In summary, the present study has shown that Gli regulates *MUC5AC* gene expression via direct protein-DNA interaction through the highly conserved 15 bp sequence between −125 and −111 bp in the promoter region of *MUC5AC*. Furthermore, immunohistochemical analysis and RT-PCR using systemic normal tissue revealed that Gli is necessary but not sufficient for *MUC5AC* expression. We conclude that *MUC5AC* expression is regulated by combination of multiple regulatory mechanisms such as universal transcription factors and epigenetic modulations.

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Author Contributions

Conceived and designed the experiments: NY NK-Y KK. Performed the experiments: NK-Y NY Y. Takahashi CN KS KI MK-S SK MF Y. Tsutsumi MI. Analyzed the data: NK-Y NY Y. Takahashi CN KS KI. Contributed to the writing of the manuscript: NK-Y NY MF KK.

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TOPIC HIGHLIGHT

Helicobacter pylori-related chronic gastritis as a risk factor for colonic neoplasms

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Abstract

To summarize the current views and insights on associations between *Helicobacter pylori* (*H. pylori*)-related chronic gastritis and colorectal neoplasm, we reviewed recent studies to clarify whether *H. pylori* infection/*H. pylori*-related chronic gastritis is associated with an elevated risk of colorectal neoplasm. Recent studies based on large databases with careful control for confounding variables have clearly demonstrated an increased risk of colorectal neoplasm associated with *H. pylori* infection. The correlation between *H. pylori*-related chronic atrophic gastritis (CAG) and colorectal neoplasm has only been examined in a limited number of studies. A recent large study using a national histopathological database, and our study based on the stage of *H. pylori*-related chronic gastritis as determined by serum levels of *H. pylori* antibody titer and pepsinogen, indicated that *H. pylori*-related CAG confers an increased risk of colorectal neoplasm, and more extensive atrophic gastritis will probably be associated with even higher risk of neoplasm. In addition, our study suggested that the activity of *H. pylori*-related chronic gastritis is correlated with colorectal neoplasm risk. *H. pylori*-related chronic gastritis could be involved in an increased risk of colorectal neoplasm that appears to be enhanced by the progression of gastric atrophy and the presence of active inflammation.

Key words: Colorectal neoplasm; Cancer risk; Pepsinogen; *Helicobacter pylori* antibody; Atrophic gastritis

Core tip: This review revealed that *Helicobacter pylori* (*H. pylori*)-related chronic gastritis plays a role in risk enhancement of colorectal neoplasm, and that this risk could be further enhanced by the progression of atrophy and the presence of active inflammation. These findings may be useful for selecting groups at high risk for colorectal neoplasm that warrant colonoscopic surveillance, particularly in areas where *H. pylori* infection is highly prevalent.

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INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) induces chronic inflammation in the stomach of both humans and ani-

mals, and *H. pylori*-related gastritis is closely associated with the development of gastric cancer[1]. Promotion of tumor development by *H. pylori* infection in extragastric target organs has recently been reported[2]. The majority of previous studies concerning correlations between colorectal neoplasm and *H. pylori* infection/*H. pylori*-related chronic gastritis have been hospital-based, showing several weaknesses in terms of limited sample size and incomplete control of confounding variables, including former colonoscopy[3-14]. In addition, results have been inconsistent, with some studies indicating a positive correlation and others finding no correlation[3-14]. Two meta-analyses combining the results of 11 and 13 case-control studies with summary OR of 1.4 (95%CI: 1.1-1.8) and 1.5 (95%CI: 1.2-1.9), respectively[15,16], have suggested modest increases in colorectal neoplasm risk due to *H. pylori* infection. However, the evidence remains limited because of significant heterogeneity among included studies and potential publication bias. A large-scale study is thus needed to confirm the increased risk of colorectal neoplasm by *H. pylori* infection owing to the relatively small OR. Several studies based on larger databases with adequate control for confounding factors were published from 2010 onward[17-20] and have demonstrated that *H. pylori* infection correlates with a moderately increased risk of colorectal neoplasm.

On the other hand, the mechanism by which *H. pylori* infection increases the risk of colorectal neoplasm is currently unclear. Progressive chronic gastritis induced by persistent *H. pylori* infection (*H. pylori*-related chronic gastritis) leads to extensive glandular atrophy and reduced acid secretion, in turn inducing hypergastrinemia, a putative trophic factor for large bowel mucosa[21]. Gastric acid reduction also alters the gastrointestinal microenvironment composed of bacterial flora[22], and thus may contribute to colorectal carcinogenesis. However, whether *H. pylori*-related chronic gastritis is associated with an increased risk of colorectal neoplasm remains inconclusive because of the limited number of epidemiological studies. This review summarizes recent findings and insights into the association between *H. pylori* infection/*H. pylori*-related chronic gastritis and colorectal neoplasm.

CORRELATION BETWEEN *H. PYLORI* INFECTION AND RISK OF COLORECTAL NEOPLASM

Since 2010, various studies have examined the correlation between *H. pylori* infection and colorectal neoplasm based on large databases with careful controls for confounding variables (Table 1). Two cross-sectional studies using the health check-up databases of Korea and Taiwan[19,20] showed that *H. pylori* infection was significantly associated with an increased risk of colorectal adenoma, with adjusted OR of 1.36 (95%CI: 1.10-1.68) and 1.37 (95%CI: 1.23-1.52), respectively. Our population-based case-control study, which investigated 478 asymptomatic male Japanese factory workers, identified *H. pylori* infection as a risk factor for colorectal adenoma (OR = 2.52; 95%CI: 1.57-4.05)[17]. In addition, a large population-based case-control study in Germany suggested a positive association between *H. pylori* infection and risk of colorectal cancer using *H. pylori* immunoglobulin (Ig)G (OR = 1.30; 95%CI: 1.14-1.50) and cytotoxin-associated gene A protein (CagA) (OR = 1.35; 95%CI: 1.15-1.59)[18]. These results clearly demonstrated an increased risk of colorectal neoplasm among patients with *H. pylori* infection.

CORRELATION BETWEEN *H. PYLORI*-RELATED CHRONIC ATROPHIC GASTRITIS DIAGNOSED ON THE BASIS OF PEPSINOGEN TEST RESULTS AND RISK OF COLORECTAL NEOPLASM

The pepsinogen method is a reliable screening method for precancerous lesions of the stomach. In addition, serum pepsinogen (PG) I and the PG I / II ratio are also valuable markers for gastric acid secretion and the extent of resultant chronic atrophic gastritis (CAG) caused by chronic gastritis. The combination of these two serum markers is the one most widely used for the detection of CAG in Japan.

Table 2 shows the correlation between CAG determined on the basis of PG test results and colorectal neoplasm. A previous hospital-based case-control study of 113 cases and 226 controls[14] and our population-based case-control study[17] in Japan showed that CAG based on the criteria of PG I $\leqslant$ 70 ng/mL and PG I / II $\leqslant$ 3.0 were not associated with a significantly increased risk of colorectal neoplasm. Since the prevalence of autoimmune gastritis is extremely low in Japan[23], the possible inclusion of autoimmune gastritis among the analyzed cases of CAG and subsequent underestimation of the risk was considered negligible. Meanwhile, subjects identified as CAG-negative based on the above-mentioned PG test criteria included not only those subjects with a *H. pylori*-free healthy stomach, but also *H. pylori*-infected subjects without CAG, which may have resulted in underestimation of colorectal neoplasm risk in CAG. A case-control study consisting of subjects with similar clinical indications for colonoscopy in Italy indicated that hypergastrinemic CAG (diagnosed by histological evaluation, fasting hypergastrinemia and low PG I levels) was not associated with an increased risk of colorectal neoplasm compared to normogastrinemic controls with healthy gastric mucosa[24]. However, in this study, most cases of hypergastrinemic CAG did not include active *H. pylori* infection and were positive for anti-parietal cell antibodies. Hypergastrinemic CAG in this study therefore may not have been equivalent to CAG resulting from *H. pylori* infection. Interestingly, a recent study indicated that other gastric pathologies likely unrelated to *H. pylori* infection, such as *H. pylori*-negative gastritis, showed

Table 1 Studies investigating correlations between *Helicobacter pylori* infection and risk of colorectal neoplasm

Ref.	Country	Year of publication	Type of study design	No. of subjects	Measure of *H. pylori* status	Outcome	Crude OR (95%CI)	Adjusted OR (95%CI)
[20]	Taiwan	2010	Cross-sectional	9311	Urease test	Adenoma	-	1.37 (1.23-1.52)
[19]	South Korea	2012	Cross-sectional	2195	IgG	Adenoma	1.35 (1.10-1.66)	1.36 (1.10-1.68)
						Advanced adenoma	2.19 (1.40-3.42)	2.21 (1.41-3.48)
[17]	Japan	2011	Population-based Case-control	478	IgG	Adenoma	2.26 (1.44-3.55)	2.52 (1.57-4.05)
[18]	Germany	2012	Population-based Case-control	3381	IgG	Cancer	-	1.3 (1.14-1.50)
					CagA	Cancer	-	1.35 (1.15-1.59)

H. pylori: *Helicobacter pylori*; IgG: Immunoglobulin G; CagA: Cytotoxin-associated gene A.

Table 2 Studies investigating correlations between *Helicobacter pylori*-related chronic atrophic gastritis diagnosed on the basis of pepsinogen tests and risk of colorectal neoplasm

Ref.	Country	Year of publication	Type of study design	No. of subjects	Measure of CAG status	Outcome	Crude OR or HR (95%CI)	Adjusted OR or HR (95%CI)
[14]	Japan	2007	Case-control	339	PG test	Cancer	-	OR = 1.56 (0.86-2.85)
[17]	Japan	2011	Population-based Case-control	478	PG test	Adenoma	OR 1.31 (0.89-1.93)	OR = 1.45 (0.97-2.17)
[24]	Italy	2012	Case-control	320	PG test + histopathology	Adenoma	-	OR = 0.59 (0.23-1.48)
						Cancer	-	OR = 1.03 (0.34-3.16)
[26]	Finland	2010	Cohort	20269	PG test + histopathology	Cancer	HR 1.00 (0.65-1.55)	HR = 0.98 (0.61-1.58)
[27]	Japan	2013	Cohort	99	PG test	Adenoma + Cancer	HR 2.02 (1.05-3.91)	HR = 2.72 (1.33-5.57)

CAG: Chronic atrophic gastritis; PG: Pepsinogen; OR: Odds ratio; HR: Hazard ratio.

no or only weak associations with the risk of colorectal neoplasm[25]. Considering these results, CAG determined by PG test results might not correlate with the risk of colorectal neoplasm. However, the heterogeneity of CAG criteria and differences in the selection of controls and other limitations of these studies, such as relatively small sample size, inadequate consideration of potential confounding variables including prescribed medication or previous history (use of proton pump inhibitors (PPIs), gastric resection, *H. pylori* eradication therapy, renal failure, *etc.*) that might affect PG test results might have influenced and distorted the results. The necessity for large studies examining the effects of *H. pylori*-related CAG on colorectal neoplasm compared to healthy gastric mucosa with adequate control of confounders should be emphasized to obtain valid results.

As for the correlation between the incidence of colorectal neoplasm and CAG diagnosed by the PG test, two relevant studies are as follows. A long-term cohort study among Finnish participants (the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study) did not indicate an increased risk of colorectal cancer by CAG based on histological findings and low PG I levels, although the investigators did not include any information on PPI therapy that might have affected serum PG levels[26]. Our study showed that CAG (diagnosed based on criteria of PG I $\leqslant$ 70 ng/mL and PG I / II $\leqslant$ 3.0) was associated with an increased risk of recurrent colorectal neoplasm after first endoscopic resection in a hospital-based cohort study, with an adjusted HR of 2.72 (95%CI: 1.33-5.57)[27]. The difference in the results of these two studies may be attributable to differences in the carcinogenic potential of the colorectal mucosa between study subjects. Subjects subsequent to colorectal neoplasm removal are considered to be at higher risk of future neoplasm, and risk enhancement with the establishment of CAG leads to the development of recurrent neoplasm; on the other hand, subjects without colorectal neoplasm on initial colonoscopy are at lower risk, and some additional factors other than CAG are required for the development of a neoplastic lesion[28,29]. The inconsistent findings between these studies might also be due to differences in study methodologies, such as differences in CAG criteria, selection of subjects, sample size, and follow-up period.

PROGRESSION OF *H. PYLORI*-RELATED CHRONIC GASTRITIS AND RISK OF COLORECTAL NEOPLASM

Once established in the stomach mucosa, *H. pylori*-related chronic gastritis is generally believed to trigger a series of events involved in stomach carcinogenesis, represented as the gastritis-atrophy-metaplasia-dysplasia-cancer sequence[30]. In addition, gastric atrophy and intestinal metaplasia, an end stage of *H. pylori*-related chronic gastritis, subsequently induce hypochlorhydria that may contribute to colorectal carcinogenesis.

Table 3 shows the correlation between the progression of *H. pylori*-related chronic gastritis and colorectal neoplasm. A recent large study using a national histopathological database in the United States indicated that

Table 3 Studies investigating correlations between progression of *Helicobacter pylori* -related chronic gatritis and risk of colorectal neoplasm

Ref.	Country	Year of publication	Type of study design	No. of subjects	Measure of *H. pylori*-related gastritis	Outcome	Crude OR (95%CI)	Adjusted OR (95%CI)
[17]	Japan	2011	Population-based Case-control	478	*H. pylori* IgG and PG test [mild CAG (Group B)]	Adenoma	2.61 (1.54-4.11)	2.81 (1.64-4.81)
[17]	Japan	2011	Population-based Case-control	478	*H. pylori* IgG and PG test [extensive CAG (Group C)]	Adenoma	2.3 (1.38-3.83)	2.7 (1.58-4.62)
					H. pylori IgG and stricter CAG criteria [more extensive CAG (Group C)]	Adenoma	3.75 (1.70-8.23)	4.2 (1.88-9.40)
[25]	United States	2012	Cross-sectional	100296	Histopathology (*H. pylori*-related gastritis)	Adenoma	1.52 (1.46-1.57)	-
				57820		Advanced adenoma	1.8 (1.69-1.92)	-
				51067		Cancer	2.35 (1.98-2.80)	-
[25]	United States	2012	Cross-sectional	90953	Histopathology (intestinal metaplasia)	Adenoma	1.82 (1.71-1.94)	-
				52802		Advanced adenoma	2.02 (1.82-2.24)	-
				46882		Cancer	2.55 (1.93-3.37)	-

H. pylori: *Helicobacter pylori*; IgG: Immunoglobulin G; PG: Pepsinogen; CAG: Chronic atrophic gastritis; OR: Odds ratio.

H. pylori-related chronic gastritis conferred increased risks of colorectal adenoma (OR = 1.52; 95%CI: 1.46-1.57) and cancer (OR = 2.35; 95%CI: 1.98-2.80) compared to normogastrinemic controls. In addition, a similar risk was found in intestinal metaplasia, a more easily recognizable form of mucosal alteration and the advanced stage of gastric atrophy most frequently associated with *H. pylori* infection[25]. However, those investigators had access only to histopathological information, so the possibility of uncontrolled confounders remains.

We stratified study subjects based on the stage of *H. pylori*-related chronic gastritis as determined by 2 serum tests (*H. pylori* antibody titer and PG)[31], then evaluated colorectal adenoma risk in each stage. The classification reflects each stage of a serial change in stomach mucosa induced by chronic *H. pylori* infection. There were 3 groups: 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 a *H. pylori*-free healthy stomach, Group B to *H. pylori*-related non-atrophic gastritis, and Group C to the presence of extensive CAG. The presence of *H. pylori*-related chronic gastritis significantly increased the risk of colorectal adenoma as a whole (Group B: adjusted OR = 2.81; 95%CI: 1.64-4.81; Group C: adjusted OR = 2.70; 95%CI: 1.58-4.62) compared to the *H. pylori*-free healthy stomach (Group A). However, no significant difference in risk existed between Groups B and C; that is, the establishment of CAG did not show any additional increase in the risk of adenoma[17]. On the other hand, stricter criteria for positive PG I ($\leqslant$ 30 ng/mL) and PG I / II ratio ($\leqslant$ 2.0) were used to detect subjects with more extensive and severe CAG[32]. These advanced-stage CAG subjects were at even higher risk for adenoma (adjusted OR = 4.20; 95%CI: 1.88-9.40) compared to CAG-positive subjects diagnosed using the less strict criteria (PG I $\leqslant$ 70 ng/mL and PG I / II $\leqslant$ 3.0) (Tables 3 and 4).

MECHANISMS BY WHICH *H. PYLORI*-RELATED CHRONIC GASTRITIS INCREASES THE RISK OF COLORECTAL NEOPLASM

Various mechanisms have been suggested to underlie the correlation between *H. pylori* infection and colorectal neoplasm. First, *H. pylori* infection increases gastrin secretion, which could contribute to colorectal carcinogenesis by inducing mucosal cell proliferation in the colon[21]. An epidemiological study of patients with *H. pylori* infection showed that mild hypergastrinemia was associated with about a 4-fold increase in the risk of colorectal neoplasm[11]. As for the correlation between colorectal neoplasm and gastrin, a limited number of epidemiological studies have been conducted with inconsistent results; some have indicated positive correlations[11,33], while others found no correlation[4,8]. The differences in these results might be attributable to non-amidated gastrins, 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[21,34].

Second, *H. pylori* infection seems likely to adversely impact the intestinal flora, contributing to colorectal carcinogenesis[35-37], as a result of the hypochlorhydria caused by *H. pylori*-related chronic gastritis. Several studies have indicated that the presence of enteric infection and overgrowth of intestinal bacteria are directly correlated with hypochlorhydria[38-40]. Our previous study demonstrated that CAG-positive asymptomatic middle-aged subjects (diagnosed on the basis of serum PG levels of PG I $\leqslant$ 70 ng/mL and PG I / II ratio $\leqslant$ 3.0) had a larger population of colonic microflora than CAG-negative subjects[22]. Hypochlorhydria was also reported to lead to an increase in unabsorbed nutrients in the lower

Table 4 Correlation between stage of *Helicobacter pylori*-related chronic gatritis and risk of colorectal neoplasm

	H. pylori CAG	Controls (*n* = 239)	Total adenoma cases (*n* =239)	Proximal adenoma (*n* = 38)	Bilateral adenoma (*n* = 78)	Distal adenoma (*n* = 123)
Group A	(-) (-)	71	35	4	15	16
Group B	(+) (-)	105	127	18	41	68
	B1 (PG1/2 > 3)	92	103	14	34	55
	B2 (PG1/2 ≤ 3)	13	24	4	7	13
Group C	(+)	63	77	16	22	39
	C1 (PG1/2 > 2, PGI > 30)	50	53	11	15	27
	C2 (PG1/2 ≤ 2, PGI ≤ 30)	13	24	5	7	12
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:B1) (95%CI)	1	2.36 (1.43-3.88)	2.73 (0.86-8.65)	1.74 (0.87-3.47)	2.86 (1.50-5.47)
	(A:B2) (95%CI)	1	3.78 (1.71-8.38)	5.32 (1.17-24.1)	2.66 (0.89-7.98)	4.36 (1.68-11.3)
Adjusted OR[1]	(A:C) (95%CI)	1	2.7 (1.58-4.62)	4.51 (1.43-14.2)	1.76 (0.83-3.74)	3.05 (1.54-6.07)
	(A:C1) (95%CI)	1	2.27 (1.29-3.99)	3.88 (1.17-12.9)	1.52 (0.67-3.45)	2.59 (1.25-5.35)
	(A:C2) (95%CI)	1	4.2 (1.88-9.40)	6.95 (1.63-29.6)	2.65 (0.87-8.05)	5.09 (1.89-13.7)

[1]Adjusted for current smoking and total cholesterol by conditional logistic regression analysis. B1: Group αβ, subgroup based on less-strict criteria for PG I (≤ 70 ng/mL) and PG I / II ratio (> 3.0) or PG I (> 70 ng/mL) and PG I / II ratio (> 3.0) to detect mild inflammation; B2: Group γ, subgroup based on stricter criteria for PG I (> 70 ng/mL) and PG I / II ratio (≤ 3.0) to detect severe active inflammation; C1: Subgroup based on less strict criteria for PG I (≤ 70 ng/mL) and PG I / II ratio (≤ 3.0) to detect extensive CAG; C2: Subgroup based on stricter criteria for positive PG I (≤ 30 ng/mL) and PG I / II ratio (≤ 2.0) to detect more extensive and severe CAG; *H. pylori*: *Helicobacter pylori*; CAG: Chronic atrophic gastritis.

intestine due to impaired gastric protein digestion[41], so some metabolites derived from bacterial fermentation of malabsorbed proteins are likely to play a role in the etiopathogenesis of colonic disorders[42,43].

Third, *H. pylori* infection might result in damage to the colorectal epithelium through inflammatory responses, such as those mediated by interleukin (IL)-8, which is associated colorectal cancer[44]. Shmuely *et al*[5] reported a 10-fold increase in colorectal cancer risk with CagA-positive strains (known to cause enhanced inflammatory response) compared to CagA-negative strains. A recent cross-sectional study showed that *H. pylori* infection-concomitant metabolic syndrome might further increase the risk of colorectal neoplasm[20] and proposed that such concomitant effects might occur secondary to common inflammatory pathways through inflammation-related factors such as tumor necrosis factor-alpha (TNF-α)[20]. Therefore, we further classified former study subjects[17] in Group B into three subgroups based on the activity of *H. pylori*-related chronic gastritis determined by serum PG levels, as described previously[32]: Group α, PG I ≤ 70 ng/mL and PG I / II > 3.0; Group β, PG I > 70 ng/mL and PG I / II > 3.0; and Group γ, PG I > 70 ng/mL and PG I / II ≤ 3.0. The activity of *H. pylori*-related chronic gastritis is considered to be higher in the order γ, β, α, and we evaluated colorectal adenoma risk at each stage. The severe active inflammation group (γ) showed an increased risk of colorectal adenoma (adjusted OR = 3.78; 95%CI: 1.71-8.38) compared to the mild inflammation groups (α and β) (adjusted OR = 2.36; 95%CI: 1.43-3.88) (Table 4), suggesting that the activity of *H. pylori*-related chronic gastritis correlates with colorectal neoplasm risk. In general, the concentrations of IL-1β and TNF-α (*i.e.*, proinflammatory cytokines that mediate host inflammatory response) have been shown to be elevated in stomach mucosa showing active inflammation[45,46]. Since both cytokines potently inhibit gastric acid secretion[47], they appear to represent an additional link between *H. pylori*-related active inflammation and colorectal neoplasm.

Correlation between location and risk of colorectal neoplasm

Accumulating evidence suggests that the risk of colorectal neoplasm associated with various environmental and genetic factors differs for proximal and distal neoplasm, probably reflecting two recently proposed tumorigenic pathways based on the molecular features of CpG island methylator phenotype (CIMP+) and microsatellite instability (MSI+) occurring predominantly in the proximal colon, and chromosomal instability (CIN) occurring in the distal colon[48]. Animal models suggest that the mitogenic action of gastrin is selective for the distal colon[49,50]. On the other hand, chronic inflammation is known to induce aberrant DNA methylation in normal tissues, and alterations in DNA methylation have been proposed to be involved in the carcinogenic process of the proximal colon[51]. In addition, colonic bacterial overgrowth is considered to lead to enhanced production of secondary bile acids, which are reported to cause DNA damage and activation of the carcinogenic pathway involving DNA methylation, particularly in the proximal colonic mucosa[52,53], thereby increasing the risk of proximal colon cancer[54].

Previous studies have classified colorectal neoplasms according to location, and examined the correlation between colorectal neoplasm and *H. pylori* infection in each group. However, the results of those studies were inconclusive, because of insufficient sample sizes to detect site-selective effects on associations; some studies indicated that the increased risk associated with *H. pylori* infection was limited to patients with proximal neoplasms[19], while other studies found the same for distal neoplasms[18]. Furthermore, the significantly increased

risk of colorectal neoplasm with *H. pylori*-related chronic gastritis has been reported to be similar for different locations of colorectal neoplasm[17,25]. Our reanalysis of previous data using serum PG levels as indices of the activity of *H. pylori*-related chronic gastritis or the resulting gastric atrophy revealed that colonic neoplasm risk in both proximal and distal regions increases with the enhancement of active inflammation or the progression of gastric atrophy (Table 4).

CONCLUSION

This review has shown that relatively few studies are available in this field, and the current evidence remains limited. Larger studies with adequate controls for confounders and that compare against normogastrinemic controls with *H. pylori*-free healthy gastric mucosa are necessary to clarify the role of *H. pylori*-related chronic gastritis in carcinogenesis of the colorectum.

In conclusion, based on critical analyses of previous studies, including our own, *H. pylori*-related chronic gastritis may well be associated with an increased risk of colorectal neoplasm. This risk appears to be further enhanced by the progression of atrophy or active inflammation. In areas where *H. pylori* infection is highly prevalent, the stage of *H. pylori*-related chronic gastritis could contribute to the identification of individuals at high risk of colorectal neoplasm. In addition, whether eradication therapy for *H. pylori*-infected subjects reduces the risk of colorectal neoplasm is a problem for future study.

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Categorization of Upper Gastrointestinal Symptoms Is Useful in Predicting Background Factors and Studying Effects and Usages of Digestive Drugs

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Abstract

Background: There have been very few reports assessing the relationship between various upper gastrointestinal (GI) symptoms or evaluating each individual upper GI symptom separately.

Methods: Based on the answers to Frequency Scale for the Symptoms of GERD from a large-scale population of healthy adults in Japan, a hierarchical cluster analysis was performed to categorize the typical 12 upper GI symptoms. The associations between the 12 symptoms and 13 background factors were systematically analyzed among the 18,097 digestive drug-free subjects, 364 proton-pump inhibitor (PPI) users, and 528 histamine H_2-receptor antagonist (H_2RA) users.

Results: The derived relationship between the 12 upper GI symptoms suggests the five symptom categories: heartburn (2), dyspepsia (4), acid regurgitation (3), pharyngo-upper esophageal discomfort (2), and fullness while eating (1). Among the digestive drug-free subjects, inadequate sleep, weight gain in adulthood, NSAID use, meals immediately prior to sleep, and frequent skipping of breakfast showed significant positive association with most upper GI symptoms. Compared to the digestive drug-free subjects, significantly associated factors for PPI and H_2RA users are respectively different in "4 of 5" and "5 of 5" symptoms in heartburn and acid regurgitation categories, "1 of 2" and "1 of 2" symptoms in pharyngo-upper esophageal discomfort category, and "0 of 5" and "3 of 5" symptoms in dyspepsia and fullness while eating categories. These differences between digestive drug-free subjects and gastric acid suppressant users seem to correlate with our experiences in clinical situations: heartburn and acid regurgitation category symptoms are effectively controlled with PPI and H_2RA whereas other category symptoms are not.

Conclusions: The 12 upper GI symptoms can be classified into five categories, which are statistically associated with various background factors. The differences of associated factors between digestive drug-free subjects and digestive drug users may be useful in studying the drug effects upon diverse upper GI symptoms.

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Introduction

The term "upper gastrointestinal (GI) symptoms" is commonly used to describe multiple complaints including heartburn, regurgitation, postprandial fullness, early satiety, epigastric pain, belching, nocturnal pain, fasting pain, nausea and vomiting, abdominal distention, and so on [1]. There have been many previous reports concerning upper GI symptoms which focused on the three symptom categories separately: gastroesophageal reflux symptoms [2,3,4,5,6,7,8], dyspeptic symptoms [7,9,10,11,12,13], and peptic ulcer related symptoms [7,14,15,16]. However, there have been very few reports assessing the relationship between various upper GI symptoms. In the present study, we therefore tried to statistically categorize the typical upper GI symptoms using a hierarchical cluster analysis.

Of the many upper GI symptoms, gastroesophageal reflux disease (GERD) symptoms are thought to be the most common [17,18]; the prevalence of reflux esophagitis (RE) and non-erosive reflux disease (NERD) are respectively 15.5% and 27.1% in Sweden [8], 6.8% and 15.9% in Japan [5]. Although GERD patients present a diverse range of symptoms including extra-esophageal symptoms [3,6,19], it is broadly accepted that the most typical symptoms of GERD are heartburn and regurgitation [4]. Dyspeptic symptoms are also thought to be very common [17,18];

the prevalence of functional dyspepsia was reported to be 14.7% in Norway [20,21] and 11.5% in England [22]. Though an accurate evaluation concerning the prevalence of dyspepsia is difficult, it is thought to be in the range of 10–40% [9]. We previously evaluated the associations of FSSG (Frequency Scale for the Symptoms of GERD) scores [19] with multiple lifestyle related factors using the data from a large-scale cohort of 19,864 healthy adults [3]. Although the FSSG questionnaire was originally developed for evaluating symptoms of GERD patients [19], the 12 questions of FSSG target not only "acid reflux-related symptoms" but also "dyspeptic (dysmotility) symptoms" [23]. Thus, it has been used for not only evaluation of GERD symptoms [3,24,25] but also for evaluating functional dyspepsia (FD) [26]. Consequently, we used the 12 symptoms included in the FSSG as the typical upper GI symptoms in the present study.

In our recent report [3], we found that the total FSSG score is significantly associated with many lifestyle related factors such as inadequate sleep, increased body weight in adulthood, meals immediately prior to sleep, midnight snacks, body mass index (BMI), frequent skipping of breakfast, lack of habitual physical exercise, quick eating, etc. However, we had not performed the thorough analyses evaluating association between individual upper GI symptoms and putative background factors separately (systemic analyses). Many questionnaires assessing diverse upper GI symptoms have been proposed [3,19,27,28,29], but detailed systemic evaluation of individual upper GI symptoms had not been executed. In this study, we therefore analyzed the individual 12 upper GI symptoms separately, together with putative background factors identified in our previous reports [3,5,30].

Based on the results from our recent analyses [3,5,31], we have decided to analyze the following 13 background factors: age, gender, BMI, serum *Helicobacter pylori (HP)* IgG, ratio of serum pepsinogen I/II reflecting atrophy of gastric mucosa, use of NSAIDs, inadequate sleep, weight gain in adulthood, intake of meals immediately prior to sleep, frequent skipping of breakfast, lack of habitual exercise, habitual alcohol drinking, and habitual smoking. In our present study, we tried to evaluate not only the persons free from digestive-drug use, but also the proton pump inhibitor (PPI) users and histamine H_2-receptor antagonist (H_2RA) users. These two drugs are the most popular gastric acid suppressants used for upper GI disorder including GERD [3,32,33], peptic ulcer disease [14,34,35], and dyspepsia [10,36,37]. Therefore, we hypothesized that a comparison of the background factors of PPI and H_2RA users with those of digestive drug-free subjects might be useful in predicting the efficacy of controlling intragastric pH upon various upper GI symptoms.

Materials and Methods

Study Subjects

All the subjects who received medical checkup at Kameda Medical Center Makuhari (Chiba-shi, Chiba, Japan) during the year 2010 were asked to participate in our study. All subjects were physically self-reliant healthy outpatients, who voluntarily applied for a complete physical examination at our institute. A total of 20,773 subjects (50.2±9.5 years of age) assented and were enrolled in our study. In cases where health checkup was performed twice in 2010, the results from the former checkup were used. Cases less than 20 years of age, with a medical history of gastrectomy, and with insufficient data for analysis were excluded from this study. This study was approved by the ethics committee of the University of Tokyo, and written informed consent was obtained from all the study participants according to the Declaration of Helsinki.

Frequency Scale for the 12 Upper GI Symptoms and Questionnaire about Lifestyles

To assay various upper GI symptoms, we analyzed the 12 symptom scores included in the Frequency Scale for the Symptoms of GERD (FSSG), which is a validated and widely used questionnaire covering various symptoms related to the upper gastrointestinal tract [3,19,23]. The frequency of each of these 12 upper GI symptoms (Figure 1A) was measured on the following scale: never = 0; occasionally = 1; sometimes = 2; often = 3; and always = 4. As the response variables for the statistical analyses, we used the 12 symptom scores derived from the study subjects.

For the explanatory variables, we adopted 13 factors based on our past research [3,5,30,31] (Figure 1B). We selected age (F1), gender (F2), and BMI (F3) as the three basic factors, and serum *HP* IgG (F4) and ratio of serum pepsinogen I/II (F5) as gastric mucosa-related factors. In addition, we selected drinking (F12), smoking (F13), and the six following yes-no questionnaire filled in by all participants (Figure 1B); (F6) Do you take any non-steroidal anti-inflammatory drugs (NSAIDs)?; (F7) Do you feel you do not have adequate sleep?; (F8) Has your body weight markedly increased in adulthood (more than 10 kg from the age of 20 years)?; (F9) Do you habitually have a midnight snack (more than three times a week)?; (F10) Do you frequently skip breakfast (more than three times a week)?; and (F11) Is your time of exercise less than 30 minutes a day?

Statistical Methods

A hierarchical cluster analysis (Ward's method with Euclidean distances) was performed in order to group the 12 upper GI symptoms based on the questionnaire answers from the digestive drug-free subjects. The results of cluster analyses were computed into a cluster dendrogram, which became the basis of our systemic categorization of multiple upper GI symptoms.

Correlation analyses were exhaustively performed, using the 12 upper GI symptoms as response variables and the above-mentioned 13 background factors as explanatory variables (systemic analyses). Digestive drug-free subjects, PPI users, and H_2RA users were analyzed separately. For univariate systemic analyses, Student's t-test or Pearson's correlation coefficient were applied. For multivariate systemic analyses, the multiple linear regression model was applied to relevant background factors for each of the 12 response variables. The effect sizes (f^2) and power of all the variables were also calculated. In both univariate and multivariate systemic analyses, two-sided p values of less than 0.005 (for digestive drug-free subjects) or 0.05 (for PPI users and H_2RA users) were considered statistically significant.

To assess the association between various background factors of digestive drug-free subjects and gastric acid suppressant (PPI or H_2RA) users, analysis of covariance (ANCOVA) was additionally performed, in which p values of less than 0.01 were considered statistically significant. All statistical analyses were performed using SAS version 8.2 (SAS Institute Inc., Cary, NC, USA) or JMP version 8.0 (SAS Institute Inc.) software.

Results

Characteristics of the Study Subjects and 12 Upper Gastrointestinal Symptoms

Of the 20,773 subjects who were originally enrolled in this study, we excluded 1,053 subjects due to an age of less than 20 years old (2), a history of gastrectomy (211), or insufficient data for analysis (840). As shown in Figure 2, the eligible 19,720 subjects comprised of 5 subjects using both PPI and H_2RA, 364 PPI users who do not use H_2RA (236 men and 128 women with a mean age

A) 12 questions for frequencies of upper gastrointestinal symptoms originated from FSSG (Frequency Scale for the Symptoms of GERD).

a) Do you get heartburn?
b) Does your stomach get bloated?
c) Does your stomach feel heavy after meals?
d) Do you subconsciously rub your chest with your hand?
e) Do you ever feel sick after meals?
f) Do you get heartburn after meals?
g) Do you have an unusual (e.g. burning) sensation in your throat?
h) Do you feel full while eating meals?
i) Do some things get stuck when you swallow?
j) Do you get bitter liquid (acid) coming up into your throat?
k) Do you burp a lot?
l) Do you get heartburn if you bend over?

The answer of symptom frequency is from 0 to 4 on each question as follows: never=0; occasionally=1; sometimes=2; often=3; and always=4.

B) 13 background factors derived from the questionnaire.

(F1) Age
(F2) Gender
(F3) Body Mass Index (BMI)
(F4) Level of serum anti-*Helicobacter pylori* antibody
A) *HP* IgG $\geq$ 10 U/ml B) 10 U/ml > *HP* IgG $\geq$ 2 U/ml
(F5) Ratio of serum pepsinogen I / pepsinogen II (PG I/II)
A) 2 $\geq$ PG I/II B) 3 $\geq$ PG I/II > 2
(F6) Use of some non-steroidal anti-inflammatory drugs (NSAIDs)
(F7) Inadequate sleep
(F8) Weight increase in adulthood (more than 10kg from age 20 years)
(F9) Habit of having dinner within two hours before going to bed
(F10) Habit of frequent skipping of breakfast (more than three times a week)
(F11) Lack of habitual physical exercise (less than 30 minutes a day)
(F12) Habit of alcohol drinking (almost every day)
(F13) Habit of smoking

Figure 1. The 12 questions for frequencies of various upper gastrointestinal symptoms (A) and 13 background factors derived from the questionnaire (B).
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of 55.6±9.9 years), 528 H_2RA users who do not use PPI (323 men and 205 women with a mean age of 52.8±9.7 years), 726 subjects using digestive drug other than PPI and H_2RA (393 men and 333 women with a mean age of 52.4±9.4 years), and 18,097 digestive drug-free subjects who do not use any digestive drugs (10,406 men and 7,691 women with a mean age of 49.8±9.3 years).

Scores of the 12 upper GI symptoms among the 18,097 digestive drug-free subjects, 364 PPI users, and 528 H_2RA users are shown in Table 1. For all the 12 upper GI symptoms, scores of PPI users and H_2RA users are significantly higher than those of digestive drug-free subjects.

Proposal of Five Categories for the 12 Upper GI Symptoms

To analyze the interrelation among the 12 upper GI symptoms, a hierarchical cluster analysis was performed based on the data from 18,097 digestive drug-free subjects. The result is visualized as a dendrogram (Figure 3), which denotes "distances" among the 12 symptoms. Although the 12 upper GI symptoms included in the

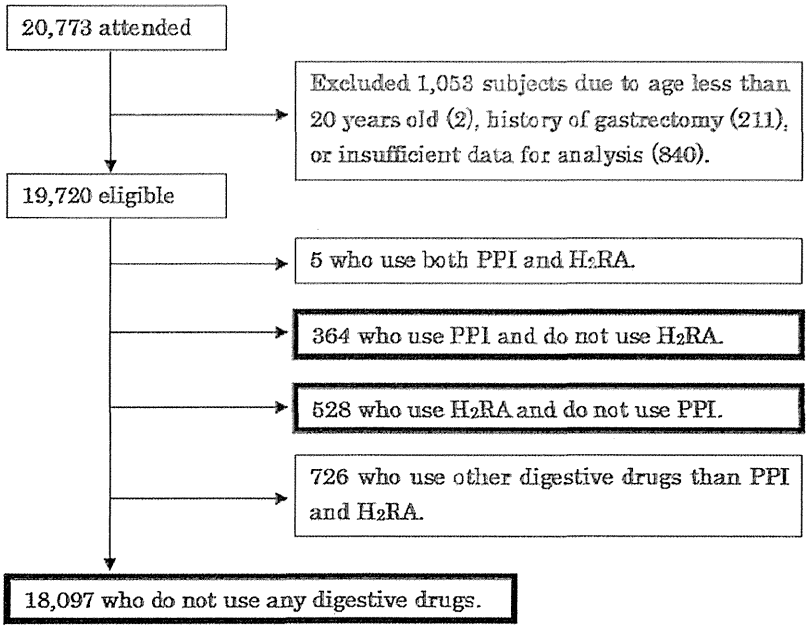

Figure 2. Study recruitment flowchart.
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FSSG [19] have been originally classified into acid reflux-related (a, d, f, g, i, j, and l) and dyspeptic (b, c, e, h, and k) symptoms [23], our results do not completely conform to this categorization. For example, "j) bitter liquid coming up to the throat" (belonging to acid reflux-related symptoms) and "k) burping a lot" (belonging to dyspeptic symptoms) are relatively close. For another example, "g) unusual sensation in the throat" and "i) some thing getting stuck in swallowing", both originally considered to be acid reflux-related symptoms, are not closely related to other acid reflux-related symptoms.

Based on the cluster dendrogram, we propose that the 12 upper GI symptoms can be classified into five categories (Figure 3): heartburn (f and a), dyspepsia (d, e, c, and b), acid regurgitation (l, j, and k), pharyngo-upper esophageal discomfort (g and i), and fullness while eating (h). Whereas heartburn and acid regurgitation are considered as two of the most typical GERD symptoms [6], our results suggest that these two should be treated separately (Figure 3). Dyspepsia, one of the most common symptoms of functional gastrointestinal disorders [9,18,20,38], includes three typical dyspeptic symptoms (b, c, and e) [23,39] and one

Table 1. Scores of the typical 12 upper GI symptoms among the 18,097 digestive drug-free subjects, 364 PPI users, and 528 H_2RA users.

12 upper gastrointestinal symptoms	18,097 digestive drug-free subjects	364 PPI users	528 H_2RA users
a) Getting heartburn	0.51±0.78	1.29±1.14	1.13±1.08
b) Stomach getting bloated	0.79±0.96	1.10±1.10	1.14±1.10
c) Stomach feeling heavy	0.57±0.81	1.08±1.13	1.17±1.07
d) Rubbing the chest with hands	0.18±0.51	0.44±0.82	0.43±0.81
e) Feeling sick after meals	0.20±0.50	0.38±0.70	0.43±0.75
f) Getting heartburn after meals	0.38±0.66	0.89±1.04	0.80±0.97
g) Unusual sensation in the throat	0.33±0.75	0.66±1.04	0.47±0.91
h) Feeling full while eating the meals	0.31±0.64	0.46±0.78	0.47±0.82
i) Some thing getting stuck in swallowing	0.19±0.52	0.38±0.76	0.26±0.57
j) Bitter liquid coming up to the throat	0.37±0.65	0.91±1.04	0.73±0.87
k) Burping a lot	0.52±0.87	0.94±1.11	0.88±1.09
l) Getting heartburn while bending over	0.13±0.44	0.48±0.94	0.32±0.70
Total of 12 symptom scores	4.46±4.95	9.00±7.35	8.23±6.57

Scores of the 12 upper GI symptoms included in the FSSG range from 0 to 4 respectively. Consequently, total symptom scores range from 0 to 48.
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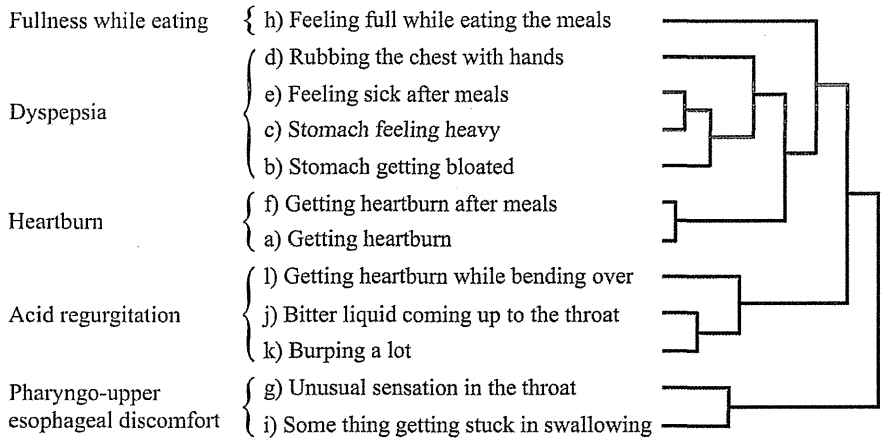

Figure 3. A dendrogram of the 12 upper GI symptoms based on the hierarchical cluster analysis (Ward's method) of the 18,097 digestive drug-free subjects. Our proposed five categorization of 12 upper GI symptoms is also denoted.
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unexpected symptom (d). Pharyngo-upper esophageal discomfort includes two close symptoms (i and g), which may be considered as extraesophageal GERD symptoms [19,40]. Contrary to prior belief, fullness while eating (h) is not closely related to any of the other dyspeptic symptoms (b, c, and e); it is in fact the most isolated symptom among the 12 upper GI symptoms (Figure 3).

Background Factors for the Individual 12 Upper GI Symptoms among the 18,097 Digestive Drug-free Subjects, 364 PPI Users, and 528 H_2RA Users

Distribution of the 12 upper GI symptom scores and 13 background factors are shown as histograms (Figure S1 and S2) based on the data of 18,097 digestive drug-free subjects. Of the 13 analyzed factors, use of NSAIDs (F6), lack of habitual exercise (F11), inadequate sleep (F7), frequent skipping of breakfast (F10), and meals immediately prior to sleep (F9) seem to be risk factors for all 12 upper GI symptoms. The other eight factors do not show unidirectional tendencies: both positive and negative associations are observed for the 12 upper GI symptoms (Figure S1 and S2).

Using the data from the 18,097 digestive drug-free subjects, univariate and multivariate analyses were further performed to evaluate associations between the 12 symptoms and 13 background factors exhaustively. The results of systematic univariate analyses (Table S1) show various associations between the 13 causative factors and the 12 upper GI symptoms. Among the 13 factors, inadequate sleep (F7) is apparently the strongest associated risk factor for most upper GI symptoms, which is consistent with our recent report [3]. The following systematic multivariate analyses (Table 2) also confirmed that inadequate sleep (F7) is the strongest risk factor for 11 of 12 upper GI symptoms. In addition, weight gain in adulthood (F8), use of NSAID (F6), meals immediately prior to sleep (F9), and frequent skipping of breakfast (F10) also showed significant positive associations with almost all 12 symptoms (Table 2). We designated these five as common risk factors for upper GI symptoms.

Next, we performed multivariate analyses on the users of gastric acid suppressants, to evaluate the thorough associations between the 12 upper GI symptoms and 13 background factors (Table 3 for PPI users, Table 4 for H_2RA users). The distributions of significant factors for PPI users and H_2RA users are apparently different from those of digestive drug-free subjects (Table 2). In particular, serum *HP* IgG (F4), serum PG I/II ratio (F5), use of NSAID (F6), and weight gain in adulthood (F8) seldom showed significant association with upper GI symptoms among either PPI and H_2RA users. Conversely, age (F1), sex (F2), and BMI (F3) among the digestive drug-free subjects and gastric acid suppressant users displayed similar associations with several upper GI symptoms (Table 2–4). It is intriguing that some significant factors for upper GI symptoms are markedly different between gastric acid suppressant users and digestive drug-free subjects, whereas others are similar.

To validate the differing results of the three groups (digestive drug-free subjects, PPI users, and H_2RA users) with different population sizes, we calculated the effect sizes and power for all variables. The statistical power proved adequate in all analyses (Table S2). Most effect sizes are >0.02 with the exception of the four symptoms (g, i, k, and l) of digestive drug-free subjects group, but these four still display sizes of more than >0.015. The effect sizes of the explanatory variables for digestive drug-free users tend to be smaller than those for PPI users and H_2RA users, but the difference is compensated by the smaller p value ($p<0.005$) for digestive drug-free users compared with gastric acid suppressant users ($p<0.05$).

Our Five Proposed Categories of 12 Upper GI Symptoms Seem to Reflect the Differences of Background Factors between Digestive Drug-free Subjects and Gastric acid Suppressant Users

To accurately evaluate the differences between the digestive drug-free subjects and gastric acid suppressant users, statistical analysis (ANCOVA) was also performed.

For PPI users (p values in Table 3), two symptoms of the heartburn category (2 of 2) and two symptoms of the acid regurgitation category (2 of 3) have markedly different background factors compared to digestive drug-free subjects. One symptom of the pharyngo-upper esophageal discomfort category (1 of 2) has also significantly but not greatly different background factors. On the contrary, no symptoms of the dyspepsia category (0 of 4) and fullness while eating category (0 of 1) have significantly different background factors; in other words, associated background factors of the five symptoms in these two categories are quite similar between PPI users and digestive drug-free subjects.

Table 2. Orders, directions, and standardized coefficients of association between the 12 upper GI symptoms and 13 background factors among the 18,097 digestive drug-free subjects.

Factors	F1	F2	F3	F4A	F4B	F5A	F5B	F6	F7	F8	F9	F10	F11	F12	F13
h) Feeling full while eating the meals	4N	2P	1N		10P			7P	3P	8P		5P		9P	6P
	0.053	0.088	0.101		0.022			0.041	0.081	0.039		0.051		0.026	0.042
d) Rubbing the chest with hands		5P	7N					4P	1P	2P	3P	6P	8P		
		0.038	0.030					0.040	0.087	0.047	0.044	0.031	0.025		
e) Feeling sick after meals	2N	3P	4N	9P				6P	1P	5P	7P	8P			
	0.094	0.084	0.051	0.035				0.047	0.106	0.050	0.043	0.038			
c) Stomach feeling heavy	4N	2P	3N	9P	10P			7P	1P	5P	6P	8P			
	0.067	0.082	0.070	0.040	0.028			0.054	0.123	0.063	0.055	0.042			
b) Stomach getting bloated	7N	2P	5N					4P	1P	3P	6P	8P	9P		
	0.043	0.114	0.061					0.065	0.130	0.069	0.054	0.032	0.022		
f) Getting heartburn after meals		8P		2P	7P	4N		5P	1P	3P	6P	9P		10P	
		0.035		0.083	0.037	0.053		0.045	0.103	0.060	0.045	0.032		0.030	
a) Getting heartburn				2P	5P	4N	11N	6P	1P	3P	7P	8P		9P	10P
				0.079	0.046	0.055	0.032	0.046	0.104	0.064	0.041	0.035		0.034	0.033
l) Getting heartburn while bending over		3P						5P	1P	2P	4P				
		0.045						0.024	0.077	0.060	0.036				
j) Bitter liquid coming up to the throat		3N		4P	11P	5N	9N	7P	1P	2P	6P	8P	10P		
		0.071		0.058	0.023	0.051	0.027	0.034	0.105	0.080	0.046	0.030	0.027		
k) Burping a lot	5N	2N	4N					6P	1P	3P	7P				
	0.036	0.077	0.038					0.031	0.081	0.044	0.028				
g) Unusual sensation in the throat	6P							3P	1P	2P	4P	8P	5P		7P
	0.027							0.044	0.095	0.045	0.033	0.024	0.031		0.026
i) Some thing getting stuck in swallowing	2P	3P						6P	1P	4P		5P	7P		
	0.044	0.043						0.025	0.090	0.034		0.030	0.024		
Total 12 upper GI symptoms	9N	7P	6N	5P	10P	12N	14N	3P	1P	2P	4P	8P	11P	13P	
	0.034	0.052	0.052	0.056	0.033	0.030	0.025	0.071	0.164	0.090	0.065	0.050	0.033	0.024	

Background factors are (F1) age, (F2) female gender, (F3) BMI, (F4A) *HP* IgG ≥10 U/ml, (F4B) 10 U/ml>*HP* IgG ≥2 U/ml, (F5A) 2≥PG I/II, (F5B) 3≥PG I/II >2, (F6) use of NSAIDs, (F7) inadequate sleep, (F8) weight gain in adulthood, (F9) meals immediately prior to sleep, (F10) frequent skipping of breakfast, (F11) lack of habitual exercise, (F12) alcohol drinking, and (F13) smoking. (F4A) and (F4B) were compared with "2> *HP* IgG", and (F5A) and (F5B) were compared with "PG I/II >3". Orders of association among the 13 background factors are shown as the upper integers for the individual 12 symptoms, in which attached "P" and "N" denote positive and negative association respectively. Standardized coefficients are shown as the lower decimal fractions. The levels of significance in these multivariate analyses were set at <0.005.

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For H_2RA users (p values in Table 4), all five symptoms of the heartburn category (2 of 2) and acid regurgitation category (3 of 3) have significantly different background factors compared to digestive drug-free subjects. As shown in Table 3 and 4, p values of these five symptoms are quite similar between H_2RA users and PPI users, with exception of "burping a lot (k)" of the acid regurgitation category. For the pharyngo-upper esophageal discomfort category, one symptom (1 of 2) has meaningfully but slightly different background factors, similar to PPI users. For the dyspepsia category, unlike PPI users, three symptoms (3 of 4) have significantly different background factors compared to digestive drug-free subjects.

Table 3. Orders, directions, and standardized coefficients of associations between the 12 upper GI symptoms and 13 background factors among the 364 PPI users.

Factors	*p* value	F1	F2	F3	F4A	F4B	F5A	F5B	F6	F7	F8	F9	F10	F11	F12	F13
h) Feeling full while eating the meals	0.3738		1P	2N						3P						4P
			0.205	0.200						0.115						0.114
d) Rubbing the chest with hands	0.0856	5N	2P	4N						3P	1P	6P				
		0.124	0.154	0.148						0.149	0.223	0.117				
e) Feeling sick after meals	0.1042	1N	3P	2N						4P						
		0.222	0.185	0.196						0.144						
c) Stomach feeling heavy	0.0100	2N	1P	3N						4P			5P			
		0.147	0.218	0.140						0.131			0.112			
b) Stomach getting bloated	0.0133	2N								1P						
		0.145								0.195						
f) Getting heartburn after meals	<0.0001*		1P										2P			
			0.153										0.134			
a) Getting heartburn	<0.0001*	1N	2P		4N								3P			
		0.171	0.134		0.117								0.124			
l) Getting heartburn while bending over	<0.0001*												1P			
													0.174			
j) Bitter liquid coming up to the throat	<0.0001*	2N	3P										1P			
		0.131	0.120										0.172			
k) Burping a lot	0.1720	1N										2P				
		0.128										0.121				
g) Unusual sensation in the throat	0.1482									1P						
										0.174						
i) Some thing getting stuck in swallowing	0.0027*									2P		1P			3N	
										0.148		0.187			0.146	
Total 12 upper GI symptoms	<0.0001*	2N	1P							3P		5P	4P			
		0.177	0.185							0.172		0.138	0.142			

Background factors are (F1) age, (F2) female gender, (F3) BMI, (F4A) *HP* IgG ≥10 U/ml, (F4B) 10 U/ml>*HP* IgG ≥2 U/ml, (F5A) 2≥PG I/II, (F5B) 3≥PG I/II >2, (F6) use of NSAIDs, (F7) inadequate sleep, (F8) weight gain in adulthood, (F9) meals immediately prior to sleep, (F10) frequent skipping of breakfast, (F11) lack of habitual exercise, (F12) alcohol drinking, and (F13) smoking. (F4A) and (F4B) were compared with "2> *HP* IgG", and (F5A) and (F5B) were compared with "PG I/II >3". Orders of association among the 13 background factors are shown as the upper integers for the individual 12 symptoms, in which attached "P" and "N" denote positive and negative association respectively. Standardized coefficients are shown as the lower decimal fractions. The levels of significance in these multivariate analyses were set at <0.05. The differences of associated background factors between PPI users and digestive drug-free subjects were calculated; *p* scores below 0.05 were set for the level of significance.

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Compared to the associated background factors of digestive drug-free subjects, those of PPI users and H_2RA users are mostly similar, particularly for the symptoms of heartburn, acid regurgitation, and fullness while eating categories (Table 3 and 4). Conversely, associated background factors among gastric acid suppressant users are somewhat different for the symptoms of dyspepsia category (*p* values in Table 3 and 4). In this category,

Table 4. Orders, directions, and standardized coefficients of associations between the 12 upper GI symptoms and 13 background factors among the 528 H_2RA users.

Factors	*p* value	F1	F2	F3	F4A	F4B	F5A	F5B	F6	F7	F8	F9	F10	F11	F12	F13
h) Feeling full while eating the meals	0.1676		2P	1N			4N			5P		3P				
			0.132	0.170			0.100			0.092		0.104				
d) Rubbing the chest with hands	0.0056*									1P		2P				
										0.123		0.104				
e) Feeling sick after meals	0.2000	3N	1P							2P						
		0.133	0.220							0.158						
c) Stomach feeling heavy	0.0078*		1P	2N						3P						
			0.174	0.174						0.170						
b) Stomach getting bloated	0.0043*	4N	2P		3N					1P						
		0.100	0.138		0.113					0.244						
f) Getting heartburn after meals	<0.0001*									1P						
										0.155						
a) Getting heartburn	<0.0001*									1P						
										0.190						
l) Getting heartburn while bending over	0.0024*					2N				1P						
						0.113				0.124						
j) Bitter liquid coming up to the throat	0.0023*									1P			2P			
										0.175			0.100			
k) Burping a lot	0.0002*	2N								1P						
		0.112								0.161						
g) Unusual sensation in the throat	0.6368									1P						
										0.100						
i) Some thing getting stuck in swallowing	0.0039*															
Total 12 upper GI symptoms	<0.0001*	3N	2P							1P						
		0.103	0.111							0.247						

Background factors are (F1) age, (F2) female gender, (F3) BMI, (F4A) *HP* IgG ≥10 U/ml, (F4B) 10 U/ml>*HP* IgG ≥2 U/ml, (F5A) 2≥PG I/II, (F5B) 3≥PG I/II >2, (F6) use of NSAIDs, (F7) inadequate sleep, (F8) weight gain in adulthood, (F9) dinner just before bedtime, (F10) frequent skipping of breakfast, (F11) lack of habitual exercise, (F12) alcohol drinking, and (F13) smoking. (F4A) and (F4B) were compared with "2> *HP* IgG", and (F5A) and (F5B) were compared with "PG I/II >3". Orders of association among the 13 background factors are shown as the upper integers for the individual 12 symptoms, in which attached "P" and "N" denote positive and negative association respectively. Standardized coefficients are shown as the lower decimal fractions. The levels of significance in these multivariate analyses were set at <0.05. The difference of associated background factors between H_2RA users and digestive drug-free subjects were calculated; *p* scores below 0.05 were set for the level of significance.

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PPI users display similar associated factors to digestive drug-free subjects, whereas those of H_2RA users are considerably different.

Discussion

Overview of Our Proposed Five Upper GI Symptom Categories and Significantly Associated Background Factors

For the heartburn category (f and a in Figure 3), strong associations with the two gastric mucosa-related factors (serum *HP* antibody (F4) and serum PG I/II ratio (F5) reflecting atrophic gastritis) observed in digestive drug-free subjects were for the most

part not significant in gastric acid suppressant users (Table 2–4). Associations of the above-mentioned five common factors (F6–F10) in digestive drug-free subjects were also mostly not significant in gastric acid suppressant users, except for the strongest lifestyle-related factor "inadequate sleep" in H_2RA users (F7 in Table 4). Associations of the three basic factors (age, gender, and BMI) for digestive drug-free subjects are similar to H_2RA users and differ from PPI users (F1–F3 in Table 2–4). As a general rule for this category, associated background factors of digestive drug-free subjects and gastric acid suppressant users are completely different, which is clearly shown in the p values in Table 3 and 4.

For the dyspepsia category (b, c, e, and d in Figure 3), associations with "inadequate sleep (F7)" and the three basic factors (F1–F3) are strong in both digestive drug-free subjects and gastric acid suppressant users (Table 2–4). Marginal associations of the gastric mucosa-related factors (F3 and F4) in digestive drug-free subjects and gastric acid suppressant users are also similar to each other. As a general rule for this category, associated background factors of digestive drug-free subjects resemble to those of gastric acid suppressant users. Judging from the p values in Table 3 and 4, this resemblance is more evident for PPI users compared with H_2RA users.

For the acid regurgitation category (l, j, and k in Figure 3), associations of the two gastric mucosa-related factors (F4–F5) and the five common factors (F6–F10) in Table 2 were mostly not significant in Table 3 and 4, except for "inadequate sleep (F7)" in H_2RA users and "frequent skipping of breakfast (F10)" in PPI users. As in the heartburn category, associated background factors of digestive drug-free subjects are considerably different from those of gastric acid suppressant users. Judging from the p values in Table 3 and 4, this difference is more obvious in PPI users than H_2RA users, except for the "burping a lot (k)" symptom.

For the pharyngo-upper esophageal discomfort and fullness while eating categories (g, i, and h in Figure 3), as in the dyspepsia category, a strong association with "inadequate sleep" is seen in both digestive drug-free subjects and gastric acid suppressant users (F7 in Table 2–4). In addition, positive and negative associations with the three basic factors of fullness while eating category are quite similar between digestive drug-free subjects and gastric acid suppressant users (F1–F3 in Table 2–4), which is also similar to the dyspepsia category to some extent.

Differences of Associated Factors between Digestive Drug-free Subjects and Gastric Acid Suppressant Users may be Useful for Studying Appropriate Usages of PPI and H_2RA upon Various Upper GI Symptoms

It is interesting that the background factors of symptoms belonging to the heartburn and acid regurgitation categories show statistically differences between digestive drug-free subjects and gastric acid suppressant users. On the contrary, background factors of symptoms belonging to the dyspepsia, pharyngo-upper esophageal discomfort, and fullness while eating categories show statistically no or small differences. In everyday clinical practice, the symptoms of heartburn and acid regurgitation tend to be well controlled with PPI or H_2RA, whereas relief of the symptoms of other three categories tend to be difficult [36,41]. The ease and difficulty to control each upper GI symptom seems to be related with similarity and difference of the significant background factors of each upper GI symptom between the digestive drug-free subjects and gastric acid suppressant users.

We are convinced that there should be some reason for these apparently different background factors of each upper GI symptom between the digestive drug-free subjects and gastric acid suppressant users. We hope that our finding will be a clue in elucidating the effects of attenuating gastric acid production or appropriate usage of PPI and/or H_2RA against individual upper GI symptoms in the future.

Study Limitations and Future Prospects

One limitation of our study is the cross-sectional design. We were therefore not able to perform accurate analyses of cause and effect. A second limitation of our study is insufficient data on the doses and types of gastric acid suppressants. More detailed information on orally taken PPIs and H_2RAs might show more accurate relationships. A third limitation is possibility of unpredicted background factors which were not taken into account. Based on the many previous reports including ours [3], we selected the possibly important 13 factors, but we cannot deny the existence of other unknown factors strongly correlated with upper GI symptoms.

We plan to follow the present study cohort for at least ten years; the upcoming large-scale prospective analyses will help us confirm the true causative factors for individual upper GI symptoms. We believe that the practicability of our proposed categorization of upper GI symptoms will also be validated together with the time-course changes of upper GI symptoms and usages of PPI and/or H_2RA. In addition, we are planning to evaluate the influence of *H. pylori* eradication [42] on our five upper GI symptom categories, because some symptom categories have significantly strong association with *H. pylori* infection status whereas others do not (Table 2). As many Japanese people with chronic *H. pylori* infection have undergone eradication therapy recently [5,43], the effect of *H. pylori* eradication on various upper GI symptoms will become clear in our next report.

Conclusions

The 12 typical upper gastrointestinal symptoms can be classified into heartburn, dyspepsia, acid regurgitation, pharyngo-upper esophageal discomfort, and fullness while eating symptom categories, which reflects various causative background factors. Differences between significantly associated factors of digestive drug-free subjects and digestive drug users may be useful for studying effects and usages of digestive drugs on various upper gastrointestinal symptoms.

Supporting Information

Figure S1 Distributions of 12 upper GI symptom scores in nine age groups and three BMI groups. Respective upper GI symptom scores (from 0 to 4) are means of the data from 18,097 digestive drug-free subjects.
(TIF)

Figure S2 Distribution of 12 upper GI symptom scores in regard of 11 background factors. Respective upper GI scores (from 0 to 4) are means of the data from 18,097 digestive drug-free subjects.
(TIF)

Table S1 Systemic univariate analyses of associations between the 12 upper GI symptoms and 13 background factors among the 18,097 digestive drug-free subjects. The levels of significance in these univariate analyses were set at $p<0.005$. R means the Pearson's correlation coefficient. Significant p-values are emphasized by shades of yellow.
(XLS)

Table S2 The effect sizes and power for the 13 variables in the three multivariate analyses concerning the 18,097 digestive drug-free users, 364 PPI users, and 528 H_2RA users.
(XLS)

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Author Contributions

Conceived and designed the experiments: NY TS YS TM KK. Performed the experiments: NY TS YS Y. Takahashi SK CN CM SO SM RM IAH KN MF Y. Tsuji CT HK OG. Analyzed the data: NY TS YS Y. Takahashi. Contributed reagents/materials/analysis tools: TS TM NY. Wrote the paper: NY YS Y. Takahashi KK.

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