Research Article

Cancer Prevention Research

Smoking, *Helicobacter Pylori* Serology, and Gastric Cancer Risk in Prospective Studies from China, Japan, and Korea

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Abstract

Smoking is an established risk factor for gastric cancer development. In this study, we aimed to assess prospectively the association of smoking with gastric cancer risk in 1,446 non-cardia gastric cancer cases and 1,796 controls from China, Japan, and Korea with consideration of *Helicobacter pylori* infection as a potential effect modifier. Applying logistic regression models stratified by study and adjusted for age and sex we found that current, but not former, smoking was significantly associated with gastric cancer risk [OR = 1.33; 95% confidence interval (CI), 1.07–1.65]. However, the association was significant only in *H. pylori* sero-positive individuals determined by 3 different sero-markers: overall sero-positivity, sero-positivity to the onco-protein CagA, and sero-positivity to the gastric cancer associated sero-marker HP0305 and HP1564. Specifically, a significant interaction was found when stratifying by HP0305/HP1564 ($P_{interaction} = 0.01$) with a 46% increased risk of gastric cancer among HP0305/HP1564 sero-positive current smokers (95% CI, 1.10–1.93) as opposed to no increased gastric cancer risk among HP0305/HP1564 sero-negative current smokers (OR = 0.93; 95% CI, 0.65–1.33). We confirmed that current smoking is associated with an increased gastric cancer risk, however, only among individuals that are simultaneously sero-positive for the leading causal factor for gastric cancer, *H. pylori*.

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Note: Supplementary data for this article are available at Cancer Prevention Research Online (http://cancerprevres.aacrjournals.org/).

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Cancer Prev Res 2019;12:667–74

doi: 10.1158/1940-6207.CAPR-19-0238

Introduction

Gastric cancer is the fifth most common cancer worldwide, and there is a large variation in incidence by geographic region, with the highest incidence occurring in East Asian countries (age-standardized incidence rate: 22.4 per 100,000) and the lowest occurring in Southern Africa (3.7 per 100,000) (1). *Helicobacter pylori* (*H. pylori*) infection is the leading risk factor for developing gastric cancer; concordantly, the infection is highly endemic in East Asian countries, with the prevalence estimated to be as high as 54% in China, Japan, Taiwan and Korea in 2015 (2, 3). However, the majority of *H. pylori*-infected individuals will not develop gastric cancer, suggesting a role for factors such as the carcinogenicity of the infecting *H. pylori* strain as well as host predisposition and environmental co-factors. Epplein and colleagues described a bio-marker, sero-positivity to *H. pylori* antigens HP0305 and HP1564 (formerly known as Omp), that was more predictive of the prevalence of precancerous gastric lesions than previously established markers, including sero-positivity to *H. pylori* in general or to virulence factor cytotoxin-associated gene A (CagA; ref. 4). No definite function has been described for HP0305 and HP1564 so far, but it was found that both

proteins are secreted by the bacterium, which may exert proinflammatory effects in the stomach (5).

Other established environmental risk factors may be important in promoting gastric carcinogenesis in concert with *H. pylori* infection (6). Case–control as well as prospective studies have reported an increased risk of developing gastric cancer with current smoking (7–33). Additionally, a dose–response relationship has been described with increasing duration of smoking and daily cigarette consumption increasing the risk of gastric cancer even further. In contrast, the risk in former smokers has been found to be similar to that in never smokers (9, 12, 14, 18, 22–26, 28). Some, but not all studies, have evaluated whether *H. pylori* infection may affect the association of smoking with gastric cancer, however, the results remain inconclusive (8, 11, 15, 20, 21, 24, 25, 29, 30, 32). Therefore, the identification of cofactors for the development of gastric cancer could help in developing targeted prevention strategies for individuals at highest risk of developing the disease (34).

In this study, we aimed to better understand the impact of smoking on risk of developing gastric cancer with consideration of *H. pylori* infection as a potential effect modifier in a large consortium of prospective cohorts in countries with a high incidence of gastric cancer—China, Japan, and Korea.

Materials and Methods

Study population

This study comprises seven prospective cohort studies [Japan Public Health Center-based Prospective Study I and II (JPHC I and II), Korean Cancer Prevention Study II (KCPS), Korean Multicenter Cancer Cohort I (KMCC), Linxian Nutrition Intervention Trial (NIT), and the Shanghai Men's and Shanghai Women's Health Studies (SMHS and SWHS)] from the *Helicobacter pylori* Biomarker Cohort Consortium (HpBCC) conducted in China, Japan, and Korea (35). At baseline, these cohorts collected information on demographic and lifestyle characteristics and drew blood samples from healthy individuals. The outcome in this study was defined as incident non-cardia gastric cancer (International Classification of Diseases for Oncology codes C16.1–C16.6, C16.8, C16.9). The median time between blood draw and diagnosis was 5.3 years (interquartile range: 2.6–8.6 years). For all cohorts except NIT, controls were chosen by incidence density sampling from the respective cohort of participants alive, free of cancer (except nonmelanoma skin cancer), and with no history of gastrectomy at the time of diagnosis of the index cases. Controls were matched to cases by sex, birth date, and date of blood collection in a ratio of 1:1 for cohorts JPHC I, JPHC II, KCPS, and KMCC and in a ratio of 2:1 for cohorts SMHS and SWHS. In case of the NIT cohort, controls were frequency matched to cases by sex.

This study was approved as nonhuman subject research by the institutional review boards of: Vanderbilt University (Nashville, TN); Duke University (Durham, NC); German Cancer Research Center (Heidelberg, Germany); Shanghai Cancer Institute (Shanghai, China); National Cancer Center (Tokyo, Japan); Chinese Academy of Medical Sciences and Peking University Cancer Hospital and Institute (Beijing, China); and Seoul University and Yonsei University (Seoul, Korea).

Smoking status assessment

At baseline, cohorts collected information on demographic characteristics of study participants including smoking status in the categories of never, former, or current smoker (35). One participant in KMCC with missing data on smoking status and the respective matched case were excluded from the current analysis resulting in a total number of 1,446 cases and 1,796 controls.

H. pylori multiplex serology

H. pylori multiplex serology was performed as described previously (35–37). Briefly, 15 *H. pylori* proteins (GroEl, UreA, HP0231, NapA, HP0305, CagM, CagD, CagA, HyuA, Catalase, VacA, HpaA, Cad, HcpC, and HP1564) were recombinantly expressed as GST-tagged proteins and affinity-purified on fluorescence-labeled polysterene beads (Luminex Corp.) coated with glutathione-casein. Antigen-loaded beads were mixed and incubated with prediluted serum (1:1,000). The amount of bound serum antibody to the respective antigen was then quantified by a Luminex flow cytometer (Luminex Corp.) through a biotin-labeled secondary antibody against human IgG, IgA, and IgM and a fluorescent reporter conjugate (Streptavidin-R-Phycoerythrin). The amount of bound serum antibody was expressed as median fluorescence intensity (MFI) and antigen-specific cut-offs for sero-positivity were defined as described previously (35, 37). Overall *H. pylori* sero-positivity was defined as being sero-positive to 4 or more out of the 15 *H. pylori* proteins included. Sero-positivity to the well-known virulence factor CagA was identified as being specifically strongly associated with an increased risk of developing gastric cancer. However, previous studies have found that dual sero-positivity to 2 other *H. pylori* proteins with so far unknown function, HP0305 and HP1564, are more suitable markers for gastric cancer risk in areas with high CagA-positive *H. pylori* prevalence like East Asia (4, 35, 38). Based on this data we included 2 additional definitions for *H. pylori* sero-positivity: (i) overall *H. pylori* plus CagA sero-positive and (ii) HP0305 and HP1564 dual sero-positivity.

Statistical analyses

Differences in study characteristics between cases and controls as well as factors associated with smoking (never/former/current) at baseline were compared using

Chi-square tests for categorical variables and Wilcoxon rank sum test for the continuous variable age.

We applied conditional logistic regression models, stratified by cohort, and adjusted for the matching variables age and sex to estimate the OR and 95% confidence interval (95% CI) for the association of smoking status at baseline (never/former/current) with gastric cancer risk.

We considered *a priori* body mass index (BMI), education, and history of gastritis as potential confounders. BMI and education were found to be associated with both the exposure and the outcome; however, adjustment with these 2 variables among those individuals not missing these variables did not alter the overall risk estimates by more than 10% and were therefore not included in the final model. Sensitivity analyses were performed including only those gastric cancer cases that were diagnosed equal to or more than 1, 2, or 5 years after blood draw to address reverse causality in the association of smoking with gastric cancer risk. Because former or current smokers were more likely to be of male sex, we further analyzed the association of smoking with gastric cancer risk stratified by sex, although power was particularly limited in the female-only analyses. Similarly, smoking status also varied by country, and we therefore assessed the association of former and current smoking separately for studies in China, Japan, and Korea.

To assess whether *H. pylori* sero-status may act as an effect modifier on the association of smoking with gastric cancer risk, we performed a stratified analysis by each of the 3 *H. pylori* sero-positivity definitions (overall *H. pylori* sero-positivity; *H. pylori* and CagA sero-positivity; dual HP0305/HP1564 sero-positivity) and inclusion of a multiplicative interaction term in the model.

All authors had the option to access the study data and had reviewed and approved the final manuscript.

Results

Study characteristics and factors associated with smoking status at baseline

Among the 1,446 prospectively ascertained non-cardia gastric cancer cases and 1,796 controls, the median age was 58.7 and 58.6 years, respectively. Cases were more likely to be of male sex, which resulted from the different matching schemes applied by individual cohorts, and of lower education, and less likely to be obese, than the controls. In terms of *H. pylori* status, cases were more likely to be positive to any of the three *H. pylori* sero-positivity definitions than controls: 92% of cases were overall *H. pylori* sero-positive compared with 81% of controls; 88% of cases were simultaneously positive for CagA as opposed to 75% controls; the gastric cancer risk specific sero-marker HP0305/HP1564 was detected in 67% of cases compared with 48% of controls (Table 1).

Never, former, and current smokers differed by age, with former smokers being the oldest group (median age 60.8 years) and current smokers the youngest (57.6 years). Of current and former smokers, 95% were of male sex as opposed to 78% females among the never smokers. Furthermore, former smokers were more likely to be of higher education than never and current smokers whereas current smokers were less likely to be obese. Ever being diagnosed with gastritis was least likely among former smokers (6%) compared with never (16%) and current smokers (14%). The majority of current and never smokers originated from China, whereas former smokers were more frequently Korean. *H. pylori* sero-status differed only for HP0305/HP1564 sero-positivity by smoking status and was most prevalent among current smokers (66%) compared with 54% in former and 52% sero-prevalence in never smokers (Table 2).

Association of smoking with gastric cancer risk with consideration of *H. pylori* infection as a potential effect modifier

Current, but not former, smoking at baseline was positively associated with a 33% increased risk of developing gastric cancer as compared with never smokers (current smoking: OR = 1.33; 95% CI, 1.07–1.65; former smoking: OR = 1.01; 95% CI, 0.77–1.32; Table 3). The strength of the association was not diminished when excluding cases that were diagnosed within 1 (OR = 1.51; 95% CI, 1.16–1.98), 2 (OR = 1.50; 95% CI, 1.13–1.97), or 5 years (OR = 1.68; 95% CI, 1.21–2.34) after blood draw (Supplementary Table S1). We further performed a stratified analysis by sex, because current smoking was common among men and rare among women. The overall risk estimate for gastric cancer in current compared with never smokers did not vary by sex. However, due to the small sample size of current smokers among women ($n = 25$ cases and $n = 26$ controls), the 95% CI was wider and thus nonsignificant (males: OR = 1.37; 95% CI, 1.08–1.73; females: OR = 1.48; 95% CI, 0.84–2.63; Supplementary Table S2). Stratification by country did not suggest significant differences, although the association of current smoking with gastric cancer was particularly strong among participants in the Korean cohorts (Supplementary Table S3).

Stratification by *H. pylori* sero-status resulted in an increased risk of developing gastric cancer with current smoking only among *H. pylori* sero-positives within all 3 definitions for *H. pylori* sero-status, and was most pronounced among HP0305/HP1564 sero-positives (OR = 1.46; 95% CI, 1.10–1.93), whereas *H. pylori* sero-negative current smokers were not at increased gastric cancer risk (OR for current smoking and gastric cancer risk among HP0305/HP1564 negatives = 0.93; 95% CI, 0.65–1.33; $P_{\text{interaction}} = 0.01$; Table 3).

Table 1. Characteristics of the study population, the HpBCC, by gastric cancer case/control status

	Cases ($n = 1,446$)	Controls ($n = 1,796$)	*P* value
Age, years [median (IQR)][a]	58.7 (51.9, 64.9)	58.6 (50.3, 65.4)	0.26
Sex[b], *n* (%)			<**0.01**
Female	631 (44)	916 (51)	
Male	815 (56)	880 (49)	
Country[b], *n* (%)			<**0.01**
China	687 (48)	1,037 (58)	
Japan	402 (28)	402 (22)	
Korea	357 (25)	357 (20)	
Study[b], *n* (%)			<**0.01**
SMHS	66 (5)	132 (7)	
SWHS	295 (20)	579 (32)	
NIT	326 (23)	326 (18)	
JPHC I	207 (14)	207 (12)	
JPHC II	195 (13)	195 (11)	
KCPS	169 (12)	169 (9)	
KMCC	188 (13)	188 (10)	
Education[b], *n* (%)			**0.03**
≤Elementary school	605 (49)	693 (44)	
Junior high school	274 (22)	405 (25)	
High school	224 (18)	288 (18)	
≥Professional education	139 (11)	205 (13)	
Missing	204	205	
BMI[b], *n* (%)			**0.02**
<18.5	59 (4)	51 (3)	
18.5–24.9	982 (69)	1,190 (67)	
≥25	388 (27)	546 (31)	
Missing	17	9	
History of gastritis[b], *n* (%)			0.87
No	843 (85)	1,148 (85)	
Yes	146 (15)	195 (15)	
Missing	457	453	
H. pylori[b], *n* (%)			<**0.01**
Sero-negative	109 (8)	341 (19)	
Sero-positive	1,337 (92)	1,455 (81)	
H. pylori and CagA[b], *n* (%)			<**0.01**
H. pylori(−) and/or CagA(−)	175 (12)	454 (25)	
H. pylori(+) and CagA(+)	1,271 (88)	1,342 (75)	
HP0305 and HP1564[b], *n* (%)			<**0.01**
HP0305(−) and/or HP1564(−)	475 (33)	938 (52)	
HP0305(+) and HP1564(+)	971 (67)	858 (48)	

NOTE: Significant associations ($P < 0.05$) are marked in bold font.
Abbreviations: (−), sero-negative; (+), sero-positive; IQR, interquartile range.
[a]Wilcoxon rank sum test.
[b]Chi-square test among nonmissing subjects.

Discussion

In this study, we report that current smoking is associated with a 33% increased risk of developing non-cardia gastric cancer. However, when *H. pylori* was considered as a potential effect modifier, current smoking increased the risk of developing gastric cancer only among study participants that were simultaneously sero-positive for *H. pylori*, particularly for the gastric cancer risk sero-marker HP0305/HP1564.

Our results on the association of current smoking with gastric cancer risk in this consortium of cohorts from East Asia is in line with other prospective studies as summarized in the monograph on the effects of tobacco smoking published by the International Agency for Research on Cancer (IARC; ref. 6). Previous studies found an approximately 2-fold increased risk with current but not former smoking, although not all of these studies were non-cardia gastric cancer only, as presented here (6). Our results are furthermore in line with previous reports that former smoking did not result in an increased risk of developing gastric cancer (6).

Most of the previous studies, however, did not take into consideration the leading risk factor for non-cardia gastric cancer, *H. pylori* infection. Those that did generally performed a combined analysis of these 2 factors with individuals who are both never smokers and *H. pylori*-negative as the reference group (8, 15, 20, 21, 24, 25, 29–32). Compared with this lowest risk group, both *H. pylori*-negative smokers and *H. pylori*-positive nonsmokers were at higher risk for developing gastric cancer and the strongest risk was observed for *H. pylori*-positive current smokers (6). Pursuing the same approach in our study would result in 26% of cases being current smokers and HP0305/HP1564 sero-positive, compared with 15% of controls. Compared with

Table 2. Characteristics of the study population, the HpBCC, by smoking status

	Never smoker ($n = 1{,}902$)	Former smoker ($n = 400$)	Current smoker ($n = 940$)	*P value*
Age, years [median (IQR)][a]	58.6 (50.4, 65.0)	60.8 (55.7, 67.0)	57.6 (50.6, 63.8)	<**0.01**
Sex[b], *n* (%)				<**0.01**
Female	1,476 (78)	20 (5)	51 (5)	
Male	426 (22)	380 (95)	889 (95)	
Country[b], *n* (%)				<**0.01**
China	1,247 (66)	55 (14)	422 (45)	
Japan	391 (21)	148 (37)	265 (28)	
Korea	264 (14)	197 (49)	253 (27)	
Study[b], *n* (%)				<**0.01**
SMHS	76 (4)	33 98)	89 (9)	
SWHS	838 (44)	3 (1)	33 (4)	
NIT	333 (18)	19 (95)	300 (32)	
JPHC I	201 (11)	70 (18)	143 (15)	
JPHC II	190 (10)	78 (19)	122 (13)	
KCPS	110 (6)	132 (33)	96 (10)	
KMCC	154 (8)	65 (13)	157 (17)	
Education[b], *n* (%)				<**0.01**
≤Elementary school	799 (47)	82 (25)	417 (52)	
Junior high school	443 (26)	74 (23)	162 (20)	
High school	299 (18)	75 (23)	138 (17)	
≥Professional education	162 (10)	91 (28)	91 (11)	
Missing	199	78	132	
BMI[b], *n* (%)				<**0.01**
<18.5	55 (3)	13 (3)	42 (5)	
18.5–24.9	1,230 (65)	249 (63)	693 (75)	
≥25	607 (32)	133 (34)	194 (21)	
Missing	10	5	11	
History of gastritis[b], *n* (%)				<**0.01**
No	1,226 (84)	226 (94)	539 (86)	
Yes	242 (16)	14 (6)	85 (14)	
Missing	434	160	316	
H. pylori[b], *n* (%)				0.63
Sero-negative	257 (14)	54 (14)	139 (15)	
Sero-positive	1,645 (86)	346 (87)	801 (85)	
H. pylori and CagA[b], *n* (%)				0.47
H. pylori(−) and/or CagA(−)	359 (19)	75 (19)	195 (21)	
H. pylori(+) and CagA(+)	1543 (81)	325 (81)	745 (79)	
HP0305 and HP1564[b], *n* (%)				<**0.01**
HP0305(−) and/or HP1564(-)	912 (48)	186 (47)	315 (34)	
HP0305(+) and HP1564(+)	990 (52)	214 (54)	625 (66)	

NOTE: Significant associations ($P < 0.05$) are marked in bold font.
Abbreviations: (−), sero-negative; (+), sero-positive; IQR, interquartile range.
[a]Wilcoxon rank sum test.
[b]Chi-square test among nonmissing subjects.

the lowest risk-group (nonsmoker and HP0305/HP1564 sero-negative) this group is at a 2.73-fold increased risk of developing gastric cancer (95% CI, 2.17–3.45).

Methods to address *H. pylori* infection in the above-mentioned studies included only conventional ELISA and/or CagA-specific ELISA. In this study, we followed a different approach by exploring *H. pylori* heterogeneity in greater depth, as we were able to do with our established HP0305/HP1564 risk marker. We first demonstrated that, concordantly with previous results, an increased risk of gastric cancer with current smoking was only detected in the *H. pylori* sero-marker positive group, although there was no significant interaction between *H. pylori* overall sero-positivity or CagA sero-positivity with current smoking. Stratifying by the gastric cancer-associated sero-marker HP0305/HP1564 for *H. pylori* sero-positivity (4, 35, 38, 39), we were able to show *H. pylori* sero-positivity does in fact act as an effect modifier. To note, we identified only one previous study that found an interaction of smoking and CagA-positive *H. pylori* infection in the association with gastric cancer (29), which is discordant with our results with CagA-sero-positivity. However, the study by Wang and colleagues (29) was different to our study in that CagA-sero-prevalence was lower in the population overall, the sero-prevalence being only 27% among controls compared to 70% among cases. Our study, which had high CagA sero-prevalence among both controls and cases (75% and 88%, respectively), is in line with the Asia-Pacific consensus that detection of *H. pylori* and CagA is not a useful marker of gastric cancer risk in the Asia-Pacific region when the majority of the population in this area is infected with CagA-positive *H. pylori* strains (39).

Table 3. Smoking and gastric cancer risk, overall and by *H. pylori* sero-marker status, the HpBCC

	Smoking			
	Never smoker	**Former smoker**	**Current smoker**	$P_{interaction}$
Overall				
n (%), cases/controls	782 (54)/1,120 (62)	185 (12)/215 (12)	479 (26)/461 (33)	
OR (95% CI)	Ref	1.01 (0.77–1.32)	**1.33 (1.07–1.65)**	
H. pylori(−)				
n (%), cases/controls	59 (54)/198 (58)	13 (12)/41 (12)	37 (34)/102 (30)	
OR (95% CI)	Ref	0.66 (0.26–1.66)	0.78 (0.40–1.54)	
H. pylori(+)				
n (%), cases/controls	723 (54)/922 (63)	172 (13)/174 (12)	442 (33)/359 (25)	
OR (95% CI)	Ref	1.06 (0.79–1.41)	**1.41 (1.12–1.78)**	0.35
H. pylori(−) and/or CagA(−)				
n (%), cases/controls	88 (50)/271 (60)	21 (12)/54 (12)	66 (38)/129 (28)	
OR (95% CI)	Ref	0.72 (0.35–1.49)	1.13 (0.65–1.96)	
H. pylori(+) and CagA(+)				
n (%), cases/controls	694 (55)/849 (63)	164 (13)/161 (12)	413 (32)/332 (25)	
OR (95% CI)	Ref	1.06 (0.79–1.43)	**1.38 (1.08–1.75)**	0.74
HP0305(−) and/or HP1564(−)				
n (%), cases/controls	302 (64)/610 (65)	67 (14)/119 (13)	106 (22)/209 (22)	
OR (95% CI)	Ref	0.96 (0.63–1.46)	0.93 (0.65–1.33)	
HP0305(+) and HP1564(+)				
n (%), cases/controls	480 (49)/510 (59)	118 (12)/96 (11)	373 (38)/252 (29)	
OR (95% CI)	Ref	1.15 (0.80–1.66)	**1.46 (1.10–1.93)**	**0.01**

NOTE: ORs and 95% CI were calculated by using conditional logistic regression stratified by cohort with further adjustment for age and sex.
Significant associations ($P < 0.05$) are marked in bold font.
Abbreviations: (−), sero-negative; (+), sero-positive, Ref, Reference.

The mechanism by which smoking increases gastric cancer risk still needs to be elucidated. A study by Hishida and colleagues in 2010 proposed that smoking contributed to gastric carcinogenesis from gastric atrophy but had no influence on earlier steps in the cascade (15). This could explain why in our results the effect of smoking on gastric cancer risk was only present when study participants were simultaneously sero-positive to HP0305/HP1564, a strong sero-marker for the presence of precancerous gastric lesions as well as progression to gastric cancer, rather than among all with a general *H. pylori* antibody response (4, 35, 38). Mechanistically, it was shown that smokers compared with nonsmokers have a suppressed innate immune system, a potential mechanism through which smoking could contribute to a more severe *H. pylori* infection and thus a higher risk of developing gastric cancer (40, 41).

There are several limitations to our study. First, smoking status was assessed only at baseline and was not updated throughout the follow-up. However, because only current smoking was associated with gastric cancer risk, potential smoking cessation among study participants during follow-up would result in a bias toward the null for our study. Similarly, information on cigarette pack-years could have added value to our study, but was not available uniformly from the cohorts in our consortium. Unfortunately, we were also lacking information on alcohol consumption, a factor that often correlates with smoking behavior. According to the IARC monograph on consumption of alcoholic beverages, there is not sufficient evidence for alcohol intake increasing the risk of developing gastric cancer (42), however, residual confounding by this variable can nonetheless not be ruled out. A further limitation is the unequal sex distribution in smoking status. To address this limitation, we performed a stratified analysis by sex and found that the overall risk estimate for gastric cancer was not different between males and females in the study, although we were limited by small numbers of smoking women. Therefore, although we acknowledge that our study does have minor limitations, we do not believe that they adversely affect our conclusions.

Furthermore, our study has several strengths, including the large number of prospectively ascertained non-cardia gastric cancer cases in a geographical region with the highest gastric cancer rates worldwide. Additionally, we were able to assess serological markers for *H. pylori* infection beyond the usually applied overall *H. pylori* and CagA sero-status. As described above, these 2 measures are considered insufficient to identify individuals at increased risk of developing gastric cancer in areas of high prevalence of CagA-positive *H. pylori* infection (39). With assessment of the effect of sero-positivity to gastric cancer risk-associated biomarker HP0305/HP1564 in a study population in East Asia we thus have added important information to the association of smoking with gastric cancer risk.

In conclusion, our results from this large prospective consortium of East Asian studies confirmed that current smoking increases the risk of developing non-cardia gastric cancer, however, only among study participants simultaneously harboring antibodies to *H. pylori*, CagA-positive *H. pylori*, or the gastric cancer risk marker *H. pylori* HP0305/HP1564. Our findings suggest that in areas of high *H. pylori* prevalence like East Asia, smoking status is a further risk marker of gastric cancer incidence, and

potentially smoking cessation could be an effective strategy to reduce gastric cancer risk.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J. Butt, T. Wang, S.H. Jee, M. Pawlita, M. Epplein
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Acknowledgments

The sample preparations for the Shanghai studies were performed at the Survey and Biospecimen Shared Resource, which is supported in part by the Vanderbilt-Ingram Cancer Center (P30 CA68485). We thank Dr. Jie Wu and M. Regina Courtney for their excellent laboratory assistance in sample handling and preparation. This work was supported by the NCI at the NIH (R01 CA174853 and K07 CA151782 to M. Epplein). The funders of the study had no role in study design; in the collection, analysis, or interpretation of the data; or writing the report. J. Butt had full access to all study data and had final responsibility for the decision to submit for publication.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received May 9, 2019; revised June 20, 2019; accepted July 22, 2019; published first July 26, 2019.

References

1. Ferlay J, Ervik M, Lam F, Colombet M, Mery L, Piñeros M, et al. 2018 Global cancer observatory: cancer today. Lyon, France: International Agency for Research on Cancer. Available from: https://gco.iarc.fr/today. Accessed February 1, 2019.
2. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the evaluation of carcinogenic risks to humans. Lyon, 7–14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994; 61:1–241.
3. Hooi JKY, Lai WY, Ng WK, Suen MMY, Underwood FE, Tanyingoh D, et al. Global prevalence of helicobacter pylori infection: systematic review and meta-analysis. Gastroenterology 2017; 153:420–29.
4. Epplein M, Butt J, Zhang Y, Hendrix LH, Abnet CC, Murphy G, et al. Validation of a blood biomarker for identification of individuals at high risk for gastric cancer. Cancer Epidemiol Biomarkers Prev 2018;27:1472–79.
5. Kim N, Weeks DL, Shin JM, Scott DR, Young MK, Sachs G. Proteins released by Helicobacter pylori in vitro. J Bacteriol 2002;184:6155–62.
6. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Tobacco smoke and involuntary smoking. IARC Monogr Eval Carcinog Risks Hum 2004;83:1–1438.
7. Batty GD, Kivimaki M, Gray L, Smith GD, Marmot MG, Shipley MJ. Cigarette smoking and site-specific cancer mortality: testing uncertain associations using extended follow-up of the original Whitehall study. Ann Oncol 2008; 19:996–1002.
8. Brenner H, Arndt V, Bode G, Stegmaier C, Ziegler H, Stumer T. Risk of gastric cancer among smokers infected with *Helicobacter pylori*. Int J Cancer 2002;98:446–9.
9. Chao A, Thun MJ, Henley SJ, Jacobs EJ, McCullough ML, Calle EE. Cigarette smoking, use of other tobacco products and stomach cancer mortality in US adults: the cancer prevention study II. Int J Cancer 2002;101:380–9.
10. Chen ZM, Peto R, Iona A, Guo Y, Chen YP, Bian Z, et al. Emerging tobacco-related cancer risks in China: a nationwide, prospective study of 0.5 million adults. Cancer 2015;121:3097–106.
11. Epplein M, Nomura AM, Hankin JH, Blaser MJ, Perez-Perez G, Stemmermann GN, et al. Association of *Helicobacter pylori* infection and diet on the risk of gastric cancer: a case-control study in Hawaii. Cancer Causes Control 2008;19:869–77.
12. Freedman ND, Abnet CC, Leitzmann MF, Mouw T, Subar AF, Hollenbeck AR, et al. A prospective study of tobacco, alcohol, and the risk of esophageal and gastric cancer subtypes. Am J Epidemiol 2007;165:1424–33.
13. Fujino Y, Mizoue T, Tokui N, Kikuchi S, Hoshiyama Y, Toyoshima H, et al. Cigarette smoking and mortality due to stomach cancer: findings from the JACC Study. J Epidemiol 2005;15:S113–9.
14. Gonzalez CA, Pera G, Agudo A, Palli D, Krogh V, Vineis P, et al. Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). Int J Cancer 2003;107:629–34.
15. Hishida A, Matsuo K, Goto Y, Naito M, Wakai K, Tajima K, et al. Smoking behavior and risk of *Helicobacter pylori* infection, gastric atrophy and gastric cancer in Japanese. Asian Pac J Cancer Prev 2010;11:669–73.
16. Jee SH, Samet JM, Ohrr H, Kim JH, Kim IS. Smoking and cancer risk in Korean men and women. Cancer Causes Control 2004;15: 341–8.
17. Kneller RW, McLaughlin JK, Bjelke E, Schuman LM, Blot WJ, Wacholder S, et al. A cohort study of stomach cancer in a high-risk American population. Cancer 1991;68:672–8.
18. Koizumi Y, Tsubono Y, Nakaya N, Kuriyama S, Shibuya D, Matsuoka H, et al. Cigarette smoking and the risk of gastric cancer: a pooled analysis of two prospective studies in Japan. Int J Cancer 2004;112:1049–55.
19. Lindblad M, Rodriguez LA, Lagergren J. Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. Cancer Causes Control 2005;16:285–94.
20. Machida-Montani A, Sasazuki S, Inoue M, Natsukawa S, Shaura K, Koizumi Y, et al. Association of Helicobacter pylori infection and environmental factors in non-cardia gastric cancer in Japan. Gastric Cancer 2004;7:46–53.

21. Moy KA, Fan Y, Wang R, Gao YT, Yu MC, Yuan JM. Alcohol and tobacco use in relation to gastric cancer: a prospective study of men in Shanghai, China. Cancer Epidemiol Biomarkers Prev 2010;19:2287–97.
22. Sasazuki S, Sasaki S, Tsugane S, Japan Public Health Center Study G. Cigarette smoking, alcohol consumption and subsequent gastric cancer risk by subsite and histologic type. Int J Cancer 2002;101:560–6.
23. Sauvaget C, Lagarde F, Nagano J, Soda M, Koyama K, Kodama K. Lifestyle factors, radiation and gastric cancer in atomic-bomb survivors (Japan). Cancer Causes Control 2005;16:773–80.
24. Shikata K, Doi Y, Yonemoto K, Arima H, Ninomiya T, Kubo M, et al. Population-based prospective study of the combined influence of cigarette smoking and *Helicobacter pylori* infection on gastric cancer incidence: the Hisayama Study. Am J Epidemiol 2008;168:1409–15.
25. Siman JH, Forsgren A, Berglund G, Floren CH. Tobacco smoking increases the risk for gastric adenocarcinoma among *Helicobacter pylori*-infected individuals. Scand J Gastroenterol 2001;36: 208–13.
26. Sjodahl K, Lu Y, Nilsen TI, Ye W, Hveem K, Vatten L, et al. Smoking and alcohol drinking in relation to risk of gastric cancer: a population-based, prospective cohort study. Int J Cancer 2007; 120:128–32.
27. Sung NY, Choi KS, Park EC, Park K, Lee SY, Lee AK, et al. Smoking, alcohol and gastric cancer risk in Korean men: the National Health Insurance Corporation Study. Br J Cancer 2007;97:700–4.
28. Tran GD, Sun XD, Abnet CC, Fan JH, Dawsey SM, Dong ZW, et al. Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. Int J Cancer 2005;113:456–63.
29. Wang XQ, Yan H, Terry PD, Wang JS, Cheng L, Wu WA, et al. Interactions between CagA and smoking in gastric cancer. World J Gastroenterol 2011;17:3330–4.
30. Wu AH, Crabtree JE, Bernstein L, Hawtin P, Cockburn M, Tseng CC, et al. Role of Helicobacter pylori CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. Int J Cancer 2003;103:815–21.
31. You WC, Zhang L, Gail MH, Chang YS, Liu WD, Ma JL, et al. Gastric dysplasia and gastric cancer: helicobacter pylori, serum vitamin C, and other risk factors. J Natl Cancer Inst 2000;92: 1607–12.
32. Zaridze D, Borisova E, Maximovitch D, Chkhikvadze V. Alcohol consumption, smoking and risk of gastric cancer: case-control study from Moscow, Russia. Cancer Causes Control 2000;11: 363–71.
33. Zendehdel K, Nyren O, Luo J, Dickman PW, Boffetta P, Englund A, et al. Risk of gastroesophageal cancer among smokers and users of Scandinavian moist snuff. Int J Cancer 2008;122:1095–9.
34. Marrone MT, Tsilidis KK, Ehrhardt S, Joshu CE, Rebbeck TR, Sellers TA, et al. When is enough, enough? When are more observational epidemiologic studies needed to resolve a research question: illustrations using biomarker-cancer associations. Cancer Epidemiol Biomarkers Prev 2019;28:239–47.
35. Cai H, Ye F, Michel A, Murphy G, Sasazuki S, Taylor PR, et al. Helicobacter pylori blood biomarker for gastric cancer risk in East Asia. Int J Epidemiol 2016;45:774–81.
36. Michel A, Waterboer T, Kist M, Pawlita M. Helicobacter pylori multiplex serology. Helicobacter 2009;14:525–35.
37. Wang T, Cai H, Sasazuki S, Tsugane S, Zheng W, Cho ER, et al. Fruit and vegetable consumption, *Helicobacter pylori* antibodies, and gastric cancer risk: a pooled analysis of prospective studies in China, Japan, and Korea. Int J Cancer 2017;140: 591–99.
38. Epplein M, Zheng W, Xiang YB, Peek RM Jr., Li H, Correa P, et al. Prospective study of *Helicobacter pylori* biomarkers for gastric cancer risk among Chinese men. Cancer Epidemiol Biomarkers Prev 2012;21:2185–92.
39. Sugano K, Tack J, Kuipers EJ, Graham DY, El-Omar EM, Miura S, et al. Kyoto global consensus report on Helicobacter pylori gastritis. Gut 2015;64:1353–67.
40. Bagaitkar J, Demuth DR, Scott DA. Tobacco use increases susceptibility to bacterial infection. Tob Induc Dis 2008;4:12.
41. Wang H, Kumar A, Lamont RJ, Scott DA. GSK3beta and the control of infectious bacterial diseases. Trends Microbiol 2014; 22:208–17.
42. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Alcohol consumption and ethyl carbamate. IARC Monogr Eval Carcinog Risks Hum 2010;96:3–1383.