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Prospective study of *Helicobacter pylori* biomarkers for gastric cancer risk among Chinese men

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Abstract

Background—*Helicobacter pylori* is the leading risk factor for gastric cancer, yet only a fraction of infected individuals ever develop neoplasia.

Methods—To identify potential predictive biomarkers, we assessed the association of 15 antibodies to *Helicobacter pylori* proteins and gastric cancer in a nested case-control study. Blood levels of antibodies were assessed using multiplex serology for 226 incident cases and 451 matched controls from the Shanghai Men's Health Study. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression.

Results—Sero-positivity to four (Omp, HP0305, HyuA, and HpaA) proteins were associated with a one-and-a-half to three-fold increased risk for gastric cancer. When excluding cases diagnosed within two years of study enrollment, sero-positivity to two additional proteins (CagA and VacA) showed significant associations with risk. Compared to individuals with ≤3 sero-positive results to the six virulent proteins identified in this population, individuals with 4–5 sero-posit ive results were at a two-fold increased risk (OR=2.08, 95% CI: 1.31–3.30) and individuals sero-positive to all 6 proteins had a three-and-a-half-fold increase in risk (OR=3.49, 95% CI: 2.00–6.11) for gastric cancer. Among individuals diagnosed at least two years after study enrollment, these associations were even stronger (OR=2.79 and OR=4.16, respectively).

Conclusions—Increasing number of sero-positives to six *H. pylori* proteins may be a risk marker for distal gastric cancer in China.

Impact—In a population with a 90% prevalence of CagA-positive *H. pylori* infection, assessment of additional virulent *H. pylori* proteins might better identify individuals at high risk for gastric cancer.

Keywords

Helicobacter pylori; biomarkers; gastric cancer; epidemiology

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INTRODUCTION

Helicobacter pylori, a gram-negative spiral bacterium that resides in the stomach of approximately half of the world's population, is the strongest known risk factor for gastric cancer (1, 2, 3, 4), the second most deadly cancer in the world (5). However, only a small percentage of *H. pylori*-infected people ever develop gastric cancer (6), and it has been postulated that disease risk involves specific interactions between pathogen and host, which, in turn, are likely dependent upon strain-specific bacterial factors. The high level of genetic variation among *H. pylori* isolates provides a unique opportunity to identify potential risk markers that could allow for the classification of individuals infected with *H. pylori* into high- and low-risk groups for targeted prevention. Currently, the most well-known *H. pylori* risk marker is the cytotoxin-associated antigen (CagA), a component of the pathogenicity island which includes a type IV bacterial secretion system, which is present in approximately 60% of *H. pylori* strains in the United States (7). By injecting the CagA into host cells, *cagA*-positive strains of *H. pylori* alter host cell physiology and impact the adaptive immune response to lower the threshold for carcinogenesis (8, 9). However, most persons infected with CagA-positive strains remain disease free. As the majority of the population in China, and other East Asian countries, is not only *H. pylori*-positive, but is infected with CagA-positive strains, the Asia-Pacific consensus is that "the prevalence of *cagA* in Asia is high, and currently identified cagA genotypes in the Asia-Pacific region are not associated with gastric cancer (10). Thus, identifying a novel risk marker for gastric cancer is a promising first step in targeting a high-risk population for prevention.

Recently, *H. pylori* multiplex serology was developed to detect antibody levels directed against 15 *H. pylori* immunogenic proteins (11), selected based on known immunogenicity in two-dimensional immunoblots and known surface exposure (UreA, Catalase, NapA, CagA, HP0231, VacA, and HpaA) (12), specific recognition in *H. pylori*-positive sera (Cagδ and CagM), and serologic association with gastric cancer (GroEl, Cad, HyuA, Omp, and HcpC) and/or gastric ulcer (HP0305 and CagM) (13, 14). The first – and to this point, only – study utilizing this technology to compare sero-positivity to these 15 proteins with gastric cancer risk identified the chaperonin GroEL as a new independent risk marker for distal gastric cancer in Germany (15). As that study used a case-control design, with biospecimens collected after gastric cancer diagnosis and even, for the majority, after starting treatment, associations with antibodies to *H. pylori* proteins could have been affected by disease-associated changes in *H. pylori* markers, disease development-related activation of *H. pylori*, and/or treatment-related loss of *H. pylori*. The current investigation is the first prospective cohort study to assess the associations between sero-positivity to 15 *H. pylori*-specific antigens and risk of distal gastric cancer. For these analyses, we performed a case-control study nested within the Shanghai Men's Health Study, with the aim of identifying novel biomarkers of gastric cancer risk in this high-incidence population.

MATERIALS AND METHODS

Study population

The Shanghai Men's Health Study (SMHS) is a population-based prospective cohort study based in urban Shanghai, China. Previous publications have described the cohort in detail (16, 17), but a summary follows here. From 2002 to 2006, the SMHS recruited 61,582 permanent male residents of Shanghai aged 40–74 years. At baseline, trained interviewers, selected from retired medical professionals, administered detailed in-person interviews using a structured questionnaire to collect information on demographic characteristics, usual diet and other lifestyle factors, disease and surgery history, family cancer history, residential history, and occupational history. Participants were also measured for height, weight, and

waist and hip circumferences at baseline. Additionally, a 10-ml blood sample was drawn from each participant, using a previously described protocol (18). After collection, samples were stored in portable, insulated ice packs and were processed within 6 hours for long-term storage at −80°C. Plasma samples were used for *H. pylori* biomarker analyses. A blood sample collection form was completed for each participant when the sample was procured. The information collected includes date and time of sample collection and time of last meal, as well as intake of selected foods, smoking, and use of any medications over the past 24 hours and during the past week. At baseline, approximately 75% of SMHS participants provided blood samples.

Case identification

Incident gastric cancer cases were ascertained through a combination of registry linkage and active follow-ups. Cancer registration is mandatory in Shanghai, with hospitals required to notify the Shanghai Cancer Registry with each incident case. Study employees then manually check all possible matches with the cohort members, followed by home visits. The Shanghai Vital Statistics Unit is also used to identify causes of death from death certificate data. Additionally, medical charts from the notifying hospitals are reviewed to confirm diagnoses and to collect pathology characteristics of the tumor. At the same time, a study interviewer visits the last known address of every living cohort member every 2 to 3 years. The response rate for the first in-person follow-up (2004–2008) of the SMHS was 97.6% and second (2008–2011) was 92.1%. For the present study, of the 255 gastric cancer cases identified between 2002 and 2009 who donated a blood sample and did not have cancer at baseline or within one month of blood sample collection, the 29 (11.4%) that were classified as gastric cardia cancer with an International Classification of Diseases for Oncology (ICD-O) code of 160 were excluded, as gastric cardia cancer appears to have multiple etiologies that vary in their association with *H. pylori* infection (19). The present study thus includes 226 incident cases of distal gastric cancer, defined as having an ICD-O code of 161–166, 168, or 169. Distal gastric cancers were diagnosed a median of 3.6 years after blood collection (range = 1 month to 8 years).

Control selection

Two controls for every case were chosen, matched on: age (within 2 years), date (within 30 days) and time (morning or afternoon) of sample collection; time interval since last meal (within 2 hours); antibiotic use in the last week (yes/no); and availability of plasma. After excluding gastric cardia cases and their matched controls, this study included 451 controls for the 226 cases of distal gastric cancer.

Helicobacter pylori multiplex serology

Sero-status of antibodies to fifteen *H. pylori* specific antigens (UreA, Catalase, GroEL, NapA, CagA, CagM, Cagδ, HP0231, VacA, HpaA, Cad, HyuA, Omp, HcpC, HP0305) was determined by *H. pylori* multiplex serology (11). The multiplex method is based on a glutathione *S*-transferase (GST) capture immunosorbent assay combined with fluorescent-bead technology (20, 21). Recombinant GST-*H. pylori* fusion proteins were used as antigens, loaded and affinity-purified on spectrally distinct glutathione-casein-coupled (GC) fluorescence-labeled polystyrene beads (SeroMap, Luminex, Austin, Texas). Antibodies bound to the beads via the GST-*H. pylori* fusion proteins were stained with biotinylated goat anti-human IgA, IgM, IgG (Dianova, Hamburg, Germany) and the reporter conjugate R-phycoerythrin-labeled streptavidin. A Luminex 100 analyzer identified the internal bead color and thus the antigen carried by the bead. The quantity of bound antibodies was determined as the median reporter fluorescence intensity (MFI) of at least 100 beads per bead set per serum. Antigen-specific cut-point values for each of the 15 antigens are base on 46 sera defined as *H. pylori* sero-negative in a previous study (22) and run within this assay.

They were calculated as the arithmetic mean MFI plus three standard deviations and excluding positive outliers. All specimens were analyzed once at 1:100 dilution within a single assay day. The assays were validated for both serum and plasma samples. *H. pylori* sero-positivity was defined as sero-positivity to >3 proteins, which has shown excellent agreement (kappa=0.70) with commercial serological assay classification (11). To test the reliability of the assay within our population, twelve quality-control samples from one pooled sample were analyzed on 4 separate plates (3 on each plate). The determination of sero-positivity for all of the 15 *H. pylori* proteins detected was highly consistent, as the results were identical on 3 plates (consisting of 135 tests), and on the fourth plate only 5 of the 45 tests (11.1%) were not identical with the others. In a previous study, we have included 5 replicates for 2 individual controls, and the results of these quality control samples were very similar, whereby only 1 (0.3%) replicate of 30 was not identical with the others in determination of sero-positivity for all *H. pylori* proteins (22).

Statistical analysis

The association of distal gastric cancer risk with sero-positivity for each of the 15 individual *H. pylori* proteins was initially assessed using conditional logistic regression to determine odds ratios (ORs) and 95% confidence intervals (CIs) for distal gastric cancer by protein-specific sero-positivity.. Potential confounders, as determined at baseline, of smoking status, regular aspirin use, and fruit intake, were considered, but these gastric cancer risk factors were not associated with *H. pylori* sub-type in this population, and did not substantially affect the main results. Additionally, markers of socio-economic status (education, income, and occupation) were also assessed as potential confounders, but were not associated with gastric cancer incidence and did not affect the associations between antibodies to specific *H. pylori* proteins and gastric cancer risk.

To assess the overall association of *H. pylori* protein-specific sero-positivity and distal gastric cancer risk, a new variable was created to indicate the number of sero-positive results for each individual to each of the six *H. pylori* proteins identified in the previous analyses to be potential virulence factors in this population. Multivariable conditional logistic regression was again used to examine the association in separate models utilizing indicator variables for category of number of sero-positive results (with sero-positivity of 0–3 as the reference category) and modeling the number of sero-positives as a continuous variable.

To minimize the influence of undiagnosed subclinical or pre-malignant lesions on levels of antibodies to *H. pylori* proteins, we assessed the association of gastric cancer with *H. pylori* protein antibodies in the subset of cases (and their matched controls) who were diagnosed with distal gastric cancer two or more years after blood collection, in the same manner as described above. All statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

RESULTS

At baseline, compared to controls, cases were more likely to be current smokers and to have been given a diagnosis of chronic gastritis, were less likely to be regular users of aspirin, and had a lower average daily fruit intake (Table 1). No differences between cases and controls were seen for the socio-economic status measures of education, income, or occupation. The overall sero-prevalence of *H. pylori* (based on the definition of sero-positivity to more than 3 *H. pylori* proteins using multiplex serology) was high (94% among controls and 98% among cases), as expected, resulting in an adjusted odds ratio for the association with distal gastric cancer risk of 2.79 (95% CI = 1.02 to 7.59).

Individually, sero-positivity to ten of the fifteen *H. pylori* proteins examined were suggestive of an increased odds of distal gastric cancer risk, although the associations reached significance for only four: Omp (OR = 2.74, 95% CI = 1.48 to 5.05); HP0305 (OR = 2.29, 95% CI = 1.57 to 3.35); HyuA (OR = 1.50, 95% CI = 1.07 to 2.08); and HpaA (OR = 1.44, 95% CI = 1.03 to 2.00) (Table 2). When excluding those cases who were diagnosed within two years of blood collection (and their matched controls), the associations with Omp, HP0305, and HpaA became even stronger, and two additional proteins – CagA and VacA – were also found to be significantly associated with gastric cancer risk.

To determine the combined effects of sero-positivity to the *H. pylori* proteins identified in these analyses, risk by number of sero-positive results to six proteins – Omp, HP0305, HyuA, HpaA, CagA, and VacA – was assessed (Table 3). Compared to individuals with 3 or fewer sero-positive results, individuals with 4 or 5 sero-positive results had a 2-fold increased risk (OR = 2.08, 95% CI = 1.31 to 3.30) and individuals sero-positive to all 6 *H. pylori* proteins had a 3-and-a-half-fold increased risk (OR = 3.49, 95% CI = 2.00 to 6.11) for distal gastric cancer. This model was a significantly better fit than that with CagA sero-positivity alone (likelihood ratio tests resulted in values of 21.1 with 2 degrees of freedom compared to 2.0 with 1 degree of freedom for the two models, respectively, $P<0.0001$ for the difference). When considered continuously, there was a 1.38-fold increased odds for distal gastric cancer for each additional sero-positive ($P < 0.0001$). When limiting the analyses to individuals who were diagnosed 2 or more years after blood draw, the association with number of sero-positives became even stronger: an almost 3-fold increased risk for individuals with 4 to 5 sero-positive results (OR = 2.79, 95% CI = 1.56 to 4.96) and a 4-fold increased risk for gastric cancer for individuals sero-positive to all 6 proteins (OR = 4.16, 95% CI = 2.08 to 8.32). This also resulted in a 1.49-fold increased odds of gastric cancer for each additional sero-positive result ($P = <0.0001$).

DISCUSSION

This is the first prospective cohort study to utilize novel *H. pylori* multiplex serology evaluating antibodies to 15 *H. pylori* proteins in association with risk of gastric cancer. We identified 6 proteins as potential novel risk markers for distal gastric cancer among Chinese men. When considering sero-positivity to these proteins collectively, the associations were much stronger than that of CagA sero-positivity alone. Among the subset of individuals who were diagnosed two or more years after blood draw (i.e., those least likely to have a pre-malignant lesion), the associations with the identified *H. pylori* serum risk markers were even stronger.

The only other published analysis that evaluated these same *H. pylori* proteins by multiplex serology are from a German case-control study, which included 123 gastric cancer cases (100 of which were distal gastric cancer) and 492 controls without a history of chronic atrophic gastritis (15). In that study, eight of the fifteen *H. pylori* proteins assessed were significantly associated with gastric cancer, and associations were strongest when cases of gastric cardia cancer were excluded. Three of the proteins found to be significantly associated with gastric cancer in the German population (HP0305, VacA, and HyuA) were also significant risk factors in the current study of a Chinese population, but one of the proteins with the strongest effect in Germany – GroEL, a bacterial chaperone – had no association with gastric cancer in China. These differences are not unexpected, as not only are the *H. pylori* infection rates in the general populations quite different (i.e., 53% of controls were *H. pylori*-positive in Germany, vs. 94% of the controls in China), as well as the prevalence of antibodies to individual *H. pylori* proteins, but the virulence of *H. pylori* strains also vary extensively by geographic origin. For example, in Colombia, Correa et al. have investigated *H. pylori* genotypes among residents in two distinct areas: a mountainous

region with a very high risk of gastric cancer (150 cases per 100,000 residents) and a coastal region with a low risk of gastric cancer (6 cases per 100,000 residents) (23). While the majority of individuals in both regions are infected with CagA+ strains of *H. pylori*, all of the strains analyzed from a high gastric cancer risk region were of European phylo-geographic origin, whereas those from the low gastric cancer risk region were predominantly of African phylo-geographic origin. Furthermore, European strain was strongly associated with greater histologic severity whereas African strain was associated with reduced severity (24). While the association of gastric cancer and sero-positivity to the most well-known virulence factor, CagA, did not reach statistical significance in the present study for the entire population, the estimated 2-fold increase in risk is consistent with that found in other studies of Chinese populations (25, 26), although the associations with the proteins Omp (an outer membrane protein), HyuA (hydantoin utilization protein A), HP0305 (a conserved hypothetical protein), and HpaA (a flagellar sheath adhesion lipoprotein) are novel biomarkers for this population. Additionally, the current analyses are the first we are aware of to consider all of these proteins together as an aggregate virulence marker of gastric cancer risk in China.

Identification of high-risk individuals based on *H. pylori* serum risk markers has great potential for cancer prevention as *H. pylori* eradication appears to substantially reduce subsequent cancer risk. Specifically, a recent meta-analysis of six randomized trials (4 in China, 1 in Japan, and 1 in Colombia) comparing eradication with no treatment in *H. pylori*-positive individuals found a significant 35% reduction in gastric cancer incidence (pooled relative risk = 0.65, 95% CI = 0.43 to 0.98) (27). Furthermore, in one of these trials, in a high gastric cancer-incidence area in China, while overall there was no significant difference in gastric cancer incidence comparing those receiving *H. pylori* eradication treatment and those receiving placebo, restriction of the analyses to individuals without precancerous lesions demonstrated that *H. pylori* eradication was significantly associated with a reduced risk in gastric cancer incidence ($P = 0.02$) (28). And, just this year, the long-term effects of a 2-week antibiotic treatment in Shandong, China were published, indicating a significant 39% reduction in gastric cancer risk (29).

The finding in the present study that newly identified *H. pylori* protein risk markers have a stronger association with gastric cancer when subjects diagnosed within two or even three years of blood collection are excluded is consistent with the notion that the progression of gastric cancer creates an environment less favorable to *H. pylori* infection (30). Similar results were found from a combined analysis that illustrated a significant trend towards an increased association between *H. pylori* sero-positivity and gastric cancer incidence by increased time between sample collection and diagnosis (31). This finding is also informative for future intervention studies of *H. pylori* eradication, as well as the possibility of future screening and treatment programs for those at high risk for gastric cancer, as it appears that earlier intervention increases the protective effect of *H. pylori* eradication treatment (32). Identifying those persons with *H. pylori* at high risk for gastric cancer is necessary as the vast majority of individuals in high-incidence areas are *H. pylori* positive, but most do not develop cancer, therefore, massive eradication campaigns are not feasible.

Of note, the present study included only men, so it is not yet known if the results apply equally to women in China. Additionally, the majority of the men in this study are current or former smokers; when we stratified our analyses by smoking status, we found no suggestion of effect modification, although a greater number of never smokers would be needed to investigate an interaction should it exist. Furthermore, adjustment for smoking as well as the other potential confounders of regular aspirin use and daily fruit intake, were investigated, and do not alter the main results. We were also able to assess socio-economic status in three ways (by education, income, and occupation) and found no differences between cases and

controls. While low socio-economic status is usually a risk indicator for gastric cancer in epidemiological studies, the null finding in the present nested case-control study is consistent with that found in the entire Shanghai Men's Cohort Study (33). This may be related to the lack of a strong association of intermediate variables such as household crowding and nutrition with educational achievement among men in Shanghai, as over time urban Chinese men have become more homogenous in terms of early exposures. The study was further strengthened by the prospective design, with blood collection taking place prior to disease diagnosis or treatment, as well as by thorough and robust outcome ascertainment by the utilization of four sources: the Shanghai Cancer Registry; the Shanghai Vital Statistics Unit; medical charts from notifying hospitals; and in-person follow-up (with a response rate of 92.1% for 3.5 years after study enrollment). A limitation, however, is that TNM staging was assessed on only 66% of the cases, so that only a preliminary examination of the association between the *H. pylori* risk markers and stage of disease was possible, the results of which did not suggest that the marker differentiates between early- and late-stage disease (data not shown).

Ideally, we would also have results from conventional testing for *H. pylori* (i.e., ELISA and immunoblot assays) for comparison to our *H. pylori* multiplex serology results, but such testing had not previously been performed on this population, and it was not feasible as a part of the present project. However, as mentioned above, *H. pylori* multiplex serology has been found to have good agreement with conventional *H. pylori* testing (sensitivity, 89%; specificity, 82%; kappa, 0.70), and may in fact even be more sensitive than conventional serology as the majority of the "false-positive" sera in the validation study recognized more than 4 *H. pylori* proteins, the established cut-off (11). This could also explain the very high prevalence of *H. pylori* sero-positivity in our population (94% of controls), although a previous examination of *H. pylori* sero-positivity, utilizing conventional ELISA and immunoblot assays, in a related population, the Shanghai Women's Health Study, found a similarly high prevalence (92%) among controls (34). Additionally, we do not have pepsinogen levels on this population, which would be interesting as this measure of atrophy is often used for gastric cancer screening as an indicator of atrophic gastritis (25). However, pepsinogen levels generally are utilized for detection of early cancer, whereby the *H. pylori* marker examined in this project is more specifically a marker of gastric cancer risk for those who have *not* begun the cascade of events leading gastric cancer, as evidenced by the stronger associations for individuals whose diagnosis came at least two years after baseline blood collection. Finally, it would be of interest to examine the association of the *H. pylori* risk marker identified in these analyses with precancerous phases of the disease, but unfortunately we do not have this information on our population.

This is the first prospective study to analyze the association of gastric cancer incidence and antibodies to *H. pylori* proteins from multiplex serology. While not comprehensive in terms of identifying all future cases of gastric cancer, as only one aspect of *H. pylori* strain variation is detected using the new methodology, our findings do suggest that number of sero-positive results to six *H. pylori* proteins represent a potential new blood biomarker for distal gastric cancer in China. Prior to investigation of these novel risk markers as an effective screening tool, replication of our results in other populations, particularly other high-risk Asian populations who may be colonized by similar Asian strains, is necessary to validate this novel finding. Should these markers predict risk in such populations, the opportunity for prevention of the second-most deadly cancer is one that could substantially change cancer morbidity and mortality rates in much of the world.

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Table 1

Baseline demographic characteristics of the gastric cancer cases and matched controls in the Shanghai Men's Health Study, 2002–2009

	Cases (n=226)	Controls (n=451)
Mean age, y ± SD	62.6 ± 9.5	62.7 ± 9.3
Mean BMI, kg/m^2 ± SD	23.5 ± 3.1	24.0 ± 3.3
Marital status, n (%)		
Married	195 (96.5)	390 (97.5)
Not married (single/widowed/divorced)	7 (3.5)	10 (2.5)
Smoking status, n (%)		
Never smoker	59 (26.1)	152 (33.7)
Former smoker	37 (16.4)	81 (18.0)
Current smoker	130 (57.5)	218 (48.3)
Alcohol drinking status, n (%)		
Never drinker	137 (60.6)	292 (64.8)
Former drinker	14 (6.2)	15 (3.3)
Current drinker	75 (33.2)	144 (31.9)
Regular physical activity, n (%)	107 (47.4)	206 (45.7)
Highest education level achieved, n (%)		
Elementary school or less	22 (9.8)	57 (12.8)
Junior High School	96 (42.9)	153 (34.4)
High School	62 (27.7)	141 (31.7)
Professional high education or above	44 (19.6)	94 (21.1)
Monthly per capita income, n (%)		
< 500 yuan	21 (9.3)	48 (10.6)
500 – < 1,000 yuan	119 (52.7)	224 (49.7)
1,000 – < 2,000 yuan	69 (30.5)	137 (30.4)
≥ 2,000 yuan	17 (7.5)	42 (9.3)
Occupation, n (%)		
Professional	62 (27.4)	142 (31.6)
Clerical	48 (21.2)	97 (21.6)
Manual	116 (51.3)	211 (46.9)
Mean fruit intake, g/day ± SD	65.4 ± 68.5	77.5 ± 72.1
Family history of gastric cancer, n (%)	19 (8.4)	35 (7.8)
Regular aspirin use, n (%)	19 (8.4)	63 (14.0)
Diagnosis of chronic gastritis, n (%)	57 (25.2)	80 (17.7)

Abbreviation: SD, standard deviation

Table 2

Risk of distal gastric cancer by sero-prevalence for antibodies to *H. pylori* proteins in the Shanghai Men's Health Study

	All subjects (227 cases/451 controls)			Excluding cases diagnosed within 2 yrs after recruitment and matched controls (158 cases /316 controls)
	Cases n (%)	Controls n (%)	Matched OR* (95% CI)	Matched OR* (95% CI)
Omp				
Sero-negative	13 (5.8)	66 (14.6)	1.00 (Reference)	1.00 (Reference)
Sero-positive	213 (94.3)	385 (85.4)	**2.74 (1.48, 5.05)**	**3.55 (1.58, 7.98)**
HP0305				
Sero-negative	47 (20.8)	169 (37.5)	1.00 (Reference)	1.00 (Reference)
Sero-positive	179 (79.2)	282 (62.5)	**2.29 (1.57, 3.35)**	**2.49 (1.56, 3.99)**
HyuA				
Sero-negative	74 (32.7)	192 (42.6)	1.00 (Reference)	1.00 (Reference)
Sero-positive	152 (67.3)	259 (57.4)	**1.50 (1.07, 2.08)**	1.32 (0.90, 1.94)
HpaA				
Sero-negative	132 (58.4)	302 (67.0)	1.00 (Reference)	1.00 (Reference)
Sero-positive	94 (41.6)	149 (33.0)	**1.44 (1.03, 2.00)**	**1.77 (1.18, 2.68)**
CagA				
Sero-negative	19 (8.4)	51 (11.3)	1.00 (Reference)	1.00 (Reference)
Sero-positive	208 (91.6)	400 (88.7)	1.67 (0.80, 3.51)	**3.31 (1.09, 10.07)**
VacA				
Sero-negative	25 (11.1)	73 (16.2)	1.00 (Reference)	1.00 (Reference)
Sero-positive	201 (88.9)	378 (83.8)	1.51 (0.94, 2.41)	**2.11 (1.18, 3.78)**
NapA				
Sero-negative	69 (30.5)	166 (36.8)	1.00 (Reference)	1.00 (Reference)
Sero-positive	157 (69.5)	285 (63.2)	1.32 (0.94, 1.85)	1.32 (0.88, 1.99)
GroEL				
Sero-negative	31 (13.7)	67 (14.9)	1.00 (Reference)	1.00 (Reference)
Sero-positive	195 (86.3)	384 (85.1)	1.09 (0.69, 1.72)	1.20 (0.69, 2.11)
Cagδ				
Sero-negative	134 (59.3)	275 (61.0)	1.00 (Reference)	1.00 (Reference)
Sero-positive	92 (40.7)	176 (39.0)	1.08 (0.77, 1.52)	1.19 (0.79, 1.77)
HcpC				
Sero-negative	92 (40.7)	185 (41.0)	1.00 (Reference)	1.00 (Reference)
Sero-positive	134 (59.3)	266 (59.0)	1.01 (0.73, 1.39)	1.08 (0.74, 1.58)
Catalase				
Sero-negative	89 (39.4)	173 (38.4)	1.00 (Reference)	1.00 (Reference)
Sero-positive	137 (60.6)	278 (61.6)	0.96 (0.69, 1.33)	0.90 (0.61, 1.33)
UreA				
Sero-negative	110 (48.7)	215 (47.7)	1.00 (Reference)	1.00 (Reference)
Sero-positive	116 (51.3)	236 (52.3)	0.96 (0.69, 1.33)	1.06 (0.71, 1.57)

	All subjects (227 cases/451 controls)			Excluding cases diagnosed within 2 yrs after recruitment and matched controls (158 cases /316 controls)
	Cases n (%)	Controls n (%)	Matched OR* (95% CI)	Matched OR* (95% CI)
HP0231				
Sero-negative	123 (54.4)	235 (52.1)	1.00 (Reference)	1.00 (Reference)
Sero-positive	103 (45.6)	216 (47.9)	0.91 (0.66, 1.26)	0.90 (0.61, 1.33)
Cad				
Sero-negative	185 (81.9)	361 (80.0)	1.00 (Reference)	1.00 (Reference)
Sero-positive	41 (18.1)	90 (20.0)	0.89 (0.59, 1.35)	1.00 (0.63, 1.59)
CagM				
Sero-negative	195 (86.3)	368 (81.6)	1.00 (Reference)	1.00 (Reference)
Sero-positive	31 (13.7)	83 (18.4)	0.70 (0.44, 1.09)	0.78 (0.46, 1.31)

*odds ratios from a conditional logistic regression model, cases and controls matched on age (within 2 years), date (within 30 days) and time (morning or afternoon) of sample collection, time interval since last meal (within 2 hours), and antibiotic use in the past week (yes/no).

Table 3

Risk of distal gastric cancer by number of sero-positives to 6 *H. pylori* proteins (Omp, HP0305, HyuA, HpaA, CagA, and VacA) for each individual, in the SMHS

	All			Excluding cases diagnosed within 2 years after recruitment and matched controls		
No. sero-positive	**Cases n (%)**	**Controls n (%)**	**Matched OR* (95% CI)**	**Cases n (%)**	**Controls n (%)**	**Matched OR* (95% CI)**
All						
0–3	29 (12.8)	114 (25.3)	1.00 (Reference)	17 (10.8)	85 (26.9)	1.00 (Reference)
4–5	141 (62.4)	273 (60.5)	**2.08 (1.31, 3.30)**	105 (66.5)	188 (59.5)	**2.79 (1.56, 4.96)**
6	56 (24.8)	64 (14.2)	**3.49 (2.00, 6.11)**	36 (22.8)	43 (13.6)	**4.16 (2.08, 8.32)**
P for trend			**<0.0001**			**<0.0001**

*odds ratios from a conditional logistic regression model, cases and controls matched on age (within 2 years), date (within 30 days) and time (morning or afternoon) of sample collection, time interval since last meal (within 2 hours), and antibiotic use in the past week (yes/no).