer tissues were histologically diagnosed according to the Japanese classification of gastric carcinoma[34] and were classified according to the Lauren classification system (11 intestinal and 41 diffuse types).[35] *H. pylori* infection status was detected by use of a serum anti-*H. pylori* IgG antibody test (SRL, Tokyo, Japan).

Cell lines and DNA extraction

Six gastric cancer cell lines, AGS, KATOIII, MKN28, MKN45, MKN74 and NUGC3, were obtained from the Japanese Collection of Research Bioresources (Tokyo, Japan) and the American Type Culture Collection (Manassas, VA). Three gastric cancer cell lines, HSC39, HSC44 and HSC57, were gifted by Dr. K. Yanagihara, National Cancer Center Research Institute, Tokyo, Japan. TMK1 was gifted by Dr. W. Yasui, Hiroshima University, Hiroshima, Japan. High molecular weight DNA was extracted by the phenol/chloroform method.

Sequencing analysis of repetitive DNA elements

Genomic DNA of a human gastric cancer cell line (AGS) was amplified by PCR with the primers for the three repetitive DNA elements (Supporting Information Table 1): Alu (AluSp from the database of the Genetic Information Research Institute: http://www.girinst.org/), LINE1 (X58075) and Satα (M38468). The PCR products were cloned into pGEM-T Easy vector (Promega, Madison, WI), and 12–41 clones for each repetitive DNA element were cycle sequenced. Sequencing was performed using a DYEnamic ET Terminator (GE Healthcare, Buckinghamshire, United Kingdom) with an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

Sodium bisulfite modification and bisulfite pyrosequencing

Bisulfite modification was performed using 1 μg of *Bam*HI-digested genomic DNA as previously described.[36] The modified DNA was suspended in 40 μl of Tris-EDTA buffer, and an aliquot of 1 μl was used for bisulfite pyrosequencing. An annealing temperature that could amplify both unmethylated and methylated DNAs was determined by comparing amplification of DNA from peripheral leukocytes (mixture of unmethylated and methylated DNA) and DNA that was fully methylated by *Sss*I methylase (New England Biolabs, Beverly, MA) (Supporting Information Table 2). The PCR product was annealed to 0.2 μM pyrosequencing primers, and pyrosequencing was carried out using the PSQ 96 Pyrosequencing System (Qiagen, Valencia, CA). A methylation level was obtained using PSQ Assay Design software (Qiagen). Two CpG sites (ALU1 and ALU2) were measured for Alu, three for LINE1 (LINE1–1, LINE1–2 and LINE1–3) and one for Satα (SATα).

Analysis of the global 5-methylcytosine content

Genomic DNA (2.5 μg) was incubated with five units of DNase I (Sigma, St. Louis, MO) and 4 mM $MgCl_2$ at 37°C for 18 hr. The sample was further treated with three units of nuclease P1 in 10 mM NaAc (pH 5.2) and 50 μg/ml $ZnSO_4$ at 37°C for 7 hr and then with 2.5 units of *Escherichia coli* alkaline phosphatase in 0.1 M NH_4HCO_3 at 37°C for 16 hr. After purification, the samples were subjected to liquid chromatography equipped with a photodiode array detector and an electrospray ionization time-of-flight mass spectrometry (LCMS; LCT premier XE, Waters). Peaks of the four deoxyribonucleotides (2′-deoxyguansine, 2′-deoxyadenosine, 2′-deoxycytidine (dC) and thymidine) were monitored with UV 260 nm, whereas that of 5-methyl-2′-deoxycytidine (5mdC) was detected by a molecular ion of 242 [M+1], retention times of which were compared with that of the authentic sample. Global 5-methylcytosine content was quantified as the fraction of 5mdC quantity in the total 5mdC and dC quantity. The LCMS analysis was performed three times for each sample, and the mean coefficient of variation was confirmed to be less than 3%. Eight of the samples were also subjected to high-performance liquid chromatography (HPLC)-UV.

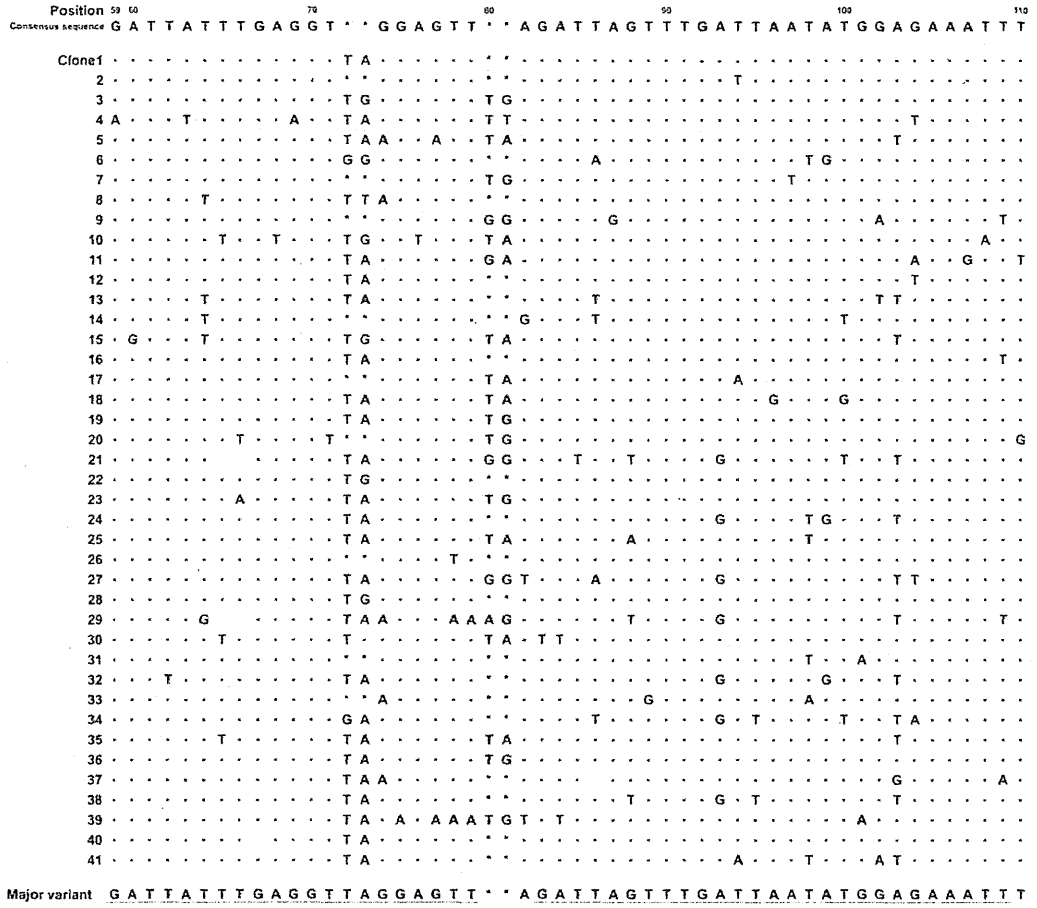

Figure 1. The most frequent Alu sequences after bisulfite conversion and location of CpG sites analyzed by pyrosequencing. The sequence obtained by virtual bisulfite conversion of a consensus sequence in the database is shown at the top. The 41 sequences are obtained by virtual bisulfite treatment of the sequences obtained. A dot shows no variation from the sequence at the top. Two consecutive asterisks show a CpG site. The most frequent sequence (shown at the bottom) was identified as the major variant. The sequences used for bisulfite pyrosequencing are underlined.

Statistical analysis

A difference in mean methylation levels or mean global 5-methylcytosine content was analyzed by the Welch t test. Correlation between the global 5-methylcytosine content by LCMS and that by HPLC-UV, correlation of methylation levels among repetitive DNA elements and correlation between age and methylation level were analyzed using Pearson's product-moment correlation coefficient. All the analyses were performed using SPSS (SPSS, Chicago, IL), and the results were considered significant when p values less than 0.05 were obtained by a two-sided test.

Results

Identification of the major variants of individual repetitive DNA elements

A major variant was identified for each of the three repetitive DNA elements to measure methylation levels of as many repeat units as possible. Based on the consensus sequence in the database, each element was amplified by PCR with low stringency, and clones obtained were sequenced (Supporting Information Fig. 1). Since Alu and LINE1 were more variable than Satα, more clones were sequenced for Alu (41 clones) and LINE1 (19 clones) than for Satα (12 clones). After virtual conversion by bisulfite treatment of the sequences obtained, the most frequent sequence was identified as the major variant for each repetitive element (Supporting Information Fig. 2).

Primers for bisulfite pyrosequencing (Alu in Fig. 1; and Supporting Information Table 2) were designed based on the major variant, covering the most frequent sequences at the CpG site. The Alu methylation level was measured at two CpG sites, ALU1 (position +80 in the consensus sequence) and ALU2 (+197), which represented five and six, respectively, of the 41 sequences. The LINE1 methylation level was measured at three CpG sites, LINE1-1 (+138), LINE1-2 (+206) and LINE1-3 (+270), which represented seven, eight and 11, respectively, of the 19 sequences. The SATα methylation level was measured at one CpG site (+360) that was common to all the 12 sequences.

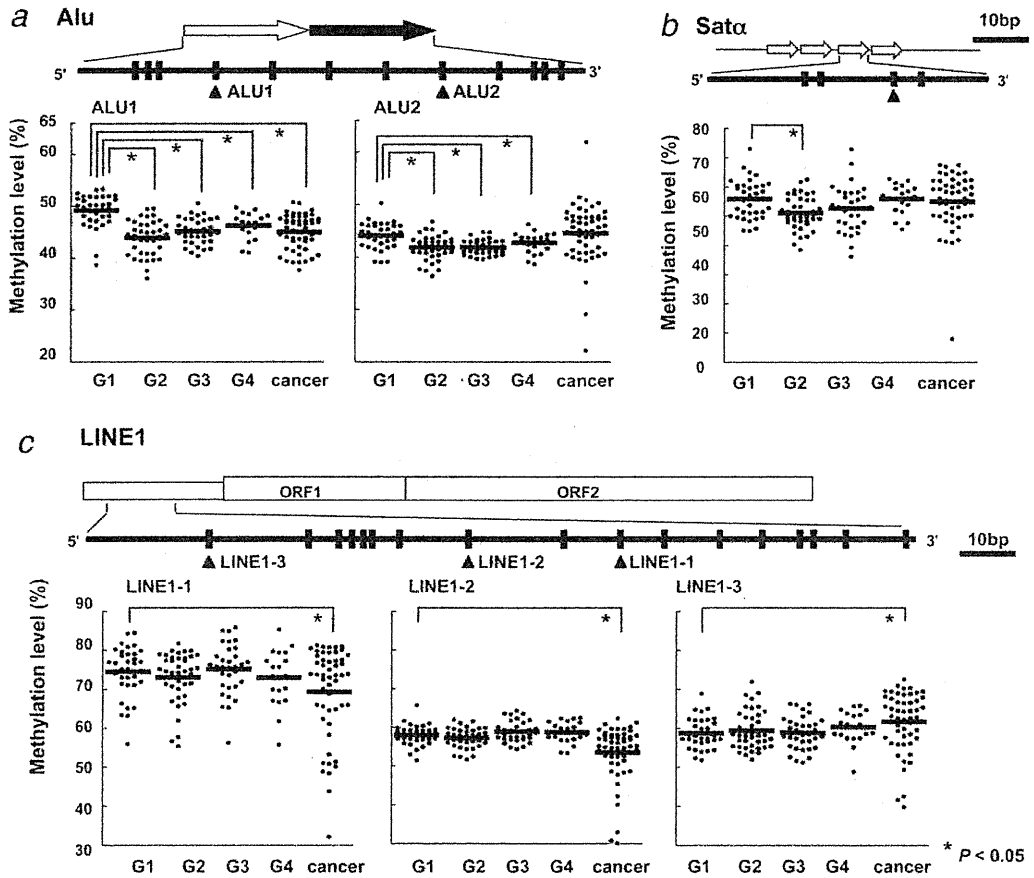

Figure 2. Methylation levels of the three repetitive DNA elements in gastric mucosae of G1-G4 and gastric cancers. Vertical ticks, individual CpG sites; arrowheads, locations of the measured CpG site. A horizontal line in a chart represents a mean methylation level for each group. (*a*) Distribution of methylation levels at two CpG sites of Alu. Compared with G1, the methylation level was decreased in G2, G3 and G4. The methylation level was decreased in cancers at ALU1 but not at ALU2. The top arrows show two duplicated arms of an Alu unit. (*b*) Distribution of methylation levels at one CpG site of Satα. Compared with G1, the methylation level was decreased in G2 and tended to be decreased in G3. The methylation level was not decreased in G4 and cancers. (*c*) Distribution of methylation levels at three CpG sites of LINE1. Compared with G1, the methylation level was not decreased in G2, G3 and G4 at LINE1-1, LINE1-2 and LINE1-3. The methylation level was decreased in cancers at LINE1-1 and LINE1-2 but was increased at LINE1-3. ORF: open reading frame.

The presence of Alu and Satα hypomethylation in *H. pylori*-infected gastric mucosae

The Alu methylation level was measured by bisulfite pyrosequencing in gastric mucosae (G1, G2, G3 and G4) and cancers (Fig. 2*a*; Supporting Information Table 3). In the normal control group (G1), the methylation level was 49.2 ± 3.2% (mean ± SD) at ALU1 and 44.1 ± 2.5% at ALU2. The mean methylation level at ALU1 was decreased in G2 (decreased to 89.2% of that in G1, $p < 0.05$), G3 (decreased to 91.9%, $p < 0.05$) and G4 (decreased to 94.1%, $p < 0.05$), and remained low in cancers (90.9% of that in G1, $p < 0.05$). Similarly, the mean methylation level at ALU2 was decreased in G2, G3 and G4 (decreased to 94.8, 95.0 and 97.1% of that in G1; $p < 0.05$, < 0.05 and < 0.05, respectively). However, a decrease was not observed in cancers (100.9% of that in G1, $p = 0.61$).

The methylation level of SATα was 55.9 ± 6.8% in G1 (Fig. 2*b*; Supporting Information Table 3). The mean methylation level was decreased in G2 (decreased to 91.6% of that in G1; $p < 0.05$) and had a tendency to be decreased in G3 (decreased to 94.3%, $p = 0.08$). However, in contrast with Alu, it was not decreased in G4 (100.0% of that in G1, $p = 0.98$) or cancers (98.2% of that in G1, $p = 0.63$).

The absence of LINE1 hypomethylation in *H. pylori*-infected gastric mucosae

The methylation levels at LINE1-1, LINE1-2 and LINE1-3 were 74.5 ± 6.6%, 58.0 ± 2.6% and 58.7 ± 3.9%, respectively, in G1 (Fig. 2*c*; Supporting Information Table 3). The mean methylation levels at LINE1-1, LINE1-2 and LINE1-3 were not decreased in G2, G3 or G4, respectively. The methylation levels at these CpG sites were highly variable in cancers, but the mean methylation levels at LINE1-1 and LINE1-2 were significantly decreased and that at LINE1-3 was significantly increased (LINE1-1, 93.2% of that in G1, $p < 0.05$; LINE1-2, 92.4% of that in G1, $p < 0.05$; LINE1-3, 105.1% of that in G1, $p < 0.05$).

No decrease of global 5-methylcytosine content in *H. pylori*-infected gastric mucosae and its high variability in gastric cancers

The global 5-methylcytosine content was measured by LCMS for five samples of G1, 17 of G2, 18 of G3, six of G4 and 27 cancer samples, because of the large amount of DNA necessary for the analysis. The global 5-methylcytosine content in G1 was 4.6 ± 0.8% and was not decreased in G2, G3 and G4 (100.3%, 92.5% and 100.7% of that in G1; $p = 0.96$, 0.31 and 0.94, respectively). In cancers, the global 5-methylcytosine content was highly variable (4.3 ± 1.4%), some showing no decrease and the others showing marked decrease (Fig. 3).

To confirm that the high variability observed was due to that of the 5-methylcytosine content in cancer cells and not due to high variability of the population of cancer cells in cancer tissues, the global 5-methylcytosine content was analyzed in gastric cancer cell lines and was again shown to have high variability (2.8–7.5%). The global 5-methylcytosine content obtained by the current LCMS method was in good accordance with that obtained by the conventional HPLC-UV method (n = 8, correlation coefficient = 0.95, $p < 0.05$).

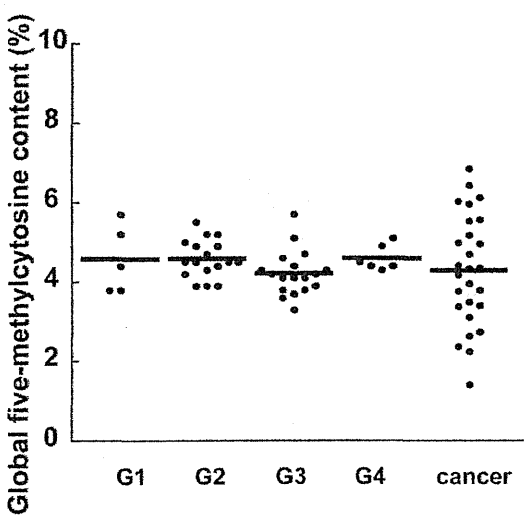

Figure 3. Distribution of global 5-methylcytosine content in G1-G4 and cancers. A horizontal line represents the mean global 5-methylcytosine content for each group. The global 5-methylcytosine content was at a similar level among G1, G2, G3 and G4. In cancers, the global 5-methylcytosine content was highly variable, some showing no decrease and others showing marked decease.

Correlation of hypomethylation among individual repetitive elements and the lack of correlation between hypomethylation and age

Correlations of the methylation levels among individual repetitive DNA elements were significant but weak (correlation coefficients = 0.15 - 0.55, $p < 0.05$; Supporting Information Table 4). Correlation between the global 5-methylcytosine content and the methylation level of individual repetitive elements was not significant, except for ALU2 (Supporting Information Table 5).

Hypermethylation of some CpG islands is known to be observed in an age-dependent manner.[37] Therefore, we analyzed association between hypomethylation of the three repetitive DNA elements and age within G1, whose members had no influence of *H. pylori* infection (Table 1). However, we observed no association. We also analyzed association between hypomethylation and gender, but again there was no association. Hypomethylation of LINE1 (LINE1-2 and LINE1-3) was marginally associated with intestinal-type histology, but that of Alu and Satα was not (Supporting Information Table 6).

Discussion

Our study showed that Alu and Satα hypomethylation was already present in *H. pylori*-infected gastric mucosae and that Alu, but not Satα, hypomethylation persisted after *H. pylori* infection discontinued and was also present in cancers. In contrast, LINE1 hypomethylation was present only in cancers. It was strongly indicated that hypomethylation is induced in gastric mucosae by *H. pylori* infection at Alu and Satα repetitive elements as an early event during gastric carcinogenesis whereas LINE1 hypomethylation is induced as a result of cellular transformation. To our knowledge, the presence of Alu and Satα hypomethylation in *H. pylori*-infected gastric mucosae during gastric carcinogenesis is shown here for the first time. Regarding hypomethylation of a specific repetitive element in noncancerous tissues that in liver, tissues exposed to hepatitis B virus[38] has been reported. Because hypomethylation is

Table 1. Lack of association between hypomethylation of the three repetitive DNA elements and age (or gender)

	ALU1	ALU2	LINE1-1	LINE1-2	LINE1-3	SATα
Age						
r	0.14	−0.30	−0.24	0.13	0.02	0.07
p	0.45	0.08	0.17	0.48	0.93	0.71
Gender						
Male (n = 16)	48.2 ± 4.1	44.0 ± 2.2	74.2 ± 7.5	58.5 ± 1.4	59.3 ± 4.1	58.4 ± 7.8
Female (n = 18)	50.1 ± 2.0	44.3 ± 2.8	74.8 ± 5.8	57.5 ± 3.3	58.3 ± 3.8	53.7 ± 5.0
p	0.12	0.74	0.79	0.25	0.47	0.05

r, correlation coefficient. To avoid confounding effects of *H. pylori* infection, the analyses were conducted in *H. pylori*-negative healthy volunteers (G1).

known to lead to genomic instability,[5] precise understanding of the timing of occurrence of hypomethylation is important as a fundamental basis to understand gastric carcinogenesis.

Alu and Satα hypomethylation showed different profiles in G1–G4, which are considered to represent the time course of gastric carcinogenesis.[19–22] Satα methylation levels were significantly decreased in G2 and tended to be decreased in G3, but not after disappearance of *H. pylori* infection (G4), whereas Alu hypomethylation persisted. The dynamics of Satα were reminiscent of hypermethylation of many protein-coding genes, which is potently induced by *H. pylori* infection and decreases after eradication of *H. pylori*.[13,39] As a mechanism for the different profiles of Alu and Satα, we can hypothesize that their hypomethylations are induced in different cell types. If methylation is induced in stem cells, it is expected to persist even after *H. pylori* infection discontinues whereas methylation induced in progenitor cells can disappear.[14] There is a possibility that Alu hypomethylation is relatively more easily induced in gastric stem cells than Satα hypomethylation. As a mechanism of how *H. pylori* infection induces hypomethylation of Alu and Satα, insufficiency of maintenance DNA methylation can be considered. It is known that expression levels of DNA methyltransferases are lower in gastric epithelial cells with *H. pylori* infection than those without in humans and gerbils.[40,41]

The finding here is important as a fundamental basis of gastric carcinogenesis associated with *H. pylori* infection. Alu is distributed throughout the genome,[40] and its hypomethylation could possibly lead to chromosomal instability as an early event during gastric carcinogenesis, as is known in mice.[4–6] From a clinical viewpoint, we initially expected that hypomethylation could be used as a cancer risk marker such as hypermethylation of CpG islands.[13,14] However, Alu hypomethylation had only low sensitivity and specificity in distinguishing healthy volunteers and gastric cancer patients among *H. pylori*-negative individuals (Fig. 2*a*), and use of hypomethylation as a risk marker was considered not to be realistic. Nevertheless, the early occurrence of Alu hypomethylation and its possible involvement in chromosomal instability suggested a possibility that suppression of hypomethylation induction can be used as a novel target of cancer prevention.

In cancers, LINE1 methylation level, which is often used as a surrogate for global hypomethylation,[42,43] was highly variable. The high variability of the LINE1 methylation level in gastric cancers was in good accordance with that reported in bladder and colon cancers.[44,45] It was considered that, because a cancer tissue is monoclonal, its methylation level reflects that of its single precursor cell and, thus, stochastically shows a low or high level. When methylation levels of the three CpG sites analyzed for LINE1 were compared in gastric cancers, methylation levels were decreased at two CpG sites while increased at another site. This suggested that there is a difference in susceptibility to hypomethylation among CpG sites. The difference of susceptibility could be related on the location of a CpG site within LINE1 because the CpG site whose methylation level was increased was located at an edge of LINE1.

The global 5-methylcytosine content in gastric cancers was also highly variable. In contrast, global hypomethylation is generally considered as one of the hallmarks of cancer cells.[1,3] In most studies, global hypomethylation is assessed by hypomethylation of repetitive sequences and not by the global 5-methylcytosine content. The 5-methylcytosine content is already reported to be variable in some cancers.[46] In gastric cancers, only our previous study[39] measured the 5-methylcytosine content, and it was highly variable not only in primary gastric cancers but also in gastric cancer cell lines. Therefore, global hypomethylation measured by the global 5-methylcytosine content was highly variable in gastric cancers.

In conclusion, our data strongly indicated that *H. pylori* infection potently induces Alu and Satα hypomethylation in gastric mucosae as an early event during gastric carcinogenesis and that global 5-methylcytosine content is not always decreased in gastric cancers.

Acknowledgements

This study was supported by Grants-in-Aid for Pioneering Basic Research and for the Third-term Comprehensive Cancer Control Strategy from the Ministry of Health, Labour and Welfare, Japan. T.A. is a recipient of the Research Resident Fellowship from the Foundation for Promotion of Cancer Research. The authors are grateful to Dr. Yoshiyuki Watanabe at St. Marianna Medical University for his critical advice.

References

1. Jones PA, Baylin SB. The epigenomics of cancer. *Cell* 2007;128:683–92.
2. Ehrlich M, Gama-Sosa MA, Huang LH, Midgett RM, Kuo KC, McCune RA, Gehrke C. Amount and distribution of 5-methylcytosine in human DNA from different types of tissues of cells. *Nucleic Acids Res* 1982;10:2709–21.
3. Feinberg AP, Ohlsson R, Henikoff S. The epigenetic progenitor origin of human cancer. *Nat Rev Genet* 2006;7:21–33.
4. Chen RZ, Pettersson U, Beard C, Jackson-Grusby L, Jaenisch R. DNA hypomethylation leads to elevated mutation rates. *Nature* 1998;395:89–93.
5. Eden A, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 2003;300:455.
6. Gaudet F, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R. Induction of tumors in mice by genomic hypomethylation. *Science* 2003; 300:489–92.
7. Holm TM, Jackson-Grusby L, Brambrink T, Yamada Y, Rideout WM III, Jaenisch R. Global loss of imprinting leads to widespread tumorigenesis in adult mice. *Cancer Cell* 2005;8:275–85.
8. Howard G, Eiges R, Gaudet F, Jaenisch R, Eden A. Activation and transposition of endogenous retroviral elements in hypomethylation induced tumors in mice. *Oncogene* 2008;27:404–8.
9. Shen L, Kondo Y, Guo Y, Zhang J, Zhang L, Ahmed S, Shu J, Chen X, Waterland RA, Issa JP. Genome-wide profiling of DNA methylation reveals a class of normally methylated CpG island promoters. *PLoS Genet* 2007;3:2023–36.
10. Roman-Gomez J, Jimenez-Velasco A, Agirre X, Cervantes F, Sanchez J, Garate L, Barrios M, Castillejo JA, Navarro G, Colomer D, Prosper F, Heiniger A, et al.

Promoter hypomethylation of the LINE-1 retrotransposable elements activates sense/antisense transcription and marks the progression of chronic myeloid leukemia. *Oncogene* 2005;24:7213–23.
11. Ushijima T. Detection and interpretation of altered methylation patterns in cancer cells. *Nat Rev Cancer* 2005;5:223–31.
12. Ushijima T. Epigenetic field for cancerization. *J Biochem Mol Biol* 2007;40:142–50.
13. Maekita T, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, Arii K, Kaneda A, Tsukamoto T, Tatematsu M, Tamura G, Saito D, et al. High levels of aberrant DNA methylation in *Helicobacter pylori*-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res* 2006;12:989–95.
14. Ando T, Yoshida T, Enomoto S, Asada K, Tatematsu M, Ichinose M, Sugiyama T, Ushijima T. DNA methylation of microRNA genes in gastric mucosae of gastric cancer patients: its possible involvement in the formation of epigenetic field defect. *Int J Cancer* 2009;124:2367–74.
15. Ohata H, Kitauchi S, Yoshimura N, Mugitani K, Iwane M, Nakamura H, Yoshikawa A, Yanaoka K, Arii K, Tamai H, Shimizu Y, Takeshita T, et al. Progression of chronic atrophic gastritis associated with *Helicobacter pylori* infection increases risk of gastric cancer. *Int J Cancer* 2004;109:138–43.
16. Lee KJ, Inoue M, Otani T, Iwasaki M, Sasazuki S, Tsugane S. Gastric cancer screening and subsequent risk of gastric cancer: a large-scale population-based cohort study, with a 13-year follow-up in Japan. *Int J Cancer* 2006;118:2315–21.
17. Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagai K, Hayashi S, Asaka M. Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *Lancet* 2008;372:392–7.
18. Yanaoka K, Oka M, Ohata H, Yoshimura N, Deguchi H, Mukoubayashi C, Enomoto S, Inoue I, Iguchi M, Maekita T, Ueda K, Utsunomiya H, et al. Eradication of *Helicobacter pylori* prevents cancer development in subjects with mild gastric atrophy identified by serum pepsinogen levels. *Int J Cancer* 2009;125:2697–703.
19. Karnes WE Jr, Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SW, Walsh JH. Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. *Gastroenterology* 1991;101:167–74.
20. Sipponen P, Kosunen TU, Valle J, Riihela M, Seppala K. *Helicobacter pylori* infection and chronic gastritis in gastric cancer. *J Clin Pathol* 1992;45:319–23.
21. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991;302:1302–5.
22. Forman D, Webb P, Parsonnet J. *H pylori* and gastric cancer. *Lancet* 1994;343:243–4.
23. Ushijima T, Nakajima T, Maekita T. DNA methylation as a marker for the past and future. *J Gastroenterol* 2006;41:401–7.
24. Nakajima T, Enomoto S, Yamashita S, Ando T, Nakanishi Y, Nakazawa K, Oda I, Gotoda T, Ushijima T. Persistence of a component of DNA methylation in gastric mucosae after *Helicobacter pylori* eradication. *J Gastroenterol* 2010;45:37–44.
25. Weiner AM. SINEs and LINEs: the art of biting the hand that feeds you. *Curr Opin Cell Biol* 2002;14:343–50.
26. Deininger PL, Moran JV, Batzer MA, Kazazian HH Jr. Mobile elements and mammalian genome evolution. *Curr Opin Genet Dev* 2003;13:651–8.
27. Lee C, Wevrick R, Fisher RB, Ferguson-Smith MA, Lin CC. Human centromeric DNAs. *Hum Genet* 1997;100:291–304.
28. Rollins RA, Haghighi F, Edwards JR, Das R, Zhang MQ, Ju J, Bestor TH. Large-scale structure of genomic methylation patterns. *Genome Res* 2006;16:157–63.
29. Schmid CW. Human Alu subfamilies and their methylation revealed by blot hybridization. *Nucleic Acids Res* 1991;19:5613–7.
30. Jordan IK, Rogozin IB, Glazko GV, Koonin EV. Origin of a substantial fraction of human regulatory sequences from transposable elements. *Trends Genet* 2003;19:68–72.
31. Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W, Funke R, Gage D, et al. Initial sequencing and analysis of the human genome. *Nature* 2001;409:860–921.
32. Jeanpierre M. Human satellites 2 and 3. *Ann Genet* 1994;37:163–71.
33. Schuffenhauer S, Bartsch O, Stumm M, Buchholz T, Petropoulou T, Kraft S, Belohradsky B, Hinkel GK, Meitinger T, Wegner RD. DNA, FISH and complementation studies in ICF syndrome: DNA hypomethylation of repetitive and single copy loci and evidence for a trans acting factor. *Hum Genet* 1995;96:562–71.
34. Japanese Gastric Cancer A. Japanese Classification of Gastric Carcinoma—2nd English Edition. *Gastric Cancer* 1998;1:10–24.
35. Lauren P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965;64:31–49.
36. Kaneda A, Kaminishi M, Sugimura T, Ushijima T. Decreased expression of the seven ARP2/3 complex genes in human gastric cancers. *Cancer Lett* 2004;212:203–10.
37. Issa JP, Ahuja N, Toyota M, Bronner MP, Brentnall TA. Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 2001;61:3573–7.
38. Lee HS, Kim BH, Cho NY, Yoo EJ, Choi M, Shin SH, Jang JJ, Suh KS, Kim YS, Kang GH. Prognostic implications of and relationship between CpG island hypermethylation and repetitive DNA hypomethylation in hepatocellular carcinoma. *Clin Cancer Res* 2009;15:812–20.
39. Kaneda A, Tsukamoto T, Takamura-Enya T, Watanabe N, Kaminishi M, Sugimura T, Tatematsu M, Ushijima T. Frequent hypomethylation in multiple promoter CpG islands is associated with global hypomethylation, but not with frequent promoter hypermethylation. *Cancer Sci* 2004;95:58–64.
40. Niwa T, Tsukamoto T, Toyoda T, Mori A, Tanaka H, Maekita T, Ichinose M, Tatematsu M, Ushijima T. Inflammatory processes triggered by *Helicobacter pylori* infection cause aberrant DNA methylation in gastric epithelial cells. *Cancer Res* 2010;70:1430–40.
41. Nakajima T, Yamashita S, Maekita T, Niwa T, Nakazawa K, Ushijima T. The presence of a methylation fingerprint of *Helicobacter pylori* infection in human gastric mucosae. *Int J Cancer* 2009;124:905–10.
42. Weisenberger DJ, Campan M, Long TI, Kim M, Woods C, Fiala E, Ehrlich M, Laird PW. Analysis of repetitive element DNA methylation by MethyLight. *Nucleic Acids Res* 2005;33:6823–36.
43. Yamamoto E, Toyota M, Suzuki H, Kondo Y, Sanomura T, Murayama Y, Ohe-Toyota M, Maruyama R, Nojima M, Ashida M, Fujii K, Sasaki Y, et al. LINE-1 hypomethylation is associated with increased CpG island methylation in *Helicobacter pylori*-related enlarged-fold gastritis. *Cancer Epidemiol Biomarkers Prev* 2008;17:2555–64.
44. Choi SH, Worswick S, Byun HM, Shear T, Soussa JC, Wolff EM, Douer D, Garcia-Manero G, Liang G, Yang AS. Changes in DNA methylation of tandem DNA repeats are different from interspersed repeats in cancer. *Int J Cancer* 2009;125:723–9.
45. Schernhammer ES, Giovannucci E, Kawasaki T, Rosner B, Fuchs C, Ogino S. Dietary Folate, Alcohol, and B Vitamins in Relation to LINE-1 Hypomethylation in Colon Cancer. *Gut* 2010;59:794–9.
46. Gama-Sosa MA, Slagel VA, Trewyn RW, Oxenhandler R, Kuo KC, Gehrke CW, Ehrlich M. The 5-methylcytosine content of DNA from human tumors. *Nucleic Acids Res* 1983;11:6883–94.

World Journal of Gastrointestinal Endoscopy

Online Submissions: http://www.wjgnet.com/1948-5190office
wjge@wjgnet.com
doi:10.4253/wjge.v3.i4.71

World J Gastrointest Endosc 2011 April 16; 3(4): 71-77
ISSN 1948-5190 (online)

Assessment of gastroesophageal reflux disease by serodiagnosis of *Helicobacter pylori*-related chronic gastritis stage

Shotaro Enomoto, Masashi Oka, Hiroshi Ohata, Chizu Mukoubayashi, Mika Watanabe, Kosaku Moribata, Yosuke Muraki, Naoki Shingaki, Hisanobu Deguchi, Kazuki Ueda, Izumi Inoue, Takao Maekita, Mikitaka Iguchi, Kimihiko Yanaoka, Hideyuki Tamai, Mitsuhiro Fujishiro, Osamu Mohara, Masao Ichinose

Shotaro Enomoto, Masashi Oka, Chizu Mukoubayashi, Mika Watanabe, Kosaku Moribata, Yosuke Muraki, Naoki Shingaki, Hisanobu Deguchi, Kazuki Ueda, Izumi Inoue, Takao Maekita, Mikitaka Iguchi, Kimihiko Yanaoka, Hideyuki Tamai, Masao Ichinose, Second Department of Internal Medicine, Wakayama Medical University, Wakayama 641-0012, Japan
Hiroshi Ohata, Osamu Mohara, Wakayama Wellness Foundation, Wakayama 640-8404, Japan
Mitsuhiro Fujishiro, Department of Endoscopy and Endoscopic Surgery, Faculty of Medicine, University of Tokyo, Tokyo 113-8655, Japan
Author contributions: Enomoto S carried out the data analyses and drafted the manuscript. Ohata H, Mukoubayashi C, Watanabe M, Deguchi H and Yanaoka K carried out endoscopic examinations of the study subjects. Moribata K, Muraki Y, Shingaki N, Ueda K, Inoue I, Maekita T, Iguchi M, Tamai H, Fujishiro M and Mohara O contributed to critical review of the article. Oka M and Ichinose M contributed to the conception and design of the study and also made a substantial contribution to data acquisition. All authors read and approved the final manuscript.
Correspondence to: Shotaro Enomoto, MD, PhD, Second Department of Internal Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama-city, Wakayama 641-0012, Japan. shoe@orion.ocn.ne.jp
Telephone: +81-73-447-1335 Fax: +81-73-445-3616
Received: December 14, 2010 Revised: February 23, 2011
Accepted: March 2, 2011
Published online: April 16, 2011

Abstract

AIM: To evaluate the association of *Helicobacter pylori* (*H.pylori*)-related chronic gastritis stage with upper gastrointestinal symptoms and gastroesophageal reflux disease (GERD).

METHODS: Subjects underwent upper gastrointestinal endoscopy, a questionnaire using a frequency scale for symptoms of GERD (FSSG), and measurements of serum *H.pylori*-antibody and pepsinogen (PG) levels. They were classified into the following 4 groups in terms of *H.pylori*-related chronic gastritis stage: Group A (n = 219), *H.pylori*(-)PG(-); Group B (n = 310), *H.pylori*(+)PG(-); Group C (n = 279), *H.pylori*(+)PG(+); and Group D (n = 17), *H.pylori*(-)PG(+).

RESULTS: Reflux esophagitis occurred in 30.6% of Group A, 14.5% of Group B, 6.8% of Group C, and 0% of Group D ($P < 0.001$). Scores for acid reflux symptoms decreased significantly with chronic gastritis stage (from Group A to D) ($P < 0.05$), while scores for dysmotility symptoms did not differ significantly. The prevalence of non-erosive reflux disease (NERD) did not differ among groups. However, in subjects with GERD, the prevalence of NERD tended to increase with chronic gastritis stage ($P = 0.081$).

CONCLUSION: Acid reflux symptoms and the prevalence of reflux esophagitis can be assessed by measuring both serum *H.pylori*-antibody and PG levels.

Key words: Gastroesophageal reflux disease; *Helicobacter pylori*; Pepsinogen; Screening and diagnosis

Peer reviewers: Young-Tae Bak, MD, PhD, Professor, Division of Gastroenterology, Department of Internal Medicine, Korea University, Guro Hospital, 97 Gurodong-gil, Guro-gu, Seoul 152-703, South Korea; Oscar Tatsuya Teramoto-Matsubara, MedCenter, Av. Paseo de las Palmas 745-102, Lomas de Chapultepec, Mexico D.F. 11000, Mexico; Peter Born, MD, Professor, D-80634 second Department of Medicine, Red Cross Hospital, Munich, Germany