

Original Contribution

Associations Between Gastric Atrophy and Its Interaction With Poor Oral Health and the Risk for Esophageal Squamous Cell Carcinoma in a High-Risk Region of China: A Population-Based Case-Control Study

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Previous findings concerning gastric atrophy as a potential risk factor for esophageal squamous cell carcinoma (ESCC) have been inconsistent. We aimed to test whether gastric atrophy and, further, its interaction with poor oral health elevated the risk of ESCC in a high-risk region of China. Our population-based case-control study in Taixing, China (2010–2014), recruited cases from local hospitals and the local cancer registry. Controls were selected randomly from the local population registry. Ultimately, 1,210 cases and 1,978 controls answered questionnaires and provided blood samples for assay of pepsinogens. Unconditional logistic regression models were used to estimate odds ratios and 95% confidence intervals. Gastric atrophy (defined as a serum level of pepsinogen I of $<55 \mu\text{g/L}$) was associated with an increased risk for ESCC (odds ratio = 1.61; 95% confidence interval: 1.33, 1.96), even after full adjustment for potential confounding factors. In addition, suggestion of an additive interaction between gastric atrophy and poor oral health was observed (relative excess risk due to interaction = 1.28, 95% confidence interval: 0.39, 2.18). We conclude that gastric atrophy appears to be a risk factor for ESCC in a high-risk region of China, and there is a suggested additive interaction with poor oral health that increases this risk even further.

esophageal squamous cell carcinoma; gastric atrophy; pepsinogens; poor oral health

Abbreviations: CI, confidence interval; ESCC, esophageal squamous cell carcinoma; PGI, pepsinogen I; PGII, pepsinogen II.

Esophageal cancer, the sixth most common cause of cancer death, is associated with very poor survival (mortality-to-incidence ratio of 0.88) and was responsible for 500,000 deaths worldwide in 2018 (1). In that year, the highest mortality (10.7 deaths per 100,000 person-years) and incidence rates were both reported in eastern Asia. In 2012, 87% of the esophageal cancer cases were esophageal squamous cell carcinoma (ESCC), 79% of which occurred in China, India, and Southeast and Central Asia (2).

Gastric atrophy caused by chronic *Helicobacter pylori* infection is already known to be involved in the development of gastric cancer (3). Moreover, Ye et al. (4) first reported an unexpected association between gastric atrophy and ESCC risk, a result subsequently confirmed by 2 case-control studies based on endoscopy findings in Japan (5, 6). One of

these also reported a rising risk with increasing severity of the gastric atrophy (histological fundic atrophy and fundic intestinal metaplasia) (5). Another retrospective, register-based study of endoscopy findings in Japan reported a higher proportion of gastric atrophy in ESCC patients compared with patients with esophageal adenocarcinoma (7).

However, although a cohort study in the Netherlands also observed a positive association between gastric atrophy and the risk for ESCC, they could not confirm that the risk increased with the severity of gastric atrophy (8). Therefore, they concluded that a causal relationship seems unlikely and that the association can be explained by confounding factors such as smoking (8). Furthermore, a prospective case-cohort study did not find an association between the level of serum pepsinogen, a marker for gastric atrophy,

and ESCC in Linxian, China, a high-risk area for this carcinoma (9).

Poor oral health has been reported to be an independent risk factor for ESCC in high-risk areas (10, 11). In a case-control study on a high-risk population in Iran by Nasrollahzadeh et al. (12), they not only once again confirmed the association between gastric atrophy (assessed on the basis of serum pepsinogens) and ESCC but also observed a potential interaction with poor oral health that led to a further increase in the risk. However, that study did not have sufficient statistical power (293 cases and 524 controls), particularly for interaction analysis, and it also had certain limitations in study design (e.g., the utilization of neighborhood controls and individual matching, which might have introduced overmatching bias and hampered appropriate analysis of interaction, respectively). The mechanism underlying the relationship between gastric atrophy and ESCC is still unknown (13); it could involve reductions in the number/activity of gastric glands and less acid secretion in the atrophic stomach, which would enable bacterial proliferation (14). Carcinogens produced by bacteria, such as nitrosamines and acetaldehyde, might then enter the esophagus through regurgitation and cause esophageal cancer (15); nonacidic reflux has also been proposed to be the missing link between gastric atrophy and ESCC (16). Moreover, poor oral health, with altered bacterial flora in the oral cavity and additional carcinogens entering the esophagus, might further elevate the risk for ESCC (10).

In the light of such inconsistent findings regarding the association between gastric atrophy and ESCC, as well as the earlier lack of sufficient statistical power to examine the possible interaction between gastric atrophy and poor oral health, we designed the present large population-based, case-control study in a high-risk region in China.

METHODS

Subject recruitment

The research design and flow of subject recruitment have been described in detail previously (17–19). In brief, we performed a population-based, case-control study from 2010–2014 in Taixing, Jiangsu province, China, where the incidence of ESCC is high (11). More than 90% of the esophageal cancer patients in this area are referred to the 4 largest hospitals (the People's Hospital of Taixing, the Second People's Hospital of Taixing, the Third People's Hospital of Taixing, and the Hospital of Traditional Chinese Medicine of Taixing).

Individuals diagnosed by the endoscopy units in these hospitals between October 2010 and September 2013 were invited to participate. This approach was designed to reduce the risk of nondifferential recall bias, given that these patients were unaware of their cancer diagnosis at the time of recruitment and data collection. We complemented this case recruitment with linkage to the local cancer registry during the same period. Altogether, 1,681 suspected cases of esophageal cancer were identified by the hospitals and

through linkage to the local cancer registry. On the basis of available sections from formalin-fixed and paraffin-embedded tissue blocks stained with hematoxylin-eosin, a pathologist histopathologically verified 1,499 cases of cancer (78.3% of the total number indicated in the local cancer registry), including 1,418 cases of ESCC and 81 tumors of other types. Here only the cases of ESCC are analyzed (Figure 1).

We utilized the Taixing Total Population Registry to randomly select control individuals who frequency-matched the patients in sex and age (within the same 5-year group) every 12 months. Simultaneously, cases of gastric cancer were enrolled. Because the age distributions for development of these 2 types of cancer are similar, a common set of controls was employed.

In total, 2,699 potential cases of gastroesophageal cancer were identified. Anticipating a moderately high nonresponse rate (20%–30%) among controls, we randomly selected 1.3 controls per case, for a total of 3,501. Of these, 643 were excluded due to death, emigration out of the study area, or inability to contact, leaving 2,858 eligible controls, of whom 2,011 provided informed consent and 1,992 were aged 40–85 years, giving a participation rate of 70.4% (2,011/2,858) (Figure 1).

Quality control

The inclusion criteria were an age of 40–85 years and residency in Taixing for at least the last 5 years. We attempted to reduce the risk of information bias by tape recording the interviews and assigning each interviewer the same number of cases and controls to balance information misclassification.

To examine the influence of potential selection bias, we compared the age and sex distribution of the enrolled (78.3%) and nonenrolled (21.7%) cases and enrolled (69.7%) and nonenrolled (30.3%) controls and found no statistically significant differences (Web Table 1, available at <https://academic.oup.com/aje>). We could not assess any potential differences in other demographic, clinical, or social characteristics, because we did not have such information concerning the nonrespondents.

Data collection and assays for biomarkers

The staff involved were specially trained to perform face-to-face interviews using a structured electronic questionnaire, covering factors proposed to be potential confounders in connection with previous studies in this area (demographic characteristics, lifestyle, family structure, and history of cancer).

Our aim was to collect 10-mL blood samples (2 tubes) from all participants, but 208 cases and 14 controls did not have available samples upon analysis. Serum prepared from 1,210 cases and 1,978 controls was stored at -80°C for later determination of pepsinogen I and II (PGI and PGII) concentrations by enzyme-linked immunosorbent assay (Pepsinogen I and II Kits; Biohit Healthcare, Helsinki, Finland) at Qilu Hospital of Shandong University, China.

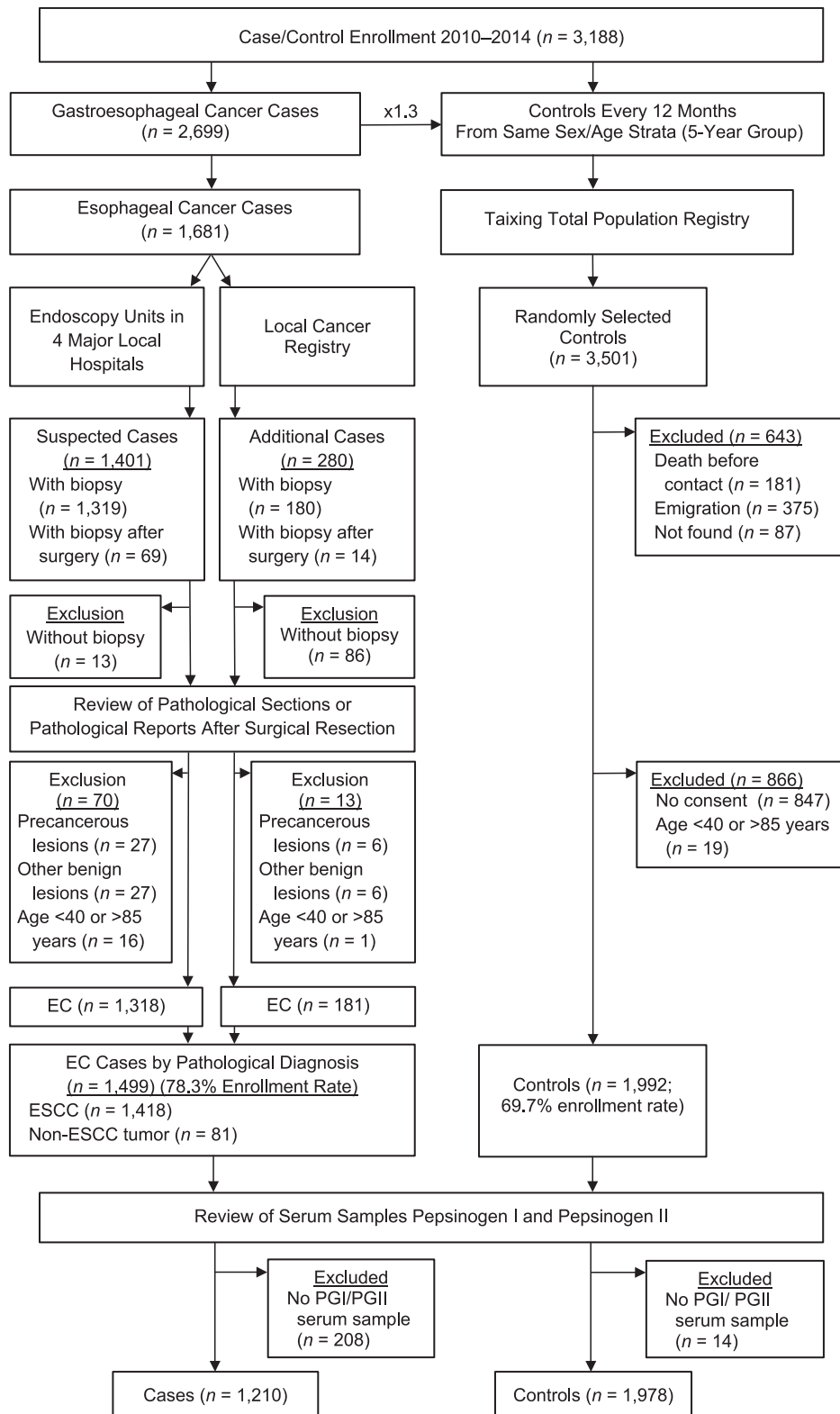


Figure 1. Flowchart illustrating the recruitment of cases of esophageal squamous cell carcinoma (ESCC) and control individuals in connection with a population-based, case-control study conducted in Taixing, China, 2010–2014. EC, esophageal cancer; PGI, pepsinogen I; PGII, pepsinogen II.

The interassay coefficients of variation were 11.3% and 14.9%, respectively. Immunoglobulin G antibodies directed against *H. pylori* were quantified by immunoblotting (*H. pylori* IgG Antibody Detection Kit; Syno Gene Digital Technology, Taizhou, China).

Statistical analysis

Although a low level of serum PGI correlates with gastric fundic atrophy (20), there are no generally accepted cutoff values for PGI, PGII, or the PGI:PGII ratio in this connection. On the basis of our previous study of an Asian population (12), we chose a serum level of PGI below 55 µg/L (sensitivity 61.9%, specificity 94.8%, positive predictive value 48.1%, negative predictive value 97.2%, and area under the curve 0.78) as the serological definition of this condition. A similar criterion was proposed on the basis of an investigation on a Chinese population (21). In addition, we examined potential associations employing other definitions of gastric atrophy (22).

The distributions of demographic and pepsinogen values for cases and controls were compared using the Mann-Whitney unpaired test for continuous variables and χ^2 test or Fisher's exact (observed number < 5) test for categorical variables (after excluding missing values). We used unconditional logistic regression models to estimate the odds ratios with 95% confidence intervals for association of ESCC with gastric atrophy as indicated by the serum concentration of PGI or PGII or PGI:PGII ratio.

Model fitting. Our multivariate analysis involved fitting 3 models. In the first model, we adjusted only for the frequency-matched variables age (continuous) and sex. For the "almost fully adjusting" model, we also adjusted for education (illiteracy, primary school, middle school, high school and above), marital status (unmarried, married, divorced or widowed), occupation (farmer, worker, service/clerk/professional/administrator), family wealth score (quartiles 1–5), body mass index 10 years before (<18.5, 18.5–23.9, 24.0–27.9, \geq 28.0), tea drinking (never/ever), history of esophageal cancer among first-degree relatives (no/yes), cigarette smoking (in pack years: never-smoker, former-smoker, current light smoker (\leq 18), current medium smoker (19–40), current heavy smoker ($>$ 41)), alcohol consumption in g/day (never drinker, former drinker, current low consumption (\leq 40), current medium consumption (41–135), current high consumption ($>$ 135)), and *H. pylori* serostatus (negative/positive). The full model also took into account the sum of missing and filled teeth (quartiles: 0, 1–3, 3–11, \geq 12) and number of daily toothbrushings (\leq 1, \geq 2). Potential confounding variables were identified through careful evaluation of risk factors for ESCC previously proposed to be important in the scientific literature (15, 23–25), together with our own background knowledge of this field. Missing data were disregarded when constructing the multivariate logistic regression models. In a separate analysis of ESCC in association with gastric atrophy, we also assessed the influence of missingness by using dummy variables indicating missing.

Assessment of interactions. We examined both potential multiplicative and additive interactions between gastric atrophy and poor oral health, alcohol consumption, cigarette smoking, and tea drinking. In the case of multiplicative interactions, a cross-product term was introduced into the regression model and the *P* value was derived from Wald test. To test additive interactions, we used the relative excess risk due to interaction (RERI), synergy index (S), and attributable proportion (AP) due to interaction, and calculations were performed using the spreadsheet developed by Andersson et al. (26). Because alcohol consumption, cigarette smoking, and tea drinking were not common among our female subjects, we examined the interaction between these factors and gastric atrophy only among the men.

A 2-sided *P* value of less than 0.05 was considered statistically significant. All statistical analyses were carried out with SAS, version 9.4 (SAS Institute, Inc., Cary, North Carolina).

Sensitivity analysis

Sensitivity analysis was attempted by restricting the analysis of combined effects of gastric atrophy and oral health to patients with a serum level of PGII of <11.8 µg/L, as proposed in our earlier investigation in an area with a high incidence of ESCC (12). To assess the potential influence of nondifferential misclassification, we also performed such an analysis only on cases identified by the endoscopy units.

Ethical considerations

This study was approved by the Institutional Review Board of the School of Life Sciences, Fudan University; the Institutional Review Board of Qilu Hospital, Shandong University; and the Regional Vetting Board of Stockholm (Dnr 2018/357–31). All participants provided written informed consent.

RESULTS

The ratio of men to women (2:1) among our cases is typical for areas with a high incidence of ESCC (Table 1). Our frequency matching ensured that there were no differences in sex or age between the cases and controls. Although male cases and controls were similar in age, marital status, occupation, missing and filled teeth, and *H. pylori* serostatus, they did differ with regard to the other variables (Table 1). Female cases and controls were similar in marital status, tea drinking, smoking, alcohol consumption, and *H. pylori* serostatus, but they differed in all other respects (Table 1). The median PGI and PGII levels and PGI:PGII ratio differed statistically between male, but not female, cases and controls (Table 2).

The odds ratio for ESCC in association with gastric atrophy (defined as a PGI level of <55 µg/L), when adjusted for age and sex, was 1.60 (95% confidence interval (CI): 1.34, 1.90) (Table 3). The estimate was quite similar when fully adjusted for age, sex, education, marital status, occupation, family wealth score, body mass index 10 years

Table 1. Demographic Information for Study Subjects Enrolled in a Case-Control Study of Esophageal Squamous Cell Carcinoma ($n = 3,188$), Taixing, China, 2010–2014

Variable	Men ($n = 2,180$)				P Value	Women ($n = 1,008$)				P Value
	Controls ($n = 1,363$)		Cases ($n = 817$)			Controls ($n = 615$)		Cases ($n = 393$)		
	No.	%	No.	%		No.	%	No.	%	
Age at interview, years ^a	65.6 (8.4)		65.5 (8.5)		0.547 ^b	67.6 (9.4)		69.5 (7.6)		0.016 ^b
Age group at interview, years					0.132 ^c					<0.001 ^c
40–49	53	3.89	25	3.06		29	4.72	5	1.27	
50–59	274	20.10	180	22.03		89	14.47	28	7.12	
60–69	584	42.85	352	43.08		208	33.82	162	41.22	
70–79	402	29.49	216	26.44		235	38.21	160	40.71	
80–85	50	3.67	44	5.39		54	8.78	38	9.67	
Educational level					0.003 ^c					<0.001 ^d
Illiterate	171	12.55	145	17.75		366	59.51	285	72.52	
Primary school	585	42.92	357	43.70		171	27.80	91	23.16	
Middle school	459	33.68	235	28.76		66	10.73	15	3.82	
High school and above	148	10.86	80	9.79		12	1.95	2	0.51	
Marital status					0.574 ^c					0.677 ^d
Unmarried	63	4.62	41	5.02		5	0.81	2	0.51	
Married	1,151	84.45	676	82.74		426	69.27	264	67.18	
Divorced/widowed	149	10.93	100	12.24		184	29.92	127	32.32	
Occupation					0.494 ^c					0.006 ^c
Farmer	709	52.02	446	54.59		545	88.62	371	94.40	
Worker	354	25.97	198	24.24		43	6.99	16	4.07	
Service/clerk/professional/administrator	300	22.01	173	21.18		27	4.39	6	1.53	
Family wealth score					<0.001 ^c					0.011 ^c
Quintile 1 (lowest)	288	21.13	238	29.13		119	19.35	110	27.99	
Quintile 2	231	16.95	135	16.52		119	19.35	74	18.83	
Quintile 3	285	20.91	191	23.38		144	23.41	91	23.16	
Quintile 4	295	21.64	150	18.36		130	21.14	72	18.32	
Quintile 5 (highest)	264	19.37	103	12.61		103	16.75	46	11.70	
Body mass index ^e 10 years prior					<0.001 ^c					0.001 ^c
<18.5	59	4.33	63	7.71		56	9.11	52	13.23	
18.5–23.9	857	62.88	555	67.93		340	55.28	219	55.73	
24.0–27.9	363	26.63	166	20.32		172	27.97	112	28.50	
≥28.0	82	6.02	30	3.67		46	7.48	9	2.29	
Missing	2	0.15	3	0.37		1	0.16	1	0.25	
Sum of missing and filled teeth					0.121 ^c					0.009 ^c
0	366	26.85	193	23.62		129	20.98	55	13.99	
1–3	349	25.61	187	22.89		137	22.28	72	18.32	
4–11	345	25.31	211	25.83		164	26.67	107	27.23	
≥12	285	20.91	197	24.11		177	28.78	140	35.62	
Missing	18	1.32	29	3.55		8	1.30	19	4.83	

Table continues

Table 1. Continued

Variable	Men (n = 2,180)				P Value	Women (n = 1,008)				P Value
	Controls (n = 1,363)		Cases (n = 817)			Controls (n = 615)		Cases (n = 393)		
	No.	%	No.	%		No.	%	No.	%	
Frequency of toothbrushing per day					<0.001 ^c					<0.001 ^c
Once or less	896	65.74	649	79.44		385	62.60	312	79.39	
Twice or more	449	32.94	141	17.26		223	36.26	69	17.56	
Missing	18	1.32	27	3.30		7	1.14	12	3.05	
Tea drinking					<0.001 ^c					0.220 ^c
Never	852	62.51	421	51.53		574	93.33	363	92.37	
Ever	491	36.02	358	43.82		31	5.04	13	3.31	
Missing	20	1.47	38	4.65		10	1.63	17	4.33	
History of esophageal cancer among first-degree relatives					<0.001 ^c					<0.001 ^c
No	1,098	80.56	532	65.12		496	80.65	257	65.39	
Yes	250	18.34	256	31.33		111	18.05	122	31.04	
Missing	15	1.10	29	3.55		8	1.30	14	3.56	
Smoking status ^f					<0.001 ^c					0.841 ^d
Never-smoker	315	23.11	171	20.93		591	96.10	374	95.17	
Former-smoker	152	11.15	61	7.47		4	0.65	2	0.51	
Current light smoker	231	16.95	104	12.73		13	2.11	10	2.54	
Current medium smoker	411	30.15	262	32.07		6	0.98	5	1.27	
Current heavy smoker	253	18.56	215	26.32		1	0.16	2	0.51	
Missing	1	0.07	4	0.49		0	0.00	0	0.00	
Alcohol drinking status ^g					<0.001 ^c					0.425 ^d
Never drinker	580	42.55	191	23.38		570	92.68	348	88.55	
Former drinker	72	5.28	34	4.16		5	0.81	2	0.51	
Current low consumption	159	11.60	106	12.97		18	2.93	19	4.83	
Current medium consumption	361	26.49	298	36.47		9	1.46	5	1.27	
Current high consumption	170	12.47	150	18.36		2	0.33	0	0.00	
Missing	21	1.54	38	4.65		11	1.79	19	4.83	
<i>Helicobacter pylori</i> serostatus					0.119 ^c					0.848 ^c
Negative	448	32.87	242	29.62		187	30.41	122	31.04	
Positive	902	66.18	566	69.28		422	68.62	268	68.19	
Missing	13	0.95	9	1.10		6	0.98	3	0.76	

^a Values are expressed as mean (standard deviation).

^b Mann-Whitney unpaired test for continuous variables was used to derive *P* values after excluding the corresponding missing value.

^c χ^2 test was used to derive *P* values after excluding the corresponding missing value.

^d Fisher exact test for categorical variables was used to derive *P* values after excluding the corresponding missing value.

^e Weight (kg)/height (m)²

^f Current smokers: light, ≤ 18 pack-years; medium, 19–40 pack-years; and heavy, ≥ 41 pack-years.

^g Current drinkers: low consumption, ≤ 40 g/day; medium consumption, 41–135 g/day; and high consumption, > 135 g/day.

before, tea drinking, history of esophageal cancer among first-degree relatives, smoking status, alcohol consumption, and *H. pylori* serostatus (odds ratio = 1.63, 95% CI: 1.35, 1.97), and even when adjusted further for the sum of missing

and filled teeth and frequency of daily toothbrushing (odds ratio = 1.61, 95% CI: 1.33, 1.96) (Table 3). Analyses stratified by sex showed statistically significant odds ratios for ESCC in association with gastric atrophy in both men and

Table 2. Serum Pepsinogen Values for Enrollees in a Case-Control Study of Esophageal Squamous Cell Carcinoma ($n = 3,188$), Taixing, China, 2010–2014

Sex and Group	No.	Missing		Mean (SD)	Median (IQR)	Range	25th–75th Percentile
		No.	%				
<i>PGI, µg/L</i>							
Women							
Cases	391	2	0.51	97.52 (75.31)	79.32 (65.23)	0.00–469.40	51.77–117.00
Controls	611	4	0.65	92.94 (50.05)	84.48 (53.37)	0.00–350.40	60.43–113.80
Men							
Cases	813	4	0.49	109.95 (84.29)	89.55 (77.84)	0.00–521.00	56.56–134.40
Controls	1,350	13	0.95	104.08 (55.82)	95.74 (59.96)	0.00–487.80	69.34–129.30
<i>PGII, µg/L</i>							
Women							
Cases	392	1	0.25	12.90 (13.81)	9.88 (12.06)	0.03–184.10	4.86–16.92
Controls	611	4	0.65	13.78 (11.64)	10.04 (14.67)	0.05–105.70	5.16–19.85
Men							
Cases	814	3	0.37	13.96 (15.09)	10.75 (11.87)	0.05–251.20	5.79–17.66
Controls	1,352	11	0.81	14.76 (12.42)	12.12 (12.89)	0.05–244.00	6.94–19.83
<i>PGI:PGII Ratio</i>							
Women							
Cases	391	2	0.51	32.42 (178.40)	8.54 (8.85)	0.00–2996.00	5.17–14.03
Controls	611	4	0.65	33.56 (163.27)	8.10 (7.99)	0.00–2244.00	5.03–13.02
Men							
Cases	813	4	0.49	39.55 (306.54)	8.87 (7.72)	0.00–6614.00	5.94–13.66
Controls	1,349	14	1.03	26.08 (155.90)	7.93 (6.75)	0.00–2732.00	5.32–12.08

Abbreviations: IQR, interquartile range; PGI, pepsinogen I; PGII, pepsinogen II; SD, standard deviation.

women (data not shown). In a subset of the cases for whom we had information about tumor location, the odds ratio for ESCC in the upper part of the esophagus was 1.35 (95% CI: 0.87, 2.09) while the odds ratio for ESCC in the mid and lower part of the esophagus was 1.87 (95% CI 1.50, 2.34). When other cutoff values were employed to define gastric atrophy, the resulting odds ratios were always greater than 1 but not always statistically significant (Web Table 2). *H. pylori* serostatus did not differ significantly between the cases and controls (Table 3).

When gastric atrophy was defined as a serum level of PGI of $<55 \mu\text{g/L}$, none of the multiplicative interactions between gastric atrophy and missing and filled teeth or number of daily toothbrushings were statistically significant (Table 4). However, indicators of additive interaction—the relative excess risk due to interaction, synergy index, and attributable proportion due to interaction—were all significant in the case of toothbrushing. When gastric atrophy was defined as a PGI of $<30 \mu\text{g/L}$, the relative excess risk due to interaction was 1.17, the synergy index was 2.38, and the attributable proportion due to interaction was 0.39 for the sum of missing and filled teeth, with corresponding values of 2.40, 2.95, and 0.52 for toothbrushing (Web Table 3). Sensitivity analysis limited to those with PGII $< 11.8 \mu\text{g/L}$ produced similar

results (Web Table 4). No significant positive interactions between gastric atrophy (defined as PGI $< 55 \mu\text{g/L}$) and alcohol consumption, cigarette smoking, or tea drinking were observed (Web Table 5). Sensitivity analysis restricted to cases identified by endoscopy units did not change the results materially (data not shown). Including subjects with missing data in the multivariate logistic regression model also did not change the estimates markedly (data not shown).

DISCUSSION

Previous reports on the potential association between gastric atrophy and ESCC have been inconsistent, probably due to inappropriate study design and small sample sizes in some studies. Although a prospective cohort study on patients diagnosed histopathologically with gastric atrophy would be ideal, such a study is almost impossible to perform because of ethical considerations and the relatively low incidence of ESCC. A population-based case-control study with a large sample size would be the next best choice, but again, endoscopic examination of all cases and control subjects is not possible. Although gastric atrophy defined serologically is an inherent limitation due to the poor sensitivity of

Table 3. Odds Ratios for Esophageal Squamous Cell Carcinoma in Association With Gastric Atrophy, Defined by Pepsinogen I Values of $<55 \mu\text{g/L}$, and *Helicobacter pylori* Seropositivity in a Case-Control Study, Taixing, China, 2010–2014

Variable	Controls		Cases		Age/Sex-Adjusted ^b			Fully Adjusted (Except MFT and Toothbrushing) ^c			Fully Adjusted ^d		
	No. ^a	%	No. ^a	%	OR	95% CI	P Value	OR	95% CI	P Value	OR	95% CI	P Value
PgI, $\mu\text{g/L}$													
≥55	1,592	83.3	841	75.1	1.00	Referent	<0.001	1.00	Referent	<0.001	1.00	Referent	<0.001
<55	319	16.7	279	24.9	1.60	1.34, 1.90		1.63	1.35, 1.97		1.61	1.33, 1.96	
<i>H. pylori</i> serostatus													
Negative	622	32.5	340	30.3	1.00	Referent	0.215	1.00	Referent	0.211	1.00	Referent	0.248
Positive	1,291	67.5	782	69.7	1.10	0.95, 1.29		1.11	0.94, 1.32		1.11	0.93, 1.31	

Abbreviations: CI, confidence interval; MFT, missing and filled teeth; OR, odds ratio; PgI, pepsinogen I.

^a Complete observations in the full model.

^b Adjusted for age (continuous) and sex.

^c Adjusted for age (continuous), sex, education, marital status, occupation, family wealth score, body mass index 10 years prior, tea drinking, history of esophageal cancer among first-degree relatives, smoking status, alcohol drinking status, and *H. pylori* serostatus (except age, all other variables are categorized as in Table 1).

^d Additionally adjusted for sum of missing and filled teeth and frequency of toothbrushing per day.

pepsinogen biomarkers, such nondifferential misclassification would bias the true association towards null. Thus, our present population-based, case-control study, with a large sample size, would provide a good possibility of detecting any potential blurred association and any interactions with other risk factors.

Our findings confirm that gastric atrophy (defined as a PGI serum level of $<55 \mu\text{g/L}$) is a risk factor for ESCC in a high-risk region in eastern China, even after adjusting for a variety of known potential confounders. The cutoff point of $<55 \mu\text{g/L}$ was validated with the same test system (Pepsinogen I and II Kits; Biohit HealthCare) in a prior study in northern Iran with similar prevalence of *H. pylori* infection and pangastritis as well as ESCC incidence and mortality as in this study.

Our conclusion is contradictory to that arrived at on the basis of a case-cohort study in another high-risk area in China (9). However, with a similar cutoff value of $\leq 50 \mu\text{g/L}$ for PGI, the point estimate of 1.64 (hazard ratio) in that earlier investigation (although only borderline statistically significant due to the small sample size) is very close to our estimate here.

However, their nonlinear continuous model, using the median of the 4th quartile of PGI:PGII ratio as a reference, showed no obvious trend. Here, we also found that the median PGI:PGII ratio was higher among cases than controls. Smoking (27) and use of proton pump inhibitors (28) (probably more common among cases than controls), might raise PGI levels and explain this difference. Moreover, because the biological meaning of a high PGI level or PGI:PGII ratio remains unclear, the usefulness of treating these as continuous variables is uncertain.

A key limitation to the present investigation is the risk of misclassification of exposure, because gastric atrophy was measured by serum pepsinogen levels and not endoscopy. Due to the population-based study design, we did not ask healthy control subjects to undergo endoscopy for ethical reasons and logistics constraints, and therefore we did not collect the histopathological reports from gastric biopsies taken for clinical purposes from ESCC patients either, although they could have been used for histopathological confirmation of gastric atrophy in a subset of the ESCC patients. We attempted to minimize this limitation by considering a broad span of cutoff values for serum levels of PGI and/or the PGI:PGII ratio. Many cutoff values proposed previously showed robust indications that gastric atrophy is a risk factor for ESCC.

Even though we made considerable efforts to reduce the risk of bias and confounding here, this risk always remains. Although the age and sex distributions of respondents and nonrespondents were similar (Web Table 1), these groups might have differed with respect to other demographic, clinical, or social characteristics. If such differences were similar for the cases and controls, the results would be biased towards null, but a differential difference could distort the findings in an unknown manner. However, such differences would have to be of a magnitude that we do not consider reasonable. The situation is similar for other forms of information bias, such as misclassification of diagnosis. The 208 cases that were unavailable for analysis were similar to the

Table 4. Estimation of Combined Effects of Gastric Atrophy (Defined by Pepsinogen I Values of < 55 µg/L), Dental Health, and Oral Hygiene on Esophageal Squamous Cell Carcinoma Risk in a Case-Control Study in Taixing, China, 2010–2014

Interaction Variable	No Gastric Atrophy				Gastric Atrophy Present				Interaction Analysis						
	Controls		Cases		Controls		Cases		Additive Interaction		Multiplicative Interaction				
	No. ^a	OR ^b	95% CI	No. ^a	OR ^b	95% CI	RER _{OR}	95% CI	S	95% CI	AP ^c	95% CI	P Value		
Missing and filled teeth ^d															
<4	824	388	1.00	Referent	143	105	1.66	1.23, 2.25							
≥4	768	453	1.55	1.18, 2.03	176	174	2.45	1.76, 3.93	0.23	-0.53, 0.99	1.19	0.67, 2.10	0.09	-0.20, 0.39	0.786
No. of toothbrushings per day ^e															
≥2	545	158	1.00	Referent	106	44	1.27	0.84, 1.93							
≤1	1,047	683	2.14	1.72, 2.65	213	235	3.67	2.80, 4.85	1.28	0.39, 2.18	1.91	1.16, 3.15	0.35	0.15, 0.55	0.203

Abbreviations: AP, attributable proportion; CI, confidence interval; OR, odds ratio; RER_{OR}, relative excess risk due to interaction; S, synergy index.

^a Complete observations.

^b All ORs were adjusted for age (continuous), sex, education, marital status, occupation, family wealth score, body mass index 10 years prior, tea drinking, history of esophageal cancer among first-degree relatives, smoking status, alcohol drinking status, *Helicobacter pylori* serostatus (except age, all other variables are categorized as in Table 1).

^c Attributable proportion due to interaction.

^d Additionally adjusted for frequency of toothbrushing per day.

^e Additionally adjusted for sum of missing and filled teeth.

1,210 cases who provided a blood sample for analysis in most aspects, except for age, BMI, missing and filled teeth, and frequency of toothbrushing (Web Table 6). If all of them did not have gastric atrophy, our estimate would be deflated to a maximally adjusted odds ratio of 1.28 (95% CI: 1.06, 1.55), which is still statistically significant. If however all of them had gastric atrophy, that would increase our estimate to a maximally adjusted odds ratio of 2.73 (95% CI: 2.30, 3.25).

The generalizability of our study results might be limited due to the fact that our study population has one of the highest incidence rates of ESCC in the world. Another limitation is lack of oral health information regarding medical conditions such as untreated decay, tooth bleeding, leukoplakia, and gingivitis, which are closely related to the risk of ESCC.

The major strengths of this investigation are its truly population-based, case-control study design and our considerable efforts to minimize the influence of selection bias and recall bias. We collected detailed information concerning potential confounding factors. Furthermore, our large sample size guaranteed sufficient statistical power to detect even small effects and examine interactions.

Our overall interpretation of present and previous findings is that gastric atrophy appears to be a risk factor for ESCC, although the evidence is not yet definitive. The 2 possible underlying causal relationships proposed previously require further elucidation. First, oxidative damage caused by nonacidic gastroesophageal reflux (16) might lead to malignant transformation of epithelial cells as it did in an animal model (29). This is partly supported by a higher odds ratio for ESCC in the mid and lower part compared with that in the upper part of the esophagus in our study. Second, alterations in the gastric microbiota might be involved, given that we have detected dysbiosis among patients with ESCC and squamous dysplasia (30). The altered gastric microbiome might harbor species that can carry out *N*-nitrosation, producing substances known to be carcinogenic towards esophageal mucosa (16).

We also found an additive interaction with poor oral hygiene, which might be due to a biological interaction mechanism. Again, altered microbiota in the oral cavity, due to poor dental health and oral hygiene, might lead to distant effects from inflammation (10) or enhanced production of carcinogenic nitrosamines (11, 31). Furthermore, gastric atrophy might act as a source of such altered microbiota (12). Another explanation for our observed findings could be that the microbiome associated with poor oral health could predispose the development of gastric atrophy and subsequent carcinogenesis in susceptible individuals, and if so, gastric atrophy might lie in the pathway between poor oral health and ESCC. However poor oral health might also be a confounder for the association between gastric corpus atrophy and ESCC. Altogether, we recommend that future research in this area employ histopathological diagnosis of gastric atrophy and explore the role of an altered gastric and oral microbiome in the development of ESCC (30).

In summary, we conclude that gastric atrophy (as defined by the serum level of pepsinogen), appears to be a risk factor for ESCC in a high-risk region of eastern China, and furthermore, there are indications of an additive interaction with poor oral hygiene.

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