Elevated risk of recurrent colorectal neoplasia with *Helicobacter pylori*-associated chronic atrophic gastritis: A follow-up study of patients with endoscopically resected colorectal neoplasia

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Abstract. In a previous population-based case-control study, we demonstrated an elevated risk of colorectal neoplasia with *Helicobacter pylori* (*H. pylori*) infection. The present study investigated the effects of *H. pylori*-associated chronic gastritis on the development of colorectal neoplasia by analyzing the recurrence of colorectal neoplasia subsequent to endoscopic resection. Ninety-nine patients who had undergone endoscopic resection of colorectal neoplasia were monitored under colonoscopy, and the recurrence of colorectal neoplasia was prospectively investigated. The stage of *H. pylori*-associated chronic gastritis in each subject was evaluated using a combination of two serum tests: *H. pylori* antibody and pepsinogen. In the present cohort, colorectal neoplasia recurred at a rate of 15,296/100,000 person-years during the study period. After adjusting for the confounding factors, chronic atrophic gastritis (CAG) was identified as an independent risk factor [adjusted hazard ratio (HR), 2.72; 95% confidence interval, 1.33-5.57], while *H. pylori*-infected non-atrophic gastritis was not identified as an independent risk factor for recurrent colorectal neoplasia. Colorectal neoplasia recurred earlier and was significantly more frequent in patients with CAG (22,573/100,000 person-years) compared to patients without CAG (11,089/100,000 person-years; P=0.029, log-rank test). Patients with more extensive CAG showed a higher risk of recurrence. These results demonstrated a significant elevation of the risk of recurrent colorectal neoplasia with the establishment and progression of CAG, indicating the involvement of *H. pylori* infection in the development of colorectal neoplasia. The two serum tests were useful clinical markers for non-invasively evaluating the risk of each individual for recurrent colorectal neoplasia subsequent to endoscopic resection.

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Introduction

An etiological association between gastric and colorectal neoplasia has been suggested (1). *Helicobacter pylori* (*H. pylori*) induces chronic inflammation in the stomach mucosa leading to long-lasting hypergastrinemia, a fact that constitutes a risk factor for stomach carcinogenesis (2). Numerous studies using various non-transformed or transformed cell lines for *in vitro* and *in vivo* animal experiments, including transgenic mouse models, have shown that non-amidated gastrins, such as progastrin and glycine-extended gastrin act as growth factors for colonic epithelia and neoplasia, and are potentially involved in colonic carcinogenesis (3-5). Chronic atrophic gastritis (CAG) and intestinal metaplasia, an end stage of chronic *H. pylori* infection, induce hypochlorhydria, which leads to bacterial overgrowth in the gastrointestinal tract and to alterations in the colonic microenvironment of the bacterial flora (6). On the basis of these findings, we hypothesized that gastric neoplasia and colorectal neoplasia share a common risk factor with regard to *H. pylori* infection and the resultant CAG, and demonstrate a significantly elevated risk of colorectal adenoma with the establishment of *H. pylori*-associated chronic gastritis in a population-based case-control study (7). In addition, the risk, especially for proximal adenomas, is further enhanced by the establishment and progression of CAG. Our previous study also showed the possibility that two serological markers,

H. pylori antibody and pepsinogen (PG), are useful for the evaluation of the risk of colorectal neoplasia, and also for the identification of individuals at high risk of colorectal neoplasia requiring colonoscopic surveillance.

Patients who have undergone endoscopic resection of colorectal neoplasia generally receive a follow-up colonoscopy due to the increased risk of metachronous neoplasia (8). However, risk factors for the recurrence of colorectal neoplasia have yet to be fully elucidated. Current consensus surveillance guidelines stratify the risk for subsequent neoplasia based on the characteristics of neoplasia at an initial colonoscopy, and various surveillance intervals are recommended depending on the risk: 5-10 years for low-risk (with 1-2 small adenomas); 3 years for intermediate-risk (with advanced neoplasia or 3-10 small adenomas) and <3 years for high-risk patients (with >10 small adenomas or large sessile adenoma) (8,9). However, these guidelines are imperfect, and 7.4% of patients classified into the low-risk group developed an advanced adenoma or invasive cancer during a follow-up evaluation (10), suggesting that additional risk factors for neoplasia recurrence should be considered together with the clinicopathological characteristics of neoplasia at the initial resection.

The present study aimed to investigate the etiological associations between *H. pylori*-associated chronic gastritis and the risk of recurrent colorectal neoplasia following the first endoscopic resection in a hospital-based cohort of patients undergoing follow-up colonoscopy.

Materials and methods

Study patients. A total of 155 patients [mean age ± standard deviation (SD), 65.1±8.6 years; range, 43-83 years] underwent endoscopic resection of a colorectal neoplasia ≥5 mm in diameter between April 2003 and March 2009, with follow-up colonoscopy at an interval of 1-2 years subsequent to resection at the Wakayama Medical University Hospital in Wakayama, which is located in the southwestern part of the main island of Japan. The patients were inhabitants of the Wakayama area presenting with high mortality rates from both colorectal and stomach cancers. The reported age-adjusted mortality rates for these two types of cancer during 2009 were 36.5/100,000 and 49.8/100,000 person-years, respectively. Data about symptoms and baseline characteristics (age, height, weight, socio-demographic characteristics, personal medical history, family history, smoking and alcohol consumption) was obtained using a questionnaire completed at the baseline examination. Subjects who had smoked cigarettes regularly for ≥6 months were classified as smokers. Alcohol consumption was defined as drinking alcohol at least once a week for the past 5 years. Four patients with a previous history of colorectal neoplasia, gastric neoplasia and inflammatory bowel disease were excluded from the study. In addition, another 40 patients, who had been prescribed medications possibly affecting gastrointestinal function, such as proton pump inhibitors, H2 blockers or non-steroidal anti-inflammatory drugs, prior to colonic examination, as well as subjects with a previous history of gastric resection, *H. pylori* eradication therapy or renal failure, were excluded due to possible inaccuracies in the PG test results. Twelve patients with colorectal cancer demonstrating histological evidence of penetration into the submucosa underwent subsequent operation and were excluded from the study. Thus, a total of 99 subjects were analyzed in this study. None of these subjects showed any indication of polyposis syndrome.

Serological evaluation of H. pylori-associated chronic gastritis. Aliquots of sera separated from blood samples collected for routine laboratory tests were stored below -20˚C for measurement of serum levels of *H. pylori* immunoglobulin (Ig)G antibody titer and PG. *H. pylori* IgG antibody titers were measured using an enzyme-linked immunosorbent assay (ELISA) kit (E Plate Eiken *H. pylori* antibody; Eiken Chemical Co., Ltd., Tokyo, Japan). Antibody titers ≥10 U/ml were classified as indicative of *H. pylori* infection. The sensitivity and specificity of the ELISA used were 90.7 and 91.5%, respectively (11). Serum PG levels were measured using PG I/PG II RIA-Bead kits (Dainabot Co., Ltd., Tokyo, Japan), using a modified radioimmunoassay method established previously (12). Subjects with extensive CAG were diagnosed on the basis of the previously described PG test-positive criteria (PG I ≤70 ng/ml; PG I/II ≤3.0) (13,14). These criteria provide 70.5% sensitivity and 97% specificity (13). In the present study, all but three CAG cases diagnosed according to the above PG test-positive criteria were considered *H. pylori* antibody-positive. Upper endoscopic examination of the three *H. pylori*-negative CAG cases (1 patient with no neoplasia on follow-up colonoscopy and 2 with neoplasia on follow-up colonoscopy) demonstrated extensive metaplastic gastritis involving both antrum and corpus. In these patients, the negative result for *H. pylori* antibody was considered to reflect spontaneous eradication of the bacteria, an end result of the progression of *H. pylori*-associated CAG. The prevalence of autoimmune gastritis is extremely low in Japan, with a reported incidence rate of 0.6/100,000 person-years (15). Therefore, we considered the possibility of autoimmune gastritis in the analyzed CAG cases, including the three *H. pylori*-negative cases, to be negligible.

H. pylori-associated chronic gastritis may be classified into three stages based on the results of the *H. pylori* antibody titer and PG serological tests (16). 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 to *H. pylori*-associated non-atrophic gastritis and group C to the presence of extensive CAG.

Serum gastrin levels were assessed using a Gastrin-RIA kit II (Dainabot), as described previously (17).

Colonoscopy and endoscopic resection of colorectal neoplasia. Subjects underwent full colonoscopy with adequate bowel preparation. The colonoscope (CF-Q260AI; Olympus, Tokyo, Japan) was inserted to the cecum, except in cases with advanced adenocarcinoma. The size of the detected neoplasia was classified as 5-9, 10-19, ≥20 mm or cancer. Subjects were classified into three groups according to the location of the detected polypoid lesions: proximal (cecum, ascending colon, hepatic flexure or transverse colon); distal (splenic flexure, descending colon, sigmoid colon or rectum) or

bilateral (lesions in both locations). The patients with polypoid lesions ≥5 mm detected during initial colonoscopy underwent endoscopic resection at second-look colonoscopy performed within several months after the initial procedure, subsequent to thorough examination of the colon and endoscopic removal of macroscopically visible polyps. This type of redundancy helps to minimize miss rates in colonoscopy.

Resected polyps were immediately fixed in 10% formalin and embedded in paraffin. Tissue sections were stained with hematoxylin and eosin (H&E) and examined under light microscopy. Routine histological evaluation was performed by staff pathologists. The removed neoplasias were classified based on architectural features (tubular, tubulovillous, villous or serrated) and epithelial dysplasia (mild, moderate, severe or intramucosal cancer). Carcinomas with invasion of cancer cells beyond the muscularis mucosa were excluded from further analysis. Analysis of clinical data was approved by the Ethics Committee of the Wakayama Medical University. Informed consent for the use of clinical data, including questionnaires, as well as laboratory and pathology results, was obtained from each patient.

Statistical analysis. Data were analyzed using the SPSS version 19.0 software (SPSS, Inc., Chicago, IL, USA) and the STATA software (Stata Corporation, College Station, TX, USA). Data for the continuous variables were expressed as the mean ± SD, and differences were tested for significance using t-tests for comparisons involving two groups and the analysis of variance for comparisons among multiple groups. Categorical variables were compared using the Chi-square test. Kaplan-Meier survival analysis was used to describe the time dependency of neoplasia recurrence with the initial endoscopic resection as time zero and the study censor as the final outcome. Log-rank testing was used to compare the cumulative probabilities of the lack of recurrence in patients. The Cox proportional hazards model was used to calculate the risk of neoplasia recurrence. The adjusted hazard ratios (HRs) and the corresponding 95% confidence intervals (CIs) were determined to examine the effect of parameters including *H. pylori* infection, CAG and neoplasia characteristics on subsequent neoplasia recurrence, while examining the effect of age, gender, body mass index (BMI), total cholesterol (TC), triglyceride (TG), blood sugar (BS) levels, smoking and drinking on baseline colonoscopy. The two-sided P<0.05 was considered statistically significant.

Results

Study patients. A total of 99 patients, who had undergone endoscopic resection of colorectal neoplasia ≥5 mm in diameter with a follow-up colonoscopy at an interval of 1-2 years subsequent to endoscopic resection, were considered eligible for analysis. In these patients, colorectal neoplasias recurred at a rate of 15,296/100,000 person-years. Subjects were classified into two groups according to the findings of the follow-up colonoscopy: patients with recurrent neoplasia on follow-up colonoscopy (n=37) and patients without neoplasia on follow-up colonoscopy (n=62). The recurrence rate of colorectal neoplasia in the former group was 43,124/100,000 person-years.

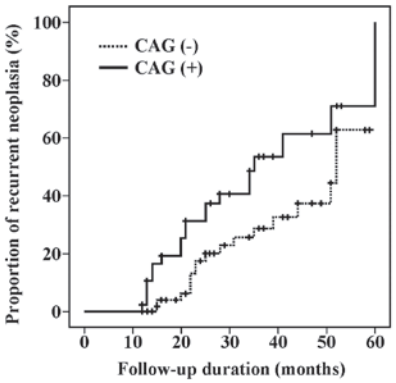

Figure 1. Effects of *H. pylori*-associated chronic atrophic gastritis (CAG) on recurrence of colorectal neoplasia subsequent to endoscopic resection are shown. Patients who had undergone endoscopic resection of colorectal neoplasia were monitored by colonoscopy, and correlations between the recurrence of colorectal neoplasia and coexisting CAG were analyzed using the Kaplan-Meier analysis. Patients with CAG demonstrated neoplasia recurrence more frequently and at a markedly earlier stage compared to patients without CAG (P=0.029, log-rank test). Recurrence rates for patients with and without CAG were 22,573/100,000 and 11,089/100,000 person-years, respectively.

Baseline characteristics of study subjects. Demographic and clinical features of patients in the two groups are shown in Table I. No significant differences were detected between the groups in terms of mean age, duration of follow-up, BMI, BS, TC, TG levels or proportion of alcohol consumption. Male gender and smoking status were more frequent among the group with neoplasia (P<0.05). Prevalence of *H. pylori* infection did not differ significantly in the groups. Although the PG I/II ratio tended to be lower in the group with compared to the group without neoplasia, there was no significant difference in serum PG levels in the two groups. The presence of CAG was significantly more frequent in the group with neoplasia (P=0.01). As for the stage of *H. pylori*-associated chronic gastritis, the presence of non-atrophic gastritis (group B) was not significantly more frequent, although the progression of chronic gastritis and the resultant CAG (group C) tended to show a higher prevalence in the group with neoplasia (P<0.1). The clinicopathological features of the neoplasia at the initial endoscopic resection for the two groups are also shown in Table I. Patients with proximal neoplasia were significantly more frequent within the neoplasia group compared to the group without neoplasia (P=0.048), whereas no significant differences in histopathology, grade of dysplasia or tumor size were detected in the two groups.

Kaplan-Meier curves for recurrence of colorectal neoplasia with or without CAG. CAG was frequently observed in patients with recurrent neoplasia. The timelines for recurrence in patients with or without CAG are shown in Fig. 1. Kaplan-Meier curves demonstrated that patients with CAG experienced significantly earlier and more frequent recurrence of neoplasia compared to patients without CAG (P=0.029, log-rank test). Recurrence rates of the two groups were 22,573/100,000 and 11,089/100,000 person-years, respectively. The clinicopathological features of the recurrent neoplasias showed no significant differences in histopathology,

Table I. Baseline characteristics of study subjects.

Characteristics	Overall (n=99)	No neoplasia on follow-up colonoscopy (n=62)	Neoplasia on follow-up colonoscopy (n=37)	P-value[a]
Person-years	242	156	86	
Follow-up (months)[b]	29.3 (14.0)	30.2 (14.5)	27.8 (13.1)	0.42
Age (years)[b]	65.1 (8.6)	64.4 (8.2)	66.2 (9.3)	0.33
Gender (male/female)	66/33	36/26	30/7	0.02
Smoker (-/+)	49/50	36/26	13/24	0.03
Alcohol use (-/+)	45/54	30/32	15/22	0.45
BMI (cm/m^2)[b]	23.2 (3.1)	23.5 (3.3)	22.6 (2.8)	0.15
BS (mg/dl)[b]	96.5 (21.4)	97.7 (24.7)	94.6 (14.4)	0.43
TG (mg/dl)[b]	122.5 (64.0)	119.5 (64.1)	127.6 (64.5)	0.55
TC (mg/dl)[b]	206.1 (33.0)	206.5 (33.0)	205.3 (33.5)	0.86
H. pylori IgG (U/ml)[b]	59.9 (71.1)	63.6 (68.8)	53.7 (75.2)	0.51
H. pylori infection (-/+)	26/73	17/45	9/28	0.73
PG I (ng/ml)[b]	62.9 (55.0)	67.8 (55.6)	54.5 (53.8)	0.25
PG II (ng/ml)[b]	22.3 (17.9)	23.4 (19.4)	20.5 (15.0)	0.42
PG I/II[b]	3.2 (1.9)	3.5 (1.9)	2.7 (1.8)	0.05
CAG (-/+)	61/38	44/18	17/20	0.01
Group A	23	16	7	
Group B[c]	38	28	10	0.73
Group C[c]	38	18	20	0.09
Gastrin (pg/ml)[b]	218.1 (209.7)	204.4 (181.1)	240.1 (251.9)	0.56
Cases/incidence rated[d]	37/15296	0	37/43124	
Tumors treated with initial endoscopic resection				
Size (mm)				
5-10/11-20/>20 (%)	49.5/34.3/16.2	48.4/33.9/17.7	51.4/35.1/13.5	
Number				
1/2/≥3 (%)	47.5/23.2/29.3	51.6/25.8/22.6	40.5/18.9/40.5	
Location				
Distal/bilateral/proximal (%)	39.4/29.3/31.3	46.8/27.4/25.8	27.0/32.4/40.5[e]	
Histopathology (%)				
Adenoma/cancer	75.8/24.2	74.2/25.8	78.4/21.6	
Ser/Ta/Villous component (+) /Ca	1/67.7/7.1/24.2	2/64.5/8.1/25.8	0/73.0/5.4/21.6	
Mild, moderate/severe/Ca	46.5/29.3/24.2	45.2/29.0/25.8	48.6/29.7/21.6	

[a]Two-sided P-values for the difference between cases and controls, based on the Chi-square test and t-test; [b]mean (SD); [c]Chi-square test, compared to group A; [d]per 100,000 person:years; [e]Chi-square test, P<0.05 compared to no neoplasia. BMI, body mass index; BS, blood sugar; TG, triglycerides; TC, total cholesterol; PG, pepsinogen; CAG, chronic atrophic gastritis; SD, standard deviation; Ser, serrated adenoma; Ta, tubular adenoma; Ca, cancer.

grade of dysplasia, number or size of colorectal neoplasias subsequent to endoscopic resection between the groups with and without CAG.

Risk factors for the recurrence of colorectal neoplasia. In order to identify the independent risk factors for the recurrence of colorectal neoplasia subsequent to endoscopic resection, a Cox proportional hazards model was used by adjusting the confounding factors, including age, gender, smoking and drinking habits, BMI, BS, TG and TC levels. Table II shows HRs for the recurrence of colorectal neoplasia subsequent to endoscopic resection. The presence of CAG represented an independent risk factor for the recurrence of colorectal neoplasia (adjusted HR, 2.72; 95% CI, 1.33-5.57), although *H. pylori* infection alone had no significant effect and was not an independent risk factor. Regarding the stages of *H. pylori*-associated chronic gastritis, *H. pylori*-associated chronic gastritis without extensive atrophy (group B) was not a risk, while progression into CAG (group C) was a significant risk factor for the recurrence of colorectal neoplasia (adjusted HR, 2.97; 95% CI, 1.10-8.00). Concerning the clinicopathological features of neoplasia at the baseline, proximal or bilateral loca-

Table II. Hazard ratios for recurrent colorectal neoplasia after endoscopic resection.

Characteristics	Crude HR (95% CI)	Adjusted HR[a] (95% CI)
H. pylori		
(-)	1	1
(+)	1.11 (0.52-2.36)	1.39 (0.61-3.14)
CAG		
(-)	1	1
(+)	2.02 (1.05-3.91)[b]	2.72 (1.33-5.57)[c]
Group A	1	1
Group B	0.89 (0.34-2.33)	1.14 (0.41-3.20)
Group C	1.88 (0.79-4.49)	2.97 (1.10-8.00)[b]
Size (mm)		
5-10	1	1
11-20	1.16 (0.56-2.40)	1.25 (0.56-2.80)
>20	0.90 (0.33-2.45)	1.05 (0.36-3.08)
Number		
1	1	1
2	0.88 (0.36-2.19)	0.86 (0.33-2.24)
≥3	2.39 (1.15-4.96)[b]	1.98 (0.83-4.75)
Location		
Distal	1	1
Bilateral	2.21 (0.92-5.27)	3.18 (1.19-8.48)[b]
Proximal	2.00 (0.87-4.57)	3.05 (1.20-7.76)[b]
Histopathology		
Adenoma	1	1
Cancer	0.79 (0.36-1.74)	0.66 (0.28-1.57)

[a]Adjusted for age, gender, smoking habit, alcohol use, BMI, BS, TG and TC by Cox proportional hazard models; [b]P<0.05; [c]P<0.001. HR, hazard ratio; CI, confidence interval; *H. pylori*, *Helicobacter pylori*; CAG, chronic atrophic gastritis; BMI, body mass index; BS, blood sugar; TG, triglycerides; TC, total cholesterol.

tion of the initially resected neoplasia showed a significantly higher risk factor for the recurrence of colorectal neoplasia compared to the distal location (proximal: adjusted HR, 3.05; 95% CI, 1.20-7.76; bilateral: adjusted HR, 3.18; 95% CI, 1.19-8.48).

When the stricter criteria of a positive PG I (≤50 ng/ml) and PG I/II ratio (≤3.0) were employed to detect subjects with more advanced and severe CAG (14) in the same analysis, 76.3% (29/38) of patients were diagnosed as CAG-positive based on the less strict criteria (PG I ≤70 ng/ml and PG I/II ≤3.0) and were considered to have a more advanced stage of CAG. These subjects with more extensive and severe CAG showed a higher risk of recurrent colorectal neoplasia (P=0.01, log-rank test) (adjusted HR, 3.16; 95% CI, 1.18-8.48) compared to the less advanced CAG. The recurrence rates of the two groups, with and without more extensive and severe CAG, were 25,352/100,000 and 11,124/100,000 person-years, respectively.

Discussion

Surveillance guidelines subsequent to colorectal adenoma removal recommend various colonoscopic surveillance intervals, based on the risk of subsequent metachronous neoplasia, as predicted by findings of studies on initial colonoscopy, while patients with larger or multiple polyps on index colonoscopy undergo more frequent surveillances (8,9). However, current knowledge concerning the correlation between host or tumor factors as demonstrated by the findings on baseline colonoscopy has indicated the risk of metachronous neoplasms is based on a rather small body of scientific evidence. One study demonstrated that neither age nor gender predicted the recurrence of neoplasia (18). In other studies, older age and/or higher BMI were associated with subsequent risk of advanced adenoma (10,19). As a result, many clinicians do not adhere to surveillance guidelines, often performing colonoscopies more frequently than recommended due to concerns about missed lesions or interval cancers (20). Consequently, the identification of clinical risk factors for the recurrence of colorectal neoplasia is undoubtedly needed. The present study investigated the correlations between *H. pylori*-associated chronic gastritis and the risk of recurrent colorectal neoplasia. When the infection is established in the stomach mucosa, *H. pylori*-associated chronic gastritis generally induces a series of events involved in stomach carcinogenesis, through the gastritis-atrophy-metaplasia-dysplasia-cancer sequence (21). Therefore, study subjects were stratified based on the stage of *H. pylori*-associated chronic gastritis as determined by the serum tests *H. pylori* antibody titer and PG, and then evaluated based on the risk of colorectal neoplasia recurrence at each stage. Results clearly confirmed *H. pylori*-associated CAG to be a risk factor for recurrent colorectal neoplasia.

A limited number of previous hospital-based case-control (22,23) and comparative studies (24-28) have reported an increased risk of colorectal neoplasia in the presence of *H. pylori* infection. As for CAG, two reports have described non-significant results for the correlation between colorectal cancer and CAG (29,30). Our previous population-based case-control study comprising middle-aged male factory workers, which included adjustment for potential confounding factors, clearly demonstrated an increase in the risk of colorectal adenoma in the presence of *H. pylori*-associated chronic gastritis (7). To the best of our knowledge, the present is the first study to assess *H. pylori*-associated chronic gastritis as a risk factor for the recurrence of colorectal neoplasia, indicating that *H. pylori* infection alone does not contribute to risk elevation, and that the establishment of CAG as evaluated by serum PG levels significantly increases the risk of recurrent colorectal neoplasia. In addition, the progression of CAG leads to further risk elevation. Generally, the subjects subsequent to colorectal neoplasia removal investigated in the present study are considered to be at higher risk of future neoplasia compared to the neoplasia-free subjects investigated in the previous study (31). These results indicate that the recurrence of neoplasia requires an additional host factor, as a more potent driving force for the carcinogenic sequence than the establishment of *H. pylori* infection. Thus, the establishment of CAG as a result of the progression of *H. pylori*-associated chronic gastritis is likely to be a risk factor. Although histopathological analysis of

the recurrent neoplasias in the present study showed no statistically significant difference between groups of patients with and without CAG, possibly due to the relatively small number of study patients and a limited follow-up period, neoplasias ≥10 mm in diameter and rapidly growing flat neoplasias were detected more frequently in patients with CAG compared to those without CAG. Considering these findings, the significant correlation between CAG and the recurrent colorectal neoplasia observed in this study reinforces the results of our previous report confirming the correlation between *H. pylori* infection and the risk of colorectal adenoma, while strongly suggesting that gastric atrophy promotes the recurrence and growth of colorectal neoplasias.

H. pylori infection increases gastrin secretion, possibly contributing to neoplastic progression in the colon (2). Gastrin-induced hyperproliferation and hyperplasia are considered to represent the early stages in the sequence of events leading to adenocarcinoma. A limited number of previously conducted epidemiological studies investigating the correlation between colorectal neoplasia and gastrin, demonstrated inconsistent results. More specifically, certain studies indicated positive correlations (32), while others including a recent large-scale nested case-control study, determined no such correlation (25,33). The differences in results reported in these studies might be attributable to the fact that most commercially available assays for gastrin detect the mature, fully-amidated form of the hormone, while gastrin precursors, such as progastrin or glycine-extended gastrin, are considered to act as more important promoters of colorectal carcinogenesis (2-5). The present study demonstrated no significant difference in the analyzed serum levels of mature amidated gastrin in groups with or without neoplasia recurrence.

H. pylori infection might also affect the normal gastrointestinal flora as a result of the reduced gastric acid secretion induced by *H. pylori*-associated chronic gastritis, contributing to colorectal carcinogenesis (34). Previous studies have indicated that the presence of enteric infection and overgrowth of intestinal bacteria are directly related to a reduction in gastric acid secretion (35-37). Our previous study demonstrated that CAG-positive asymptomatic middle-aged subjects, as determined by the serum PG levels of PG I ≤70 ng/ml and a PG I/II ratio of ≤3.0, were found to have larger colonic microflora compared to CAG-negative subjects, with an increase in 83% of the genera or groups comprising intestinal microflora (6). Gastric acid reduction is also reported to lead to increases in unabsorbed nutrients in the lower intestine, due to impaired gastric protein digestion (38), therefore leading to the alteration of the microflora with an increase in bacterial species utilizing these malabsorbed nutrients. Certain metabolites derived from the bacterial fermentation of malabsorbed proteins are also likely to be involved in the etiopathogenesis of colonic disorders, including epithelial neoplasia (39). In addition, *H. pylori* urease might transform urea in the gastric juice into ammonia and carbon dioxide (40). Ammonia is a known cytotoxic agent and mediator of mucosal cell damage, as well as a carcinogen. The higher luminal pH levels induced by *H. pylori* infection may also increase the absorption of ammonia, thus contributing to enhanced genetic damage, leading to colorectal carcinogenesis (41).

Consistent with several previously conducted studies (10,19,42), in our study, patients who had proximal neoplasia were more likely to develop recurrent neoplasia. Although the underlying mechanisms are uncertain, this finding suggests differences in the tumorigenic process based on the site of the large bowel. Accumulated evidence suggests that the molecular mechanisms involved in the development of colorectal neoplasia varies in the proximal and distal colon; the molecular features of the CpG island methylator phenotype (CIMP+) and microsatellite instability (MSI+) are predominant in the proximal colon, while chromosomal instability (CIN) occurs in the distal colon (43).

However, our previous study demonstrated that extensive CAG was more strongly correlated with the prevalence of proximal compared to the prevalence of distal neoplasia. The alterations in DNA methylation has recently been observed during inflammation and chronic inflammation-associated carcinogenesis in various organs (44). Thus, CAG-induced colonic bacterial overgrowth may trigger aberrant DNA methylation and activate the carcinogenic process, to which the proximal colon is more susceptible. Furthermore, colonic bacterial overgrowth is considered to lead to the enhanced production of secondary bile acids, reportedly increasing the risk of proximal colon cancer (45). In addition to causing DNA damage, these bile acids are presumed to activate the carcinogenic pathway involving DNA methylation (46,47). As a result, these findings indicate the possibility that the recurrence of neoplasias among patients with proximal neoplasia and patients with *H. pylori*-associated CAG are likely to have a common background predisposition to the development of neoplasias, such as altered DNA methylation-induced cancerization in normal tissues.

Certain limitations should be considered for this study. Firstly, neoplasias detected on follow-up colonoscopy subsequent to endoscopic resection at times might represent lesions missed during baseline colonoscopy. Rex *et al* (48) demonstrated tandem colonoscopy miss rates of 24% for polyps of any size, ranging from 27 to 6% for adenomas <5 and ≥10 mm, respectively. Hixson *et al* (49) found a miss rate of 15% for polyps <10 mm. Since neoplasia miss rates were the highest for small adenomas (48), the size of the initial resected neoplastic lesion was restricted to ≥5 mm. In addition, the removal of neoplasia was performed during second-look colonoscopy to minimize the effect of missed lesions on our findings. Thus, the proportion of missed lesions during baseline colonoscopy is considered likely to be small.

Secondly, since our study relied on observational data from a relatively small sample at a single hospital, the potential for patient selection bias remains. In the present study, the recurrence rate of colorectal neoplasia after endoscopic resection was 15,296/100,000 person-years. Of the individuals with one or more adenomas removed at colonoscopy, 20-50% are likely to be found to have a metachronous lesion on follow-up colonoscopy within 3-5 years (10). In addition, the rate of cancer development in the present study (1.24 cancers/1000 person-years) was similar to the results reported in several other clinical trials with colonoscopic follow-up of patients following the endoscopic resection of colorectal adenomas (0.6-2.4 cancers/1000 person-years) (50). Although we do not claim a complete absence of selection bias, the recurrence rate

for colorectal neoplasia in the present study lies within a range similar to the recently reported values, as described above. Thirdly, with respect to the misclassification of exposures, diagnoses of *H. pylori* infection and atrophic gastritis were based on serological test results. The fact that results similar to our previous report were obtained despite the use of different cohorts indicates the reliability of the serological tests.

The establishment of CAG was associated with an increased risk of recurrent colorectal neoplasia after endoscopic resection, and the progression of this CAG increased the risk. Approximately 67% of patients with CAG developed colorectal neoplasia within the 3-year follow-up period. By contrast, almost the same percentage of patients without CAG showed no recurrence. The stage of *H. pylori*-associated chronic gastritis, as determined by the two serological markers *H. pylori* antibody and PG, are useful for the evaluation of the risk of colorectal interval neoplasia, while allowing identification of individuals at high risk subsequent to endoscopic resection, especially in countries with a high prevalence of *H. pylori* infection. Whether or not the results of the present study apply to other populations with low prevalences of *H. pylori* infection, and particularly to the inhabitants of Western countries, has yet to be elucidated. To determine the involvement of *H. pylori* infection in the carcinogenesis of the colorectum and whether the eradication therapy for *H. pylori*-infected subjects reduces the risk of colorectal neoplasia further investigations are required.

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