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Cancer development based on chronic active gastritis and resulting gastric atrophy as assessed by serum levels of pepsinogen and *Helicobacter pylori* antibody titer

Takeichi Yoshida[1], Jun Kato[1], Izumi Inoue[1], Noriko Yoshimura[2], Hisanobu Deguchi[1], Chizu Mukoubayashi[1], Masashi Oka[1], Mika Watanabe[1], Shotaro Enomoto[1], Toru Niwa[1], Takao Maekita[1], Mikitaka Iguchi[1], Hideyuki Tamai[1], Hirotoshi Utsunomiya[1], Nobutake Yamamichi[3], Mitsuhiro Fujishiro[3], Masataka Iwane[4], Tatsuya Takeshita[5], Toshikazu Ushijima[6] and Masao Ichinose[1]

[1] Second Department of Internal Medicine, School of Medicine, Wakayama Medical University, Wakayama City, Wakayama, Japan
[2] Department of Joint Disease Research, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan
[3] Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan
[4] Wakayama Wellness Foundation, Wakayama City, Wakayama, Japan
[5] Department of Public Health, School of Medicine, Wakayama Medical University, Wakayama City, Wakayama, Japan
[6] Division of Epigenomics, National Cancer Center Research Institute, Chuo-ku, Tokyo, Japan

Our study investigated the relationship between gastric cancer development and activity of *Helicobacter pylori*-associated chronic gastritis or the resulting chronic atrophic gastritis (CAG). A cohort of 4,655 healthy asymptomatic subjects, in whom serum pepsinogen (PG) and *H. pylori* antibody titer had been measured to assess the activity and stage of *H. pylori*-associated chronic gastritis, was followed for up to 16 years, and cancer development was investigated. In subjects with a serologically diagnosed healthy stomach (*H. pylori*-negative/CAG-negative), cancer incidence rate was low, at 16/100,000 person-years. With the establishment of *H. pylori* infection and progression of chronic gastritis, significant stepwise cancer risk elevations were seen from CAG-free subjects (*H. pylori*-positive/CAG-negative) [hazard ratio (HR) = 8.9, 95% confidence interval (CI) = 2.7–54.7] to subjects with CAG (*H. pylori*-positive/CAG-positive) (HR = 17.7, 95% CI = 5.4–108.6) and finally to subjects with metaplastic gastritis (*H. pylori*-negative/CAG-positive) (HR = 69.7, 95% CI = 13.6–502.9). In *H. pylori*-infected CAG-free subjects, significantly elevated cancer risk was observed in the subgroup with active inflammation-based high PG II level or potent immune response-based high *H. pylori* antibody titer; the former was associated with a particularly high risk of diffuse-type cancer, and both subgroups showed high cancer incidence rates of around 250/100,000 person-years, comparable to that in subjects with CAG. No such risk elevation was observed in *H. pylori*-infected subjects with CAG. These results clearly indicate that gastric cancer develops mainly from the gastritis-atrophy-metaplasia-cancer sequence and partly from active inflammation-based direct carcinogenesis, and that serum levels of PG and *H. pylori* antibody titer provide indices of cancer development in *H. pylori*-infected subjects.

Despite worldwide declines in the incidence of gastric cancer and associated mortality over the past 50 years, this pathology remains one of the leading causes of cancer-related death in Eastern Asia, including Japan, South America and Eastern Europe.[1–4] In Japan, more than 100,000 new cases of gastric cancer are diagnosed every year, and the Japanese Ministry of Health, Labor and Welfare reported that 50,136 deaths attributed to the cancer in 2010.[5] Gastric cancer thus remains a major health problem in Japan. Development of gastric cancer represents a classical example of host–genetic and environmental interactions and is characterized by a multistep process of molecular and morphological events known as the gastritis-atrophy-metaplasia-dysplasia-cancer sequence.[6,7] Based on a large number of epidemiological and clinicopathological studies and also on animal experiments using Mongolian gerbils, this sequence, which predominantly leads to intestinal-type cancer, is considered to represent a major route of stomach carcinogenesis, particularly in areas of high cancer risk, such as Japan. Although other environmental and lifestyle factors together with genetic predisposition also play roles in cancer development *via* this sequence, *Helicobacter pylori* is known as the single most common factor.[6–9] This bacterium colonizes

Key words: atrophic gastritis, *Helicobacter pylori*, cohort study, pepsinogen, cancer high-risk, gastric cancer

Abbreviations: CAG: chronic atrophic gastritis; CI: confidence interval; DR: digital radiography; ELISA: enzyme-linked immunosorbent assay; H. pylori: *Helicobacter pylori*; HR: hazard ratio; PG: pepsinogen; SD: standard deviation

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Correspondence to: Masao Ichinose, Second Department of Internal Medicine, School of Medicine, Wakayama Medical University, 811-1 Kimiidera, Wakayama City, Wakayama 641-0012, Japan, Tel.: +81-73-447-1335, Fax: +81-73-445-3616, E-mail: ichinose@wakayama-med.ac.jp

What's new?

Infection with the bacteria *H. pylori* is the most common risk factor for developing gastric cancer. The infection causes chronic inflammation leading to a sequence of gastritis, atrophy, metaplasia, dysplasia, cancer. This study follows up a cohort of 6,000 men after 16 years and showed that cancer risk increased with the progression of inflammation and atrophy, which can be measured by serum tests. These tests, the authors suggest, would be useful in assessing risk among people infected with *H. pylori*.

the stomach mucosa, probably during childhood, triggering the carcinogenic sequence by inducing life-long chronic inflammation and potently driving the sequence leading eventually to cancer development after several decades. Previous prospective studies, including our own, have indicated *H. pylori* infection as the single largest risk factor for cancer development, and the establishment of chronic atrophic gastritis (CAG) significantly increases the risk of cancer.[10–12] However, the role of *H. pylori* in this process of stomach carcinogenesis is not fully known. In addition, several studies have indicated the possibility that highly active inflammation induced by *H. pylori* is deeply involved in the cancer development, particularly of diffuse-type cancer with higher malignant potential, without passing through the above-described sequence.[7,9,13] One previous long-term follow-up study provided supportive evidence for the hypothesis that incomplete and unstable CAG in the process of extensive atrophy is directly associated with cancer development.[14] Furthermore, our recent study revealed that a group of *H. pylori*-infected CAG-negative subjects with highly active inflammation were at high risk of diffuse-type cancer after a mean follow-up of 5.4 years.[15] As long-term follow-up studies reporting the relationship between activity of *H. pylori*-associated chronic gastritis or the extent of CAG and cancer development are limited, this area requires further study.

In 1994, a cohort of around 6,000 asymptomatic middle-aged male factory workers was established to investigate these problems relating to gastric cancer development in *H. pylori*-associated chronic gastritis. In the cohort, serum levels of pepsinogen (PG) and *H. pylori* antibody titer were measured to evaluate the stage of *H. pylori*-associated gastritis, and interim results on the association between cancer development and stage of gastritis were reported after 8 years of follow-up, showing elevated cancer risk with the progression of gastritis.[12] In our study, we report on the latest findings on gastric cancer development in this cohort after 16 years of follow-up and the risk for gastric cancer based on inflammatory activity and resulting gastric atrophy during the course of *H. pylori*-associated chronic gastritis.

Subjects and Methods

Study population

Our study was conducted in Wakayama City, Wakayama Prefecture, located in the southwestern part of the main island of Japan. The gastric cancer mortality rate in Wakayama Prefecture is among the highest in Japan; in 2010 it ranked fifth among the 47 administrative divisions of Japan, with a mortality rate of 51.6/100,000 person-years, compared to 39.7/100,000 person-years for all of Japan.[16] Details of the present longitudinal cohort study have been described previously.[12] Briefly, participants comprised 5,706 middle-aged (40–59 years old) male employees of a certain workplace, who underwent annual general health checkups to detect incident diseases in the early stage between April 1994 and March 1995. Participants were essentially asymptomatic and could be considered representative of healthy middle-aged men in the general population. The ethics committee of Wakayama Medical University approved the protocol and informed consent was obtained from all participants before enrolment.

Serological analysis

Aliquots of separated sera from fasting blood samples collected as routine laboratory tests for the aforementioned general health checkup program were used for the measurement of serum levels of PG and *H. pylori* antibody titer. Serum PG I and PG II levels were measured using a modification (RIA-Beads Kit; Dainabot, Tokyo, Japan) of our previously reported radioimmunoassay.[17] Subjects with extensive CAG were diagnosed by serum PG levels based on PG test-positive criteria of PG I $\leq$70 ng/ml and PG I/II ratio $\leq$3.0.[18] These criteria offer 70.5% sensitivity and 97% specificity for the diagnosis of extensive CAG.[19] Serum *H. pylori* antibody titers were measured using enzyme-linked immunosorbent assay (ELISA) (MBL, Nagoya, Japan).[20] Subjects were classified as: *H. pylori*-infected, antibody titer >50 U/ml; infection-negative, antibody titer <30 U/ml and indeterminate, antibody titer $\geq$30 U/ml but $\leq$50 U/ml. The sensitivity and specificity of the ELISA used in our study were 93.5 and 92.5%, respectively.[20]

As previously described, *H. pylori*-associated chronic gastritis can be classified into the following four stages based on the results of the two serological tests, *H. pylori* antibody titers and PG.[12] The four groups were as follows: Group A, *H. pylori*-negative/CAG-negative subjects; Group B, *H. pylori*-positive/CAG-negative subjects; Group C, *H. pylori*-positive/CAG-positive subjects and Group D, *H. pylori*-negative/CAG-positive subjects. Groups A, B, C and D thus represent subjects with *H. pylori* infection-free healthy stomachs, *H. pylori*-infected subjects without extensive CAG, subjects with *H. pylori*-induced extensive CAG and subjects with extensive CAG together with widespread intestinal metaplasia

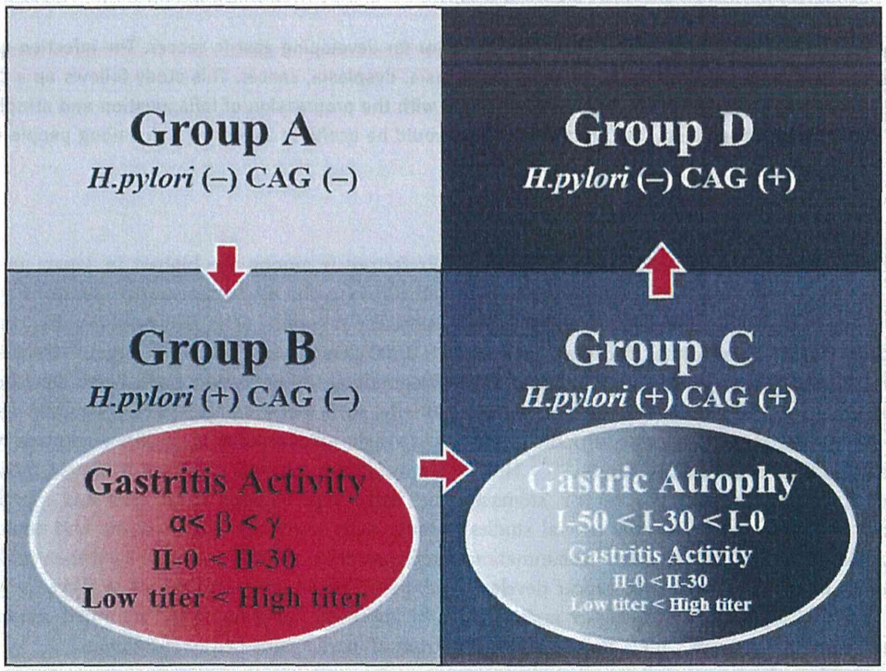

Figure 1. Schematic presentation of groups representing the stages of *H. pylori*-associated chronic gastritis and subgroups representing the gastritis activity or extent of gastric atrophy used in our study. Based on the results of the two serological tests, pepsinogen (PG) and *H. pylori* antibody titer, *H. pylori*-associated chronic gastritis was classified into four groups (A–D). The disease process advances from Group A to D, as described in the Subjects and Methods section. Groups A to D reflect each stage of *H. pylori*-associated chronic gastritis; Group A including subjects with an *H. pylori* infection-free healthy stomach, Group B including *H. pylori*-infected subjects without CAG, Group C including subjects with *H. pylori*-induced CAG and Group D including subjects with extensive CAG together with widespread intestinal metaplasia. Groups B and C were further classified into several subgroups; Group B subjects were stratified into Groups Bα, Bβ and Bγ based on gastritis activity, while Group C subjects were stratified into Groups CI-50, CI-30 and CI-0 based on gastric atrophy, as described in the Subjects and Methods section. Gastritis activity was considered to increase in the order of Groups Bγ, Bβ and Bα, and gastric atrophy was considered to be more extensive in the order of Groups CI-0, CI-30 and CI-50. Similarly, both Groups B and C were stratified into the following subgroups based on gastritis activity: II-30 and II-0, or *H. pylori* antibody high- and low-titer. Gastritis activity was considered to be higher in II-30 (*H. pylori* antibody high-titer) than that in II-0 (*H. pylori* antibody low-titer).

(*i.e.*, metaplastic gastritis), respectively.[12] In addition, our previous study clearly demonstrated that, based on the same PG test-positive criterion used in our study, the test-negative group includes three distinct subgroups from the perspectives of gastritis activity and cancer risk[21]: α, with PG I ≤70 ng/ml and PG I/II ratio >3.0; β, with PG I >70 ng/ml and PG I/II ratio >3.0 and γ, with PG I >70 ng/ml and PG I/II ratio ≤3.0. Thus, in the analysis of Group B (*i.e.*, *H. pylori*-infected subjects with negative PG test), subjects were further classified into the three subgroups of Bα, Bβ and Bγ, and gastritis activity was considered to be higher in the order of Bγ, Bβ and Bα. Furthermore, results of our previous longitudinal cohort study indicated a positive, dose-dependent association between gastric cancer risk and serum levels of PG II or *H. pylori* antibody titer, as markers reflecting the gastritis activity, and that subjects with serum PG II level >30 ng/ml or serum *H. pylori* antibody titer >500 U/ml were at significantly elevated risk of gastric cancer.[22] In analyses of Group B or Group C, subjects were stratified into two subgroups based on serum PG II level (II-0 with PG II ≤30 ng/ml and II-30 with PG II >30 ng/ml) or on *H. pylori* antibody titer (low-titer group with titer ≤500 U/ml and high-titer group with titer >500 U/ml). Gastritis activity was considered to be higher in the II-30 and high-titer groups than in the II-0 and low-titer groups, respectively. Meanwhile, in the analysis of Group C, subjects were stratified into three subgroups according to the extent of *H. pylori*-induced gastric atrophy as assessed by its specific serum marker, PG I, as follows: Group CI-50, PG I >50 ng/ml; Group CI-30, PG I >30 ng/ml and ≤50 ng/ml and Group CI-0, PG I ≤30 ng/ml. Gastric atrophy was considered to be more extensive in the order of Groups CI-0, CI-30 and CI-50. These classifications of stages of *H. pylori*-associated chronic gastritis are illustrated in Figure 1.

Surveillance method

Subjects were screened annually to identify incident gastric cancer cases during the 16-year period between April 1994 and March 2011. Surveys for gastric cancer were conducted using a combination of screening methods, as described

Table 1. Baseline characteristics of study subjects and gastric cancer development according to stage of *H. pylori* -associated chronic gastritis

		H. pylori		CAG		Stage of *H. pylori*–associated chronic gastritis				
Study subjects	**Total**	**Negative**	**Positive**	**Negative**	**Positive**	**Group A**	**Group B**	**Group C**	**Group D**	***p* (trend)**
Subjects (*n*)	4655	998	3657	3293	1362	965	2328	1329	33	
Follow-up years (years)[1]	11.6 (4.3)	13.3 (4.0)	11.2 (4.3)[2]	11.8 (4.3)	11.2 (4.3)[2]	13.3 (3.9)	11.2 (4.3)[3]	11.2 (4.3)[3]	11.1 (5.6)[3]	
Person-years	54,140	13,231	40,909	38,880	15,260	12,865	26,015	14,894	366	
Age (years)[1]	49.5 (4.6)	48.3 (4.5)	49.8 (4.6)[2]	49.1 (4.7)	50.4 (4.3)[2]	48.3 (4.5)	49.5 (4.7)[3]	50.4 (4.3)[3]	49.3 (4.7)	
Alcohol drinking (%)	67.6	68.7	67.3	68.2	66.2	68.7	68.0	66.1	69.7	
Smoking (%)	59.3	61.0	58.8	59.3	59.3	61.4	58.4	59.5	51.5	
H. pylori Ab titer (U/mL)[1]	314.5 (458.1)	19.0 (5.7)	395.1 (486.6)[2]	269.6 (415.3)	423.0 (532.7)[2]	19.0 (5.6)	373.5 (455.2)[3]	433.0 (535.4)[3]	20.1 (6.4)	
PG I (ng/mL)[1]	61.2 (30.4)	58.2 (20.7)	62.0 (32.5)[2]	71.0 (29.2)	37.6 (17.7)[2]	59.4 (19.7)	75.7 (31.1)[3]	37.9 (17.5)[3]	23.1 (19.2)[3]	
PG II (ng/mL)[1]	17.8 (10.8)	9.6 (4.4)	20.0 (11.0)[2]	17.1 (11.7)	19.4 (8.0)[2]	9.5 (4.2)	20.3 (12.4)[3]	19.5 (7.8)[3]	12.2 (9.0)	
PG I/II[1]	4.1 (2.1)	6.4 (1.8)	3.4 (1.6)[2]	4.9 (1.8)	1.9 (0.7)[2]	6.6 (1.5)	4.3 (1.4)[3]	2.0 (0.7)[3]	1.7 (0.8)[3]	
Total gastric cancer										
cases/incidence[4]	87/161	6/45	81/198	39/100	48/315	2/16	37/142	44/295	4/1093	
HR (95%CI)[5]		1	4.1 (1.9–10.6)[2]	1	3.0 (1.9–4.6)[2]	1	8.9 (2.7–54.7)	17.7 (5.4–108.6)	69.7 (13.6–502.9)	<0.0001
Intestinal-type gastric cancer										
cases/incidence[4]	59/109	3/23	56/137	25/64	34/223	1/8	24/92	32/215	2/546	
HR (95%CI)[5]		1	5.4 (2.0–22.2)[2]	1	3.1 (1.9–5.3)[2]	1	11.0 (2.3–197.5)	23.8 (5.1–423.7)	66.6 (6.4–1433.9)	<0.0001
Diffuse-type gastric cancer										
cases/incidence[4]	28/52	3/23	25/61	14/36	14/92	1/8	13/50	12/81	2/546	
HR (95%CI)[5]		1	2.4 (0.8–10.2)	1	2.7 (1.3–5.7)[2]	1	6.7 (1.3–120.9)	11.1 (2.2–203.3)	72.9 (7.0–1568.6)	<0.001

Group A, *H. pylori* (-)/CAG(-); Group B, *H. pylori* (+)/CAG(-); Group C, *H. pylori* (+)/CAG(+); Group D, *H. pylori* (-)/CAG(+).
[1]Mean (SD).
[2]Significantly different from the respective negative group ($p < 0.05$).
[3]Significantly different from Group A ($p < 0.05$).
[4]Per 100,000 person-years.
[5]The adjusted HR was calculated in each group according to Cox proportional-hazards modeling. Adjusted for age, alcohol drinking, and smoking status.

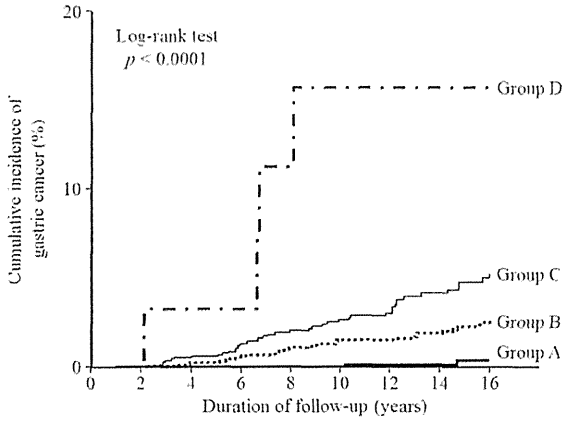

Figure 2. Gastric cancer development according to the stage of *H. pylori*-associated chronic gastritis. Subjects were classified into one of four stages (A–D) of gastritis based on the results of the two serological tests for pepsinogen (PG) and *H. pylori* antibody titer, as described in the Subjects and Methods section. Cumulative incidences of gastric cancer in these four groups were plotted using Kaplan–Meier analysis. Differences among groups were assessed using the log-rank test. Significant stepwise increases in cancer development were evident from Group A to D ($p < 0.0001$) and cancer incidence rates for Groups A, B, C and D were 16/100,000, 142/100,000, 295/100,000 and 1,093/100,000 person-years, respectively.

previously.[12] In short, all subjects were screened using the PG test and double-contrast barium digital radiography (DR).[23] Subjects with positive X-ray findings and/or a positive PG test result based on the above-mentioned criteria were further examined by panendoscopy (Olympus, Tokyo, Japan). Histopathological assessment of the detected cancers was performed on resected specimens obtained by endoscopy or surgery. On the basis of the location from which the specimen was obtained, cancer locations were classified as cardia and noncardia. Pathologically, gastric cancer cases were classified as intestinal- or diffuse-type, according to Lauren's classification.[24] The incident day of gastric cancer was defined as the day of the health checkup on which the cancer was first detected. The length of the observation period was calculated for each subject from the time of the baseline survey to the diagnosis of gastric cancer.

Statistical analysis

Data were analyzed using SPSS version 19.0 software (SPSS, Chicago, IL) and STATA (STATA Corp., College Station, TX). Differences were tested for significance using *t*-test for comparisons between two groups, analysis of variance (ANOVA) for comparisons among multiple groups and Scheffe's least significant difference test for comparisons of pairs of groups. For comparisons of categorical variables, the χ^2 test was used. Long-term effects of serologically diagnosed stages of *H. pylori*-associated chronic gastritis and their subgroups on gastric cancer development were analyzed using the Kaplan–Meier method and statistical differences between curves were tested using the log-rank test. Hazard ratios (HRs) were calculated using Cox proportional hazards modeling. For all comparisons, probability values less than 5% ($p < 0.05$) were considered statistically significant.

Results

After excluding those who met the exclusion criteria, 4,655 middle-aged male subjects [mean (standard deviation, SD) age; 49.5 (4.6) years at the start of the study] remained as the original cohort as described previously,[12] and were followed for up to 16 years [mean (SD), 11.6 (4.3) years]. Table 1 shows the baseline characteristics of study subjects and the details of gastric cancer development. Most subjects (78.6%; 3,657/4,655) were infected with *H. pylori* and 29.3% (1,362/4,655) were CAG-positive. During 54,140 person-years of follow-up, a total of 87 subjects developed gastric cancer, resulting in an incidence rate of gastric cancer in the cohort of 161/100,000 person-years. Seventy-two cases (82.8%) were in the early stage, with tumors confined to the mucosa or submucosa, whereas the remaining 15 cases (17.2%) were in the advanced stage. To date, over 2 years after the end of follow-up, no cases of cancer appear to have escaped detection by the annual screening during the study period. Macroscopically, only four cancers were located in the cardia; most (95.4%) were noncardia cancers. Histologically, 67.8% (59/87) of detected cancers were intestinal-type and the remaining 32.2% (28/87) were diffuse-type. Among the incident cancers, 93.1% (81/87) developed in a *H. pylori*-positive group and 55.2% (48/87) in a CAG-positive group (Table 1). Incidence rates of gastric cancer in the *H. pylori*-positive and -negative groups were 198/100,000 and 45/100,000 person-years, respectively, and those for CAG-positive and -negative groups were 315/100,000 and 100/100,000 person-years, respectively. *H. pylori* infection or coexisting extensive CAG as diagnosed by PG test were thus associated with a significantly increased risk of gastric cancer, particularly intestinal-type, and the respective adjusted HRs were 4.1 [95% confidence interval (CI) = 1.9–10.6] and 3.0 (95% CI = 1.9–4.6), respectively.

Next, cancer development based on the stage of *H. pylori*-associated chronic gastritis was investigated. Figure 2 shows the Kaplan–Meier analysis of subjects in each of the four stages (Groups A–D). After 2 years of the study, subjects in Groups B, C and D developed stomach cancer continuously and steadily, whereas those in Group A developed only two cancers after 10 years of follow-up. Incidence rates of gastric cancer were 16/100,000, 142/100,000, 295/100,000 and 1,093/100,000 person-years for Groups A, B, C and D, respectively (Table 1). As a result, a significant stepwise increase in adjusted HR for gastric cancer was observed among groups from Group A to D, reaching the highest ratio of 69.7 (95% CI = 13.6–502.9) in Group D. This trend was also observed irrespective of the two histological types, intestinal and diffuse, of the cancer.

Table 2. Gastric cancer development among subgroups of Group B subjects stratified by serum levels of pepsinogen or *H. pylori* antibody titer

		Stratification by serum PG level				Stratification by serum PG II level		Stratification by *H. pylori* antibody titer	
Group B subjects	**Total**	**Bα**	**Bβ**	**Bγ**	***p* (trend)**	**BII-0**	**BII-30**	**Low titer**	**High titer**
Subjects (*n*)	2328	1094	898	339		1948	380	1814	514
Follow-up years (years)[1]	11.2 (4.3)	11.5 (4.3)	10.7 (4.3)[2]	11.4 (4.3)		11.2 (4.3)	11.1 (4.4)	11.5 (4.3)	9.9 (4.2)[3]
Person-years	26015	12561	9620	3834		21797	4218	20922	5093
Age (years)[1]	49.5 (4.7)	48.3 (4.7)	49.6 (4.6)[2]	49.9 (4.7)[2]		49.4 (4.7)	49.8 (4.5)	49.5 (4.7)	49.5 (4.6)
Alcohol drinking (%)	68.0	70.8	64.9[2]	67.0		68.3	66.3	68.7	65.6
Smoking (%)	58.4	59.5	61.0	47.9[2]		61.0	45.0[3]	60.8	50.2[3]
H. pylori Ab titer (U/mL)[1]	373.5 (455.2)	298.6 (368.9)	407.9 (486.6)[2]	525.6 (557.8)[2]		347.5 (433.6)	507.2 (533.6)[3]	200.4 (121.8)	984.6 (638.0)[3]
PG I (ng/mL)[1]	75.7 (31.1)	53.4 (11.2)	95.4 (32.5)[2]	96.0 (29.4)[2]		68.4 (22.2)	113.5 (41.3)[3]	74.2 (131.2)	81.2 (30.3)[3]
PG II (ng/mL)[1]	20.3 (12.4)	12.4 (4.0)	22.2 (8.3)[2]	40.6 (14.2)[2]		16.0 (6.0)	42.2 (13.3)[3]	19.3 (11.9)	23.7 (13.4)[3]
PG I/II[1]	4.3 (1.4)	4.6 (1.3)	4.5 (1.2)	2.2 (0.4)[2]		4.6 (1.3)	2.8 (0.8)[3]	4.4 (1.5)	3.8 (1.2)[3]
Total gastric cancer									
cases/incidence[4]	37/142	17/135	9/94	11/287		27/124	10/237	23/110	14/275
HR (95%CI)[5]		1	0.7 (0.3–1.6)	2.1 (1.0–4.6)	0.08	1	2.0 (0.9–4.0)	1	2.7 (1.3–5.2)
Intestinal-type gastric cancer									
cases/incidence[4]	24/92	14/111	5/52	5/130		20/92	4/95	15/72	9/177
HR (95%CI)[5]		1	0.5 (0.2–1.2)	1.1 (0.4–3.0)	0.37	1	1.0 (0.3–2.8)	1	2.6 (1.1–5.9)
Diffuse-type gastric cancer									
cases/incidence[4]	13/50	3/24	4/42	6/156		7/32	6/142	8/38	5/98
HR (95%CI)[5]		1	1.9 (0.4–9.6)	7.1 (1.9–33.7)	0.002	1	4.8 (1.5–14.7)	1	2.8 (0.8–8.4)

Bα, PG I ≤70 ng/mL and PG I/II ratio >3.0; Bβ, PG I >70 ng/mL and PG I/II ratio >3.0; Bγ, PG I >70 ng/mL and PG I/II ratio ≤3.0.
BII-0, PG II ≤30 ng/mL; BII-30, PG II >30 ng/mL; Low titer, *H. pylori* antibody titer ≤500 U/mL; High titer, *H. pylori* antibody titer >500 U/mL.
[1]Mean (SD).
[2]Significantly different from group Bα ($p < 0.05$).
[3]Significantly different from the respective control group ($p < 0.05$)
[4]Per 100,000 person-years.
[5]The adjusted HR was calculated in each group according to Cox proportional-hazards modeling. Adjusted for age, alcohol drinking, and smoking status.

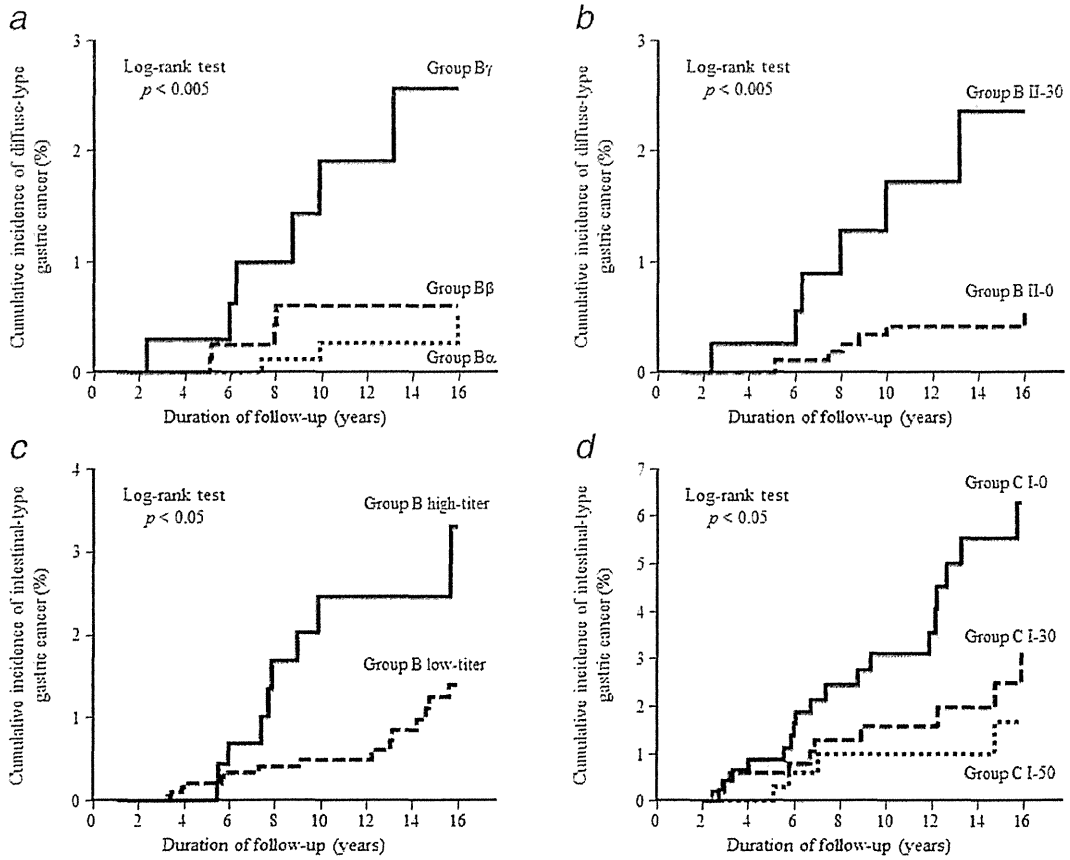

Figure 3. Gastric cancer development according to the activity of *H. pylori*-associated chronic gastritis and also to the extent of the resulting gastric atrophy. (*a*) *H. pylori*-infected subjects without extensive chronic atrophic gastritis (CAG) (Group B) were stratified into three subgroups (Bα, Bβ and Bγ) based on serum PG levels, as described in the Subjects and Methods section. Diffuse-type cancer development in the three subgroups was analyzed by Kaplan–Meier analysis. Cancer incidence rates for Groups Bα, Bβ and Bγ were 24/100,000, 42/100,000 and 156/100,000 person-years, respectively, showing a significant difference between Groups Bβ and Bγ ($p < 0.005$; log-rank test). (*b*) Group B subjects were stratified into two groups based on serum PG II level (BII-0 and BII-30, as in the Subjects and Methods section). Diffuse-type cancer development in each group was analyzed by Kaplan–Meier analysis. Cancer incidence rates for Groups BII-0 and BII-30 were 32/100,000 and 142/100,000 person-years, respectively, showing a significant difference between groups ($p < 0.005$; log-rank test). (*c*) Group B subjects were stratified into two groups based on serum *H. pylori* antibody titer (high- and low-titer groups, as in the Subjects and Methods section). Intestinal-type cancer development in each group was analyzed by Kaplan–Meier analysis. Cancer incidence rates for high- and low-titer groups were 177/100,000 and 72/100,000 person-years, respectively, showing a significant difference between groups ($p < 0.05$; log-rank test). (*d*) *H. pylori*-infected subjects with extensive CAG (Group C) were stratified into three groups based on serum PG I level (CI-50, CI-30 and CI-0, as in the Subjects and Methods section). Intestinal-type cancer development in the three groups was analyzed using Kaplan–Meier analysis. Cancer incidence rates for Groups CI-50, CI-30 and CI-0 were 97/100,000, 174/100,000 and 359/100,000 person-years, respectively. The difference between Groups CI-50 and CI-0 was significant ($p < 0.05$; log-rank test).

As described in the Subjects and Methods section, Group B subjects were further classified into the Bα, Bβ and Bγ subgroups. Table 2 shows baseline characteristics and adjusted HRs for gastric cancer in these three subgroups. Serum levels of PG, particularly PG II, or *H. pylori* antibody titers, which are considered to reflect gastritis activity,[25–28] were higher in the order of Bγ, Bβ and Bα; the differences between Bα and Bβ, and between Bα and Bγ were significant. Of the 37 cancers that developed in Group B, 45.9% (17/37) were from Bα, 24.3% (9/37) from Bβ and 29.7% (11/37) from Bγ. Cancer development was most frequent in Group Bγ, reflecting development of diffuse-type cancer. Figure 3*a* shows the Kaplan–Meier analysis of diffuse-type cancer development in the three subgroups of Group B; incidence rates of diffuse-type cancer were 24/100,000, 42/100,000 and 156/100,000 person-years for Groups Bα, Bβ and Bγ, respectively, showing a significant difference between Groups Bα and Bγ (adjusted HR = 7.1; 95% CI = 1.9–33.7) (Table 2). Percentages of diffuse-type cancer that developed in Bα, Bβ and Bγ were 17.6% (3/17), 44.4% (4/9) and 54.5% (6/11), respectively.

Table 3. Gastric cancer development among subgroups of Group B subjects stratified by serum levels of pepsinogen together with *H. pylori* antibody titer

	Stratification by serum PG level							Stratification by serum PG II level				
	Bα		Bβ		Bγ			BII-0		BII-30		
Group B subjects	Low titer	High titer	Low titer	High titer	Low titer	High titer	*p* (trend)	Low titer	High titer	Low titer	High titer	*p* (trend)
Total gastric cancer												
Cases/subjects	11/906	6/188	6/686	3/212	6/222	5/114		18/1561	9/387	5/253	5/127	
Incidence[1]	103	314	79	150	226	426		100	235	169	397	
HR (95%CI)[2]	1	3.3 (1.1–8.8)	0.8 (0.3–2.1)	1.6 (0.4–5.0)	2.2 (0.7–5.8)	4.6 (1.5–12.8)	0.03	1	2.5 (1.1–5.5)	1.7 (0.6–4.4)	4.4 (1.4–11.2)	0.005
Intestinal-type gastric cancer												
Cases/subjects	9/906	5/188	4/686	1/212	2/222	3/114		14/1561	6/387	1/253	3/127	
Incidence[1]	85	262	53	50	75	255		78	156	34	238	
HR (95%CI)[2]	1	3.4 (1.0–9.9)	0.6 (0.2–2.0)	0.6 (0.0–3.3)	0.9 (0.1–3.3)	3.3 (0.7–11.2)	0.43	1	2.1 (0.8–5.3.4)	0.4 (0.0–2.2)	3.3 (0.8–10.3)	0.19
Diffuse-type gastric cancer												
Cases/subjects	2/906	1/188	2/686	2/212	4/222	2/114		4/1561	3/387	4/253	2/127	
Incidence[1]	19	52	26	100	150	170		22	78	135	159	
HR (95%CI)[2]	1	3.1 (0.1–32.1)	1.5 (0.2–12.6)	6.1 (0.7–51.2)	8.6 (1.7–62.4)	10.5 (1.3–88.0)	0.001	1	3.8 (0.17–4)	6.6 (1.5–28.1)	8.5 (1.2–44.1)	0.001

Bα: PG I ≤70 ng/mL and PG I/II ratio >3.0; Bβ: PG I >70 ng/mL and PG I/II ratio >3.0; Bγ: PG I >70 ng/mL and PG I/II ratio ≤3.0.
BII-0, PG II ≤30 ng/mL; BII-30, PG II >30 ng/mL; Low titer, *H. pylori* antibody titer ≤500 U/mL; High titer, *H. pylori* antibody titer >500 U/mL.
[1]Per 100,000 person-years.
[2]The adjusted HR was calculated in each group according to Cox proportional-hazards modeling. Adjusted for age, alcohol drinking, and smoking status.

Table 2 also shows cancer development in Group B subjects stratified into two groups based on serum PG II level (*i.e.*, Group BII-0 or Group BII-30) or on *H. pylori* antibody titer (*i.e.*, high- or low-titer), as described in the Subjects and Methods section. Gastric cancer of diffuse-type, but not intestinal-type, was significantly more frequent in Group BII-30 (142/100,000 person-years) than in Group BII-0 (32/100,000 person-years) throughout the study period and diffuse-type cancer was the dominant histological type in Group BII-30. The risk of diffuse-type cancer was significantly higher in Group BII-30 than in Group BII-0 (adjusted HR = 4.8; 95% CI = 1.5–14.7) (Fig. 3*b*; Table 2). Meanwhile, in the group with high *H. pylori* antibody titer, gastric cancer development reflecting mainly intestinal-type (275/100,000 person-years) was significantly more frequent than in the low-titer group (110/100,000 person-years) after 6 years of follow-up. The risk of intestinal-type cancer was significantly higher in the high-titer group (adjusted HR = 2.6; 95% CI = 1.1–5.9) (Fig. 3*c*, Table 2).

As inclusion in Group Bγ or presence of a high serum PG II level, and high serum *H. pylori* antibody titer were independent risk factors for gastric cancer by Cox proportional hazards modeling, Groups Bα, Bβ and Bγ, and Groups BII-0 and BII-30 were further stratified by serum *H. pylori* antibody titer. This led to identification of groups of subjects with still higher cancer incidence rate: Group Bγ subjects with high *H. pylori* antibody titer (426/100,000 person-years) and Group BII-30 subjects with high *H. pylori* antibody titer (397/100,000 person-years) (Table 3). Both of these groups were at particularly high risk for diffuse-type cancer.

Table 4 shows cancer development in Group C according to the progression of *H. pylori*-induced gastric atrophy. As described in the Subjects and Methods section, Group C subjects were further stratified into three subgroups based on serum PG I levels: Groups CI-50, CI-30 and CI-0. Gastritis activity as reflected by serum level of PGII or *H. pylori* antibody titer was lower, and the extent of gastric atrophy as reflected by serum level of PGI was greater in the order of Groups CI-0, CI-30 and CI-50. Gastric cancer development, especially that of intestinal-type, increased in a stepwise manner from Group CI-50 to Group CI-0 (Table 4). Figure 3*d* shows the Kaplan–Meier analysis of Group C subjects in the three subgroups. After 3 years of follow-up, the development of intestinal-type cancer in Group CI-0 was highest, followed by Group CI-30 and Group CI-50. Incidence rate of intestinal-type cancer ranged from 97/100,000 person-years in Group CI-50 to 359/100,000 person-years in Group CI-0, showing a significant risk elevation in Group CI-0 (adjusted HR = 2.9; 95% CI = 1.1–10.1). This stepwise increase in incidence rate and HR with reduction in the PG I level was not observed in diffuse-type cancer. Although the stratification of Group C subjects by serum level of PG II or *H. pylori* antibody titer revealed that cancer development in Group C was more frequent in the group of subjects with higher serum level, irrespective of histological type, the cancer risk elevation was not significant (Table 4).

Discussion

Our study analyzed gastric cancer development in healthy asymptomatic middle-aged subjects in a high-risk area in Japan. A cohort of 4,655 male subjects, whose serum PG and *H. pylori* antibody titer had been assessed, was followed for up to 16 years [mean (SD) follow-up, 11.6 (4.3) years], and cancer development according to the four stages of *H. pylori*-associated chronic gastritis as determined by the two serum tests was investigated. During the study period, 87 cancers were detected at an incidence rate of 161/100,000 person-years, lower than the incidence rates reported in other hospital-based studies[10,11,14] and slightly higher than that reported by the follow-up study in the general population (130/100,000 person-years), in which subjects were self-referred for endoscopy and appeared to be more health conscious.[29] Our figure is higher than that reported by population-based cancer registration in Japan in 2007 (128.7/100,000 person-years),[30] probably reflecting the fact that our study region was in a high cancer risk area in Japan.

Our previous study based on the same cohort, analyzing gastric cancer development after 8 years of follow-up, revealed that *H. pylori* infection is deeply involved in the development of gastric cancer, and that progression of *H. pylori*-associated chronic gastritis significantly increased cancer risk.[12] The present results after 16 years of follow-up confirmed these findings and clearly demonstrated that cancer development increased significantly and dramatically with the establishment of *H. pylori* infection (Group B: HR = 8.9; 95% CI = 2.7–54.7), and thereafter, steady stepwise increases in cancer development, irrespective of histological type, were observed as the stage of *H. pylori*-associated chronic gastritis advanced from Group B (incidence rate, 142/100,000 person-years), through Group C with extensive CAG (incidence rate, 295/100,000 person-years) and finally to Group D with metaplastic gastritis (incidence rate, 1,093/100,000 person-years). Further stratification of Group C subjects based on serum PG I level as an index for the extent of gastric atrophy also revealed a significant stepwise risk elevation of intestinal-type cancer as CAG became more widespread. The present results clearly indicate that in the cohort, cancer development occurred steadily following the sequence of gastritis-atrophy-metaplasia-cancer triggered and driven by *H. pylori* infection, confirming this sequence as the main route of gastric cancer development, particularly for intestinal-type, in this high cancer risk area. In addition, during the study period of 16 years, 97.7% (85/87) of cancers developed from stomachs exposed to *H. pylori* infection, while only 2.3% (85/3,690) of *H. pylori*-infected subjects developed cancer. Our results thus strongly indicate that *H. pylori* infection is a necessary factor for cancer development, but by itself is not sufficient to cause cancer, suggesting the involvement of a complex interplay among the bacterium, host genetics and other environmental factors.

Of note is the finding that among the 965 subjects with a *H. pylori* infection-free healthy stomach (Group A), no cancer development at all was seen until 10 years after the start

Table 4. Gastric cancer development among subgroups of Group C subjects stratified by serum levels of pepsinogen or *H. pylori* antibody titer

		Stratification by serum PG I level				Stratification by serum PG II level		Stratification by *H.pylori* antibody titer	
Group C subjects	**Total**	**CI-50**	**CI-30**	**CI-10**	***p* (trend)**	**CII-0**	**CI-30**	**Low titer**	**High titer**
Subjects (*n*)	1329	363	508	458		1204	125	1004	325
Follow-up years (years)[1]	11.2 (4.3)	11.4 (4.3)	11.3 (4.2)	10.9 (4.3)		11.2 (4.3)	11.2 (4.6)	11.4 (4.3)	10.6 (4.4)[2]
Person-years	14,894	4127	5757	5010		13,497	1397	11,441	3453
Age (years)[1]	50.4 (4.3)	49.6 (4.4)	50.2 (4.3)[3]	51.3 (4.1)[3]		50.5 (4.3)	49.9 (4.5)	50.5 (4.3)	50.1 (4.5)
Alcohol drinking (%)	66.1	63.6	67.1	67.0		66.1	66.4	66.3	65.5
Smoking (%)	59.5	50.4	60.6	65.5[3]		59.3	61.6	58.2	63.7
H. pylori Ab titer (U/mL)[1]	433.0 (535.4)	487.4 (644.7)	415.4 (460.9)	409.4 (514.7)[3]		4186 (509.4)	571.7 (729.4)[2]	221.7 (121.5)	1085.6 (750.9)[2]
PG I (ng/mL)[1]	37.9 (17.5)	59.6 (5.8)	40.3 (5.8)[3]	18.1 (7.5)[3]		36.1 (17.0)	55.7 (11.3)[2]	37.6 (17.5)	39.1 (17.7)
PG II (ng/mL)[1]	19.5 (7.8)	26.6 (6.8)	19.9 (6.0)[3]	13.6 (5.2)[3]		17.8 (5.8)	36.1 (5.1)	519.2 (7.7)	20.5 (8.1)[2]
PG I/II[1]	2.0 (0.7)	2.3 (0.5)	22 (0.5)[3]	1.4 (0.6)[3]		2.0 (0.7)	1.6 (0.4)[2]	2.0 (0.7)	1.9 (0.7)
Total gastric cancer									
cases	44	8	13	23		38	6	31	13
Incidence[4]	295	194	226	459		282	429	271	376
HR (95%CI)[5]		1	1.1 (0.5–2.8)	2.1 (1.0–5.0)	0.008	1	1.6 (0.6–3.5)	1	1.4 (0.7–2.7)
Intestinal-type gastric cancer									
cases	32	4	10	18		28	4	23	9
Incidence[4]	215	97	174	359		207	286	201	261
HR (95%CI)[5]		1	1.6 (0.5–5.9)	2.9 (1.1–10.1)	0.003	1	1.5 (0.4–3.8)	1	1.3 (0.6–2.8)
Diffuse-type gastric cancer									
cases	12	4	3	5		10	2	8	4
incidence[4]	81	97	52	100		74	143	70	116
HR (95%CI)[5]		1	0.6 (0.1–2.6)	1.2 (0.3–5.0)	0.43	1	2.0 (0.3–7.6)	1	1.7 (0.5–5.5)

CI-50, PG I >50 ng/mL; CI-30, PG I >30 ng/mL and ≤50 ng/mL; CI-0, PG I ≤30 ng/mL; CII-0, PG II ≤30 ng/mL; CII-30, PG II >30 ng/mL; Low titer, *H. pylori* antibody titer ≤500 U/mL; High titer, *H. pylori* antibody titer >500 U/mL.
[1]Mean (SD).
[2]Significantly different from the respective control group ($p < 0.05$).
[3]Significantly different from group CI-50 ($p < 0.05$).
[4]Per 100,000 person-years.
[5]The adjusted HR was calculated in each group according to Cox proportional-hazards modeling. Adjusted for age, alcohol drinking, and smoking status

of follow-up, and after that only two cancers developed by the end of the study period, resulting in an extremely low incidence rate of 16/100,000 person-years. A comparable, but slightly higher annual cancer incidence rate of 0.04% in Group A-equivalent subjects was reported by Watabe *et al.*,[29] probably owing to lower sensitivity of the antibody detection test, *i.e.*, ELISA, and resultant inclusion of some infected subjects in their equivalent of Group A. Matsuo *et al.* investigated the status of *H. pylori* infection in 3,161 gastric cancer cases resected endoscopically or surgically, and reported that the prevalence of gastric cancers developing in the stomach without *H. pylori* infection was 0.66%.[31] The present results together with those previous findings strongly indicate that in the present epidemiological situation in Japan, development of any histological type of gastric cancer in a healthy *H. pylori*-free stomach is quite rare.

Despite the importance of the gastritis-atrophy-metaplasia-cancer sequence producing mainly intestinal-type cancer, the present results revealed that a considerable proportion (42.5%) of cancers was derived from the group of *H. pylori*-infected subjects without extensive CAG (Group B). The percentage of diffuse-type cancers developing in Group B (35.1%) was significantly higher than that (27.3%) among *H. pylori*-infected subjects with extensive CAG (Group C), and nearly half (46.4%) of the total diffuse-type cancers developed in Group B. These results are consistent with the hypothesis that in addition to induction of the above-described carcinogenic sequence, *H. pylori*-induced inflammation in the stomach leads to direct cancer development, particularly of diffuse-type lesions, without passing thorough an intermediate step of atrophic gastritis together with intestinal metaplasia.[7,9,13] However, precancerous lesions or high-risk conditions for cancer developing from the stomach without extensive CAG have not been fully clarified. Our previous long-term follow-up study based on an endoscopic gastric cancer screening cohort revealed a group at high risk of cancer within subjects without extensive CAG.[15] Among the three distinct subgroups of Group B subjects identified by serum levels of PG and *H. pylori* antibody titer (that is, Groups Bα, Bβ and Bγ), Group Bγ was characterized by highly active gastric inflammation as indicated by the low serum PG I/II ratio (owing to high PG II level) together with high antibody titer and, more importantly, showed a high risk for diffuse-type cancer.[15] The same analysis in our cohort confirmed these findings, and clearly demonstrated Group Bγ as being at the highest risk for cancer among the three subgroups, showing an incidence rate of 287/100,000 person-years, comparable to that of *H. pylori*-infected subjects with extensive CAG (Group C: 295/100,000 person-years). The observed high incidence rate reflects mainly the development of diffuse-type cancer (156/100,000 person-years), and more than half (54.5%) of the cancers developing from Group Bγ were diffuse-type. Previous studies have indicated that *H. pylori* infection in subjects without extensive CAG leads to elevated serum levels of PG, particularly PG II, together with *H. pylori* antibody titer, and the extent of elevation correlates positively with histopathological changes reflecting the activity of mucosal inflammation.[25–28] These elevated serum levels are reversed by *H. pylori* eradication and are thus considered to represent indices for the activity of *H. pylori*-associated gastritis in a stomach without extensive CAG. The observed incidence rate and percentage of diffuse-type cancer, but not intestinal-type, among the three subgroups of Group B subjects thus appear to increase in the order of the gastritis activity as indicated by respective serum levels of PG II or *H. pylori* antibody titer, according well with the above-described hypothesis of diffuse-type cancer development. Along this line, stratification of Group B subjects by serum PG II level also led to the identification of a group of subjects with higher cancer risk, mainly reflecting the risk for diffuse-type cancer. However, contrary to our expectations, stratification of Group B subjects by *H. pylori* antibody titer led to the identification of a high-risk group for intestinal-type cancer. The reason for the difference in histological type of cancer observed between these two different high-risk groups is unclear, but the two serum tests appear to reflect two different aspects of *H. pylori* infection. Although *H. pylori* antibody titer reflects the complex interaction between bacterial infection and host immune response, PG II level appears to reflect the inflammatory process localized to the stomach mucosa. Interestingly, our data are consistent with the results of previous experiments using *H. pylori*-infected Mongolian gerbils, which indicated that animals showing high serum antibody levels predominantly developed intestinal-type cancer.[32–34] Further stratification of Group B subjects with the combination of two serum tests also led to identification of a group of subjects with still higher cancer incidence rate.

Generally, chronic active inflammation associated with *H. pylori* infection is considered to induce host immune response and cellular damage in the infected stomach mucosa resulting from oxidative stress, which will lead to a series of intracellular molecular events and various subsequent genetic and epigenetic alterations.[9,35] In the development of intestinal-type cancer, a wide variety of genetic and epigenetic events including chromosome 1q loss of heterozygosity (LOH), point mutation, microsatellite instability and hypermethylation are reported to be highly prevalent,[36,37] whereas LOH at chromosome 17p and mutation or epigenetic silencing of the E-cadherin/*CDH1* gene are reportedly involved in the development of diffuse-type cancer.[38,39] In addition, our recent study revealed that alteration in DNA methylation level in stomach mucosa correlated closely with activity of *H. pylori*-associated gastritis as assessed by serum levels of PG II and the antibody titer.[40] The present results therefore strongly support the notion that *H. pylori*-induced active inflammation, together with a strong host immune response, induces a series of genetic and epigenetic events that directly lead to the development of gastric cancer, particularly diffuse-type cancer.

In a more advanced stage of *H. pylori*-associated chronic gastritis where CAG is extensive (*i.e.*, Group C), stratification based on serum levels of PG II or *H. pylori* antibody titer did not allow identification of a group of subjects with significantly higher cancer risk. In terms of general tendencies, as the stage of gastritis advances and gastric atrophy together with intestinal metaplasia become extensive, a gradual reduction in *H. pylori* bacterium colonization of gastric epithelia occurs.[41,42] This eventually leads to spontaneous eradication of the bacterium with the establishment of metaplastic gastritis and also to subsequent disappearance of serum antibody specific for the bacterium (*i.e.*, Group D). In this final stage of *H. pylori* infection, the carcinogenic potential reaches the maximal level in the absence of the bacterium together with the absence of active mucosal inflammation. Taken together with the observed enhancement of cancer risk with the progression of *H. pylori*-induced CAG, an inverse correlation exists between bacterial load in the stomach and cancer risk. These results probably reflect that once gastric atrophy and the accompanying intestinal metaplasia become extensive, carcinogenesis based on the mucosal alteration becomes dominant, and thereafter, with the progression of CAG, the power of inflammation-mediated carcinogenesis directly induced by the bacterium becomes less potent. This strongly indicates that in the stage where CAG is extensive, the mucosal alterations induced as a result of long-lasting *H. pylori* infection are more important than the bacterium itself for the development of cancer. Consistent with this, previous studies investigating the effect of *H. pylori* eradication on cancer development have revealed that eradication is not beneficial in subjects with extensive CAG,[43,44] whereas a significant preventive effect was observed in subjects without extensive CAG. Our previous study has also demonstrated a significant reduction in cancer development with eradication therapy in Group B subjects, but not in Group C subjects, strongly indicating the existence of a point of no return during the course of *H. pylori*-induced carcinogenesis.[45] Taken together, subjects in Groups C and D, in which carcinogenic potential is independent of *H. pylori*-induced active inflammation, appear to be beyond the point of no return in the carcinogenic sequence. As a result, without the recovery of mucosal alteration, risk reduction should not be expected following *H. pylori* eradication. From the perspective of the screen-and-treat strategy for *H. pylori* in the prevention of gastric cancer, the above-described subgroups of Group B subjects with highly active gastritis and high risk of cancer appear to be good targets for *H. pylori* eradication.

The present long-term follow-up study confirmed that chronic *H. pylori* infection plays pivotal roles in gastric cancer development along two different pathways. One is based on altered mucosal lesions or conditions generated after long-lasting inflammation, as represented by the atrophy-metaplasia-dysplasia-cancer sequence. The other is active inflammation-based direct carcinogenesis. Our results also clearly demonstrate that serum levels of PG and *H. pylori* antibody titer provide an index of cancer development in these two different pathways of *H. pylori*-induced carcinogenesis, and that use of these two serum markers allows objective estimation of cancer risk in middle-aged individuals in the general population. Although the prevalence of *H. pylori* infection varies widely by geographic area, age, race, ethnicity and socioeconomic status, the reported annual cancer incidence rates in subjects with extensive CAG in several other countries were at comparable levels (0.2–1.1%) to that in our study, whether they are from higher or lower incidence regions.[46–49] The present data could thus be the basis for individually based cancer risk assessments not only in Japan, but also in other countries around the world, particularly where *H. pylori* infection is highly prevalent. As the assessment uses simple serum tests that are reproducible, easy to accept and relatively inexpensive,[18] this approach could be used for risk assessment of large populations and provides significant information not only for cancer development but also for prevention and control of the cancer, including target setting for cancer screening or surveillance, chemoprevention of the cancer by *H. pylori* eradication[45] or administration of selective cyclooxygenase 2 inhibitors.[50]

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厚生労働科学研究委託費

革新的がん医療実用化研究事業

高精度エピゲノム胃がんリスク診断の確立と多層的食道がんリスク診断の開発に関する研究

平成２６年度　委託業務成果報告書

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Genetic polymorphisms of *ADH1B*, *ADH1C* and *ALDH2*, alcohol consumption, and the risk of gastric cancer: the Japan Public Health Center-based prospective study

Akihisa Hidaka[1], Shizuka Sasazuki[1,*], Keitaro Matsuo[2], Hidemi Ito[3], Norie Sawada[1], Taichi Shimazu[1], Taiki Yamaji[1], Motoki Iwasaki[1], Manami Inoue[1,4], Shoichiro Tsugane[1], and for the JPHC Study Group[†]

[1]Epidemiology and Prevention Group, Research Center for Cancer Prevention and Screening, National Cancer Center, Tokyo 104-0045, Japan,
[2] Department of Preventive Medicine, Kyushu University Faculty of Medical Sciences, Fukuoka 812-8582, Japan, [3] Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya 464-8681, Japan, and [4] Graduate School of Medicine, The University of Tokyo, Tokyo 113-0033, Japan

*To whom correspondence should be addressed. Tel: +81 3 3542 2511; Fax: +81 3 3547 8578; Email: ssasazuk@ncc.go.jp

†The members of JPHC Study Group are listed under Appendix.

Abstract

The association between alcohol consumption, genetic polymorphisms of alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) and gastric cancer risk is not completely understood. We investigated the association between *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms, alcohol consumption and the risk of gastric cancer among Japanese subjects in a population-based, nested, case–control study (1990–2004). Among 36 745 subjects who answered the baseline questionnaire and provided blood samples, 457 new gastric cancer cases matched to 457 controls were used in the analysis. The odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated using logistic regression models. No association was observed between alcohol consumption, *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms and gastric cancer risk. However, considering gene–environmental interaction, *ADH1C* G allele carriers who drink ≥150 g/week of ethanol had a 2.5-fold increased risk of gastric cancer (OR = 2.54, 95% CI = 1.05–6.17) relative to AA genotype carriers who drink 0 to <150 g/week (*P* for interaction = 0.02). *ALDH2* A allele carriers who drink ≥150 g/week also had an increased risk (OR = 2.08, 95% CI = 1.05–4.12) relative to GG genotype carriers who drink 0 to < 150 g/week (*P* for interaction = 0.08). To find the relation between alcohol consumption and gastric cancer risk, it is important to consider both alcohol consumption level and *ADH1C* and *ALDH2* polymorphisms.

Introduction

Alcohol consumption is a strong risk factor for some cancers of the head and neck, liver, breast and colon and rectum (1). However, based on many epidemiological studies, the association between alcohol consumption and gastric cancer risk was reported as inconsistent by the World Cancer Research Fund/ American Institute for Cancer Research (2).

In general, the metabolism of ethanol (alcohol) by alcohol dehydrogenases (ADH) is converted into the generation of acetaldehyde, and acetaldehyde is oxidized into nontoxic acetate by aldehyde dehydrogenases (ALDH (3)). Among all classes of ADH and ALDH isoenzymes, ADH1B, ADH1C and ALDH2 are the main ethanol-metabolizing enzymes (4,5). It has been suggested that

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Abbreviations

ADH	alcohol dehydrogenases
ALDH	aldehyde dehydrogenases
BMI	body mass index
CagA	cytotoxin-associated gene A
CI	confidence interval
DM	diabetes mellitus
DR	dietary records
FFQ	food frequency questionnaire
ICD-O	International Classification of Diseases for Oncology
JPHC study	Japan Public Health Center-based prospective study
OR	odds ratio
PHC	public health center.

the metabolism of ethanol leads to accumulation of acetaldehyde (acetaldehyde associated with alcoholic beverages) that is toxic and classified as a group 1 carcinogen in humans by the International Agency for Research on Cancer (IARC (6)). Accumulation of acetaldehyde differs according to functional enzymatic *ADH1B*, *ADH1C* and *ALDH2* genetic polymorphisms. In previous studies, active *ADH1B* allele metabolizes ethanol into acetaldehyde ~40 times more than inactive allele, and active *ADH1C* allele metabolizes ~2.5 times more than inactive allele (5). Furthermore, light drinkers with inactive homozygote *ALDH2* genotype and with heterozygote genotype have 18 times and 5 times higher, respectively, average peaks of acetaldehyde concentrations in blood than moderate drinkers with active homozygote genotypes (7). Therefore, it is important to consider alcohol consumption level and functional genetic polymorphisms of ethanol-metabolizing enzymes to clarify the association between alcohol consumption and gastric cancer risk.

The genotype frequencies of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms differ according to race. The genotype frequencies of *ADH1B* and *ALDH2* polymorphisms are unevenly distributed in Caucasians, but not in Asians (8). Thus, we suggest that it is necessary to evaluate the association of *ADH1B* and *ALDH2* polymorphisms in Asians. In contrast, the genotype frequencies of the *ADH1C* polymorphism are unevenly distributed in Asians, but not in Caucasians (8). However, this polymorphism is also an important gene in alcohol metabolism, and there is no published study regarding the association between the *ADH1C* polymorphism and gastric cancer risk in Asians.

In our study, we selected genetic polymorphisms *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671), which are functionally established single nucleotide polymorphisms, and aimed to clarify the association between these genetic polymorphisms, alcohol consumption and gastric cancer risk in a large-scale Japanese population-based study. Our hypothesis was that drinkers with inactive *ADH1B* and *ADH1C* G alleles would have an increased risk for gastric cancer compared with those with active A alleles. Because inactive allele carriers cannot metabolize ethanol into acetaldehyde, they are less prone to the effects of acetaldehyde such as nausea, increased heart rate and flushing (9). International Agency for Research on Cancer classifies ethanol in alcoholic beverages as a group 1 carcinogen in humans, the same classification as acetaldehyde (6). In addition, drinkers with inactive *ALDH2* A alleles would be at increased risk compared with those with active G alleles because inactive allele carriers cannot oxidize acetaldehyde.

Materials and methods

Study population

The Japan Public Health Center-based prospective study (JPHC study) was launched in 1990 for cohort I (subject age range, 40–59 years) and in 1993 for cohort II (subject age range, 40–69 years) and investigated cancer, cardiovascular disease and other lifestyle-related diseases (10). The JPHC study consisted of 11 public health centers (PHCs) throughout Japan with a total of 140 420 subjects (68 722 men and 71 698 women). Among study subjects, those who registered at two PHC areas (Tokyo and Osaka) were excluded from this study because data regarding cancer incidence was not available or selection of subjects was defined differently from that of other cohort subjects. A population-based cohort of 123 576 subjects (61 009 men and 62 567 women) was established. This study was approved by the Institutional Review Board of the National Cancer Center, Tokyo, Japan.

Baseline survey

In the baseline survey, the study subjects were asked to reply to a self-administered questionnaire about various lifestyle factors, such as socio-demographic characteristics, personal medical history, family history, smoking and drinking habits, dietary habits and physical activity. A total of 99 808 subjects (47 525 men and 52 283 women) responded, giving a response rate of 80.8%.

We excluded subjects who self-reported cancer at baseline (n = 2136), who were not Japanese (n = 18) and who did not live in the area at the baseline (n = 11), which left 97 644 eligible subjects (46 803 men and 50 841 women). One subject reported having cancer at baseline and was also not Japanese. Among the eligible subjects, 36 745 subjects (13 467 men and 23 278 women) provided a 10-ml blood sample at the time of the health check-up conducted by each PHC area. These blood samples were stored at −80°C until analysis. Blood samples were collected from 1990 to 1992 for cohort I and from 1993 to 1995 for cohort II. Following the standard protocol, subjects were asked to avoid having a meal after 21:00 hours on the day before the health check-up and they recorded the last time of caloric intake (including a meal and/or drinking).

Follow-up and cancer registry for JPHC Study

Subjects were observed until 31 December 2004. In Japan, residence and death registration are required by law, and residence status, survival and death were identified annually through residential registries in each area. Among the 36 745 subjects, 3.9% moved outside the study area, 4.4% died and 0.03% were lost to follow-up during the study period, which left 33 701 subjects.

Incidence data regarding gastric cancer cases were identified from two major sources: local major hospitals in the study area and population-based cancer registries. Death certificate information was also used as an information source. In our cancer registry system, 7.6% of gastric cancer cases were based on information first notified via death certificate and 2.1% were registered based on information from the death certificate alone.

Selection of cases and controls

The anatomic site of each case was coded according to the International Classification of Diseases for Oncology (ICD-O), 3rd edition, codes C16.0–16.9 (11). A tumor located in the upper third of the stomach was classified as proximal gastric cancer 'cardia site' (ICD-O code C16.0–16.1), and that in the lower position of the stomach was classified as distal gastric cancer 'noncardia site' (ICD-O code C16.2–16.7). The other cases were tumors that could not be classified because of overlapping lesions (ICD-O code C16.8) or no information (ICD-O code C16.9). The subdivisions by histological type were based on classification derived by Lauren (12). For each case, we selected one control subject who had no history of gastric cancer when the case was diagnosed. Each control was matched to the case for age (±3 years), sex, PHC area, fasting time at blood donation (±5 h) and blood donation date (±2 months). Among 1681 cases diagnosed histologically and registered in cohort I or cohort II (study period from 1990 to 2004), 512 cases replied to a self-administered questionnaire and provided blood. Furthermore, among the 512 new gastric cancer cases, one case was excluded because of a technical error in the measurement of *Helicobacter pylori* (*H.pylori*) and 45 cases for one PHC area in Osaka were excluded because buffy coat was not available. Another nine cases were excluded because of an inadequate concentration of buffy coat for DNA extraction. The final analysis included 457 matched sets of cases and controls. A flowchart of the study subjects is presented in Figure 1.

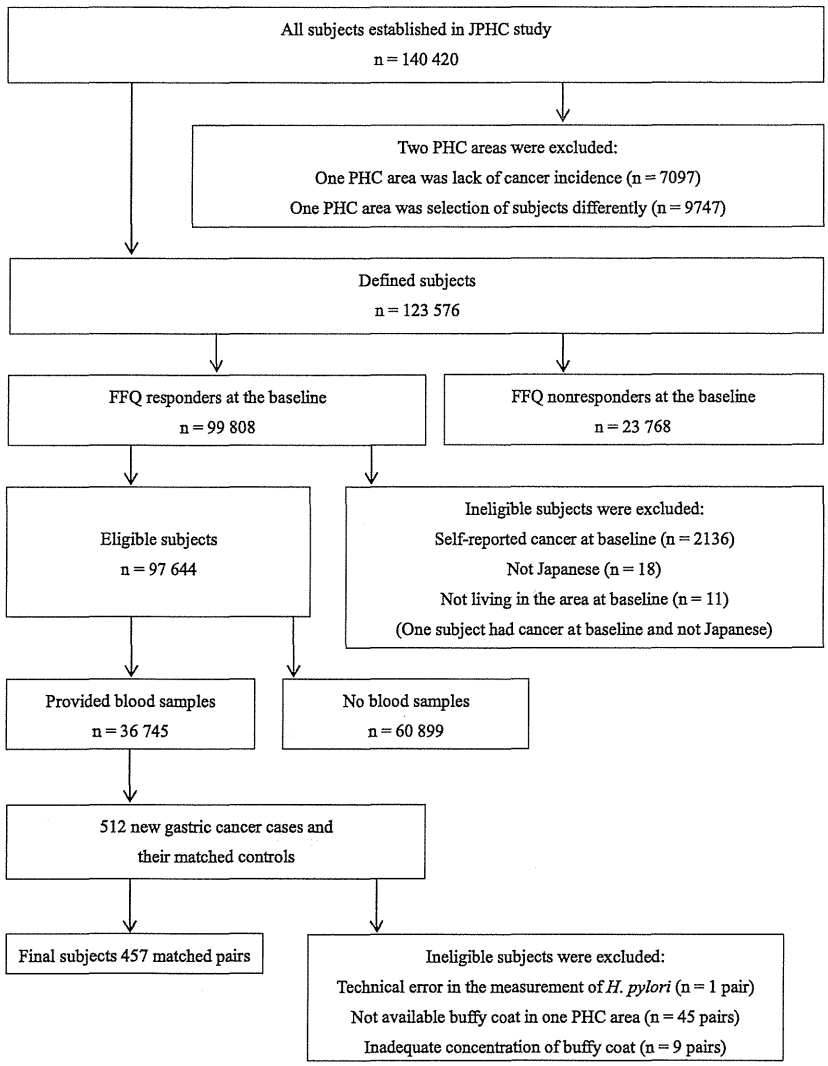

Figure 1. Flowchart of the study subjects.

Assessment of alcohol consumption

Information regarding alcohol consumption was assessed based on the frequency and amount using a validated self-administered food frequency questionnaire (FFQ). During the baseline survey, cohort I and cohort II used slightly different FFQ. In cohort I, the average frequency of alcohol consumption was reported in six categories (almost never, 1–3 days per month, 1–2 days per week, 3–4 days per week, 5–6 days per week and every day). Subjects who drank at least once per week were also asked about the average amount and types of drinks. In cohort II, alcohol consumption status (never, former and current drinkers) was asked first, and then former and current drinkers were asked for more information, similar to cohort I. We then assigned a score to each category of the average frequency of consumption as follows: 1.5 for 1–2 days per week, 3.5 for 3–4 days per week, 5.5 for 5–6 days per week and 7 for every day in cohort I; and 1.5 for 1–2 days per week, 3.5 for 3–4 days per week and 6 for almost every day in cohort II. The amount of alcohol consumption was quantified in grams of ethanol by each type of beverage as follows: 180 ml of sake classified as 23 g of ethanol, 180 ml of shochu or awamori classified as 36 g, 633 ml of beer classified as 23 g, 30 ml of whiskey or brandy classified as 10 g and 60 ml wine classified as 6 g. Finally, we calculated the weekly ethanol intake, which was estimated by multiplying the quantity by the score. In our study, alcohol consumption was classified into three groups: never or occasional drinker; ethanol <150 g per week and ethanol ≥150 g per week. Alcohol consumption levels were defined by the unit *go*, the standard measure of ethanol content of alcoholic beverages in Japan. This unit equals 23 g of alcohol, the amount contained in 180 ml of sake. If a subject drinks 1 *go* every day, he or she is consuming ~150 g of ethanol per week. Validity of this FFQ-based estimated alcohol consumption was evaluated in a subsample of the JPHC study subjects who completed 28-day dietary records (DR). In cohort I, Spearman rank correlation coefficients between the FFQ and DR were 0.79 (n = 94) for men and 0.44 (n = 107) for women, respectively ( ). In cohort II, these results were 0.59 (n = 176) for men and 0.40 (n = 178) for women, respectively ( ).

Assessment of other potential confounding factors

Smoking status was divided into four groups: never smoker, former smoker, current smoker using ≤20 cigarettes per day and current smoker using ≥21 cigarettes per day. Body mass index (BMI) status was divided into three groups: BMI <22 kg/m², 22 kg/m² ≤ BMI <25 kg/m² and BMI ≥25 kg/m². According to a previous prospective study of the association with gastric cancer risk in Japan ( ), the classifications for smoking status and BMI are reasonable. Total calorie intake and salt intake were treated as continuous variables. Family history of gastric cancer was considered positive if at least one parent or sibling had gastric cancer. The *H.pylori* infection status was regarded as positive if subjects had either *H.pylori* antibody ≥10 U/ml or cytotoxin-associated gene A (CagA) antibody >10. Atrophy was regarded

as positive if pepsinogen I was ≤70 ng/ml and pepsinogen I:pepsinogen II ratio was ≤3 (16). History of diabetes mellitus (DM) was considered positive if subjects reported a history of DM and/or drug use for DM at baseline.

Genotyping of ADH1B, ADH1C and ALDH2 polymorphisms

DNA of each subject was extracted from white blood cells in the buffy coat using a FlexiGene DNA kit (Qiagen, Hilden, Germany). Genotyping of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms was analyzed by using TaqMan single nucleotide polymorphism genotyping assays (Applied Biosystems Inc, Foster City, CA). In this assay, fluorescently labeled sequence-specific primers were used in polymerase chain reaction. These measurements were performed with blinding of case and control status. The genotype distributions of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms among controls were all in agreement with Hardy–Weinberg equilibrium ($P > 0.05$).

Statistical analysis

The chi-square test was used to compare baseline characteristics between cases and controls. Matched odds ratios (OR) and their corresponding 95% confidence intervals (CIs) were calculated to indicate the association between alcohol consumption, *ADH1B*, *ADH1C* and *ALDH2* polymorphisms, and gastric cancer risk using conditional logistic regression models. OR1 was matched for age (±3 years), sex, PHC area, blood donation date (±2 months) and fasting time at blood donation (±5 h). OR2 was further adjusted for potential confounding factors such as smoking status, alcohol consumption, total calorie intake, salt intake, BMI, family history of gastric cancer, *H.pylori* infection status, atrophy and history of DM. Data for subjects who were missing values for BMI ($n = 8$), total calorie intake ($n = 1$) and salt intake ($n = 1$) were deleted from the study when adjusting for these confounding factors. When we calculated the effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption, and that of these polymorphisms combined, unconditional logistic regression models were used. We conducted the effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms associated with alcohol consumption with further adjustment for these polymorphisms mutually. Reported P values were two-sided, and $P < 0.05$ was defined as statistically significant. All statistical analyses were performed with SAS software version 9.3 (SAS Institute, Cary, NC).

Results

Baseline characteristics of cases and controls are shown in Table 1. Higher BMI was more frequently distributed among controls than patients with gastric cancer. In contrast, history of DM, family history of gastric cancer, *H.pylori*, CagA positivity and atrophy were more frequently distributed among patients. These results generally agree with previous reports, including the JPHC study (15,17–19).

Table 2 presents the association between alcohol consumption, *ADH1B*, *ADH1C* and *ALDH2* polymorphisms and gastric cancer risk. Alcohol consumption was marginally associated with an increased risk of gastric cancer in the OR1 group compared with never to occasional drinkers; drinkers with ethanol <150 g/week had OR of 0.89 and with ≥150 g/week had OR of 1.29 (P for trend = 0.15). However, after further adjustment for potential confounding factors, the association became null (OR2 group). Compared with *ALDH2* GG genotype, GA and AA genotypes were marginally associated with an increased risk, with OR2 values of 1.09 (95% CI = 0.77–1.54) and 2.01 (95% CI = 0.91–4.48), respectively (P for trend = 0.18). However, *ALDH2* A allele carriers had no risk association compared with GG genotype carriers. We found no association between alcohol consumption and *ADH1B* and *ADH1C* polymorphisms. *ADH1C* GG genotype was rare in this Japanese population.

Table 3 shows the effect modification of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption (gene–environmental interaction). Compared with *ADH1C* AA genotype carriers who drink 0 to <150 g/week, G allele carriers who drink ≥150 g/week had an increased risk, with OR2 value of 2.54 (95% CI = 1.05–6.17); the interaction between alcohol consumption and G allele carriers was statistically significant (P for interaction = 0.02). *ALDH2* A allele carriers who drink ≥150 g/week had an increased risk compared with GG genotype carriers who drink 0 to <150 g/week, with OR2 value of 2.08 (95% CI = 1.05–4.12). A trend toward a positive interaction between alcohol consumption and A allele carrier status was shown (P for interaction = 0.08). No association was shown for *ADH1B* polymorphism and alcohol consumption.

We further examined the effect modification of the combination of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk associated with alcohol consumption (gene–gene–environmental interaction) in Table 4. Compared with the combination of *ADH1B* AA and *ALDH2* GG genotype carriers who drink 0 to <150 g/week, each combination of *ADH1B* AA genotype and *ALDH2* A allele, *ADH1B* G allele and *ALDH2* A allele carriers who drink ≥150 g/week showed a trend toward an increased risk for gastric cancer, with OR2 values of 2.16 (95% CI = 0.83–5.63) and 1.66 (95% CI = 0.66–4.16), respectively. However, the interaction between *ADH1B* G allele and *ALDH2* A allele and alcohol consumption was not statistically significant (P for interaction = 0.40). In addition, compared with the combination of *ADH1C* AA and *ALDH2* GG genotype carriers who drink 0 to <150 g/week, the combination of *ADH1C* G and *ALDH2* A alleles in carriers who drink 0 to <150 g/week showed a statistically significant decreased risk (OR = 0.43, 95% CI = 0.21–0.91). Each combination of *ADH1C* AA genotype and *ALDH2* A, *ADH1C* G and *ALDH2* A alleles in carriers who drink ≥150 g/week showed a marginally increased risk, with OR2 values 1.92 (95% CI = 0.95–3.87) and 8.95 (95% CI = 0.62–129.25), respectively. Moreover, the interaction between *ADH1C* G allele and *ALDH2* A allele and alcohol consumption seemed to be marginally statistically significant (P for interaction = 0.13).

We performed stratified analyses by sex regarding the association of each polymorphism with gastric cancer risk and observed no differences by stratification (data not shown). In addition, the gene–environmental interaction analysis was repeated with stratification by gastric atrophy. Among the subjects with gastric atrophy, *ALDH2* A allele carriers who drink ≥150 g/week had an increased risk of gastric cancer compared with those with GG genotype who drink 0 to <150 g/week (OR2 = 2.71, 95% CI = 1.18–6.27). An interaction between alcohol consumption and A allele was shown (P for interaction = 0.02). However, the subjects without gastric atrophy and *ALDH2* polymorphism did not show a positive association with risk. *ADH1B* and *ADH1C* polymorphisms also did not show any positive association with risk when stratified by atrophy. We also evaluated the combination effects of *ADH1B*, *ADH1C* and *ALDH2* polymorphisms on gastric cancer risk. Compared with *ADH1B* AA, *ADH1C* AA and *ALDH2* GG genotype carriers, OR2s were 1.15 (95% CI = 0.75–1.76) (P for interaction = 0.13) for *ADH1B* G and *ALDH2* A allele carriers and 0.59 (95% CI = 0.30–1.15) (P for interaction = 0.02) for *ADH1C* G and *ALDH2* A allele carriers. Although the interaction between *ADH1C* and *ALDH2* polymorphisms was statistically significant, a chance finding cannot be ruled out because *ADH1C* GG genotype was rare among our study subjects. Analyses considering anatomic site and histological type of gastric cancer were also performed. Cardia site ($n = 76$) was not robustly evaluated because of the small number of subjects. When limited to distal site and intestinal or diffuse type of gastric cancer, *ADH1C* G allele and *ALDH2* A allele carriers who drink ≥150 g/week showed a trend