TABLE 2
Hazard ratios (HRs) and 95% CIs for total cancer and cardiovascular disease according to quintiles (Q) of sodium and salted food consumption: Japan Public Health Center–based Prospective Study, 1995 and 1998–2004 (n = 77,500)

		Total cancer				Cardiovascular disease			
	Median intake[1]	Person- years	No. of cases	HR[2] (95% CI)	HR[3] (95% CI)	Person- years	No. of cases	HR[2] (95% CI)	HR[3] (95% CI)
	g								
Sodium									
Q1	3084	113,841	876	1.00 (reference)	1.00 (reference)	114,800	416	1.00 (reference)	1.00 (reference)
Q2	4005	117,226	881	1.01 (0.92, 1.11)	1.02 (0.93, 1.13)	118,218	428	1.03 (0.90, 1.17)	1.11 (0.96, 1.29)
Q3	4709	118,923	906	1.05 (0.95, 1.15)	1.07 (0.96, 1.18)	119,983	386	0.93 (0.81, 1.07)	1.02 (0.87, 1.19)
Q4	5503	121,521	882	1.02 (0.93, 1.12)	1.01 (0.91, 1.12)	122,609	403	0.97 (0.84, 1.11)	1.10 (0.94, 1.29)
Q5	6844	122,109	931	1.08 (0.99, 1.19)	1.04 (0.93, 1.16)	123,153	433	1.04 (0.90, 1.19)	1.19 (1.01, 1.40)
P for trend				0.10	0.61			0.78	0.06
Pickled vegetables									
Q1	3.3	115,909	833	1.00 (reference)	1.00 (reference)	116,584	458	1.00 (reference)	1.00 (reference)
Q2	12	116,861	844	1.04 (0.95, 1.15)	1.04 (0.94, 1.15)	117,789	373	0.83 (0.73, 0.96)	0.88 (0.76, 1.01)
Q3	23	118,303	844	1.03 (0.93, 1.13)	1.01 (0.91, 1.12)	119,163	424	0.93 (0.81, 1.06)	0.98 (0.85, 1.13)
Q4	39	120,344	978	1.15 (1.05, 1.26)	1.15 (1.04, 1.27)	121,649	397	0.83 (0.72, 0.95)	0.88 (0.76, 1.02)
Q5	85	122,203	977	1.13 (1.03, 1.25)	1.08 (0.97, 1.20)	123,579	414	0.85 (0.74, 0.97)	0.92 (0.79, 1.07)
P for trend				<0.01	0.10			0.07	0.48
Dried and salted fish									
Q1	0.5	115,798	901	1.00 (reference)	1.00 (reference)	116,548	499	1.00 (reference)	1.00 (reference)
Q2	6.4	121,052	924	1.10 (1.01, 1.21)	1.08 (0.98, 1.19)	122,066	433	0.93 (0.82, 1.06)	0.97 (0.85, 1.12)
Q3	13	119,264	896	1.09 (0.99, 1.20)	1.05 (0.95, 1.16)	120,540	366	0.80 (0.70, 0.91)	0.84 (0.72, 0.97)
Q4	23	118,975	820	1.01 (0.92, 1.11)	0.99 (0.89, 1.10)	119,998	380	0.84 (0.73, 0.95)	0.88 (0.76, 1.02)
Q5	43	118,531	935	1.13 (1.03, 1.24)	1.11 (1.00, 1.22)	119,610	388	0.83 (0.73, 0.95)	0.86 (0.74, 0.99)
P for trend				0.10	0.19			0.01	0.04
Salted fish roe									
Q1	0.0	113,158	880	1.00 (reference)	1.00 (reference)	114,116	420	1.00 (reference)	1.00 (reference)
Q2	0.2	113,450	875	1.07 (0.97, 1.17)	1.08 (0.96, 1.22)	114,249	424	1.08 (0.95, 1.24)	1.02 (0.86, 1.21)
Q3	0.7	118,606	877	1.05 (0.96, 1.16)	1.05 (0.94, 1.17)	119,674	421	1.05 (0.92, 1.20)	1.00 (0.86, 1.17)
Q4	1.6	121,082	874	1.14 (1.04, 1.26)	1.12 (1.01, 1.25)	122,147	378	1.03 (0.90, 1.19)	1.00 (0.85, 1.17)
Q5	4.7	127,323	970	1.18 (1.07, 1.29)	1.15 (1.04, 1.27)	128,577	423	1.06 (0.93, 1.22)	1.08 (0.93, 1.25)
P for trend				<0.01	0.01			0.71	0.27
Miso soup									
Q1	42	113,632	765	1.00 (reference)	1.00 (reference)	114,404	380	1.00 (reference)	1.00 (reference)
Q2	132	114,185	876	1.09 (0.99, 1.21)	1.08 (0.97, 1.20)	115,262	374	0.93 (0.81, 1.07)	0.99 (0.85, 1.16)
Q3	218	119,013	934	1.10 (1.00, 1.21)	1.09 (0.98, 1.20)	120,142	403	0.94 (0.82, 1.08)	0.97 (0.83, 1.13)
Q4	313	122,733	932	1.05 (0.95, 1.15)	1.03 (0.93, 1.14)	124,078	390	0.87 (0.75, 1.00)	0.90 (0.77, 1.04)
Q5	458	124,057	969	1.01 (0.92, 1.11)	0.99 (0.89, 1.10)	124,876	519	1.07 (0.94, 1.22)	1.09 (0.95, 1.26)
P for trend				0.60	0.36			0.30	0.35
Cooking and table salt									
Q1	2.3	117,214	973	1.00 (reference)	1.00 (reference)	118,305	437	1.00 (reference)	1.00 (reference)
Q2	3.4	117,997	884	0.95 (0.87, 1.04)	0.97 (0.88, 1.07)	118,906	430	1.03 (0.90, 1.18)	1.05 (0.91, 1.21)
Q3	4.4	118,323	896	0.99 (0.91, 1.09)	1.02 (0.92, 1.12)	119,392	409	1.01 (0.88, 1.15)	1.06 (0.92, 1.23)
Q4	5.6	119,446	845	0.94 (0.86, 1.03)	0.96 (0.86, 1.06)	120,547	370	0.91 (0.79, 1.05)	0.98 (0.84, 1.15)
Q5	8.0	120,640	878	1.02 (0.92, 1.12)	1.00 (0.89, 1.12)	121,613	420	1.08 (0.94, 1.23)	1.21 (1.03, 1.42)
P for trend				0.68	0.94			0.60	0.05

[1] Values for sodium intake are provided in milligrams.

[2] HRs were adjusted for sex and age (5-y groups). Linear trends across quintiles of sodium or salted food intake were tested with the use of the median consumption for each quintile as an ordinal variable.

[3] HRs were further adjusted for BMI (in kg/m^2 ; <19, 19–22.9, 23–24.9, 25–26.9, ≥27), smoking status (never, past, current), alcohol consumption (none; occasional; 1–149, 150–299, 300–449, ≥450 g ethanol/wk), physical activity in metabolic equivalent task-hours/d (<30, 30–34.9, 35–39.9, ≥40), and quintiles of energy, potassium, and calcium.

Although the results for total cancer and major site-specific cancers were not substantially changed (data not shown), multivariate HRs of CVD and stroke for the highest compared with lowest quintiles of sodium intake were greater than those before the exclusion of these patients (HR for CVD: 1.30, 95% CI: 1.06, 1.60; *P* for trend: 0.01; HR for stroke: 1.36, 95% CI: 1.09, 1.71; *P* for trend < 0.01).

DISCUSSION

In this population-based prospective cohort study in Japan, we observed that higher consumption of sodium as a whole was associated with an increased risk of CVD but not of cancer. In contrast, higher consumption of salted fish roe was associated with a higher risk of cancer but not of CVD. Moreover, higher consumption of dried and salted fish was associated with a lower

TABLE 3
Hazard ratios (HRs) and 95% CIs for major site-specific cancers and stroke or myocardial infarction according to quintiles (Q) of sodium and salted food consumption: Japan Public Health Center–based Prospective Study, 1995 and 1998–2004 (n = 77,500)

	Gastric cancer		Colorectal cancer		Stroke		Myocardial infarction	
	No. of cases	HR[1] (95% CI)	No. of cases	HR[1] (95% CI)	No. of cases	HR[1] (95% CI)	No. of cases	HR[1] (95% CI)
Sodium								
Q1	177	1.00 (reference)	164	1.00 (reference)	355	1.00 (reference)	64	1.00 (reference)
Q2	175	1.05 (0.84, 1.31)	161	1.05 (0.84, 1.33)	349	1.05 (0.90, 1.24)	83	1.50 (1.05, 2.14)
Q3	167	1.06 (0.84, 1.34)	163	1.08 (0.85, 1.37)	315	0.97 (0.82, 1.14)	73	1.34 (0.92, 1.96)
Q4	174	1.05 (0.83, 1.34)	171	1.08 (0.84, 1.37)	343	1.08 (0.92, 1.28)	64	1.26 (0.85, 1.88)
Q5	174	1.07 (0.83, 1.38)	177	1.10 (0.85, 1.42)	383	1.21 (1.01, 1.43)	54	1.09 (0.71, 1.68)
P for trend		0.64		0.51		0.03		0.91
Pickled vegetables								
Q1	95	1.00 (reference)	163	1.00 (reference)	375	1.00 (reference)	86	1.00 (reference)
Q2	175	1.91 (1.47, 2.48)	136	0.91 (0.72, 1.15)	315	0.92 (0.78, 1.07)	62	0.74 (0.52, 1.04)
Q3	168	1.70 (1.30, 2.22)	190	1.20 (0.96, 1.50)	354	1.00 (0.86, 1.17)	75	0.94 (0.67, 1.31)
Q4	212	2.14 (1.65, 2.77)	182	1.11 (0.88, 1.40)	343	0.92 (0.79, 1.08)	55	0.69 (0.48, 1.00)
Q5	217	2.24 (1.71, 2.93)	165	0.95 (0.74, 1.22)	358	0.96 (0.81, 1.13)	60	0.75 (0.51, 1.10)
P for trend		<0.01		0.71		0.80		0.22
Dried and salted fish								
Q1	129	1.00 (reference)	158	1.00 (reference)	416	1.00 (reference)	86	1.00 (reference)
Q2	195	1.56 (1.22, 1.98)	156	1.12 (0.88, 1.42)	362	0.96 (0.83, 1.12)	74	1.06 (0.76, 1.48)
Q3	199	1.57 (1.24, 2.00)	171	1.18 (0.93, 1.49)	301	0.83 (0.71, 0.97)	69	0.93 (0.65, 1.31)
Q4	173	1.48 (1.15, 1.89)	157	1.15 (0.90, 1.46)	319	0.87 (0.74, 1.02)	63	0.97 (0.68, 1.38)
Q5	171	1.46 (1.14, 1.88)	194	1.40 (1.11, 1.77)	347	0.90 (0.77, 1.06)	46	0.66 (0.44, 0.98)
P for trend		0.12		<0.01		0.22		0.03
Salted fish roe								
Q1	146	1.00 (reference)	158	1.00 (reference)	343	1.00 (reference)	80	1.00 (reference)
Q2	144	1.14 (0.85, 1.51)	135	0.90 (0.68, 1.20)	354	1.05 (0.87, 1.27)	78	0.95 (0.63, 1.43)
Q3	181	1.35 (1.06, 1.72)	162	1.01 (0.79, 1.29)	345	1.02 (0.86, 1.21)	76	0.85 (0.58, 1.23)
Q4	170	1.39 (1.09, 1.77)	178	1.22 (0.96, 1.56)	333	1.08 (0.91, 1.28)	47	0.63 (0.42, 0.95)
Q5	226	1.66 (1.32, 2.09)	203	1.35 (1.07, 1.69)	370	1.14 (0.97, 1.34)	57	0.80 (0.55, 1.16)
P for trend		<0.01		<0.01		0.09		0.21
Miso soup								
Q1	145	1.00 (reference)	140	1.00 (reference)	317	1.00 (reference)	67	1.00 (reference)
Q2	158	1.04 (0.82, 1.33)	159	1.12 (0.88, 1.42)	302	0.95 (0.81, 1.13)	73	1.17 (0.82, 1.67)
Q3	170	1.09 (0.86, 1.39)	180	1.11 (0.88, 1.41)	350	1.00 (0.85, 1.18)	55	0.80 (0.54, 1.17)
Q4	181	1.06 (0.84, 1.35)	177	1.05 (0.82, 1.33)	335	0.91 (0.78, 1.08)	59	0.83 (0.57, 1.21)
Q5	213	1.10 (0.87, 1.39)	180	1.01 (0.80, 1.28)	441	1.12 (0.96, 1.31)	84	0.98 (0.69, 1.39)
P for trend		0.45		0.73		0.14		0.47
Cooking and table salt								
Q1	210	1.00 (reference)	180	1.00 (reference)	368	1.00 (reference)	75	1.00 (reference)
Q2	173	0.96 (0.78, 1.19)	178	1.09 (0.88, 1.36)	349	1.02 (0.87, 1.20)	84	1.17 (0.84, 1.64)
Q3	175	1.01 (0.81, 1.26)	155	0.98 (0.77, 1.23)	345	1.06 (0.90, 1.24)	66	1.02 (0.71, 1.47)
Q4	143	0.86 (0.68, 1.09)	156	0.97 (0.76, 1.23)	316	0.98 (0.83, 1.16)	56	0.97 (0.66, 1.43)
Q5	166	1.03 (0.80, 1.33)	167	1.01 (0.78, 1.31)	367	1.21 (1.02, 1.44)	57	1.12 (0.74, 1.69)
P for trend		1.00		0.78		0.05		0.88

[1] HRs were adjusted for sex, age (5-y groups), BMI (in kg/m^2; <19, 19–22.9, 23–24.9, 25–26.9, ≥27), smoking status (never, past, current), alcohol consumption (none; occasional; 1–149, 150–299, 300–449, ≥450 g ethanol/wk), physical activity in metabolic equivalent task-hours/d (<30, 30–34.9, 35–39.9, ≥40), and quintiles of energy, potassium, and calcium. Linear trends across quintiles of sodium or salted food intake were tested with the use of the median consumption for each quintile as an ordinal variable.

risk of CVD. To our knowledge, this is the first prospective cohort study to simultaneously examine associations between sodium and salted foods and the risk of cancer and CVD.

Results from 7 previous prospective cohort studies that examined the association between sodium intake and CVD risk have been poorly consistent: 3 studies showed significant associations between sodium and risk (8, 9, 11), 1 showed a significant inverse association (12), and 3 showed no association (10, 13, 14). These studies used different methods to assess exposure: 2 used 24-h urinary sodium excretion (9, 10), 1 used a validated FFQ that consisted of 35 items (8), and 4 used a single 24-h dietary recall method (11–14). Our results are consistent with 2 of the 3 studies that used an FFQ or 24-h urinary excretion to assess habitual salt intake (8, 9).

Only one previous study has examined the association between salted foods and total cancer risk (35), although at a small scale (155 cases). Results showed no association between consumption of salted fish, Japanese pickles, and miso soup with total

cancer mortality. With regard to gastric cancer, the 6 prospective studies of the association of this cancer with total salt intake after adjustment for other risk factors (6, 7, 36–39) were inconsistent, although the report of a joint World Cancer Research Fund/American Institute for Cancer Research Expert Consultation identified salt as a "probable" risk of gastric cancer (1): 2 of 3 studies conducted in Japan showed a positive association between salt intake and gastric cancer incidence (6, 38) and 3 conducted in Western countries [Norway (7), Netherlands (37), and the United States (39)] showed no association, whereas the third study conducted in Japan reported an inverse association between total salt intake and gastric cancer mortality. Of these studies, one conducted in the Japan Public Health Center–based cohort reported positive associations with the risk of gastric cancer for both salt intake and salted foods but did not take table salt or cooking method into consideration when total salt intake was calculated (6). In contrast, the present study did include table salt or cooking method variables, which correlated strongly with total sodium intake ($r = 0.71$), and showed them to represent 40% of total sodium intake. Nevertheless, we observed no association between total sodium consumption and the risk of either total cancer or gastric cancer.

The major strength of the present study was its prospective design, which avoided exposure recall bias. Other strengths included the following: study subjects were defined as the general population; response rate to the questionnaire (85%) was acceptable for study settings such as this; and the proportion of losses to follow-up (0.02%) was negligible. Furthermore, the use of a general population in Asia and an FFQ enabled sodium intake to be estimated from not only salted foods but also salty seasonings from dietary and cooking behaviors, which likely represented a relatively large portion of sodium intake. In the present study, this strength may have eliminated the possibility that the observed absence of an association between sodium intake as a whole salt equivalent and gastric cancer incidence was attributable to any inability to take into account salty seasonings from dietary and cooking behaviors in the estimation of total sodium intake.

The present results suggest that the associations of cancer with specific foods with high salt concentrations, such as salted fish roe, are not due to the amount of salt per se, but rather to other causes. Consistent with our results, Tsugane et al (6) reported that, after adjustment for total salt intake, higher consumption of salted fish roe among men and women and of dried or salted fish among men was associated with a higher risk of gastric cancer. One potential explanation for this may have been the presence of chemical carcinogens such as *N*-nitroso compounds in dried fish or salted fish roe, which can be formed by the reaction of nitrate or nitrite in the curing process or in the body (1, 4, 5), or heterocyclic amines, which have been detected in fish or meat cooked in high temperatures, such as grilling (40), which is commonly used for dried and salted fish in Japan. An additional, inseparable explanation is the destruction of the gastric mucosal barrier by a high intragastric salt concentration, which leads to inflammation, diffuse erosion, and degeneration. The subsequent proliferative change may exacerbate the effect of food-derived carcinogens (3, 41). In contrast, the decreased risk of CVD with dried and salted fish intake might reflect beneficial cardiovascular effects of n–3 polyunsaturated fatty acids in fish in the inhibition of platelet aggregation, modulation of the inflammatory system, and lowering of blood pressure (27). Salted foods might thus not be as precise a surrogate marker of total salt in the investigation of the influence on cancer and CVD as whole sodium chloride consumption.

Our study has several potential limitations. First, with the use of multiple 24-h urinary sodium excretion as a reference, the validity of the FFQ for sodium intake was moderate at best [$r = 0.30$–0.42 (20)]. Some misclassification in the FFQ was unavoidable, and it is possible that the accuracy of salt intake was less than that of salted food intake (6). If this biased the association between sodium and cancers toward the null, then the observed association would have underestimated the true magnitude of the association between sodium and CVD as well as cancers. Second, variation in sodium consumption among subjects was also moderate at best, with median intake in the highest quintile group (6844 mg) only 2.2-fold that in the lowest (3084 mg; Table 1). However, this range was similar to that of a study based on 24-h urinary excretion, which identified positive associations between sodium intake and CVD [1.6-fold difference (9)]. Given that this variation was sufficient to detect an association between sodium intake and CVD risk, the possibility that the lack of association between sodium intake and total and gastric cancer was due to insufficient variation therefore appears unlikely. In addition, we did not obtain information on infection with *Helicobacter pylori*, a strong risk factor for gastric cancer (42). Because salted food intake may increase the risk of *H. pylori* infection, the prevalence of infection would have been higher in subjects with higher intakes of salt or salted food (43). Therefore, even if *H. pylori* infection causes gastric cancer, we believe it unlikely that the failure to account for *H. pylori* infection masked a positive association between the consumption of sodium and the risk of gastric cancer.

In conclusion, this population-based prospective cohort study in Japan showed that the amount of sodium as a whole salt equivalent was not associated with the risk of cancer but was associated with an increased risk of CVD. In contrast, the intake of highly salt-concentrated preserved foods may increase the risk of cancer. Our findings support the notion that sodium and salted foods have differential influences on the development of cancer and CVD.

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Plasma levels of C-reactive protein and serum amyloid A and gastric cancer in a nested case–control study: Japan Public Health Center-based prospective study

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Gastric carcinogenesis may be under the combined influence of factors related to the host, *Helicobacter pylori* bacterial virulence and the environment. One possible host-related factor is the inflammatory or immune response. To clarify this point, we investigated the association between plasma levels of C-reactive protein (CRP) and serum amyloid A (SAA) and the subsequent risk of gastric cancer in a population-based nested case–control study. Subjects were observed from 1990 to 2004. Among 36 745 subjects who answered the baseline questionnaire and provided blood samples, 494 gastric cancer cases were identified and matched to 494 controls for our analysis. The overall distribution of CRP and SAA was not apparently associated with the development of gastric cancer. However, a statistically significant increased risk was observed when subjects were categorized dichotomously. The adjusted odds ratio (OR) for the development of gastric cancer for the CRP-positive group (CRP > 0.18 mg/dl) compared with the CRP-negative group was 1.90 [95% confidence interval (CI): 1.19–3.02, $P = 0.007$]. The OR for the SAA-positive group (SAA > 8 μg/ml) compared with the SAA-negative group was 1.93 (95% CI: 1.22–3.07, $P = 0.005$). In conclusion, our results suggest that those who react strongly to inflammation or who have a high host immune response, as reflected by extremely elevated plasma levels of CRP and SAA, are at a high risk to develop gastric cancer.

Introduction

It is well established that cancer arises in chronically inflamed tissue, and one of the classic examples is *Helicobacter pylori*-associated gastric cancer (1). *Helicobacter pylori* persistently colonizes the gastric mucosa, leading to chronic inflammation, atrophic gastritis and, finally, gastric cancer. There are high interindividual differences in the extent of gastric inflammation among *H.pylori*-infected subjects, and only a small proportion of them develop clinical consequences. This indicates that gastric carcinogenesis may be under the combined influence of factors related to the host, bacterial virulence and the environment. One possible host-related factor is the inflammatory or immune response. Many studies have reported an association between serum proinflammatory cytokines [e.g. interleukin (IL)-6, IL-8 and IL-1β] levels (2–4) or polymorphisms (such as IL-1, IL-2 and IL-8) and gastric cancer risk (5–8), but the results are controversial. The lack of consensus may be partly due to the nature of cytokines, which are components of a large, complex signaling network, and difficulties in measuring their levels and interactions. Measurement of cytokines in plasma is difficult because of their short plasma half-lives and the presence of blocking factors (9). Additionally, combinations of cytokines have been found to have additive, inhibitory or synergistic effects. Therefore, more useful or systematic indicators of host inflammatory or immune response are needed.

C-reactive protein (CRP) is a well-established indicator of inflammation in the body (10). It is an acute-phase reactant that reflects low-grade systemic inflammation and has been studied in a variety of cardiovascular diseases. CRP production by the liver is regulated by cytokines, principally IL-6 and tumor necrosis factor α, which is the main trigger for the production of IL-6 by a variety of cells. In fact, strong positive associations between IL-6, tumor necrosis factor α and CRP were observed (11). Serum amyloid A (SAA) is another major acute-phase reactant. It is a putative serum precursor of the amyloid A protein, which constitutes amyloid fibrils in secondary amyloidosis and is an apolipoprotein associated with the high density lipoprotein 3 fraction of serum (12). In most studies, a parallel increase of SAA and CRP has been observed, although some studies have delineated acute-phase SAA as the more sensitive parameter (13,14). Therefore, to indicate the host inflammatory or immune response systematically, CRP and SAA may be useful markers.

In this large-scale nested case–control study, we aimed to examine whether the host inflammatory or immune response has any association with the development of gastric cancer. To clarify this point, we explored the relation of plasma levels of CRP and SAA to risk of developing gastric cancer. As far as we know, this is the first study to prospectively seek this association in a population.

Abbreviations: BMI, body mass index; CagA, cytotoxin-associated gene A; CI, confidence interval; CRP, C-reactive protein; ICD-O, International Classification of Diseases for Oncology; Ig, immunoglobulin; IL, interleukin; JPHC, Japan Public Health Center; OR, odds ratio; PG, pepsinogen; PHC, public health center; SAA, serum amyloid A.

Materials and methods

Study population

The Japan Public Health Center-based prospective study (JPHC Study) is an ongoing cohort study to investigate cancer, cardiovascular disease and other lifestyle-related diseases. The first group (Cohort I) of the JPHC Study was started in 1990 and the second group (Cohort II) in 1993 (15). The JPHC Study included 140 420 subjects (68 722 men and 71 698 women), defined as all inhabitants in the study areas [27 cities, towns or villages served by 11 public health centers (PHCs)] who were 40–59 years old (Cohort I) or 40–69 years old (Cohort II). Among the study subjects, those registered at one PHC area in Cohort I were excluded from the present analysis because data on cancer incidence were not available. Additionally, one subcohort in Cohort II was excluded because the selection of subjects differed from that of other cohort subjects, i.e. random sampling of residents from a municipality population registry for one city, stratified by 10 year age–gender groups. We thus defined 123 576 subjects (61 009 men and 62 567 women) for the present study. The JPHC Study was approved by the institutional review board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In 1990 for Cohort I and in 1993–1994 for Cohort II, subjects were asked to reply to a lifestyle questionnaire that covered sociodemographic characteristics, medical history, smoking and drinking habits, diet and so on. Details of the food frequency questionnaire included in the baseline survey have been described previously (16). A total of 99 808 (81%) subjects—47 525 men and 52 283 women—responded to the questionnaires.

We excluded subjects who self-reported cancer at baseline ($n = 2136$), those who were not Japanese ($n = 18$) and those who were later discovered to have moved away at baseline ($n = 11$). This left 97 644 eligible subjects (46 803 men and 50 841 women). Among them, 36 745 subjects (38%; 13 467 men and 23 278 women) donated blood samples at health checkups conducted by the PHC in each area. Each subject voluntarily provided 10 ml of blood during the health checkups. As customary, subjects were asked to avoid having a meal later than 21:00 on the day before the examination. The last time of either consuming a meal or drinking water or tea was recorded. The plasma and buffy layer were divided into four tubes, with each tube holding 1.0 ml (3 tubes for plasma and 1 for the buffy layer) and stored at 80°C. Blood was collected from 1990 to 1992 in Cohort I and from 1993 to 1995 in Cohort II.

Follow-up and identification of gastric cancer

In Japan, at the time the study was conducted, a PHC played a role as an organization that provided primary health care, including health checkups,

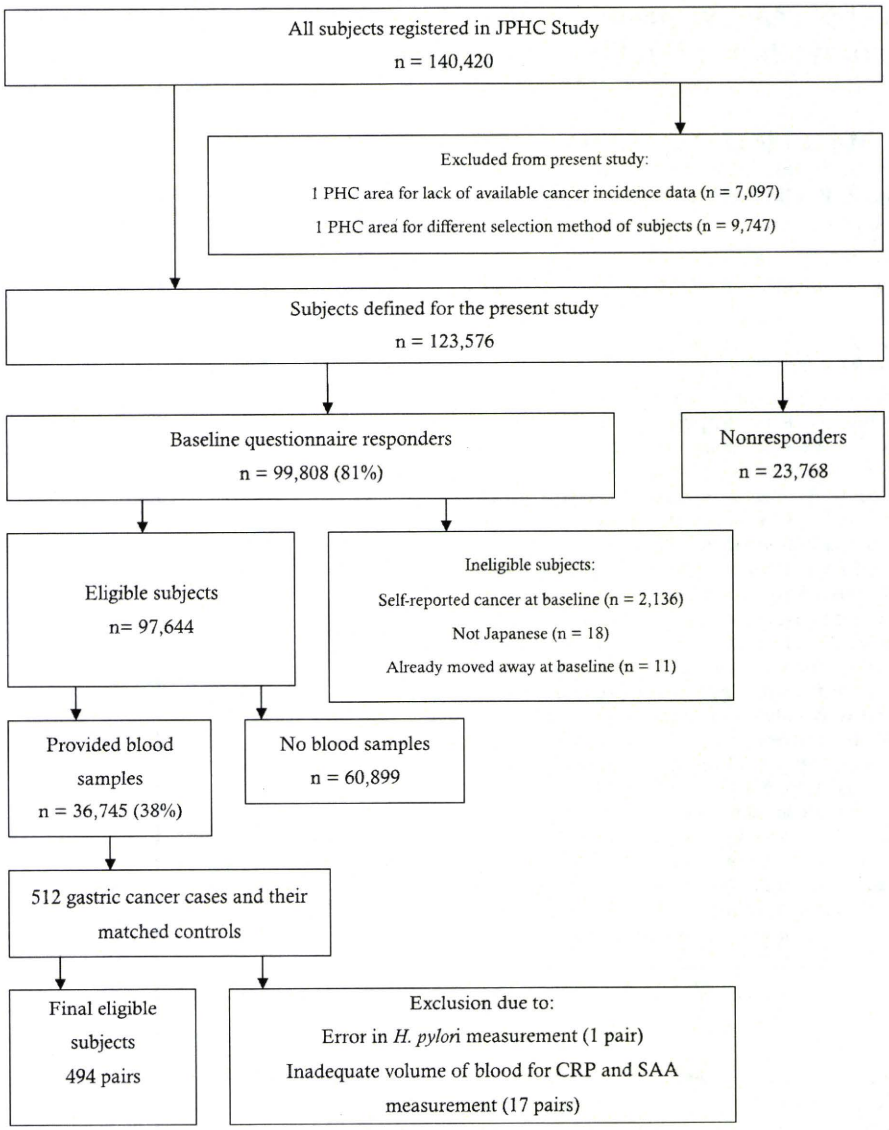

Fig. 1. Flow of study population.

or other health promotion activities for all inhabitants of the municipalities supervised by the PHC. In this study, the main role of the PHC was to collect and report data on mortality, relocation and cancer cases.

Death and relocation

We observed study subjects until 31 December 2004. The changes in residency status, including death, were identified annually through the residential registry in each area. To confirm causes of death, we used mortality data from the Ministry of Health, Labour and Welfare. Residence and death registration are required by law in Japan, and the registries are believed to be complete. Among 36 745 study subjects, 1423 (3.9%) moved away from the study area, 1610 (4.4%) died and 11 (0.03%) were lost to follow-up within the study period.

Cancer registry for JPHC Study

Data on newly diagnosed cases of cancer were collected from two sources: active patient notification from the local major hospitals in the study area and data linkage with population-based registries (usually prefecture-wide). Death certificate information was used as a supplementary information source. In our cancer registry system, the proportion of cases of gastric cancer for which information was based on death certificate notification was 7.6% and on information available from death certificates only was 2.1%. This level of quality for the information was considered satisfactory for the present study.

Identification of gastric cancer and selection of control subjects

Cases of gastric cancer were extracted from the cancer registry for the JPHC Study on the basis of site [International Classification of Diseases for Oncology (ICD-O) code C160–169] (17). Up to the end of the study period, 512 new gastric cancer cases were identified. Until quite recently in Japan, the upper third of the stomach has been called the 'cardia' on the basis of the guidelines for gastric cancer classification (18). Because it seemed difficult to distinguish the cardia, which is mainly located in the esophagogastric junction, from the upper third of the stomach, we combined tumors at these sites into one group for analysis (ICD-O code C160–161). A tumor located on the lower side of the stomach was classified as distal gastric cancer (ICD-O code C162–167). Subsites that could not be classified because of a diffuse lesion (ICD-O code C168) or those with no information (ICD-O code C169) were categorized as an unclassified subsite. Histologic classification was based on one author's (S.S.) review, in consultation with a pathologist, of the record reported by each hospital. The subdivisions were made on the basis of a classification derived by Lauren (19). For each case, one control was selected from subjects who had no history of gastric cancer and who lived in the study area when the case was

diagnosed. Each control was matched to a case for gender, age (±3 years), PHC area, blood donation date (±2 months) and fasting time at blood donation (±5 h). Because of a technical error in measurement of *H.pylori* and inadequate volume of blood available for CRP and SAA measurements, 1 case with its matched control and another 17 pairs (8 cases with their matched controls and 10 controls with their matched cases) were excluded. Finally, we had 494 sets each of cases and controls for use in the present analysis. A flowchart of the study subjects is provided in Figure 1.

Laboratory analysis

CRP and SAA concentrations were determined by the latex agglutination nephelometric immunoassay test (LZ test 'Eiken' CRP-HG; Eiken Kagaku Co. Ltd, Tokyo, Japan; and LZ test 'Eiken' SAA; Eiken Kagaku Co. Ltd, respectively). For the CRP test, based on 10 replicated measurements of three concentrations of blood samples (0.07, 0.50 and 4.41 mg/dl) at the time of analyses, the coefficients of variation were 1.69%, 0.59% and 0.76%, respectively. For SAA, 10 replicated measurements of two concentrations of blood samples (22 μg/ml and 110 μg/ml) yielded a coefficient of variation of reproducibility values of 1.53% and 1.17%. Normal values for the examined parameters were <0.18 mg/dl for CRP and <8 μg/ml for SAA according to the kit's protocol. Both cutoff values were based on data from reports for the same kit. The cutoff value of CRP was set by the iterative truncation method among 478 health checkup samples (20). In brief, after repeated deletion of outliers, mean ± 1.96 SD was considered the normal range. For SAA, after being converted to a logarithm, the value was set as the upper 95th percentile of the distribution of 1056 normal subjects (0–70 years old) (21).

Immunoglobulin (Ig) G antibodies to *H.pylori* were measured with a direct enzyme-linked immunosorbent assay kit (E Plate 'Eiken' *H.pylori* Antibody; Eiken Kagaku Co. Ltd). Levels of IgG were categorized as seropositive and seronegative for *H.pylori* according to a selective cutoff value (≤10 or >10). The cutoff value was based on the results of sensitivity and specificity calculated with the urea test, which is the gold standard (report by company). Assays of cytotoxin-associated gene A (CagA) were performed with the use of an enzyme-linked immunosorbent assay kit, in which horseradish peroxidase was used as the enzyme tracer (CagA IgG EIA; Sceti Co. Ltd, Rome, Italy). According to the manufacturer's protocol, samples with IgG values ≤10 RU/ml must be considered non-reactive for anti-CagA IgG antibodies; samples with IgG values within 10–15 RU/ml must be considered weakly reactive and samples with IgG values >15 RU/ml must be considered reactive for anti-CagA IgG antibodies. With regard to interpretation of these results, reactive and/or questionable samples are considered positive for anti-CagA IgG antibodies, i.e. values >10 are regarded as CagA positive. Serum levels of pepsinogen I and II (PGI and PGII, respectively) were measured by commercial kits based on a two-step enzyme immunoassay (E Plate 'Eiken' Pepsinogen I; Eiken Kagaku Co. Ltd; and E Plate 'Eiken' Pepsinogen II; Eiken Kagaku Co. Ltd). Results were defined as 'atrophic' when the criteria of both PGI level ≤70 ng/ml and PGI : PGII ratio ≤3.0 were fulfilled. Comparing the PG levels between gastric cancer cases and healthy controls retrospectively, Miki (22) reported that applying a PGI level ≤70 ng/ml and a PGI : PGII ratio ≤3.0 as cutoff values was most effective in distinguishing cases from controls. Using these criteria, other authors have showed an extremely high correlation (r = 0.999) between atrophy and age-adjusted gastric cancer mortality among inhabitants of five areas in Japan (23). Among atrophic cases, more severe cases with a PGI level ≤30 ng/ml and PGI : PGII ratio ≤2.0 were defined as severe atrophy.

All measurements were conducted by a person blinded to the case–control situation.

Statistical analysis

Statistical analysis included chi-square test, analysis of variance, analysis of covariance and conditional logistic model. Multiple conditional logistic regression analyses were conducted to control for potential confounding factors. For cardia cancer, smoking status, alcohol consumption (for SAA analysis), intake of salt, body mass index (BMI), family history of gastric cancer, history of infectious or inflammatory disease (i.e. cardiovascular disease, ischemic heart disease, liver disease and kidney disease) and current use of analgesics for lumbago, neuralgia, common cold, arthrosis and joint pain were controlled. For all gastric cancer, all non-cardia cancer, differentiated-type non-cardia cancer and undifferentiated-type non-cardia cancer further adjustment was applied for *H.pylori* infection, atrophy and CagA seropositivity. Smoking status was divided into four groups: never smoker, past smoker, current smoker with <20 cigarettes per day and current smoker with ≥20 cigarettes per day). Alcohol consumption was defined as drinker (>1 day/week) and non-drinker (<1 day/week). BMI was categorized into three groups so that each category included an approximately equal number of controls. Salt was treated as a continuous variable. Family history of gastric cancer was regarded as positive if at least one parent or sibling had gastric cancer. CRP and SAA status (positive/negative) were determined according to the protocol's normal value. Additionally, the non-linear continuous models of the association between CRP and SAA and gastric cancer risk were tested by PROC GAM. Odds ratios (ORs) were calculated relative to the cutoff points of CRP and SAA. Because the distribution was skewed, log transformation was conducted for CRP, SAA, *H.pylori* titer, CagA titer, PGI level and PGII and PGI : PGII ratio, which altered the distribution close to normal in comparisons of the mean values between groups.

Reported P-values were two sided, and all statistical analyses were done with SAS software version 9.1 (SAS Institute Inc., Cary, NC).

Results

Baseline characteristics of cases and controls are shown in Table I. Among listed factors, predominance of *H.pylori* positivity, CagA status, atrophy and family history of gastric cancer were apparent in cases compared with controls.

Table II summarizes the distribution of lifestyle factors and plasma biomarkers according to the CRP and SAA status among controls. Forty-seven (9.5%) and 63 (12.8%) subjects met the criteria for being positive for plasma CRP and SAA, respectively. For CRP status, no factors were differently distributed other than SAA levels; the mean value of SAA among CRP-positive subjects was >10 times that of CRP-negative subjects ($P < 0.0001$). Plasma CRP level among SAA-positive subjects was 13 times that among SAA-negative subjects ($P < 0.0001$). Correlation of the log-transformed CRP and SAA was 0.55 ($P < 0.0001$). Mean daily salt intake was higher in SAA-negative subjects compared with SAA-positive subjects. This may be due to the predominance of male gender and alcohol consumption among SAA-negative subjects, which contribute to high salt intake. When gender and alcohol consumption were adjusted (analysis of covariance), the difference in salt intake was no longer significant ($P = 0.40$). Compared with positive subjects, SAA-negative subjects had a significantly higher *H.pylori* titer against IgG antibody and more frequent distribution of male gender, alcohol consumption, *H.pylori* positivity and atrophy.

Table I. Baseline characteristics of cases and controls

	Case	Control	P-value[a]
n	494	494	
Age	57.3 (0.3)	57.3 (0.3)	Matching value
Men (%)	329 (66.6%)	329 (66.6%)	Matching value
Cigarette smoking			
Never smoker (%)	228 (46.2%)	245 (49.6%)	
Past smoker (%)	91 (18.4%)	98 (19.8%)	
Current smoker with <20 cigarettes per day (%)	133 (26.9%)	109 (22.1%)	
Current smoker with ≥20 cigarettes per day (%)	42 (8.5%)	42 (8.5%)	0.35
Alcohol consumption			
Never or occasional (%)	245 (49.6%)	244 (49.4%)	
≥1 day, <300 g/week (%)	187 (37.9%)	203 (41.1%)	
≥1 day, ≥300 g/week (%)	62 (12.6%)	47 (9.5%)	0.26
BMI			
<25	396 (80.2%)	369 (74.7%)	
25–29.9	89 (18.0%)	113 (22.9%)	
≥30	9 (1.8%)	12 (2.4%)	0.12
Family history of gastric cancer (%)	60 (12.2%)	40 (8.1%)	0.03
Salt (g/day)	5.3 (0.1)	5.1 (0.1)	0.40
Helicobacter pylori positive (%)[b]	463 (93.7%)	371 (75.1%)	<0.0001
Helicobacter pylori positive (%)[c]	489 (99.0%)	445 (90.1%)	<0.0001
CagA (+) (%)	375 (75.9%)	346 (70.0%)	0.04
Atrophy (%)	406 (82.2%)	285 (57.7%)	<0.0001

Values are mean (SE) except where specified otherwise.
[a]Based on chi-square test or analysis of variance.
[b]Based on IgG antibody.
[c]Based on CagA positive and/or *Helicobacter pylori* IgG antibody positive.

Table II. Distribution of lifestyle factors and plasma biomarkers according to CRP and SAA status among control

	CRP status			SAA status		
	Negative (CRP ≤ 0.18 mg/dl)	Positive (CRP > 0.18 mg/dl)	*P*-value[a]	Negative (SAA ≤ 8 μg/ml)	Positive (SAA > 8 μg/ml)	*P*-value[a]
n	447	47		431	63	
Age	57.1 (0.3)	58.6 (1.1)	0.20	57.2 (0.3)	58.1 (0.9)	0.35
Men (%)	296 (66.2%)	33 (70.2%)	0.58	296 (68.7%)	33 (52.4%)	0.01
BMI						
<25	336 (75.2%)	33 (70.2%)		327 (75.9%)	42 (66.7%)	
25–29.9	100 (22.4%)	13 (27.7%)		93 (21.6%)	20 (31.8%)	
≥30	11 (2.5%)	1 (2.1%)	0.71	11 (2.6%)	1 (1.6%)	0.19
Cigarette smoking						
Never smoker (%)	225 (50.3%)	20 (42.6%)		207 (48.0%)	38 (60.3%)	
Past smoker (%)	91 (20.4%)	7 (14.9%)		90 (20.9%)	8 (12.7%)	
Current smoker with <20 cigarettes per day (%)	97 (21.7%)	12 (25.5%)		98 (22.7%)	11 (17.5%)	
Current smoker with ≥20 cigarettes per day (%)	34 (7.6%)	8 (17.0%)	0.12	36 (8.4%)	6 (9.5%)	0.23
Alcohol consumption						
Never or occasional (%)	218 (48.8%)	26 (55.3%)		204 (47.3%)	40 (63.5%)	
≥1 day, <300 g/week (%)	184 (41.2%)	19 (40.4%)		185 (42.9%)	18 (28.6%)	
≥1 day, ≥300 g/week (%)	45 (10.1%)	2 (4.3%)	0.39	42 (9.7%)	5 (7.9%)	0.05
Family history of gastric cancer (%)	36 (8.1%)	4 (8.5%)	0.91	36 (8.4%)	4 (6.4%)	0.85
Salt (g/day)	5.2 (0.1)	4.9 (0.3)	0.43	5.2 (0.1)	4.6 (0.3)	0.04
CRP (mg/dl)/SAA (μg/ml)[b]	3.6 (1.8)	38.6 (5.6)	<0.0001[a]	0.05 (0.03)	0.65 (0.07)	<0.0001[a]
Helicobacter pylori positive (%)[c]	338 (75.6%)	33 (70.2%)	0.42	332 (77.0%)	39 (61.9%)	0.01
Helicobacter pylori positive (%)[d]	403 (90.2%)	42 (89.4%)	0.86	390 (90.5%)	55 (87.3%)	0.43
Helicobacter pylori titer	43.9 (2.3)	36.1 (7.1)	0.31[e]	44.0 (2.3)	37.1 (6.1)	0.02[e]
CagA (+) (%)	314 (70.3%)	32 (68.1%)	0.76	302 (70.1%)	44 (69.8%)	0.97
CagA titer	85.1 (4.2)	74.7 (12.9)	0.72[e]	84.6 (4.3)	80.8 (11.1)	0.82[e]
PGI	28.6 (0.8)	29.7 (2.5)	0.55[e]	28.5 (0.8)	30.1 (2.1)	0.52[e]
PGII	11.2 (0.3)	10.8 (1.0)	0.60[e]	11.2 (0.3)	11.0 (0.8)	0.71[e]
PGI : PGII	3.5 (0.6)	2.9 (1.8)	0.83[e]	3.5 (0.6)	3.2 (1.6)	0.28[e]
Atrophy (%)	260 (58.2%)	25 (53.2%)	0.51	256 (59.4%)	29 (46.0%)	0.04
Severe atrophy (%)	122 (27.3%)	9 (19.2%)	0.23	119 (27.6%)	12 (19.1%)	0.15

Values are mean (SE) except where specified otherwise.
[a]Based on chi-square test or analysis of variance.
[b]Mean plasma CRP level for SAA status and mean plasma SAA level for CRP status.
[c]Based on IgG antibody.
[d]Based on CagA positive and/or *Helicobacter pylori* IgG antibody positive.
[e]Based on analysis of variance of log biomarkers.

In Table III, ORs and 95% confidence intervals (CIs) of CRP positivity for development of gastric cancer are presented by tumor subsite and histologic types. CRP ranged from 0 to 19.1 mg/dl (mean: 0.14 mg/dl, median: 0.033 mg/dl) among cases and from 0 to 9.3 mg/dl (mean: 0.13 mg/dl, median: 0.032 mg/dl) among controls. The risk of developing gastric cancer increased by ~36% among those who were CRP positive; the crude OR equaled 1.36 (95% CI: 0.91–2.02, $P = 0.13$), although with no significance. After being adjusted for potential confounding variables, the point estimate altered substantially and reached the level of statistical significance; the adjusted OR equals 1.90 (95% CI: 1.19–3.02, $P = 0.007$). Among the adjusted covariates, *H.pylori* infection contributed the most to the elevation of risk; adding only *H.pylori* infection to the model elevated the OR to 1.67, which was much higher than the OR for adding CagA seropositivity (adjusted OR = 1.39), atrophy (adjusted OR = 1.48) or even all other lifestyle factors [i.e. cigarette smoking, BMI, family history, history of infectious or inflammatory disease, current drug use of analgesics and salt intake (adjusted OR = 1.47)]. When the cancers were stratified by tumor location and histologic type, the largest OR was demonstrated for cardia cancers, but it failed to reach statistical significance; adjusted OR equaled 3.14 (95% CI: 0.51–19.39, $P = 0.22$). Among non-cardia cancers, the association did not differ much by histologic type. When the analyses were repeated with subjects divided into quartiles according to control distribution of the CRP level (<0.012, 0.012–0.032, 0.032–0.081 and ≥0.081 mg/dl), no apparent association was observed. Compared with the lowest (reference) group, the adjusted ORs (95% CIs) for development of gastric cancer for the second, the third and the highest group were 0.85 (0.56–1.29), 0.96 (0.62–1.47) and 1.35 (0.88–2.07), respectively (P for trend = 0.0496). When CRP was treated as a continuous measure, the adjusted OR for development of gastric cancer was 1.06 (0.87–1.28), for 1 mg/dl increase of log-transformed CRP. Furthermore, non-linear continuous models did not reveal any evidence of dose response.

SAA among cases and controls ranged from 0 to 319.7 μg/ml (mean: 5.9 μg/ml, median: 2.6 μg/ml) and from 0 to 847.5 μg/ml (mean: 7.0 μg/ml, median: 2.5 μg/ml), respectively. For SAA positivity, about a 2-fold increased risk was observed for total gastric cancer and non-cardia cancer; the adjusted ORs (95% CIs) were 1.93 (1.22–3.07, $P = 0.005$) and 2.13 (1.14–3.98, $P = 0.02$), respectively (Table IV). Among adjusted covariates, atrophy as well as *H.pylori* infection contributed most of the elevation of risk. Among non-cardia cancers, no difference was observed by histologic type. The largest OR was demonstrated for cardia cancers, although it failed to reach the level of statistical significance; the adjusted OR equaled 3.84 (95% CI: 0.82–17.99, $P = 0.09$). When results for SAA status were shown separately for men and women, there was no material difference; the adjusted ORs for developing total gastric cancer were 1.95 and 2.15 for men and women, respectively. The adjusted OR for cardia cancer among women could not be calculated because of the small sample size; therefore, all analyses were conducted for men and women combined. No apparent association was observed when SAA

Table III. ORs and 95% CIs of CRP positivity (CRP > 0.18 mg/dl) for development of gastric cancer by tumor subsite and histologic type

	No. of CRP-positive cases/controls	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)[a]	*P*-value
All (494 pairs)	62/47	1.36 (0.91–2.02)	0.13	1.90 (1.19–3.02)	0.007
Cardia (39 pairs)	7/2	3.50 (0.73–16.85)	0.12	3.14 (0.51–19.39)	0.22
Non-cardia (355 pairs)	44/33	1.36 (0.85–2.16)	0.20	2.18 (1.24–3.84)	0.007
Differentiated type (232 pairs)	30/23	1.32 (0.76–2.29)	0.33	1.77 (0.89–3.52)	0.10
Undifferentiated type (107 pairs)	9/8	1.14 (0.41–3.15)	0.80	2.01 (0.53–7.62)	0.30

[a]Cardia cancers, adjusted for cigarette smoking, BMI, family history of gastric cancer, history of infectious or inflammatory disease, current drug use of analgesics and salt intake. All gastric cancers, all non-cardia cancers, differentiated-type non-cardia cancer and undifferentiated-type non-cardia cancer, further adjusted for *Helicobacter pylori* infection, CagA positivity and atrophy.

Table IV. ORs and 95% CIs of SAA positivity (SAA > 8 μg/ml) for development of gastric cancer by tumor subsite and histologic type

	No. of SAA-positive cases/controls	Crude OR (95% CI)	*P*-value	Adjusted OR (95% CI)[a]	*P*-value
All (494 pairs)	75/63	1.26 (0.86–1.86)	0.24	1.93 (1.22–3.07)	0.005
Cardia (39 pairs)	11/5	3.00 (0.81–11.08)	0.10	3.84 (0.82–17.99)	0.09
Non-cardia (355 pairs)	45/39	1.21 (0.74–2.00)	0.45	2.13 (1.14–3.98)	0.02
Differentiated type (232 pairs)	27/25	1.11 (0.59–2.06)	0.75	1.73 (0.81–3.72)	0.16
Undifferentiated type (107 pairs)	12/11	1.14 (0.41–3.15)	0.80	1.80 (0.41–7.92)	0.44

[a]Cardia cancers, adjusted for cigarette smoking, alcohol consumption, BMI, family history of gastric cancer, history of infectious or inflammatory disease, current drug use of analgesics and salt intake. All gastric cancers, all non-cardia cancers, differentiated-type non-cardia cancer and undifferentiated-type non-cardia cancer, further adjusted for *Helicobacter pylori* infection, CagA positivity and atrophy.

level was divided into quartiles (<1.3, 1.3–2.5, 2.5–5.1 and ≥5.1 μg/ml). Compared with the lowest (reference) group, the adjusted ORs (95% CIs) for development of gastric cancer for the second, the third and the highest group were 0.81 (0.53–1.24), 1.06 (0.70–1.61) and 1.19 (0.77–1.85), respectively ($P = 0.20$). When SAA was treated as a continuous measure, the adjusted OR for development of gastric cancer was 1.00 (0.995–1.00) for 1 mg/dl increase of log-transformed SAA. Similar to the analysis of CRP, non-linear continuous models did not reveal any evidence of dose response.

Because of the high correlation between CRP and SAA, we included only the values for the marker being analyzed (Tables III and IV). When CRP and SAA were included in the model simultaneously, the OR was attenuated and was no longer significant for CRP, but was still significant for SAA (data not shown). This may not contradict previous reports that suggest overlapping of the roles of the two markers and delineation of SAA as the more sensitive parameter (13,14).

The observed association did not differ for stratification by smoking status (never/past + current) for SAA; however, for CRP, the association was clearer among never smokers [2.50 (1.13–5.53)] compared with past and current smokers [1.15 (0.56–2.33)]. Using the World Health Organization category to adjust BMI did not alter the results essentially. When the interactions between each covariate in the model and CRP and SAA status were tested, no significant interaction was observed.

When all analyses were repeated in only those who were *H.pylori* positive (seropositive for IgG antibody and/or CagA), the associations were slightly attenuated, although they did not differ essentially; the adjusted ORs (95% CIs) for developing total gastric cancer were 1.72 (1.07–2.78, $P = 0.03$) for CRP-positive status and 1.82 (1.13–2.94, $P = 0.01$) for SAA-positive status, respectively.

Discussion

In this study, the overall distributions of CRP and SAA were not apparently associated with the development of gastric cancer. However, when subjects were divided on the basis of dichotomous categorization of positive versus negative, an increased risk was observed for positive subjects. The association was statistically significant even after adjustment for *H.pylori* infection, CagA status, atrophy and lifestyle factors. Elevated levels of CRP and SAA reflect a generalized host reaction that is either localized or systematic with regard to the initial event. Mechanisms of inflammation-associated tumor development are well described. These include stimulation of cellular proliferation (e.g. in cellular proto-oncogenes, DNA and cellular repair), inhibition of apoptosis, cellular adhesion, stimulation of angiogenesis and cellular transformation (1). In our data set, under the conditions that most subjects were infected with *H.pylori*, only those who reacted strongly to inflammation or had a high host immune response, as reflected by extremely elevated plasma levels of CRP and SAA, showed an elevated risk of developing the malignancy. The proportions of those who were categorized as positive were small; therefore, the findings should be interpreted with caution. However, this may be one of the explanations for why only a small proportion of *H.pylori*-infected subjects develop clinical consequences. CRP and SAA were useful markers to detect these high-risk groups.

Several clinical studies have shown that, compared with controls, gastric cancer patients have elevated CRP levels (24–26). Previous studies have even revealed that CRP has an impact on gastric cancer prognosis (24,27). It has been observed in previous studies that the SAA level increases in patients with stomach, lung, renal, colorectal, breast and other forms of cancers (28–35). With regard to gastric cancer, Chan *et al.* (28) demonstrated that patients with gastric cancer have higher SAA concentrations than do patients with gastric ulcers and healthy subjects and that levels of SAA correlate with tumor status, prognosis and recurrence. In our study, the average duration between blood donation and cancer diagnosis among cases was 5.4 years. When subjects who developed gastric cancer within 2 years of blood donation and their matched controls were excluded, the observed associations were strengthened; the adjusted ORs (95% CIs) for the association between development of gastric cancer and CRP and SAA positivity were 2.25 (1.31–3.85, $P = 0.003$) and 2.29 (1.32–3.95, $P = 0.003$), respectively. Furthermore, when subjects were stratified by the median duration between blood donation and

diagnosis (5.12 years), the adjusted ORs (95% CIs) for the association between development of gastric cancer CRP and SAA positivity within 5.12 years were 1.38 (0.69–2.73, $P = 0.36$) and 1.59 (0.82–3.09, $P = 0.17$), respectively. The values for CRP and SAA diagnosed after 5.12 years were 2.42 (1.23–4.77, $P = 0.01$) and 2.25 (1.12–4.52, $P = 0.02$), respectively. Therefore, our findings cannot be explained by the effect of preclinical samples among cases. Rather, our findings suggest that CRP and SAA may be useful markers for predicting the malignancy.

In our study, *H.pylori* seropositivity, *H.pylori* titer and atrophy were not distributed differently according to CRP status. Surprisingly, *H.pylori* seropositivity and atrophy were more frequent, and higher *H.pylori* titer was observed among SAA-negative subjects than among SAA-positive subjects. When the values were compared on the basis of tumor location, CRP did not show any difference; mean value (SE) was 0.09 (0.16) for cardia and 0.15 (0.05) for non-cardia cancer, respectively ($P = 0.75$). The value for SAA was 6.77 (1.87) for cardia, which was higher than that for non-cardia, 5.12 (0.62) ($P = 0.03$). High SAA level with an upper tumor site compared with a middle or a lower site was also observed by Chan *et al.* (28). Furthermore, the largest OR was observed for cardia cancer for both CRP and SAA. It is well known that *H.pylori* infection is related to non-cardia gastric cancer. As the majority of our subjects were infected with *H.pylori*, we were unable to show the results among *H.pylori*-seronegative subjects. Therefore, we cannot clarify whether the observed phenomenon was independent of *H.pylori*. We can state only that the observed elevated risk of gastric cancer with high levels of CRP and SAA is probably a phenomenon that cannot be totally explained by *H.pylori*; this conclusion is in line with that of previous studies (26,36). Comparing 153 preoperative gastric cancer patients with 19 healthy subjects, Tsavaris *et al.* (26) observed high serum levels of CRP, ceruloplasmin and a1-acid glycoprotein in cancer patients; however, among cancer patients, CRP level did not differ by status of *H.pylori* infection. Also, Delanghe *et al.* (36) showed that neither SAA nor other acute-phase proteins, including CRP, correlated with *Chlamydia pneumoniae* IgG, *H.pylori* IgG and IgA and cytomegalovirus IgG. On the other hand, the reason for the large OR observed in the cardia for both CRP and SAA positivity is unknown. One recent study reported that plasma CRP levels were associated with high BMI and other indicators of obesity (37). On the other hand, some studies, but not all, have proposed that elevated body weight may increase the risk of gastroesophageal reflux, which has been associated with adenocarcinomas of the gastroesophageal junction (38). Therefore, it is possible that elevated CRP and SAA were strongly associated with cardia cancer because of BMI status. However, in our data set, BMI did not differ by either CRP status or SAA status. The observed high OR in cardia cancer may be due to factors other than BMI or may be a mere chance finding.

On the basis of self-reported information, we adjusted for any condition that might alter the plasma levels of CRP or SAA. When these subjects were deleted (61 pairs; corresponds to 12% of total subjects), the overall findings did not change essentially, except when CRP values were divided into quartiles; the P for trend then became not significant ($P = 0.44$). Alternatively, when subjects with an extremely high level of CRP (>0.5 mg/dl) or SAA (>16.5 μg/ml) were excluded (55 pairs; corresponds to 11% of total subjects), the observed ORs became slightly higher, although the overall findings did not change essentially. To ensure the generalizability of findings and statistical power, we retained these subjects in the analyses.

Our study has several limitations. First, among 97 644 eligible subjects of the JPHC Study cohort, 36 745 (38%) men and women participated in the survey and provided blood samples. As reported previously, compared with non-participants, participants in the health checkup survey, especially women, had a different socioeconomic status and a favorable lifestyle profile, such as less smoking and alcohol consumption, greater participation in physical exercise and greater consumption of fruits or green vegetables (39). These findings mean that caution is needed in generalizing or interpreting the results in this report. Second, because of the relatively small sample size, further studies are needed to test our findings in analyses conducted by tumor location and histologic subtype.

The advantage of this study is its population-based prospective design and analysis of prediagnosed blood samples. Also, detailed information including *H.pylori* infection, CagA status, atrophy and environmental factors contributed to the detection of the relationships independent of these factors. Other strengths include negligible loss to follow-up and the satisfactory quality of our cancer registry system during the study period.

In conclusion, the overall distribution of CRP and SAA was not apparently associated with the development of gastric cancer. However, it was suggested that those who react strongly to inflammation or who have high host immune response, as reflected by extremely elevated plasma levels of CRP and SAA, were at high risk to develop gastric cancer.

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

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

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

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

Key words: colorectal adenoma, pepsinogen, chronic atrophic gastritis, case-control study, cancer risk

Abbreviations: BMI: body mass index; CAG: chronic atrophic gastritis; CI: confidence interval; CIMP+: CpG island methylator phenotype; CIN: chromosomal instability; ELISA: enzyme-linked immunosorbent assay; IgG: immunoglobulin G; MSI+: microsatellite instability; OR: odds ratio; PG: pepsinogen; SD: standard deviation; TC: total cholesterol; TG: triglyceride

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

Material and Methods

Subjects

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

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

Questionnaire

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

Evaluation of CAG and *H. pylori* infection

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

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

Screening for colorectal neoplasia

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

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

Table 1. Baseline characteristics of study subjects

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

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

Statistical analysis

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

Results

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Discussion

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

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

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

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

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

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

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

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

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

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