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Helicobacter pylori Infection, Serum Pepsinogen Level and Gastric Cancer: A Case-Control Study in Japan

Haruhiko Fukuda,[1] Daizo Saito,[1] Shuya Hayashi,[1] Hiroyuki Hisai,[1] Hiroyuki Ono,[1] Shigeaki Yoshida,[1] Yanao Oguro,[1] Takeshi Noda,[1] Toshiya Sato,[2] Masaru Katoh,[3] Masaaki Terada[3] and Takashi Sugimura[3]

[1]*Endoscopy Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104,* [2]*Institute of Statistical Mathematics, 4-6-7 Minami-azabu, Minato-ku, Tokyo 106 and* [3]*Genetic Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104*

We conducted a case-control study to evaluate the effect of *Helicobacter pylori* (HP) infection on the risk of gastric cancer in Tokyo, Japan. The sera at the time of diagnosis from 282 gastric cancer cases and 767 sex- and age-matched cancer-free controls were tested for the presence of anti-HP IgG antibody (HM-CAP ELISA kit) and serum pepsinogen (PG) level (PG I and PG II Riabead). No significant association was observed in all sets [matched odds ratio (OR)=1.04, 95% confidence interval: 0.73–1.49]. In subgroup analyses, however, an association was suggested in females [OR=1.57], a younger population (<50 years) [OR=1.86], early cancer [OR=1.53] and small cancer (<40 mm) [OR=1.55]. Furthermore, we observed a tendency for odds ratios to decrease with an increase in age or cancer growth (depth of tumor invasion and tumor size). Considering that the spontaneous disappearance of HP due to extended mucosal atrophy may lead to these decreasing odds ratios, we applied the conditional logistic model adjusted for the PG I/II ratio as a measure of atrophic gastritis. This analysis showed a positive association with HP infection in all sets [OR=1.69; 1.01–2.81], distal cancer [OR=1.88; 1.07–3.31] and intestinal-type cancer [OR=3.76; 1.39–10.18]. We concluded that the risk of cancer associated with HP infection may be underestimated in studies with cross-sectional exposure because of spontaneous disappearance of HP due to extended mucosal atrophy.

Key words: *Helicobacter pylori* — Stomach neoplasm — Serum pepsinogen — Atrophic gastritis — Case-control study

Although the incidence of gastric cancer has declined worldwide in recent decades, gastric cancer is still one of the leading causes of cancer-death in Japan.[1,2] Several risk factors for gastric carcinogenesis, such as salt, nitrates, low intake of fresh vegetables and β-carotene, have been reported.[1,3,4] Recently, an association has been reported between *Helicobacter pylori* (*H. pylori*) infection and gastric cancer in several prospective and cross-sectional studies.[5–12] *H. pylori* is linked with gastritis and has received widespread attention among gastroenterologists and epidemiologists as a new risk factor of gastric carcinogenesis. Since both the incidence of gastric cancer and the prevalence of *H. pylori* infection are much higher in Japan than in Western countries, examining the association between them would be of practical value.

Blaser *et al.* reported a significant association between *H. pylori* infection and gastric cancer in a putatively high-risk subgroup (non-cardia tumors and age $\leq$70 years) in Japanese patients.[13] In their report, however, conclusive results were not obtained because of the small sample size. Therefore, we conducted a case-control study to evaluate the association between the incidence of gastric cancer and *H. pylori* infection in Tokyo, Japan.

MATERIALS AND METHODS

Study population Four hundred and ninety-nine gastric cancer patients were gastrectomized from 1989 to 1990 in the National Cancer Center Hospital. Of these, 428 patients without multiple stomach cancer or a history of gastrectomy, and for whom stored serum samples were available, were selected for the potential case pool. For the potential control pool, 1295 cancer-free patients were selected out of 18,361 out-patients during the same period, with the following restrictions: no cancerous lesions detected clinically in any organ, stored serum available, a visit within the previous 3 to 6 months and no history of hospitalization in our hospital. Controls were matched to each case according to sex, age (within 3 years) and date of blood sampling (within 3 months) until the available control pool was exhausted. We obtained 297 matched sets with at least one control, and up to 16 controls (297 cases and 786 controls). Of the 297 matched case-control sets, the stored serum samples from 282 cases and 767 controls were available and were included in the analyses. All sera examined in this study had been stored at -20°C.

Assay for serum anti-*H. pylori* IgG antibody and serum pepsinogen level Stored serum samples were tested for anti-*H. pylori* IgG antibody using an HM-CAP ELISA kit (Enteric Products Co., Italy)[14] and patients with a titer of antibody higher than the cut-off value of 2.2 were considered to have *H. pylori* infection. Serum pepsinogen levels in the same samples were assayed for diagnosis of atrophic gastritis using pepsinogen I Riabead and pepsinogen II Riabead radioimmunoassay kits (Dainabot, Tokyo).

Statistical methods Matched analyses were performed. The prevalence of *H. pylori* infection in cases and controls was compared using the Mantel-Haenszel test.[15] Odds ratios, which approximate the relative risk, and 95% confidence intervals (CI) were determined using the Mantel-Haenszel method and a conditional logistic model. All calculations were performed using SAS software (SAS Institute Inc.).

RESULTS

The characteristics of the 282 cases and 767 controls are shown in Table I. Although a simple comparison shows an imbalance, since we permitted unequal matching, all statistical estimates were calculated in mached comparisons. The difference in mean age between cases and controls indicates that more controls were available for younger cases than for older cases.

Histopathological features of the cases are summarized in Table II. Histological subtypes were determined as either intestinal type or diffuse type according to Lauren's classification[16] and tumor locations were classified as either proximal or distal. When the main tumor was located in the upper third of the stomach, the tumors were defined as "proximal cancer"; otherwise they were considered "distal cancer." Tumor size was divided into 4 classes by considering similar numbers of cases (25% quartile, median, and 75% quartile were 24, 40 and 64 mm, respectively).

Table I. Characteristics of Cases and Controls

Characteristic		Cases (N=282)	Controls (N=767)
Age (yrs)	mean	57.1	53.4[a]
	median	58	53
	range	23–83	25–84
Female	(%)	105 (37.2)	416 (54.2)
Male	(%)	177 (62.8)	351 (45.8)
With anti-*H. pylori* antibody (%)		215 (76.2)	567 (73.9)

a) Mean of the mean age of controls in a matched set: 56.5.

Association between *H. pylori* infection and cancer In all sets, the matched odds ratio was 1.04, with a 95% confidence interval of 0.73 to 1.49 (the *P*-value by the Mantel-Haenszel test was 0.820). No significant association was observed.

The results of subgroup analyses by sex, age, histological subtype, tumor location, depth of tumor invasion and tumor size are shown in Table III. The matched odds ratios for each histological subtype and tumor location were similar (1.21 vs. 0.90 and 0.86 vs. 1.09). In contrast, the odds ratios in the other subgroups were quite different.

For age, depth of invasion and tumor size, we recategorized the subjects into 4 classes (Table IV). The odds ratios for each age class decreased with increasing age. In the sets under 50 years old, the odds ratio was high, 1.86 (95%CI: 0.95–3.65), and the odds ratio in the sets over 70 years old was significantly low, 0.33 (95% CI: 0.12–0.90). Odds ratios also decreased in proportion to the depth of tumor invasion. The sets with intramucosal cancer showed a high odds ratio (1.81; 95%CI: 0.94–3.51) and the sets with cancers which invaded the serosal layer showed a low odds ratio (0.61; 95%CI: 0.32–1.15). Odds ratios also decreased roughly in proportion to tumor size.

Next, we examined the associations among these three factors. The depth of invasion was closely associated with tumor size ($P<0.001$, trend test). Thus, these two variables were considered to reflect cancer growth. However, neither the depth of invasion nor the tumor size was associated with age ($P=0.971$ and $P=0.689$, respectively; trend test). Thus, cancer growth and age might contribute independently to the association between *H. pylori* infection and the increased risk of gastric cancer.

Table II. Histopathological Features of the Cases

Features	Number of cases (%)
Histological subtype	
intestinal type	142 (50.4)
diffuse type	140 (49.6)
Location	
proximal	52 (18.4)
distal	230 (81.6)
Depth of invasion	
intramucosa	81 (28.7)
submucosa	72 (25.5)
proper muscle-subserosa	45 (16.0)
serosa	84 (29.8)
Size (mm)	
–24	74 (26.2)
25–39	60 (21.3)
40–64	75 (26.6)
65–	73 (25.9)

Table III. Matched Odds Ratios of *Helicobacter pylori* Infection and Gastric Cancer (2 Levels)

Subgroup		No. of cases (No. of controls)	*P*-value[a)]	Matched odds ratio	95% confidence interval
All matched sets		282 (767)	0.820	1.04	0.73–1.49
Sex	female	105 (416)	0.120	1.57	0.89–2.79
	male	177 (351)	0.281	0.78	0.49–1.23
Age (years)	<50	76 (270)	0.072	1.86	0.95–3.65
	≥50	206 (497)	0.312	0.80	0.52–1.23
Histological type	intestinal type	142 (342)	0.464	1.21	0.73–2.02
	diffuse type	140 (425)	0.691	0.90	0.55–1.49
Location	proximal	52 (112)	0.711	0.86	0.38–1.92
	distal	230 (655)	0.676	1.09	0.73–1.62
Depth of invasion	early cancer	153 (402)	0.093	1.53	0.93–2.49
	advanced cancer	129 (365)	0.119	0.65	0.38–1.12
Tumor size (cm)	<4	134 (351)	0.122	1.55	0.89–2.69
	≥4	148 (416)	0.277	0.77	0.48–1.24

a) The *P*-values and odds ratios were based on the Mantel-Haenszel method.

Table IV. Matched Odds Ratios of *Helicobacter pylori* Infection and Gastric Cancer (4 Levels)

Subgroup	No. of cases (No. of controls)	*P*-value[a)]	Matched odds ratio	95% confidence interval
Age (years) –49	76 (270)	0.072	1.86	0.95–3.65
50–59	86 (266)	0.873	1.06	0.55–2.04
60–69	78 (172)	0.862	0.94	0.46–1.90
70–	42 (59)	0.030	0.33	0.12–0.90
Depth of invasion				
intramucosa	81 (213)	0.077	1.81	0.94–3.51
submucosa	72 (189)	0.557	1.25	0.60–2.61
proper muscle-subserosa	45 (132)	0.608	0.76	0.27–2.14
serosa	84 (233)	0.124	0.61	0.32–1.15
Tumor size (mm)				
–24	74 (191)	0.320	1.43	0.71–2.90
25–39	60 (160)	0.227	1.73	0.71–4.21
40–64	75 (186)	0.998	1.00	0.50–2.00
65–	73 (230)	0.134	0.61	0.31–1.17

a) The *P*-values and odds ratios were based on the Mantel-Haenszel method.

Association between serum pepsinogen level and cancer Serum pepsinogen I level (PG I) ranged from 0 to 163.4 ng/ml (median: 42.1 ng/ml) among all of the cases and from 0 to 217.3 ng/ml (median: 45.4 ng/ml) among all of the controls. Serum pepsinogen II level (PG II) ranged from 0 to 55.2 ng/ml (median: 16.8 ng/ml) among all of the cases and from 0 to 55.2 ng/ml (median: 17.7 ng/ml) among all of the controls. The ratio of pepsinogen I to pepsinogen II (PG I/II ratio) ranged from 0 to 9.1 (median: 2.7) among all of the cases and from 0 to 58.3 (median: 3.9) among all of the controls. We examined the correlation among PG I, PG II, PG I/II ratio and serum anti-*H. pylori* antibody titer (Table V). Among the cases, a significant correlation was observed in all of the possible combinations except that between PG I/II ratio and antibody titer. However, correlation coefficients were high only for the correlation between PG I and PG II (0.5441–0.7019). PG II tended to correlate better with antibody titer in controls ($r=0.4313$) than in the cases ($r=0.1881$).

Matched odds ratios of serum pepsinogen level and gastric cancer are shown in Table VI. Low PG I (<30 ng/ml; OR, 1.42), high PG II (>20 ng/ml; OR, 2.14) and low PG I/II ratio (<3.0; OR, 2.56) were all significantly associated with cancer in all matched sets. Although a significant association was also observed in most of the subgroups, a negative association was observed in 3 subgroups: low PG I in the sets under 50 years

Table V. Correlation Coefficients among Serum Pepsinogen Level and Anti-*Helicobacter pylori* Antibody Titer

	All subjects	Cases	Controls
PG I vs. PG II	0.5774	0.7019	0.5441
PG I vs. HM-CAP	0.1572	0.1523	0.1606
PG II vs. HM-CAP	0.3538	0.1881	0.4313
PG I/II ratio vs. HM-CAP	0.3137	0.0755	0.3797

PG I: Serum pepsinogen I level. PG II: Serum pepsinogen II level. PG I/II ratio: The ratio of serum pepsinogen I to serum pepsinogen II. HM-CAP: Serum anti-*Helicobacter pylori* IgG antibody titer.

Table VI. Matched Odds Ratios of Serum Pepsinogen Level and Gastric Cancer

Subgroup		No. of cases (No. of controls)	Low pepsinogen I (<30 ng/ml)	High pepsinogen II (≥20 ng/ml)	Low pepsinogen I/II ratio (<3.0)
All matched sets		282 (767)[a)]	1.42 (1.03–1.96)	2.14 (1.58–2.90)	2.66 (1.98–3.56)
Sex	female	105 (416)	1.01 (0.59–1.72)	3.06 (1.96–4.78)	2.43 (1.54–3.85)
	male	177 (351)	1.75 (1.16–2.63)	1.64 (1.08–2.50)	2.81 (1.92–4.12)
Histological type	intestinal	142 (342)	1.68 (1.05–2.70)	2.17 (1.38–3.41)	4.16 (2.61–6.64)
	diffuse	140 (425)	1.22 (0.79–1.90)	2.12 (1.41–3.19)	1.89 (1.29–2.76)
Location	proximal	52 (112)	1.46 (0.63–3.38)	2.22 (1.02–4.85)	5.21 (2.49–10.91)
	distal	230 (655)	1.42 (1.00–2.00)	2.13 (1.53–2.96)	2.33 (1.69–3.22)
Age (years)	–49	76 (270)	0.86 (0.44–1.67)	2.99 (1.64–5.47)	2.20 (1.35–3.61)
	50–59	86 (266)	1.83 (1.05–3.16)	1.98 (1.17–3.34)	3.62 (2.08–6.30)
	60–69	78 (172)	1.30 (0.72–2.36)	2.58 (1.50–4.46)	2.06 (1.16–3.66)
	70–	42 (59)	2.65 (1.00–7.00)	0.75 (0.26–2.11)	3.69 (1.51–9.04)
Depth of invasion					
intramucosa		81 (213)	1.33 (0.70–2.54)	2.52 (1.39–4.55)	3.31 (1.79–6.13)
submucosa		72 (189)	1.61 (0.89–2.90)	1.76 (0.99–3.14)	2.30 (1.32–4.03)
proper muscle-subserosa		45 (132)	0.87 (0.39–1.95)	3.21 (1.56–6.59)	1.66 (0.80–3.44)
serosa		84 (233)	1.76 (0.96–3.21)	1.77 (1.00–3.14)	3.26 (1.96–5.44)
Tumor size (mm)	–24	74 (191)	1.47 (0.77–2.81)	2.82 (1.49–5.33)	2.17 (1.22–3.86)
	25–39	60 (160)	1.07 (0.56–2.05)	1.09 (0.59–2.03)	1.79 (0.94–3.41)
	40–64	75 (186)	1.30 (0.70–2.41)	2.68 (1.46–4.94)	2.35 (1.30–4.13)
	65–	73 (230)	2.02 (1.03–3.96)	2.49 (1.39–4.47)	5.21 (2.91–9.35)

a) Matched odds ratio and its 95% confidence interval.

old or with proper muscle or subserosal invasion and high PG II in the sets over 70 years old. Odds ratios tended to be higher with a low PG I/II ratio.

Multivariate regression analyses using the conditional logistic model To evaluate the association between *H. pylori* infection and gastric cancer with regard to the effect of gastric mucosal atrophy, we applied the conditional logistic regression model to our case-control data. This model contains three terms: 1) low PG I or low PG I/II ratio, 2) positive anti-*H. pylori* antibody, and 3) their interaction. The results are shown in Tables VII and VIII. Table VII shows the results of calculations with low PG I (<30 ng/ml). All of the interaction terms were less than one and odds ratios higher than those determined by univariate analysis (Table III) were obtained in all of the columns on the left (positive anti-*H. pylori* antibody). However, these odds ratios were not statistically significant.

Table VIII shows the results of the same calculations using a low PG I/II ratio. Odds ratios in all of the interaction terms except for that of proximal diffuse-type cancer were lower than those in Table VII. The odds ratios for positive anti-*H. pylori* antibody in females, proximal cancer and diffuse type cancer were similar to those in Table VII. In contrast, significantly high odds ratios were observed in distal cancer, intestinal-type cancer and distal intestinal-type cancer.

DISCUSSION

Contrary to several previous prospective studies which have shown a positive association between *H. pylori*

Table VII. Matched Odds Ratios with Conditional Logistic Regression Model, *Helicobacter pylori* Infection, Low Serum Pepsinogen I Level and Gastric Cancer

	With anti-*H. pylori* antibody	Low PG I[a] (PG I < 30)	Interaction between anti-*H. pylori* antibody and low PG I
Total	1.34[b] (0.87–2.08)	2.38 (1.26–4.52)	0.50 (0.24–1.05)
Female	1.98 (0.97–4.05)	1.75 (0.65–4.73)	0.50 (0.16–1.62)
Male	1.05 (0.60–1.83)	3.71 (1.48–9.32)	0.39 (0.14–1.08)
Proximal	1.11 (0.42–2.97)	2.64 (0.54–12.93)	0.42 (0.06–2.80)
Distal	1.41 (0.87–2.29)	2.38 (1.17–4.82)	0.51 (0.23–1.14)
Intestinal type	1.73 (0.89–3.39)	3.05 (1.22–7.63)	0.45 (0.16–1.28)
Diffuse type	1.11 (0.62–1.96)	1.98 (0.80–4.94)	0.52 (0.18–1.52)
Proximal			
intestinal type	2.31 (0.58–9.24)	5.56 (0.74–41.65)	0.23 (0.02–2.32)
diffuse type	0.44 (0.09–2.23)	0.91 (0.05–18.57)	0.59 (0.01–33.13)
Distal			
intestinal type	1.58 (0.74–3.39)	2.58 (0.92–7.28)	0.54 (0.17–1.75)
diffuse type	1.30 (0.69–2.45)	2.31 (0.87–6.10)	0.47 (0.15–1.43)

a) Serum pepsinogen I level.
b) Matched odds ratio and its 95% confidence interval.

Table VIII. Matched Odds Ratios with Conditional Logistic Regression Model, *Helicobacter pylori* Infection, Low Pepsinogen I/II Ratio and Gastric Cancer

	With anti-*H. pylori* antibody	Low PG I/II ratio[a] (I/II ratio < 3.0)	Interaction between anti-*H. pylori* antibody and low PG I/II ratio
Total	1.69[b] (1.01–2.81)	10.92 (5.29–22.54)	0.18 (0.08–0.40)
Female	1.75 (0.83–3.68)	5.46 (1.83–16.26)	0.35 (0.10–1.19)
Male	1.66 (0.82–3.36)	18.37 (6.46–52.22)	0.11 (0.04–0.34)
Proximal	0.96 (0.28–3.30)	11.68 (2.26–60.48)	0.38 (0.06–2.35)
Distal	1.88 (1.07–3.31)	10.85 (4.81–24.44)	0.16 (0.07–0.38)
Intestinal	3.76 (1.39–10.18)	27.50 (8.13–93.02)	0.09 (0.02–0.32)
Diffuse	1.14 (0.62–2.11)	6.48 (2.40–17.52)	0.26 (0.09–0.75)
Proximal			
intestinal	2.39 (0.44–12.98)	30.88 (2.22–430.72)	0.09 (0.01–1.50)
diffuse	0.18 (0.02–1.97)	4.79 (0.51–44.79)	3.36 (0.17–65.99)
Distal			
intestinal	4.60 (1.31–16.15)	30.03 (6.94–130.0)	0.08 (0.02–0.36)
diffuse	1.36 (0.71–2.63)	7.09 (2.30–21.92)	0.20 (0.06–0.66)

a) Ratio of serum pepsinogen I level to serum pepsinogen II level.
b) Matched odds ratio and its 95% confidence interval.

infection and gastric cancer,[8, 9, 17] a positive association was not observed in any of the case-control sets in this study. However, differences between the odds ratios of some of the subgroups suggest that we should not consider these results conclusive: these difference raise the possibility that *H. pylori* infection has different effects on different populations, i.e., it is a positive risk in one subgroup (under 50 years old, female, early cancer and small cancer) and a negative risk in others. Furthermore, the tendency for odds ratios to decrease in the 4 class subgroup analyses, especially with regard to the depth of tumor invasion, made it difficult to interpret these results.

If *H. pylori* infection is a risk factor in the early stage of cancer, it should be associated with a positive risk in the more advanced stages of cancer. Therefore, these strange results can not be interpreted unless some factor(s) which decrease the antibody titer against *H. pylori* in proportion to cancer growth are taken into account.

Some investigators have pointed out that extended atrophy may cause spontaneous disappearance of *H. pylori* from the stomach.[12, 18–20] Therefore, we considered the hypothesis that mucosal atrophy which extends parallel to cancer growth causes us to underestimate the real cancer risk associated with *H. pylori* infection.

Serum pepsinogen levels (low PG I and low PG I/II ratio) are considered to be reliable markers for chronic atrophic gastritis (gastric mucosal atrophy)[21–24] and are useful for serological screening of gastric cancer.[19, 25–32] The extent and severity of atrophic gastritis have been reported to be well-correlated to serum PG I or the PG I/II ratio.

Therefore, we examined the serum pepsinogen level to diagnose extended mucosal atrophy. If our hypothesis is correct and the serum pepsinogen level truly reflects extended mucosal atrophy, the odds ratio of cancer risk can be explained in terms of 1) *H. pylori* infection, which is represented by the presence of serum antibody, 2) mucosal atrophy, as reflected by the PG I level or PG I/II ratio, and 3) their interaction. This final contribution should be less than one, and the odds ratio due to *H. pylori* infection closely estimates the odds ratio between *H. pylori* infection and gastric cancer if mucosal atrophy has not occurred. In our multivariate analyses, especially with regard to the PG I/II ratio (Table VIII), almost all of the interaction terms were less than one. The main effect of *H. pylori* infection, when mucosal atrophy had not occurred, was estimated to be 1.69. *H. pylori* infection was associated with a 70% increase in the risk of cancer, and this was statistically significant at the 5% level. Interestingly, we observed significant odds ratios with distal cancer (1.88), intestinal-type cancer (3.76) and distal intestinal-type cancer (4.60). The odds ratios were not significant in females and diffuse-type cancer in this multivariate analysis. These results indicate that the association between *H. pylori* infection or mucosal atrophy and gastric cancer may differ by sex and histological subtype. These findings are consistent with those in previous prospective studies.[8, 9, 17]

We conclude that *H. pylori* infection is associated with an increased risk of gastric cancer. Moreover, it is possible that extended mucosal atrophy may lead to the spontaneous disappearance of *H. pylori*, which may have caused us to underestimate the cancer risk in this study. Furthermore, the fact that no association was observed in cross-sectional studies in countries with a high prevalence of *H. pylori* infection[33–36] and a cohort study,[37] can be explained by the possibility that the case population may include more individuals who have severe mucosal atrophy, and thus spontaneous disappearance of *H. pylori*, than the control population, and the prevalence of *H. pylori* infection may still be high in the control population at the time of diagnosis in these countries.

We may still underestimate the real risk of *H. pylori* infection for cancer development because patients with intramucosal cancer should show some degree of mucosal atrophy. If we chose patients with gastric dysplasia as cases in a similarly designed case-control study, a higher odds ratio may be revealed. From this perspective, the results of our study are fully compatible with those of previous prospective studies with higher odds ratios which used sera obtained decades before the diagnosis of gastric cancer.[9, 32]

It is important to determine whether PG I, PG II or PG I/II ratio is the most reliable marker for atrophic gastritis or screening of gastric cancer. In the light of the strong correlation between PG I and PG II (Table V) and the results of the multivariate analysis with the PG I/II ratio, which were more compatible with previous reports than the results with PG I, we believe that the PG I/II ratio is a more reliable marker than PG I. Indeed, the PG I level primarily decreased in proportion to the extension of mucosal atrophy. However, taking PG II into account should reduce inter-patient variability and show the association with cancer risk more clearly.

The results obtained in this study encourage us to conduct a large-scale intervention trial for the eradication of *H. pylori*, aimed at decreasing the risk of gastric cancer.

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REFERENCES

1) Correa, P. Human gastric carcinogenesis: a multistep and multifactorial process. First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res.*, **52**, 6735–6740 (1992).
2) Parkin, M. D., Stjernsward, J. and Muir, C. Estimates of the worldwide frequency of twelve major cancers. *Int. J. Cancer*, **41**, 184–197 (1988).
3) Palli, D., Decarli, A., Cipriani, F., Forman, D., Amadori, D., Avellini, C., Giacosa, A., Manca, P., Russo, A., Salkeld, R. M., Samloff, I. M., Fraumoni, J. F., Jr., Blot, W. J. and Buiatti, E. Plasma pepsinogens, nutrients, and diet in areas of Italy at varying gastric cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **1**, 45–50 (1991).
4) Tsugane, S., Kabuto, M., Imai, H., Gey, F., Tei, Y., Hanaoka, T., Sugano, K. and Watanabe, S. *Helicobacter pylori*, dietary factors, and atrophic gastritis in five Japanese populations with different gastric cancer mortality. *Cancer Causes Control*, **4**, 297–305 (1993).

5) Correa, P., Fox, J., Fontham, E., Ruiz, B., Lin, Y. P., Zavala, D., Taylor, N., Mackinley, D., de Lima, E., Portilla, H. and Zarama, G. *Helicobacter pylori* and gastric carcinoma. Serum antibody prevalence in populations with contrasting cancer risks. *Cancer,* **66,** 2569–2574 (1990).
6) Forman, D., Sitas, F., Newell, D. G., Stacey, A. R., Boreham, J., Peto, R., Campbell, T. C., Li, J. and Chen, J. Geographic association of *Helicobacter pylori* antibody prevalence and gastric cancer mortality in rural China. *Int. J. Cancer,* **46,** 608–611 (1990).
7) Forman, D. *Helicobacter pylori* infection: a novel risk factor in the etiology of gastric cancer [editorial]. *J. Natl. Cancer Inst.,* **83,** 1702–1703 (1991).
8) Nomura, A., Stemmermann, G. N., Chyou, P. H., Kato, I., Perez, P. G. and Blaser, M. J. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N. Engl. J. Med.,* **325,** 1132–1136 (1991).
9) Parsonnet, J., Friedman, G. D., Vandersteen, D. P., Chang, Y., Vogelman, J. H., Orentreich, N. and Sibley, R. K. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N. Engl. J. Med.,* **325,** 1127–1131 (1991).
10) Parsonnet, J., Vandersteen, D., Goates, J., Sibley, R. K., Pritikin, J. and Chang, Y. *Helicobacter pylori* infection in intestinal- and diffuse-type gastric adenocarcinomas. *J. Natl. Cancer Inst.,* **83,** 640–643 (1991).
11) Talley, N. J., Zinsmeister, A. R., Weaver, A., DiMagno, E. P., Carpenter, H. A., Perez, P. G. and Blaser, M. J. Gastric adenocarcinoma and *Helicobacter pylori* infection. *J. Natl. Cancer Inst.,* **83,** 1734–1739 (1991).
12) Sipponen, P., Kosunen, T. U., Valle, J., Riihelar, M. and Seppala, K. *Helicobacter pylori* infection and chronic gastritis in gastric cancer. *J. Clin. Pathol.,* **45,** 319–323 (1992).
13) Blaser, M. J., Kobayashi, K., Cover, T. L., Cao, P., Feurer, I. D. and Perez, P. G. *Helicobacter pylori* infection in Japanese patients with adenocarcinoma of the stomach. *Int. J. Cancer,* **55,** 799–802 (1993).
14) Evans, D. J., Evans, D. G., Graham, D. Y. and Klein, P. D. A sensitive and specific serologic test for detection of *Campylobacter pylori* infection. *Gastroenterology,* **96,** 1004–1008 (1989).
15) Breslow, N. E. and Day, N. E. "Statistical Methods in Cancer Research. Vol.1 — The Analysis of Case-Control Studies" (1980). Oxford University Press, New York.
16) Lauren, P. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. *Acta Pathol. Microbiol. Scand.,* **64,** 31–49 (1965).
17) Forman, D., Newell, D. G., Fullerton, F., Yarnell, J. W., Stacey, A. R., Wald, N. and Sitas, F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *Br. Med. J.,* **302,** 1302–1305 (1991).
18) Siurala, M., Sipponen, P. and Kekki, M. *Campylobacter pylori* in a sample of Finnish population: relation to morphology and function of the gastric mucosa. *Gut,* **29,** 909–915 (1988).
19) Asaka, M., Kimura, T., Kudo, M., Takeda, H., Mitani, S., Miyazaki, T., Miki, K. and Graham, D. Y. Relationship of *Helicobacter pylori* to serum pepsinogens in an asymptomatic Japanese population. *Gastroenterology,* **102,** 760–766 (1992).
20) Karnes, W. J., Samloff, I. M., Siurala, M., Kekki, M., Sipponen, P., Kim, S. W. and Walsh, J. H. Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. *Gastroenterology,* **101,** 167–174 (1991).
21) Samloff, I. M., Varis, K., Ihamaki, T., Siurala, M. and Rotter, J. I. Relationship among serum pepsinogen I, serum pepsinogen II and gastric mucosal histology: a study in relatives of patient with pernicious anemia. *Gastroenterology,* **83,** 204–209 (1982).
22) Borch, K., Axelsson, C. K., Halgreen, H., Damkjaer, N. M., Ledin, T. and Szesci, P. B. The ratio of pepsinogen A to pepsinogen C: a sensitive test for atrophic gastritis. *Scand. J. Gastroenterol.,* **24,** 870–876 (1989).
23) Kekki, M., Samloff, I. M., Varis, K. and Ihamaki, T. Serum pepsinogen I and serum gastrin in the screening of severe atrophic corpus gastritis. *Scand. J. Gastroenterol. Suppl.,* **26,** 109–116 (1991).
24) Sitas, F., Smallwood, R., Jewell, D., Millard, P. R., Newell, D. G., Meuwissen, S. G., Moses, S., Zwiers, A. and Forman, D. Serum anti-*Helicobacter pylori* IgG antibodies and pepsinogens A and C as serological markers of chronic atrophic gastritis. *Cancer Epidemiol. Biomarkers Prev.,* **2,** 119–123 (1993).
25) Huang, S. C., Miki, K., Furihata, C., Ichinose, M., Shimizu, A. and Oka, H. Enzyme-linked immunosorbent assays for serum pepsinogens I and II using monoclonal antibodies with data on peptic ulcer and gastric cancer. *Clin. Chim. Acta,* **175,** 37–50 (1988).
26) Miki, K., Ichinose, M., Kawamura, N., Matsushima, M., Ahmad, H. B., Kimura, M., Sano, J., Tashiro, T., Kakei, N., Oka, H., Furihara, C. and Takahashi, K. The significance of low serum pepsinogen levels to detect stomach cancer associated with extensive chronic gastritis in Japanese subjects. *Jpn. J. Cancer Res.,* **80,** 111–114 (1989).
27) Farinati, F., Di, M. F., Plebani, M., Cielo, R., Fanton, M. C., Valiante, F., Masiero, M., De Boni, M., Della, L. G., Burlina, A. and Naccarato, R. Pepsinogen A/pepsinogen C or pepsinogen A multiplied by gastrin in the diagnosis of gastric cancer? *Ital. J. Gastroenterol.,* **23,** 194–196 (1991).
28) Varis, K., Kekki, M., Harkonen, M., Sipponen, P. and Samloff, I. M. Serum pepsinogen I and serum gastrin in the screening of atrophic pangastritis with high risk of gastric cancer. *Scand. J. Gastroenterol. Suppl.,* **26,** 117–123 (1991).
29) Matsukura, N., Onda, M., Tokunaga, A., Fujita, I., Okuda, T., Mizutani, T., Kyono, S. and Yamashita, K. Significance of serum markers pepsinogen I and II for chronic atrophic gastritis, peptic ulcer, and gastric cancer.

J. Clin. Gastroenterol. Suppl. 1, **17,** 146–150 (1993).
30) Miki, K., Ichinose, M., Ishikawa, K. B., Yahagi, N., Matsushima, M., Kakei, N., Tsukada, S., Kido, M., Ishihama, S., Shimizu, Y., Suzuki, T. and Kurokawa, K. Clinical application of serum pepsinogen I and II levels for mass screening to detect gastric cancer. *Jpn. J. Cancer Res.,* **84,** 1086–1090 (1993).
31) You, W. C., Blot, W. J., Zhang, L., Kneller, R. W., Li, J. Y., Jin, M. L., Chang, Y. S., Zeng, X. R., Zhao, L., Fraumeni, J. J., Xu, G. and Samloff, I. M. Serum pepsinogens in relation to precancerous gastric lesions in a population at high risk for gastric cancer. *Cancer Epidemiol. Biomarkers Prev.,* **2,** 113–117 (1993).
32) Parsonnet, J., Samloff, I. M., Nelson, L. M., Orentreich, N., Vogelman, J. H. and Friedman, G. D. *Helicobacter pylori,* pepsinogen, and risk for gastric adenocarcinoma. *Cancer Epidemiol. Biomarkers Prev.,* **2,** 461–466 (1993).
33) Sierra, R., Munoz, N., Pena, A. S., Biemond, I., van Duijn, W., Lamers, C. B., Teuchmann, S., Hernandez, S. and Correa, P. Antibodies to *Helicobacter pylori* and pepsinogen levels in children from Costa Rica: comparison of two areas with different risks for stomach cancer. *Cancer Epidemiol. Biomarkers Prev.,* **1,** 449–454 (1992).
34) Palli, D., Decarli, A., Cipriani, F., Sitas, F., Forman, D., Amadori, D., Avellini, C., Giacosa, A., Manca, P., Russo, A., Samloff, I. M., Fraumeni, J. F., Blot, W. J. and Buiatti, E. *Helicobacter pylori* antibodies in areas of Italy at varying gastric cancer risk. *Cancer Epidemiol. Biomarkers Prev.,* **2,** 37–40 (1993).
35) Takahashi, S., Igarashi, H., Ishiyama, N., Nakamura, K., Masubuchi, N., Ozaki, M., Saito, S., Aoyagi, T., Itoh, T. and Hirata, I. Is *Helicobacter pylori* a causal agent in gastric carcinoma? *Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis.,* **280,** 144–149 (1993).
36) Fukao, A., Komatsu, S., Tsubono, Y., Hisamichi, S., Ohori, H., Kizawa, T., Ohsato, N., Fujino, N., Endo, N. and Iha, M. *Helicobacter pylori* infection and chronic atrophic gastritis among Japanese blood donors: a cross-sectional study. *Cancer Causes Control,* **4,** 307–312 (1993).
37) Estevens, J., Fidalgo, P., Tendeiro, T., Chagas, C., Ferra, A., Leitao, C. N. and Mira, F. C. Anti-*Helicobacter pylori* antibodies prevalence and gastric adenocarcinoma in Portugal: report of a case-control study. *Eur. J. Cancer Prev.,* **2,** 377–380 (1993).