Thoracic Cancer

Open Access

Thoracic Cancer ISSN 1759-7706

MINI REVIEW

What have we learned from Linxian esophageal cancer etiological studies?

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Keywords
Epidemiology; esophageal squamous cell carcinoma; etiology; Linxian; China.

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Received: 14 February 2019;
Accepted: 15 March 2019.

doi: 10.1111/1759-7714.13058

Thoracic Cancer **10** (2019) 1036–1042

Abstract

Esophageal cancer is the sixth leading cause of cancer death in the world. Esophageal squamous cell carcinoma (ESCC) accounts for 90% of esophageal cancer cases, over half of which occur in China. Linxian, a county located in the North Central Taihang Mountain range, has the highest ESCC mortality rate, which may be the leading cause of death in this area. In a decades-long research program in Linxian, Chinese and international scientists have exerted great efforts to describe the epidemiological characteristics and elucidate the etiology of ESCC. A systematic review and summary of the current knowledge gained from previous research is informative for future ESCC prevention and control in similar populations, and may be translated to other high-incidence countries, such as Brazil, Iran, Malawi, and South Africa. As ESCC is a major cause of cancer death, more research is required in China and in other high-incidence countries to deepen our understanding of the etiology of ESCC and develop preventative strategies.

Background

Esophageal cancer (EC) is the sixth leading cause of cancer death in the world.[1] There are two different histologic types of EC, esophageal squamous cell carcinoma (ESCC) and adenocarcinoma (EADC), which have distinct geographic and population distributions and risk factors. According to data from the International Agency for Research on Cancer, ESCC accounts for approximately 88% of EC cases and the majority occur in less economically-developed regions.[2] The highest ESCC incidence rates occur in Eastern to Central Asia, and along the Indian Ocean coast of Africa along the Great Rift Valley.[3] The recognized ESCC hotspots exist within sharply defined regions, including Linxian, China; Golestan Province in Iran; Western Kenya and south to Malawi; the Eastern Cape province of South Africa; Calvados in France; Southern Brazil; and Uruguay.[4]

China has the highest ESCC incidence, accounting for 53% of ESCC cases worldwide.[2] EC mortality rates differ across different geographic areas in China, with significant gender discrepancy. This was well documented in the 1970s in county-level EC mortality maps stratified by gender.[5] According to recent cancer registry data, both EC incidence (men 320.8/100 000 vs. women 157.2/100 000) and mortality (men 253.8/100 000 vs. women 121.3/100 000) rates in men are double those in women.[6]

Linxian, a county located in the North Central Taihang Mountain range, has the highest ESCC mortality rate, and thus may the leading cause of death in this area. Previous studies have found that Barrett's esophagus and esophageal adenocarcinoma are rare in this population, and almost all EC cases in Linxian are squamous cell carcinoma.[7] Gastric cardia adenocarcinoma also occurs at an epidemic rate in Linxian and shares some etiologic risk factors with ESCC. Before the widespread use of endoscopy and biopsy, ESCC and cardia cancer were diagnosed as a single disease, referred to as "esophageal cancer" or "hard of swallowing

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disease."[8] For the past few decades, Chinese and international scientists have conducted numerous studies to describe the epidemiological characteristics elaborate the etiology of EC in this high-risk area.

Questionnaire-based risk factor study

In 2016, the World Cancer Research Fund separately evaluated the risk factors of subtypes of EC for the first time, using different grades of evidence: "convincing" evidence of tobacco use and alcoholic beverage intake, "probable" evidence of the consumption of Maté (an herbal infusion consumed in parts of South America), and "limited-suggestive" evidence of the consumption of processed meat. There is also limited-suggestive evidence of the protective effect of consuming vegetables and fruits, and physical activity.[9] For the other risk factors, there is still "limited-no conclusion" evidence that requires further investigation.

The etiology of ESCC is multifactorial and certain risk factors are strongly population-dependent. In Linxian and other Taihang Mountain Grand Junction areas of the Henan, Hebei, and Shanxi Provinces in China, pickled vegetables are popular foods and were convincing confirmed as carcinogens of ESCC decades ago.[10,11] We conducted a series of surveys and took advantage of the detailed data to investigate some inconclusive possible risk factors of esophageal squamous dysplasia (ESD, the precursor lesion) and ESCC in Linxian residents. In a cross-sectional study, we found that more household members, a family history of cancer, higher systolic blood pressure, heating the home without a chimney, and the loss of more (but not all) teeth were factors associated with a higher risk of ESD.[12] In addition, higher socioeconomic status (SES), evaluated by household income, was associated with a lower risk of ESD.[12] Further consistent results were found in a prospective study of the association between SES and the risk of ESCC. We found that higher general SES, assessed by formal education; having water piped into the home; increased consumption of meat, eggs, and fresh fruits; and an increased body mass index (BMI), were associated with a lower risk of ESCC in Linxian.[13] In addition, we conducted a 21-year prospective analysis to further investigate the association between body fat and ESCC, and the results showed that compared to people with a normal BMI (18.5–24 kg/m^2) at baseline, underweight people had a 21% higher risk and overweight people had a 13% lower risk of ESCC.[14]

Another important finding was the association between oral health and the risk of ESCC. We found that poor oral health, measured by greater tooth loss, less frequent teeth brushing, and poorer periodontal health, were related to an increased risk of ESCC.[12,15] This raised the hypothesis that tooth loss may be related to alterations in oral bacterial flora and subsequent increases in the in vivo production of carcinogens, either directly or through inflammation. A later analysis on the association between oral leukoplakia and upper gastrointestinal (UGI) cancers provided supportive evidence that patients with oral leukoplakia have a significantly higher risk of ESCC.[16] Overall, these results have gradually attracted worldwide interest on the association between microbiome and the risk of ESCC.

Randomized controlled intervention trial

General population nutrition intervention trial

Nutrition deficiency has been investigated for decades as a possible risk factor of ESCC, but until now there has been "limited with no conclusion" evidence of the role of micronutrients, including vitamin C, vitamin E, folate, beta-carotene, vitamin A, retinol, thiamin, riboflavin, calcium, iron, zinc, provitamin A carotenoids, or beta-cryptoxanthin in the carcinogenesis of ESCC.[9] Over 20 major randomized controlled trials (RCTs) have been conducted worldwide to test the effects of nutritional intervention on cancer prevention, but few reported significant effects of the nutrients tested, and even fewer reported their effects on EC.

The Linxian General Population Nutrition Intervention Trial (NIT), which commenced in 1985, was one of the first-generation NITs on cancer prevention. Approximately 30 000 residents were randomly assigned into one of eight intervention arms to investigate the effects of nine nutrients combined into four multi-agent factors on UGI cancer prevention using a one-half replicate of a 2^4 fractional factorial design.[17] The intervention lasted for 5.25 years (1986–1991) and the cohort was followed post-supplementation for an additional 25 years. The trial results showed that daily riboflavin and niacin supplementation for 5.25 years reduced the risk of ESCC by 14%, although this result was not statistically significant.[18] The protective effect on ESCC became apparent in a later 25-year post-trial follow-up analysis (hazard ratio [HR] 0.92, 95% confidence interval [CI] 0.85–1.00; $P = 0.04$).[19] In addition, our 10-year post-trial follow-up analysis showed that selenium, β-carotene, and α-tocopherol supplementation significantly reduced the risk of ESCC by 17%, but only in participants aged < 55 years at study entry.[8] Further analyses of the same trial participants suggest that the main protective agent in this population was selenium;[20–22] thus a series of studies were conducted to investigate the association between selenium and ESCC.

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Dysplasia population nutrition intervention trial

Parallel with the NIT study of the general population, our team conducted another NIT including approximately 3300 subjects with esophageal dysplasia in Linxian, which randomized subjects to daily multivitamin and mineral supplements for six years. However, no effect of multivitamin supplementation was found on total or cause-specific mortality during six years of multivitamin supplementation or during the 20 years of post-intervention follow-up.[23] This raised the hypothesis that nutrient supplementation may only prevent or slow progression prior to the existence of preneoplastic lesions. A similar result was found for the association between folate supplementation and colorectal cancer;[24–27] however, further basic studies are needed to test this hypothesis.

Chemoprevention trial

In Linxian, the standard of care for mild or moderate esophageal dysplasia is to monitor by surveillance. Previous studies suggested that several promising agents may favor dysplasia regression, including selenomethionine (a synthetic form of organic selenium) and celecoxib (a selective inhibitor of cyclooxygenase 2 [COX2]).[28] Thus, a randomized, controlled chemoprevention trial was designed to assess the effects of selenomethionine and celecoxib among asymptomatic adults with mild or moderate ESD. A 10-month intervention of selenomethionine or celecoxib did not inhibit esophageal squamous carcinogenesis in high-risk subjects; however, in patients with mild ESD at baseline, the administration of selenomethionine did yield higher regression and lower progression rates ($P = 0.02$).[29] This study was the first to report the possible beneficial effect of any candidate ESCC chemopreventive agent in an RCT. Together with the beneficial effects of selenium found in NIT studies, our data warrant the further pursuit of the effect of selenium on ESCC prevention.

Biomarker exploration

Nutritional biomarker

The NIT study collected longitudinal data on the potential risk factors and fasting blood samples at baseline, thus we took advantage of this cohort and conducted a series of studies to explore the etiology of ESCC.

Selenium

Considering the promising protective effect of selenium on cancer prevention identified in the NITs, we conducted a case-cohort study to further investigate the risks of ESCC in patients with different serum selenium concentrations. The results showed a significant inverse association of serum selenium levels with the incidence of ESCC ($P_{trend} < 10^{-4}$).[20] Patients in the highest serum selenium quartile had nearly half the risk compared to those in the lowest quartile (HR 0.56, 95% CI 0.44–0.71). A quarter (26.4%) of the ESCC cases in this population were attributed to low selenium levels.[20] In later 15-year follow-up analysis of the subcohort in this study, we found that a higher serum selenium level had a consistent protective effect on death from ESCC (relative risk [RR] 0.83, 95% CI 0.71–0.98) and additional benefits to heart disease.[30] Given these results, the effect of selenium on ESCC cancer prevention merits serious consideration.

Cysteine

Cysteine is an important non-essential amino involved in a myriad of immunomodulatory, anti-oxidant, and anticarcinogenic pathways; however, limited studies have investigated the etiology of EC. We conducted a case-cohort study in Linxian and found that higher concentrations of serum cysteine were significantly associated with a lower risk of ESCC ($HR_{Q4\ vs.\ Q1}$ 0.70, 95% CI 0.51–0.98), and the association was dose dependent ($P_{trend} = 0.006$).[31] This promising result indicates that cysteine may act as a potential chemopreventive agent for EC and thus requires further investigation.

25-hydroxyvitamin D

A previous study reported that geographic areas with lower solar radiation had higher EC rates, which led to the hypothesis that a higher risk of EC is related to low vitamin D status.[32] Linxian is an area in which the traditional diet provides very little vitamin D because the residents eat very little fatty fish, liver, or eggs, and fortified products are not available.[33] Therefore, we explored the association between serum 25 hydroxyvitamin (OH) D and the risk of ESCC and ESD in Linxian residents with a low vitamin D status. Results from a cross-sectional study showed that higher serum 25(OH)D concentrations were associated with a significantly increased risk of squamous dysplasia.[32] The results of another prospective study concurred with this finding, reporting that the risk of ESCC was significantly increased in men with higher serum 25(OH)D levels.[34]

B vitamins

B vitamins play important roles in DNA synthesis and methylation and may affect the risk of ESCC. We investigated the associations between B vitamins and related genes with ESCC using serum samples collected in the NIT cohort. People with a higher level of riboflavin ($HR_{Q4\ vs.\ Q1}$ 0.56, 95% CI 0.41–0.75) or B12 had a

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significantly lower risk of ESCC.[35] In another investigation on three polymorphisms in two genes that code for enzymes that require folate and B12 as cofactors, *MTRR* A66G, *MTHFR* C677T, and *MTHFR* A1298C, we found that individuals with the *MTHFR* 677TT genotype had significantly higher combined ESCC/gastric cardia cancer risks (RR 1.45, 95% CI 1.02–2.05) than those with CC or CT genotypes. Compared to subjects with the *MTRR* 66AA genotype, subjects with the AG or GG genotypes had a significantly higher risk of ESCC (RR 1.59, 95% CI 1.04–2.42).[36]

Microbiome

Microbiome is an emerging area in the etiological exploration of various diseases; however, limited studies have focused on the association between microbiota and UGI diseases. A study was conducted in Linxian to evaluate the UGI microbiota using a microarray chip (Human Oral Microbe Identification Microarray) from DNA extracted from sample cells from the stomach, esophagus, and saliva collected using a balloon device. In a comparison of 142 patients with ESD and 191 without, the odds of this precancerous lesion were 0.74 (95% CI 0.58–0.95) per one standard deviation increase in the number of detected bacterial genera.[37] This finding suggests that people with lower esophageal microbial richness and microbial diversity in saliva may be more prone to developing ESD; however, these results warrant future validation in prospective cohort studies.

Human papillomavirus

Since the human papillomavirus (HPV) was suspected as an etiological agent of ESCC in the 1980s, numerous studies have been conducted to assess the role of HPV in the etiology of ESCC. Enthusiasm particularly increased after the development of the HPV vaccine. We conducted a series of studies in Linxian of ESCC cases using a variety of different HPV assessment measures, including a prediagnostic serological HPV antibody test, cytological HPV DNA test, PCR assay in tissue samples, and serological E6/E7 antibody test, but found no consistent evidence of the role of HPV in the etiology of ESCC.[38–40] These null results were consistent with results from a large international consortium project on serologic analyses of HPV L1 and E6/E7 and ESCC, which found only 0.3% of the total ESCC cases were positive for HPV 16 E6 and E7.[41] Thus, the current evidence indicates that HPV does not play a significant role in the etiology of ESCC, and the number of ESCC cases caused by this virus, if any, is limited.

H. pylori

Barrett's esophagus and esophageal adenocarcinomas are rare in Linxian residents. Our research team conducted a case-cohort study in Linxian residents to investigate the role of *H. pylori* in the carcinogenesis of ESCC. We measured immunoglobulin G antibodies to whole-cell and CagA *H. pylori* antigens, and found that *H. pylori* did not affect the risk of ESCC.[42] Considering the possible roles of *H. pylori* in different esophageal and gastric cancer subtypes, more studies are required in diverse populations to elucidate the role of *H. pylori* in the etiology of different UGI cancers.

Pepsinogen

A low serum pepsinogen I (PGI) level and a low serum PGI/PGII ratio (PGI/II ratio) are markers of gastric fundic atrophy, and were thought to be possible risk factors of ESCC. We investigated the associations in two study populations in Linxian. In patients with esophageal dysplasia, we found that a lower serum PGI/II ratio had a dose–response association with an increased risk of ESD ($OR_{Q4\ vs.\ Q1}$ 2.12, 95% CI 1.08–4.18), and a lower serum PGI/II ratio was linearly associated with a higher risk of ESD ($P = 0.03$).[43] However, when we explored the associations in patients with ESCC, little evidence was found, except for a marginal association (HR 1.56, 95% CI 0.99–2.47) in people with a PGI/II ratio ≤ 4.[44] Further study is required to understand the role of pepsinogen in the etiology of ESCC.

Genetic factors

Previous studies have shown that people with family history of cancer have an increased risk of developing UGI cancers. Linxian has the highest ESCC mortality in China and over 30% of the residents have a family history of UGI cancers,[8] which enabled us to explore the associations between generic factors and ESCC. Several large genome-wide association studies (GWAS) have been conducted in Linxian alone or in combination with other high-risk areas in China.[45–47] Several important variants in *PLCE1* and a polymorphism in the *TP*53 gene region are related to the risk of ESCC.[45,47] In addition, joint analysis of three published Chinese GWAS found that a significant variant in the *HLA 2* genome region is related to ESCC risk, especially in residents of the high-risk Taihang Mountain areas.[47] However, better elucidation of the role of the *HLA* region is required to understand the complex and long-range linkage disequilibrium patterns of this specific region.

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Clinical relevant precursor identification

For a long period, there was no consistent understanding of the histological origins, progression, or definition of clinically relevant precursor lesions of ESCC. To better elucidate the histological progression process and identify the clinical endpoints for treatment, our research team conducted a 13.5-year prospective analysis to compare ESCC RRs in patients with different histological diagnoses at baseline. The results showed that squamous dysplasia and carcinoma in situ were the only histological lesions associated with a significantly increased risk of developing ESCC. Increasing grades of dysplasia were strongly associated with an increased risk of ESCC.[48] Notably, the results of our study provided robust evidence that severe dysplasia and carcinoma in situ present the same risk of developing ESCC and should be treated immediately. This led to a nationwide guideline of the clinical endpoint definition for the screening, early detection, and treatment of ESCC across China.

Current gaps and future directions

After decades-long research in Linxian and other high-risk areas worldwide, many risk factors of ESCC have been confirmed, suspected, or ruled out.[9] Sustained efforts to control tobacco, reduce heavy alcohol consumption, reduce lifestyle risk behaviors (i.e. less consumption of pickled vegetables), as well as screening and early detection projects, have led to a decrease in the incidence of ESCC in Linxian and in many western countries in recent decades.[49] However, ESCC remains a major cause of cancer death worldwide and has not been sufficiently investigated. There are still large unknown areas in the etiology of ESCC. More research is warranted to understand the associations between ESCC and suspected risk factors, including polycyclic aromatic hydrocarbons, diet, nutrition, high-temperature food, and diverse cooking and eating styles. In addition, further research in emerging areas of microbiome and metabolomics, and large GWAS are required in China and in other high-incidence countries in Africa, Asia, and South America to obtain to a deeper understanding of the etiology of ESCC and develop preventative strategies.

Acknowledgments

This work was supported by the Young Scientists Fund of the National Natural Science Fund of China (No. 81502864) and the Intramural Research Program (No. HHSN261201700047C) of the National Cancer Institute, National Institute of Health, USA.

Disclosure

No authors report any conflict of interest.

References

1 Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray, F GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. Lyon, France: IARC; 2013. [Cited 25 Dec 2015.] Available from URL: http://globocan.iarc.fr. 2015.
2 Arnold M, Soerjomataram I, Ferlay J, Forman D. Global incidence of oesophageal cancer by histological subtype in 2012. *Gut* 2015; **64**: 381–7.
3 Abnet CC, Arnold M, Wei WQ. Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology* 2018; **154**: 360–73.
4 Murphy G, McCormack V, Abedi-Ardekani B *et al.* International cancer seminars: A focus on esophageal squamous cell carcinoma. *Ann Oncol* 2017; **28**: 2086–93.
5 Editorial Committee for the Atlas of Cancer Mortality. *Atlas of Cancer Mortality in the People's Republic of China*. China Map Press 1979; 31–8; Beijing, China.
6 Chen W, Zheng R, Baade PD *et al.* Cancer statistics in China, 2015. *CA Cancer J Clin* 2016; **66**: 115–32.
7 Dawsey SM, Mark SD, Taylor PR, Limburg PJ. Gastric cancer and H pylori. *Gut* 2002; **51**: 457–8.
8 Qiao YL, Dawsey SM, Kamangar F *et al.* Total and cancer mortality after supplementation with vitamins and minerals: Follow-up of the Linxian General Population Nutrition Intervention Trial. *J Natl Cancer Inst* 2009; **101**: 507–18.
9 World Cancer Research Fund International/American Institute for Cancer Research. Continuous Update Project Report: Diet, Nutrition, Physical Activity and Oesophageal Cancer. 2016. [Cited 10 December 2018.] Available from URL: wcrf.org/oesophageal-cancer-2016.
10 Islami F, Ren JS, Taylor PR, Kamangar F. Pickled vegetables and the risk of oesophageal cancer: A meta-analysis. *Br J Cancer* 2009; **101**: 1641–7.
11 Kamangar F, Chow WH, Abnet CC *et al.* Environmental causes of esophageal cancer. *Gastroenterol Clin North Am* 2009; **38**: 27–57, vii.
12 Wei WQ, Abnet CC, Lu N *et al.* Risk factors for oesophageal squamous dysplasia in adult inhabitants of a high risk region of China. *Gut* 2005; **54**: 759–63.
13 Tran GD, Sun XD, Abnet CC *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.
14 Wang SM, Fan JH, Jia MM *et al.* Body mass index and long-term risk of death from esophageal squamous cell carcinoma in a Chinese population. *Thorac Cancer* 2016; **7**: 387–92.

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15 Abnet CC, Qiao YL, Mark SD, Dong ZW, Taylor PR, Dawsey SM. Prospective study of tooth loss and incident esophageal and gastric cancers in China. *Cancer Causes Control* 2001; **12**: 847–54.
16 Fan JH, Wang JB, Qu CX *et al.* Association between oral leukoplakia and upper gastrointestinal cancers: A 28-year follow-up study in the Linxian General Population Trial. *Oral Oncol* 2014; **50**: 971–5.
17 Li B, Taylor PR, Li JY *et al.* Linxian Nutrition Intervention Trials. Design, methods, participant characteristics, and compliance. *Ann Epidemiol* 1993; **3**: 577–85.
18 Blot WJ, Li JY, Taylor PR *et al.* Nutrition intervention trials in Linxian, China: Supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. *J Natl Cancer Inst* 1993; **85**: 1483–92.
19 Wang SM, Taylor PR, Fan JH *et al.* Effects of nutrition intervention on total and cancer mortality: 25-year post-trial follow-up of the 5.25-year Linxian Nutrition Intervention Trial. *J Natl Cancer Inst* 2018; **110**: 1229–38.
20 Mark SD, Qiao YL, Dawsey SM *et al.* Prospective study of serum selenium levels and incident esophageal and gastric cancers. *J Natl Cancer Inst* 2000; **92**: 1753–63.
21 Taylor PR, Qiao YL, Abnet CC *et al.* Prospective study of serum vitamin E levels and esophageal and gastric cancers. *J Natl Cancer Inst* 2003; **95**: 1414–6.
22 Abnet CC, Qiao YL, Dawsey SM *et al.* Prospective study of serum retinol, beta-carotene, beta-cryptoxanthin, and lutein/zeaxanthin and esophageal and gastric cancers in China. *Cancer Causes Control* 2003; **14**: 645–55.
23 Wang JB, Abnet CC, Fan JH, Qiao YL, Taylor PR. The randomized Linxian Dysplasia Nutrition Intervention Trial after 26 years of follow-up: No effect of multivitamin supplementation on mortality. *JAMA Intern Med* 2013; **173**: 1259–61.
24 Ulrich CM, Potter JD. Folate and cancer: Timing is everything. *JAMA* 2007; **297**: 2408–9.
25 Cole BF, Baron JA, Sandler RS *et al.* Folic acid for the prevention of colorectal adenomas: A randomized clinical trial. *JAMA* 2007; **297**: 2351–9.
26 Song J, Medline A, Mason JB, Gallinger S, Kim YI. Effects of dietary folate on intestinal tumorigenesis in the apcMin mouse. *Cancer Res* 2000; **60**: 5434–40.
27 Song J, Sohn KJ, Medline A, Ash C, Gallinger S, Kim YI. Chemopreventive effects of dietary folate on intestinal polyps in Apc+/−Msh2−/− mice. *Cancer Res* 2000; **60**: 3191–9.
28 Steinbach G, Lynch PM, Phillips RK *et al.* The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. *N Engl J Med* 2000; **342**: 1946–52.
29 Limburg PJ, Wei W, Ahnen DJ *et al.* Randomized, placebo-controlled, esophageal squamous cell cancer chemoprevention trial of selenomethionine and celecoxib. *Gastroenterology* 2005; **129**: 863–73.
30 Wei WQ, Abnet CC, Qiao YL *et al.* Prospective study of serum selenium concentrations and esophageal and gastric cardia cancer, heart disease, stroke, and total death. *Am J Clin Nutr* 2004; **79**: 80–5.
31 Murphy G, Fan JH, Mark SD *et al.* Prospective study of serum cysteine levels and oesophageal and gastric cancers in China. *Gut* 2011; **60**: 618–23.
32 Abnet CC, Chen W, Dawsey SM *et al.* Serum 25(OH)-vitamin D concentration and risk of esophageal squamous dysplasia. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 1889–93.
33 Zou XN, Taylor PR, Mark SD *et al.* Seasonal variation of food consumption and selected nutrient intake in Linxian, a high risk area for esophageal cancer in China. *Int J Vitam Nutr Res* 2002; **72**: 375–82.
34 Chen W, Dawsey SM, Qiao YL *et al.* Prospective study of serum 25(OH)-vitamin D concentration and risk of oesophageal and gastric cancers. *Br J Cancer* 2007; **97**: 123–8.
35 Ren J, Murphy G, Fan J *et al.* Prospective study of serum B vitamins levels and oesophageal and gastric cancers in China. *Sci Rep* 2016; **6**: 35281.
36 Stolzenberg-Solomon RZ, Qiao YL, Abnet CC *et al.* Esophageal and gastric cardia cancer risk and folate- and vitamin B(12)-related polymorphisms in Linxian, China. *Cancer Epidemiol Biomarkers Prev* 2003; **12**: 1222–6.
37 Yu G, Gail MH, Shi J *et al.* Association between upper digestive tract microbiota and cancer-predisposing states in the esophagus and stomach. *Cancer Epidemiol Biomarkers Prev* 2014; **23**: 735–41.
38 Gao GF, Roth MJ, Wei WQ *et al.* No association between HPV infection and the neoplastic progression of esophageal squamous cell carcinoma: Result from a cross-sectional study in a high-risk region of China. *Int J Cancer* 2006; **119**: 1354–9.
39 Kamangar F, Qiao YL, Schiller JT *et al.* Human papillomavirus serology and the risk of esophageal and gastric cancers: Results from a cohort in a high-risk region in China. *Int J Cancer* 2006; **119**: 579–84.
40 Koshiol J, Wei WQ, Kreimer AR *et al.* No role for human papillomavirus in esophageal squamous cell carcinoma in China. *Int J Cancer* 2010; **127**: 93–100.
41 Sitas F, Egger S, Urban MI *et al.* InterSCOPE study: Associations between esophageal squamous cell carcinoma and human papillomavirus serological markers. *J Natl Cancer Inst* 2012; **104**: 147–58.
42 Kamangar F, Qiao YL, Blaser MJ *et al.* Helicobacter pylori and oesophageal and gastric cancers in a prospective study in China. *Br J Cancer* 2007; **96**: 172–6.
43 Kamangar F, Diaw L, Wei WQ *et al.* Serum pepsinogens and risk of esophageal squamous dysplasia. *Int J Cancer* 2009; **124**: 456–60.
44 Ren JS, Kamangar F, Qiao YL *et al.* Serum pepsinogens and risk of gastric and oesophageal cancers in the General Population Nutrition Intervention Trial cohort. *Gut* 2009; **58**: 636–42.

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45 Abnet CC, Freedman ND, Hu N *et al*. A shared susceptibility locus in PLCE1 at 10q23 for gastric adenocarcinoma and esophageal squamous cell carcinoma. *Nat Genet* 2010; **42**: 764–7.
46 Abnet CC, Wang Z, Song X *et al*. Genotypic variants at 2q33 and risk of esophageal squamous cell carcinoma in China: A meta-analysis of genome-wide association studies. *Hum Mol Genet* 2012; **21**: 2132–41.
47 Wu C, Wang Z, Song X *et al*. Joint analysis of three genome-wide association studies of esophageal squamous cell carcinoma in Chinese populations. *Nat Genet* 2014; **46**: 1001–6.
48 Wang GQ, Abnet CC, Shen Q *et al*. Histological precursors of oesophageal squamous cell carcinoma: Results from a 13 year prospective follow up study in a high risk population. *Gut* 2005; **54**: 187–92.
49 Sun XB, Lian SY, Liu SZ, Li BY, Quan PL, Lu JB. [The trends on the mortality for esophagus and stomach cancers in Linzhou city from 1988 to 2003]. *China J Prev Med* 2007; **41** (Suppl 66-9):4. (In Chinese.)

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