The Etiology of Esophageal Cancer in High– and Low– Risk Areas of Jiangsu Province, China

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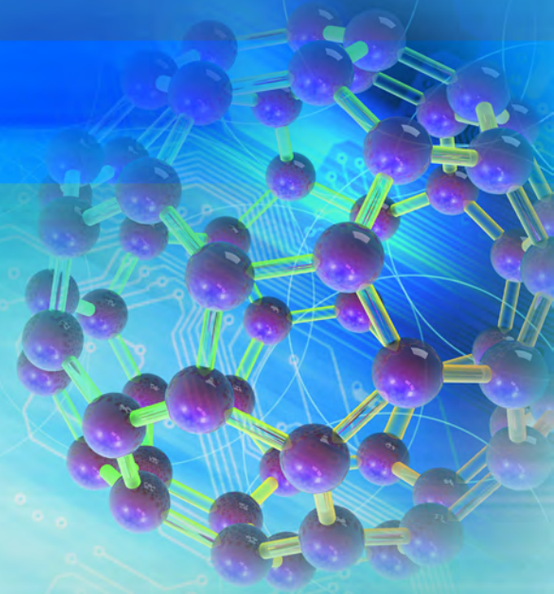

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Thesis committee

Thesis supervisors
Prof. dr. ir. E. Kampman
Personal Chair at the Division of Human Nutrition, Wageningen University

Prof. dr. ir. P. van 't Veer
Professor of Nutrition and Epidemiology, Wageningen University

Thesis co-supervisor
Dr. J.K. Zhao
Associate Professor of Epidemiology,
Jiangsu Provincial Center for Disease Control and Prevention, Nanjing, China

Other members
Prof. dr. P.D. Siersema, University Medical Center Utrecht
Prof. dr. J.W. Coebergh, Erasmus University Medical Center, Rotterdam
Prof. dr. ir. I.M.C.M. Rietjens, Wageningen University
Dr. L. J. Schouten, Maastricht University Medical Centre

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Thesis

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ABSTRACT

[Background] Esophageal cancer (EC) is one of the most common and fatal malignancies in the world. The geographic variation in EC occurrence is striking, and China is one of the highest incidence areas worldwide. A number of epidemiological studies have been conducted on EC in the past decades, results suggested that tobacco smoking, alcohol drinking, unhealthy dietary factors and chronic injuries of the esophageal mucosa are important in the development of this disease. Genetic polymorphisms in enzymes involved in metabolism of carcinogens may also influence individual susceptibility. However, the effects of major lifestyle and hereditary risk factors on the development of EC remain poorly understood in China. Moreover, little attention has been paid to the etiological heterogeneity between similar areas with great risk gradient.

[Methods] From 2003 to 2007, a large population-based case-control study of EC has been conducted in a selected high-risk area (Dafeng) and a selected low-risk area (Ganyu) of Jiangsu Province, one of the high cancer risk areas in China. In total, 1,520 cases and 3,879 controls were recruited. In this thesis, we evaluated the role of major lifestyle factors such as tobacco smoking, alcohol drinking and dietary factors, as well as inherited determinants including family history of cancer and genetic polymorphisms of alcohol-metabolizing related genes on the risk of EC. In addition, we investigated how much of the risk gradient between the two areas could be explained by variation in the distributions of major risk factors.

[Results] Tobacco smoking and alcohol drinking moderately increased the risk of EC, while the positive associations were only found among men but not among women. Dietary factors were observed to play important roles in the development of EC. Specific dietary habits i.e., fast eating speed, and hot eating and/or drinking substantially elevated EC risk and could explain more than 20% of EC cases each. High intake of salty foods and fried foods, low consumption of raw garlic were also observed to increase the risk of EC. In addition to environmental and lifestyle factors, we confirmed that a positive family history can significantly increase EC risk, and found the inheritance may modify the effects of some unhealthy lifestyles. Moreover, we further explored the relationship between EC and single nucleotide

polymorphisms of ADH1B, ADH1C and ALDH2 genes. Results showed that the slow metabolizing ADH1B and ADH1C G allele, and ALDH2 A allele were associated with EC risk among moderate-to-heavy alcohol drinkers, and a significant interaction was observed between ALDH2 gene and alcohol consumption. Lastly, we found that more than 60% of EC cases could be attributable to major lifestyle risk factors in the study population; furthermore, dissimilar distribution of several lifestyle factors, together with variations of hereditary factors may be largely responsible for the incidence difference between the two regions.

[Conclusion] The findings in this thesis confirm that unhealthy lifestyles including smoking, alcohol drinking and some dietary factors are the predominant risk factors of EC in China, and a large proportion of incidence difference between regions at varying risk could be attributed to the different prevalence of lifestyle factors. As most of the identified risk factors are modifiable, these could be translated into risk reduction prevention programs in China. A substantial proportion of new EC cases are expected to be prevented by eliminating or avoiding these risk factors in the population.

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Chapter 1

General Introduction

Chapter 1

Esophageal cancer (EC) remains one of the most common cancers and an increasing health problem in the world.[1] This aggressive malignancy is normally characterized by rapid development and poor prognosis in most cases. Even when the tumor is surgically removed at its early and operable stage, five-year survival is still unfavorable.[2]

Comprehensive studies have been conducted toward EC in the past decades. Based on multidisciplinary approaches of clinical, laboratory and field investigations, epidemiological evidence suggests that tobacco smoking, alcohol drinking, diets deficient in vitamins/protective antioxidant, high intake of carcinogens such as mycotoxins and nitrosyl compounds, thermal injuries and possible infections (Human papilloma virus and Helicobacter pylori) are important in the development of this disease.[3-6] Genetic polymorphisms in enzymes involved in metabolism of carcinogens also may modify the risk of behavior and environmental factors, and influence individual susceptibility to cancer. However, EC remains one of the least studied cancers and its etiology still needs to be further elucidated.[7]

DESCRIPTIVE EPIDEMIOLOGY

Worldwide

EC ranks the 8th most common cancer worldwide and about 481,000 new cases are diagnosed in 2008 (3.8% of the total cancer). Among all new cases of EC, 71.49% are males. More than 80% of the cases and of the deaths occur in developing countries.[8] The age-standardized incidence is 10.2 and 4.2 per 100,000 for male and female respectively, according to the estimation of GLOBOCAN 2008 Database.[8]

Although a combination of screening and treatment is increasingly effective in reducing the mortality of EC nowadays, it remains one of the most deadly diseases, 75% of patients die within 1 year after diagnosis, only 16% of cases in the United States and 10% cases in Europe can survive more than five years. The prognosis is much poorer in developing countries.[1] Estimated by the GLOBOCAN 2008, EC is the 6th fatal cancer and causes about 406,000 deaths every year in the world (5.4% of the total cancer). The global

average age-standardized mortality is estimated to be 8.6 and 3.4 per 100,000 for male and female, respectively.[8]

Worldwide, the geographic variation in the occurrence of EC is striking, more than for almost any other cancer (Figure 1-1). Although accurate cancer registry information is limited, the highest risk areas of the world are in the Asian EC belt (stretching from Northern Iran through the Central Asian republics to North-Central China). Other areas of relatively high-risk are southern and eastern Africa, south-central Asia and Northern France.[1,9] At the global level, a 20-fold variation is observed between high-risk China and low-risk western Africa. This geographic variability is even more marked when smaller areas are studied--for example, between countries or even within countries. In most areas, EC occurs 2-4 times higher among males than females, however, in the high-risk areas, the cancer appears almost as often in women as men. [1]

There are two major histological types of EC, squamous cell carcinoma (ESCC) and adenocarcinoma (EAC). Although the incidence of ESCC appears to be the same or decrease, it remains the dominant type of esophageal malignancy in the world. On the other hand, both relative and absolute numbers of EAC have been shown to increase dramatically, particularly in Western countries.[10] In the USA, for example, the incidence of EAC among white men was 0.5-0.9/100,000 in the 1970s, but have increased to 3.2-4.0/100 000 over the next two decades, and now EAC accounts the majority of total esophageal malignancies in the US.[11]

China

China is one of the highest risk areas of EC worldwide, about half of the cases occurring in the world are estimated to occur in China each year.[8] As information on cancer incidence in China is rather sparse, national patterns and trends of EC incidence are not completely clear yet. Data of routine mortality surveillance, together with the results from three national mortality surveys (1973-1975, 1990-1992 and 2004-2005) were used to address the distribution of EC in China.[12] Since the survival from this disease is poor, mortality and incidence rates should be comparable.

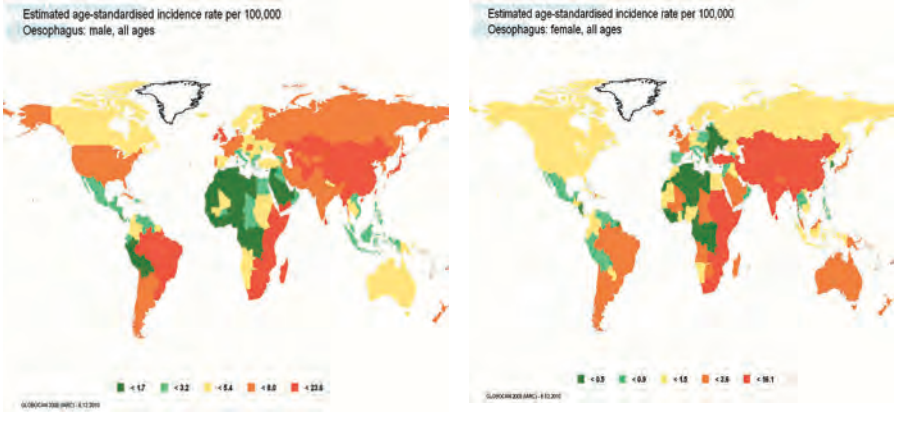

Figure 1-1 Age-standardized incidence of EC worldwide. *(Source: GLOBCAN 2008(IARC)-6.12.2010)*

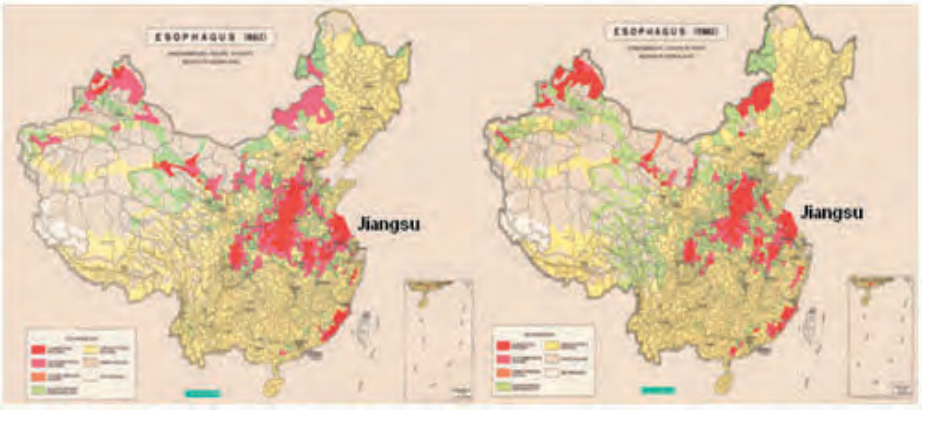

Figure 1-2 Gender specific age-standardized mortality of EC in China, 1973-1975. *(Source: Atlas of cancer mortality in the people's Republic of China)* [14]

The mortality rates of EC in China are considerably higher in rural areas than in urban areas and higher among males than females. Based on the latest death retrospective survey (2004-2005), EC is the 4th leading cause of death from cancer, accounted for 11.2% of total cancer death in China. The age-standardized mortality is 13.2 per 100,000 on average (male:

18.6; female: 7.5).[12] ESCC accounts for more than 95% of EC in China[13] and is characterized by a remarkable geographic difference over the country (Figure 1-2). The mortality is extremely high in some high-risk areas around the Taihang Mountains (more than 60/100,000), including Linxian in Henan Province, Cixian in Hebei province and Yangcheng in Shanxi province.[14] Jiangsu Province, south-eastern part of China, is one of the developed coastal areas in China, however, but it is also a high-risk area of EC.[12,15]

Comparing the three national death retrospective surveys, both crude and age-standardized mortality of EC decreases since 1970s (Figure 1-3).[12] This steadily decline is possibly largely related to the dramatic development in socioeconomic status, living conditions, nutrition and health care services in China during the past three decades. Another reason might be the changing survival rates, possible as a consequence of improvement in early diagnosis and treatment.[12,16]

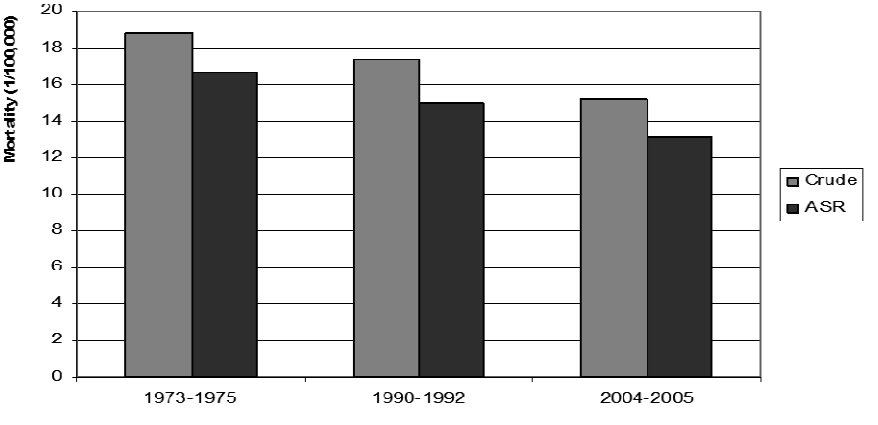

Figure 1-3 Age standardized mortality of EC in China according to three national death retrospective surveys

Jiangsu Province

According to the results of the latest death survey, during 2003-2005, the annual average age-standardized mortality of this disease was 19.7 per 100,000 in Jiangsu Province (male: 23.8; female: 15.2), about 50% higher than the national average. EC

remains the 3rd most common cause of cancer death and accounts for 17% of total death from cancer in this province.[17]

In contrast to the situation in China as a whole, the age-standardized mortality of EC in Jiangsu has decreased about 50% in the past 30 years, but the crude mortality slightly increased in the beginning of 21st century (Figure 1-4) as compared to the 1970s and 1990s. This may indicate that ageing has become an important characteristic of the population in Jiangsu, and could explain a large proportion of the mortality increase in this province. Thus, if it is not effectively controlled, EC will cause a substantial disease burden due to ageing and population growth.

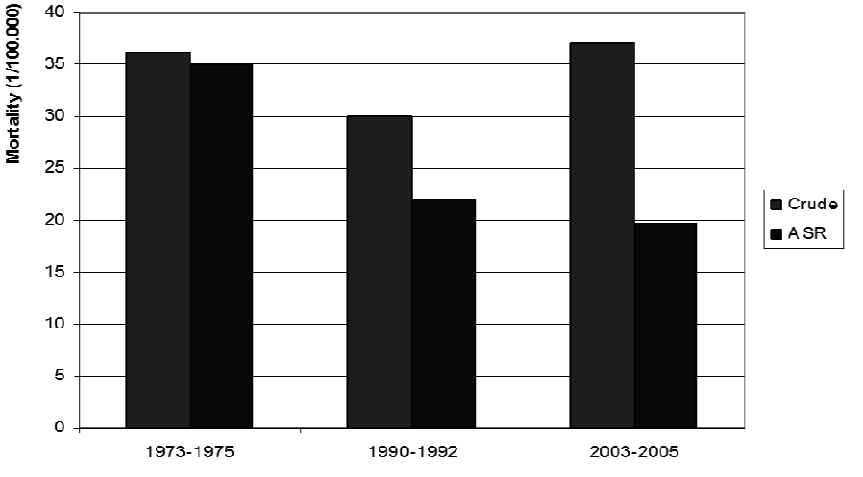

Figure1-4 Mortality of EC in Jiangsu according to three death retrospective surveys

Significant geographic risk gradients of EC are observed within Jiangsu. Most high-risk counties of EC are found to aggregate in the middle part of this province (Figure 1-5), with the age-standardized mortality may exceed 50/100,000 in some high-risk counties. Risks are relatively low in the northern and southern part of this province, mortalities in most counties of those parts are lower than 20/100,000 and even less than 10/100,000 in several regions.[18]

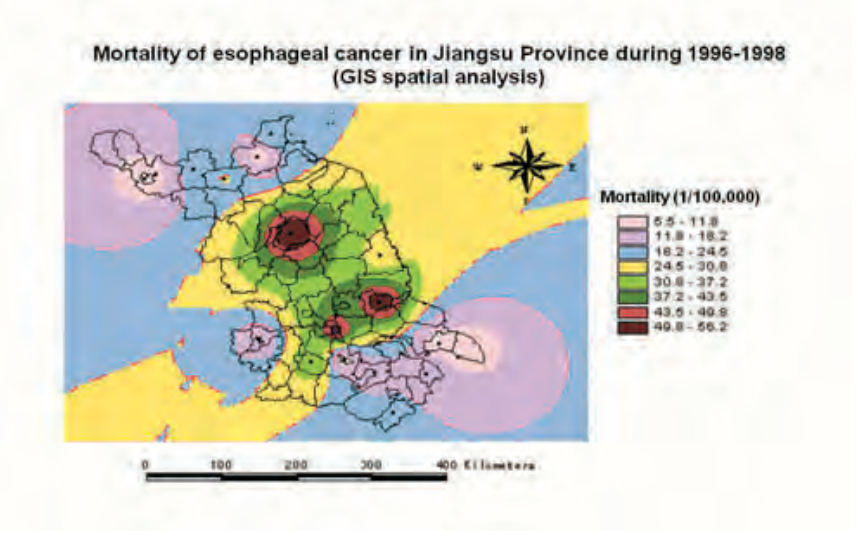

Figure 1-5 Mortality of EC in Jiangsu province during 1996-1998（Source: *Hu et al, 2002*）[18]

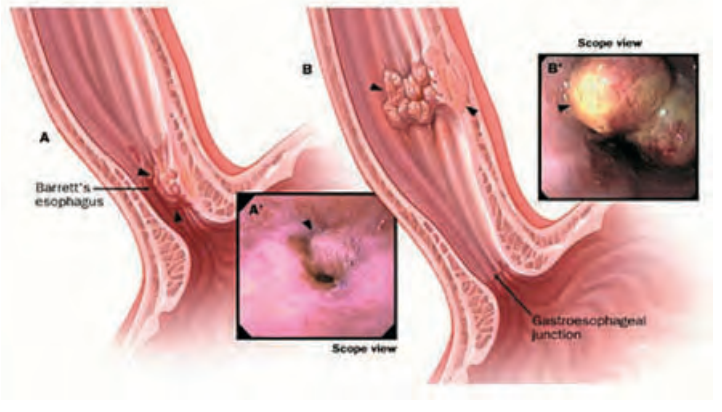

Figure 1-6 Esophageal adenocarcinoma (A) and squamous cell carcinoma (B) *(Source: Johns Hopkins Medicine Gastroenterology and Hepatology)*[23]

Chapter 1

BIOLOGICAL SYNOPSIS

Histological type

The esophagus is the muscular tube through which food passes from the pharynx to the stomach. For descriptive purposes the esophagus is referred to as cervical (~6 cm), thoracic (~25 cm) and abdominal (~4 cm). Surgeons often refer to the esophagus in divisions of one-third, upper, middle and distal.[19] Esophagus is lined over most of its length by squamous epithelial cells, while the portion just above the gastric junction is lined by columnar epithelial cells.[20]

Epithelial tumors of the esophagus including ESCC and EAC are responsible for more than 95% of all esophageal carcinomas. Non-epithelial cell carcinomas (e.g., metastatic tumors, lymphomas, sarcomas) are rare.[21] ESCC and EAC normally present in different parts of the esophagus. About 50~60% of ESCC occur in the middle third of the esophagus, approximately 30% occur in the lower third, and 10~20% in the upper third. EAC is usually located in the distal end of the esophagus (Figure 1-6).[22,23]

The epidemiology, etiology, tumor biology between ESCC and EAC are quite different. Although EAC increased dramatically in Western countries, ESCC remains the vast majority of EC in the world and accounted for more than 95% of esophageal malignancy in China. Therefore, ESCC will be the main focus in this thesis.

Pathogenesis

The pathogenesis of EC remains unclear. Any factor that causes chronic irritation, inflammation and increased cell turnover of the esophageal mucosa appears to increase the incidence of this lethal disease.[7] ESCC is believed to develop progressively through a slow multistep dysplasia-carcinoma sequence. An early indicator of ESCC is the increased proliferation of esophageal epithelial cells due to chronic esophagitis, including basal cell hyperplasia or simple hyperplasia. The hyperplasia may evolve into dysplasia, and in sequence, into in situ carcinoma, early invasive cancer and become invasive carcinoma finally.[24]

Dysplasia is a main precancerous lesion of the esophagus. Traditionally, dysplasia has been classified as mild, moderate and severe (and carcinoma in situ).[25] Now a two-grade system for dysplasia in the gastro-intestinal tract is preferred, with mild and moderate atypia being classified as low grade, and severe dysplasia and carcinoma in situ as high grade.[26] The risk of ESCC dramatically increases with the severity of dysplasia. In a 14-year follow-up study, Wang et al. reported that the relative risk of ESCC was 2.9 for mild dysplasia, 9.8 for moderate dysplasia, 28.3 for severe dysplasia and 34.4 for carcinoma in situ.[27]

Once cancer develops, it may spread rapidly. It has been estimated that 14 to 21% of submucosal cancers and 38 to 60% of cancers that invade muscle are associated with spread to lymph nodes. At the time of diagnosis, more than 50% of patients have either unresectable tumors or radiographically visible metastases.[7]

ETIOLOGY

The large risk difference in a small geographic area and changes in incidence over time suggest a predominant role of external environmental factors in the etiology of EC.[1,3] Migrant studies also confirm that persons from high-risk areas diminished their elevated risks relatively shortly after migration to low risk areas.[28] Comprehensive studies on the etiology of EC have been conducted in the past decades. Evidence shows that tobacco use, alcohol consumption and dietary factors are strongly associated with the occurrence of EC.[2-7] However, the individual susceptibility is also influenced by endogenous factors. A variety of genes have been suggested to be associated with esophageal carcinogenesis including genes involved in alcohol metabolism, carcinogen metabolism, DNA repair, cell cycle control and oncogenes.[29]

Lifestyle and environmental risk factors

Tobacco smoking

The mainstream and sidestream smoke generated when cigarette tobacco is burnt contains more than 4000 constituents including about 60 carcinogens, e.g. polycyclic

aromatic hydrocarbons (PAHs), aromatic amines and N-nitrosamines.[30] Numerous epidemiological studies have identified tobacco smoking as a main risk factor for EC, the risk among smokers is 2-5 fold in general, while for heavy smokers the risk may exceed 10-fold when compared to non-smokers.[31] All quantitative aspects of tobacco use seem to be dose-dependently related to EC risk, such as smoking intensity and years of smoking, but some studies observed that the risk of smoking depends mainly on the duration of tobacco consumption rather than smoking intensity. [3,31-33]

A beneficial risk reduction of EC after cessation of smoking has been reported. Most studies showed that ex-smokers have a lower risk than current smokers, but their risk after stopping smoking remains elevated for several years (at least 10 years) as compared to never smokers. This pattern suggests that tobacco has a strong role both in the early and late stages of carcinogenesis (cancer initiation and promotion). The risk reduction after abstaining from smoking may be associated with the intensity and duration of tobacco consumption.[32,34,35] For instance, Castellsague et al. reported that the decreased risk for ESCC after stopping smoking was greater among population who smoked in high daily quantity.[32]

Although the risk between tobacco use and EC has been well established in many areas of the world, some variation between areas does exist. It is estimated that 42% of the total death of EC can be attributed to smoking worldwide. However, this proportion is estimated to be about 70% in high-income counties, while it is about 35% in low- and middle- income countries.[36] Different from Western countries, smoking appears to play a less important role in the development of EC in China, despite the high smoking rate especially in rural areas of this country. In a meta-analysis, Yu et al. estimated that in China the population attributable fraction (PAF) of EC caused by smoking is 23.2%.[37] Another large-scale case-control study conducted in 103 areas of China reported that the proportion of EC deaths attributable to smoking was 27.6%~31.3% in urban areas and 13.4%~ 21.1% in rural areas.[38] The less substantial attributable fraction could partly be explained by the shorter smoking history and low prevalence among females, but still needs further clarification.

Alcohol drinking

The consumption of alcoholic beverages has been shown to increase the risk of several cancers including EC in many epidemiological studies. A linear dose-response relationship was found between average daily alcohol intake and EC risk, a 4% increased risk per drink/week has been estimated by a meta-analysis,[4] but the risk ratio may also over 10-fold among heavy drinkers.[32] Results regarding the type of alcoholic beverages (e.g., beer, wine and spirits) are contradictory. Some studies found hard liquor consumption to be most strongly associated with EC, while several others reported that wine and/or beer drinkers have the highest risks.[4] The specific type of alcoholic drink and duration of drinking are reported less importantly associated with EC risk than the weekly or daily dose of ethanol consumed.[39] The consequences of drinking cessation have been studied less frequently than smoking cessation and results are more controversial, a beneficial risk reduction has been found in some studies particularly 10 years after stopping drinking,[40,41] whereas other studies have shown either a non beneficial effect or a higher risk among former drinkers.[42,43]

The possible mechanism by which alcohol intake increases EC risk includes:[4] 1) Ethanol metabolism may generate acetaldehyde within the esophageal mucosa, this is known to form adducts with macromolecules (for example DNA) and may act as a tumor promoter by increasing the proliferation of the epithelium; 2) Alcohol may act as a direct irritant to the esophageal epithelium; 3) Alcoholic beverages may contain carcinogens and other compounds, and may act as solvent to facilitate the absorption of carcinogens and enhance their penetration in to cells; 4) Alcohol may affect the metabolism of carcinogens, particular those in tobacco. There is abundant evidence that the risk of EC is significantly increased when alcohol and smoking coexist, their joint effects are approximately multiplicative, risk in the highest joint level of alcohol and cigarette smoking may increase 130- fold.[33]

The proportion of EC attributable to alcohol drinking is also diverse in different areas. Daniel estimated that 26% of death from EC can be attributed to alcohol use worldwide, the PAF was 41% in high-income counties and 24% in low- and middle- income countries.[36]

Similar to smoking, alcohol consumption appears to be a less important contributor in China as compared to Western countries, it has been reported that only 16% of EC cases can be attributable to alcohol drinking in China.[37] The multiplicative joint effects between smoking and alcohol drinking were also observed considerably weaker or even absent according to some previous studies.[44-46]

Alcohol and tobacco appear to be strong and independent risk factors of EC in Western countries, 90% or more of the risk of EC can be attributed to these two factors. Their less strongly associations with EC in China could be partly explained by the relatively short exposure history and low exposures to both factors among Chinese women; moreover, there might be some other strong risks factors that account for the majority of cases and weaken the effects of smoking and alcohol drinking.

Foods and nutrients

Since the fraction of EC attributable to smoking and alcohol appears to be small in some high-risk areas, diet and nutrition emerge as important factors in esophageal carcinogenesis in those areas. Furthermore, a common denominator in high-risk areas of ESCC is poverty and lack of variation in diet, indicating that malnutrition or micronutrient deficiency can undoubtedly result in an increased sensitivity to EC.

A protective effect on EC has been shown for high intake of fruits and fresh vegetables by a large quantity of data. Fruits and vegetables are sources of dietary fiber, vitamin C and other antioxidants, such as carotenoids, phenols, and flavonoids, as well as other potentially bioactive phytochemicals.[4] Consumption of fruits and fresh vegetables is typically low in the areas with high EC incidence;[47] even in well nourished populations, epidemiological studies suggest that diet low in fruit and vegetables confer on average a 2-fold increased risk.[48] Meta analysis in WCRF report showed a 22% and 31% decreased risk of EC per 50g of fruit and raw vegetables per day, respectively.[4] A few studies found fruit to be more protective than vegetables, and citrus fruit to be especially beneficial.[49] However, the results on fruits and vegetables with EC remain inconclusive; the inconsistence between studies may result from discrepancies in the types of fruits and vegetables, or in their methods of preparation.[4]

Limited evidence from case-control studies suggests that high intake of meat, especially red meat (pork, beef, lamb etc) and processed meat (salted or barbecued meat) is a cause of EC. The potential mechanisms for a positive association with high meat intake include the generation of N-nitroso compounds, the production of heterocyclic amines and polycyclic aromatic hydocarbons (PAH) when cooked at high temperatures, production of free radicals by haem iron and free iron in the meat.[4] Results on meat intake and EC are conflicting, with ORs ranging from 0.2 to 4.7 for different types of meat, but most studies have reported red meat as a risk for ESCC, and cooking method appears to play a role in esophageal carcinogenesis.[50] Unlike meat, frequent consumption of fresh fish has been suggested to decrease risk as fish and fish oils are rich dietary sources of n-3 fatty acids.[51]

Frequent intake of carcinogens such as N-nitroso compounds, fungi toxins from foods and water has been postulated to be the primary determinants of EC in China.[5] Pickled vegetable was once a popular food item in many areas of China, it is prepared by fermenting the vegetables under water in ceramic containers up to several months until they are covered with mold, thus some mycotoxins including aflatoxins, T-2 toxin and nitroso compounds may be generated during this process. High intake of pickled vegetables has been reported positively associated with EC by many studies in high-incidence areas of China, and was thought to be a major risk factor for EC in those areas.[52] However, several studies in China including a cohort study were unable to verify this positive association in their study populations.[53] Pickled or fermented meat and fish also have been shown to be related to EC by a few Chinese studies.[54] Besides preserved foods, drinking water is also a possible source for nitrosamines and other nitroso compounds in some regions.[52]

Various nutrients have been observed to be related to the risk of EC. Inverse associations have been reported most notably for vitamins C and E and β-carotene.[4,55] Deficiency of some minerals such as selenium, zinc and calcium may also increase EC risk.[4] Therefore, malnutrition and a monotonous diet in the sense of lack of protective factors may lead to an increased susceptibility of EC. However, in Linxian, China, one of the highest EC incidence areas worldwide, about 30,000 adults were randomized to receive eight mineral/vitamin combinations for 5 years. According to the latest follow-up, no

significant reduction in incidence/mortality from EC was observed. Whereas, a small and non-significant decline in deaths from esophageal/gastric cardia cancer for those esophageal dysplasia patients who received multivitamin supplementation was observed in this trial.[56]

The role of foods and nutrients in the etiology of EC is complex, partly because diet encompasses a wide variety of foods and the different methods for measurements. Moreover, people normally eat combinations of foods rather than particular food or group of foods; therefore, the whole dietary pattern may play a more important role in EC occurrence.

Unhealthy dietary habits

Unhealthy dietary habits such as frequent consumption of high temperature foods/drinks and fast eating speed have been found to be possible risk factors of EC.[3,4,57,58] Eating fast and high temperature foods/drinks are common physical stimulations to the esophagus, and can impair the barrier function of the esophageal epithelium, thus can lead to chronic esophagitis. Esophagitis has been considered the earliest tissue perturbation in the progressive process of malignant transformation of the squamous epithelium. Moreover, the increasing cell turnover by chronic irritants could increase the contact between carcinogens and dividing target cells in esophagus.[3,59]

De Jong and colleagues showed that intake of hot drinks could substantially increase the intraesophageal temperature, and this increase was a function of the initial drinking temperature and size of the sip. For example, drinking 65 °C coffee may increase the intraesophageal temperature by 6–12 °C, depending on the sip size.[60] In a systematic review, Islami et al. reported that among 59 eligible studies they found, the majority of studies showed an increased risk of EC associated with higher drinking temperature, which was statistically significant in most of them.[57] However, there are several limitations in establishing the association between hot drinks and EC: 1) Most of the evidence comes from case-control studies which may suffer from potential selection and recall biases; 2) In some studies, consuming various types of hot drinks have been asked or analyzed together;

3) Little has been done to measure sip size or the actual temperature of the drinks. The relationship between eating speed and EC is less frequently studied and results remain inconclusive. Zhang et al. observed that fast eating habit was associated with 1.54- to 4.10-fold risk of EC in China.[61] Wang et al. reported that eating fast significantly increased the risk of EC with an OR of 3.39 (95% CI: 1.15–9.95).[46]

Some dietary habits such as consumption of dried and smoked meat, excessive use of chilies and spices, chewing of betel nuts were also found to increase the risk of EC in some studies conducted in different areas.[4]

Green tea drinking

Tea is the most frequently consumed beverage worldwide after water, especially in Asian countries such as China, Japan, and India. Depending on the manufacturing process, tea is classified into green tea (non-fermented), oolong tea (half-fermented) and black tea (fermented). Green tea is derived from *Camellia sinesis*, an evergreen shrub of the Theaceae family.[62] A number of studies have provided evidence that the polyphenolic antioxidants present in green tea, including epigallo-cathechin-3 gallate (EGCG), epigallo-cathechin (EGC) and epicathechin-3 gallate (ECG) may be capable of affording protection against cancer.[63,64]

Several epidemiological studies also suggested a protective effect of green tea on EC, but results remain inconclusive. Gao et al. and Wang et al. reported that green tea drinking reduced the risk of EC among women, but the dose-response relationship was inconsistent.[46,65] In an intervention trial conducted in China, subjects with esophageal precancerous lesions were supplemented with decaffeinated green tea (DGT) 5 mg/day for 12 months, but the results did not show an apparent difference between treatment and placebo groups.[66] A pooled analysis of two prospective cohorts in Japan found that drinking more than 5 cups of green tea/day significantly increased the risk of EC (HR = 1.67), the author explained that high tea temperature could be a plausible explanation for the increased risk.[67]

Socioeconomic status

Results of ecological studies indicated that ESCC is a disease mainly occurring in middle and low income countries. Epidemiologic studies also show that within a population, ESCC is more frequent among people with lower socioeconomic status (SES).[3] Although socioeconomic status itself is not a direct biological causal factor, it can act as a surrogate for a set of lifestyle and environmental factors, and may influence the access to health care. Low SES may be associated with low income, poor housing, unemployment, workplace hazards, poor nutrition and limited access to medical care. In a case-control study conducted among African American and white men in the United States, significantly elevated EC risks were observed for individuals with the lowest level of annual income, the ORs were 4.3 for whites and 8.0 for African Americans.[68] The association for SES with ESCC and EAC is different, low SES was found to be related to ESCC while high SES may increase the risk of EAC.[11]

Infection

Human papillomavirus (HPV), especially HPV type 16 and type 18 has been considered as a potential etiological agent in EC.[6] The presence of HPV was reported as 20–70% in patients with EC, but the association between HPV and EC remains controversial and the evidence is inadequate.[69] Some studies have found 2-5 fold positive associations with HPV 16 or HPV 18, while some others have found no association or even inverse associations. The inconsistency of these results may be due to differences in study design, geographic variation, differences in positivity cut-points used in different studies, lack of appropriate adjustment for tobacco use or alcohol consumption, or simply chance fluctuation due to the small number of cases in some studies.[6]

Although Helicobacter pylori (H. pylori) is now a known cause of gastric and duodenal ulcers and may increase the risk of gastric adenocarcinoma, several studies found the infection of H. pylori is associated with a decreased risk for EAC by reducing acid production in the stomach and hence reducing acid reflux to the esophagus.[6] It may also reduce EAC risk by decreasing the production of ghrelin, a hormone that is mostly

produced in the stomach and stimulates appetite, thus may lead to lower rates of obesity.[70,71] In contrast to EAC, H. pylori have not shown a consistent association with ESCC.[6]

Summary of diet and other lifestyle factors in the etiology of EC

Table 1-1 Summary of lifestyle and environmental associated factors of EC

	Decreases risk	Increases risk
Convincing		- Tobacco smoking - Alcohol drinks - Chronic injury to the esophagus
Probable	- Non-starchy vegetables - Fruits - Foods containing β-carotene - Foods containing Vitamin C	- High temperature foods/drinks - Poor socioeconomic status (ESCC) - Obesity (EAC)
Limited-suggestive	- Green tea - Foods containing dietary fiber - Foods containing folate - Foods containing pyridoxine - Foods contain Vitamin E - H. Pylori infection (EAC)	- Red meat - Processed meat - Micronutrient deficiency - HPV infection
Limited- No conclusion	- Cereals (grain) and their products - Starchy roots tubers and plantains - Soya and soya products - Herbs, spices and condiments - Salt - Energy intake (EAC) - Frying, grilling and barbequing - Spices - Pickled vegetables	

The relationship between aforementioned lifestyle and environmental determinants and EC are summarized in Table 1-1. Tobacco smoking, alcohol drinks and chronic injury

to the mucosa of esophagus are convinced to increase the risk of EC. While there is limited evidence for other associated factors such as food consumption and micronutrient deficiency.

Although China is one of the highest EC incidence areas in the world, there are both high- and low-risk areas in this country. Nutrition deficiencies, N-nitrosamines and fungi toxins are considered to be the major causative factors of EC in high-risk areas of China; while diets rich in garlic and other allium vegetables has been observed in some low-risk areas in China.

Hereditary factors and genetic susceptibility

Family history of cancer

Family members normally share a common genetic background, individuals with a positive family history of cancer (FH) was found to be associated with an increased risk of EC in some high-risk areas such as China and Iran.[72,73] In general, such a risk was at 2-3 fold for those with affected first-degree relatives (FDR, e.g. parents, siblings and children), especially for cancer of the same histological type. Unlike high risk areas of the world, the associations of FH-FDR and EC were less consistent in Western countries.[74,75] This inconsistency in different areas might be due to variation in the frequency of esophageal susceptibility alleles, or due to variation in major attributable environmental or lifestyle risk factors, or the combination of both.[73] Although cancer among FDRs can be regarded as a representation of genetic predisposition, experiencing similar environmental influences and lifestyle risk factors of family members may also partly contributed to the familial propensity of disease occurrence.[76]

Genetic polymorphisms

The process of carcinogenesis is affected in a number of ways, including mitochondrial metabolism and oxidative stress, DNA damage and repair, protein synthesis and cell proliferation, disturbance of immune functions etc.[77] Over the past decades, many laboratory and epidemiological studies have concentrated on the identification of genes

whose roles are to metabolize and excrete potentially carcinogens and to repair subtle mistakes in DNA. Findings in molecular biology also found that a large number of genes coding for molecules (enzyme and receptors) involved in xenobiotic metabolism show polymorphisms.[78] Polymorphisms are sequence variations such as nucleotide substitutions, deletions/insertions and gene duplications/deletions. Most polymorphisms are located outside gene boundaries and have no apparent effects. If a polymorphism is within a gene's coding region, in an exon, amino acid substitution may occur and result in changes in protein activity ranging from slight to significant.[79] For example, polymorphisms characterized by whole gene deletions will clearly eliminate any functional enzyme activity, while polymorphisms which are duplications of the entire gene may result in higher levels of activity.[80]

Individual variations in cancer risk have been found to be associated with specific polymorphisms of different genes that are present in a significant proportion of the normal population. Many studies have suggested that genetic polymorphisms may clarify the causes and events involved in alcohol metabolism, folate metabolism, carcinogen metabolism, DNA repair and cell cycle control and oncogenes.[81,82] Genetic host factors can also interact with environmental carcinogens, i.e., carcinogens in the diet, tobacco smoke and ambient air due to environmental or occupational sources.[29] In addition, genes may influence individual behaviour, such as smoking, alcohol drinking and the preference of diet, thus, may potentially to substantially affect cancer risk.[83] Therefore, genetic polymorphisms and gene-environmental interactions can place an individual at a greater or lesser risk of a particular cancer than another individual.

Alcohol-metabolizing genes and EC

As aforementioned, the association between alcohol drinking and EC has been well established in many areas of the world, but remarkable regional variation does exist.[3,4] Alcohol metabolism involves two steps of enzymatic oxidation, upon the consumption of alcoholic beverage, ethanol is first catalytically oxidized into acetaldehyde, which is known to form DNA adducts and can act as a tumor promoter. Acetaldehyde is subsequently metabolized into harmless acetate.[4,84,85]

Functional polymorphisms have been observed at various genes encoding enzyme proteins, all of which act to alter the rate of synthesis of the toxic metabolite acetaldehyde, or decrease its further oxidation. In particular, molecular genetic research into the causes of alcoholism and alcohol-related cancer has drawn attention to the potentially important role of alcohol- and acetaldehyde-metabolizing enzymes, alcohol dehydrogenases (ADHs) and aldehyde dehydrogenases (ALDHs).[86-88]

Alcohol dehydrogenases(ADHs)

The *ADH* genes are mapped to chromosome 4p 21-25. There are seven genes for human *ADHs*, namely *ADH4, ADH5, ADH6, ADH7, ADH1A, ADH1B* and *ADH1C*.[89] Most *ADH* genes are expressed in the liver, but some are found in other tissues including lung, stomach, cornea and esophagus. It is likely that the type and content of the polymorphic isoenzymes subunit encoded at *ADH1B* and *ADH1C* are contributing factors to the genetic variability in ethanol elimination and individual risk among alcohol drinkers.[90]

The protein subunits at the *ADH1B* locus differ by one amino acid from each other, three different subunits are encoded by the *ADH1B**1 (48 Arg), *ADH1B**2 (48 His) and *ADH1B**3 (48 Arg + 370 Cys) genes, respectively. The *ADH1B**2 is very prevalent among East Asians but is rare in Caucasians. *ADH1B**3 has been identified only among Africans.[91] Both *ADH1B**1 and *ADH1B**2 have a low K_m for ethanol, but the V_{max} of *ADH1B**2 is much higher than that of *ADH1B**1.[84,86,90] Yin et al. reported that the homodimer of *ADH1B* encoded by *ADH1B**1/*1 has only 1/100 and 1/200 of the ethanol oxidation capacity of the isozymes encoded by *ADH1B**1/*2 and *ADH1B**2/*2, respectively.[92]

Although the *ADH1B**1 allele appears to metabolize ethanol to acetaldehyde less actively than *ADH1B**2, it has been reported to be more strongly associated with the development of EC in both alcoholics and general populations. Yokoyama et al. found the presence of *ADH1B**1/*1 significantly increased the risk of EC whereas there were no different effects on cancer risk between the *ADH1B**1/2*2 and *ADH1B**2/*2 genotypes.[93] A recent meta-analysis on most published studies showed that as compared to *ADH1B**2/*2, the risk of EC among *ADH1B**1/*1 carriers was 1.56 (95% CI: 0.93-2.61) for never/rare

drinkers, 2.71 (95% CI: 1.37-5.35) for moderate drinkers and 3.22 (95% CI: 2.27-4.57) for heavy drinkers.[94] In contrast, the increased risk among *ADH1B**1 carriers might result from an absence of alcohol flushing response after drinking, including facial flushing, tachycardia, headaches and other unpleasant symptoms. While *ADH1B* active type may cause high concentration of acetaldehyde after drinking alcohol and produce ethanol intolerance at low doses, thus, could prevent people from heavy drinking and reduce the exposure of esophageal mucosa to ethanol.[86]

Polymorphisms at the *ADH1C* locus involve two different subunits corresponding to the *ADH1C**1 (Arg 272, Ile 350) and *ADH1C**2 (Gln 272, Val 350) alleles.[91] The homodimer *ADH1C**1/*1 was observed to have a two-fold greater Vmax than the *ADH1C**2/*2 homodimeric form.[84] In those of white ethnic origin, 45-70% are heterozygous *ADH1C**1/*2; by contrast, the frequency of *ADH1C**1 allele is 75-90% in Africans and 85%-100% in Asian populations.[95] Similar to *ADH1B*, alcohol drinkers possessing less active *ADH1C**2 allele were observed to be at greater risk of EC.[93] However, *ADH1B* and *ADH1C* genes are located closely in the short arm of chromosome 4. It was reported that linkage disequilibrium exists between these two genes, and the polymorphisms of *ADH1B*, rather than *ADH1C*, have the stronger association with the development of alcoholism.[96] But a comprehensive epidemiologic study in Europe showed that ADH1B and *ADH1C* had a significant independent association with upper-aerodigestive cancer (including EC), despite of strong linkage disequilibrium.[97] Results on *ADH1C* and EC remain sparse and inconsistent.

Aldehyde dehydrogenases (ALDHs)

The *ALDHs* are a group of enzymes catalyzing the conversion of aldehyde to the corresponding acids. *ALDHs* exhibit a rather broad substrate specificity and have been considered as general detoxifying enzymes which eliminate toxic biogenic and xenobiotic aldehydes.[98]

Nine major families of *ALDH* have been identified in humans, whereas *ALDH2* is believed to be responsible for the majority of acetaldehyde oxidation and play a central role

in acetaldehyde detoxification because of its very high affinity for acetaldehyde.[99] *ALDH2* gene is located on chromosome 12, the normal allele is designated as *ALDH2**1 (Glu 487), but a point mutation in the gene produces a variant allele with deficient activity, designated as *ALDH2**2 (Lys 487). Subjects who are both homozygous and heterozygous for *ALDH2**2 lack detectable *ALDH2* activity in the liver.[100,101] The blood acetaldehyde concentrations after consuming alcoholic beverages in the individuals having *ALDH2* *2/*2 and *ALDH2* *1/*2 genotypes were found as 19- and 6-fold higher than in *ALDH2* *1/*1, respectively.[102]

ALDH2 deficiency is relatively common among Asians, the frequency of *ALDH2**2 allele may be up to 40% in Asians, whereas it does not exceed 5% in European and African populations.[103] Its failure to promptly metabolize the ethanol metabolite acetaldehyde leads to excessive acetaldehyde accumulation and is associated with facial flush and other unpleasant symptoms after alcohol is consumed. Chromosome alterations also have been observed more frequently in lymphocytes from drinkers with inactive *ALDH2* allele.[104] Studies of various Chinese and Japanese drinking populations have shown that inactive form of *ALDH2* is a risk factor of EC.[87,93,105] Results of a meta-analysis showed that the overall risk was increased in *ALDH2* heterozygotes (OR=3.19) but decreased in *ALDH2**2 homozygotes (OR=0.36; 95% CI: 0.16–0.80), as compared to *ALDH2**1 homozygotes.[106] This can be explained by that *ALDH2* heterozygotes may result in excessive accumulation of acetaldehyde after alcohol intake due to the low enzymatic activity, while *ALDH2**2 homozygotes are characterized by a facial flushing which may prevent them from heavy drinking.

A combined influence of *ADH1B* and *ALDH2* genotypes has also been observed by several studies.[94] Yokoyama reported that for patients with both *ADH1B**1/*1 and *ALDH2**1/*2, the risks for EC were enhanced in a multiplicative fashion (OR=40.40).[104]

Summary of risk factors of EC

The etiology of EC is multifactorial. Tobacco smoking and alcohol consumption are responsible for a high fraction of EC occurrence, especially in Western countries. Dietary

factors such as ingestion of hot foods and drinks, fast eating speed, nutrition deficiency, and high intake of carcinogens from pickled vegetables have been suggested to play an important role in China. Moreover, genetic predispositions may also influence the inherited susceptibility to EC and could modify the risk of environmental and lifestyle factors.

Scientific evidence suggests both environmental factors and human genetic variants have a large regional variability, the variation in both lifestyle/ environmental factors, hereditary factors and clinical factors ultimately determine the individual risk to EC.

RATIONALE AND OUTLINE OF THIS THESIS

Rationale of the study

Although, in the past decades, numerous studies have been conducted on the etiology of EC worldwide, the effects of major lifestyle and inherited risk factors on the development of this fatal disease remain poorly understood in China. Moreover, little attention has been paid to the difference in the etiology between similar areas with great risk gradient. From 2003 to 2007, a large population-based case-control study has been conducted in two counties of Jiangsu province, Dafeng and Ganyu. Both Dafeng and Ganyu are less developed rural counties in northern Jiangsu; however, Dafeng has a much higher incidence of EC than Ganyu (Table 1-2). This study aims to provide further evidence on the effect of major lifestyle and inherited risks on EC in China, and the simultaneously evaluation of two populations at different risk may be a potentially insightful approach in understanding both etiology and prevention of EC.

Outline of this thesis

In order to describe the study design and evaluate the associations between EC and tobacco smoking, alcohol drinking, dietary habits and major food consumption in general, **Chapter 2** firstly presents the results of a preliminary analysis with major risk factors in a subset of the population. **Chapter 3** describes the overall and gender specific effects of smoking and alcohol drinking on EC in detail, including the independent and joint effects

Table 1-2 The general information of Dafeng and Ganyu

	Dafeng	Ganyu
Geographical location	Coasts of the Yellow Sea in North-east of Jiangsu. (N33°, E 120°)	Coasts of the Yellow Sea in North-east of Jiangsu. (N35°, E 119°)
Land square	2,367 Km^2	1,427 Km^2
Total population	0.7 Million	1.1 Million
Minority ethnicity	0.29%	0.09%
Annual income in rural area	8,750 RMB	6,599 RMB
Annual income in urban area	14,860 RMB	12,731 RMB
Agricultural population (%)	64.8%	55.1%
Annual mean temperature	14.5℃	13.2℃
Annual amount of precipitation	1,189.8 mm	976.4 mm
Mortality/SMR in 2008 (1/100,000)	711.1/567.2	530.1/406.9
Incidence/SIR of total cancer in 2008(1/100,000)	262.2/206.0	115.4/88.5
Mortality/SMR of total cancer in 2008 (1/100,000)	229.4/147.2	84.6/63.8
Incidence/SIR of EC during 2006-2008 (1/100,000)	46.0/36.3	31.2/24.4
Mortality/SMR of EC during 2006-2008 (1/100,000)	43.6/33.9	23.5/17.5

SIR: age-standardized incidence rate. SMR: age-standardized mortality rate

of these two well-known risk factors. **Chapter 4** evaluates the association of EC with green tea drinking and temperature at drinking. **Chapter 5** systematically investigates the relationship between family history of cancer and the risk of EC, and explores the effect modification between heredity risks and major lifestyle factors (i.e. smoking, alcohol drinking and some dietary factors). **Chapter 6** explores how the single nucleotide polymorphisms of *ADH1B, ADH1C* and *ALDH2* genes could modify the risk of EC among

alcohol drinkers and the general population, as well as gene-environmental and gene-gene interactions. **Chapter 7** describes the role of major lifestyle risk factors and heredity factors on the attributable fraction of EC in two counties respectively, and shows what proportion of the risk gradient between the two areas could be explained by differences in the distribution of those risk factors. This thesis ends with **Chapter 8**, in which the main research findings, epidemiologic considerations and public health implications are discussed in a broader context, while recommendations for future research and developments are formulated as well.

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Chapter 2

The association of smoking, alcohol drinking and dietary factors with esophageal cancer in high- and low-risk areas of Jiangsu province, China

Ming Wu, Jin-Kou Zhao, Xiao-Shu Hu,
Pei-Hua Wang, Yu Qin, Yin-Chang Lu,
Jie Yang, Ai-Min Liu, De-Lin Wu,
Zuo-Feng Zhang, Frans J Kok , Pieter van 't Veer

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ABSTRACT

AIM: To study the main environmental and lifestyle factors that account for the regional differences in esophageal cancer (EC) risk in low- and high-risk areas of Jiangsu province, China.

METHODS: Since 2003, a population-based case-control study has been conducted simultaneously in low-risk (Ganyu county) and high-risk (Dafeng county) areas of Jiangsu province, China. Using identical protocols and pre-tested standardized questionnaire, following written informed consent, eligible subjects were inquired about their detail information on potential determinants of EC, including demographic information, socio-economic status, living conditions, disease history, family cancer history, smoking, alcohol drinking, dietary habits, frequency and amount of food intake etc. Conditional logistic regression with maximum likelihood estimation was used to obtain Odds ratio (OR) and 95% confidence interval (95% CI), after adjustment for potential confounders.

RESULTS: In the preliminary analysis of the ongoing study, we recruited 291 pairs of cases and controls in Dafeng and 240 pairs of cases and controls in Ganyu, respectively. In both low-risk and high-risk areas, EC was inversely associated with socio-economic status, such as level of education, past economic status and body mass index. However, this disease was more frequent among those who had a family history of cancer or encountered misfortune in the past 10 years. EC was also more frequent among smokers, alcohol drinkers and fast eaters. Furthermore, there was a geographic variation of the associations between smoking, alcohol drinking and EC risk despite the similar prevalence of these risk factors in both low-risk and high-risk areas. The dose-response relationship of smoking and smoking related variables, such as age of first smoke, duration and dosage were apparent only in high-risk areas. On the contrary, a dose-response relationship on the effect of alcohol drinking on EC was observed only in low-risk areas.

CONCLUSION: The environmental risk factors, together with genetic factors and gene-environmental interactions might be the main reason for this high risk gradient in Jiangsu province, China.

Key words: Esophageal cancer, Case-control study, Smoking, Alcohol drinking, Dietary factors

Introduction

Esophageal cancer (EC) is the sixth most common cause of cancer mortality worldwide. The incidence of this disease shows a striking geographic variation in the world; a 20-fold variation is observed between high-risk China and low-risk western Africa.[1] Jiangsu province, south-eastern China is one of the highest EC incidence areas with a mortality rate of 30.0/100000 in 1990~1992, which was significantly higher than the national average of 17.0/100000.[2,3] EC has been the third leading cause of cancer mortality in Jiangsu province since the 1970's.[3] Although the mortality rates of EC are high in most counties of Jiangsu, national surveys conducted in the 1970's and 1990's have shown that rates differ considerably between different counties within the province, despite their similar geographic characteristics and socioeconomic status.[3,4]

Comprehensive studies on the etiology and carcinogenesis of EC in high-risk areas have been carried out during the past decades. Epidemiological evidence suggests that the independent risk factors, tobacco smoking and alcohol drinking, are strongly associated with EC risk and have approximately multiplicative joint effects.[5-7] Dietary factors were also found to play an important role in the development of EC. Increased risk of EC was found to be associated with low intake of raw vegetables and fresh fruits, a deficiency in vitamins or protective antioxidants (e.g. Vitamin C and E, β-carotene, and selenium), high intake of carcinogens (frequent consumption of pickled vegetables and fungi toxins) and mucosa injuries (fast eating speed for hot drinks and soups).[8-10] Although the contributory factors of EC are the high consumption of tobacco and alcoholic beverages in Western countries, the causative factors of EC in high-risk areas of China are nutrition deficiency, N-nitrosamines, fungi toxins and genetic factors.[11]

Numerous epidemiological studies have been conducted to explore the associations between environmental, lifestyle, dietary factors and the risk of esophageal cancer, but very few studies have been conducted to compare the association between risk factors and EC in apparently similar areas with a high risk gradient. Thus, a population-based case-control study has been conducted since 2003 in both low and high-risk areas of Jiangsu province, China to study the main environmental and lifestyle factors that account for regional differences in EC risk.

This paper reports the preliminary results on the independent and joint effects of smoking, alcohol drinking and dietary factors on EC risk and compares their associations with EC in both high-risk and low-risk areas.

Material and methods

Study area

This study has been conducted in Dafeng county and Ganyu county since late 2003. Both counties are less developed coastal areas in northern Jiangsu Province, China, with 0.7 million and 1.1 million inhabitants, respectively. Dafeng is a high cancer incidence area and has a much higher mortality rate of EC than Ganyu. From 1996~2002, the yearly average age-adjusted mortality rate of EC in Dafeng was 36/100000, whereas Ganyu had a considerably lower age-adjusted EC mortality rate of 24/100000 during the same period(P<0.01).[12]

Selection of cases and controls

Cases

Newly diagnosed patients with primary esophageal cancer were recruited using data from regional cancer registry agencies. The cancer registry agencies in both counties were established in the late 1990's and are connected to the local Center for Disease Control and Prevention (CDC). All cases were coded according to the International Classification of Diseases, tenth revision (ICD-10, code C15.0 to C15.9). Secondary and recurrent cancers were excluded. All cases were restricted to local inhabitants of the two counties who have lived in either area for at least 5 years.In 2004, 45 and 72% of all newly registered EC cases were recruited and interviewed in Dafeng and Ganyu, respectively. The comparatively low response rate in Dafeng was partly due to the low involvement of local hospitals during the beginning of the study. A small number of cases were also unwilling to participate. Presently, the response rate in Dafeng is much higher. A system of rapid case recognition was used in the study. All regional hospitals were required by the local health authorities to report new EC patients shortly after diagnosis. As the cancer registry agencies are connected to the local CDC, the field investigators from the local CDC were able to identify and interview most patients within one month after their diagnosis. Of all the EC cases in Dafeng, 46% were histologically confirmed, 40 and 13% were diagnosed by endoscopy and radiology, respectively. In Ganyu, 30% of EC cases were histologically confirmed, 50 and 16% were diagnosed by endoscopy and radiology, respectively.

Controls

Cases and controls were individually matched and derived from the same county. The criteria for the eligibility of controls were established as: controls had to be the same gender and within 5 years of age as the case, had to have lived in the area for at least 5 years, and had to have had good physical and mental health to answer questions reliably. Controls were randomly selected by a computer from the demographic database of the general population in the county police station. Local interviewers were responsible for locating and interviewing controls. If a selected control refused to participate, a replacement was

found using the same recruitment criteria. The response rate of controls in both Dafeng and Ganyu was around 70%. Till March 2005, more than 400 EC cases were recruited in each county. However, as the control recruitment lags behind case identification, only 291 and 240 pairs of cases and their matched controls were used in this analysis.

Data collection

Identical protocols and pre-tested standardized questionnaires were used in both counties. Data collection included a written informed consent, a face-to-face interview, a physical examination and a 5 ml blood sample taken by professional interviewers from the local CDC in both counties. The questionnaire elicited detailed information on potential determinants of EC, including demographic information, socio-economic status, living conditions, disease history, family cancer history (any malignant neoplasm in first-degree relatives), smoking, alcohol drinking, dietary habits, and frequency and amount of food intake.

In our study, never-smokers were defined as having smoked fewer than 100 cigarettes in their lifetime. Current smokers and drinkers were defined as those who had the habit during the time of interview or those who stopped the habit because of health problems within one year. The dietary questionnaire used in this study included 90 food items. For each food item, the amount and frequency of consumption over the past year were inquired. For cases, the amount and frequency of consumption referred to the year prior to the onset of the disease. In the final analysis, foods were categorized into several major groups: staple foods, preserved foods, meat, fish, eggs, soybean, and fruits and vegetables.

An anthropometric measurement and physical examination also took place at the time of interview to evaluate the subject's health status. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared (i.e., kg/m^2). BMI was grouped into quartiles according to the Chinese national standard (underweight: <18.5, normal: 18.5~23.9, overweight: 24~27.9, obesity: ≥28).[13]

Statistical analysis

Data were double entered using Epidata 2.1b and cleaned and analyzed using SAS v8.2. Chi-square and student t-tests were used to compare the distribution of relevant factors among the control groups between the two counties. Conditional logistic regression with maximum likelihood estimation of parameters was applied for both univariate and multivariate analyses. This was done by transforming each matched pair into a single observation, where the explanatory variable value was the difference between the corresponding values for the case-control pair.[14] Continuous variables such as income level and amount of food intake were divided into quartiles based on the frequency distribution among control groups.

The strength of the association was quantified as odds ratio (OR) obtained from conditional logistic regression. Statistical significance was set at 0.05, and accordingly, 95% confidence interval (CI) around the OR were used to address precision.

Results

Subject's characteristics

There were 291 pairs of cases and controls (200 male and 91 female pairs) in Dafeng and 240 pairs (181 male and 59 female pairs) in Ganyu, respectively. By design, cases and controls had similar distributions in terms of gender and age in both two counties (Table 2-1). The differences in the distribution of above-mentioned variables between the two counties were examined by comparing the two control groups. The proportion of patients who were older than 70 years of age and the proportion of illiteracy in Ganyu were higher than that of Dafeng (P=0.002 and P<0.001, respectively). There was also a geographic variation between the two counties with respect to the proportion of BMI (P=0.014), occurrences of misfortunes such as fire disasters, loss of family members, divorces, etc in the past 10 years (P=0.008) and family history of cancer (P<0.001). On the other hand, there was no significant difference in past economic status, ever-smoking and ever-alcohol drinking habits between the two counties.

Socio-economic status

EC occurred less in higher socio-economic groups which are characterized by high levels of education and high economic status in both counties (Table 2-2). On the contrary, low levels of education, low economic status, family history of cancer in first-degree relatives (Dafeng OR: 1.53, Ganyu OR: 2.07), and occurrences of misfortune in the past 10 years (Dafeng OR: 1.26, Ganyu OR: 1.64) increased the risk of developing EC in both areas. In Dafeng, when compared to the lowest quartile (underweight people) of BMI, the second quartile (normal weight people, OR=0.45) and the third quartile (overweight people, OR=0.26) significantly showed a reduced risk of EC; whereas the OR increased in the highest quartile (obese people, OR=0.49). A similar association between BMI and EC risk was also found in Ganyu, although the trend was not significant.

Tobacco smoking and alcohol drinking

Consistent smoking elevated the risk of developing EC in both counties (Table 2-3). In Dafeng, former smokers and current smokers have a 1.93- and 2.42- fold higher risk of

Table 2-1 Characteristics among EC cases and controls in Dafeng and Ganyu [1] n (%)

Characteristics	Dafeng (high-risk)		Ganyu (low-risk)		*P* value [2]
	Case (n=291)	Control (n=291)	Control (n=240)	Case (n=240)	
Gender					
Male	200 (68.7)	200 (68.7)	181 (75.4)	181 (75.4)	0.088
Female	91 (31.3)	91 (31.3)	59 (24.6)	59 (24.6)	
Age (yrs) Mean±SD	64.8 ± 8.6	64.6 ± 8.9	65.4 ± 10.3	65.6 ± 10.4	
<50	14 (4.8)	17 (5.8)	19 (7.9)	17 (7.1)	
50~59	61 (30.0)	59 (20.3)	48 (20.0)	51 (21.3)	0.002
60~69	137(47.1)	137 (47.1)	78 (32.5)	76 (31.7)	
70~79	71 (24.4)	69 (23.7)	77 (32.1)	78 (32.5)	
≥80	8 (2.8)	9 (3.1)	18 (7.5)	18 (7.5)	
Level of education					
Illiterate	156 (53.6)	130 (44.7)	138 (57.7)	164 (68.6)	<0.001
Primary school	95 (32.7)	119 (40.9)	63 (26.4)	54 (22.6)	
Secondary school & above	40 (13.7)	42 (14.4)	38 (15.9)	21 (8.8)	
Past economic status (By separate cut-off points)					
Median (CNY/yr)	1250	1500	1000	775	
1 (lowest)	97 (33.5)	55 (19.0)	38 (16.2)	47 (20.3)	
2	68 (23.5)	64 (22.1)	71 (30.2)	87 (37.5)	0.235
3	73 (25.2)	96 (33.1)	71 (30.2)	59 (25.4)	
4 (highest)	52 (17.9)	75 (25.9)	55 (23.4)	39 (16.8)	
Smoking status [3]					
Never-smoker	92 (31.6)	122 (41.9)	95 (39.6)	82 (34.2)	0.067
Ex-smoker	71 (24.4)	64 (22.0)	19 (7.9)	17 (7.1)	
Current smoker	128 (44.0)	105 (36.1)	126 (52.5)	141 (58.7)	
Alcohol drinking status [4]					
Never drinker	175 (60.1)	181 (62.2)	143 (59.6)	131 (54.6)	0.076
Former drinker	5 (1.7)	7 (2.4)	7 (2.9)	7 (2.9)	
Current drinker	111 (38.1)	103 (35.4)	90 (37.5)	102 (42.5)	
Encountered misfortune in past 10 yr					
No	54 (18.6)	41 (14.2)	33 (14.0)	54 (23.3)	0.008
Yes	237 (81.4)	248 (85.8)	203 (86.0)	178 (76.7)	
Family history of cancer					
No	112 (38.5)	86 (29.6)	16 (6.7)	29 (12.1)	<0.001
Yes	179 (61.5)	205 (70.5)	224 (93.3)	211 (87.9)	
Body mass index					
<18.5	60 (20.8)	27 (9.3)	18 (7.6)	31 (13.4)	
18.5-23.9	182 (63.0)	192 (66.2)	161 (67.9)	155 (67.1)	0.014
23.9-27.9	34 (11.8)	60 (20.7)	46 (19.4)	28 (12.1)	
≥28	13 (4.5)	11 (3.8)	12 (5.1)	17 (7.4)	

[1] Some strata do not match the total because of missing values. [2] The *P*-value for comparing the distribution of factors between the two counties. [3] Never-smokers and ever-smokers were used for comparing the smoking habits between the two counties. [4] Because of the few numbers of former drinkers in both two counties, alcohol drinking status was categorized to never-drinkers and ever-drinkers for the comparison between the two counties and following analyses.

Table 2-2 OR [1] and 95% CI of socio-economic status in EC of Dafeng and Ganyu

Socio-economic status	Dafeng (high-risk)	Ganyu (low-risk)
Level of education		
Illiterate	1.00 (Referent)	1.00 (Referent)
Primary school	0.54 (0.35-0.84)	0.58 (0.33-1.03)
Secondary school & above	0.74 (0.39-1.41)	0.42 (0.21-0.83)
P value for trend	*0.080*	*0.008*
Past economic status		
1 (lowest)	1.00 (Referent)	1.00 (Referent)
2	0.67 (0.43-1.06)	1.03 (0.61-1.75)
3	0.44 (0.28-0.68)	0.76 (0.44-1.31)
4 (highest)	0.39 (0.23-0.65)	0.73 (0.41-1.28)
P value for trend	*<0.001*	*0.024*
Encountered misfortune in past 10 yrs		
No	1.00 (Referent)	1.00 (Referent)
Yes	1.26 (0.80-1.98)	1.64 (0.98-2.73)
Family history of cancer		
No	1.00 (Referent)	1.00 (Referent)
Yes	1.53 (1.06-2.19)	2.07 (1.03-4.17)
Body mass index		
<18.5	1.00 (Referent)	1.00 (Referent)
18.5-23.9	0.45 (0.26-0.76)	0.50 (0.28-0.90)
23.9-27.9	0.26 (0.13-0.50)	0.36 (0.17-0.75)
≥28	0.49 (0.18-1.33)	0.80 (0.33-1.98)
P value for trend	*0.002*	*0.376*

[1] Matched by age and gender, further adjusted for education level and past economic status (quartile).

Table 2-3 OR [1] and 95% CI of tobacco smoking in EC of Dafeng and Ganyu

Tobacco smoking	Dafeng(high-risk)	Ganyu (low-risk)
Smoking status [2]		
Former smoker	1.93 (0.91-4.08)	1.28 (0.28-5.83)
Current smoker	2.42 (1.28-4.56)	2.36 (0.89-6.26)
P value for trend	*0.005*	*0.070*
Age at first smoke [2]		
<20	2.02 (0.93-4.38)	1.60 (0.31-7.90)
20-34	2.32 (1.15-4.67)	2.25 (0.80-6.35)
≥35	1.80 (0.62-5.24)	0.98 (0.29-3.24)
P value for trend	*0.016*	*0.249*
Duration of smoking (yrs) [2]		
1-29	1.61 (0.67-3.86)	1.44 (0.46-4.42)
30-49	2.65 (1.28-5.49)	2.04 (0.60-6.92)
≥50	2.04 (0.78-5.35)	1.98 (0.43-9.11)
P value for trend	*0.009*	*0.194*
Dosage of smoking (Cig/d) [2]		
1-9	1.36 (0.50-3.74)	1.12 (0.27-4.68)
10-19	2.21 (1.01-4.80)	1.56 (0.42-5.78)
≥20	2.04 (1.00-4.18)	0.91 (0.32-2.61)
P value for trend	*0.015*	*0.915*
Total consumption of cigarettes [2]		
1 (lowest)	1.40 (0.61-3.21)	0.96 (032-2.82)
2	2.55 (1.06-6.14)	3.50 (0.37-32.8)
3	1.88 (0.79-4.49)	1.94 (0.25-14.7)
4 (highest)	1.81 (0.57-5.74)	0.74 (0.19-2.81)
P value for trend	*0.029*	*0.959*

[1] Matched by age and gender, further adjusted for level of education, past economic status (group) and alcohol drinking. [2] Never-smokers were used as the reference group.

developing EC than never-smokers. In Ganyu former smoking and current smoking also increased the risk of developing EC (OR=1.28 and 2.36 respectively). We found in Dafeng that smoking at an earlier age (for trend *P*=0.016), long durations of smoking (for trend *P*=0.006), and large amounts of cigarettes per day (for trend *P*=0.029) were significantly associated with increased EC risk, with an apparent dose-response relationship. However, these associations were not significant in Ganyu.

Table 2-4 OR [1] and 95% CI of alcohol drinking in EC of Dafeng and Ganyu

Alcohol drinking	Dafeng (high-risk)	Ganyu (low-risk)
Alcohol drinking		
Never	1.00 (Referent)	1.00 (Referent)
Ever	1.01 (0.70-1.46)	1.71 (1.02-2.88)
P value	*0.964*	*0.043*
Age of first drink [2]		
<20	0.83 (0.44-1.58)	2.59 (1.03-6.50)
20-34	1.23 (0.79-1.91)	1.95 (1.08-3.53)
≥35	0.81 (0.48-1.35)	1.18 (0.56-2.47)
P value for trend	*0.815*	*0.012*
Duration of drinking (yrs) [2]		
1-24	0.96 (0.56-1.59)	1.28 (0.58-2.79)
25-34	0.89 (0.48-1.64)	1.48 (0.75-2.94)
35-44	1.57 (0.92-2.70)	1.47 (0.71-3.01)
≥45	0.77 (0.43-1.40)	1.88 (0.95-3.75)
P value for trend	*0.834*	*0.061*
Alcohol consumption 10 years ago [2] (pure ethanol mL/wk)		
1-249	0.87 (0.49-1.54)	0.79 (0.36-1.74)
250-499	1.06 (0.60-1.89)	0.61 (0.30-1.25)
500-749	0.97 (0.52-1.79)	1.63 (0.77-3.43)
≥750	1.10 (0.63-1.93)	1.27 (0.71-2.28)
P value for trend	*0.740*	*0.223*

[1] Matched by age and gender, further adjusted for level of education, past economic status (group) and tobacco smoking.[2] Never-drinkers were used as the reference group.

In Ganyu, subjects who ever drank alcohol tended to have a higher risk of EC (OR=1.71, 95% CI: 1.02-2.88). Moreover, drinking at an early age (for trend *P*=0.012) and long durations of drinking (for trend *P*=0.061) showed an increased association with EC (Table 2-4). A high consumption of pure ethanol per week 10 years ago slightly elevated the risk of EC, but no significant dose-response relationship was apparent. We did not find any significant association between alcohol drinking and EC in Dafeng, despite its similar alcohol drinking prevalence as Ganyu. The joint effects between smoking and alcohol drinking were also explored in both counties, but no significant interaction was observed either in Dafeng (*P*=0.900) or in Ganyu (*P*=0.870).

Table 2-5 OR and 95% CI of dietary habits, food consumption with EC in Dafeng and Ganyu

Dietary factor	County	Category				*P value for trend*
		1(lowest)	2	3	4(highest)	
Food temperature [1]	Dafeng	1.00	0.51(0.24-1.09)	-	-	*0.080*
(1-Normal; 2- Hot)	Ganyu	1.00	1.14(0.55-2.41)	-	-	*0.714*
Eating Speed [1]	Dafeng	1.00	4.01(1.87-8.62)	-	-	*<0.001*
(1-Normal; 2-Fast)	Ganyu	1.00	3.09 (1.2-7.70)	-	-	*0.015*
Self reported grain fungi pollution [1]	Dafeng	1.00	2.27(0.79-6.54)	-	-	*0.131*
(1-Likely;2-Not likely)	Ganyu	1.00	1.18(0.45-3.11)	-	-	*0.741*
Fresh garlic/wk [2 3]	Dafeng	1.00	0.64(0.26-1.60)	-	-	*0.337*
	Ganyu	1.00	1.17(0.57-2.41)	-	-	*0.664*
Staple foods [1]	Dafeng	1.00	0.45(0.19-1.10)	0.54(0.21-1.38)	0.73(0.26-2.04)	*0.474*
	Ganyu	1.00	0.98(0.10-9.77)	0.45(0.05-4.38)	0.54(0.05-4.34)	*0.324*
Meat [4]	Dafeng	1.00	0.73(0.29-1.85)	1.66(0.68-4.10)	1.93(0.64-5.77)	*0.160*
	Ganyu	1.00	0.54(0.12-2.42)	1.17(0.28-4.92)	0.65(0.11-3.67)	*0.305*
Fish and seafood products [4]	Dafeng	1.00	1.14(0.64-2.03)	2.11(1.12-3.96)	1.91(1.00-3.64)	*0.023*
	Ganyu	1.00	0.98(0.42-2.28)	0.64(0.28-1.44)	1.04(0.46-2.33)	*0.794*
Eggs [4]	Dafeng	1.00	0.53(0.20-1.44)	1.23(0.54-2.80)	1.99(0.72-5.49)	*0.146*
	Ganyu	1.00	0.69(0.28-1.73)	0.30(0.10-1.10)	0.95(0.41-2.22)	*0.936*
Soybean [4 5]	Dafeng	1.00	1.81(0.88-3.74)	-	-	*0.110*
	Ganyu	1.00	1.31(0.37-4.59)	-	-	*0.677*
Preserved foods [4]	Dafeng	1.00	0.26(0.09-0.75)	0.49(0.16-1.46)	0.94(0.37-2.36)	*0.635*
	Ganyu	1.00	1.05(0.37-2.97)	0.56(0.21-1.48)	1.21(0.46-3.20)	*0.932*
Vegetables [4]	Dafeng	1.00	1.26(0.50-3.16)	0.94(0.39-2.30)	1.37(0.49-3.83)	*0.720*
	Ganyu	1.00	0.34(0.08-1.54)	0.80(0.20-3.18)	0.76(0.15-3.72)	*0.889*

Table 2-5 OR and 95% CI of dietary habits, food consumption with EC in Dafeng and Ganyu (continued)

Dietary factor	County	Category				Dietary factor
		1(lowest)	2	3	4(highest)	
Fruits [2]	Dafeng	1.00	1.02(0.42-2.47)	0.42(0.16-1.12)	1.23(0.51-2.98)	*0.802*
	Ganyu	1.00	1.61(0.68-3.80)	1.13(0.43-2.95)	1.17(0.41-3.37)	*0.746*

[1] Matched by age and gender, further adjusted for level of education, past economic status (group), smoking, alcohol drinking, BMI group, cancer family history, eating speed, food temperature and self-reported grain fungi pollution. [2] Matched by age and gender, further adjusted for education level, past economic status (group), smoking, alcohol drinking, BMI group, eating speed and family history of cancer. [3] Less than 3 times per week=1, 3 times per week and above=2. [4] Matched by age and gender, further adjusted for level of education, past economic status (group), smoking, alcohol drinking, BMI group, cancer family history, eating speed and food temperature. [5] Categorized by median among controls.

Dietary factors

After adjusting for potential confounders in both Dafeng and Ganyu, subjects with fast eating speed showed an increased risk of developing EC (Dafeng: OR=4.01; Ganyu: OR=3.09). On the other hand, high food temperatures, the possibility of being exposed to grain fungi pollution, and frequent intake of fresh garlic did not influence EC risk significantly (Table 2-5).

With regard to the consumption of major food groups, high consumptions of fish and seafood products significantly elevated the risk of developing EC in Dafeng (for trend P=0.024). Staple foods, preserved foods, fruits and vegetables, and soybean, however, were not apparently associated with EC risk in either county.

Discussion

This population-based case-control study, conducted in high- and low-risk areas of Jiangsu province, China, demonstrated the associations between tobacco smoking, alcohol drinking, dietary factors and EC. These associations were compared separately in the two regions which had similar socioeconomic statuses and geographic characteristics. From our awareness, this is the first comparative population-based case-control study conducted in low-risk and high-risk areas simultaneously to compare the different associations of risk factors and EC in similar areas with high-risk gradients. In consistent with other epidemiological researches, our study showed that EC was inversely associated with socioeconomic status, such as level of education and income level. However, this disease was more frequent among subjects with smoking and alcohol drinking habits and unhealthy dietary factors. Furthermore, a geographic variation of some associations was observed between the low-risk and high-risk areas. Smoking elevated the risk of EC in both areas concordantly, but the dose-response relationship of smoking and smoking related variables (age of first smoke, duration and dosage) was apparent only in the high-risk area. On the contrary, the effect of alcohol drinking on EC and a dose-response relationship was only observed in the low-risk area.

Supporting previous studies, the risk of EC was inversely associated with socio-economic status in the present study.[15,16] People with higher levels of education and better financial situations tend to have a lower risk of developing EC due to good living conditions and better health care access. Increased risk was found in people who had encountered misfortune in the past 10 years (Dafeng OR: 1.26, Ganyu OR: 1.64), or had a family history of cancer in first-degree relatives (Dafeng OR: 1.53, Ganyu OR: 1.57). These results were consistent in both high-risk and low-risk areas.

Associations between body mass index (BMI) and EC have been explored in several studies. Chow et al.[17] reported a tendency towards a decreasing risk of esophageal squamous cell carcinoma with increasing BMI. Engeland found that low BMI increased the risk of esophageal squamous cell carcinoma, while high BMI increased the risk of esophageal adenocarcinoma. In general, lowest BMI had the highest risk of EC.[18] Our study found similar results in both low-risk and high-risk areas. The risk of developing EC was significantly lower in normal and overweight groups when compared to the underweight group. However, the OR was high in the obese group. An increased risk of esophageal adenocarcinoma among obese persons has been explained by a dose-dependent association between increasing BMI and the risk of gastro-esophageal reflux symptoms, as observed by Nilsson et al.[19]

In conformity with other epidemiological studies shown in Western countries and some areas of Asia and Africa,[20-22] increased risks of EC among former smoking and current smoking subjects were observed in both areas of our study. Tobacco smoke contains over 3000 constituents including 30 carcinogens, such as polycyclic aromatic hydrocarbons (PAHs), aromatic amines, and N-nitrosamines. The metabolites of these carcinogens may lead to gene mutation and cancer.[23] Age of first smoke, duration and dosage of tobacco use were also strongly associated with an elevated risk of developing EC in Dafeng, with an apparent dose-response relationship. Although Ganyu had a similar smoking prevalence, these time and dosage dependent results were not statistically significant.

Several studies have reported a strong correlation between EC and alcohol abuse.[24,25] Alcoholic beverages also contain carcinogens and other compounds, and may facilitate the absorption of esophageal mucosal cells and make them more susceptible to chemical carcinogens.[26] On the contrary of tobacco smoking, the positive association between alcohol drinking and EC was only found in Ganyu in our study (OR=1.71). Several studies have reported a linear relationship between an overall daily ethanol consumption and EC risk.[27, 28] However, in our study only the age of initial drinking and years of alcohol drinking were found to be associated with EC risk in Ganyu.

The interaction between tobacco smoking and alcohol drinking has been studied in many researches. It has been suggested that alcohol and tobacco may interact in a multiplicative way.[29,30] In a large scale study, Castellsagué reported that the risk of EC in the highest joint level of alcohol and cigarette smoking increased 50.85-fold and 35.34-fold among men and women, respectively.[7] However, the joint effect of smoking and alcohol was not found to be statistically significant in our study. The link between smoking, alcohol and EC in China are not as apparent as in Western countries. Several previous studies conducted in other high-risk areas of Jiangsu, China either did not find any relation or found only a weak association between smoking, alcohol drinking and EC.[31,32]

Dietary factors are thought to play an important role in the pathogenesis of EC. Some

epidemiological studies have suggested that the risk of EC is inversely associated with a higher intake of fruits and vegetables,[33,34] while a detrimental effect was observed among high intake of certain types of meat, butter and saturated fatty acids.[35] Increased risk was found to be related to N-nitrosamine compounds (mainly from preserved foods), foods contamination by fungus and the presence of toxins. Some unhealthy dietary habits such as fast eating speed, consumption of hot foods and soups can cause the injury of esophageal mucosa and render the mucosa more susceptible to carcinogens.

An increased OR was found among fast eating subjects in both areas of this study (Dafeng OR: 4.01, Ganyu OR: 3.09). However, the associations between high food temperatures, the possibility of fungi pollution of grain, frequent intake of fresh garlic and EC was not statistically significant. In the food group analysis, after adjusting for potential confounders, we did not find any significant association between major food consumption and EC risk in either area. A positive association of fish and seafood product intake in Dafeng was found (for trend P=0.024). Fish is a rich dietary source of n-3 fatty acids. It has been reported that this long chain of fatty acid can suppress mutation, inhibit cell growth, and enhance cell apoptosis; thus reduce the risk of developing cancer.[36] The contradictory result found in our study for the increased risk between fish consumption and EC in Dafeng was probably due to water contamination or other unidentified confounders. However, this hypothesis needs to be further clarified and studied. Moreover, it may be more reasonable to study food composition and micronutrients in our future analysis rather than to use individual food or food groups.[37]

Ganyu has a high proportion of ageing and illiterate residents. The economic status of residents in Ganyu is also lower than Dafeng, although the difference is not significant (Table 2-1). As disease is more prevalent among ageing populations, and the level of education and economic situation are inversely associated with the risk of developing EC, it can be expected that the two counties would have a far higher risk gradient if they had a similar distribution of age and socio-economic related factors.

As mentioned above, a heterogeneous association between smoking, alcohol drinking and EC was observed in the low- and high-risk areas in our study, despite their similar geographic characteristics and general socioeconomic statuses. A malignant tumor is the result of a series of DNA alterations in a single cell, which leads to a loss of normal functioning. A large number of gene coding for enzymes and receptors are involved in xenobiotic metabolism, with many of them showing polymorphisms. Many molecular epidemiological studies have proved that polymorphisms in activation and detoxification enzymes can interact with environmental carcinogens. It has been reported that GSTM1 null carriers may be especially susceptible to the action of tobacco with regards to EC,[32] while the inactive ALDH2 genotype may increase the risk of EC in alcoholics.[38] Genetic polymorphisms can interact with dietary factors. For example, cruciferous vegetables can

inhibit the metabolic activation of phase I enzymes and induce the detoxification of carcinogens via phase II enzymes.[39] The polymorphism of one gene may also have an effect on other genes. Gene-gene interactions between GSTM1 0/0 and CYP1A1 and CYP1A2 enzyme induction have been observed in smokers.[40] Another example is that individuals with CYP1A1 Ile/Val alleles have greater CYP1A2 activity than those with wild type CYP1A1.[41] Furthermore, it has been suggested that genes can influence individual behaviours such as smoking, alcohol drinking and excess calorie intake, thereby having the potential to affect cancer risk. [42]

Both environmental factors and human genes can show considerable regional variability. The variation in these factors, together with their separate and joint effects, ultimately determine the risk of cancer in different regions and may be the main reason for the large EC risk gradient between the counties in Jiangsu province. Unfortunately, scientific evidence in genetic polymorphisms, gene-environmental and gene-gene interactions remains inconsistent and inconclusive because of low statistical power and few candidate genes in previous studies. Moreover, there was no study has ever been conducted to compare the association between gene-environmental interaction and EC risk in apparently similar areas with a high risk gradient. Therefore, our future study will focus on genetic polymorphisms and their interactions with different environmental, lifestyle and dietary factors in the etiology of EC in high- and low-risk areas, with a sufficient sample size and multiple candidate genes.

Our present population-based case-control study has some limitations. Differences in the etiological factors between esophageal adenocarcinoma and squamous cell carcinoma may exist. Because of the low histological examination rate in China, it is difficult to differentiate the subtypes of EC in this population-based study. Additionally, most risk factors in our study are based on self-reported data and may be subject to recall bias. Moreover, the relationship between BMI and EC was examined by using height and weight measurements obtained at the time of interview. Some cases might have begun to lose weight at an earlier time because of the disease. This could also have caused bias in our study.

In summary, the present study demonstrated the association between smoking, alcohol drinking, dietary factors and EC risk in the low-risk and high-risk areas of Jiangsu Province, China. Heterogeneous effects of smoking and alcohol drinking were found between the two areas, despite their similar geographic characteristics and general socio-economic statuses. The variation in environmental risk factors, together with gene-environment and gene-gene interactions may be the main reason for these heterogeneous associations and may contribute to the large risk gradient of EC mortality.

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Chapter 3

Smoking and alcohol drinking increased the risk of esophageal cancer among Chinese men but not women in a high-risk population

Ming Wu, Jin-Kou Zhao, Zuo-Feng Zhang,
Ren-Qiang Han, Jie Yang, Jin-Yi Zhou,
Xu-Shan Wang, Xiao-Feng Zhang, Ai-Min Liu,
Pieter van' t Veer, Frans J Kok, Ellen Kampman

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ABSTRACT

Although the association for esophageal cancer with tobacco smoking and alcohol drinking has been well established, the risk appears to be less strong in China. To provide more evidence on the effect of smoking and alcohol consumption with esophageal cancer in China, particularly among Chinese women, a population-based case-control study has been conducted in Jiangsu, China from 2003 to 2007. A total of 1,520 cases and 3,879 controls were recruited. Unconditional multivariate logistic regression analysis was applied. Results showed that odds ratio (OR) and confidence interval (CI) for ever smoking and alcohol drinking was 1.57 (95% CI: 1.34-1.83) and 1.50 (95% CI: 1.29-1.74). Dose-response relationships were observed with increased intensity and longer duration of smoking/drinking. Risk for smoking and alcohol drinking at the highest joint level was 7.32 (95% CI: 4.58-11.7), as compared to those never smoked and never drank alcohol. Stratifying by genders, smoking and alcohol drinking increased the risk among men with an OR of 1.74 (95% CI: 1.44-2.09) and 1.76 (95% CI: 1.48-2.09); however, neither smoking nor alcohol consumption showed a significant association among women. In conclusion, smoking and alcohol drinking increased the risk of esophageal cancer among Chinese men, but not among Chinese women.

Key words: Esophageal cancer; Smoking; Alcohol; Case-control studies; China

Introduction

Esophageal cancer remains one of the most common and fatal malignancies in the world. In 2005, about 497,700 new cases occurred worldwide and the prevalence is expected to increase by approximately 140% by 2025.[1] The actual etiology of esophageal cancer remains unclear, but extensive evidence in the past decades has demonstrated that tobacco smoking and alcohol drinking are prominent risk factors of this disease.[2-6] The risk for esophageal cancer among smokers is 2-5 fold in general when compared to non-smokers, while for heavy smokers the risk may exceed tenfold.[3] The average risk of esophageal cancer with alcohol drinking is estimated to be 1.04-fold per drink/week,[4] and the risk ratio may also over tenfold among heavy drinkers.[5] Some previous studies reported that the associations significantly increased when alcohol and smoking coexist, their joint effects are approximately multiplicative and may increase 130-fold in the highest joint level.[6]

Although the association for esophageal cancer with tobacco smoking and alcohol drinking has been well established in many areas of the world, some striking variation between regions does exist.[2] In Western countries, smoking and alcohol consumption seem to be the primary risk factors of esophageal squamous cell cancer, the population attributable fraction (PAF) of smoking and alcohol was estimated to be 71 and 41% respectively.[7] China is one of the areas with the highest incidence of esophageal cancer worldwide, about half of the cases that occur in the world each year are estimated to be in this country.[8] Whereas, the strong association of smoking and alcohol as well as their multiplicative joint effects were observed considerably weaker or even absent according to some previous studies.[9-12] The proportion of esophageal cancer attributed to smoking and alcohol in China was estimated to be only 23 and 16% respectively.[13] Previous studies showed that smoking and alcohol are strong and important risk factors in both genders in Western populations,[5,14-16] however, because of the rather low prevalence of smoking and alcohol drinking in Chinese women, very few studies explored the etiology of these two risk factors among women in this country.

During 2003-2007, a large population-based case-control study on esophageal cancer has been carried out in Jiangsu province, an area with the third highest esophageal cancer mortality in China.[17] The study design has previously been described in detail.[18,19] In brief, this study was conducted in two counties, Dafeng and Ganyu. Both of these counties are less-developed rural areas in northern Jiangsu, and farming remains the main occupation (round 60%) of the local population. The annual average age-standardized incidence during 2006-2008 by China standard population was 36 and 24 per 100,000 in Dafeng and Ganyu, respectively. In this present analysis, we analyzed the overall and gender specific effects of smoking and alcohol drinking on esophageal cancer in this high-risk Chinese population. It aims to provide further data on the independent and joint effects of these two well-known risk factors in China, especially among Chinese women.

Materials and methods

Subject recruitment and data collection

Eligible subjects were restricted to local inhabitants who have lived in the study area for at least 5 years. Newly diagnosed primary esophageal cancer patients were recruited as cases, using the data from local population-based cancer registries. From 2003-2007, 68 and 75% of all newly registered patients were recruited and interviewed in Dafeng and Ganyu, respectively. Because of the low proportion of pathological examination in the less developed rural areas (39%), patients who were diagnosed by other sophisticated methods such as endoscopic examination (40%) or radiology (11%) were also included.

Controls were derived from the same county as cases, randomly selected from the county demographic database. Controls were frequency matched with cases by gender and age (±5 years). The participation rate of controls was 87% in Dafeng and 85% in Ganyu, respectively.

With written informed consent, epidemiological data were obtained by face-to-face interviews using a pre-tested standardized questionnaire. The questionnaire included detailed information on factors known or suspected to be associated with esophageal cancer, including demographic information, socio-economic status, living conditions,

environmental exposures and dietary habits. Details on lifetime smoking and alcohol drinking habits were collected, questions included age at starting smoking/drinking, years of consumption, the average number of cigarettes smoked per day, weekly frequency and the amount of drinking different types of alcohol beverages (e.g. beer, wine, and liquor). If the subject had quit smoking/drinking habit at time of interview, the duration of cessation was also recorded. This study was approved by the Institutional Review Board of the Jiangsu Provincial Health Department.

Statistical analysis

Data were entered into the computer by Epidata 3.0, cleaned and analyzed using SAS v9.1 software (SAS Institute, Inc., Cary, NC). In the present analysis, never smokers were defined as having smoked fewer than 100 cigarettes in their lifetime; while never drinkers were those who drank less than once per month. Current smokers and current drinkers were defined as those who had the habit at the time of interview, or stopped the habit within 1 year before interview. Pack-years of smoking and weekly consumption of pure ethanol (grams/week) on average were calculated. Since no marked difference was observed regarding the effect of smoking and alcohol drinking between the two counties, data were pooled to improve statistical power.

Confounders were selected based on the previous knowledge on esophageal cancer and our preliminary results,[20] including age, gender, education level, previous income, body mass index (using Chinese recommendation standard),[21] family history of cancer in first-degree relatives (any malignancy) and study area. After adjusting for confounders, the overall and gender specific effects for smoking and alcohol drinking were evaluated by unconditional logistic regression. The strength was quantified as odds ratios (OR), and 95% confidence intervals (CI) around the OR were used to quantify precision. The trend test of ordered variables was performed by assigning scores to different exposure levels and treating the categorical variable as a continuous variable in the logistic regression model. Effect modifications were evaluated by stratification, statistical interaction was assessed by including main effect variables and their product terms in the logistic regression model.

Chapter 3

Results

In total, 1,520 cases (637 in Dafeng and 883 in Ganyu) and 3,879 controls (1,938 in Dafeng and 1,941 in Ganyu) were recruited. Table 3-1 shows the demographical information and socio-economic characteristics of cases and controls by gender. Cases were more frequently men and older people, and more frequently occurred in the population with lower socio-economic status, i.e., lower education level, lower previous income, lower BMI and with cancer-affected relatives.

Table 3-1 The demographic information and socio-economic status of cases and controls

	Men		Women	
	Case (%) (N=1,191)	**Control (%) (N=2,916)**	**Case (%) (N=329)**	**Control (%) (N=963)**
Study area				
Dafeng	426 (35.8)	1,368 (46.9)	211 (64.1)	570 (59.2)
Ganyu	765 (64.2)	1,548 (53.1)	118 (58.1)	393 (40.8)
Age (years)				
Mean (SD)	65.3 (9.6)	64.2 (11.0) [3]	67.4 (9.1)	64.9 (11.7) [3]
<60	344 (28.9)	945 (32.4)	56 (17.0)	274 (28.5)
60-	405 (34.0)	933 (32.0)	142 (43.2)	311 (32.3)
70-	366 (30.7)	857 (29.4)	99 (30.1)	300 (31.2)
≥80	76 (6.4)	181 (6.2)	32 (9.7)	78 (8.1)
Education level [1]				
Illiteracy	608 (51.1)	1,302 (44.7) [3]	288 (87.5)	755 (78.4) [3]
Primary school	409 (34.4)	1,028 (35.3)	35 (10.6)	142 (14.8)
Middle school & above	173 (14.5)	584 (20.0)	6 (1.8)	66 (6.8)
Previous Income[1]				
<1000	364 (31.0)	691 (24.0) [3]	97 (29.7)	207 (22.0) [3]
1000~	250 (21.3)	541 (18.8)	63 (19.3)	206 (21.9)
1500~	305 (26.0)	764 (26.6)	92 (28.1)	269 (28.6)
≥2500	254 (21.7)	882 (30.6)	75 (22.9)	260 (27.6)
Body Mass Index (BMI)[1,2]				
Mean (SD)	21.7 (3.7)	22.8 (5.4) [3]	21.3 (4.7)	22.7 (3.5) [3]
18.5~23.9	843 (71.4)	1,954 (67.2)	174 (53.0)	539 (56.3)
<18.5	153 (13.0)	186 (6.4)	87 (26.5)	92 (9.6)
24~27.9	149 (12.6)	638 (21.9)	49 (14.9)	264 (27.6)
≥28	36 (3.0)	132 (4.5)	18(5.5)	63 (6.6)
Family history of cancer				
No	865 (72.6)	2,243 (76.9) [3]	212 (64.4)	689 (71.6) [3]
Yes	326 (27.4)	673 (23.1)	117 (35.6)	274 (28.4)

[1] Sum does not add up because of missing values. [2]Chinese recommendation standard was used for the cut-off points of overweight and obesity.[21 3] For the comparison between cases and controls, P<0.01.

Table 3-2 shows the associations for esophageal cancer with selected smoking-related variables. Ever smoking significantly increased the risk of esophageal cancer with an OR of

1.57 (95% CI: 1.34-1.83) compared to never smokers. ORs for former smoking and current smoking were 1.74 (95% CI: 1.38-2.21) and 1.54 (95% CI: 1.31-1.80), respectively. Positive dose-response relationships were observed with increased daily amount, duration and pack-years of smoking (for trend, P<0.001 each), except for age at starting the habit. Quitting smoking was found associated with a decreased cancer risk, OR for those who had quit for more than 10 years was similar to never smokers (OR=0.90, 95% CI: 0.62-1.30); however, quitting less than 5 years still increased risk 1.64-fold when compared to never smokers. Stratifying for gender, OR for ever smoking men and women was 1.74 (95% CI: 1.44-2.09) and 1.13 (95% CI: 0.83-1.54), respectively. Results showed that the magnitude of the effects among women was much smaller than the corresponding ones among men, and all associations were observed not statistically significant.

Table 3-3 presents the overall and gender specific OR and 95% CI for esophageal cancer with selected alcohol-related variables. Ever drinking alcohol significantly increased cancer risk (OR=1.50, 95% CI: 1.29-1.74), and ORs were observed positively associated with increased drinking frequency, longer duration and high weekly consumption of alcohol (for trend, P<0.001 each), but not for age at starting drinking. A substantially elevated OR was found among former drinkers (OR = 5.16, 95% CI: 4.23-6.29), even those who had quit drinking for more than 10 years still had a 1.80-fold risk (95% CI: 1.14-2.85) when compared to never drinkers. Similar to smoking, the associations for alcohol consumption were found generally lower among women than that of men, OR for ever drinking was 1.76 (95% CI: 1.48-2.09) for men but was 0.82 (95% CI: 0.59-1.16) for women, and no dose-response association was observed among women.

Table 3-2 The OR and 95% CI for smoking-related variables with esophageal cancer among men and women[1]

	ALL		Men		Women	
	Case/Control	**OR (95% CI)** [2]	**Case/Control**	**OR (95% CI)**[3]	**Case/Control**	**OR (95% CI)**[3]
Smoking status						
Never smoking	415/1,549	1.00 (referent)	187/824	1.00 (referent)	228/725	1.00 (referent)
Ever smoking	1,105/2,330	1.57 (1.34-1.83)	1,004/2,092	1.74 (1.44-2.09)	101/238	1.13 (0.83-1.54)
Former	162/337	1.74 (1.38-2.21)	142/287	2.01 (1.53-2.63)	20/50	1.15 (0.65-2.02)
Current	943/1,993	1.54 (1.31-1.80)	862/1,805	1.69 (1.40-2.05)	81/188	1.13 (0.81-1.57)
Daily amount of smoking (Cig/day)						
Never smoking	415/1,549	1.00 (referent)	187/824	1.00 (referent)	228/725	1.00 (referent)
<10	373/803	1.35 (1.12-1.63)	338/718	1.42 (1.14-1.78)	35/85	1.37 (0.97-1.94)
10-	231/543	1.52 (1.24-1.88)	187/472	1.58 (1.24-2.01)	44/71	
20-	370/807	1.63 (1.35-1.97)	351/736	1.87 (1.50-2.32)	19/71	0.70 (0.42-1.18)
30-	131/177	2.84 (2.16-3.74)	128/166	3.25 (2.41-4.37)	3/11	
P for trend		<0.001		<0.001		0.693
Duration of Smoking (years)						
Never smoking	415/1,549	1.00 (referent)	187/824	1.00 (referent)	228/725	1.00 (referent)
<20	151/361	1.50 (1.18-1.90)	132/323	1.60 (1.22-2.09)	19/38	1.23 (0.81-1.84)
20-	282/594	1.70 (1.39-2.08)	256/528	1.87 (1.48-1.79)	26/66	
35-	419/855	1.61 (1.34-1.93)	386/776	1.80 (1.45-2.22)	33/79	1.05 (0.71-1.54)
50-	253/520	1.40 (1.13-1.75)	230/465	1.59 (1.24-2.05)	23/55	
P for trend		<0.001		<0.001		0.782
Pack-years of smoking						
Never smoking	415/1,549	1.00 (referent)	187/824	1.00 (referent)	228/725	1.00 (referent)
<15	406/904	1.38 (1.15-1.66)	362/805	1.45 (1.17-1.80)	44/99	1.20 (0.84-1.72)
15-	208/502	1.45 (1.17-1.80)	178/437	1.56 (1.22-2.00)	30/65	
30-	241/463	1.85 (1.49-2.29)	223/422	2.05 (1.62-2.61)	18/41	0.95 (0.58-1.56)
45-	250/461	1.91 (1.54-2.37)	241/428	2.21 (1.74-2.80)	9/33	
P for trend		<0.001		<0.001		0.765

Table 3-2 The OR and 95% CI for smoking-related variables with esophageal cancer among men and women[1] (Continued)

	ALL		Men		Women	
	Case/Control	**OR (95% CI)** [2]	**Case/Control**	**OR (95% CI)**[3]	**Case/Control**	**OR (95% CI)**[3]
Age at starting smoking (years)						
30-	211/447	1.00 (referent)	164/353	1.00 (referent)	47/94	1.00 (referent)
25-	184/381	1.24 (1.00-1.53)	174/357	1.23 (0.99-1.54)	10/24	
20-	373/780	1.26 (1.07-1.49)	358/727	1.30 (1.09-1.55)	15/53	0.71 (0.43-1.18)
<20	244/547	1.23 (1.02-1.49)	219/490	1.28 (1.04-1.58)	25/57	
P for trend		0.752		0.998		0.189
Time since stopping smoking (years)						
Never smoker	415/1,549	1.00 (referent)	187/824	1.00 (referent)	228/725	1.00 (referent)
10-	41/130	0.90 (0.62-1.30)	33/107	0.92 (0.61-1.39)	6/16	
5-	41/81	1.33 (0.90-1.97)	35/70	1.34 (0.87-2.05)	6/11	1.20 (0.68-2.14)
<5	80/126	1.64 (1.21-2.20)	74/110	1.70 (1.24-2.33)	8/23	
P for trend		<0.001		<0.001		0.524

[1] Missing data were excluded from the analysis.[2] Adjusted for age (continuous), gender, study area, previous income (continuous), BMI (continuous), pure ethanol intake (continuous) and family history of cancer. [3] Adjusted for above mentioned variables except gender.

Table 3-3 The OR and 95% CI for alcohol-related variables with esophageal cancer among men and women

	ALL		Men		Women	
	Case/Control	**OR (95% CI)[2]**	**Case/Control**	**OR (95% CI)[3]**	**Case/Control**	**OR (95% CI)[3]**
Alcohol drinking						
Never drinking	490/1,631	1.00 (referent)	221/864	1.00 (referent)	269/767	1.00 (referent)
Ever drinking	1,030/2,248	1.50 (1.29-1.74)	970/2,052	1.76 (1.48-2.09)	60/196	0.82 (0.59-1.16)
Former	454/293	5.16 (4.23-6.29)	424/256	6.43 (5.14-8.04)	30/37	2.19 (1.30-3.71)
Current	576/1,955	0.94 (0.80-1.10)	546/1,796	1.10 (0.92-1.33)	30/159	0.52 (0.34-1.02)
Frequency of drinking						
Never drinking	490/1,631	1.00 (referent)	221/864	1.00 (referent)	269/767	1.00 (referent)
Occasionally	281/727	1.32 (1.09-1.59)	259/631	1.60 (1.29-1.99)	22/96	0.62 (0.38-1.02)
Often	278/574	1.70 (1.40-2.06)	259/501	1.94 (1.56-2.42)	19/46	1.14 (0.64-2.03)
Every day	471/974	1.54 (1.29-1.84)	452/920	1.77 (1.45-2.15)	19/54	0.94 (0.54-1.65)
P for trend		<0.001		<0.001		0.674
Duration of drinking (years)						
Never drinking	490/1,631	1.00 (referent)	221/864	1.00 (referent)	269/767	1.00 (referent)
<20	102/314	1.04 (0.81-1.34)	85/252	1.27 (0.94-1.70)	17/62	0.83 (0.53-1.29)
20-	181/476	1.16 (0.94-1.44)	168/436	1.31 (1.03-1.67)	13/40	
30-	271/565	1.36 (1.12-1.63)	260/532	1.56 (1.26-1.93)	11/33	0.75 (0.46-1.23)
40-	414/812	1.30 (1.10-1.54)	401/765	1.57(1.29-1.91)	13/47	
P for trend		<0.001		<0.001		0.204
Ethanol intake (ml/Week)						
0	624/1,929	1.00 (referent)	343/1,121	1.00 (referent)	281/808	1.00 (referent)
1-	125/331	1.21 (0.96–1.53)	106/267	1.37 (1.05-1.79)	19/64	0.79 (0.46-1.36)
250-	198/519	1.19 (0.97-1.45)	182/466	1.29 (1.04-1.60)	16/53	
500-	204/478	1.30 (1.06-1.59)	197/456	1.37 (1.11-1.69)	7/22	0.92 (0.57-1.46)
750-	354/562	1.90 (1.58-2.28)	349/550	1.96 (1.62-2.38)	5/12	
P for trend		<0.001		<0.001		0.898

Table 3-3 The OR and 95% CI for alcohol-related variables with esophageal cancer among men and women (Continued)

	ALL		Men		Women	
	Case/Control	OR (95% CI)[2]	Case/Control	OR (95% CI)[3]	Case/Control	OR (95% CI)[3]
Age at start drinking						
30-	391/895	1.00 (referent)	347/770	1.00 (referent)	44/125	1.00 (referent)
25-	244/447	1.45 (1.20-1.75)	239/430	1.46 (1.20-1.77)	5/17	
20-	280/652	1.25 (1.05-1.49)	273/621	1.28 (1.07-1.54)	7/31	0.54 (0.22-1.35)
<20	104/231	1.24 (0.96-1.61)	102/215	1.33 (1.02-1.74)	2/16	
P for trend			0.966		0.739	0.190
Tume since stopping drinking (years)						
Never drinking	490/1,631	1.00 (referent)	221/864	1.00 (referent)	269/767	1.00 (referent)
10-	32/502	1.80 (1.14-2.85)	32/43	2.26 (1.40-3.63)	0/7	
5-	27/35	2.22 (1.32-3.75)	26/32	2.33 (1.36-4.02)	1/3	1.66 (0.79-3.49)
<5	237/13	5.28 (4.19-6.65)	223/122	5.46 (4.29-6.96)	14/10	
P for trend		<0.001		<0.001		0.184

[1] Missing data were excluded from the analysis.[2] Adjusted for age (continuous), gender, study area, previous income (continuous), BMI (continuous), pack-years of smoking (continuous) and family history of cancer. [3] Adjusted for above mentioned variables except gender.

Table 3-4 The joint effects of smoking and alcohol drinking on esophageal cancer among men and women

Smoking	Alcohol	All		Men		Women	
		Case/control	OR (95% CI)[1]	Case/control	OR(95% CI)[2]	Case/Control	OR (95% CI)[2]
Never	Never	276/984	1.00 (referent)	69/358	1.00 (referent)	207/626	1.00 (referent)
Never	Ever	139/565	1.03 (0.80-1.32)	118/466	1.41 (1.00-1.98)	21/99	0.63 (0.38-1.05)
Ever	Never	214/647	1.20 (0.96-1.51)	152/506	1.48 (1.06-2.05)	62/141	1.04 (0.73-1.49)
Ever	Ever	891/1,683	2.10 (1.72-2.56)	852/1,586	2.75 (2.07-3.65)	39/97	1.03 (0.68-1.56)
P for interaction			<0.001		0.170		0.212

[1] Adjusted for age (continuous), gender, study area, previous income (continuous), BMI (continuous) and family history of cancer.[2] Adjusted for above mentioned variables except gender.

Table 3-5 The overall OR and 95% CI for independent and joint effects of smoking and alcohol drinking on esophageal cancer risk[1]

Smoking (cig/day)	Ethanol intake (ml/week)				P for trend
	never	1-249	250-499	≥500	
Never	1.00	1.04 (0.68-1.59)	0.98 (0.64-1.51)	1.38 (0.97-1.98)	0.043
1-9	1.40 (1.07-1.83)	1.41 (0.94-2.12)	1.52 (1.07-2.17)	1.89 (1.45-2.46)	0.100
10-19	1.50 (1.10-2.04)	2.51 (1.53-4.10)	1.89 (1.27-2.82)	1.85 (1.35-2.55)	0.238
20-39	1.39 (1.06-1.83)	1.70 (0.99-2.90)	1.89 (1.34-2.66)	2.74 (2.15-3.48)	0.003
≥40	2.45 (1.20-4.96)	2.28 (0.36-14.2)	2.04 (0.53-7.89)	7.32 (4.58-11.7)	0.021
P for trend	0.001	0.024	0.021	<0.001	<0.001
P for interaction			0.016		

[1] Adjusted for age (continuous), gender, study area, previous income (continuous), BMI (continuous) and family history of cancer.

The joint effects of ever smoking and ever drinking alcohol on esophageal cancer are shown in Table 4. Although no significantly increased OR was observed for ever exposed to either smoking (OR=1.20, 95% CI: 0.96-1.51) or alcohol (OR=1.03, 95% CI: 0.80-1.32) alone, being exposed to both factors increased risk 2.10-fold (95%CI: 1.72-2.56) when compared to never smoking and drinking group, with a more than multiplicative interaction (P<0.001). Gender specific results showed that either smoking or drinking alcohol alone significantly increased the risk among men, but not among women. OR for ever smoking and drinking was 2.75 (95%CI: 2.07-3.65) for men but was 1.03 (95%CI: 0.68-1.56) for women. No significant interaction term was observed for either men or women on a multiplicative scale.

Table 3-5 summarized the joint effects for different levels of daily smoking and average weekly ethanol intake, while gender specific results were not estimated, given the few numbers in some categories. Among never smokers, light or moderate ethanol intake (<500 ml/week) was not found to increase the risk of esophageal cancer, an elevated OR was observed for heavy drinkers (≥500 ml/week) but did not reach statistical significance (OR=1.38, 95% CI: 0.97-1.98). On the other hand, among those who described themselves as never drinkers, even a low daily smoking amount (<10 cig/day) increased risk 1.40-fold (95% CI: 1.07-1.83). Those who smoked more than 40 cig/day have a 2.45-fold risk (95% CI: 1.20-4.96) even without drinking alcohol (for trend p<0.001). Apparent positive dose-response relationships were observed for most levels of smoking intensity and amount of alcohol intake. A significant interaction was observed on multiplicative scale (P = 0.016), OR for exposed to the highest consumption level of smoking and alcohol was 7.32 (95% CI: 4.58-11.7), when compared to those who never smoked and never drank alcohol.

Discussion

In this large population-based case-control study, we confirmed that tobacco smoking and alcohol drinking were associated with esophageal cancer development in a high-risk Chinese population, and found the positive dose-response trends with both intensity and

duration of consumption during lifetime. In agreement with some previous studies in China, their independent and joint effects seem to be less strong when compared to that of Western countries.[9-13] Whereas, neither smoking nor alcohol drinking was found to be associated with esophageal cancer among Chinese women in the present analysis.

Tobacco smoking and alcohol have been claimed as strong risk factors of esophageal cancer for a long time, both of them have been categorized into group I carcinogens (carcinogenic to human) by the working group of International Agency for Research on Cancer (IARC).[22] Strong associations for smoking and alcohol consumption with esophageal cancer were observed in Western countries (including South-American and African populations); however, the risks appear to be much lower in China, especially in some high incidence regions.[9-11] In a large prospective study in Linxian China, one of the highest esophageal cancer risk areas in the world, Tran et al. only found modest elevations in the risk of esophageal cancer among current smokers (OR=1.3), while alcohol drinking was not associated with esophageal cancer (OR=0.86).[9] In a meta-analysis of seven Chinese studies, the pooled OR for smoking and alcohol drinking on esophageal cancer were estimated to be 1.84 and 1.50, respectively.[13] Similarly, we observed a moderate increased risk for smoking (OR=1.57) and alcohol drinking (OR=1.50) in our study, supporting that the risks of these two well-known risk factors are less strong in China.

The reasons for the weaker association could be partly explained by the short exposure history and considerably low prevalence among Chinese women.[7] Although China is currently the largest producer and consumer of tobacco in the world and there is evidence of a striking increase in alcohol consumption, tobacco, and alcohol use became more prevalent in China just from 1980s, and traditionally it is more acceptable for Chinese men to smoke and drink than for women. It has been reported that about 66.9% of men but only 4.2% of women are smokers in China, whereas the prevalence of smoking among men and women was estimated to be 35 and 22% in developed countries, 50 and 9% in developing countries.[23,24] The annual ethanol consumption among Chinese adults was also reported much lower than in industrialized countries, and men drink 13.4 times more than women.[25]

Another explanation for the weak association is that there might be some other strong risks factors that account for the majority of cases in those high-risk areas; thus, the effect of smoking and alcohol drinking is diluted.[26] For instance, nutrition deficiency, exposure to N-nitrosamines, and fungi toxins have been summarized as the major causative factors in some high-risk areas of China.[27]

Several studies have indicated that smoking and alcohol consumption are strong risk factors for both men and women in Western populations,[5,14-16] but very few studies explored their effects among Chinese women because of the considerably low prevalence. Gao et al. reported that the risk of smoking among women was 1.6 (95% CI: 1.0-2.4) in a case-control study (902 cases and 1,552 controls), but no elevated risk was observed among female alcohol drinkers.[28] Another case-control study (355 cases and 408 controls) found no association between alcohol drinking and esophageal cancer among women (OR=0.83, 95% CI: 0.22-3.09); given the limited number of female smokers, the author did not estimate the effect of smoking among women.[29] In this present analysis, we found that neither smoking nor alcohol drinking was associated with the risk of esophageal cancer among women in a Chinese population.

Some quantitative aspects of tobacco and alcohol use were demonstrated to be dose-dependently related to the risk of esophageal cancer, such as the intensity and years of consumption.[2-6] It has been suggested that the risk of smoking depends mainly on the duration of tobacco consumption rather than smoking intensity; on the contrary, the duration of alcohol drinking is less important than the weekly or daily dose of ethanol intake.[30] In our analysis, the risk of esophageal cancer was observed not only positively related to smoking duration and weekly amount of ethanol intake but also associated with smoking intensity and drinking years. Results are similar to the findings of Castellsague et al. and Fan et al.[5,31] The association between age at starting smoking or drinking alcohol with esophageal cancer remains inconsistent,[5,6,31,32] and we observed that an earlier age at starting smoking or drinking elevated the risk when compared to those who began to smoke or drink later than 30-year old, but no trend was apparent for age and cancer risk among

early smokers or drinkers.

In our analysis, an increased risk was observed in men both for smoking among non-drinkers and for drinking alcohol among non-smokers, confirmed that smoking and alcohol alone may play independent role in the etiology of esophageal cancer. We also found that light alcohol drinking without smoking has relatively low effect on esophageal cancer risk, whereas smoking among never drinkers increased the occurrence of esophageal cancer significantly, suggesting smoking is more strongly related to esophageal cancer than alcohol in China. The synergistic interaction when alcohol and smoking coexist has been reported previously, their joint effects are approximately multiplicative and risk in the highest consumption level may increase 130-fold.[2,5,6] Although a statistical interaction between smoking and alcohol drinking was observed in the present analysis, exposure to the highest joint level caused a 7.32-fold risk compared to those who neither smoke nor drink alcohol, indicating less strong effects of these two well-known factors in the high-risk Chinese population.

A beneficial reduction in the risk of esophageal cancer after cessation of smoking and alcohol consumption was observed by some previous studies, but it may take decades to decrease the risk to the level as never exposed individuals.[33-37] Several case-control studies reported that those who had stopped smoking for less than 10 years had an OR similar to that of current smokers, while quitting for 10 years or more dropped risk to that of never smokers.[33-35] Similarly, we observed that quitting smoking for less than 5 years has a similar OR (OR=1.64) compared to current smokers (OR=1.54), while quitting more than 10 years decreased the OR to the level of never smokers (OR=0.90). Different from smoking, significantly elevated ORs were observed among former alcohol drinkers, even for quitting drinking for more than 10 years (OR=1.80). The consequences of drinking cessation have been studied less frequently than smoking cessation, results are more controversial, and beneficial effect has been found in some studies particularly 10 years after giving up drinking,[36-37] whereas other studies have shown either a non-beneficial effect or a higher risk among former drinkers.[6,32,33.38,39] It is possible that cases were more

prone to quit drinking because of digestive tract symptoms long before cancer occurrence; thus, the higher proportion of former drinkers among cases may cause an inflated OR in this group. Another reason is that some heavy drinker cases may under-report their habit, declaring no consumption when in fact they were still drinking and consequently exhibited a higher risk than never drinkers.[32] These two reasons could also explain the increased OR among former smokers.

There are several limitations to this present analysis. Firstly, although the questionnaire had been pre-tested in some previous studies and all interviewers had been trained to explain questions to participants more clearly, the exposure level of smoking and alcohol drinking was reported by study participants without accurate measurements, and thus, subjective judgement and recall bias may exist and cause non-differential misclassification of exposures. However, the strength of the associations for esophageal cancer with smoking and alcohol consumption, particularly the dose-response trends indicate good validity and sensitivity of our study. Secondly, when evaluating the risk of intensity and duration of smoking/drinking, the same cut-off point was used for both men and women. Given the relatively smaller number in some categories among women, it may cause an unstable OR. However, similar results were observed when gender specific cut-off points were used separately (data not shown). Thirdly, we were not able to determine the pathological type for all cases in this population-based study because of the low proportion of histological confirmed cases in less-developed rural areas, however, more than 95% of esophageal cancer in China are esophageal squamous cell carcinoma (ESCC) according to previous reports.[40,41]

In conclusion, our study indicates that smoking and alcohol drinking are associated with the risk of esophageal cancer among Chinese men but not among Chinese women in a high-risk population, with less strong independent and joint effects when compared with that in Western countries. Nevertheless, the elimination of these modifiable lifestyle risk factors should be part of the primary prevention strategy and control activities on esophageal cancer in China.

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Chapter 4

Green tea drinking, high tea temperature and esophageal cancer in high and low risk areas of Jiangsu Province, China: a population-based case-control study

Ming Wu, Ai-Min Liu, Ellen Kampman,
Zuo-Feng Zhang, Pieter van' t Veer,
De-Lin Wu, Pei-Hua Wang , Jie Yang,
Yu Qin, Li-Na Mu, Frans J Kok, Jin-Kou Zhao

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ABSTRACT

Epidemiological studies suggested drinking green tea is inversely associated with esophageal cancer but results remain inconclusive. Moreover, inconsistent observations found high temperature drinks are associated with esophageal cancer. A population-based case-control study was conducted in a high-risk area (Dafeng) and a low-risk area (Ganyu) of esophageal cancer in Jiangsu province China from 2003-2007. It aimed to explore green tea drinking and tea temperature with the risk of esophageal cancer, and to compare the difference between different risk regions. Using identical protocols, 1520 cases and 3879 healthy controls were recruited as study subjects in two regions. Detailed information was collected to assess green tea drinking habits. Unconditional logistic regression was used to obtain OR and 95% CI. Results showed that ever drinking green tea elevated OR in both counties (Dafeng OR=1.2, 95%CI=0.9-1.5; Ganyu: OR=1.9, 95% CI=1.4-2.4). Drinking tea at high temperature was found to increase cancer risk in both areas (Dafeng: OR=1.9, 95%CI=1.2-2.9; Ganyu OR=3.1 95%CI=2.2-4.3). However, after further adjustment for tea temperature, ever drinking tea was not related to cancer in either county (Dafeng: OR=1.0, 95% CI=0.7-1.3; Ganyu: OR=1.3, 95% CI=0.9-1.7). For dose-response relationships, we observed positive relationship with monthly consumption of tea (p for trend=0.067) and tea concentration (p for trend=0.006) after further adjustment for tea temperature. In conclusion, green tea drinking was not inversely associated with esophageal cancer in this study. However, drinking tea at high temperatures significantly increased esophageal cancer risk. There was no obvious difference of green tea drinking between low-risk and high-risk areas.

Key words: Green tea; Hot drinking; Esophageal cancer; Case-control studies; Smoking; Alcohol drinking

Introduction

Esophageal cancer is the sixth most common cause of cancer mortality worldwide. The number of new esophageal cancer cases in China accounted for 53% of all new cases in the world in 2002. The incidence and mortality rates (per 100,000) in China are 27.4 and 21.6 for men and 12.0 and 9.6 for women, respectively.[1] Jiangsu Province, located in South-Eastern China, is one of the highest incidence areas of the disease. According to the Second National Death Cause Retrospective Survey, the mortality of esophageal cancer was 30/100,000 from 1990 to 1992 in Jiangsu province, much higher than the national average of 17/100,000.[2] Although the mortality of esophageal cancer is high in most counties in Jiangsu, it differs considerably between counties, despite their similar geographic characteristics and socioeconomic status.[3]

Numerous epidemiological studies have demonstrated that environmental and lifestyle factors such as tobacco smoking, alcohol drinking, and dietary habits are associated with the development of esophageal cancer.[4,5] It is also suggested that the consumption of green tea may help prevent esophageal cancer in humans.[6,7] Tea is currently grown in at least 30 countries, and it is the most frequently consumed beverage worldwide after water, especially in Asian countries such as China, Japan, and India.[8] The per capita worldwide consumption of tea is approximately 120 ml brewed tea per day.[9] Depending on the manufacturing process, tea is classified into three major types: green tea (non-fermented), oolong tea (half-fermented) and black tea (fermented). Green tea is derived from *Camellia sinesis*, an evergreen shrub of the Theaceae family. It contains many polyphenols known as cathechins, including epigallo-cathechin-3 gallate (EGCG), epigallo-cathechin (EGC) and epicathechin-3 gallate (ECG) .[10] A number of studies have provided evidence that the polyphenolic antioxidants present in tea may be capable of affording protection against cancer.[11-13]

A few epidemiological studies have addressed the association between green tea and esophageal cancer, but results remain inconclusive.[14-17] Moreover, inconsistent observations suggest that high-temperature drinks are associated with esophageal cancer.[18] Since 2003, a population-based case-control study has been conducted in selected high- and low-risk

areas of esophageal cancer in Jiangsu, China. In this analysis, we evaluate the association of esophageal cancer with green tea drinking and tea temperature in high- and low-risk areas. The results may help us improve the current understanding of the effects of green tea drinking and high-temperature drinking on the development of esophageal cancer.

Material and methods

Study areas

A population-based case-control study has been conducted in two counties of Jiangsu province, Dafeng and Ganyu from 2003-2007. Both Dafeng and Ganyu are less developed, coastal, rural counties in northern Jiangsu province. The total population in Dafeng and Ganyu are approximately 0.7 million and 1.1 million, respectively. Dafeng has a higher mortality of esophageal cancer than Ganyu. From 1996 to 2002, the yearly average age-adjusted mortality of esophageal cancer was 36/100,000 in Dafeng, but was 24/100,000 in Ganyu during the same period.[19]

Study subjects

All subjects were restricted to local inhabitants who have lived in either area for at least 5 years. Newly diagnosed primary esophageal cancer patients from local adult residents were recruited as cases, using the data from local population based cancer registry agencies. The cancer registry agencies in both counties were established in the late 1990s and are part of the local Center for Disease Control and Prevention (CDC). All cases were identified by International Classification of Diseases, tenth revision (ICD-10, code C15). Second primary and recurrent cancers were excluded. A system of rapid case recognition was used in the study. All regional hospitals were required by the local health authorities to report new patients shortly after diagnosis. As the cancer registry agencies are attached to local CDC, investigators from local CDC could identify and interview the cases as quickly as possible. In this study, 68% and 75% of newly registered esophageal cancer cases were identified and interviewed in Dafeng and Ganyu counties, respectively. Because of the low proportion of pathological examination in less developed rural areas (Dafeng 61%, Ganyu

30%), patients who were diagnosed by other sophisticated methods such as endoscopic examination or radiology were also included.

Controls were derived from the same county as cases. Eligible controls were randomly selected from the general population, using the data of the county demographic database. Controls and cases were frequency matched by gender and age (±5 years). Individuals with history of cancer were not eligible as controls. The responding rate of control was 87% in Dafeng and 85% in Ganyu.

By study design, 600 cases and 600 controls in each county were required for the study. For Dafeng and Ganyu, recruitment of cases and controls was finished in 2006 and in 2007, respectively. As identical case-control studies on stomach, liver and lung cancer were also conducted in these two counties at the same time, controls for all cancer sites were used in this analysis. In total, 1,520 cases (637 in Dafeng County and 883 in Ganyu County) and 3,879 controls (1,938 in Dafeng County and 1,941 in Ganyu County) were recruited for this study.

Data collection

Using standard protocols and a pre-tested standardized epidemiologic questionnaire, with written informed consent, we collected epidemiological data by face-to-face interviews in both counties. Five millilitres blood samples were collected at the time of interview.

The questionnaire included detailed information on known or potential risk or protective factors for esophageal cancer, including demographic information, socio-economic status, living conditions, environmental exposure, tobacco smoking, alcohol drinking, dietary habits, disease history etc. Ever drinking green tea was defined as drinking at least one cup of green tea per week for more than 6 months. We collected lifetime general consumption of green tea drinking, and change of drinking pattern one year before diagnosis for cases or one year before interview for controls. Details of tea drinking habits included drinking status (current, former or never drinking), age when the person began to drink tea regularly, number of years drinking tea, monthly consumption of tea

(grams/month), tea concentration and temperature of the water (boiling or not boiling) used to brew tea at the time of drinking. To validate the variables above, the questionnaire also collected information about the number of new cups of tea made each day (times the person changed the leaves in the tea cup), subsequent brewing of each cup (times the person poured new water into each cup without changing leaves).

In the rural areas of Jiangsu Province, China, seldom do people drink oolong tea or coffee, therefore, we did not include information on these two beverages in the questionnaire.

Statistical analysis

Data were entered into the computer by Epidata 2.1b, cleansed and analyzed using SAS v8.2 software. In the analysis, ever drinking green tea was further categorized into former drinking and current drinking; individuals who quit drinking because of health reasons but quit in less than 1 year at the time of interview were considered current drinkers. Smoking was categorized into ever smoking and never smoking. Pack-years of smoking was also calculated. Alcohol drinking was categorized into never or seldom drinking and often drinking. For body mass index (BMI), the Chinese recommended standard was used for the definition of overweight and obese: low weight (BMI<18.5), overweight (BMI>= 24 and BMI<28), obesity (BMI>=28) .[20]

Chi-square and Student t-tests were used to compare the distribution of potential risk or protective factors among control groups between the two counties. Unconditional logistic regression with a maximum likelihood estimation of parameters was applied for both univariate and multivariate analyses. The strength of the association was quantified as odds ratios (OR), and 95% confidence intervals (CI) around the OR were used to quantify precision. Dummy variables were used in the logistic regression to estimate OR for each exposure category. The trend test of ordered variables was performed by assigning scores to different exposure levels and treated the categorical variable as a continuous variable in the logistic regression model. Effect modification was analyzed by stratification. Statistical interaction was assessed by including main effect variables and their product terms in the

logistic regression model.

On the basis of prior knowledge and confounding assessment, the effect of green tea was evaluated adjusting for age (continuous), gender (female=0, male=1), education level (ordered), income 10 years before (continuous), cancer family history of first degree relatives (No=0, yes=1), BMI (continuous), pack-years of smoking (continuous), and alcohol drinking (never or seldom=0, often=1). The effect after further adjustment for tea temperature was also presented (never drinker and normal tea temperature=0, high tea temperature=1).

Results

The demographic characteristics of cases and controls are shown by county in Table 4-1, together with socio-economic related variables, cancer family history of first degree relatives, as well as smoking and alcohol drinking status.

Comparing the two counties, Ganyu has a higher proportion of male cases than Dafeng. Ganyu also has a lower educational level and lower previous income than Dafeng, as well as a lower prevalence of cancer family history ($p<0.01$). Prevalence of smoking and green tea drinking in Dafeng is much lower than in Ganyu ($p<0.01$). Although alcohol drinking appears to be higher in Dafeng, the difference was not statistically significant ($p=0.18$). Within both counties, cases were older and more often male.

The OR and 95% CI for esophageal cancer with socio-economic status, cancer family history, smoking and alcohol drinking were also shown in Table 4-1. Cases more frequently occurred in the population with lower socio-economic statuses, i.e. lower education level, lower previous income, and lower BMI. Cancer family history in first degree relatives was found to significantly increase the risk of esophageal cancer (OR: Dafeng=1.4, Ganyu=2.1). An increased risk was observed among smokers in both counties (OR: Dafeng=1.4, Ganyu=1.5) as compared to non-smokers. An apparent dose-response relationship was also found between esophageal cancer and pack-years of smoking (p for trend<0.05). Similar to smoking, people who often drink alcohol tend to have a higher risk of esophageal cancer (OR: Dafeng=1.3, Ganyu=1.6) as compared to those who never or seldom drink alcohol.

Table 4-1 Demographic information and epidemiologic characteristics of study subjects in high and low risk areas

	Dafeng (High)			Ganyu (Low)			*p-value*[1]
	Case (%) (N=637)	Control (%) (N=1938)	OR(95% CI)	Case (%) (N=883)	Control (%) (N=1941)	OR(95% CI)	
Gender							
Males	426 (66.9)	1368 (70.6)	-	765 (86.6)	1548 (79.8)	-	*<0.01*
Females	211 (33.1)	570 (29.4)		118 (11.4)	393 (20.2)		
Age							
Mean±SD (years)	65.4 ± 9.0	63.6 ±11.0		66.0 ± 9.9	65.1± 11.3		
<50	26 (4.1)	227 (11.7)		42 (4.8)	165 (8.5)		
50~60	131 (20.6)	401 (20.7)	-	201 (22.8)	426 (22.0)	-	*<0.01*
60~70	266 (41.8)	681 (35.1)		281 (31.8)	563 (29.0)		
70~80	177 (27.8)	525 (27.1)		288 (32.6)	632 (32.6)		
≥80	37 (5.8)	104 (5.4)		71 (8.0)	155 (8.0)		
Education level							
Illiteracy	338 (53.1)	827 (42.7)	1.0 (referent)	558 (63.3)	1230 (63.4)	1.0 (referent)	
Primary school	214 (33.6)	703 (36.3)	0.8 (0.6-1.0)	230 (26.1)	467 (24.1)	1.1 (0.9 -1.3)	*<0.01*
Middle school & above	85 (13.3)	407 (21.0)	0.6 (0.4-0.8)	94 (10.7)	243 (12.5)	0.9 (0.6-1.2)	
p for trend			*<0.001*			*0.689*	
Income 10 years ago	1792 ± 2026	2293 ± 2366		1493 ± 1812	1773 ± 1953		
Mean±SD	148 (23.3)	266 (13.8)	1.0 (referent)[3]	313 (36.2)	632 (33.4)	1.0 (referent)[3]	
<1000	123 (19.3)	353 (18.3)	0.7 (0.5-1.0)	190 (22.0)	394 (20.8)	1.0 (0.8-1.3)	*<0.01*
1000~1500	194 (30.5)	589 (30.6)	0.6 (0.5-0.8)	203 (23.5)	444 (23.4)	1.0 (0.8-1.2)	
1500~2500	171 (27.0)	718 (37.3)	0.5 (0.4-0.7)	158 (18.3)	424 (22.4)	0.8 (0.6-1.0)	
>2500			*<0.001*			*0.062*	
p for trend (continuous)							

Table 4-1 Demographic information and epidemiologic characteristics of study subjects in high and low risk areas (Continued)

	Dafeng (High)			Ganyu (Low)			*p-value*[1]
	Case (%) (N=637)	Control (%) (N=1938)	OR(95% CI)	Case (%) (N=883)	Control (%) (N=1941)	OR(95% CI)	
Body Mass Index (BMI)[2]							
Mean±SD	20.8 ± 3.7	22.7 ± 6.0		22.2 ±4.0	22.9 ± 3.6		
Low (<18.5)	162(25.5)	174 (9.0)	1.0(referent)[3]	78 (8.9)	104 (5.4)	1.0 (referent)[3]	
Normal (18.5~23.9)	381 (60.0)	1175 (60.7)	2.8 (2.2-3.6)	636 (72.8)	1318 (68.2)	1.5 (1.1-2.1)	*0.27*
Overweight (24~27.9)	73 (11.5)	471 (24.3)	0.5 (0.4-0.6)	125 (14.3)	431 (22.3)	0.6 (0.5-0.8)	
Obesity (>=28)	19 (3.0)	115 (5.9)	0.5 (0.3-0.9)	35(4.0)	80 (4.1)	1.0 (0.7-1.6)	
p for trend (continuous)			*<0.001*			*<0.001*	
Cancer family history							
No	348 (54.4)	1178 (60.8)	1.0 (referent)[3]	730 (82.7)	1759 (90.6)	1.0 (referent)[3]	
Yes	289 (45.4)	760 (39.2)	1.4 (1.1-1.6)	153 (17.3)	182 (9.4)	2.1 (1.6-2.6)	*<0.01*
Smoking status							
Never smoke	200 (31.4)	817 (42.2)	1.0(referent)[4]	215 (24.4)	732 (37.7)	1.0(referent)[4]	*<0.01*
Ever smoke	437 (68.6)	1121 (57.8)	1.4 (1.1-1.8)	668 (75.6)	1209 (62.3)	1.5 (1.2-1.9)	
Pack-years of smoking							
Never smoker	200 (31.4)	817 (42.2)	1.0(referent)[4]	215 (24.4)	732 (37.7)	1.0(referent)[4]	
<30 yrs	187 (29.6)	554 (28.6)	1.2 (1.0-1.6)	427 (48.4)	852 (43.9)	1.4 (1.1-1.8)	*<0.01*
>=30 yrs	250 (39.2)	567 (29.3)	1.6 (1.3-2.1)	241 (33.4)	357 (18.4)	1.8 (1.4-2.4)	
p for trend (continuous)			*0.029*			*0.011*	
Alcohol drinking status							
Never or seldom	354 (55.6)	1158(59.8)	1.0(referent)[4]	417 (47.2)	1200 (61.8)	1.0 (referent)[4]	*0.18*
Often drinking	283 (44.4)	780 (40.2)	1.3 (1.0-1.6)	466 (52.8)	741 (38.2)	1.6 (1.3-1.9)	

[1] P-value for comparing control groups between two counties.[2] Chinese recommend standard was used for the definition of overweight and obesity:low weight (BMI<18.5),overweight (BMI>= 24 and BMI<28), obesity (BMI>=28).[3] Adjusted for age (continuous) and gender. [4] Adjusted for age (continuous), gender education level, income 10 years before (continuous), cancer family history and BMI (continuous).

Table 4-2 The OR and 95% CI of esophageal cancer risk with green tea drinking in high- and low- risk areas

Variables	Dafeng (High)			Ganyu (Low)		
	Case/ Control	OR[1] (95% CI)	OR[2] (95% CI)	Case/ Control	OR[1] (95% CI)	OR[2] (95% CI)
Green tea drinking						
Never drinking	467 / 1401	1.0 (referent)	1.0 (referent)	384 / 1132	1.0 (referent)	1.0 (referent)
Ever drinking	170 / 537	1.2 (0.9-1.5)	1.0 (0.7-1.3)	499 / 809	1.9 (1.4-2.4)	1.3 (0.9-1.7)
Former drinking	33 / 42	3.4 (1.9-6.1)	2.2 (1.6-5.3)	95 / 44	6.4 (3.6-11.5)	4.2 (2.3-7.6)
Current drinking	137 / 495	1.0 (0.8-1.3)	0.8 (0.6-1.1)	404 / 765	1.6 (1.2-2.1)	1.1 (0.8-1.5)
Tea temperature						
Never drinking	467 / 1401	1.0 (referent)	-	384 / 1132	1.0 (referent)	-
Normal temperature	118 / 431	1.0 (0.7-1.3)		244 / 553	1.3 (0.9-1.7)	
High temperature	51 / 103	1.9 (1.2-2.9)		252 / 248	3.1 (2.2-4.3)	
Age at start drinking						
Never drinking	467 / 1401	1.0 (referent)	1.0 (referent)	384 / 1132	1.0 (referent)	1.0 (referent)
<25	20 / 79	1.0 (0.6-1.8)	0.8 (0.4-1.5)	117 / 177	1.8 (1.2-2.6)	1.2 (0.8-1.9)
25~34	53 / 151	1.4 (1.0-2.2)	1.2 (0.8-1.8)	255 / 374	1.9 (1.4-2.6)	1.3 (1.0-1.9)
35~44	37 / 105	1.4 (0.9-2.2)	1.2 (0.7-1.9)	77 / 164	1.6 (1.0-2.6)	1.1 (0.7-1.8)
45~	58 / 202	0.9 (0.6-1.4)	0.8 (0.6-1.2)	44 / 90	1.3 (0.6-2.6)	0.9 (0.4-1.9)
p value for trend		0.543	0.701		<0.001	0.469

Table 4-2 The OR and 95% CI of esophageal cancer risk with green tea drinking in high- and low- risk areas (Continued)

Variables	Dafeng (High)			Ganyu (Low)		
	Case/ Control	OR[1] (95% CI)	OR[2] (95% CI)	Case/ Control	OR[1] (95% CI)	OR[2] (95% CI)
Years of tea drinking						
Never drinking	467 / 1401	1.0 (referent)	1.0 (referent)	384 / 1132	1.0 (referent)	1.0 (referent)
<20 yrs	65 / 238	1.1 (0.8-1.5)	1.0 (0.6-1.4)	51 / 146	1.2 (0.7-2.0)	0.8 (0.5-1.5)
20yrs~34yrs	57 / 176	1.1 (0.7-1.6)	0.9 (0.6-1.4)	227 / 330	2.0 (1.5-2.8)	1.4 (1.0-2.0)
35yrs~	45 / 123	1.4 (0.9-2.1)	1.1 (0.7-1.8)	209 / 325	1.6 (1.2-2.3)	1.1 (0.8-1.6)
p value for trend		0.161	0.817		<0.001	0.189
Monthly consumption of tea (g/month)						
Never drinking	467 / 1401	1.0 (referent)	1.0 (referent)	384 / 1132	1.0 (referent)	1.0 (referent)
1g~149g	109 / 382	1.1(0.8-1.4)	1.0(0.7-1.3)	117 / 244	1.5(1.0-2.3)	1.1(0.7-1.7)
150g~249g	29 / 88	1.3 (0.8-2.1)	1.0 (0.6-1.8)	111 / 185	1.4 (1.0-2.1)	1.0 (0.7-1.6)
250g~	27 /62	1.4 (0.8-2.6)	1.0 (0.6-2.0)	264 / 361	2.2 (1.7-3.0)	1.6 (1.1-2.2)
p value for trend		*0.140*	*0.926*		*<0.001*	*0.014*
Tea concentration						
Never drinking	467 / 1401	1.0 (referent)	1.0 (referent)	384 / 1132	1.0 (referent)	1.0 (referent)
Low	29 / 152	0.7 (0.4-1.1)	0.6 (0.4-1.0)	53 / 137	1.3 (0.8-2.3)	1.0 (0.6-1.7)
Moderate	75 / 243	1.1 (0.8-1.6)	1.0 (0.7-1.4)	273 / 452	1.7 (1.2-2.3)	1.3 (0.9-1.8)
High	66 / 142	1.7 (1.2-2.5)	1.4(0.9-2.1)	171 / 213	2.4 (1.7-3.4)	1.4(0.9-2.1)
p value for trend		*0.016*	*0.235*		*<0.001*	*0.059*

[1]Adjusted for age (continuous), gender, education level, income 10 years before (continuous), cancer family history, BMI (continuous), pack-year of smoking (continuous), alcohol drinking .[2] Adjusted for above mentioned variables, further adjusted for tea temperature (never drinker & normal temperature=0, high temperature=1).

Table 4-3 The OR and 95% CI of esophageal cancer risk with green tea drinking in different groups in high- and low risk areas

Variables	Former drinking		Current drinking		Ever drinking [3]	
	OR[1](95% CI) [1]	OR[2](95%CI)	OR[1](95% CI)[1]	OR[2](95% CI)[2]	OR[1](95%CI)[1]	OR[2] (95% CI)
Tea temperature						
Never drinking	1.0 (referent)	-	1.0 (referent)	-	1.0 (referent)	-
Normal temperature	3.2 (2.0-5.1)		0.9 (0.8-1.2)		1.1 (0.9-1.3)	
High temperature	10.2 (4.8-21.6)		2.1 (1.6-2.8)		2.5 (1.9-3.2)	
Age at start drinking						
Never drinking	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<25	5.4 (2.4-12.4)	3.1 (1.2-7.9)	1.2 (0.9-1.7)	0.9 (0.6-1.3)	1.4 (1.0-1.9)	1.0 (0.8-1.5)
25~34	6.4 (3.4-12.3)	4.6 (2.3-9.3)	1.5 (1.1-1.9)	1.1 (0.8-1.4)	1.7 (1.3-2.1)	1.2 (1.0-1.6)
35~44	4.4 (1.7-11.1)	3.1 (1.2-8.2)	1.3 (0.9-1.8)	1.0 (0.7-1.4)	1.4 (1.0-2.0)	1.1 (0.8-1.5)
45~	2.1 (0.9-5.0)	1.6 (0.7-4.1)	0.9 (0.6-1.2)	0.7 (0.5-1.0)	1.0 (0.7-1.3)	0.8 (0.6-1.1)
p value for trend	*<0.001*	*0.003*	*0.276*	0.241	*0.100*	*0.172*
Years of drinking						
Never drinking	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
<20 yrs	3.0 (1.5-5.8)	2.3 (1.2-4.7)	0.9 (0.7-1.2)	0.7 (0.52-1.0)	1.1 (0.8-1.4)	0.9 (0.6-1.2)
20yrs~34yrs	5.0 (2.8-9.1)	3.6 (1.9-6.5)	1.3 (1.01-1.7)	1.0 (0.74-1.3)	1.5 (1.2-1.9)	1.1 (0.9-1.4)
35yrs~	6.8 (3.0-15.7)	3.9 (1.5-10.0)	1.3 (1.01-1.7)	1.0 (0.73-1.3)	1.5 (1.1-1.9)	1.1 (0.8-1.4)
p value for trend	*<0.001*	*<0.001*	*0.009*	0.965	*0.404*	*0.666*
Monthly consumption of tea leaves (g/month)						
Never drinking	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
1g~149g	3.9(2.3-6.8)	3.0(1.6-5.4)	1.0(0.8-1.2)	0.8(0.6-1.0)	1.2 (0.9-1.5)	1.0 (0.7-1.2)
150g~249g	3.4 (1.3-8.4)	2.6 (1.0-6.9)	1.2 (0.9-1.7)	0.9 (0.6-1.3)	1.3 (1.0-1.8)	1.0 (0.7-1.4)
250g~	7.4 (3.5-15.5)	4.4 1.9-10.3)	1.8 (1.4-2.3)	1.3 (1.0-1.8)	2.0 (1.5-2.5)	1.4 (1.1-1.9)
p value for trend	*<0.001*	*0.043*	*<0.001*	*0.206*	*0.023*	*0.067*
Tea concentration						
Never drinking	1.0 (referent)	1.0(referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)	1.0 (referent)
Low	2.1 (1.0-4.8)	1.8 (0.8-4.2)	0.8 (0.5-1.1)	0.6 (0.4-0.9)	0.9 (0.6-1.2)	0.7 (0.5-1.0)
Moderate	3.9 (2.3-6.7)	2.9 (1.6-5.4)	1.2 (0.9-1.5)	1.0 (0.8-1.2)	1.3 (1.1-1.7)	1.1 (0.9-1.4)
High	14.9 (5.8-38.1)	9.7(3.5-26.7)	1.6 (1.3-2.2)	1.1(0.8-1.5)	1.9 (1.5-2.4)	1.3(1.0-1.8)
p value for trend	*<0.001*	*<0.001*	*<0.001*	*0.212*	*<0.001*	*0.006*

[1] Adjusted for age (continuous variable), gender, education level, income 10 years before (continuous variable), cancer family history, BMI (continuous variable), pack-year of smoking (continuous variable), alcohol drinking and counties.[2] Adjusted for above mentioned variables, further adjusted for tea temperature (never drinker & normal temperature=0, high temperature=1).[3] Ever drinking is the combination of former drinking and current drinking.

Table 4-2 shows the association between esophageal cancer and green tea drinking in each county. After adjusting for potential confounders including age, gender, education level, previous income, cancer family history, BMI, pack-years of smoking and alcohol drinking, we found that ever drinking green tea significantly increased esophageal cancer risk in Ganyu (OR=1.9, 95%CI=1.4-2.4), but it was not significant in Dafeng (OR=1.2, 95%CI=0.9-1.5). Former drinking was observed to be strongly associated with increasing OR in both counties (Dafeng: OR=3.4, 95% CI=1.9-6.1; Ganyu: OR=6.4, 95%CI=3.6-11.5), whereas for current drinking, increased risk was found in Ganyu (OR=1.6, 95% CI=1.2-2.1) but not in Dafeng (OR=1.0, 95% CI=0.8-1.3). Tea temperature was found to be positively related to esophageal cancer risk in both counties, OR of drinking tea at high temperature was 1.9 in Dafeng (95%CI=1.2-2.9) and 3.1 in Ganyu (95%CI=2.2-4.3), as compared to never drinkers. When further adjusted tea temperature in the logistic regression model, we found ever drinking tea was not significantly related to esophageal cancer in either county (Dafeng: OR=1.0, 95% CI=0.7-1.3; Ganyu: OR=1.3, 95% CI=0.9-1.7). A positive association was still found in the former drinking group after adjusting tea temperature (Dafeng: OR=2.2, 95% CI=1.6-5.3; Ganyu: OR=4.2, 95% CI=2.3-7.6), but no significant association was observed among current green tea drinkers in either Dafeng or Ganyu.

Dose-response relationships for esophageal cancer risk with green tea-drinking related variables such as age at starting drinking, years of drinking, monthly consumption of tea (grams/month) and tea concentration were explored by county (Table 4-2). We found earlier age at starting drinking, long years of drinking, higher grams of monthly tea consumption, and high tea concentration were positively associated with cancer risk in both counties, though the trends were more apparent in Ganyu. However, after further adjustment for tea temperature, we only observed a positive dose-response relationship with monthly consumption of tea (p for trend=0.014), and a borderline positive relationship with tea concentration (p for trend=0.059) in Ganyu. No other apparent dose-response relationships were found.

Table 4-3 shows the effects of green tea drinking and dose-response relationships

among the former drinking, current drinking group and their combination (ever drinking). As we did not find big difference between two counties (data not shown) and limited by the page width, pooled results of two counties are presented. In the former drinking group, green tea drinking significantly increased ORs despite the tea temperature. In the current drinking group, drinking in normal temperature did not increase the cancer risk (OR=0.9, 95% CI=0.8-1.2), but hot drinking elevated OR significantly (OR=2.1, 95% CI=1.6-2.8). Similar results were found in the ever drinking group, high tea temperature was found significantly increased OR (OR=2.5, 95% CI=1.9-3.2) but normal temperature did not (OR=1.1, 95% CI=0.9-1.3).

Positive dose-response relationships were observed among former drinkers. Earlier age at starting drinking, long years of drinking, higher monthly consumption of tea and high tea concentration were found increasing ORs. Similar positive relations among the current drinking group were observed only before adjusting for tea temperature. For ever drinking, we found higher monthly consumption of tea (p for trend=0.067) and usually drinking tea in high concentration (p for trend=0.006) showed a positive tendency with cancer risk after adjusting for tea temperature.

Effect modification between green tea drinking and smoking status, pack-years of smoking, and alcohol drinking were evaluated by stratified analysis, pooled results of two counties are shown in Table 4-4. Former drinking individuals who had smoked, or had pack-years of smoking larger than 30, or had often drunk alcohol have the highest risk of esophageal cancer, but the interactions were not statistically significant.

Table 4-5 shows the effect modification of hot drinking by smoking status, pack-years of smoking, and alcohol drinking. Additive effects were found between drinking tea at high temperature and ever smoking, pack-years of smoking, and ever drinking alcohol. The highest ORs were among hot drinking individuals who had smoked, or had pack-years of smoking larger than 30, or had often drunk alcohol, but these effect modifications were not statistically significant again.

Table 4-4 The effect modification of esophageal cancer risk between green tea drinking and smoking, pack-years of smoking,alcohol drinking

	Green tea drinking		
	Never	**Former**	**Current**
Ever-smoking [1]			
No	1.0 (referent)	1.6 (0.8-3.2)	0.7 (0.5-0.9)
Yes	1.3 (1.1-1.6)	5.2 (3.6-7.5)	1.3 (1.0-1.6)
p-value for Interaction	*0.058*		
Pack Years of smoking [1]			
<30	1.0 (referent)	3.1 (2.1-4.6)	0.9 (0.7-1.0)
>=30	1.4 (1.1-1.6)	5.2 (3.1-8.7)	1.4 (1.1-1.8)
p-value for Interaction	*0.224*		
Alcohol drinking [2]			
No	1.0 (referent)	1.9 (0.9-3.9)	0.8 (0.6-1.1)
Yes	1.2 (0.9-1.5)	5.0 (3.0-8.4)	1.1 (0.9-1.5)
p-value for Interaction	*0.450*		

[1] Adjusted for age (continuous), gender, education level, income 10 years before (continuous), cancer family history, BMI (continuous), alcohol drinking, tea temperature (never drinker & normal temperature=0, high temperature=1) and counties.[2] Adjusted for age (continuous), gender, education level, income 10 years before (continuous), cancer family history, BMI (continuous), pack-year of smoking (continuous), tea temperature (never drinker & normal temperature=0, high temperature=1) and counties.

Table 4-5 The effect modification of esophageal cancer risk between high tea temperature and smoking, pack-years of smoking, alcohol drinking

	High tea temperature [1]	
	NO	**YES**
Ever-smoking [2]		
No	1.0 (referent)	1.9 (1.2-2.9)
Yes	1.4 (1.2-1.7)	3.2 (2.5-4.0)
p-value for Interaction	*0.459*	
Pack Years of smoking [2]		
<30	1.0 (referent)	2.1 (1.7-2.6)
>=30	1.4 (1.2-1.7)	3.6 (2.6-4.8)
p-value for Interaction	*0.253*	
Alcohol drinking [3]		
No	1.0 (referent)	2.2 (1.5-3.3)
Yes	1.2 (1.0-1.5)	3.2 (2.3-4.2)
p-value for Interaction	*0.616*	

[1] No=Never drinker and normal tea temperature; Yes=drinking tea in high tea temperature. [2] Adjusted for age (continuous variable), gender, education level, income 10 years before (continuous variable), cancer family history, BMI (continuous variable), alcohol drinking and counties.[3] Adjusted for age (continuous variable), gender, education level, income 10 years before (continuous variable), cancer family history, BMI (continuous variable), pack-year of smoking (continuous variable) and counties.

Chapter 4

Discussion

This population-based case-control study, conducted in high- and low-risk areas of Jiangsu Province, China explored the association between green tea, hot tea drinking and esophageal cancer. Compared to previous studies, this study has the largest sample size and has addressed the association in different risk areas simultaneously. In the presented analysis, however, no obvious association between green tea drinking and esophageal cancer was observed in either high- or low- risk areas. On the contrary, drinking tea at high temperature was significantly related to the occurrence of esophageal cancer consistent in both high and low risk counties.

Green tea has been considered an herbal medicine and a healthy beverage since ancient times. It is considered as a potential cancer preventive agent on the basis of numerous *in vitro, in vivo* and epidemiological studies.[11-15] It has been suggested that the anti-oxidative and anti-inflammatory properties of green tea make it a promising agent for human cancer prevention.[6] Tea polyphenols are known to be strong antioxidants. Cao et al. reported that green tea even has a much higher antioxidant activity against peroxyl radicals than some vegetables.[21]

Only a few studies have reported the relationship between green tea drinking and esophageal cancer with conflicting results. Some case-control studies carried out in Jiangsu and Shanghai, China, reported inverse association.[14,15,22,23] Gao et al. found that green tea drinking reduced the risk of esophageal cancer among women (OR= 0.50, 95% CI = 0.30-0.83) in Shanghai, and this risk decreased as tea consumption increased (p for trend < 0.01); the OR were also below 1.00 among men but were not statistically significant.[14] Wang et al. reported that green tea drinking showed a protective effect in women (OR=0.26; 95% CI=0.07-0.94) in Jiangsu, but no dose-response relationship was found for tea-drinking duration.[15] Another case-control study was also conducted in Jiangsu did not find an obvious protective effect.[16] An intervention trial was conducted in He'nan, another high risk province of China, where subjects with esophageal precancerous lesions were supplemented with decaffeinated green tea (DGT) 5 mg/day for 12 months. The results did

not show an apparent difference between treatment and placebo groups.[24] In consistence with our study, some studies conducted in western country also reported no association between tea drinking and esophageal cancer. Tavani reported OR=0.9 (95%CI=0.7-1.2) in a hospital-based case control study.[25] La Vecchia found no association (RR=1.0, 95%CI=0.7-1.4) in another study in Italy.[26]

In this study, ever drinking green tea was positively associated with esophageal cancer risk in both low- and high- risk areas (Dafeng: OR=1.2, 95%CI=0.9-1.5; Ganyu OR=1.9, 95%CI=1.4-2.4). But after further adjusting for tea temperature, no significant association was observed either in Dafeng (OR=1.0, 95%CI=0.7-1.3) or in Ganyu (OR=1.3, 95%CI=0.9-1.7). Drinking beverages at high temperatures has been suggested as a cause of esophageal cancer by a number of studies.[18] Hot drinking can cause thermal injury of esophageal mucosa and make it more susceptible to carcinogenesis. Our findings show that drinking tea at high temperatures had a 1.9- and 3.1- fold elevated risk in Dafeng and Ganyu. Additive effects between hot tea drinking and smoking, pack-years of smoking, as well as alcohol drinking were also observed in our study. Individuals who drank green tea at a high temperature, but who also smoked cigarettes or drank alcohol had the highest odds ratio for esophageal cancer, although the effect modifications were not statistically significant.

Kinjo et al. reported similar associations in a cohort study, the rate ratio was 1.6 (95%CI=1.2-2.0) for hot tea (drinking green tea at high temperature) in comparison with non-hot tea (drinking green tea at moderate temperature) .[27] Another pooled analysis of two prospective cohorts in Japan found that as compared to never drinkers, drinking> or =5 cups of green tea/day significantly increased the risk of esophageal cancer (HR=1.67, p for trend=0.04). The population attributable fraction of esophageal cancer incidence attributed to green tea consumption was 22.1%, but as mentioned by the author, tea temperature could be a plausible explanation for the increased OR.[17] Some cohort studies and case-control studies reported no association for hot drinks.[28-30] In this population-based case-control study with relatively large sample size, we observed a strong association between drinking tea at a high-temperature and esophageal cancer.

We observed substantially elevated ORs in the former drinking group in two counties (Dafeng: OR=2.2, Ganyu: OR=4.2), even after adjusting for tea temperature. However, no significant association was found for current drinkers in either Dafeng (OR=0.8) or Ganyu (OR=1.1). This could be explained by cases who may be more likely to quit drinking tea because of early digestive tract symptoms. This higher proportion of former tea drinkers among cases may have caused an inflated OR in the former drinking group. However, in the current drinking group, the proportion of tea drinkers was lower than the fact because some cases quit drinking at an early time before disease onset, therefore the association might have been underestimated in this group. We even found that ORs were significantly changed if we used the combination of never drinkers and former drinkers as a reference group, then compared them with current drinkers (Dafeng: OR=0.74, 95% CI=0.54-0.99 ; Ganyu OR=0.84, 95% CI= 0.63-1.22), but this change was attributed to potential bias. Therefore, how to avoid this kind of information bias should be carefully considered in future studies. In the presented analysis, as few numbers of former drinkers, the results of current drinking are more close to the real associations. The results of ever drinking (combination of former and current drinking) are similar to those in the current drinking group, and could be a better way to estimate the real associations.

After adjusting for potential confounders and tea temperature, earlier age at starting drinking, long drinking years, higher amount and higher concentration of tea drinking increased ORs apparently in the former drinking group; no clear tendency was observed in current drinkers, but again, there was a possibility of over- or under-estimation of ORs in the former drinking and current drinking groups, respectively. When former and current drinking are combined together, positive dose-response trends were found with higher monthly consumption of tea (p for trend=0.067) and high tea concentration (p for trend=0.006) even after adjustment for tea temperature. A plausible explanation is drinking green tea is often accompanied by tobacco smoking and alcohol drinking among the Chinese population, people who frequently drink a high concentration of green tea are often heavy smokers or alcohol drinkers.[31] Mu et al. reported a more than multiplicative interaction between green tea drinking and alcohol drinking (OR=4.57; 95% CI=1.62-12.89)

in a study on stomach cancer.[32] However, smoking and alcohol drinking were not observed as significant effect modifiers to tea drinking in the present study.

Several methodological issues need to be discussed. Potential selection bias and information bias may exist in any case-control study. A population-based study design and a random control selection method were used to minimize selection bias in our study. Cases were identified from the cancer registry data rather than from certain hospitals, controls were randomly selected from the county population demographic database, the response rate of cases and controls were 68 and 87% in Dafeng, 75 and 85% in Ganyu respectively. To reduce information bias, investigators were well trained to collect epidemiologic data in detail. Moreover, green tea related variables such as tea temperature, drinking years, monthly consumption of tea and tea concentration were also investigated and analyzed to avoid misclassification of exposure.

Confounding also has been considered in our analysis. Although the frequency matching method was applied in the study, controls for stomach, liver and lung cancers were also used in the present analysis. Therefore, differences of age and gender between case and control group were enlarged and might cause residual confounding, even after adjusting them in the logistic regression model. When sensitivity analysis was carried out with only esophageal cancer cases and their matched controls, the results were similar as for the overall analysis, the OR and 95% CI of former drinking and current drinking green tea was 2.2 (1.0-4.6) and 0.7 (0.5-1.0) in Dafeng, 3.6 (1.7-7.7) and 1.3 (0.9-1.8) in Ganyu, after adjusting for confounders and tea temperature.

There might be differences in the etiological factors between esophageal adenocarcinoma and squamous cell carcinoma. Because of the low pathological examination rate in less developed rural counties, it is difficult to differentiate between the subtypes of esophageal cancer in this population-based study. However, it has been reported that esophageal squamous cell carcinoma represents more than 95% of esophageal cancer cases in China.[33]

In conclusion, green tea drinking was not inversely associated with esophageal cancer in this study in Jiangsu province, China. However, drinking tea at high temperatures is strongly associated with esophageal cancer. There was no obvious difference for the effects of green tea drinking between low- and high-risk areas.

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Chapter 5

Does family history of cancer modify the effects of lifestyle risk factors on esophageal cancer? A population-based case-control study in China

Ming Wu, Zuo-Feng Zhang, Ellen Kampman, Jin-Yi Zhou, Ren-Qiang Han, Jie Yang, Xiao-Feng Zhang, Xiao-Ping Gu , Ai-Min Liu, Pieter van' t Veer, Frans J Kok, Jin-Kou Zhao

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ABSTRACT

A population-based case-control study on esophageal cancer has been conducted since 2003 in Jiangsu Province, China. The aim of this analysis is to provide further evidence on the relationship between family history of cancer in first-degree relatives (FH-FDR) and the risk of esophageal cancer, and to explore the joint effects for FH-FDR with major lifestyle risk factors. A total of 1,520 cases and 3,879 controls were recruited. Unconditional logistic regression was applied for evaluating independent association, as well as potential interactions between FH-FDR and lifestyle risk factors on the risk of esophageal cancer. Population attributable fraction (PAF) was calculated to quantify the proportion of cases attributable to risk factors. Results showed that with a FH-FDR of any malignant tumor or esophageal cancer, there is a 1.64- and 2.22-fold risk of esophageal cancer, respectively. Association was increased when there was more than one affected first-degree relative (OR = 3.14) and younger age at diagnosis of relatives. Exposure of both FH-FDR and lifestyle risk factors strongly associated with esophageal cancer. Significant super-additivity interaction was found for FH-FDR with fast eating speed and diets low in fruits and vegetables. The estimation of PAF indicated that the majority of cases were attributed to lifestyle risk factors. In conclusion, it was found that FH-FDR significantly increases the risk of esophageal cancer and could modify the effect of certain lifestyle risk factors. If comprehensive lifestyle interventions are carried out within high-risk populations, there is a high probability of curbing occurrences of esophageal cancer.

Key words: Esophageal cancer; Family history of cancer; Lifestyle; Interaction; China

Introduction

Esophageal cancer is one of the most common cancers worldwide, with approximately 462,000 new cases and 386,000 deaths each year. China is an area with one of the highest incidences of esophageal cancer worldwide. Each year, about half of the cases of esophageal cancer that occur in the world are estimated to be in China.[1] According to the results of a national mortality retrospective survey conducted in 2006, esophageal cancer was the fourth leading cause of cancer death in China, with a national average age-standardized mortality of 15.2/100,000.[2] Squamous cell carcinoma of the esophagus remains the predominant histological subtype, representing more than 95% of total cases in the Chinese population.[3]

The etiology of esophageal cancer shows that it is multifactorial. A number of studies have suggested that lifestyle factors are significant to the development of this disease. Tobacco smoking and alcohol consumption are responsible for a high fraction of esophageal cancer occurrence; more than 90% of cases could be attributed to these two factors in Western countries.[4] Dietary factors such as ingestion of hot foods and drinks, fast eating speed, nutrition deficiency, and high intake of carcinogens from pickled vegetables have been suggested to contribute to most cases of esophageal cancer in high-risk areas such as China and Iran.[5,6] Moreover, genetics and other endogenous factors may also influence the inherited susceptibility towards cancer. Studies of gene-environmental interactions suggest that the risk of environmental and lifestyle factors could be modified by genetic predispositions.[7]

Several epidemiological studies have pointed to the familial aggregation of esophageal cancer,[8-12] and reported that having a positive family history of esophageal cancer could increase the risk of the disease, with a two- to three-fold risk among those with affected first-degree relatives (FDR) observed in most studies, especially for cancer in the same histological type. Family members normally share a common genetic background, and thus, a family history of cancer (FH), especially FH among first-degree relatives (FH-FDR), may be considered as a marker for genetic predisposition. However, sharing environmental and lifestyle risk factors similar with other family members may also play a partial role in the

familial propensity of the disease.[13]

Although positive FH-FDR has been suggested to play a role in the etiology of esophageal cancer, only a few studies have investigated the risk and FH-FDR in detail with a large sample size. Furthermore, the joint effect of FH-FDR and lifestyle risk factors has been studied for certain cancers such as colon cancer and breast cancer,[14-18] but to date has been rarely reported for esophageal cancer. In this study, we took advantage of a large population-based case-control study in Jiangsu Province, one of the high-risk areas for esophageal cancer in China,[2] to investigate in depth the relationship between family history of cancer and the risk of esophageal cancer, and to explore the effect modification between FH-FDR and major lifestyle risk factors.

Materials and methods

Subject recruitment and data collection

The study design has previously been described in detail.[19] In brief, a population-based case-control study was conducted in two counties of Jiangsu Province, Dafeng and Ganyu, from 2003 to 2007. Both of these counties are less developed rural areas in northern Jiangsu; however, Dafeng shows a 50% higher incidence of esophageal cancer than Ganyu.

All newly diagnosed esophageal cancer patients in local inhabitants were eligible cases, using the information from local population-based cancer registries. Because of the low proportion of pathological examination in rural areas (39% in average), patients who were diagnosed by endoscopic examination (40%) or radiology (11%) were also included. Eligible controls were randomly selected from the general population in the same county, frequency matched with cases by gender and age (±5 years). The participation rate of cases and controls was 68 and 87% in Dafeng, 75 and 85% in Ganyu, respectively.

With written informed consent, epidemiological data were obtained by face-to-face interviews using a standardized questionnaire. The questionnaire elicited information on known or potentially associated factors of esophageal cancer in detail, including demographic information, number of family members, socio-economic status, living

conditions, duration and amount of smoking, alcohol consumption, habitual dietary habits, and frequency and portion of major foods (via a pre-tested Food Frequency Questionnaire). Detailed information on family history of cancer was collected for each relative that was affected by any malignant tumor (both blood and non-blood relatives i.e. spouse). Information that was collected included the type of relationship, site of cancer, and age at diagnosis of affected relatives.

Statistical analysis

Data were entered into the computer by Epidata 3.0, cleaned and analyzed using SAS v9.1 software (SAS Institute, Inc., Cary, NC). Unconditional logistic regression with a maximum likelihood estimation of parameters was applied for multivariate analyses. Confounders were selected based on the previous knowledge on esophageal cancer and our results of preliminary analysis,[20] including age, gender, area of study, education level, previous income, body mass index (BMI), pack-years of smoking, weekly consumption of ethanol and family size (represented by number of siblings). After adjusting for confounders, the strength of the association was quantified as odds ratios (OR), and 95% confidence intervals (CI) around the OR were used to quantify precision.

Family history of cancer in first-degree relatives (FH-FDR) was regarded as positive when at least one of first-degree relatives (FDR) e.g. parent, sibling or child had been diagnosed with *any type of cancer*. A positive FH-FDR of esophageal cancer was restricted to having at least one FDR affected with *esophageal cancer*. Pack-years of smoking and weekly consumption of ethanol in average were both calculated and categorized into three categories. Intake of red meat, fruits and vegetables were divided into quartiles based on the distribution among controls. Missing values for some variables such as educational level, BMI, alcohol consumption and food intake were excluded for relevant analyses.

Interactions between FH-FDR and lifestyle risk factors were tested on an additive scale. Rothman noted that when biological interaction or public health relevance is examined in epidemiological studies, interaction as departure from additivity should be the focus, rather than departure from multiplicativity.[21] He had shown how interaction as

departure from additivity of two dichotomous variables can be quantified in a logistic regression model, and recently this method has been extended to estimate interactions for multi-level or continuous determinants.[22] In the present analysis, the three measures of biological interaction – relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP), and synergy index (SI) – were calculated as follows:

$$RERI = e^{(\beta_A + \beta_B + \beta_{A*B})} - e^{\beta_A} - e^{\beta_B} + 1$$

$$AP = \frac{RERI}{e^{(\beta_A + \beta_B + \beta_{A*B})}} \qquad SI = \frac{e^{(\beta_A + \beta_B + \beta_{A*B})} - 1}{[e^{\beta_A} - 1] + [e^{\beta_B} - 1]}$$

β_A, β_B and β_{A*B} represent the regression coefficients derived from the logistic regression model with determinants A, B and the product of A and B, after adjusting for potential confounders. RERI can be interpreted as the risk that is excess to the expected risk on the basis of the ORs under exposure; AP is the proportion of cases due to interaction among persons with both exposures; SI is the excess risk of exposure to both risk factors with interaction, relative to the excess risk from both exposures without interaction. The 95% CI of RERI, SI, and AP were estimated by delta method.[23,24] In the absence of an interaction, RERI and AP amount to 0 and SI amounts to 1.

The population attributable fraction (PAF) was calculated using the method suggested by Bruzzi, et al.[25] It provides adjusted PAF estimates by combining adjusted OR derived from logistic regression models and the observed prevalence of risk factors among case patients. To estimate the joint PAF due to FH-FDR and lifestyle factor [26], subjects were included in a new dichotomous variable: 1) exposed to neither FH-FDR nor lifestyle risk factor, 2) exposed to both FH-FDR and lifestyle risk, or at least one of them. Then the joint PAF was calculated using this newly defined dichotomous variable by combining the adjusted OR and the prevalence among cases in the way according to Bruzzi et al. The 95% CI of PAF was calculated by the method based on the Bonferonni inequality.[27] In order to avoid a negative PAF for known protective factors such as eating raw garlic and intake of fruits and vegetables, we defined the highest exposure category as a reference.

Although Dafeng has a 50% higher incidence of esophageal cancer than Ganyu, we did not find a large difference in the effect of FH-FDR between the two counties. Therefore, data from subjects of the two regions were pooled to improve the statistical power in our analysis.

Results

Table 5-1 The demographic information and socio-economic status of study subjects[1]

	Cases (%) (N=1,520)	**Controls (%) (N=3,879)**	***p*-Value[3]**
Study area			
Dafeng	637 (41.9)	1,938 (50%)	-
Ganyu	883 (58.1)	1,941 (50%)	
Gender			
Male	1,191 (78.4)	2,916 (75.2)	*0.014*
Female	329 (21.6)	963 (24.8)	
Age			
Mean±SD (years)	65.7±9.6	64.4±11.2	
<50	68 (4.5)	392 (10.1)	
50~	332 (21.8)	827 (21.3)	*<0.001*
60~	547 (36.0)	1,244 (32.1)	
70~	465 (30.6)	1,157 (29.8)	
≥80	108 (7.1)	59 (6.7)	
Education level			
Illiteracy	896 (59.0)	2,057 (53.1)	*<0.001*
Primary school	444 (29.2)	1,170 (30.2)	
Middle school & above	179 (11.8)	650 (16.8)	
Previous income (RMB)	461 (30.7)	898 (23.5)	
<1000	313 (20.9)	747 (19.6)	*<0.001*
1000~1500~	397 (26.5)	1,033 (27.0)	
2500~	329 (21.9)	1,142 (29.9)	
Body Mass Index (BMI)[2]			
Mean±SD	21.6±3.9	22.8±5.0	
Low (<18.5)	240 (15.9)	278 (7.2)	*<0.001*
Normal (18.5~23.9)	1,017 (67.4)	2,493 (64.5)	
Overweight (24~27.9)	198 (13.1)	902 (23.3)	
Obesity (≥28)	54 (3.6)	195 (5.0)	

[1] Missing data were excluded from analysis for those variables that the total numbers of cases or controls were less than the total number. [2]Chinese recommend standard was used for the cut-off points for overweight and obesity. [3] p-value comparing cases and controls.

Table 5-2 The distribution of lifestyle risk factors and their associations with esophageal cancer[1]

	Case (%) (N=1,520)	Control (%) (N=3,879)	OR (95% CI) [2]
Smoking			
Never	415 (27.3)	1,549 (39.9)	1.00 (referent)
<30 pack-years	614 (40.4)	1,406 (36.2)	1.38 (1.17-1.63)
≥30 pack-years	491 (32.3)	924 (23.8)	1.84 (1.53-2.20)
p for trend			*<0.001*
Alcohol consumption (Ethanol intake)			
Never	624 (42.0)	1,929 (50.5)	1.00 (referent)
1~499 ml/week	323 (21.3)	850 (22.3)	1.16 (0.98 -1.38)
≥500 ml/week	558 (36.7)	1,040 (27.2)	1.59 (1.36-1.86)
p for trend			*<0.001*
Fast eating speed			
Normal	958 (63.0)	3,055 (78.8)	1.00 (referent)
Fast	562 (37.0)	824 (21.2)	2.40 (2.09-2.76)
Hot foods/drinks			
Normal	605 (39.8)	2,146 (55.4)	1.00 (referent)
Hot	808 (53.2)	1,635 (42.2)	1.75 (1.54-1.99)
Extremely hot	107 (7.0)	96 (2.5)	4.04 (2.98-5.47)
p for trend			*<0.001*
High-sodium foods intake			
Less frequent	893 (58.8)	2,624 (67.7)	1.00 (referent)
Normal	549 (36.1)	1,128 (29.1)	1.38 (1.21-1.58)
Frequent	78 (5.1)	124 (3.2)	1.99 (1.46-2.72)
p for trend			*<0.001*
Fried foods intake			
Normal	1,027 (67.6)	2,825 (72.8)	1.00 (referent)
Frequent	493 (32.4)	1,054 (27.2)	1.37 (1.18-1.58)
Rawgarlic consumption			
≥ 2 times week	148 (9.7)	441 (11.4)	1.00 (referent)
<2 times week	640 (42.1)	1,471 (38.0)	1.26 (1.02-1.57)
Never	731 (48.1)	1,957 (50.6)	1.37 (1.08-1.74)
p for trend			*0.019*
Fruits & vegetables intake			
Q4 (Highest)			
Q3	354 (23.5)	963 (25.0)	1.00 (referent)
Q2	371 (24.6)	963 (25.0)	1.10 (0.92-1.31)
Q1 (Lowest)	392 (26.0)	963 (25.0)	1.18 (0.99-1.41)
p for trend	392 (26.0)	962 (25.0)	1.11 (0.93-1.34)
			0.226
Red meat intake			
Q1 (Lowest)	369 (24.7)	905 (23.7)	1.00 (referent)
Q2	356 (23.8)	968 (25.4)	1.01 (0.84-1.20)
Q3	406 (27.2)	972 (25.4)	1.18 (0.99-1.40)
Q4 (Highest)	364 (24.4)	974 (25.5)	1.13 (0.94-1.36)
p for trend			*0.116*

[1] Missing data were excluded from analysis for these variables that the total numbers of cases or controls were less than the total number. - [2] Adjusted for age (continuous), gender, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous, except for smoking model), weekly ethanol intake (continuous, except for alcohol model) and study area.

In total, 1,520 cases (637 in Dafeng and 883 in Ganyu) and 3,879 controls (1,938 in Dafeng and 1,941 in Ganyu) were recruited. Table 5-1 gives their demographical information and socio-economic characteristics. More often than not, it is apparent that cases were older and male, and more frequently occurred in the population with lower socio-economic statuses, i.e. lower education level, lower previous income, and lower BMI.

Table 5-2 shows the ORs and 95% CI of major lifestyle risk factors with esophageal cancer risk, including smoking, alcohol consumption, and certain dietary factors. Smoking and alcohol consumption significantly increased the risk of esophageal cancer; positive dose-response relationships were observed with increased pack-years of smoking and higher weekly consumption of ethanol (p for trend<0.001 each). For dietary factors, elevated ORs were found for fast eating speed (OR = 2.40, 95% CI = 2.09-2.76), hot foods/drinks (OR = 4.04 for the highest exposure group), frequent intake of high-sodium foods (p for trend<0.001) and fried foods (OR=1.37, 95%CI = 1.18-1.58). It was found that raw garlic consumption was inversely related to the occurrence of esophageal cancer, with a dose-response relationship. Individuals who never ate raw garlic had a 1.37-fold risk, compared to those who ate raw garlic more than twice per week. Weak positive associations were observed with high intake of red meat and low intake of fruits and vegetables, but associations were relatively statistically insignificant.

Table 5-3 describes the distribution of family history of cancer (FH), and its association with the risk of esophageal cancer. The prevalence of having FH of any malignant tumor among first-degree relatives (FH-FDR) was 25.3% in the case group and 19.6% among controls. Parents affected with cancer was the most common type of FH in both cases and control groups. Esophageal cancer was the predominant cancer type among affected first-degree relatives (FDR), much higher than the occurrence of stomach cancer, liver cancer, and lung cancer. Among individuals with FH-FDR of esophageal cancer, again the prevalence of FH in the case group (16.0%) was higher than that of the control group (8.8%), and there was a greater proportion of having affected parents than affected siblings. Most of the affected relatives were diagnosed with esophageal cancer after turning 50-years old, and a few individuals had more than one affected FDR.

Table 5-3 The distribution of family history of cancer and its association with esophageal cancer risk

Family history of cancer (FH)	Case (%) (N=1,520)	Control (%) (N=3,879)	OR1 (95% CI) [3]	OR2 (95% CI) [4]
FH of any malignant tumor				
Type of affected relatives				
No FH	1,077 (70.8)	2,932 (75.6)	1.00 (referent)	1.00 (referent)
First-degree relatives (FDR)	384 (25.3)	761 (19.6)	1.68 (1.45-1.96)	1.64 (1.40-1.92)
Other relatives	59 (3.9)	186 (4.8)	1.03 (0.76-1.41)	1.04 (0.76-1.43)
Affected relatives [1,2]				
Parent	283 (18.6)	564 (14.5)	1.67 (1.41-1.99)	1.64 (1.37-1.96)
Sibling	131 (8.6)	223 (5.7)	1.84 (1.46-2.33)	1.78 (1.39-2.27)
Child	11 (0.7)	33 (0.8)	0.95 (0.48-1.90)	0.94 (0.46-1.90)
Spouse	48 (3.2)	125 (3.2)	1.15 (0.81-1.63)	1.11 (0.77-1.60)
Number of affected FDRs [2]				
1	301 (19.8)	634 (16.3)	1.57 (1.33-1.84)	1.52 (1.28-1.79)
2	70 (4.6)	113 (2.9)	2.17(1.58-2.97)	2.15 (1.56-2.98)
3-	13 (0.8)	14 (0.4)	3.35 (1.567.19)	3.33 (1.50-7.41)
p for trend			*<0.001*	*<0.001*
Age at diagnosis of FDRs [2]				
60~	205 (13.5)	461(11.9)	1.44 (1.19-1.74)	1.40 (1.15-1.70)
50~59	96 (6.3)	176 (4.5)	1.81 (1.38-2.36)	1.71 (1.30-2.26)
40~49	48 (3.2)	83 (2.1)	1.93 (1.34-2.79)	1.90 (1.30-2.78)
<40	22 (1.4)	31 (0.8)	2.22 (1.27-3.88)	2.26 (1.26-4.04)
p for trend			*<0.001*	*<0.001*
Cancer type of affected FDRs [1,2]				
Esophagus	244 (16.0)	340 (8.8)	2.27 (1.88-2.74)	2.22 (1.83-2.70)
Stomach	66 (4.3)	171 (4.4)	1.30 (0.96-1.75)	1.18 (0.86-1.62)
Liver	39 (2.6)	124 (3.2)	1.01 (0.70-1.47)	1.01 (0.68-1.48)
Lung	23 (1.5)	63 (1.6)	1.19 (0.73-1.94)	1.23 (0.75-2.03)
Other sites	50 (3.3)	144 (3.7)	1.10 (0.79-1.54)	1.09 (0.77-1.54)
FH of esophageal cancer				
Type of affected relatives [1,2]				
Parents	181 (11.9)	264 (6.8)	2.20 (1.78-2.72)	2.16 (1.74-2.69)
Father	126 (8.3)	175 (4.5)	2.27 (1.77-2.91)	2.19 (1.70-2.82)
Mother	66 (4.3)	101 (2.6)	2.17 (1.56-3.01)	2.22 (1.58-3.11)
Siblings	67 (4.4)	74 (1.9)	2.64 (1.88-3.72)	2.57 (1.80-3.67)
Bother	61 (4.0)	59 (1.5)	2.97 (2.06-4.30)	2.93 (2.00-4.30)
Sister	11 (0.7)	16 (0.4)	2.13 (0.98-4.64)	2.09 (0.94-4.63)
Spouse	18 (1.2)	30 (0.8)	1.86 (1.023.38)	1.72 (0.93-3.21)
FH-FDR of esophageal cancer				
Probands' gender [2]				
Male	180 (11.8)	240 (6.2)	2.36 (1.89-2.94)	2.34 (1.86-2.94)
Female	64 (4.2)	100 (2.6)	1.91 (1.32-2.76)	1.78 (1.21-2.60)
Probands' age [2]				
<50	13 (0.9)	31 (0.8)	1.20 (0.63-2.32)	1.45 (0.74-2.84)
50~	231(15.2)	309 (8.0)	2.37 (1.95-2.88)	2.29 (1.87-2.80)

Table 5-3 The distribution of family history of cancer and its association with esophageal cancer risk (continued)

FH-FDR of esophageal cancer			**OR1 (95% CI)** [3]	**OR2 (95% CI)** [4]
Number of affected FDRs [2]				
1	200 (13.2)	294 (7.6)	2.14 (1.75-2.62)	2.09 (1.69-2.57)
2-	44 (2.9)	46 (1.2)	3.12 (2.03-4.78)	3.14 (2.03-4.86)
p for trend			*<0.001*	*<0.001*
Age at diagnosis of FDRs [2]				
60~	142 (9.3)	229 (5.9)	1.95 (1.55-2.46)	1.92 (1.51-2.43)
50~59	69 (4.5)	77 (2.0)	2.87 (2.04-4.03)	2.72 (1.92-3.86)
40~49	20 (1.3)	27 (0.7)	2.33 (1.30-4.20)	2.28 (1.25-4.16)
<40	6 (0.4)	3 (0.1)	5.82 (1.44-23.6)	5.02 (1.23-20.5)
p for trend			*<0.001*	*<0.001*

[1] Add up exceeds the total number because some subjects have more than one type of family cancer history. - [2] Subject without family history of cancer was used as reference. [3] - Adjusted for age (continuous), gender of proband and study area. – [4] Further adjusted for education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous), weekly ethanol intake (continuous) and number of siblings.

After adjusting for potential confounders and family size (represented by number of siblings), we found subjects with positive FH-FDR of any type of cancer had a 1.64-fold risk of esophageal cancer, compared to individuals without FH; while FH among other relatives e.g. spouse and second-degree relatives apparently did not affect the risk. Significantly increased OR was found with any cancer in parents (OR = 1.64, 95% CI = 1.37-1.96) and siblings (OR = 1.78, 95% CI = 1.39-2.27), but not in children (OR = 0.94, 95% CI=0.46-1.90). The risk of esophageal cancer was stronger if any FDR had cancer at the same site (OR = 2.22, 95%CI = 1.83-2.70). No elevated OR was observed for a FH-FDR of stomach cancer, liver cancer, or lung cancer.

For individuals with FH of esophageal cancer, the risk of having an affected sibling (OR = 2.57, 95% CI = 1.80-3.67) was slightly higher than having one affected parent (OR=2.16, 95% CI=1.74-2.69), with the highest OR observed being among individuals with an affected brother (OR = 2.93; 95% CI = 2.00-4.30). Having an affected spouse also increased disease risk but was not statistically significant (OR = 1.72, 95% CI = 0.93-3.21). Among the population with a positive FH-FDR of esophageal cancer, the association was higher for male probands (OR=2.34, 95% CI = 1.86-2.94) or probands aged 50 years and above (OR = 2.29, 95% CI = 1.87-2.80). A 3.14-fold risk of esophageal cancer was observed if more than one FDR had cancer at the same site. Dose-response relationship was also found associated with younger age at diagnosis of affected FDR (*p* for trend<0.001).

Table 5-4 The joint effects and test of interactions between FH-FDR of esophageal cancer and major lifestyle risk factors

	FH-FDR of esophageal cancer				Additive Interaction		
	No		Yes				
	Case/ Control	OR (95% CI)[1]	Case/ Control	OR (95% CI)[1]	RERI (95% CI)	AP (95% CI)	SI (95% CI)
Smoking							
Never smoking	352/1,433	1.00 (referent)	3/116	2.44 (1.73-3.44)			
<30 pack-years	522/1,296	1.38 (1.16-1.64)	92/110	3.46 (2.52-4.76)	0.07(-0.49~0.64)	0.02(-0.16~0.21)	1.04(0.78~1.38)
≥30 pack-years	402/810	1.80 (1.48-2.20)	89/114	3.33 (2.41-4.61)			
Alcohol (Ethanol intake)							
Never	525/1,761	1.00 (referent)	99/168	2.22 (1.68-2.94)			
1~499 ml/week	266/773	1.18 (0.99-1.42)	57/77	2.86 (1.97-4.17)	0.38(-0.10~0.86)	0.13(-0.03~0.29)	1.25(0.93~1.68)
≥500 ml/week	470/945	1.68 (1.42-1.98)	88/95	3.72 (2.68-5.15)			
Fast eating speed							
Normal	828/2,800	1.00 (referent)	130/255	2.05 (1.62-2.61)	2.05(0.47~3.91)	0.40(0.19~0.60)	1.93(1.26~2.95)
Fast	448/739	2.31 (1.98-2.69)	114/85	5.55 (4.08-7.56)			
Hot foods/drinks							
Normal	487/1,976	1.00 (referent)	118/170	3.26 (2.49-4.28)			
Hot	698/1,471	1.96 (1.70-2.25)	110/164	3.06 (2.32-4.28)	-0.40(-1.41~0.62)	-0.11(-0.40~0.18)	0.87(0.61~1.25)
Very Hot	91/90	4.25 (3.08-5.88)	16/6	12.4 (4.71-32.7)			
High-sodium foods							
Less frequent	725/2,394	1.00 (referent)	168/230	2.69 (2.14-3.38)			
Normal	487/1,027	1.52 (1.32-1.76)	62/101	2.35 (1.66-3.31)	-0.23(-1.09~0.62)	-0.08(-0.40~0.24)	0.89(0.56~1.39)
Frequent	64/115	2.02 (1.44-2.82)	14/9	6.04 (2.48-14.7)			
Fried foods							
Normal	840/2,538	1.00 (referent)	187/287	2.16 (1.75-2.68)	1.06(-0.39~2.50)	0.30(-0.02~0.60)	1.71(0.92~3.20)
Frequent	436/1,001	1.32 (1.13-1.53)	7/53	3.54 (2.37-5.26)			
Raw garlic							
≥2 times/ wk	132/425	1.00 (referent)	16/16	3.39 (1.62-7.09)	0.15(-0.48~0.78)	0.06(-0.19~0.31)	1.11(0.70~1.76)
<2 times/ wk	576/1,378	1.37 (1.09-1.73)	64/93	2.62 (1.77-3.88)			
Never	567/1,727	1.43 (1.11-1.83)	64/230	3.36 (2.43-4.64)			
Fruits & vegetables							
Q3 & Q4	627/1,756	1.00 (referent)	8/170	1.86 (1.41-2.46)	0.92 (0.10~1.73)	0.32(0.09~0.56)	2.01(1.04~3.92)
Q1 & Q2	638/1,755	1.06 (0.92-1.21)	46/170	2.84 (2.19-3.67)			
Red meat							
Q1 & Q2	612/1,707	1.00 (referent)	113/166	2.22 (1.70-2.92)	0.25(-0.60~1.10)	0.10(-0.21- 0.40)	1.18(0.67~2.09)
Q3 & Q4	642/1,776	1.15 (1.00-1.32)	128/170	2.63 (2.02-3.42)			

[1] Adjusted for age (continuous), gender, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous, except for smoking model), weekly ethanol intake (continuous, except for alcohol model) and number of siblings and study area.

Table 5-5 The population attributable fraction (PAF) of lifestyle risk factors among people with and without FH-FDR of esophageal cancer and the joint PAF of FH-FDR with lifestyle risk factors

Risk factors	FH-FDR of esophageal cancer				Risk factors and FH-FDR of esophageal cancer	
	No		Yes			
	Case(%)	PAF(95% CI) [1]	Case(%)	PAF (95% CI) [1]	Case (%)	PAF (95% CI) [1]
FH-FDR of EC	-	-	-	-	16.0	9.0 (6.3~11.7)
Smoking	72.4	25.8 (15.3~35.2)	74.2	13.9 (-21.0~39.9)	76.8	32.8 (23.3~41.3)
Alcohol consumption	58.4	17.1 (8.9~24.8)	59.4	19.2 (-2.6~37.4)	65.1	25.2 (17.6~32.4)
Fast eating speed	35.1	19.9 (15.6~24.2)	46.7	29.5 (17.1~41.0)	45.5	27.2 (22.6~31.6)
Hot foods/drinks	61.8	32.2 (25.8~38.3)	51.6	1.2 (-20.0~20.2)	68.0	38.3 (32.2~44.0)
High-sodium foods	43.2	15.6 (10.1~21.0)	31.1	0.4 (-12.5~13.4)	52.3	23.2 (17.7~28.6)
Fried foods	34.2	8.1 (2.7~13.4)	23.4	8.9 (-1.4~19.0)	44.7	17.1 (11.8~22.2)
Raw garlic [2]	44.5	3.3 (-4.7~10.9)	67.2	16.0 (-18.1~40.9)	53.4	11.8 (3.9~19.3)
Fruit & vegetable	50.4	2.5 (-5.3~10.0)	59.8	20.8 (1.0~37.9)	58.4	10.8 (3.1~18.2)
Red meat	51.2	6.1 (-1.6~13.5)	53.1	6.3 (-15.3~25.1)	59.1	15.0 (7.5~22.2)
All lifestyle factors	99.6	6.1 (-48.8~87.2)	100	NA	99.7	58.2 (41.7~87.8)

[1] Adjusted for age (continuous), gender, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous, except for smoking model), weekly ethanol intake (continuous, except for alcohol model), number of siblings and study area. - [2] Subjects were categorized into never or ever eating raw garlic giving the few numbers in the group of ≥2 times/week.

Table 5-4 presents the joint effects of FH-FDR of esophageal cancer and major lifestyle risk factors by stratified analysis, along with the results of the test of interactions on the additive scale. Compared to the reference category, an increased risk was observed among subjects either exposed to a lifestyle risk factor or FH-FDR of esophageal cancer; OR was substantially elevated among those who had exposures to both. However, regarding the consumption of raw garlic, it was found that people with positive FH-FDR who ate raw garlic more than twice a week had a high risk of esophageal cancer (OR = 3.39, 95% CI = 1.62-7.09).

A strong supra-additive interaction was observed between FH-FDR of esophageal cancer and fast eating speed (RERI = 2.05, AP = 0.40, SI = 1.93). This could be interpreted as there appeared to be a synergistic effects between two risk factors: the coexist of having FH-FDR and eating quickly caused an excess 2.05-fold risk of esophageal cancer due to their interaction, and 40% of cases exposed to both determinants could be attributed to interaction. Significant supra-additive interaction was also observed for positive FH-FDR and diets low in fruits and vegetables (RERI = 0.92, AP = 0.32, SI=2.01). Modest super-additivity was found for FH-FDR with alcohol consumption, high intake of fried foods and red meat, but was determined statistically insignificant, their RERI and AP ranged between 0.25-1.06 and 0.10-0.30, respectively. An additive effect was found for FH-FDR with smoking (RERI = 0.07, AP = 0.02), less consumption of raw garlic (RERI =0.15, AP = 0.06), hot foods/drinks (RERI = -0.40, AP = -0.11) and frequent intake of high-sodium foods (RERI = -0.23, AP = -0.08).

The population attributable fraction (PAF) of risk factors and FH-FDR of esophageal cancer is shown in Table 5-5. Among those without a FH-FDR of esophageal cancer, PAF of preferring hot foods/drinks was the highest (32.2%); smoking, alcohol consumption, fast eating speed, and frequently eating foods high in sodium accounted for modest PAF with a range of 15.6-25.8%. In total, 56.1% of cases can be attributed to selected lifestyle risk factors in this population. Among individuals with a FH-FDR of esophageal cancer, fast eating speed accounted for 29.5% of the cases; alcohol consumption and a diet low in fruits

and vegetables accounted for about 20% each; and a modest PAF was observed for smoking, never ate raw garlic, and high intake of fried foods and red meat, but was found to be statistically insignificant. As all cases with FH-FDR of esophageal cancer were exposed to at least one lifestyle risk factor, the combined PAF for all lifestyle factors in this group could not be calculated because of the lack of a reference group.

The individual PAF of FH-FDR and its joint PAF with lifestyle risk factors are also shown in Table 5-5. We found that FH-FDR of esophageal cancer accounted for 9% of cases alone. More than 30% of esophageal cancer cases could be attributed to FH-FDR, with the addition of either smoking (PAF = 32.8%) or hot foods/drinks (PAF = 38.3%), while FH-FDR plus alcohol consumption caused a PAF of 25.2%. The combined PAF of FH-FDR with other dietary risk factors was in the range between 10.8-27.2%. Moreover, 58.2% of all cases in the population could be explained by the presence of FH-FDR of esophageal cancer and unhealthy lifestyles.

Discussion

In the present analysis, we observed that the individual risk of esophageal cancer was significantly elevated if any first-degree relative (FDR) presented with cancer at the same site. This risk was increased with a greater number of affected FDR and younger age of those relatives at time of diagnosis. We also found that the effects of some lifestyle risk factors could be modified by the presence of familial risk. Significant supra-additive interactions were found for FH-FDR of esophageal cancer with fast eating speed and diets low in fruits and vegetables. Additionally, results showed that more than 50% of esophageal cancer cases are preventable through lifestyle interventions in the study area, indicating that primary prevention methods still stand as good options for reducing the occurrence of this disease.

When considering the genetic effect of FH on the development of cancer, the same risky habits may be aggregated within the same household, resulting in a close relationship between FH and cancer occurrence.[10,13,15] A likely aggregation of esophageal cancer with

spouses (OR = 1.72 95% CI = 0.93-3.21) and weak association in probands younger than 50-years old (OR = 1.45; 95% CI = 0.74-2.84) in our analysis also suggested the potential effect of shared exogenous risk factors. Due to lack of exposure information in cancer affected relatives, we were unable to distinguish the effect of genetic susceptibility and environmental exposures shared by family members, but several findings in our study indicated that genetic factors still play an important role in the familial aggregation of esophageal cancer. First, consistent familial risks were observed among individuals who were not exposed to lifestyle risk factors, e.g. never smokers and those who rarely consume alcohol. Secondly, an apparent dose-response relationship was observed with multiple affected relatives, suggesting a role of genetic susceptibly in the etiology.[7] Additionally, we found a younger age at diagnosis of FDR to be related to increased risk, the larger risk observed in younger affected relatives, indicating the role of genetic components in disease rather than environmental exposures.[28] Lastly, the risk of esophageal cancer was not significantly increased in individuals with a FH-FDR of other environmental and lifestyle-related cancers, such as stomach cancer (related to dietary factors), liver cancer (alcohol-related), and lung cancer (smoking-related), this could also be partially attributed to heredity risks. All of these supported the view that FH-FDR of esophageal cancer plays a significant role in the occurrence of this disease and could be used as the proxy for genetic predisposition.

The relationship between FH-FDR and esophageal cancer has been studied by a number of epidemiological studies around the world. A positive association was found in high-risk areas such as China and Iran.[8-11] Chang-Claude et al. reported that the standardized mortality ratio of esophageal cancer among people with FH-FDR of this disease was 2.36 in a cohort study in China.[8] Another large case-control study conducted in China (600 cases and 1514 controls) showed ORs of 1.72 and 2.84 among people with FH-FDR of any malignant tumor and esophageal cancer, respectively.[10] Akbari et al. found a hazard ratio of 2.3 among individuals whose FDR was diagnosed with esophageal cancer in Iran.[11] In line with these previous studies, we observed a 1.64- and 2.22-fold risk for FH-FDR of any malignant tumor and esophageal cancer, respectively. Unlike high-risk

areas of the world, the associations of FH-FDR and esophageal cancer were less consistent in Western countries. Dhillon et al. found no association between either esophageal squamous cell carcinoma (ESCC) or adenocarcinoma (EAC) and FH of any digestive cancer in the United States.[29] A case–control study in Sweden reported no significant association between FH-FDR of esophageal cancer and the risk of either ESCC or EAC,[30] while another Swedish study based on the nation-wide family-cancer database reported the SIRs for EAC were 4.05 and 3.52 when a parent was diagnosed with ESCC and any esophageal cancer, respectively.[12] The inconsistency in different areas might be due to variation in the frequency of esophageal susceptibility alleles and differences in major attributable environmental or lifestyle risk factors, but this still needs further study and clarification.[11] Only a few studies systematically investigated the association between esophageal cancer and FH. Gao et al. reported that the OR with affected father, mother, and sibling was 2.01, 3.27 and 4.66, respectively; the risk was greater if more than one FDR was affected.[10] Similar results were reported by Tran et al. and Akbari et al.[9,11] In agreement with their findings, we observed elevated risk of esophageal cancer among people with affected parents (OR = 2.16) or siblings (OR= 2.57), and a 3.14-fold risk if more than one FDR had the same type of cancer. OR for siblings was slightly higher than for parents, indicating that recessive or X-linked susceptibility genes may be important in the development of esophageal cancer,[31] or that siblings share more environmental exposures than children do with their parents.[32]

Lifestyle risk factors such as smoking, alcohol consumption, and unhealthy dietary habits were confirmed to be associated with esophageal cancer in the present analysis. A much greater risk was observed in individuals exposed to both FH-FDR of esophageal cancer and lifestyle risk factors. On an additive scale, we observed significant super-additivity interaction for FH-FDR with fast eating speed and diet low in fruits and vegetables, indicating that the joint risks when lifestyle with genetic predisposition coexists were significantly enlarged and were more than additive because of the interaction. Fast eating speed has been suggested to be associated with the risk of esophageal cancer by some epidemiological studies in China. Zhang et al. observed that fast eating habit was

associated with 1.54 to 4.10 folds risk of esophageal cancer.[33] Wang et al. reported that eating fast significantly increased the risk of esophageal cancer with an OR of 3.39 (95% CI: 1.15 – 9.95).[34] Eating too quickly can cause irritation of the esophageal mucosa and lead to chronic esophagitis, which has been considered an early sign of malignant transformation of the squamous epithelium.[35] Moreover, irritation may increase cell turnover and increase the contact between carcinogens and dividing target cells, thus making the esophagus more susceptible to cancer.[36] Fruits and vegetables are rich in antioxidants, dietary fiber, and micronutrients, and have been confirmed to reduce the risk of certain types of cancers, including esophageal cancer. Effect modifications between FH-FDR and intake of fruits and vegetables were observed by some studies related to breast cancer and colon cancer, but results remain inconclusive and most of them studied interactions on multiplicative scale.[16-18]

Modest supra-additive effects were observed for FH-FDR, with high intake of fried foods and red meat. Fried foods may contain carcinogens such as N-nitroso compounds and heterocyclic amines (HCA), while red meat also generates N-nitroso compounds and can produce free radicals by heme iron and free iron in the meat. Both of these two factors have been suggested to increase the risk of esophageal cancer.[37] Marchand et al. showed a significant multiplicative interaction between high intake of beef and FH-FDR in colorectal cancer cases.[15]

No marked interactions were observed for FH-FDR of esophageal cancer with smoking and alcohol consumption in our study, which suggests that they may act independently from the presence of FH-FDR. Smoking and alcohol consumption have been identified as major risk factors of esophageal cancer in Western countries, with a nearly multiplicative joint effect. However, their strong independent and joint effect was shown to be relatively smaller or even absent in some studies conducted in China.[36] In the present analysis, moderate increased ORs were observed for smoking and alcohol, as well as an additive effect with inherit risks. The relatively small effects of smoking and alcohol in China remain largely unknown, but could be partly explained by the short exposure history

and low prevalence among females, as compared to those in more developed countries.[38] Garavello et al. reported that FH-FDR of esophageal cancer appeared to act in a multiplicative way, with alcohol and tobacco in the pooled analysis of three case-control studies conducted in northern Italy and Switzerland, but test of interaction was not statistically significant.[39] Since the joint effects between FH-FDR and lifestyle risk factors are rarely reported for esophageal cancer, we are providing our results for other future studies to test.

A protective effect was observed for frequent eating raw garlic in FH-FDR negative group and the general population in our analysis; however, an increased risk was also found from frequent intake of raw garlic in FH-FDR positive individuals (OR = 3.39). Garlic contains high levels of flavonols and organosulfur compounds, and it has been suggested by a few epidemiological studies that it reduces the risk of esophageal cancer.[40] The potentially adverse effect of raw garlic has been reported by a few studies. For instance, raw garlic was found to present an irritating effect on esophageal and gastric mucosal surfaces;[41] high concentration of garlic extract has been shown to be clastogenic in mice.[42] Due to lack of previous epidemiological data and the few numbers in several groups in our analysis, this result should be interpreted with caution.

In the present study, we observed FH-FDR of esophageal cancer to account for 9% of total cases alone, showing that the majority of esophageal cancer cases were attributed to environmental and lifestyle risk factors. From a public health view, the substantially increased risk of lifestyle risk factors in FH-FDR positive individuals indicated that it is important to properly manage the disease by changing unhealthy lifestyles within this population. We found that more than 20% of cases in FH-FDR positive individuals could be prevented by either eliminating fast eating speed or increasing the intake of fruits and vegetables; however, no single risk factor was found with a particularly high PAF, therefore it may be better to lower the exposure of all aforementioned lifestyle risk factors in order to achieve a major reduction of esophageal cancer in this high-risk population. For those without a FH-FDR of esophageal cancer, more than 50% cases could also be

prevented by changing major unhealthy lifestyles.

Several methodological issues and limitations need to be discussed here. First, whether or not interaction is present depends on the choice of model or scale, biological interactions were predominantly explored on additive scale in the present analysis. However, even if interaction as departure from additivity is observed, risk factors may act independently on multiplicative scale; on the other hand, departure from multiplicity may be observed even if risk factors act independently on additive scale. Next, the family history of cancer was reported by study subjects, without medical confirmation. It is possible that cases were more aware of a family history of cancer and more liable to report both true-positive and false-positive disease history than the controls, resulting in inflated estimates of the relative risk.[43] Moreover, there might be misclassification of cancer type due to the lack of medical confirmation of affected relatives. Thirdly, due to the relative low proportion of histological confirmed cases in rural areas, it was not possible to determine the pathological type of all cases in this population-based study. However, based on previous reports, more than 95% of esophageal cancers are expected to be of the type of ESCC in our analysis.[3] Fourthly, although the questionnaire had been pre-tested in some previous studies and all interviewers had been trained to explain questions to subjects more clearly, the exposure level of most lifestyle risk factors were reported by study subjects without accurate measurements, and thus subjective judgement and recall bias (e.g. eating speed, temperatures of foods and drinks) may exist in this retrospective study and cause misclassification of exposures. Lastly, there is a large difference in the incidence of esophageal cancer between two study areas, which indicates the possible heterogeneity in the association of risk factors. In our another analysis, we found that although there were some variations regarding the strength of ORs, effects of major risk factors including family history of cancer did not show big differences between counties. On the other hand, differences in the prevalence of several lifestyle risk factors were attributable to a large fraction of the total risk gradient (data not shown).

Despite of the limitations, to our best knowledge, this is one of the largest

population-based case-control studies addressing the relationship between esophageal cancer and FH in detail, and exploring the interaction between FH-FDR and lifestyle risk factors. The results provide further evidence on the role of FH-FDR in the etiology of esophageal cancer, and indicate that the risks of certain unhealthy lifestyle factors such as fast eating speed and low intake of fruits and vegetables could be exaggerated by the presence of hereditary risk. The substantially increased risk of unhealthy lifestyles and the estimation of PAF suggest that it is important to control lifestyle risk factors as much as possible, especially for individuals with FH-FDR of esophageal cancer. By choosing comprehensive lifestyle intervention methods including intensive health education and health promotion activities, certain beneficial effects on esophageal cancer may be anticipated by this population.

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Chapter 6

Genetic polymorphisms of ADH1B, ADH1C and ALDH2 are associated with esophageal cancer risk among alcohol drinkers

Ming Wu, Shen-Chih Chang, Ellen Kampman,
Jie Yang, Xu-Shan Wang, Xiao-Ping Gu,
Ren-Qiang Han, Ai-Min Liu, Frans J Kok,
Jin-Kou Zhao and Zuo-Feng Zhang

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ABSTRACT

Epidemiologic studies and genome-wide association study (GWAS) have indicated that genetic polymorphisms in alcohol dehydrogenase 1B gene (ADH1B) and aldehyde dehydrogenase 2 (ALDH2) are associated with the risk of esophageal cancer. Using a population-based case-control study of esophageal cancer conducted from 2003 to 2007 in Jiangsu Province, China, we are replicating these results from GWAS in Chinese population. The aim of this analysis is to provide further information on the relationship between of ADH1B, ADH1C and ALDH2 polymorphisms on the development of esophageal cancer. A total of 858 cases and 1,081 controls were interviewed by a standard questionnaire and biological specimens from those participants were collected. DNAs were isolated from blood samples and empolyed in genotyping of proposed assays. Unconditional logistic regression was applied for evaluating the main association of different genotypes, as well as potential gene-gene and gene-environmental interactions. Results showed that the ADH1B G allele, ADH1C G allele and ALDH2 A allele significantly increased the risk of esophageal cancer among moderate/heavy drinkers, with an OR of 1.89 (95% CI: 1.40-2.55), 1.73 (95% CI: 1.15-2.62) and 1.76 (95% CI: 1.20-2.60) when compared to never/light drinkers carrying the ADH1B, 1C A/A genotype and ALDH G/G genotype, respectively. A significant interaction was observed between ALDH2 and alcohol drinking on both additive and multiplicative scale. Alcohol drinkers harboring an ALDH2 A allele and ADH1B G allele were at the highest risk of esophageal cancer, whereas no gene-gene interaction was observed for ALDH2 with either ADH1B or ADH1C. In conclusion, genetic polymorphisms of ADH1B, ADH1C and ALDH2 are associated with the risk of esophageal cancer among Chinese alcohol drinkers. Genetic predispositions, together with the variation in lifestyle factors may ultimately determine the individual risk of esophageal cancer in this high-risk Chinese population.

Key Words: Alcohol dehydrogenase (ADH); aldehyde dehydrogenase (ALDH); Polymorphisms; Esophageal cancer; China

Introduction

The causal link between alcohol consumption and several types of cancer has been well established including esophageal cancer, which remains one of the most common and fatal malignancies nowdays.[1,2] It is estimated that 26% of death from esophageal cancer could be attributed to alcohol use worldwide, the attributable fraction was 41% in high-income countries and 24% in low- and middle income countries, respectively.[3] Although the biological mechanisms underlying alcohol-induced carcinogenesis have not been fully understood, the metabolism of ethanol has been suggested to play an important role in the development of esophageal cancer.[4,5] Ethanol is first oxidized by alcohol dehydrogenases (*ADHs*) to acetaldehyde, which is known to form DNA adducts and can act as a tumor promoter, then further oxidized to harmless acetate by aldehyde dehydrogenases (*ALDHs*).[6]

In humans, *ADHs* are encoded by seven different genes. Single-nucleotide polymorphisms (SNPs) of these ADH-related genes may lead to structure and function changes of ADHs.[7] The SNP (rs1229984) in *ADH1B* is the alteration of arginine (*ADH1B**1, G) to histidine (*ADH1B**2, A) at codon 47 in exon 3.The super-active *ADH1B* A/A homodimer has siginifactly 40-fold higher enzyme activity for ethanol metabolism than the *ADH1B* G/G form.[8] The functionally important polymorphic sites for *ADH1C* are Ile350Val (rs698) and Arg272Gln (rs1693482); valine at codon 350 and glutamine. at codon 272 constitute the *ADH1C**1 allele.[9] *ADH1C**1 may result in 2.5-time higher capacity to ethanol oxidation compared to those encoded by *ADH1C**2 (i.e., isoleucine at amino acid position 350, G).[8] Nine major families of ALDH have been identified in humans, whereas *ALDH2* plays the central role in acetaldehyde metabolism.[10] A single-nucleotide transition for *ALDH2* from glutamic acid (*ALDH2**1, G) to lysine (*ALDH2**2, A) at codon 487 of exon 12 has been frequently studied (rs671). *ALDH2**2 genotype encoded an inactive subunit and restrained the ability to metabolize acetaldehyde. Blood acetaldehyde concentrations after consuming alcoholic beverages in the individuals having *ALDH2* A/A

and *ALDH2* A/G genotypes were found as 19- and 6-fold higher than in those with *ALDH2* G/G genotype, respectively.[11]

Epidemiologic studies have suggested that genetic variants in alcohol metabolizing genes may lead to different exposure to alcohol and acetaldehyde, and may influence the risk of esophageal cancer. The active *ADH1B* A and inactive *ALDH2* A allele were observed to be rare in Western populations but highly prevalent among Asians,[7,9] studies in Japanese and Chinese studies have reported they were associated with increased risks of esophageal cancer among alcohol drinkers.[12-16] Whereas, most previous studies were conducted with a relatively small sample size and less sufficient statistical power to identify potential gene-environmental and gene-gene interactions. Moreover, few studies have investigated the *ADH1C* polymorphism and esophageal cancer among Asians. From 2003 to 2007, a large population-based case-control study on esophageal cancer has been carried out in Jiangsu province, an area with one of the highest esophageal cancer mortality in China.[17] In this present analysis, we evaluated how polymorphisms of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) genes influence the individual risk of esophageal cancer, in addition, we explored their joint effects and interactions with alcohol intake among this high-risk Chinese population.

Materials and Methods

Study subjects

The study design has been previously described in detail.[18-19] In brief, a population-based case-control study has been conducted from 2003 to 2007 in two counties, Dafeng and Ganyu in Jiangsu province, south-eastern part of China. Both counties are less developed, coastal, rural areas in northern Jiangsu. The annual average age-standardize incidence of esophageal cancer was 36 and 24 per 100,000 in Dafeng and Ganyu during 2006-2008, respectively.

Eligible subjects were restricted to local inhabitants who have lived in the study area

for at least 5 years. Newly diagnosed primary esophageal cancer patients were recruited as cases, using the information from local population-based cancer registries. From 2003-2007, 68% and 75% of newly registered patients were recruited and interviewed in Dafeng and Ganyu, respectively. Because of the low proportion of histologically confirmed cases in rural areas (39%), patients who were diagnosed by endoscopic examination (40%) or radiology (11%) were also included, whereas, more than 95% of esophageal cancers are supposed to be squamous cell carcinoma based on previous reports.[20] Controls were derived from the same county as cases, randomly selected from the county demographic database. Cases and controls were frequency matched by gender and age (±5 years). The responding rate of controls was 87% in Dafeng and 85% in Ganyu, respectively.

This study was approved by the Institutional Review Board of Jiangsu Provincial Health Department. With written informed consent, epidemiological data were obtained by face-to-face interviews using a pre-tested standardized questionnaire. The questionnaire elicited information on known or potentially associated factors for esophageal cancer, including demographic information, socioeconomic status, living conditions, duration and amount of smoking etc. Details on lifetime alcohol drinking habits were also collected, questions included age at starting drinking, drinking frequency, years of consumption, weekly frequency and amount of drinking different types of alcohol beverages (e.g. beer, wine and liquor). If the subject had quit drinking habit at time of interview, the duration of cessation was also recorded. A 5 ml non-fasting venous blood sample was collected by vacuum tube at the time of interview.

Genotyping analysis

DNA was extracted from blood clots using phenol-chloroform methods. SNPs were genotyped using the Taqman assay (Applied Biosystems [ABI], Foster City, CA). Briefly, 10ng of dried DNA in a total volume of 5 μl PCR reaction mix of fluorescent-labeled sequence-specific primers and probe were used with the following protocol: denaturation at 92°C for 10 min followed by 60 cycles at 92°C for 15 sec and extension at 62°C for 80 sec.

Genotype detection was performed on ABI 7900HT sequence detection system with SDS2.3 software. Around 10% of the samples were randomly repeated for quality control. Call rates were >95% and reproducibility was 0.993. Genotype distributions for all three SNPs were accordant with the Hardy-Weinberg equilibrium among controls (P>0.05).

Statistical analysis

Data were entered into the computer by Epidata 3.0, cleaned and analyzed using SAS v9.1 software (SAS Institute, Inc., Cary, NC). In the present analysis, never drinkers were referred to those who drank less than once per month. Weekly consumption of pure ethanol (grams/week) on average were converted according to the average frequency and amount of drinking different types of alcohol beverages (e.g. beer, wine, and liquor) according to average frequency and amount of drinking.

Pearson χ^2 test and student's t test were used to compare the differences in distributions of selected demographic factors among cases and controls. Unconditional logistic regression with a maximum likelihood estimation of parameters was applied for estimating the associations between SNPs and the risk of esophageal cancer. Confounders were selected based on the previous knowledge of esophageal cancer and our results of previous analyses, including age (continous), gender, education level, previous income (continous), body mass index (BMI, continous), family history of cancer (any malignancy in first-degree relatives) and study area. After adjusting for confounders, the associations between smoking, drinking, SNPs and esophageal cancer were estimated with odds ratios (ORs) and correspondent 95% confidence intervals (CIs).

Dummy variables were used to estimate OR for each exposure category. The trend test of ordered variables was performed by assigning scores to different exposure levels and treating the categorical variable as a continuous variable in the logistic regression model. Effect modifications were evaluated by stratification, Gene-environmental and gene-gene interaction was assessed on both additive and multiplicative scale. The multiplicative interaction was assessed by including main effect variables and their product terms in the

logistic regression model. For additive interaction, the three measures – relative excess risk due to interaction (RERI), attributable proportion due to interaction (AP), and synergy index (SI) were calculated suggested by Knol, et al.[21] The 95% CI of RERI, SI, and AP were estimated by delta method.[22,23] In the absence of an interaction, RERI and AP amount to 0 and SI amounts to 1.

Results

From 2003 to 2007, 1,520 cases and 1,683 corresponding controls were recruited for this study, whereas, considering the duration and storage of blood samples, genotyping analysis was only performed among subjects who were recruited after 2004. In total, 858 esophageal cancer cases and 1,081 controls were involved in the present analysis. The distributions of selected demographic characteristics of cases and controls are shown in Table 6-1. Compared to population controls, cases were more likely to be male and older, and with lower socio-economic status, i.e. lower education level, lower previous income and lower BMI. Smoking was also found to be related to the occurrence of esophageal cancer with an apparent dose-response relationship.

Table 6-2 presents the associations between esophageal cancer and alcohol drinking among the study population. After adjusting for potential confounders, OR for ever drinking was 1.39 (95%: CI 1.09-1.78). Positively dose-response relationships were observed with higher frequency and amount of drinking alcohol (p for trend <0.01, each). OR for drinking ethanol ≥250ml/week and ≥500ml/week was 1.28 (95% CI: 0.91 -1.79) and 1.56 (95% CI: 1.19-2.04), respectively.

Genotype distributions of the three SNPs (*ADH1B*, *ADH1C* and *ALDH2)* and their associations with esophageal cancer are shown in Table 6-3. The distributions for all three SNPs were in agreement with the Hardy-Weinberg equilibrium among controls (P>0.05). Compared to individuals carrying *ADH1B* (rs1229984) A/A genotype (active form), the inactive *ADH1B* G/G homozygotes were associated with increased risk of esophageal

cancer with an adjusted OR of 1.91 (95% CI: 1.38-2.64), while the A/G heterozygotes showed a borderline OR of 1.22 (95% CI: 0.98-1.53). The OR for G allele carriers (A/G+G/G) compared to homozygote those with A/A was 1.34 (95% CI: 1.09-1.67). No strong association was observed between the risk of esophageal cancer and *ADH1C* (rs698) and *ALDH2* (rs671) SNPs.

Table 6-1 Demographic information and socio-economic status of cases and controls[1]

	Cases (%) (N=858)	**Controls (%) (N=1081)**	***p*-Value[3]**
Gender			
Male	673 (78.4)	782 (72.3)	*0.002*
Female	185 (21.6)	299 (27.7)	
Age			
Mean±SD (years)	63.6±9.5	63.7±10.3	
<50	63 (7.3)	101 (9.3)	
50~	221 (25.8)	231 (21.4)	*0.122*
60~	338 (39.4)	426 (39.4)	
70~	202 (23.5)	273(25.2)	
≥80	34 (4.0)	50 (4.6)	
Education level			
Illiteracy	488 (56.9)	496 (45.9)	*<0.001*
Primary school	267 (31.1)	387 (35.8)	
Middle school & above	103 (12.0)	198 (18.3)	
Previous income (RMB)			
<1000	197 (23.3)	164 (15.3)	
1000~	165 (19.5)	197 (18.3)	*<0.001*
1500~	149 (17.6)	154 (14.3)	
2500~	335 (39.6)	559 (52.1)	
Body Mass Index (BMI)[2]			
Mean±SD	21.5±3.6	22.7±7.4	
Low (<18.5)	139 (16.2)	84 (7.8)	*<0.001*
Normal (18.5~23.9)	578 (67.4)	695 (64.4)	
Overweight (24~27.9)	112 (13.1)	250 (23.2)	
Obesity (≥28)	28 (3.3)	51 (4.7)	
Smoking			
Never	214 (24.9)	421 (39.0)	*<0.001*
Ever	644 (75.1)	660 (61.0)	
Pack-years of Smoking			
Never	214 (24.9)	421 (39.0)	*<0.001*
<30 pack-years	344 (40.1)	345 (31.9)	
≥30 pack-years	300 (34.5)	315 (29.1)	
Family history of caner			
No	590 (68.8)	692 (64.0)	*0.028*
Yes	268 (31.2)	389 (36.0)	

[1] Missing data were excluded from analysis. - [2] Chinese recommend standard was used for the cut-off points for overweight and obesity. - [3] p-value comparing cases and controls.

Table 6-2 Distribution of alcohol drinking and their associations with esophageal cancer[1]

	Case (%) (N=858)	Control (%) (N=1,081)	OR (95% CI) [2]	OR (95% CI) [3]
Alcohol consumption				
Never	268 (31.2)	459 (42.5)	1.00(referent)	1.00 (referent)
Ever	590 (68.8)	622 (57.5)	1.54(1.22-1.95)	1.39 (1.09-1.78)
Drinking frequency				
Never	268 (31.2)	459 (42.5)	1.00 (referent)	1.00 (referent)
Occasional	137 (16.0)	168 (15.5)	1.25 (0.91-1.72)	1.22 (0.88-1.69)
Often	159 (18.5)	137 (12.7)	1.61 (1.16-2.23)	1.46 (1.04-2.04)
Everyday	294 (34.3)	317 (29.3)	1.70 (1.29-2.23)	1.48 (1.12-1.97)
p for trend			*<0.001*	*0.005*
Average ethanol intake (ml/week)				
0	332 (39.2)	543 (50.4)	1.00 (referent)	1.00 (referent)
1-	68 (8.0)	104 (9.7)	1.16 (0.80-1.70)	1.15 (0.78-1.69)
250-	115 (13.6)	134 (12.4)	1.39(1.00 -1.94)	1.28(0.91 -1.79)
≥500	331 (39.1)	296 (27.5)	1.76 (1.36-2.27)	1.56 (1.19-2.04)
p for trend			*<0.001*	*0.001*

[1] Missing data were excluded from analysis.- [2] Adjusted for age (continuous), gender and study area.- [3] Further adjusted for education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous) and family history of cancer.

Table 6-3 Distribution of ADH1B, ADH1C and ALDH2 polymorphisms and their associations with esophageal cancer[1]

	Case (%) (N=858)	Control (%) (N=1,081)	OR (95% CI) [2]	OR (95% CI) [3]
ADH1B (rs1229984)				
A/A (fast)	359 (44.1)	512 (50.0)	1.00 (referent)	1.00 (referent)
A/G	313 (38.4)	410 (40.1)	1.20 (0.97-1.49)	1.22 (0.98-1.53)
G/G (slow)	142 (17.4)	101 (9.9)	1.93 (1.41-2.66)	1.91 (1.38-2.64)
A/G+GG	*455 (55.8)*	*511 (50.0)*	1.34 (1.09-1.65)	1.34 (1.09-1.67)
ADH1C (rs698)				
A/A (fast)	680 (82.9)	846 (82.4)	1.00 (referent)	1.00 (referent)
A/G	127 (15.5)	171 (16.6)	1.00 (0.75-1.31)	1.01 (0.76-1.34)
G/G (slow)	13 (1.6)	10 (1.0)	1.22 (0.48-3.14)	1.16 (0.44-3.07)
A/G+GG	*140 (17.1)*	*181 (17.6)*	1.00 (0.76-1.31)	1.01 (0.76-1.33)
ALDH2 (rs671)				
G/G (fast)	531 (65.3)	645 (62.7)	1.00 (referent)	1.00 (referent)
A/G	249 (30.6)	337 (32.8)	0.95 (0.76-1.18)	1.04 (0.82-1.31)
A/A (Slow)	33 (4.1)	47 (4.6)	0.64 (0.38-1.09)	0.69 (0.40-1.19)
A/G+A/A	282 (34.7)	384 (34.7)	0.90 (0.73-1.12)	0.99 (0.78-1.24)

[1] Missing data were excluded from analysis. [2] Adjusted for age (continuous), gender and study area. [3] Further adjusted for previous income (categorical), BMI (categorical), pack-years of smoking (categorical), ethanol intake (categorical) and family history of cancer.

Table 6-4 shows the joint effects of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) polymorphisms and alcohol consumption on the risk of esophageal cancer. We found that inactive G allele of *ADH1B* was associated with an elevated risk of esophageal cancer among both never-to-light (<250 ml/week) and moderate-to-heavy drinkers (≥250 ml/week). Those who consumed ethanol of more than 250 ml/week and with *ADH1B* G/G genotype had a 3.12-fold increased cancer risk compared to never-to-light drinkers with ADH1B A/A genotype. Similar association was observed for *ADH1C* G allele carriers, but only among moderate-to-heavy drinkers (OR=1.73, 95% CI: 1.15-2.62). For *ALDH2* polymorphism, an elevated OR was found for moderate-to-heavy drinkers harboring the A allele (OR=1.76, 95% CI: 1.20-2.60), and the risk was the highest for those with A/G genotype (OR=1.88, 95% CI: 1.26-2.80). Interaction was observed between alcohol drinking and *ALDH2* rs671 polymorphism on both additive scale and multiplicative scale, but not for *ADH1B* and *ADH1C*.

The joint impacts and interactions between *ALDH2* (rs1229984) and *ADH1B* (rs698) and *ADH1C* (rs671) polymorphisms on the risk of esophageal cancer were also evaluated, and the results are summarized in Table 6-5. We found that among moderate-to-heavy drinkers, the G allele (A/G+G/G) of both *ADH1B* and *ADH1C* was associated with increased risks when compared to the A/A type, despite of the ALDH2 genotype. On the other hand, moderate-to-heavy alcohol drinkers harboring the *ALDH2* A allele (A /G +A/A) conferred a higher risk independent of their ADH genotypes. Compared to never-to-light drinkers with fast metabolizing type (*ALDH2* G/G and *ADHs* A/A), moderate-to-heavy drinkers carrying both inactive *ALDH2* A allele and inactive *ADHs* G allele have the highest risk, with ORs of 2.60 (95% CI: 1.60-4.25) for *ADH1B* and 2.06 (95% CI: 1.01-4.20) for *ADH1C*, respectively. However, no obvious interactions were observed beween *ALDH2* and *ADHs* polymorphisms, either on additive or multiplicative scale.

Table 6–4 Joint effects on esophageal cancer between alcohol drinking with ADH1B, ADH1C and ALDH2 polymorphisms[1]

Genotype	Alcohol Drinking			
	Case/ Control	Never/light (<250ml/week)	Case/ Control	Moderate/heavy (≥250ml/week)
ADH1B (rs1229984)				
A/A (fast))	169/328	1.00 (referent)	190/184	1.53 (1.11-2.10)
A/G	164/236	1.36 (1.01-1.83)	149/174	1.56 (1.13-2.16)
G/G (slow)	57/56	1.68 (1.06-2.67)	85/45	3.12 (1.97-4.93)
A/G+G/G	221/292	1.42 (1.08-1.88)	234/219	1.89 (1.40-2.55)
Interaction	Additive: RERI=0.19 (95% CI: -0.30-0.68); AP=0.09 (95% CI: -0.14-0.32); SI=1.21 (95% CI:0.72-2.02)			
	Multiplicative: OR = 1.00 (95% CI: 0.72-1.38)			
ADH1C (rs698)				
A/A (fast)	329/509	1.00 (referent)	351/337	1.37 (1.07-1.76)
A/G	60/107	0.85 (0.58-1.25)	67/64	1.68 (1.10-2.57)
G/G(slow)	5/7	0.74 (0.19-2.84)	8/3	2.65 (0.59-12.0)
A/G+G/G	65/114	0.84 (0.58-1.23)	75/67	1.73 (1.15-2.62)
Interaction	Additive: RERI=0.57 (95% CI: -0.21-1.35); AP=0.30 (95% CI: -0.03-0.64); SI=2.87 (95% CI:0.51-16.3)			
	Multiplicative: OR = 1.53 (95% CI: 0.89-2.63)			
ALDH2 (rs671)				
G/G (fast)	231/320	1.00 (referent)	300/325	1.10 (0.83-1.44)
A/G	130/260	0.72 (0.53-0.97)	119/77	1.88 (1.26-2.80)
A/A (Slow)	23/41	0.60 (0.33-1.12)	10/6	0.76 (0.24-2.44)
A/G+A/A	153/301	0.70 (0.53-0.93)	129/83	1.76 (1.20-2.60)
Interaction	Additive: RERI=0.76 (95% CI: 0.11-1.41); AP=0.41 (95% CI: 0.15-0.67); SI=9.21 (95% CI: 0.05-∞)			
	Multiplicative: OR = 1.79 (95% CI:1.15-2.78)			

[1]Missing data were excluded from analysis.- [2]Adjusted for age (continuous), gender, study area, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous) and family history of cancer. - [3]Synergy index was not calculated because of the heterogeneity of associations between Never/light and moderate/heavy drinkers.

Table 6-5 Joint effects on esophageal cancer for ALDH2 with ADH1B and ADH1C

ALDH2	ADH1B	Alcohol Drinking			
		Case/ Control	Never/light (<250ml/week)	Case/ Control	Moderate/heavy (≥250ml/week)
G/G	A/A	87/176	1.00	136/152	1.25 (0.87-1.80)
A/G+A/A	A/A	75/149	0.94 (0.64-1.39)	50/31	1.85 (1.03-3.33)
G/G	A/G+G/G	137/138	1.83 (1.28-2.62)	155/169	1.48 (1.04-2.10)
A/G+A/A	A/G+G/G	77/150	0.87 (0.59-1.23)	75/48	2.60 (1.60-4.25)
Interaction					
Additive	RERI (95% CI)		-0.94(-1.88-0.01)		0.42(-0.80-1.65)
	AP (95% CI)		-0.87 (-1.77-0.03)		0.22(-0.36-0.78)
	SI (95% CI)		0.08 (0.00-16.7)		1.77 (0.26-12.2)
Multiplicative	OR (95% CI)		0.52 (0.28-1.02)		1.23 (0.55-2.76)
ALDH2	**ADH1C**				
G/G	A/A	186/261	1.00	243/272	1.05 (0.78-1.40)
A/G+A/A	A/A	129/242	0.75 (0.55-1.02)	102/62	1.71 (1.11-2.63)
G/G	A/G+G/G	44/55	1.05 (0.64-1.70)	48/47	1.32 (0.80-2.17)
A/G+A/A	A/G+G/G	21/58	0.48 (0.26-0.86)	26/19	2.06 (1.01-4.20)
Interaction					
Additive	RERI (95% CI)		-0.23 (-0.88-1.62)		0.58(-1.22-2.38)
	AP (95% CI)		-0.44 (-1.77-0.88)		0.26(-0.39-0.91)
	SI (95% CI)		1.98 (0.15-26.6)		1.88 (0.30-12.0)
Multiplicative	OR (95% CI)		0.69 (0.30-1.55)		1.28 (0.49-3.38)

[1] Missing data were excluded from analysis. [2] Adjusted for age (continuous), gender, study area, education level, previous income (categorical), BMI (categorical), pack-years of smoking (categorical) and family history of cancer.

Discussion

The variant *ADH1B* rs1229984 and *ALDH2* rs671 alleles are rare in Western populations but are highly prevalent among Eastern Asians. Therefore, their associations with cancer were mostly studied in those of Asian ethnic origisn, especially among Chinese and Japanese populations. Results from most previous studies consistently showed that the inactive *ADH1B* G allele and *ALDH2* A allele could increase the risk of esophageal cancer among alcohol drinkers.[12-16] Different from *ADH1B* and *ALDH2*, *ADH1C* rs698 is the rate-limiting factor in alcohol metabolism among most Western populations. Studies from European origins have focused on *ADH1C* polymorphism but mainly for their associations

with head and neck cancer.[24,25] Studies for *ADH1C* with esophageal cancer remain sparse and there results are inconclusive.[26] In this large population-based case-control study, we confirmed that genetic polymorphisms in *ADH1B* gene (rs1229984) and *ALDH2* (rs671) were associated with esophageal cancer risk among moderate-to-heavy alcohol drinkers. We further found similar association for *ADH1C* (rs698) polymorphism in this high-risk Chinese population. Moreover, strong interaction between alcohol drinking and *ALDH2* polymorphism was observed, whereas no apparent gene-gene interactions were detected between *ALDH2* and *ADHs* on esophageal cancer risk.

Although the *ADH1B* rs1229984 G allele is known to metabolize ethanol to acetaldehyde less actively than the A allele, it has been shown to be associated with an increased risk of esophageal cancer unexpectedly. Chen et al found that individuals with *ADH1B* G/G genotype had a 3.99-fold risk (95% CI: 2.13–7.48) of esophageal cancer compared to those with A/A genotype.[27] Yang et al reported that the adjusted OR for *ADH1B* G allele carriers was 1.65 (95%CI: 1.02-2.68) compared to those with A/A genotype.[28] A meta-analysis on showed that when compared to those with *ADH1B* A/A genotype, the risk of esophageal cancer among G/G carriers was 1.56 (95% CI: 0.93-2.61) for never/rare drinkers, 2.71 (95% CI: 1.37-5.35) for moderate drinkers and 3.22 (95% CI: 2.27-4.57) for heavy drinkers.[29] In agreement with these findings, we found that *ADH1B* G allele was associated with an increased risk of esophageal cancer, and the risk for individuals with G/G genotype was 1.68-fold higher among never/light drinkers and 3.12-fold higher among moderate/heavy drinkers compared to never/light drinkers with A/A genotype; however, no strong interaction with alcohol drinking was detected. The increased risk among *ADH1B* active type carries might result from an absence of alcohol flushing response after drinking, which includes facial flushing, tachycardia, headaches and other unpleasant symptoms.[30] *ADH1B* G allele may cause high concentration of acetaldehyde after drinking alcohol and produce ethanol intolerance at low doses. Therefore, it could prevent people from heavy drinking and reduce the exposure of esophageal mucosa to ethanol. In contrast, alcohol drinkers with inactive *ADH1B* G/G genotype tend to experience binge-drinking and withdrawal syndrome more than those with other

genotypes.[31] Several studies, including our study, have demonstrated that *ADH1B* G/G genotype was more prevalent among alcoholic cases than healthy controls in Asians.[32,33].

Similar to *ADH1B*, we observed that alcohol drinkers possessing *ADH1C* rs698 G allele appears to be at higher risk for esophageal cancer. However, *ADH1B* and *ADH1C* genes are located closely in the short arm of chromosome 4, and strong linkage disequilibrium between these two genes has been reported by previous studies.[34,35] A Japanese study reported that *ADH1C* rs698 G allele was associated with increased risk of esophageal cancer, and the ORs for A/G and G/G genotype were 13.32 (95% CI: 5.28–33.63) and 28.83 (95% CI: 7.67–74.06) among moderate and heavy drinkers, respectively. However, no relationship was observed after the adjustment of *ALDH2* and *ADH1B* genotypes in multiple logistic model.[36] Macgregor et al. found that linkage disequilibrium exists between *ADH1B* and *ADH1C*, and the genetic polymorphisms of *ADH1B*, rather than *ADH1C*, have a stronger association with the development of alcoholism.[35] However, a comprehensive epidemiologic study in Europe showed that *ADH1B* and *ADH1C* had a significant independent association with upper aero-digestive cancer (including esophageal cancer), despite of strong linkage disequilibrium.[34] After adjusting for *ADH1B* polymorphism, we found that the ORs were 1.37 (95% CI: 0.88-2.14) for *ADH1C* A/G and 1.40 (95% CI: 0.91-2.17) for A/G+G/G among moderate/heavy drinkers, indicating the impact of *ADH1C* may be partly associated with *ADH1B* gene because of linkage disequilibrium. Results on *ADH1C* rs698 polymorphism and esophageal cancer remain sparse and inconsistent, and still need to be further elucidated.

Various studies have been conducted on the polymorphism of *ALDH2* rs671 and its association with esophageal cancer among Asians, and most of them reported increased risk among alcohol drinkers with inactive *ALDH2* A/G or A/A genotype. Interactions between alcohol consumption and *ALDH2* polymorphisms have also been observed.[29] Interestingly, it has been indicated that the associations appeared to be particularly high for heavy drinkers who had *ALDH2* A/G genotype rather than those with A/A genotype.[12] Results from one meta-analysis showed that the overall risk increased in *ALDH2* A/G

heterozygotes (OR=3.19; 95% CI: 1.86–5.47) but decreased in A/A homozygotes (OR=0.36; 95% CI: 0.16–0.80), when compared to G/G homozygotes.[37] *ALDH2* heterozygotes may result in excessive accumulation of acetaldehyde after alcohol intake due to the low enzymatic activity. On the other hand, *ALDH2* A/A homozygotes, characterized by a facial flushing after alcohol intake, may prevent them from heavy drinking.[12] Results from another meta-analysis suggested that the OR for *ALDH2* heterozygotes was not so high in moderate drinkers in the high-incidence regions of China (OR = 1.98).[29] We found a similar OR for *ALDH2* heterozygous among moderate-to-heavy drinkers in this high-risk Chinese population (OR=1.88, 95% CI: 1.26-2.80), as well as the interaction between *ALDH2* and alcohol drinking on both additive scale and multiplicative scale. However, inconsistent with some previous studies, a negative association was observed among never-to-light drinkers with heterozygous *ALDH2* gene, these might be attributed to the relatively low minor allele frequency in this high-risk study population. Another case-control study conducted in Jiangsu also reported similar findings.[38]

The joint effect of *ADH1B* and *ALDH2* on esophageal cancer has been reported by a few studies.[13,15,27,28,31] An increased risk was observed in alcohol drinkers who carried both *ALDH2* (rs671) A allele and *ADH1B* (rs1229984) G allele, as carrying these two alleles simultaneously indicates a longer exposure time to alcohol and highly-concentrated acetaldehyde.[28] It has been reported that 52% of alcoholic Japanese men with esophageal cancer have both the *ALDH2* A/G and the *ADH1B* G/G genotypes, and never reported alcohol flushing.[38] Yang et al. found that the OR for esophageal cancer among alcohol drinkers with both *ADH1B* G allele and *ALDH2* A allele was 9.86 (95% CI = 3.10-31.38).[28] Wu et al. reported that the increased risk of esophageal cancer was greater when subjects carried both *ADH1B* G/G and *ALDH2* A/G (OR = 36.79, 95%CI = 9.36-144.65), compared to those with *ADH1B* A/G or A/A and *ALDH2* G/G genotype.[40] In our study, we also found that moderate-to-heavy drinkers harboring the *ALDH2* A allele and *ADH1B* G allele were at the highest risk of esophageal cancer (OR=2.60, 95% CI=1.60-4.25). Similar increased risk was observed for the combination of the *ALDH2* A allele and *ADH1C* G allele (OR=2.06, 95% CI=1.01-4.20). However, no apparent gene-gene interactions were observed for

ALDH2 with either *ADH1B* or *ADH1C* polymorphism.

There are several limitations in this present analysis. Firstly, although the questionnaire had been pre-tested in previous studies, the self-reported exposure level of alcohol drinking may be vulnerable to subjective judgement and recall bias which could cause misclassification of exposures. However, the strength of the associations for esophageal cancer with alcohol consumption, particularly the dose-response trends indicate good validity and sensitivity of our study. Secondly, cases were in mixed histologies in this population-based study because of the low proportion of pathological examinations in less developed rural areas; however, more than 95% of esophageal cancer cases in China are esophageal squamous cell carcinoma (ESCC) according to previous reports.[20] Lastly, only subjects recruited after 2004 were involved in this study, whereas, we did not find marked difference between this study population and the population at large.

In conclusion, the present study found that genetic polymorphisms of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) are associated with the risk of esophageal cancer among Chinese alcohol drinkers. A substantially increased risk was observed among moderate-to-heavy drinkers carry the *ADH1B* and *ADH1C* G allele or *ALDH2*A allele. In addition, a strong gene-environmental interaction was observed between alcohol drinking and *ALDH2* rs671 polymorphism. Genetic predispositions, together with the variation in lifestyle factors may ultimately determine individual's risk of esophageal cancer in this high-risk Chinese population.

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Chapter 7

A large proportion of esophageal cancer cases and the incidence difference between regions are attributable to lifestyle risk factors in China

Ming Wu, Pieter van' t Veer, Zuo-Feng Zhang,
Xu-Shan Wang, Xiao-Ping Gu, Ren-Qiang Han,
Jie Yang, Xiao-Feng Zhang, Ai-Min Liu, Frans J Kok,
Ellen Kampman, Jin-Kou Zhao

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ABSTRACT

Esophageal cancer remains one of the most common cancers with striking geographic variation worldwide. This study aimed to quantify and compare the role of major risk factors by examining attributable risks in two populations at varying risk, and to explore what proportion of the risk gradient between areas could be explained by differences in the distribution of risk factors. A population-based case-control study was conducted between 2003-2007 in a high-risk area (Dafeng) and a low-risk area (Ganyu) of Jiangsu province, China. A total of 1,520 cases and 3,879 controls were recruited. The population attributable fraction (PAF) was calculated to quantify the etiology of risk factors. The relative attributable risk was applied to explore how much of the incidence difference between two areas is explained by variations in the distribution of risk factors. Results showed that smoking and alcohol drinking accounted for a PAF of 25.4% and 15.6% respectively. PAF of fast eating speed, hot eating/drinking, high intake of salty foods and family history of cancer was 21.6, 28.0, 12.5 and 9.7%. The combination of six lifestyle risk factors accounted for more than 60% of total cases. Moreover, the difference in the prevalence of eating raw garlic and family history of cancer accounted for 37.7% and 29.6% incidence difference between two counties. In conclusion, unhealthy lifestyles accounted for a high fraction of esophageal cancer in China. Dissimilar distribution of several lifestyle factors, together with hereditary variations may be largely responsible for the incidence difference between areas.

Key words: Esophageal neoplasms; Life style; Population attributable fraction (PAF); Relative attributable risk (RAR) ; Case-control studies; China

Introduction

Esophageal cancer is one of the most common cancers worldwide. Although the combination of screening and treatment is increasingly effective in reducing the mortality of this disease nowadays, survival rate is still very low, especially in developing countries.[1] Therefore, primary prevention through lifestyle interventions might stands for a good option for reducing the risk of esophageal cancer.

Many studies have been conducted to evaluate the role of lifestyle factors in the etiology of esophageal cancer. Extensive epidemiologic evidence shows that tobacco and alcohol use are major risk factors especially in Western counties.[2-4] Dietary factors are also important in esophageal carcinogenesis. Increased risks are associated with low intake of vegetables and fruits, high intake of carcinogens from pickled vegetables and fried foods, and injuries of esophageal mucosa e.g., fast eating speed and high temperature foods and beverages.[4-6] From a public health perspective, risk factors with a high risk ratio but a low prevalence may be relatively less important in the population context. On the contrary, factors having a moderate risk ratio but with a high prevalence can be associated with a substantial fraction of the population disease burden. Therefore, it is of importance not only to identify specific risk factors and their risk ratios for a disease, but also to quantify the risk attributable to specific factors in the population. The *Population attributable fraction* (PAF) is a useful method to measure the burden of disease that can be attributed to particular exposures in a whole community, and provides an important link between etiology and public health relevance. PAF is defined as the proportion of cases in a population that is attributable to one or more specific risk factors and, thus, could be prevented if those risk factors were eliminated.[7-9]

The geographic variation in esophageal cancer incidence is very striking worldwide, and even more marked in small geographic areas. However, relatively little attention has been paid to the fraction of the difference in risk between populations that may possibly be due to differences in the distribution of risk factors. Such a fraction was first discussed by Cornfield et al and was described as the *relative attributable risk* (RAR) by Breslow and Day.[10,11] The RAR was elegantly extended from PAF and can estimate how much of the

difference in incidence (or prevalence) between two populations can be explained by the difference in patterns of exposure to a particular risk factor or a group of risk factors, and thus reflects the extent to which the excess incidence between two populations could be reduced if they had the same distribution of risk factors.[12, 13]

Jiangsu province, in South-east China, is one of the highest cancer incidence areas but the mortality of esophageal cancer differs considerably between counties according to the results of previous surveys.[14,15] Since 2003, a population-based case-control study on cancer has been conducted simultaneously in a selected high-risk area (Dafeng) and a low-risk area (Ganyu). While these two counties are both less developed rural areas in northern Jiangsu, Dafeng has a 50% higher esophageal cancer incidence than Ganyu. In a recently published paper, we have shown the PAF among populations with and without a family history of esophageal cancer.[16] In this present analysis, we evaluated the role of major lifestyle risk factors and heredity factors on the PAF of esophageal cancer in two populations at varying risk respectively, and explored what proportion of the risk gradient between two areas could be explained by differences in the distribution of risk factors. The simultaneously evaluation of two populations at different risk may provide a potentially insightful approach in understanding both etiology and prevention of esophageal cancer.

Materials and Methods

Subject recruitment and data collection

This study has been previously described in detail.[16,17] In brief, a population-based case-control study was conducted in two counties of Jiangsu province, Dafeng and Ganyu from 2003 to 2007. Both of these counties are coastal, less developed rural areas in northern Jiangsu, with similar geophysical characteristics and population size (0.7 million in Dafeng and 1.1 million in Ganyu). However, there appears to be a significant difference in esophageal cancer incidence between two counties, the annual average age-standardized incidence during 2006-2008 by China standard population was 36 and 24 per 100,000 in Dafeng and Ganyu, respectively.

All newly diagnosed esophageal cancer patients in local inhabitants were eligible cases,

using the information from local population-based cancer registries. Because of the low proportion of pathological examination in rural areas (39% in average), patients who were diagnosed by endoscopic examination (40%) or radiology (11%) were also included. Although we were unable to determine the histological type of all cases, more than 95% of esophageal cancers in China are squamous cell carcinoma based on previous reports.[18] Eligible controls were randomly selected from the general population in the same county, frequency matched with cases by gender and age (±5 years). The participation rate of cases and controls was 68 and 87% in Dafeng, 75 and 85% in Ganyu, respectively.

This study was approved by the Institutional Review Board of Jiangsu Provincial Health Department. With written inform consent, epidemiological data were obtained by face-to-face interviews using a standardised questionnaire. The questionnaire included detailed information on known or potential risk factors of esophageal cancer, including demographic information, socio-economic status, living conditions, duration and amount of smoking, alcohol consumption, passive smoking of non-smokers, habitual dietary habits such as eating speed, eating/drinking of high or normal temperature foods/liquids and family history of any malignant cancer. Frequency and portion size of major foods intake was acquired by a pre-tested Food Frequency Questionnaire.[19]

Statistical analysis

Data were entered using Epidata 3.0, cleaned and analyzed using SAS v9.1 package. Unconditional logistic regression was applied for multivariate analyses. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were used to quantify the strength of associations. Confounders were selected based on the previous knowledge on esophageal cancer and our results of preliminary analysis,[20] including age, gender, education level, previous income, body mass index (BMI), pack-years of smoking, weekly intake of pure ethanol and study area (for pooled analysis). Pack-years of smoking and weekly consumption of ethanol in average were both calculated and categorized into three categories. Intake of red meat, fruits and vegetables were divided into quartiles based on the distribution among controls of both counties.

The population attributable fraction (PAF) was calculated by the method described by Bruzzi et al,[21] which allows estimating adjusted PAF in case–control studies. The 95% CI of PAF was calculated using the method based on the Bonferonni inequality.[22] For some potential protective factors e.g., consumption of raw garlic and intake of fruits and vegetables, the high exposure category was defined as reference in order to avoid a negative PAF. Based on the results of individual PAF estimation, several risk factors were combined to see their various joint effects, the PAF for a combination of risk factors is the proportion of the disease that can be attributed to any of the risk factors or their combinations.[23]

The relative attributable risk (RAR) and corresponding adjusted rate ratio (ARR) of particular risk factors were calculated as suggested by Lele and Whittemore,[13] briefly:

$$RAR = \frac{1-(1-PAF_H)(\sum_{j=1}^{J} q_j \quad _j)}{1-r} \qquad ARR = \frac{(1-PAF_H)(\sum_{j=1}^{J} q_j \quad _j)}{r}$$

where PAF_H is the PAF associated with the exposures in the high-risk population, q_j denotes the distribution of exposures among controls in the low-risk population in stratum j (j=1 denotes the referent category), ψ_j denotes the rate ratio for exposure category j relative to the referent category in the high-risk population, and r is the ratio of overall incidence between the two populations (I_{low}/ I_{high}). As denoted in Fig.1, RAR measures the fraction of excess incidence in the high-risk population that would vanish if it had the same distribution of risk factors as the low-risk population. ARR is the rate ratio that would be observed (I_{high} / I_{low}) if the two populations had the same distribution of risk factors. Sometimes a negative RAR might be observed in practice. A negative RAR means that the prevalence of this risk factor is higher in the low-risk area, and the incidence difference would be larger if the distribution of this factor in high-risk area more closely resembled that of the low-risk area. Negative RAR does not explain the higher incidence of disease in the high-risk area.

As mentioned previously, the average age-standardized incidence rate was 36 and 24 per 100,000 in Dafeng and Ganyu, respectively. Therefore, in this analysis, the risk ratio of high- relative to low-risk area was 36/24 = 1.50, and r = 24/36 = 0.67.

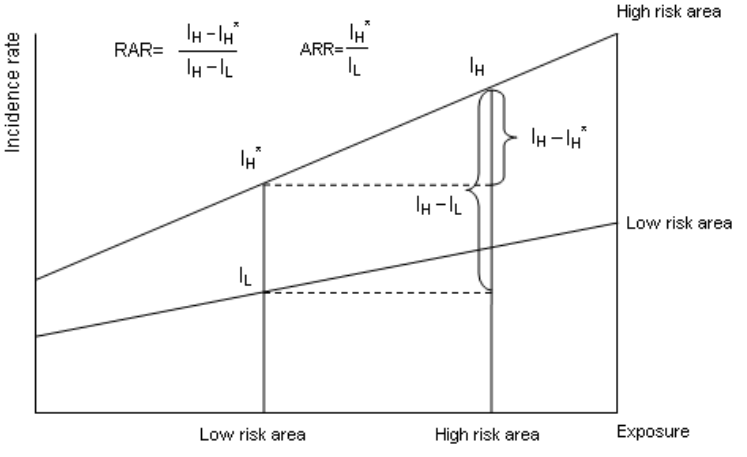

Fig. 7-1 The relative attributable risk (RAR) and adjusted rate ratio (ARR). Let I_H denotes the incidence rate in the high risk population, I_L is the rate in the low–risk population, and I_H* is the hypothetical rate that would prevail in the high-risk population if its exposure prevalence equalled that of the low-risk population, then $RAR=(I_H - I_H^*)/(I_H - I_L)$, $ARR=I_H^*/I_L$.

Results

Table 7-1 describes the demographic information and socio-economics status of study subjects by county. In both counties, cases were more likely to be male and older, and more frequently occurred in the population with lower socio-economic status, i.e. lower education level, lower previous income and lower BMI.

Table 7-2 shows the association between risk factors and esophageal cancer. Consistent in both counties, smoking and alcohol drinking significantly increased the risk of esophageal cancer, positive dose-response relationships were observed with increased pack-years of smoking and weekly consumption of ethanol (*P* for trend <0.001 each). Passive smoking also elevated risk among non-smokers (OR = 1.25, 95%CI: 1.00, 1.57) but the OR was significant only in Ganyu. Unhealthy dietary habits were associated with

Table 7-1 Demographic Information and Socio-economic Status of Cases and Controls

	Dafeng (High)		Ganyu (Low)		
	Case (%) (N=637)	Control (%) (N=1,938)	Case (%) (N=883)	Control (%) (N=1,941)	P Value[1]
Gender					
Male	426 (66.9)	1,368 (70.6)	765 (86.6)	1,548 (79.8)	*<0.01*
Female	211 (33.1)	570 (29.4)	118 (11.4)	393 (20.2)	
Age (years)					
Mean (SD)	65.4 (9.0)	63.6 (11.0)	66.0 (9.9)	65.1 (11.3)	
<50	26 (4.1)	227 (11.7)	42 (4.8)	165 (8.5)	
50~	131 (20.6)	401 (20.7)	201 (22.8)	426 (22.0)	*<0.01*
60~	266 (41.8)	681 (35.1)	281 (31.8)	563 (29.0)	
70~	177 (27.8)	525 (27.1)	288 (32.6)	632 (32.6)	
≥80	37 (5.8)	104 (5.4)	71 (8.0)	155 (8.0)	
Education level					
Illiteracy	338 (53.1)	827 (42.7)	558 (63.3)	1,230 (63.4)	*<0.01*
Primary school	214 (33.6)	703 (36.3)	230 (26.1)	467 (24.1)	
Middle school & above	85 (13.3)	407 (21.0)	94 (10.7)	243 (12.5)	
Previous Income (RMB)					
Mean (SD)	1,792(2026)	2,293 (2366)	1,493(1812)	1,773 (1953)	
<1000	148 (23.3)	266 (13.8)	313 (36.2)	632 (33.4)	*<0.01*
1000~	123 (19.3)	353 (18.3)	190 (22.0)	394 (20.8)	
1500~	194 (30.5)	589 (30.6)	203 (23.5)	444 (23.4)	
≥2500	171 (27.0)	718 (37.3)	158 (18.3)	424 (22.4)	
Body Mass Index (BMI)[2]					
Mean (SD)	20.8 (3.7)	22.7 (6.0)	22.2 (4.0)	22.9 (3.6)	
<18.5	162 (25.5)	174 (9.0)	78 (8.9)	104 (5.4)	0.27
18.5~23.9	381 (60.0)	1,175(60.7)	636 (72.8)	1,318 (68.2)	
24~27.9	73 (11.5)	471 (24.3)	125 (14.3)	431 (22.3)	
≥28	19 (3.0)	115 (5.9)	35 (4.0)	80 (4.1)	

[1] P Value for comparing control groups between two counties.[2] Chinese recommend standard was used for the cut-off points of overweight and obesity .[24]

Table 7-2 The Association of Major Risk Factors with Esophageal Cancer in High- and Low Risk Areas of Jiangsu, China

China	Both centers		Dafeng (High)		Ganyu (Low)	
	Case/Control	OR (95% CI)[1]	Case/Control	OR (95% CI)[1]	Case/Control	OR (95% CI)[1]
Smoking						
Never smoking	415/1,549	1.00	200/817	1.00	215/732	1.00
<30 pack-years	614/1,406	1.38 (1.17-1.63)	187/554	1.23 (0.96-1.57)	427/852	1.40(1.10-1.76)
≥30 pack-years	491/924	1.84 (1.53-2.20)	250/567	1.65 (1.28-2.12)	241/357	1.86(1.42-2.44)
P for trend		*<0.001*		*<0.001*		*<0.001*
Passive smoking among non-smokers						
No	216/900	1.00	110/473	1.00	106/427	1.00
Yes	199/649	1.25 (1.00-1.57)	90/344	1.00 (0.72-1.38)	09/305	1.55(1.11-2.14)
Alcohol drinking (Pure ethanol)						
Never	624/1,929	1.00	301/970	1.00	323/959	1.00
1~499 ml/week	323/850	1.16 (0.98-1.38)	140/454	1.05 (0.82-1.35)	183/396	1.24(0.99-1.56)
≥500 ml/week	558/1,040	1.59 (1.36-1.86)	196/508	1.44 (1.12-1.84)	362/532	1.74(1.41-2.14)
P for trend		*<0.001*		*<0.001*		*<0.001*
Fast eating speed						
Normal	958/3,055	1.00	357/1,471	1.00	601/1,58	1.00
Fast	562/824	2.40 (2.09-2.76)	280/467	2.74 (2.24-3.35)	4282/357	2.13(1.75-2.58)
Hot eating/drinking						
Normal	605/2,146	1.00	303/1,056	1.00	302/1,090	1.00
Hot	808/1,635	1.75 (1.54-1.99)	291/826	1.19(0.98-1.44)	517/809	2.39(2.00-2.85)
Extremely hot	107/96	4.04 (2.98-5.47)	43/56	2.78 (1.79-4.32)	64/40	5.65(3.67-8.70)
P for trend		*<0.001*		*<0.001*		*<0.001*
Salty foods						
Less frequent	893/2,624	1.00	423/1,317	1.00	470/1,307	1.00
Normal	549/1,128	1.38 (1.21-1.58)	176/555	0.98 (0.80-1.21)	373/573	1.77(1.48-2.11)
Frequent	78/124	1.99 (1.46-2.72)	38/66	1.91(1.24-2.96)	40/58	2.01(1.29-3.14)
P for trend		*<0.001*		*<0.001*		*<0.001*
Fried foods						
Normal	1,027/2,825	1.00	576/1,699	1.00	451/1,126	1.00
High	493/1,054	1.37 (1.18-1.58)	61/239	1.02 (0.75-1.40)	432/815	1.47(1.24-1.74)

Table 7-2 The Association of Major Risk Factors with Esophageal Cancer in High- and Low Risk Areas of Jiangsu, china (Continued)

China	Both centers		Dafeng (High)		Ganyu (Low)	
	Case/Control	**OR (95% CI)[1]**	**Case/Control**	**OR (95% CI)[1]**	**Case/Control**	**OR (95% CI)[1]**
Raw garlic consumption						
≥2times week	148/441	1.00	10/36	1.00	138/405	1.00
<2times week	640/1,471	1.26 (1.02-1.57)	100/398	1.38 (0.51-2.28)	540/1,073	1.39 (1.10-1.75)
Never	731/1,957	1.37 (1.08-1.74)	527/1,496	1.46 (0.67-2.87)	204/461	1.29 (0.99-1.69)
P for trend		*0.019*		*0.066*		*0.097*
Red meat intake[2]						
Q1 (Lowest)	369/905	1.00	134/356	1.00	235/549	1.00
Q2	356/968	1.01 (0.84-1.20)	164/545	0.92 (0.70-1.20)	192/423	1.09 (0.86-1.38)
Q3	406/972	1.18 (0.99-1.40)	189/553	1.07 (0.82-1.41)	217/419	1.27 (1.01-1.60)
Q4 (Highest)	364/974	1.13 (0.94-1.36)	142/467	1.16 (0.87-1.56)	222/507	1.17 (0.92-1.49)
P for trend		*0.116*		*0.158*		*0.143*
Fruit&vegetable[2]						
Q4 (Highest)	354/963	1.00	78/310	1.00	276/653	1.00
Q3	371/963	1.10 (0.92-1.31)	175/509	1.26 (0.92-1.73)	196/454	0.98 (0.78-1.22)
Q2	392/963	1.18 (0.99-1.41)	191/554	1.26 (0.92-1.71)	201/409	1.14 (0.91-1.44)
Q1 (Lowest)	392/962	1.11 (0.93-1.34)	193/564	1.14 (0.83-1.56)	199/398	1.15 (0.91-1.45)
P for trend		*0.226*		*0.733*		*0.182*
Family cancer history *(First degree relatives)*						
No	1,136/3,118	1.00	81/1,318	1.00	755/1,800	1.00
Yes	384/761	1.62 (1.39-1.89)	256/620	1.47 (1.22-1.79)	128/141	2.02 (1.54-2.63)

[1] Adjusted for age (continuous), gender, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous), weekly ethanol consumption (continuous) and study center, when appropriate.[2]Variable was divided into 4 categories using the quartiles of combined control groups of the two counties.

increased risk in both counties: fast eating speed (OR = 2.40, 95%CI: 2.09, 2.76), eating/drinking hot foods/liquids (OR = 4.04 for the highest group), frequently eating salty foods (p for trend <0.001) and fried foods (OR = 1.37, 95%CI: 1.18, 1.58). Raw garlic consumption was inversely associated with risk of esophageal cancer; those who never ate raw garlic had a 1.37- fold risk as compared to those who ate raw garlic more than twice per week. A weak positive association was observed between esophageal cancer and high red meat and low fruits and vegetables intake. In addition to lifestyle risk factors, we observed that family cancer history in first-degree relatives increased OR in both counties.

Table 7-3 presents the individual PAF of each risk factor with both pooled and county specific results. Results indicate that ingestion of hot foods/liquids was the most important contributor of esophageal cancer with a PAF of 28.0%. Smoking and fast eating speed accounted for 25.4% and 21.6% of total cases, respectively. Alcohol drinking, family cancer history, high intake of salty foods and fried foods, and diet rich in red meat caused modest PAF with a range of 7.4-15.6%. A weak PAF was observed for passive smoking, never ate raw garlic and diet low in fruits and vegetables but not statistically significant.

Looking into separate counties, we found that in Dafeng, eating quickly had the highest PAF of 27.9%, while in Ganyu, PAF for hot eating/drinking was the highest (PAF= 40.0%). Smoking accounted for 20.0% and 25.7% of cancer cases in Dafeng and Ganyu respectively. Passive smoking also contributed 18.0% to the excess risk among non-smokers in Ganyu but not in Dafeng. The PAF of alcohol drinking in Ganyu was 20.6% and higher than that in Dafeng (9.4%), while high intakes salty foods and fried foods explained 20.6% and 15.6% of cases respectively, in Ganyu but very little in Dafeng. Moreover, family cancer history accounted for about 10% of cases in both counties.

The RAR and ARR of each risk factor are shown in Table 7-3 as well. The RAR of never eating raw garlic was the highest among all risk factors (RAR = 37.7%) with a corresponding ARR of 1.31, meaning that 37.7% of the difference in esophageal cancer incidence between the two risk regions could be explained by the unequal distribution of raw garlic consumption, the rate ratio of Dafeng relative to Ganyu would decline from 1.50 to 1.31 if the distribution in the two counties were similar. Family history of cancer was the

Table 7-3 Individual Population Attributable Fraction (PAF), Relative Attributable Risk (RAR) and Adjusted Rate Ratio (ARR) of Risk Factors

	Both centers		Dafeng (High)		Ganyu (Low)			High vs. Low	
	Case (%)	PAF (95% CI)[1]	Case (%)	PAF (95% CI)[1]	Case (%)	Cont[2] (%)	PAF (95% CI)[1]	RAR (%)	ARR
Smoking	72.7	25.4 (15.7-34.2)	68.6	20.0 (5.8-32.6)	75.6	62.3	25.7 (10.6-38.6)	10.7	1.45
Passive smoking[3]	48.0	9.6 (-1.5-20.5)	45.0	-0.2 (-17.1-16.4)	50.7	41.7	18.0 (3.0-32.3)	0.0	1.50
Alcohol drinking	58.5	15.6 (7.7-23.0)	52.7	9.4 (-2.1-20.2)	62.8	49.2	20.6 (9.7-30.5)	-4.7	1.52
Fast eating speed	37.0	21.6 (17.5-25.6)	44.0	27.9 (21.4-34.3)	31.9	18.4	16.9 (11.8-22.1)	14.6	1.43
Hot eating/drinking	60.2	28.0 (22.0-33.8)	52.4	11.7 (1.8-21.2)	65.8	43.8	40.0 (32.4-47.0)	4.1	1.48
Salty foods intake	41.2	12.5 (7.4-17.5)	33.6	2.4 (-4.9-9.8)	46.8	32.6	20.6 (13.8-27.3)	0.6	1.50
Fried foods	32.4	8.7 (4.0-13.4)	9.6	0.2 (-2.8-3.9)	48.9	42.0	15.6 (7.8-23.1)	-2.4	1.51
Never ate raw garlic[4]	48.1	5.3 (-2.5-12.8)	82.7	18.0 (-2.6-35.0)	23.1	23.8	0.0 (-5.1-5.4)	37.7	1.31
Red meat[5]	51.5	7.4 (0.4-14.2)	52.6	7.7 (-3.2-18.0)	50.7	48.8	7.0 (-2.4-16.0)	0.0	1.50
Vegetable & fruit[5]	52.0	4.1 (-3.1-11.2)	60.3	1.4 (-12.1-13.9)	45.9	42.2	5.9 (-2.4-14.0)	1.2	1.49
Family cancer history	25.2	9.7 (6.0-13.4)	40.2	12.9 (5.5-20.3)	14.5	7.3	7.3 (3.9-10.9)	29.6	1.35

[1] Adjusted for age (continuous), gender, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous) , weekly ethanol consumption (continuous) and study center, when appropriate.[2]The prevalence of exposure among control group in low-risk area was used for RAR calculation.[3] Passive smoking among non-smokers.[4] Never and ever eating garlic was used for the analysis, given the few numbers of eating raw garlic more than 2 times/week. [5]Subjects were divided into two groups: Q1+Q2 vs. Q3+Q4.

Table 7-4 Population Attributable Fraction (PAF), Relative Attributable Risk (RAR) and Adjusted Rate Ratio (ARR) for the Combination of Risk factors.

	Both centers		Dafeng (High)		Ganyu (Low)			High vs. Low	
	Case (%)	PAF (95% CI)[†]	Case (%)	PAF (95% CI)[1]	Case (%)	Cont. (%)[2]	PAF (95% CI)[1]	RAR (%)	ARR
Smoking									
+ Alcohol drinking	80.2	31.4 (19.9-41.4)	76.1	16.1 (-2.8-32.1)	83.2	71.3	40.9 (25.0-53.8)	0.2	1.50
+ Fast eating speed	82.2	43.6 (34.4-51.8)	80.7	40.4 (26.2-52.3)	83.4	68.2	43.0 (28.8-54.7)	-1.0	1.50
+ Hot eating/drinking	89.1	50.8 (40.4-59.6)	85.2	36.5 (18.8-50.9)	92.0	78.0	61.4(48.0-71.8)	-1.8	1.51
+ Salty foods	83.4	36.4 (25.7-45.7)	78.5	21.9 (4.5-36.7)	87.0	73.1	46.4 (32.0-58.1)	-0.6	1.50
+ Raw garlic[3]	88.2	29.7 (15.0-42.2)	96.1	41.6 (5.4-64.4)	82.6	73.3	19.3 (0.9-34.7)	26.7	1.37
+ Family cancer history	79.0	32.3 (22.2-41.3)	81.3	32.9 (17.0-46.4)	77.3	64.3	27.3 (12.0-40.2)	10.7	1.45
Alcohol drinking									
+ Fast eating speed	72.0	34.0 (25.3-40.8)	70.6	32.0 (19.7-43.0)	72.9	57.2	33.7 (22.6-43.6)	-0.7	1.50
+ Hot eating/drinking	82.1	33.6 (23.1-42.9)	76.3	14.8 (-3.2-30.3)	86.2	72.0	47.8 (34.9-58.6)	0.1	1.50
+ Salty foods	74.6	25.3 (15.7-34.1)	67.5	12.3 (-2.5-25.6)	79.8	65.4	36.9 (24.3-47.9)	-1.4	1.51
+ Raw garlic[3]	81.5	21.6 (9.1-32.7)	92.8	22.8(-10.9-46.8)	73.2	63.6	16.5 (2.6-28.8)	20.4	1.40
+ Family cancer history	68.9	24.1(15.5-32.0)	72.2	23.6 (9.6-36.1)	66.5	47.9	23.1 (11.7-33.3)	17.5	1.41
Fast eating speed									
+ Hot eating/drinking	72.2	40.7 (34.2-46.8)	72.2	35.5 (24.4-45.5)	72.2	51.7	44.0 (35.8-51.6)	9.7	1.45
+ Raw garlic[3]	66.6	26.7 (18.6-34.2)	91.7	44.6 (23.6-60.4)	48.4	38.2	17.1 (9.7-24.4)	73.6	1.13
+ Salty foods	59.1	27.9 (21.9-33.6)	59.3	25.5 (15.9-34.5)	58.9	41.5	29.8 (22.0-37.1)	6.6	1.47
+ Family cancer history	51.2	28.2 (23.0-33.2)	65.5	36.6 (27.5-45.1)	40.9	24.1	22.4 (16.5-28.2)	51.8	1.24
Hot eating/drinking									
+ Raw garlic[3]	81.6	40.8 (31.6-48.9)	92.3	23.0 (-9.2-46.2)	73.8	56.7	39.9 (30.6-48.4)	25.5	1.37
+ Salty foods	72.4	31.8 (24.2-39.0)	69.2	15.3 (1.4-27.9)	74.6	56.7	42.6 (33.5-50.8)	5.0	1.47
+ Family cancer history	72.0	39.7 (33.1-46.0)	72.4	26.6 (13.8-38.2)	71.7	47.4	46.7 (39.0-53.8)	19.1	1.40

Table 7-4 Population Attributable Fraction (PAF), Relative Attributable Risk (RAR) and Adjusted Rate Ratio (ARR) for the Combination of Risk factors (continued)

	Both centers		Dafeng (High)		Ganyu (Low)			High vs. Low	
	Case (%)	PAF (95% CI)[†]	Case (%)	PAF (95% CI)[1]	Case (%)	Cont. (%)[2]	PAF (95% CI)[1]	RAR (%)	ARR
Never ate raw garlic[3]									
+ Salty foods	70.4	19.2 (9.6-28.0)	88.2	10.9(-18.1-33.4)	57.5	48.4	17.6 (8.6-26.2)	15.5	1.42
+ Family cancer history	57.2	12.8 (4.2-20.8)	88.8	20.4 (-6.0-40.8)	34.4	29.3	7.4 (1.2-13.7)	40.4	1.29
Salty foods									
+ Family cancer history	57.8	24.6 (18.4-30.5)	60.8	16.4 (5.2-26.9)	55.7	37.0	29.3 (22.1-36.2)	14.8	1.42
All lifestyle factors[4]	98.0	62.6 (41.6-76.3)	-	-	-	-	-	-	-

[1]Adjusted for age (continuous), gender, education level, previous income (continuous), BMI (continuous), pack-years of smoking (continuous), weekly ethanol consumption (continuous) and study center, when appropriate. [2]The prevalence of exposure among control group in low-risk area was used for RAR calculation.[3] Never and ever eating garlic was used for the analysis, given the few numbers of eating raw garlic more than 2 times/week. [4] All lifestyle risk factors mentioned in Table 7-4 (excluding family cancer history). Given the few number in unexposed group, county specific PAF and corresponding RAR were not calculated.

second most important factor contributing to the incidence difference (RAR = 29.6%, ARR = 1.35). Differences in the distribution of smoking and fast eating speed could also explain 10.7% and 14.6% of the incidence gradient, respectively. A negative RAR was observed for some variables such as alcohol drinking and intake of fried foods, indicating that they did not explain the incidence difference between counties.

Based on the results of individual estimations, risk factors with either PAF or RAR above 20% were selected and combined to see their various joint effects, with results summarized in Table 7-4. Most often when two risk factors were combined, their joint PAFs were higher than the individual PAF of each risk factor, but less than the sum of individual factors. The joint exposure of smoking and eating/drinking hot foods/liquids accounted for 50.8% of total cases in the two counties, and could further explain more than 60% cases in Ganyu, however, the corresponding RAR was negative, thus was unable to explain the risk difference between regions. Fast eating speed plus never eating raw garlic has the highest PAF in Dafeng (PAF= 44.6%), moreover, difference in the joint distribution of these two factors accounted for more than 70% of the risk gradient between two counties (RAR = 73.6%, ARR = 1.13). Family cancer history plus either fast eating speed or never eating raw garlic also explained 51.8% and 40.4% of the incidence difference, respectively. The PAF of the combination of all six lifestyle risk factors mentioned in Table 7-4 was 62.6%. County specific PAF for combination of all lifestyle risk factors was not calculated given the very few numbers in unexposed group.

Discussion

The present population-based case-control study, one of the largest studies on esophageal cancer, found that more than 60% of total cases could be attributable to lifestyles risk factors. Consistent with other studies, the present analysis also confirmed that smoking, alcohol consumption and unhealthy dietary factors such as fast eating speed, eating/drinking hot foods/liquids play important roles in the carcinogenesis of esophageal cancer in the Chinese population. Moreover, between high- and low- risk regions, individual and joint variations on the distribution of some lifestyle risk factors accounted

for large fraction of the incidence gradients, such as fasting eating speed, hot eating/drinking and raw garlic consumption. This indicates that a substantial reduction of esophageal cancer could be achieved by eliminating certain lifestyle risk factors, especially in the high-risk area.

Dietary factors were found to be important in the etiology of esophageal cancer in our study. Fast eating speed and eating/drinking hot foods/liquids strongly increased the risk of esophageal cancer and accounted for the highest PAF in Dafeng and Ganyu, respectively. Both eating quickly and frequent ingestion of hot foods and drinks are common physical stimulations to the esophagus and can lead to chronic esophagitis, which has been considered the earliest tissue perturbation in the progression of malignant transformation of the squamous epithelium.[33] Moreover, irritants may increase cell turnover, which could then increase contact between carcinogens and dividing target cells in the esophagus.[4] We found that the joint PAF of smoking plus either fast eating speed (43.6%) or hot eating/drinking (50.8%) was one of the highest PAF combinations of two risk factors, suggesting that these factors might act jointly, causing a high fraction of esophageal cancer in China.

In our study, diets rich in salty foods and fried foods were positively associated with esophageal cancer occurrence. There is evidence that salty foods and fried foods contain carcinogens such as N-nitroso compound and heterocyclic amines (HCAs).[5,34] However, high intake of fried foods only accounted for a PAF of less than 10% because of its relatively low prevalence. We also observed a weak association between esophageal cancer and high intake of red meat and low intake of fruits and vegetables; their PAFs and RARs were relatively low due to small odds ratios. The potential mechanisms that could possibly explain a positive association with red meat include the generation of N-nitroso compounds, production of free radicals by heme iron and free iron in the meat.[5] Several studies have suggested that fruit and vegetable intake may reduce the risk of esophageal cancer, but results remain inconsistent, such inconsistencies may result from differences in the types of fruits and vegetables or in their methods of preparation.[5]

Never eating raw garlic was observed to be associated with an increased risk of esophageal cancer. Garlic contains high levels of flavonols and organosulfur compounds

and may therefore inhibit the initiation and promotion processes of carcinogenesis.[35] Some epidemiologic studies have reported an inverse association between garlic intake and esophageal cancer risk.[36,37] A previous study found that in the low-epidemic area of esophageal cancer in Jiangsu, diets are usually rich in garlic and other allium vegetables.[38] In agreement with this finding, we found that the different prevalence of raw garlic consumption accounted for 37.7% of the incidence difference between the two risk regions. The joint distribution of never eating raw garlic and fast eating speed can also explain more than 70% of the total risk gradient, suggesting that a major reduction of esophageal cancer in the high-risk area could be expected if it had a similar distribution as the low-risk area regarding these two factors.

Although family history of cancer is an unmodifiable risk factor and accounted for about 10% of esophageal cancer cases, the increased PAF of lifestyle risk factors among people with a family cancer history indicates that it is vital to advocate a healthy lifestyle in those with genetic predispositions. Moreover, about 30% of the incidence difference between two regions could be attributed to the different distribution of family cancer history, and the difference in joint exposure of family cancer history with fast eating speed and raw garlic consumption explaining 51.8% and 40.4% of the risk gradient, respectively. This suggests that differences in genetic susceptibility may also be important in the risk difference between regions. Variations in both lifestyle and hereditary factors ultimately determine individual risk of cancer, and may be the main reason for the large difference in esophageal cancer risk between different populations.

Several methodological issues warrant discussion. Firstly, to improve readability, joint PAFs of the various combinations of three to five risk factors were not presented, but we can expected that the combinations of more risk factors will have a larger PAF because of the broad definition of exposure. The PAF will always increase with more risk factors provided that newly included individuals under the broader definition have a relative risk for disease greater than 1.0 when compared to the remaining unexposed group.[23] However, there may be a loss of precision since the standard error of the PAF increases.[39] Furthermore, the corresponding RAR will decrease since the difference in exposure

distribution between regions will diminish (more exposed and less unexposed subjects in both regions). Secondly, the PAF for the combination of risk factors is usually less than the sum of the PAFs individually since a diseased case can simultaneously be exposed to several risk factors and therefore, be counted more than once. Another important reason is that in the logistic model, the OR for a combined exposure is approximately multiplicative for the individual ORs, thereby exceeding the OR for the additive excess risk model. It is possible for the sum of the individual PAFs to exceed 100%, although an individual PAF will never exceed that value.[40,41]

There are some limitations to this present analysis. First, the calculation of PAF and RAR assumes cases and controls are reasonably representative for the population; however, potential selection bias may exist in any case-control study. A population-based study design, a random control selection method and a high response rate of cases and controls enabled us to minimize selection bias in this study. Second, additive or multiplicative interactions may exist between different risk factors, and will influence the OR and PAF when combining risk factors. We did not look into interactions in detail as our main purpose in this analysis was to examine the proportion of disease that can be explained by a single or a set of risk factors. Thirdly, one of the major assumptions for PAF calculation is the casual association between exposures and the outcome of interest, however there is limited evidence for causality of some risk factors, e.g., fast eating speed and eating garlic, therefore some of the results should be interpreted with caution. Lastly, although the questionnaire had been pre-tested in previous studies, the exposure level of most lifestyle risk factors were reported by study subjects without accurate measurements, and thus subjective judgement and recall bias may exist in this retrospective study and may cause non-differential misclassification of exposures.

Despite these limitations, our study indicates that lifestyle risk factors e.g. smoking, alcohol drinking, fast eating speed and eating/drinking hot foods/liquids account for the majority of esophageal cancer cases in a Chinese population. Differences in the distribution of several lifestyle factors such as garlic consumption and fast eating speed, together with the variation of hereditary factors may be the main reason causing large differences in

esophageal cancer risk between high- and low-risk areas in China. A substantial proportion of cases in the population could be prevented by eliminating or avoiding these lifestyle risk factors through corresponding health education and health promotion methods.

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Chapter 8

General Discussion

In this large population-based case-control study, we explored the role of major lifestyle factors such as tobacco smoking, alcohol drinking and dietary factors, as well as hereditary determinants including family history of cancer and genetic polymorphisms of alcohol-metabolizing related genes on the development of esophageal cancer (EC) in Jiangsu Province, one of the high incidence regions in China. Moreover, we simultaneously evaluated the attributable fractions of major risk factors in two counties with large incidence difference, to investigate how much of the risk gradient could be explained by variation in their distributions. It aims to shed further light on both etiology and prevention of EC in the high-risk areas of China. In this chapter, the main findings of this thesis are summarized, the major epidemiological considerations and public health implications of this study are discussed, and recommendations for future research are given.

MAIN FINDINGS

The main findings in this thesis are summarized in Table 8-1. In agreement with many epidemiologic studies,[1-5] we found that unhealthy lifestyle factors such as smoking, alcohol drinking and some dietary factors are positively associated with the risk of EC, while heredity factors also play a role in esophageal carcinogenesis and could modify the effects of some lifestyle risk factors.

Unlike in Western countries, smoking and alcohol appear to be less strongly associated with EC in China.[6,7] Our results confirmed that these two well-known risk factors moderately increased the risk of EC in China **(Chapters 2 and 3)**, and the positive associations were only found among Chinese men but not among Chinese women **(Chapter 3)**. Moreover, smoking and alcohol could explain more than 90% of EC cases in western populations,[1] while their population attributable fraction (PAF) is estimated to be only 25% and 16% in our study, indicating there might be other strong determinants causing the high risk of EC in China **(Chapters 5 and 7)**.

Consistent with previous studies, dietary factors were found to play important roles in the development of EC in this study.[1,2] Specific dietary habits i.e., fast eating speed, and hot

eating and/or drinking substantially elevated EC risk and could explain more than 20% of EC cases each. High intake of salty foods and fried foods, low consumption of raw garlic are also found to increase the risk of EC **(Chapters 2, 5 and 7)**. Although it has been suggested in previous studies that a diet rich or poor in certain food groups is associated with EC occurrence, e.g., high intake of red meat and low intake of fruits and vegetables,[2] no significant association was found for the majority of foods intake with EC in this Chinese population **(Chapter 2)**.

Green tea is one of the most frequently consumed beverages in China, and previous epidemiological studies have suggested that the consumption of green tea may help prevent EC.[8,9] In the case-control study described in this thesis, however, no protective effect was observed. On the other hand, drinking tea at high temperature significantly increased the risk of EC development **(Chapter 4)**.

In addition to environmental and lifestyle factors, we confirmed that a positive family history can significantly increase EC risk. Individuals with a positive family history of EC, especially among first-degree relatives, have a higher risk of getting the disease. Moreover, inheritance may also modify the risk of unhealthy lifestyles, i.e., a significant super-additive interaction was found for family history of EC with fast eating speed and diets low in fruits and vegetables **(Chapter 5)**.

As alcohol consumption has been confirmed to be positively associated with EC in our study **(Chapter 3)**, we further explored the relationship between EC and genetic polymorphisms of several alcohol-metabolizing related genes, including alcohol dehydrogenases (ADHs) *ADH1B* (rs1229984), *ADH1C* (rs698) and aldehyde dehydrogenase 2 (*ALDH2*, rs671). Results showed that the slow metabolizing *ADH1B G* allele, *ADH1C* G allele and *ALDH2* A allele significantly increased the risk of EC among moderate-to-heavy alcohol drinkers, and a significant interaction was observed between *ALDH2* polymorphism and alcohol consumption **(Chapter 6)**.

Table 8-1 Summary of the main results in this thesis regarding the OR, population attributable fraction (PAF) of major risk factors, and the corresponding relative attributable risk (RAR)

Risk factors	Exposure	OR (95% CI)	PAF (95% CI)	RAR(%)	Chapter
Smoking	Ever vs. Never	1.57 (1.34-1.83)	25.4(15.7-34.2)	10.7	2, 3, 5, 7
Men		1.74 (1.44-2.09)			
Women		1.13 (0.83-1.54)			
Alcohol drinking	Ever vs. Never	1.50 (1.29-1.74)	15.6 (7.7-23.0)	-4.7	2, 3, 5, 7
Men		1.76 (1.48-2.09)			
Women		0.82 (0.59-1.16)			
Fast eating speed	Fast eating	2.40 (2.09-2.76)	21.6 (17.5-25.6)	14.6	2, 3, 5, 7
Hot eating/ drinking	Hot Extremely hot	1.75 (1.54-1.99) 4.04 (2.98-5.47)	28.0 (22.0-33.8)	4.1	2, 3, 4, 5, 7
Raw garlic intake	Never vs. Ever	1.37 (1.08-1.74)	5.3 (-2.5-12.8)	37.7	2, 5, 7
Fried foods	High vs. Normal	1.37 (1.18-1.58)	8.7 (4.0-13.4)	-2.4	2, 5, 7
Red meat	Q4 vs. Q1	1.17 (0.92-1.49)	7.4 (0.4-14.2)	0.0	5, 7
Fruits and vegetables	Q4 vs. Q1	1.51 (0.91-1.45)	4.1 (-3.1-11.2)	1.2	5, 7
Staple foods	High vs. Low	NS[1]	NE[2]	NE[2]	2
Soybean	High vs. Low	NS[1]	NE[2]	NE[2]	2
Eggs	High vs. Low	NS[1]	NE[2]	NE[2]	2
Preserved foods	High vs. Low	NS[1]	NE[2]	NE[2]	2
Fish and seafood	Q4 vs. Q1	1.91(1.00-3.64)[3]	NE[2]	NE[2]	2
Green tea drinking	Ever vs. Never	DF:1.0 (0.7-1.3)[4] GY:1.3 (0.9-1.7)[4]	NE[2]	NE[2]	4
Family history of EC (First-degree relatives)	Yes vs. No	1.64 (1.40-1.92)	9.7 (6.0-13.4)	29.6	2, 5, 7
ADH1B	G allele	1.89(1.40-2.55)[5]	NE[2]	NE[2]	6
ADH1C	G allele	1.73(1.15-2.62)[5]	NE[2]	NE[2]	6
ALDH2	A allele	1.76(1.20-2.60)[5]	NE[2]	NE[2]	6

[1.] NS - Not statistically significant. [2.] NE - Not evaluated. [3.] Positive association was observed only in Dafeng. [4.] DF- Dafeng; GY-Ganyu. [5.] OR for moderate/heavy alcohol drinkers vs. never/light drinkers.

Although both Dafeng and Ganyu are less developed rural areas in northern Jiangsu with similar geophysical characteristics, there is a 50% risk incidence gradient for EC between the two counties. Lastly, we evaluated the role of major lifestyle and heredity risk factors on the attributable fraction of EC in these two counties separately, and explored

what proportion of the risk gradient between the two areas could be explained by differences in the distribution of risk factors. Results showed that more than 60% of EC cases could be attributable to major lifestyle risk factors; furthermore, dissimilar distribution of several lifestyle factors such as raw garlic consumption and fast eating speed, together with variations of hereditary factors may be largely responsible for the incidence difference between two study areas **(Chapter 7)**.

EPIDEMIOLOGICAL CONSIDERATIONS

Being a retrospective observational study, this case-control study is subject to epidemiologic considerations regarding internal and external validity that need to be taken into account, such as representativeness of cases and controls, exposure measurement, confounding, bias and generalizability. Most epidemiologic issues and limitations for individual studies have been separately discussed in previous chapters. This section integrates and discusses relevant considerations for the whole thesis.

Internal validity

Selection of cases and controls

In our study, all newly diagnosed esophageal cancer patients identified by local population-based cancer registries were eligible to be included as cases. In both Dafeng and Ganyu, the cancer registries are attached to local Center for Disease Control and Prevention (CDCs) and were established in the late 1990s. For this study, a rapid reporting system to identify newly diagnosed cancer cases was created, each EC patient was reported by the local hospitals to the CDC shortly after diagnosis, after which staff in CDC organized face-to-face interviews and sample collections, either in hospitals or at the home of subjects. We were not able to recruit all eligible cases because: 1) Some cases were treated outside the county; 2) Some cases refused to participate in this study because of blood sampling and; 3) Some cases were in poor physical or mental conditions and could not be

interviewed; however, the participation rate of cases was still relatively high (68% in Dafeng and 75% in Ganyu).

Controls were randomly selected from the general population in the same county as cases, and 1:1 individually matched to cases by age (within a 5-year category) and gender. For all residents in the study areas, personal information such as name, address, date of birth, sex and other demographic variables is available in the local demographic information database. Therefore, eligible controls were randomly identified from this database according to the age and gender of corresponding cases. For each control chosen, two additional subjects were selected as backup at the same time. When the first control could not be interviewed, an alternative was enrolled in the study. The selection procedure was repeated until an eligible subject was interviewed. The participation rate of the first control in Dafeng and Ganyu was 87 and 85%, respectively.

As responding rates for both cases and controls are relatively high, we believe that cases and controls were able to represent the total population of incident EC patients and the population at large, respectively.

Incomplete matching in data analysis

As we mentioned previously, EC cases and corresponding controls were originally individually matched by age and gender originally. Because studies on stomach, lung and liver cancer with identical protocols and questionnaires were conducted in both Dafeng and Ganyu simultaneously, we used those additional controls in our analyses to improve statistical power and precision, although it may have caused incomplete matching between case and control groups.

It is generally agreed that in the presence of confounding, matching on known risk factors may enhance the power to reveal effects of other risk factors in case-control studies.[10] Incomplete or inexact matching may lead to bias, but can be entirely avoided by appropriate analysis.[11] Greenland and Friedlander evaluated the effects of partial matching

on the efficiency of individually matched case-control studies and concluded that partial matching achieves most of the benefits (or shortcomings) of full matching.[12,13] Sturmer demonstrated that imperfect matching of controls neither impedes the validity nor substantially decreases the precision of estimation of the study, and perfect agreement in the distribution of the matching factors in cases and controls is neither necessary nor efficient.[14] In our analysis, matching factors and potential confounders were included in the logistic regression model. Moreover, a sensitivity analysis with only EC cases and their matched controls was also carried out for validation; the results of the conditional logistic regression analysis were similar to the overall analysis but with wider confidence intervals because of a smaller sample size. Therefore, we concluded that the use of additional controls improved the statistical power and precision rather than biased our true findings.

Low proportion of histologically confirmed cases

As stated earlier, both Dafeng and Ganyu are less developed rural areas, and pathological tests are less frequently performed for diagnosis because of the costs. In our analysis, only 39% of EC cases were histologically confirmed. Cases who were diagnosed by endoscopy (40%) and radiology (11%) were also included. As a result, some tumors may be esophageal adenocarcinomas (EAC), which have a different etiology as compared to esophageal squamous cell carcinoma (ESCC).[4] Also, a small number of patients may be wrongly diagnosed as having carcinomas because of lack of pathological information.

Although we were unable to confirm all EC cases and differentiate their histological types, it has been reported that ESCC remains predominant form of EC and accounts for more than 95% of esophageal malignancy in China.[15] Moreover, 92% of our cases were diagnosed at a county hospital or at higher levels (e.g., city hospital or provincial hospital), and no marked difference was observed between participants diagnosed by pathology and other methods with respect to demographic information and socio-economic status. Therefore, it is highly possible that the low proportion of histologically confirmed cases does not affect much the reliability of our findings.

Inaccurate measurement of exposures

Misclassification of exposure

Interviewer-administered questionnaire

Although not the only possible method, use of questionnaires is the cheapest and most efficient method for assessing exposures in large scale studies. In this thesis, data were collected by a structured standardized questionnaire used in face-to-face interviews in both Dafeng and Ganyu. This questionnaire has been tested by previous studies.[16,17] All interviewers came from local township hospitals, and were trained to explain questions in a proper way to avoid interviewer-induced errors. Although interviewer bias cannot be totally avoided, when adjusting for interviewers in the logistic regression model, no remarkable changes of the ORs were observed.

According to our results, the majority (more than 60%) of EC cases could be explained by the selected risk factors in the study areas **(Chapter 6)**, however, there might be other important determinants contributing to the occurrence of EC in the study areas which were not covered by the questionnaire.

Food frequency questionnaire

To evaluate the association between diet and disease, the food frequency questionnaire (FFQ) has been most frequently used in large-scale epidemiologic studies for many years. However, the usefulness, validity and reproducibility of the FFQ have been debated widely in recent years.[18-20] It has been estimated that the FFQ can be up to 50% inaccurate depending on the food items of interest.[21] Whereas, the FFQ is low-cost and relatively easy to administer than other dietary assessment methods such as 24-h dietary recall or food records, and can evaluate long-term diet rather than short period exposures.[22] In this thesis, dietary data were also obtained using a pre-tested semi-quantitative FFQ. Controls were asked to report how often they consumed a specified amount of a certain food item in the previous year, while cases were asked to report food consumption in the year before their

diagnosis. Although this FFQ has been tested by previous studies, measurement errors cannot be ruled out in the analysis. Moreover, recall bias may also exist since cases are more prone to change their dietary patterns because of symptoms or complaints **(Chapters 2, 5 and 7)**.

Other differential and non-differential misclassifications

Misclassification of exposure in case-control studies is usually a potential source of bias.[21] In this thesis, the exposure level of most risk factors was reported by study subjects without accurate measurements; therefore, subject judgments and measurement errors may lead to both non-differential and differential misclassification of exposures. For some risk factors, e.g., raw garlic consumption and vegetable intake, misclassification seems to be independent of case-control status, thus, may cause non-differential misclassification and weaken the associations **(Chapters 5 and 7)**. On the other hand, differential misclassifications may also exist. For instance, when evaluating the association between alcohol drinking and EC, cases were more prone to quit drinking because of early digestive symptoms, which may subsequently cause an inflated OR among former drinkers **(Chapter 3)**.

Confounding adjustment: selection and categorization

In observational studies, confounding can easily obscure the association of real scientific interest and lead the unwary astray. Therefore, confounders that cannot be controlled in the study design must be adjusted for in the analysis.[22] In this thesis, confounders were selected according to previous knowledge on EC and our preliminary analysis,[23] including age, gender, study area, education level, previous income, BMI, pack-years of smoking and weekly consumption of ethanol, etc.

The covariates mentioned above should be considered as confounders undoubtedly when evaluating the associations between lifestyle risk factors and EC, as they have been

shown to be associated with both outcome and exposures by previous epidemiologic studies as well as our preliminary analysis. When studying the association between genotype and EC **(Chapter 6)**, those above mentioned socioeconomic indicators were still included in the logistic regression model, although they were unlikely to be associated with individuals' genotypes (exposure), thus, did not fulfill the criteria of confounders. According to the comparability definition, if a covariate is only a determinant of the outcome and has no association with the exposure, its control, though, irrelevant for research validity, may nevertheless reduce the residual variance of the outcome variate and thus enhances the statistical efficiency.[24] Also, we did not observe remarkable differences between the crude OR and adjusted OR. Therefore, socio-economic related variables were kept in the logistic regression model even though they were unlikely to be associated with genotype and act as confounders.

Some potential confounders were continuous variables with less accurate measurements, e.g., income, pack-years of smoking and weekly alcohol intake. It has been suggested that for continuous variables without reliable measurements, categorization may reduce the effects of differential variability.[25] However, Brenner observed that categorization may often be inadequate when controlling for continuous confounders, and may lead to serious residual confounding if the number of categories is small.[26] Nurminen found that control for confounding can be very ineffective with classification of individuals into five or less categories.[25] Becher demonstrated that residual confounding arises when a continuous confounder is divided into a categorical variable for use in logistic regression.[27] Moreover, categorization of continuous covariates will lead to loss of information and inflation of the type I error rate.[28] Therefore, those above-mentioned potentially confounding variables were continuously adjusted in our analyses, although misclassification of exposures may exist.

Though ORs remained stable after adjustment for most potential confounding variables, residual confounding can never be completely ruled out because of unmeasured confounding variables and inaccurate measurement for some exposures.

Additive interaction vs. Multiplicative interaction

In this thesis, the focus was predominantly on additive interaction when exploring the joint effects for positive family history of EC with major lifestyle risk factors **(Chapter 5)**, and genetic polymorphisms of *ADH1B, ADH1C* and *ALDH2* with alcohol drinking **(Chapter 6)**. On the other hand, when evaluating the interaction between smoking and alcohol consumption, we showed their combined effects on a multiplicative scale for comparison, as the vast majority of previous studies have reported multiplicative interactions **(Chapter 3)**.

Whether interaction should be determined by a statistical model or should be assessed on an additive scale irrespective of the underlying statistical model has been a long-standing debate in epidemiology.[29] It is now generally accepted that the additive interaction is more appropriate and meaningful than multiplicative interaction with respect to indicating the underlying biological mechanism of a particular disease, and because of this, additive interaction has also been termed as biological interaction.[30]

Although in logistic regression, models are constructed exponentially and are therefore inherently multiplicative, Rothman showed how interactions as departure from additivity of two dichotomous variables can be quantified,[31] and this method has been extended to estimate interactions for multi-level or continuous determinants by Knol et al.[32] One drawback of calculating indices for additive interaction from logistic regression models in case-control studies is that adjustment for additional covariates will yield only an approximation of the relative excess risk due to interaction (RERI) and attributable proportion due to interaction (AP), because RERI and AP may vary across strata defined by covariates. Conversely, the synergy index (S) for additivity has been demonstrated to be more resistant to this problem.[33] In this thesis, the three measures of additive interaction were also derived from logistic regression model after adjusting for potential confounders **(Chapters 5 and 6)**. Although there might be deviations for the point estimation of RERI and AP, we did not find marked differences in direction and significance of these two

indices as well as for the synergy index, before and after adjustment for potential confounding variables.

Another practical consideration is that the measures of additive interaction may be difficult to interpret when the OR of one of the factors is below 1 (inverse association); therefore, the joint category at lowest risk should be chosen as the reference category in order to result in a positive risk difference.[34,35] However, this may seem counterintuitive when a variable has been confirmed as a risk factor by previous studies.

Causality of risk factors

In this thesis, we evaluated the effects of a set of risk factors on EC development, including the calculation of their PAFs and the evaluation of interactions on an additive scale. Both estimation of PAF and examination of additive interaction are based on the assumption that risk factors are causally associated with the outcome of interest.[31,36] However, there is only little or limited evidence for causal associations between some risk factors and EC at this moment, such as fast eating speed and eating raw garlic. Furthermore, some risk factors are surrogate factors rather than causal risk factors, e.g., family history of cancer. Although these results are indicative and therefore should be interpreted with caution, they may have important implications from a public health perspective.

External validity

China has one of the highest incidences of EC worldwide. In some counties, the incidence of EC may exceed 60/100,000. Therefore, whether the findings of this thesis can be applied to other high EC risk areas in China, and generalized to the population at large need to be discussed.

This study was conducted in a selected high-risk area (Dafeng) and a relatively low-risk area (Ganyu) in Jiangsu, China. Both of these two counties are less developed rural areas. The population sizes are 0.7 and 1.1 million in Dafeng and Ganyu, respectively.

Similar to other rural areas in China, farming remains the main occupation of the local population (See **Chapter 1**).

As compared to the strong associations in Western countries, the risk for smoking and alcohol drinking was observed to be much weaker in China. Our results are comparable to most previous studies conducted in other high-risk regions in China, showing that ever smoking or drinking alcohol moderately increased the risk of EC **(Chapter 3)**. Furthermore, it has been estimated by a meta-analysis that smoking and alcohol drinking explained 23.2% and 16.4% of EC cases in China, and a large study conducted in 103 areas of China reported the fraction of EC attributable to smoking to be 27.6%~31.3% in urban areas and 13.4%~21.1% in rural areas.[37] In line with these findings, we observed that smoking and alcohol contributed 25.4% and 15.6% to EC respectively in this thesis **(Chapter 7)**. Dietary factors were found to be important in esophageal carcinogenesis in many areas of the world.[2] Similarly, we found that unhealthy dietary habits such as fast eating speed, hot eating/drinking, frequent intake of fried foods and salty foods significantly increased the risk of EC. Besides lifestyle risk factors, our results for family cancer history are also comparable with other studies in China and other countries: a positive family history of EC may increase the risk of EC 2-3 fold, and can modify the effect of some lifestyle risk factors. In both Dafeng and Ganyu, Han Chinese are the vast majority of local inhabitants (>99%), the genotype distributions of *ADH1B* (rs1229984), *ADH1C* (rs698) and *ALDH2* (rs671) in this thesis are similar to the genotype distribution in Hapmap consortium of Han Chinese in Beijing, indicating that our study subjects do represent the general population in these counties.[38]

In conclusion, based on internal and external comparisons, we conclude that our study population was representative for Chinese rural areas, and our findings are close to those findings in other high-risk regions in China. However, the age-standardized incidence of EC in Dafeng and Ganyu was 36 and 24/100,000 during 2006-2008, respectively, higher than the national average which was 13.2/100,000. In other words, even relatively low in

Jiangsu, the incidence of EC in Ganyu is still higher than the national average while remaining relatively low in Jiangsu. Moreover, both Dafeng and Ganyu are less developed rural areas. Considering the study population, our findings should be cautiously extrapolated to urban areas and some low-risk areas of EC in China, and are only applicable for ESCC since the majority of EC cases are in the type of ESCC in China.

PUBLIC HEALTH IMPLICATIONS

Comprehensive cancer control includes integrating and coordinating different approaches to reducing cancer morbidity and mortality through prevention, early detection, treatment, rehabilitation and palliation (Figure 8-1).[39,40] From a public health perspective, although the development of treatment is increasingly effective in reducing the mortality of EC nowadays, the survival rate is still very low, especially in developing countries. Therefore, primary prevention through lifestyle interventions still holds promise for reducing the occurrence of EC. In addition, secondary prevention through screening and early detection may also offer a good way to reduce the mortality associated with EC.

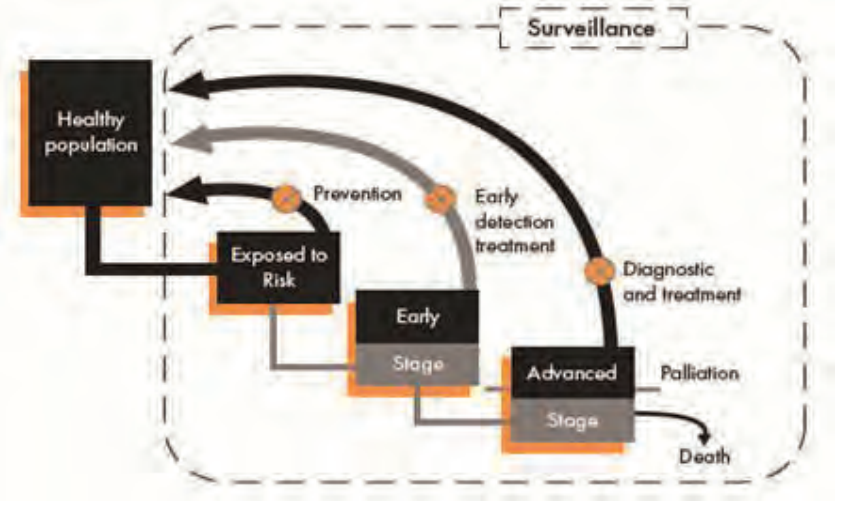

Fig 8-1 WHO comprehensive approach to cancer prevention (*Source: World Cancer Report, 2008)*

Primary prevention (Population-wide strategy)

It is widely agreed that up to perhaps 90% of human cancer may be attributable to environmental factors, and thus, are potentially preventable if those external causative factors can be identified and avoided.[40] In this thesis, we found that smoking, alcohol drinking and several dietary factors such as fast eating speed, hot eating/drinking and high intake of salty foods are important determinates of EC **(Chapters 2, 5 and 7)**, and more than 60% of cases are attributable to these major lifestyle risks **(Chapter 7)**. Furthermore, differences in the distribution of lifestyle factors may primarily explain the large differences in EC risk between high- and low-risk areas **(Chapter 7)**. Our results indicate that a large proportion of EC cases are expected to be prevented by eliminating or avoiding those unhealthy lifestyle factors.

Tobacco Control

China is now the leader in both tobacco production and consumption.[41] Although smoking was found to be modestly associated with EC in China, given the huge number of smokers (350 million)[41] and attributable fraction of EC (25%) **(Chapter 3 and 7)**, achieving tobacco abstinence will undoubtedly bring large beneficial effects not only on EC control but also on the prevention of other smoking-related cancers and other diseases. Moreover, general tobacco control activities will reduce exposure to secondhand smoke, which has also been suggested to increase the risk of EC among non-smokers by several previous studies and this thesis **(Chapter 7)**.

A comprehensive approach to tobacco control includes both reducing tobacco use and supply, such as improving public awareness through health education, total or partial ban of smoking in work and public places, enforcement of smoking restrictions, bans on smoking advertisement, large health warning labels and raising of the price of cigarettes.[40] The implementation of the WHO Framework Convention on Tobacco Control (FCTC) has

provided a unique opportunity for tobacco control campaigns in China;[42] however, the implementation still needs to be intensified and some policies are waiting to be formulated.

Reducing alcohol consumption

There is clear evidence that alcohol consumption and alcohol-related problems have strikingly increased in China in past decades, and more than 80% of Chinese men and nearly 30% of Chinese women are current drinkers. Whereas, as compared to tobacco control, few comprehensive public health policies on reducing alcohol consumption has been formulated in China, e.g., access to alcohol has few restrictions, alcohol advertising is widespread in all kinds of media and there is no limit to the age at which it can be bought or consumed.[43] In this thesis, we showed an apparent association between alcohol drinking and EC **(Chapter 3)**, and estimated that about 16% of EC cases could be attributed to alcohol consumption **(Chapter 7).** Although the association between alcohol drinking and EC is less strong as compared to Western countries, considering the high prevalence among Chinese men, reducing alcohol consumption is still important for EC prevention in China. A population approach in alcohol control should aim at reducing the level of consumption across the whole population, which may include improving public awareness, increasing tax on alcohol, advertising control, occupational health strategies in the workplace, and limiting or controlling the availability of alcohol.[44]

Different from tobacco smoking which is the primary risk factor of many diseases, consuming appropriate amounts of alcohol has been found to protect against some cardiovascular diseases,[45,46] therefore, an evidence-based recommendation for safe and healthy drinking needs to be developed for both cancer control and cardiovascular disease prevention in China.

Dietary modification

From this thesis, we found that dietary factors play much more important roles in esophageal carcinogenesis than smoking or alcohol does **(Chapter 7)**; therefore,

modifications in diet will provide a practical and cost-effective way for EC prevention and may have substantial impact in the high-risk areas of China.

Unhealthy dietary habits such as eating fast and hot eating/drinking was observed to be strongly associated with EC risk and attributable to a large fraction of cases **(Chapter 7)**, while these two important determinants can be efficiently avoided through intensive health educations in the population. Consistent with many previous studies,[2] high intake of foods containing carcinogens such as fried foods, salty foods and red meat, as well as a diet low in fruits and vegetables are also found to be associated with EC in this thesis **(Chapter 7)**. How to eat a healthy diet to prevent EC should be another important topic in health education. One thing we have noted is that in the low-risk area, garlic consumption is much more prevalent than in high-risk area, and this difference explained a large proportion of the risk gradient between the two counties **(Chapter 7)**. A previous study also reported that in the low-epidemic areas of EC, diets are usually rich in garlic and other allium vegetables.[47] Although this needs to be further elucidated, eating more garlic and allium vegetables may potentially have largely beneficial effects on EC prevention in high-risk areas of China.

Secondary prevention (High-risk strategy)

Esophageal carcinogenesis is a multi-factorial and multistage process which may take more than 10 years.[48] Though primary prevention strategies including tobacco control, alcohol reduction and dietary modification should be the highest priority for reducing EC occurrence, it may take a long time to decrease EC deaths and bring beneficial effects mainly to younger generations. Secondary prevention to reduce mortality by diagnosing disease at an earlier and more curable stage is thus logical and crucial for decreasing the deaths caused by EC in high mortality areas. Nowadays, endoscopic examination with iodine staining and biopsy is one of the most effective methods for early detection of EC,[49] whereas, taking limited resources in less developed areas and cost-effectiveness into account, screening and early detection by endoscopies among high-risk populations are more realistic and practical.

As the identification of high-risk subjects for secondary prevention is important to optimize the utilization of limited resources, using demographic information and exposure to risk factors may provide a simple and practical way to target individuals for endoscopy in the population. From this thesis, we propose that high-risk populations should include either 1) Male, above 50 years old; or female, above 55 years old; 2) Living under poor socioeconomic conditions; 3) Having a family history of EC in first degree relatives; 4) Currently smoking or drinking moderately/heavily, or former smokers/drinkers who quit less than 10 years ago; 5) Habitually eating fast and eating/drinking hot foods/liquids; 6) Frequently eating salty foods and fried foods (more than twice per week); 7) Diet low in fruits and vegetables, and high in red meat (compared to the Dietary Recommend Intake in China); 8) Have been tested with cancer susceptible genes, such as ADHs or ALDH2 less active variants, if applicable. These high-risk individuals are recommended to take an endoscopic examination every 3-4 years, whether it is achieved by personal actions or through participation in screening programs.

RECOMMENDATION FOR FUTURE RESEARCH

Identification of unknown determinants

Although many studies have been conducted in the past decades, the actual etiology of EC in China remains poorly understood. Besides those frequently studied lifestyle and inherited risk factors, there might be other unknown determinants that strongly increase the risk of EC in China, such as environmental carcinogens, micronutrient deficiency and cultural risk factors. To identify these important contributors is of great importance in both understanding the etiology and preventing occurrence of EC. In this context, case-control studies may be conducted as the first step since they can provide a low-cost and effective approach in finding the clues. Considering that bias and confounding may subsequently lead to spurious associations in case-control studies, large-scale prospective studies may be performed to further test the hypotheses.

Further elucidation of pronounced risk factors

Some unhealthy dietary habits such as fast eating speed and high temperature of foods and drinks at consumption have been suggested to substantially increase EC risk by previous studies and this thesis, but evidence remains limited and inconclusive.[1,2,50] Their exposures were usually assessed by a self-administrated or an interviewer-administrated questionnaire without accurate measurements. Further studies with more accurate quantitative measures need to be conducted to provide further evidence on the associations between esophageal carcinogenesis and these important predictors. An example would be to apply a visual analog scale (VAS) and actual eating time to assess the actual eating speed,[51] and to use food thermocouple thermometers to test the real temperature of foods/drinks at eating/drinking instead of using a questionnaire.[52]

Diet and EC

Dietary pattern approach

Currently, research on diet and EC primarily focused on individual food items or food groups, while people normally eat combinations of foods containing a mixture of nutrients and non-nutrients, therefore, not a particular food or group of foods, but rather the whole dietary pattern plays an important role in disease occurrence. To explore the patterns of dietary intake through principle component analysis or factor analysis instead of focusing on individual dietary components has been recommended as an appropriate approach in nutritional epidemiology.[53,54] To date, studies on dietary pattern and EC have been mainly conducted in the Western countries;[55] therefore, it could become an interesting topic for future research in China. One concern in evaluating dietary patterns and EC risk is that, the disease may influence the types of foods tolerated and consumed because of symptoms or extensive examinations at an earlier stage, so with this in mind, prospective studies would be more appropriate than retrospective studies.[56]

Accurate assessment of dietary intake

For future studies, more reliable dietary assessment approaches are required, such as collecting food information based on 24-hour dietary recall method for several consecutive days in combination with a short food propensity questionnaire, or incorporating specific biomarkers.[57] One issue that warrants discussion is that this does not necessarily mean that the FFQ is useless and should be abandoned in future studies. Although the use of food records and biomarkers for dietary assessment in diet-cancer studies will undoubtedly enhance the study design, they are more likely to reflect short-term exposures rather than long-term exposures, and biomarkers in some cases assess only one nutrient at time. Therefore, these methods are complementary to, rather than a replacement for FFQs in large epidemiologic studies.[20] A high quality FFQ with more detailed information to focus on specific hypotheses, together with more accurate dietary assessment methods and incorporation of biomarkers may be an ideal design for future studies on diet and EC.

Short-term randomized-controlled clinical trials

Due to the limitations of observational studies, such as uncontrolled confounding, findings from randomized controlled trials are generally believed to provide the highest level of evidence.[58] In this thesis, we found that raw garlic consumption significantly reduced the risk of EC, and may explain a remarkable fraction of incidence difference between the two study areas at varying risk. To further test this finding, a large-scale prospective study may be conducted among people frequently eating raw garlic and seldom eating raw garlic. A randomized-controlled clinical trial with garlic or garlic contents (mainly flavonol and organosulfur compounds) may be also applied for this purpose. An important matter is that, many clinical trials use cancer incidence as endpoints, while it requires a study period of many years, very large sample sizes and great expense. Therefore, short-term, smaller trials that use surrogate endpoint biomarker (SEB) could be applied for future studies. A SEB may be defined as an early change during the intraepithelial, pre-invasive phase of neoplastic progression, at the molecular, celluar or tissue level, such as markers and indices of proliferation, oncogene mutation or amplification, and allelic loss

and other alterations.[59-61] The progression/regression of esophageal precancerous lesions also could be considered for future studies.[48]

Cancer susceptible genes and gene-environmental interactions

The process of carcinogenesis is affected in a number of different ways, including mitochondrial metabolism and oxidative stress, epigenetic changes, DNA damage and repair, protein synthesis and cell proliferation, and disturbance of immune functions etc. In this thesis, we explored the relationship between polymorphisms of several alcohol metabolism-related genes and EC (ADHs and ALDH2).[62] Future studies on the multiple pathways with sufficient statistical power would be warranted to identify more cancer susceptible genes and their interactions with environmental risk factors.

The advent of high-throughput genotyping technologies has allowed fast evaluation of single nucleotide polymorphisms (SNPs) on a genome-wide scale at a relatively low cost. Genome wide association studies (GWAS) have recently emerged as a powerful approach to identify lower penetrance common variants associated with cancer susceptibility.[63] Several previous GWAS studies have been conducted on EC and have found some novel genetic markers.[64-65] Although the genomic revolution has produced a comprehensive map of genetic variation that has enabled research to scan the genome, the ability to survey environmental and lifestyle exposures is not nearly as advanced, thus hampering the opportunity to explore the dynamic relationship between genomic variants and the environment.[66] In this context, GWAS studies incorporating environmental-wide association studies (EWAS) [67] might be an insightful method to shed further light on understanding the etiology of EC and to evaluate individual risk and prognosis. For future research, a well-designed GWAS study with a large sample size based on prospective studies could be conducted among the high-risk Chinese populations to screen susceptible markers of EC, to provide clues for the identification of novel regions and new pathways, and to explore the relationship between genomic variants and the environment.

Chapter 8

CONCLUSION

The findings in this thesis confirm that unhealthy lifestyles including smoking, alcohol drinking and some dietary factors are the predominant risk factors of EC in high-risk areas of China, and a large proportion of incidence difference between regions at varying risk could be attributed to the different prevalence of lifestyle factors. As most of the identified risk factors are modifiable, these could be translated into risk reduction prevention programs in China, and a substantial proportion of new EC cases are expected to be prevented by eliminating or avoiding these risk factors in the population.

As the etiology of EC still needs to be further elucidated, well-designed case-control studies and prospective studies with sufficient sample size are warranted to be conducted to identify other pronounced risk factors. GWAS study incorporating with advanced methods for the assessment of environmental and lifestyle exposures also could be conducted to provide clues for the identification of new cancer pathways and to explore the relationship between genomic variants and the environment.

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SUMMARY

Summary

Esophageal cancer (EC) remains one of the most common cancers in the world, and the 5-year survival rate is among the lowest, especially in developing countries. China is an area with high incidence of EC, about half of the new cases in the world are diagnosed in China each year, and squamous cell carcinoma is the predominant histological type. Epidemiological studies have suggested that tobacco smoking, alcohol drinking, a diet low in fruits and vegetables, high exposure to carcinogens from preserved foods and red meat, and chronic injuries of the esophageal mucosa are important in the development of this disease. Genetic polymorphisms in enzymes involved in metabolism of carcinogens may also modify the risk of lifestyle and environmental exposures, and influence individual susceptibility to cancer **(Chapter 1)**. However, EC remains one of the least studied cancers, and the effects of major lifestyle and hereditary risk factors on the development of this fatal disease remain poorly understood in Chinese population. Moreover, the geographic variation in EC occurrence is striking, while little attention has been paid to the etiological heterogeneity between similar areas with great risk gradient.

Jiangsu province, in south-east China, is one of the highest cancer incidence areas but the mortality of EC differs considerably between counties. From 2003 to 2007, a large population-based case-control study of EC has been conducted in two counties of Jiangsu, Dafeng and Ganyu. Both counties are less developed rural areas in northern Jiangsu with similar geophysical conditions; however, Dafeng has a much higher incidence of EC than Ganyu. In total, 1,520 cases (637 in Dafeng and 883 in Ganyu) and 3,879 controls (1,938 in Dafeng and 1,941 in Ganyu) were recruited. In this thesis, we evaluated the role of major lifestyle factors such as tobacco smoking, alcohol drinking and dietary factors, as well as inherited determinants including family history of cancer and genetic polymorphisms of alcohol-metabolizing related genes on the risk of EC. In addition, we simultaneously evaluated the attributable fractions of major risk factors in two counties, in order to investigate how much of the risk gradient could be explained by variation in the

distributions of major risk factors. It aims to shed further light on both the etiology and prevention of EC in China.

During the study period, we performed a preliminary analysis with 291 pairs of cases and controls in Dafeng and 240 pairs of cases and controls in Ganyu in year 2 **(Chapter 2)**. In both low- and high-risk areas, we found that EC was inversely associated with socio-economic status and body mass index. Positive associations were observed for EC with family history of cancer, encountered misfortune in the past 10 years, smoking, alcohol drinking and fast eating speed. Furthermore, there appears to be a geographic variation in the association of smoking, alcohol drinking and EC risk between low and high-risk areas: dose-response relationship of smoking and smoking related variables, such as age of starting smoking, duration and dosage of smoking were apparent only in high-risk areas; whereas, a dose-response relationship between alcohol drinking and EC was observed only in low-risk area.

Although the associations for EC with tobacco smoking and alcohol drinking have been well established worldwide, their risks appear to be less strong in China. To provide more evidence on the effect of smoking and alcohol consumption with EC in China, particularly among Chinese women, in **Chapter 3**, we analyzed the overall and gender specific effects of these two well-established risk factors. Different from the results of the preliminary analysis, no marked differences in associations were observed between the two counties; therefore, data were pooled to improve statistical power. Results showed that smoking and alcohol drinking moderately increased the risk of EC in the study population. Dose-response relationships were observed with increased intensity and longer duration of smoking as well as alcohol drinking. The joint odds ratio (OR) for individuals at the highest joint level was 7.32, when compared to those who never smoked and never drank alcohol. Stratifying by gender, ever smoking and drinking alcohol significantly increased the OR among men, whereas, neither was found to be associated with EC among women. This study confirmed that the independent and joint associations of smoking and alcohol

drinking are less strongly associated with EC in China, partly because of the relatively short exposure history and low exposures to both factors among Chinese women.

Green tea is one of the most frequently consumed beverages in China. Some previous epidemiological studies suggest that drinking green tea is inversely associated with EC but results remain contradictory; furthermore, inconsistent observations found high temperature drinks are associated with the risk of EC. Therefore, we explored green tea drinking and tea temperature with the risk of EC occurrence in **Chapter 4**, and compared the difference between the two different risk regions. Our results showed that drinking green tea at high temperature significantly elevated OR in both Dafeng and Ganyu. After further adjustment for tea temperature in the logistic regression model, green tea drinking was not associated with EC in either county, and there was no obvious difference in the effect of green tea drinking between low- and high-risk areas.

Although positive family history of cancer in first-degree relatives (FH-FDR) has been hypothesized to play a role in the etiology of EC, only a few large studies have investigated the risk and FH-FDR in detail. Furthermore, to date the joint effect of FH-FDR and lifestyle risk factors has been rarely reported for EC. In **Chapter 5**, we evaluated the relationship between FH-FDR and the risk of EC, and explored the joint effects for FH-FDR with major lifestyle risk factors. We found that a positive FH-FDR significantly elevated the individual risk of EC development. The association was stronger when there was more than one affected first-degree relative and when the age at which the tumor was diagnosed was lower among the relatives. We also found that FH-FDR could modify the effect of certain lifestyle risk factors. Super-additivity interaction was found for FH-FDR with fast eating speed and diet low in fruits and vegetables. The substantially increased risk of lifestyle risk factors in FH-FDR positive individuals indicates that it is important to properly prevent the disease by changing unhealthy lifestyles within this high-risk population.

In this thesis we have found that, although moderate, alcohol drinking significantly increased the risk of EC in the study population **(Chapter 3)**. Epidemiologic studies also have indicated that genetic polymorphisms in alcohol-metabolizing related genes such as alcohol dehydrogenases (ADHs) and aldehyde dehydrogenase 2 (ALDH2) may be associated with EC occurrence. In order to provide further information on the relationship between EC and single nucleotide polymorphisms (SNPs) of ADH1B, ADH1C and ALDH2 genes, and to explore the possible gene-environment interaction and gene-gene interactions, a relevant analysis was conducted **(Chapter 6)**. Results showed that the SNPs of ADH1B, ADH1C and ALDH2 genes were associated with EC among moderate-to-heavy drinkers. A significant interaction was observed between ALDH2 and alcohol drinking on multiplicative scale. Although no obvious gene-gene interaction was observed for ALDH2 with either ADH1B or ADH1C, we observed that alcohol drinkers harboring the inactive ALDH2 A allele and ADH1B G allele were at the highest risk of EC. Our findings confirm that genetic polymorphisms of ADH1B, ADH1C and ALDH2 could modify the risk of EC among Chinese alcohol drinkers, and suggest that genetic predispositions, together with the variation in lifestyle factors may ultimately determine the individual risk of EC in the Chinese population.

In **Chapter 7**, we evaluated the role of major lifestyle risk factors and heredity factors on the population attributable fraction (PAF) of EC in the two counties respectively, and explored what proportion of the risk gradient between the two areas could be explained by differences in the distribution of major risk factors. The simultaneous evaluation of two populations at different risk may provide a potentially insightful approach in understanding both etiology and prevention of EC in China. Results showed that smoking and alcohol drinking accounted for a PAF of 25.4% and 15.6% respectively. PAF of fast eating speed, hot eating/drinking, high intake of salty foods and family history of cancer was 21.6, 28.0, 12.5 and 9.7%, respectively. The combination of six lifestyle risk factors accounted for more than 60% of total EC cases. Moreover, although no significant difference was

observed in the association for major lifestyle risk factors with EC, the difference in the prevalence of eating raw garlic and family history of cancer accounted for 37.7% and 29.6% of the incidence difference between the two counties. These findings, if confirmed, may imply that major unhealthy lifestyles could explain a large fraction of EC occurrence in China, and dissimilar distribution of several lifestyle factors, together with hereditary variations may be mainly responsible for the incidence difference between areas.

At the end of this thesis **(Chapter 8)**, the main findings of this thesis are summarized, the major epidemiological considerations and public health implications of this study are discussed, and recommendations for future research are given. In conclusion, the findings in this thesis indicate that unhealthy lifestyles including smoking, alcohol drinking and some dietary factors are the predominant risk factors of EC in China. Hereditary determinants such as family history of cancer and genetic polymorphisms of alcohol related genes may also influence the individual susceptibility of cancer and could modify the risk of lifestyle factors. Moreover, a large proportion of incidence difference between regions could be attributed to the different prevalence of several risk factors. As the majority of risk factors found in this thesis are modifiable, together with the protective factors identified in this thesis and other studies, these could be translated into risk reduction programmes in the high-risk areas of EC in China. It is expected that a substantial proportion of new EC cases could be prevented by eliminating or avoiding these risk factors in the population. Screening among highly susceptible individuals to detect the disease at an earlier and more curable stage is also of importance to reduce the mortality of EC in high-risk areas in China.

SAMENVATTING

Slokdarmkanker is één van de meest voorkomende kankers in de wereld en de 5-jaars overleving behoort, voornamelijk in ontwikkelingslanden, tot één van de laagste. China is een land met één van de hoogste incidenties van slokdarmkanker; ongeveer de helft van de nieuwe ziektegevallen in de wereld wordt elk jaar gediagnosticeerd in China, waarbij het plaveiselcelcarcinoom het dominante histologische type is. Epidemiologische studies suggereren dat roken, alcoholconsumptie, een voeding laag in fruit en groente, een hoge blootstelling aan carcinogenen uit geconserveerd voedsel en rood vlees, en chronische beschadiging aan de mucosa van de slokdarm belangrijk zijn in de ontwikkeling van deze ziekte. Genetische polymorfismen in enzymen, die betrokken zijn bij het metabolisme van carcinogenen kunnen ook het risico van leefstijl en blootstelling aan omgevingsfactoren modificeren en de individuele vatbaarheid voor kanker beïnvloeden (Hoofdstuk 1). Echter, slokdarmkanker is één van de minst bestudeerde kankers en de effecten van belangrijke leefstijl- en erfelijke factoren op de ontwikkeling van deze fatale ziekte zijn nauwelijks bekend in de Chinese populatie. Bovendien is de geografische variatie in het ontstaan van slokdarmkanker opvallend, terwijl er weinig aandacht wordt gegeven aan de etiologische heterogeniteit tussen gelijke gebieden met een grote risicogradiënt.

De Jiangsu provincie in het zuidoosten van China is een gebied met één van de hoogste slokdarmkankerincidentie. Tussen 2003 tot 2007 is een patiënt-controle onderzoek naar slokdarmkanker uitgevoerd in twee regio's van Jiangsu, namelijk Dafeng en Ganyu. Beide regio's zijn minder ontwikkelde landelijke gebieden in het noorden van Jiangsu met vergelijkbare geofysische condities; echter, Dafeng heeft een veel hogere slokdarmincidentie dan Ganyu. In totaal zijn er 1.520 ziektegevallen (637 in Dafeng en 883 in Ganyu) en 3.879 controles, afkomstig uit dezelfde populatie, (1.938 in Dafeng en 1.941 in Ganyu) gerekruteerd. In dit proefschrift hebben we de rol van belangrijke leefstijlfactoren, zoals roken, alcoholconsumptie en voedingsfactoren op het risico van

slokdarmkanker geëvalueerd, evenals de mogelijk modificerende effecten van overgeërfde determinanten waaronder familiegeschiedenis van kanker en genetische polymorfismen in enzymen betrokken bij de omzettng van alcohol . Tevens hebben we het attributief risico van belangrijke risicofactoren in twee regio's geëvalueerd om te onderzoeken hoeveel van de risicogradiënt door de variatie van de verdelingen van belangrijke risicofactoren kan worden verklaard. Het doel is om inzicht te geven in de etiologie én de preventie van slokdarmkanker in China.

Tijdens de studieperiode hebben we een eerste analyse in het tweede jaar uitgevoerd met 291 paren van ziektegevallen en controles in Dafeng en 240 paren van ziektegevallen en controles in Ganyu (Hoofdstuk 2). In beide gebieden, namelijk laag- en hoogrisicogebieden, vonden we een inverse associatie tussen sociaal-economische status, body mass index en slokdarmkanker. Er zijn positieve associaties gevonden voor slokdarmkanker met familiegeschiedenis van kanker, ondervonden ongeluk in de afgelopen 10 jaar, roken, alcoholconsumptie en een hoge eetsnelheid. Verder is gebleken dat er een geografische variatie is tussen laag- en hoogrisicogebieden bij de associatie tussen roken, alcoholconsumptie en het risico op slokdarmkanker. Een dosis-effect relatie van roken en variabelen gerelateerd aan roken, zoals de leeftijd waarop men begon met roken, de totale tijdsduur en de hoeveelheid die men rookt, waren alleen zichtbaar in het hoogrisicogebied (Dafeng), terwijl een dosis-effect relatie tussen alcoholconsumptie en slokdarmkanker alleen was aangetoond in het laagrisicogebied (Ganyu).

Hoewel de associaties voor slokdarmkanker met roken en alcoholconsumptie wereldwijd goed zijn onderzocht, blijken hun risico's minder sterk te zijn in China. Om meer bewijs te verkrijgen over het effect van roken en alcoholconsumptie op slokdarmkanker in China, en in het bijzonder bij Chinese vrouwen (Hoofdstuk 3), hebben we de totale en geslachtsspecifieke effecten van deze risicofactoren geanalyseerd. In vergelijking met de resultaten uit de eerste analyse zijn er geen verschillen in associaties

gevonden tussen de regio's. Daarom zijn de data samengevoegd om de statistische power te verhogen. Roken en alcoholconsumptie laten beiden een matig verhoogd risico van slokdarmkanker in de studiepopulatie. Er zijn dosis-effect relaties met verhoogde intensiteit en een langere tijdsduur van roken en alcoholconsumpties gevonden. De gecombineerde odds ratio voor mensen in de hoogste categorie voor roken en alcoholconsumptie was 7,3 vergeleken met mensen die nooit hebben gerookt of alcohol hebben geconsumeerd. Stratificatie voor geslacht liet een significant verhoogde odds ratio zien voor mannen, terwijl er geen associaties zijn gevonden voor slokdarmkanker bij vrouwen. Deze studie bevestigt dat de onafhankelijke en gezamenlijke associaties van roken en alcoholconsumptie minder sterk geassocieerd zijn met slokdarmkanker in China, deels door de relatief korte blootstelling en lage blootstelling aan beide factoren onder Chinese vrouwen.

Groene thee is één van de meest geconsumeerde dranken in China. Sommige epidemiologische studies suggereren dat consumptie van groene thee invers geassocieerd is met slokdarmkanker, maar de resultaten spreken elkaar tegen. Dranken met een hoge temperatuur zijn mogelijk positief geassocieerd met het risico van slokdarmkanker, maar resultaten van studies zijn ook hier inconsistent. Daarom hebben we de rol van de consumptie van groene thee en de temperatuur van de thee op het risico van slokdarmkanker onderzocht in hoofdstuk 4, en hebben we het verschil tussen de twee verschillende risicogebieden vergeleken. Onze resultaten laten zien dat consumptie van groene thee gedronken op een hoge temperatuur het risico op slokdarmkanker significant verhoogt in zowel Dafeng en Ganyu. Nadat we verder hebben gecorrigeerd voor de temperatuur van de thee met behulp van multivariate logistische regressie, bleek dat groene thee consumptie niet was geassocieerd met slokdarmkanker in beide gebieden. Er was geen duidelijk verschil in het effect van groene thee consumptie tussen laag- en hoogrisicogebieden.

Summary in Dutch

Ondanks dat wordt verondersteld dat een positieve familiegeschiedenis van kanker in eerstegraads familieleden (FH-FDR) een rol speelt in de etiologie van slokdarmkanker, hebben slechts een paar grote studies het effect van FH-FDR op het risico in detail onderzocht. Op dit moment is het gezamenlijke effect van FH-FDR en leefstijlfactoren op slokdarmkanker nauwelijks gerapporteerd. In hoofdstuk 5 hebben we de relatie tussen FH-FDR en het risico op slokdarmkanker geëvalueerd en onderzochten we de gezamenlijke effecten van FH-FDR met de belangrijkste leefstijlfactoren. We hebben gevonden dat een positieve FH-FDR van slokdarmkanker het individuele risico op de ontwikkeling van slokdarmkanker significant verhoogt. De associatie was sterker indien er meer dan één eerstegraads familielid slokdarmkanker had gehad en de leeftijd waarop de tumor was aangetroffen lager was bij de familieleden. Tevens hebben we gevonden dat FH-FDR het effect van verschillende leefstijlfactoren kan modificeren. Een super-additieve interactie werd gevonden voor FH-FDR met een hoge eetsnelheid en een voeding laag in fruit en groente. Het aanzienlijk verhoogde risico van leefstijlfactoren bij FH-FDR positieve personen indiceert dat het belangrijk is om op de juiste manier de ziekte te voorkomen door ongezonde leefstijlen bij hoogrisico groepen te veranderen.

In dit proefschrift hebben we laten zien dat matige alcoholconsumptie het risico op slokdarmkanker significant verhoogt in de studiepopulatie (Hoofdstuk 3). Epidemiologische studies indiceren ook dat genetische polymorfismen in enzymen betrokken bij de omzetting van alcohol in het lichaam , zoals alcohol dehydrogenases (ADHs) en aldehyde dehydrogenase 2 (ALDH2) geassocieerd kunnen zijn met het ontstaan van slokdarmkanker. Een relevante analyse is uitgevoerd om meer informatie te verkrijgen over de relatie tussen slokdarmkanker en zogenaamde single nucleotide polymorphisms (SNPs) van ADH1B, ADH1C en ALDH2 genen, en om de mogelijke gen-omgeving interactie en gen-gen interacties te onderzoeken (Hoofdstuk 6). Resultaten tonen aan dat SNPs van ADH1B, ADH1C en ALDH2 genen geassocieerd zijn met slokdarmkanker bij matige tot zware drinkers. Een significante interactie is gevonden tussen ALDH2 en

alcoholconsumptie op een multiplicatieve schaal. Ondanks dat er geen duidelijke gen-gen interactie is aangetoond voor ALDH2 met zowel ADH1B of ADH1C, zagen we dat alcoholgebruikers, die het inactieve ALDH2 allel en ADH1B G allel dragen, een hoger risico hebben op slokdarmkanker. Onze resultaten bevestigen dat genetische polymorfismen van ADH1B, ADH1C en ALDH2 het risico op slokdarmkanker bij Chinese alcoholgebruikers kunnen modificeren. Tevens suggereren onze resultaten dat genetische predispositie in combinatie met de variatie in leefstijlfactoren het individuele risico op slokdarmkanker in de Chinese populatie uiteindelijk kunnen bepalen.

In hoofdstuk 7 hebben we de rol van de belangrijke leefstijlfactoren en erfelijke factoren op de populatie attributieve fractie (PAF) van slokdarmkanker in de twee regio's geëvalueerd. Tevens onderzochten we welke proportie van de risicogradiënt tussen de twee gebieden kan worden verklaard door verschillen in de verdeling van belangrijke risicofactoren. De vergelijkbare evaluatie van twee populaties met een verschillend risico kunnen een mogelijk inzicht geven in het begrijpen van de etiologie en de preventie van slokdarmkanker in China. Resultaten toonden aan dat roken en alcoholconsumptie bijdroegen met een PAF van respectievelijk 25,4% en 15,6%. De combinatie van zes leefstijlfactoren verklaarden meer dan 60% van het totale aantal slokdarmkankergevallen. Ondanks dat er geen significant verschil is gevonden in de associatie tussen de belangrijke leefstijlfactoren met slokdarmkanker, verklaarde het verschil in de prevalentie van het eten van rauwe knoflook en een familiegeschiedenis van kanker 37,7% en 29,6% van de verschillen in incidentie tussen de twee regio's. Deze bevindingen impliceren, als ze worden bevestigd, dat een ongezonde leefstijl een groot deel van het ontstaan van slokdarmkanker in China kan verklaren. Tevens kunnen ongelijke verdelingen van verschillende leefstijlfactoren in combinatie met erfelijke variaties verantwoordelijk zijn voor het verschil in incidentie tussen de gebieden.

Aan het eind van dit proefschrift (Hoofdstuk 8) zijn de belangrijkste bevindingen van dit proefschrift samengevat, de belangrijke epidemiologische overwegingen en de

implicaties voor de volksgezondheid bediscussieerd, en de aanbevelingen voor toekomstig onderzoek weergegeven. Concluderend tonen de bevindingen in dit proefschrift aan dat ongezonde leefstijlfactoren, zoals roken, alcoholconsumptie en enkele voedingsfactoren, belangrijke risicofactoren zijn voor slokdarmkanker in China. Erfelijke determinanten, zoals familiegeschiedenis van kanker en genetische polymorfismen in enzymen betrokken bij de omzetting van alcohol kunnen ook het individuele risico op slokdarmkanker beïnvloeden en het risico van leefstijlfactoren modificeren. Bovendien kan een groot deel van het verschil in incidentie tussen regio's worden toegeschreven aan het verschil in prevalentie van verschillende risicofactoren. Omdat de meerderheid van de risicofactoren in combinatie met beschermende factoren, die beschreven zijn in dit proefschrift en andere studies, kunnen worden veranderd, kunnen ze vertaald worden naar preventieprogramma's in de hoogrisicogebieden van slokdarmkanker in China. Er wordt verwacht dat een substantieel deel van nieuwe gevallen van slokdarmkanker kan worden voorkomen door het vermijden van deze risicofactoren in de populatie. Het screenen van personen met een hoog risico is van groot belang om de ziekte in een vroeg en geneesbaar stadium te detecteren, zodat de mortaliteit van slokdarmkanker in hoogrisicogebieden in China kan worden verminderd.

总　　结

食管癌是常见的癌症之一，它的 5 年生存率仍然很低，尤其在发展中国家。中国是食管癌高发地区，每年全世界的新发病例大约有一半出现在中国，鳞状上皮细胞癌仍然是中国食管癌的主要病理类型。流行病学研究结果表明：吸烟、饮酒、饮食中缺乏蔬菜水果、经常食用腌制食品以及慢性食管粘膜损伤是导致食管癌发生的重要危险因素。此外，与致癌物代谢相关基因的多态性也会影响到生活方式和环境危险因素的作用，从而影响个体对癌症的易感性（第 1 章）。目前，关于食管癌的研究仍然相对较少，中国人群中生活方式和遗传因素与食管癌的关系也需要进一步研究。食管癌发病、死亡的地理分布差异巨大，而很少有人在地理、社会环境相似，发病差别巨大的地区间针对食管癌病因学差异开展对比研究。

江苏省位于中国东南沿海，是中国食管癌高发地区之一，但省内各地的食管癌发病、死亡分布也存在较大差异。2003-2007 年，一个大样本、以人群为基础的病例-对照研究在该省的大丰市和赣榆县开展。这两个地区都是位于苏北沿海的欠发达农村地区，地理环境相似，但是大丰的食管癌发病率要明显高于赣榆。本研究总共收集了 1520 例有效病例（大丰 637 人，赣榆 883 人）和 3879 名对照（大丰 1938 人，赣榆 1941 人）。本篇论文中，我们研究了主要生活方式危险因素，包括吸烟、饮酒、饮食因素等，以及遗传因素如癌症家族史、饮酒相关基因多态性与食管癌发病风险的关系。此外，我们还分析了主要危险因素在两个地区的归因危险百分比，以探讨地区间发病差异与危险因素流行水平的关系。研究将进一步为中国的食管癌病因研究和预防提供科学依据。

在研究进行的第二年，我们使用部分收集到的数据进行了初步分析，包括大丰的 291 对病例和对照，以及赣榆的 240 对病例和对照（第 2 章）。我们发现，在高发和低发地区，食管癌和社会经济因素及体重指数（BMI）均呈负相关，而阳性癌症家族史、吸烟、饮酒、进食速度快和遭受过重大变故显著增加

了发病风险。另外，吸烟、饮酒和食管癌的联系在高、低发区之间存在一定差异：我们只在高发区观察到吸烟相关变量和食管癌的剂量反应关系，如开始吸烟年龄、每日吸烟量和吸烟年数等；相反，饮酒和食管癌的剂量反应关系只出现在低发区。

尽管吸烟、饮酒和食管癌的关联在世界上很多地方得到了很多验证，这种联系在中国表现的相对较弱。为了进一步研究吸烟和饮酒与食管癌在中国人群中的关系，尤其是在中国女性中的情况，我们在第 3 章分析了这两个危险因素合计和分性别的结果。和前一章初步分析的结果略微不同，我们发现两个地区间吸烟、饮酒和食管癌的联系没有显著差别，因此对数据进行了合并以提高分析的统计效率。结果表明：吸烟和饮酒中度增加了研究人群中的食管癌发病风险，吸烟/饮酒的量和时间长短与癌症的发生存在明显的剂量反应关系。和既不吸烟也不饮酒组相比，吸烟、饮酒量同时最高者的 OR 值为 7.32。分性别结果显示，曾经吸烟和曾经饮酒明显增加了男性的 OR 值，但在女性人群中没有发现任何关联。本研究证明了在中国人群中，吸烟和饮酒与食管癌的单独效应和联合作用相对较低，部分因为暴露时间较短和中国女性的低吸烟、饮酒率。

在中国，绿茶是最常饮用的饮料之一，一些流行病学研究发现饮用绿茶可以降低食管癌的发病危险，但研究结论仍不一致。另外，饮茶温度高也被提示和食管癌发病之间存在关联。因此，我们在第 4 章探讨了饮用绿茶、茶温与食管癌的联系，并且比较了两个地区间的差别。结果在大丰和赣榆均发现饮茶温度较高显著增加了食管癌的发病危险。在 Logistic 回归模型中进一步调整饮茶温度后，在两个地区均没有发现饮绿茶和食管癌间存在关联，也没有发现绿茶饮用的效果在高发和低发地区间存在明显差异。

尽管具有一级亲属阳性癌症家族史（FH-FDR）是食管癌的一个病因，只有一小部分大样本研究系统分析了癌症家族史的作用，此外，FH-FDR 和生活方式危险因素的联合作用很少见到报道。在第 5 章，我们分析了 FH-FDR 对

食管癌发生的影响，并探讨了 FH-FDR 和主要生活方式危险因素的联合作用。我们发现 FH-FDR 显著升高了个体食管癌的发病危险。当不止一个一级亲属患癌以及亲属发现癌症的年龄较低时，联系的强度逐渐加大。我们还发现 FH-FDR 可以影响生活方式危险因素的作用效果，例如 FH-FDR 和进食速度快、低蔬菜水果摄入间存在协同作用。阳性 FH-FDR 人群中，生活方式危险因素的作用显著加强，提示在这些高危人群中通过改变生活方式预防食管癌十分重要。

在第 3 章中我们提到，尽管强度较弱，饮酒仍然显著增加了本研究人群中食管癌的发生危险。流行病学研究发现酒精代谢相关基因的多态性，如乙醇脱氢酶（ADHs）、乙醛脱氢酶 2（ALDH2）对食管癌的发生会产生一定影响。为了进一步探讨 ADH1B，ADH1C 和 ALDH2 基因单核苷酸多态性与食管癌的关系，并且研究潜在的基因-环境和基因-基因交互作用，我们在第 6 章进行了相关的分析。结果表明 ADH1B，ADH1C 和 ALDH2 基因多态性在中度和重度饮酒人群中和食管癌存在联系，并且 ALDH2 和饮酒之间存在着交互作用。尽管没有发现 ALDH2 和 ADH1B 或 ADH1C 间存在基因-基因交互作用，我们观察到饮酒人群携带 ALDH2 A 等位基因和 ADH1B G 等位基因具有最高的发病危险。本研究结果证实了 ADH1B，ADH1C 和 ALDH2 基因多态性会影响饮酒人群中的食管癌发生危险，并且提示了基因和生活方式危险因素共同决定了个体的发病风险。

第 7 章我们分别评价了两个地区主要生活方式危险因素和遗传因素对于食管癌的人群归因危险百分比（PAF），并且探讨了两个地区间的发病差异有多大程度可以归因于危险因素流行水平的差异。这种同时在两个不同危险人群中的对比研究对于了解中国食管癌的病因和开展预防具有特殊的意义。分析结果表明吸烟和饮酒的 PAF 分别为 25.4% 和 15.6%。进食速度快、热烫饮食、喜吃咸食和癌症家族史的 PAF 分别是 21.6，28.0，12.5 和 9.7%；而六种主要危险因素的联合归因危险百分比则超过了 60%。另外，尽管我们发现主要危险因素和食管癌的联系在高发和低发地区之间没有明显不同，但两地人群中吃

生蒜和癌症家族史的不同流行水平分别解释了 37.7%和 29.6%的发病率差异。这样的结果如果能够得到进一步的证实，将会证明中国不同地区的食管癌危险差别主要是生活方式危险因素引起的。并且一些主要危险因素，以及遗传的差别，最终导致了不同地区间的巨大发病率差别。

在论文的最后部分（第 8 章），我们总结了主要的研究发现，讨论了主要的流行病学方法以及研究的公共卫生意义，并且对今后的主要研究方向进行了建议。总之，本文的研究结果表明不健康的生活方式，包括吸烟、饮酒以及部分饮食因素是中国人群食管癌的主要危险因素；而遗传因素如阳性癌症家族史和饮酒相关基因的基因多态性也与个体患癌易感性有关，并且会影响生活方式危险因素的作用大小。此外，研究地区间的发病率差异在很大程度上归结于一些危险因素流行水平的不同。本研究中发现的主要危险因素都是可以通过干预改变的，加上本研究和其他研究已经发现的保护因素，这些结果均可以用于在中国食管癌高发地区开展危险因素干预。可以预计，在人群中降低或消除上述危险因素可以预防大量的食管癌发生。而在高危人群中进行筛查，早期发现可治愈的癌症病人对于降低高发地区的食管癌死亡率也具有非常重要的意义。

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About eight years ago, I met Prof. Dr. Frans Kok for the first time when he was visiting Nanjing. I discussed with him about the possibility of carrying out research projects collaboratively, and studying as a PhD student at Wageningen University. Although I was interested in pursuing my PhD degree abroad, I was hesitant for several reasons: Would I be able to get a proper research project for my PhD thesis? Do I have sufficient English skills to follow English courses and write scientific papers? What happens if none of my papers get accepted and published? When I talked with Frans about my worries, I got enthusiastic encouragement from him. Frans also recommended that I contact Prof. Pieter van't Veer because of Pieter's expertise in nutrition and epidemiology. With the inspiration and help from Frans and Pieter, I made one of the most important decisions in my life. In 2004, I successfully applied for a fellowship from Wageningen University and was formally admitted as a PhD candidate. And because my research topic was cancer-related, I was so lucky that Prof. Ellen Kampman became the first promoter of my PhD study.

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武 鸣

2011 年 2 月

About the Author

ABOUT THE AUTHOR

WU Ming was born on July 7th 1974 in Quanjiao County, Anhui Province, China. After finishing his secondary school education, he started his bachelor study at the Department of Preventive Medicine in Anhui Medical University in 1991, and received his Bachelor degree in Medicine in 1996. Shortly after that, he became a Master student at the School of Public Health at South-East University (formally called Nanjing Railway Medical College) with a specialization in epidemiology and biostatistics. In July 1999, he received his Master's degree in Biostatistics.

From September 1999, he has been working at the Department of Chronic Disease Control at the Jiangsu Provincial Center for Disease Control and Prevention (Jiangsu CDC). During this period, he was involved in several research projects such as "the application of geographical information systems in cancer prevention", "the etiologic heterogeneity of the top four cancers in high- and low-risk areas of Jiangsu, China" and "a comprehensive study on the prevention of metabolic disorders and metabolic syndrome". He was also in charge of other studies titled "a study on the relationship between genetic polymorphisms of peroxisome proliferator–activated receptors and metabolic syndrome" and "the third death retrospective survey (2003-2005) in Jiangsu Province". He is currently the director of the Department of Chronic Disease Control at the Jiangsu CDC and is responsible for organizing and implementing control and prevention activities on chronic diseases such as cancer, cardiovascular diseases, diabetes, and others in the whole province. In 2004, he was formally admitted as a sandwich PhD student in the Division of Human Nutrition at Wageningen University in the Netherlands.

List of selected publications

As First Author

1. **Wu M**, Zhao JK, Zhang ZF, et al. Smoking and alcohol drinking moderately increased the risk of esophageal cancer among Chinese men but not women in a high-risk population. *Cancer Causes Control*. DOI: 10.1007/s10552-011-9737-4
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As Co-author

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2. Kang G, Guo L, Guo Z, Hu X, **Wu M**, Zhou Z, Zhou H, Liu S, Chen F. Impact of blood pressure and other components of the metabolic syndrome on the development of cardiovascular disease. *Circ J.* 2010;74(3): 456-61
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OVERVIEW OF COMPLETED TRAINING ACTIVITIES

➢ Courses

✧ Analytical Epidemiology, VLAG, Wageningen, 2003

✧ The 6th International Advanced Course on Nutrition and Lifestyle Epidemiology, VLAG, Wageningen, 2003

✧ Advanced Training Course on Nutrition and Lifestyle Epidemiology, VLAG & Jiangsu CDC, Nanjing, China, 2004

✧ Cancer Epidemiology, NIHES Course, Amsterdam, 2005

✧ The 7th International Advanced Course on Nutrition and Lifestyle Epidemiology, VLAG, Wageningen, 2005

➢ Meetings and workshops

✧ International Forum on Frontiers of Cancer Research, Gene-environment Interaction & Cancer,Nanjing Medical University, Nanjing, China, 2006

✧ International workshop on Drowning Prevention, WHO & China CDC, Beijing, 2006

✧ International Training Workshop in Cancer Epidemiology, Prevention and Control, UCLA-Fogarty AITRP & Fudan Univeristy, Shanghai, China (Presentation), 2008

✧ International Seminar on Diabetes Epidemiology, Oslo University & Jiangsu CDC,Nanjing, China (Presentation), 2008

✧ Workshop on International Cancer of the Head and Neck Genetics and Environment Study (InterCHANGE), IARC & CICAMS, Beijing, China, 2009

✧ International Conference on Physical Activity and Health, WHO & China CDC, Beijing, 2010

➢ General courses

✧ Administration and management, Jiangsu Provincial Health Department, 2004

✧ Community Health and Chronic Disease Control, Queensland University of Technology, Brisbane, Australia, 2005

✧ Incorporating Non-communicable Chronic Disease Control into Community-based Health Service, WHO & Jiangsu CDC, Suzhou, 2005

✧ National Training Course on Hypertension and Diabetes Control, China CDC, Wuxi, 2005

✧ Project Management, China CDC, Beijing, China, 2006

✧ Presentation and Communication skills, China CDC, Suzhou, China, 2006

✧ Comprehensive Prevention of Non-communicable Chronic Disease Control in Community, China CDC, Wuhan, China, 2006

✧ Public Health Administration and Health Inspections, Connecticut University, Connecticut, USA, 2007

✧ Public Health and Epidemiology, Rollins School of Public Health, Emory University & CDC, Atlanta, USA, 2008

➢ Optional

✧ Preparation PhD research proposal

✧ Literature study program, Wageningen, 2004, 2005, 2008, 2009, 2010

✧ Study Tour on Prospective Study and Bio-bank Establishment, Oxford, UK, 2008

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