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Polymorphisms in the stem cell pathway and esophageal cancer in a Chinese population

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Polymorphisms in the stem cell pathway and esophageal cancer in a Chinese population

A dissertation submitted in partial satisfaction of the requirements for the Doctor of Philosophy in Epidemiology

By

Gina Maria Wallar

2013

ABSTRACT OF THE DISSERTATION

Polymorphisms in the Stem Cell Pathway and Esophageal Cancer in A Chinese Population

by

Gina Maria Wallar

Doctor of Philosophy in Epidemiology

University of California, Los Angeles, 2013

Professor Zuo-Feng Zhang, Chair

Few studies have explored the association between stem cell pathways and esophageal cancer and potential interactions between genetic markers and environmental exposures. We hypothesize that genetic susceptibility markers in the stem cell pathway might be associated with esophageal cancer and there might be interaction between these markers and environmental exposures. In a population-based case-control study conducted in Jiangsu, China, we evaluate the associations between esophageal cancer and various environmental factors and genetic markers using logistic regression model. The focus was on independent associations of cancer stem cell-related genes and their potential modification of the associations of known risk factors. In our analysis, we found that environmental tobacco smoking was associated with non-smokers with an adjusted OR of 1.29 (95% CI: 1.04-1.59) with a corresponding PAR of 11.4% (0.90%-21.7%).Garlic consumption was found to be inversely associated with esophageal cancer risk with an adjusted OR of 0.67 (95% CI: 0.53-0.85) for those in the entire study that self-reported often consumption of raw garlic. This

inverse association was seen across stratum of drinking status and smoking status with an adjusted OR of 0.67 (95% CI: 0.48-0.96) among the group of frequent drinkers who were ever smokers.

We analyzed 5 SNPs of the Wnt pathway and its association with esophageal cancer. We found rs4730775 (Wnt2) to have a potential weak inverse association of 0.89 (95% CI: 0.75-1.07 T/T+C/T vs C/C) in the study population overall. The association for Wnt2 (rs4730775) varied by smoking status; never smokers had a negative association with esophageal cancer with an aOR of 0.72 (95% CI: 0.53-0.98; T/T+C/T vs. C/C). When stratified by alcohol status, never and infrequent drinkers had inverse of associations with Wnt2 rs4730775 (T/T+C/T vs C/C aOR: 0.79 95% CI: 0.62-1.00) while there was no association for frequent and daily drinkers (T/T+C/T vs C/C aOR: 1.03 95% CI: 0.79-1.34). Likewise, the inverse association of Wnt2 was seen in the non-smoking/non-drinking category for rs4730775 (aOR: 0.67, 95% CI: 0.46-0.98 T/T+C/T vs C/C, respectively). No association was observed in the drinkers/smokers group for rs4730775 (Wnt 2).

Similarly, rs4835761 (Wnt8A) had an inverse association with an aOR of 0.82 (95% CI: 0.64-1.05, G/G+A/G vs A/A) in never and infrequent drinkers while there was no association observed in frequent and daily drinkers (aOR 0.97 95% CI: 0.74-1.28, G/G+A/G vs A/A. For rs222851 (DVL2) we observed a potential weak association for never and infrequent drinkers with an aOR of 1.19 (95% CI: 0.93-1.52, G/G+A/G vs A/A) and no association in frequent and daily drinkers aOR of 0.88 (95% CI: 0.74-1.06, G/G+A/G vs A/A). For non-drinkers and non-smokers, there was an inverse association observed with the rs2241802 (FZD3) with an adjusted OR of 0.68 (95% CI: 0.46-1.00; G/A+A/A vs. G/G) while there was no association observed in the drinkers/smokers group (aOR=1.00, 95% CI: 0.82-1.49; G/A+A/A vs. G/G).

We did not observe an association of rs2953 miRNA589 (CTNNB1 binding site) with esophageal cancer with an adjusted OR of 0.95 (95% CI: 0.80-1.13; G/T+G/G vs. T/T). When stratified by smoking status, we observed a potential inverse association among smokers with an adjusted OR of 0.84 (95% CI: 0.69-1.03; G/T+G/G vs. T/T) while there was no association observed among never smokers. Joint effects with rs2953 miRNA589 and other Wnt pathway SNPs did not appear to have an association when including the entire study population. However, when performing a stratified analysis of smokers and non-smokers the aROR for rs2953 miRNA589 and rs3729629 Wnt2 demonstrated an inverse association in smokers (aROR=0.58, 95% CI=0.35-0.96; G/G+A/G vs A/A for rs2953 and C/C+G/C vsG/G for rs3729629). This association remained when we stratified on both drinking and smoking status. For ever smokers and frequent/daily drinkers, the aROR was 0.48 (95% CI: 0.26-0.885 G/G+A/G vs A/A for rs2953 and C/C+G/C vsG/G for rs3729629).

In summary, we have identified the protective association between garlic intake and esophageal cancer and observed moderate association between environmental tobacco smoking (ETS) and esophageal cancer. The observed associations between genetic markers and esophageal cancer are relatively weak and there are some indications of potential gene-environmental interaction. This is the first time that ETS is associated with esophageal cancer in a large population-based case-control study in a Chinese population and garlic intake is inversely associated with esophageal cancer. Our observations suggest that in addition to tobacco cessation and alcohol control, the prevention strategy for esophageal cancer should include avoidance of ETS and increasing intake of raw garlic.

The dissertation of Gina Maria Wallar is approved.

Janet Sinsheimer

Frank Sorvillo

Roger Detels

Zuo-Feng Zhang, Committee Chair

University of California, Los Angeles

2013

DEDICATION

For Carter.

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Education

MPH, Epidemiology and Biostatistics, Boston University SPH, May 2001
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Experience and Skills

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- Provide consultation to clients to effectively design research proposals meeting their clinical and pre-clinical research needs. Perform preliminary literature research to assess feasibility of study and present findings to clients.
- Develop proposals to present to client including cost of service, timelines, and study methods. Perform cost analyses on cost of service for the purpose of developing budgets included in proposals.
- Oversee research projects to ensure project timelines, accuracy of budget, timely completion of projects, regulatory (GCP, GLP) compliance, and client satisfaction.
- Manage projects from conception to closure including proposal design and statistical analysis. Manage data to ensure validity of results.
- Analyze raw data and summarize findings in final reports; perform statistical analyses when needed.
- Report and track monthly volume and revenue to project future revenue in budget planning.

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- Oversaw daily activities of a large epidemiological study; responsible for designing and maintaining an orderly system of project organization assuring proper collection and timely receipt of data, as well as data management.
- Designed study-related instruments, materials, operations manuals, interviewing materials, case report forms and implemented Standard Operating Procedures for the study.
- Supervised staff involved with data entry and data management tasks and served as a liaison between the data management center, the Investigators, and various data collection components of projects.
- Provided oversight to insure cost-effective study conduct, meeting contractual budgetary commitments.

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- Managed clinical trial coordination for the Parkinson's Disease clinic ensuring compliance with good clinical practices and the sponsor protocol. Prepared IRB applications as well as consent forms.
- Managed the referral hotline for the American Parkinson Disease Association and ensured counseling to the elderly community.
- Hired and supervised research assistants to work on clinical trials

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- Assisted in the large epidemiological study of post-menopausal women by maintaining the lab and processing lab specimens in congruence with FDA guidelines.
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Chapter 1.

Introduction

1.1 Epidemiology of Esophageal Cancer

The esophagus is a muscular tube structure that is lined with squamous epithelial cells in the upper, middle, and some areas of the lower thirds. Location of the cancer differs between the 2 common types. Squamous cell carcinomas are found in the upper and middle third of the esophagus; most adenocarcinomas are found in the lower third or distal esophagus. Ninety percent of cancer in the esophagus comprise of either adenocarcinoma or squamous-cell carcinoma histologies. The remaining 10% represent a combination of rare cancer types such as leiomyosarcoma, melanoma and lymphoma.[1] Although the biological mechanisms of esophageal cancer remain not fully identified, it appears as though the cancer progresses and spreads quickly. By the time of diagnosis, a high proportion of esophageal cancer cases have already metastasized.

The etiology of the 2 histological types appears to vary as well. Whereas reflux disease, Barrett's esophagus, and obesity are associated with adenocarcinoma, they are not associated with squamous cell carcinoma which is strongly associated with tobacco smoking, alcohol drinking, socioeconomic status, physical injury, hot tea consumption, and radiation exposure. Tobacco smoking and alcohol drinking remain risk factors for both types but both are stronger risk factor for squamous cell carcinoma.

Squamous cell esophageal cancer used to represent the majority of cases in the United States until the past several decades when adenocarcinoma has been on the rise[2]. From 1973 to 2002, data from the Surveillance, Epidemiology and End Results (SEER) observed a 30% decline in the incidence of squamous cell carcinoma with the greatest declines observed in black males [3].

Conversely, incidence of adenocarcinoma has increased 4 times in this same time period with the greatest increase in white males.[4] The 2 major histological types of esophageal cancer are now equally represented in the United States leaving researchers perplexed as to the reason for the shift. Some have proposed the rise in obesity is partly to blame for this shift, but other factors may also contribute to this change.[3]

Primary esophageal cancer is the 8th most common cancer, with over 481,465 new cases occurring annually worldwide, and is the 6th most common cause of death from cancers, with over 406,533 deaths annually.[5] The mortality to incidence ratio, which is often used as an indicator of case fatality, is close to one. Worldwide, esophageal cancer accounts for 3.8% of all new cancers (except skin) and 5.4% of cancer deaths. Among men, however, esophageal cancer accounts for 4.9% of new cancers and 6.5% of cancer deaths. Worldwide, rates among men are 2-4 times higher in men than women and may be up to 15 times greater among men in high-risk areas, like Western Africa and 20 times greater among women in Southern Africa. In addition to the disparity among genders, over 80% of new cases occur in developing countries. In developing countries, esophageal cancer is the fourth most common cancer and second most common cause of deaths from cancers. Areas in the world with the highest mortality rates include Eastern and Southern Africa, and in Eastern Asia. [3]

In the US, 16,640 new cases and 14,500 deaths from esophageal cancer are estimated to occur in 2011, and the five year survival rate for all stages combined is 17%. Localized cancer has a 5 year survival rate of 37%. Esophageal cancer remains relatively rare in the US and accounts for 1% of all cancer cases. Rates for esophageal cancer in men are 3 times that of women which may have to do

with lifestyle (e.g. increased exposure to tobacco smoking and alcohol drinking) Most cases are presented when the cancer has spread to other sites thus, making 5-year survival rates low.

China accounts for 53.6% of all new cases and 51.7% of all deaths from esophageal cancer worldwide. In 2008, 258,000 new cases and 210,000 deaths from esophageal cancer are estimated to occur in China with it ranking as the 4th leading cause of cancer and cancer death. The age standardized incidence rates among males and females are 22.9 and 10.5 per 100,000 persons per year, respectively. Among men, esophageal cancer is the 4th most common cancer and common cause of death from cancer; yet among women, it is the 7th most common cause of death from cancer and 4th most common new cancer in the area.[5] The five-year survival in China remains low as well with the mortality rates highest among rural communities[6]. Mortality rates have been on the decline since the 1970s which is potentially due to the urban development and transformation of China's health care services in recent decades[7].

1.2 Risk and Protective Factors

The major risk factors for primary esophageal cancer include age, male gender, tobacco smoking, and alcohol drinking. The predominance of esophageal cancer in males is hypothesized to be attributed to lifestyle exposures such as higher exposure to tobacco smoking and alcohol drinking, male sex hormones, and altered carcinogen metabolism. Various genes involved in DNA repair, inflammation and alcohol metabolism have been implicated in elevated risk for the disease as well. For the 2 histological types—squamous cell and adenocarcinoma—the risk factors vary. For squamous cell, risk factors include tobacco smoking, alcohol drinking, achalasia, physical injury to the esophagus, tylosis, Plummer-Vinson syndrome, history of head and neck cancer, history of breast cancer with radiation, consumption of extremely hot beverages, and socioeconomic status.

For adenocarcinoma, reflux disease appears to be on the causal pathway; other potential risk factors include obesity, history of breast cancer treated with radiation, and prior use of beta-blockers, anticholinergic agents and aminophylines, in addition to tobacco smoking and alcohol drinking[1]. The trend in incidence of the disease suggests that the alterations of environmental exposures may play an important role in the esophageal carcinogenesis.

1.2.1 Tobacco Smoking and Alcohol Drinking

Tobacco smoking has long been identified as a risk factor for both adenocarcinoma and squamous cell esophageal carcinoma.[8] Smoking has been observed to increase the risk from 2 to 10 fold dependent on the study methods used for a particular publication. While the association seems to be dose-dependent on the intensity and length of exposure, there has been evidence to suggest that the duration of exposure poses great risk over the intensity. [9]

When alcohol drinking is present in addition to tobacco smoking the interaction appears to be multiplicative. [10, 11] In one meta-analysis of smoking in esophageal cancer, Castellsague et al found that duration of smoking and amount of smoking as well as duration and amount of alcohol drinking a dose-response on risk for esophageal cancer.[12] In a population-based case cohort study in Shanghai, Gao et al found the OR to be 12.0 among those that smoke a pack or more a day and had over 750g per week while the OR in men for current smokers was 2.1 and 1.4 among drinkers. [13] On its own, esophageal cancer deaths attributed to alcohol is estimated at 26% worldwide with an attributable fraction of 41% in high-income counties. Biological markers for alcohol-induced carcinogenesis include polymorphisms of the alcohol metabolic pathway.

Lifestyle factors including the level of tobacco smoking influences levels of indoor air pollution and have been hypothesized to increase risk of tobacco-related cancers. Environmental tobacco smoking has been studied widely for lung cancer with meta-analyses demonstrating associations for its effects for lung cancer risk[14] [15]. It has been established as a group one carcinogen by IARC for lung cancer .[16] However, few studies have been conducted to explore potential association between exposure to ETS and esophageal cancer in Chinese population, except reports from two case-control studies with small sample sizes in China.[17, 18]

1.2.2 Gastroesophageal reflux disease (GERD)

Evidence to date suggests that gastroesophageal reflux disease plays a causal role in the development of adenocarcinoma of esophagus, specifically. Individuals with GERD have an increased risk of esophageal adenocarcinoma with odds ratios estimated at 8 times the risk. [19] The size of the point estimates appear to be dose-responsive with the length and severity of reflux. Approximately 30% of adenocarcinoma of esophagus can be attributed to GERD. Obesity has also been suggested to serve as a potential risk factor though its association with GERD which could explain some of the observed association with esophageal adenocarcinoma. However, the association does not appear with squamous cell carcinoma of esophagus.

When GERD occurs in the lower esophagus, it may cause a condition known as Barrett's esophagus where the normal squamous cell lining is replaced with glandular cells. Barrett's esophagus can be found in 10 to 15% of white men aged 50 and over with chronic heartburn. Cases of Barrett's have 30-60 times the risk of developing esophageal cancer, and it is thought it may be a precursor to adenocarcinoma. Though there has been some argument as to whether Barrett's is on the causal

pathway to carcinogenesis, most agree that surveillance should be applied to those with the condition given their heightened risk.[20]

1.2.3 Diet and Other Risk Factors

A diet high in fruits and vegetables appears to have an inverse association with esophageal cancer.[21-23] Estimates suggest that approximately 15% of esophageal cancer can be attributed to a diet low in fruits and vegetables. Additionally, it has been suggested that diets high in animal origin along with processed meat may increase the risk for esophageal cancer.[24] As a result, studies have investigated the link between nitrosamines of processed meat and esophageal cancer with findings of a possible association. In addition to nitrosamines and their precursors, moldy food and pickled vegetables have long been suspected to be potential risk factors for the disease in China.[11] In a more recent study by Tran et al, pickled vegetables, moldy food and hot tea were not found to be associated with esophageal cancer. On the other hand, family history appeared to be associated as well though it is not clear whether the association is due to environmental or genetic factors.[25] The link between diet and esophageal cancer may partially be observed in its association with obesity and GERD in adenocarcinoma as a result of overeating. Additionally, poor diet may be a marker of low socioeconomic status in squamous cell carcinoma.[21]

Drinking extremely hot beverages as a risk factor for squamous cell esophageal cancer has also been examined given it is a common practice in areas of increased incidence and can cause physical damage to the lining of the esophagus. In a study performed in South America, extremely hot mate drinking was associated with an increased risk of 4 times that of a person who did not drink the extremely hot beverage.[26] It is unclear as to whether the association came from the chemicals in the tea or the temperature at which it was consumed. The study of EC in Iran has suggested that

alcohol drinking has less of an effect and that hot temperature and low intake of fruits and vegetables play a larger role.[27]

Past studies of the role of HPV in risk of EC has produced mixed results—though the presence of HPV has been confirmed, its association has been observed as an inverse association at times. [28-39] Some have questioned the method in which HPV was detected. [40-42]Others have suggested that its role differs by the pathology of EC (squamous cell versus adenocarcinoma) or the geographical location.[43-52] Given the mixed results, researchers often suggest that HPV plays a role in EC when it originates from an area with high incidence rates.

1.3 Cancer Stem Cells

Tumors are well-known for the heterogeneity among cells and the ability of those cells to divide indefinitely. Stem cells have the ability to self-renew though they act in a highly regulated fashion in comparison to cancer cells that appear to lack control. Stem cells also have the ability to differentiate to normal, mature cells whereas as cancer cells differentiate abnormally. There are 2 types of stem cells; placental and chord blood stem cells and adult stem cells. Placental and chord blood stem cells have the ability to differentiate mature into the 200 different somatic cells as well as germ cells that make up humans. Adult stem cells have a more limited ability to differentiate though they play a regulation and regeneration of organs and tissues. [53]

Cancers are comprised of a small population of highly proliferative cells, stem cells as well as more differentiated cancer cells that do not contain the same proliferation potential. This was first determined by various in vitro and in vivo assays where only a small portion of cells were highly proliferative. More recently, a model has been proposed to suggest that tumor cells and their

different genetic characteristics could arise from a small population of cancer stem cells. Though ongoing mutagenesis and epigenetic influences can explain part of this ability to differentiate and replicate, the cancer stem cell theory allows for the vast heterogeneity observed among cells of a tumor. [54-56]

Cancer stem cells were first observed in acute myeloid leukemia where surface markers could be used to distinguish AML stem cells from the other AML cells with limited potential for proliferation. Later, human breast cancer cells were separated into factions with different surface molecules and injected into immunodeficient mice. A small portion of the mice were able to induce tumor formation with cells that expressed CD44 but expressed little to no CD24. These CD44+CD24- cells could be serially passed from one mouse to another but they also gave rise to different cells with other phenotypes. Researchers found that CD133+ cells were responsible for the observed in vitro proliferation. In culture, CD133+ cells gave rise to cells s that expressed phenotypes in proportions that were representative of the original tumor and were more likely to be cancer cells than normal CNS stem cells.[57, 58]

Various pathways are involved with stem cell signaling to regulate self-renewal, proliferation, and differentiation among these cells. Upregulation or silencing in the genes encoding stem cell signaling molecules due to mutations or polymorphisms can contribute to the multiple steps involved in carcinogenesis. SNPs of genes encoding components of the stem cell regulatory network have been found to be associated with gastric cancer.[59] Genome-Wide Association Studies have revealed SNPs in germ line cells to be associated with esophageal cancer.[60]

1.3.1 Wnt Pathway

The Wnt pathway was identified about 20 years ago and observed to play roles in embryogenesis and carcinogenesis.[61-63] The origin of its name comes from the combination of Int, a gene near the mouse mammary tumor virus, and wg, the wingless gene in Drosophila. The Wnt protein is a secreted protein that helps regulate proliferation during development. It binds to the transmembrane Frizzled receptor which activates the Dishevelled (Dsh), a disrupter of glycogen synthase kinase 3B (GSK-3B) protein complex to casein kinase 1 (CK1), Axin, and adenomatosis polyposis coll (APC).[64] (AKT also inhibits GSK-3.) GSK-3 signals the Beta-Catenin/APC complex for degradation.[65-68] Presence of DSH therefore allows the accumulation of B-catenin that translocates to the nucleus. In the nucleus, B-catenin binds to LEF/TCF complex which activates the transcription of downstream genes. Two downstream pathways regulated by WNT are EPH and c-myc—responsible for differentiation and proliferation, respectively. [69, 70] B-catenin promotes the self-renewal of CNS and kartinocyte stem cells and is thought to lead to cancer of the CNS and skin.

Activating mutations in the Wnt pathway have been found in several cancer types including colon, prostate and ovary cancers [71, 72] as well as esophageal cancer cell lines.[73] The Wnt pathway has also been implicated in haemotopoietic stem cells and lymphoblastic leukemia, intestinal epithelial stem cells and colorectal cancer, and cerebellar granule cell progeneitors and medulloblastomas. [71, 72] Some have postulated that activation of Wnt causes normal stem cells to over-activate their self-renewal pathway and cause neoplasms.

1.3.1.1 Wnt Proteins

There are 19 wingless-type MMTV integration site Wnt proteins found in humans located on multiple chromosomes. Wnt proteins are highly conserved and secreted glycoproteins which have transforming properties in vitro and promote the upregulation of ß-catenin in cells.[74] Wnt protiens are believed to interact with different receptors dependent on the time of development and are sometimes dependent on additional promoters and co-factors. [75] For the Wnt/B-catenin pathway, they generally bind to frizzled transmembrane proteins and other receptors to activate Wnt signaling.

Various Wnt proteins have been implicated in carcinogenesis.[76] Wnt-2 has been found to be overexpressed in an immunohistochemical analysis in colorectal carcinoma.[72] Wnt2B/13 was found to be upregulated in gastric cancer though the study saw only 2 of 8 cases display this upregulation in an expression array analysis. [71, 72] Wnt-7A was found to be overexpressed in an immunohistochemical analysis of ovarian tumors as compared to normal ovary tissue and benign suggesting that it may represent a poor prognostic indicator. Wnt-7B was found to be overexpresssed in a breast cancer cell line.[77] Wnt-8A also appears to be upregulated in gastric cancer cell lines. [78] Data suggest wnt-16 is involved in leukemogenesis[71] and Wnt3a protein has been observed to self-renew haematopeoietic stem cells.[79]

1.3.1.2 Frizzled

Eleven frizzled genes have been identified in humans and not yet fully characterized. Though their mechanisms have not been fully elucidated to date, extracellular proteins containing cysteine-rich domain with homology to frizzled (sFRP) bind to wnt ligands. When overexpressed, sFRPs will inhibit wnt signaling pathways. One previous study looked at sFRPs and observed an increase in

intracellular concentration of ß-catenin while preventing apoptosis in MCF-7 cells in culture. Thus, while sFRPs have not yet been found to be associated with cancer in humans, it is possible that its interference with apoptosis could lead to tumorigenesis. Recently, FzD3 expression was found to be increased in esophageal cancer but not in normal esophageal tissue. [80]

Several studies have looked at mutant phenotypes of frizzled proteins in mice. Work in a mouse models has identified FzD3, a human frizzled receptor that activates TCF/LEF and results in nuclear localization of ß-catenin. Interestingly, Wang et al found esophageal defects when a mutation is present in FzD4.[77] Various researchers have found frizzleds to stimulate PKC activity and believe this to be the case in humans as well.[81]

1.3.1.3 Dishevelled

Four disheveled proteins have been identified in humans. Though its activity is not well-understood, data suggest that it has multiple functions in the Wnt pathway as well as other pathways. It shares similar characteristics with the DIX domain in the axin protein suggesting that they 2 proteins function together in the pathway. Dishevelled has been found to interact with CK-1 and Par-1 in the Wnt pathway. Par-1 can phosphorylate ß-catenin directly making it an upregulator of the pathway. The DEP domain of the protein has been shown to have downstream effects on the pathway as well. Mutations of this domain inhibit transcription of LEF-1. Mutations in the mouse genome have consequences such as open neural tube defects to gating abnormalities.

In a screen for proteins interacting with Dishevelled, Dapper has identified as a protein to bind with Dishevelled and the complex with axin, GSK-3, CK1, and ß-catenin. When Dapper is overexpressed, the amount of soluble ß-catenin decreases. Conversely, inhibition of Dapper

activates ß-catenin suggesting that it serves as an antagonist to Dishevelled. Very few studies have been published on Dishevelled and its potential association with cancer. However, in one study, DVL2 had been found to be overexpressed in colorectal cancer via its activation of B-catenin.[82]

1.3.1.4 GSK3ß

GSK3ß can be considered a potential tumor suppressor as it down regulates wnt signaling by binding to and phosphorylating several proteins in the pathway. Located on chromosome 3, it is an enzyme that was initially identified to inactivate glycogen synthase. When Wnt is absent, GSK3 constitutively phosporylates the ß-catenin protein which, as a result, causes a decrease in soluble ß-catenin. However, mutations in the gene encoding for GSK3ß were not observed to have an association in a study of colorectal cancer. [83] Additionally, a complete deletion of the gene is probably unlikely given its role in other pathways and, thus, cell survival suggesting that inactivation may occur via other means. Recently, GSK3ß has been seen to bind and phosporlate Axin, inhibiting B-catenin transcription.[84] One protein, Frat-1, has been identified in mice and observed in vitro to compete with axin for GSK3ß binding. One Frat-1 homolog, GBP was observed to inhibit phosphorylation of ß-catenin.

1.3.1.5 Axin

Axin 1 is located on chromosome 16 at 16p13.3. The axin gene encodes a cyoplasmic protein that acts as a negative regulator of the wnt pathway. Axin contains similarities to Dishevelled proteins as well as a regulators of G-Protein Signaling and In Xenopus embryos, it was observed to bind to APC, ß-catenin, disheveled and GSK3ß. Evidence has suggested that it aids in the phorsphorylation of ß-catenin as well as APC via GSK3ß.[85] [86]Mutations have been identified in hepatocellular cancers and were observed to remove the ß-catenin binding site.

1.3.1.6 ß-catenin

ß-catenin is encoded by the CTNNB1 gene and is a cadherin-associated protein. Its activation either by mutation or by Wnt expression has been associated with many tumors.[87, 88] It serves as part of a complex of proteins called the adherens junctions that are responsible for cell growth and adhesion of epithelial cell layers in tissues. [89] It serves as an integral component in the Wnt pathway and can interact with other proteins such as ICAT and APC. ß-catenin mutations in carcinogenesis appear to inactivate APC. Observed mutations alter specific sites which are required for targeted degradation of ß-catenin. [90]Various human cancers are found to lack the normal interaction of ß-catenin and a component of an E3 ubiquitin ligase, ß-TRCP. In a study of colon cancer, samples without APC mutations were more likely to have ß-catenin mutations. Individuals with familial adenomatous polyposis coli (FAP) contain mutations in the APC within their germline and are at increased risk of desmoids tumors (fibromatosis). [91] Desmoids have also been observed to contain ß-catenin mutations without overlap with APC mutations. [92]

Studies have shown that approximately 20% of gastric cancers, a cancer associated with FAP, contain ß-catenin mutations and that nuclear accumulation of ß-catenin is associated with a poorer prognosis. [93] Some tumors have been found to contain up to 4 independent mutations. [65] FAP individuals are also at higher risk for thyroid cancers which have been found to contain mutations. Other non-FAP related cancers such as ovarian tumors have been found to have an association with ß-catenin mutations. It too, has been observed to have a poor prognosis when ß-catenin accumulation is observed in the nucleus. In some studies, the ß-catenin mutation has co-existed with other known tumorigenic mutations. In endometroid tumors, it has co-occurred with a PTEN

mutation. [94] [95] Additionally, mutations have been found in 15% of pediatric kidney cancers which co-occur with WT1. [96]

1.3.1.7 Tcf-Lef genes

In the nucleus, B-catenin binds to LEF/TCF complex which activates the transcription of downstream genes. When there is no Wnt signal, TCF acts as repressor of the pathway. Recently, B-catenin/TCF4 complex has been shown to bind to the binding element of the STAT3 gene promoter regulating B-catenin at the transcriptional level.[97] The STAT3 activation allows for the accumulation of B-catenin in the nucleus[98, 99]. There are 4 TCF genes identified in humans to date; TCF1, LEF1, TCF3 and TCF4. Mutant phenotypes in knockout mice for TCF1 and LEF1 include various developmental defects such as the addition of neural tubes, and formation of limb buds as well as the formation of mammary tumors. SNPs in the TCF1 gene have been previously reported to increase risk of Type 1 diabetes. TCF3 was found to be essential for head formation. A microsatellite within intron 3 of TCF4 was found to be associated with type 2 diabetes in US and Danish cohorts studies.

1.4 microRNA

Hundreds of microRNAs have recently been found to exist in animals, plants and viruses.[100] They are short RNAs consisting of about 22 single-stranded ribonucleotides. miRNAs base-pair to their target mRNA which can lead to mRNA cleavage or inhibition of translation. [101] One miRNA can bind to many mRNA targets. Additionally, different miRNA can cooperatively act on one mRNA target suggesting that they are act as part of a gene regulatory network. [102] They are non-coding, functional and endogenous and it has been estimated that 30% of genes in humans are regulated by miRNA.

MicroRNAs bind to 3'-UTR of target mRNA which can lead to inhibition of translation. Their activity has been observed in a variety of biological activities including cell differentiation and proliferation, apoptosis, and tissue development. MiRNA genes are mostly located in the introns or in antisense orientation to other genes. miRNAs have sequence and function conservation between organisms which indicate they are essential to cell processes.

Long primary miRNA are initially transcribed and then cut into pre-miRNAs by nuclear RNase III Drosha. [103, 104] The processing intermediate is exported out of the nucleus into the cytoplasm where is is cleaved by RNase III Dicer into a 22 nt duplex. One strand is degraded while the other becomes the mature miRNA. [105, 106] This step determines the mature miRNA sequences are thought to regulate gene expression post-transcription in certain events including carcinogenesis. It has been estimated that 50% of miRNAs are found in regions of the genes that are associated with cancer.

To date, miRNAs have been implicated in carcinogenesis across cancer types via several mechanisms.[107-113] miRNSs have been observed to be deregulated and have aberrant expression profiles in cancer.[114-119] One study has observed expression profiles of miRNAs for esophageal cancer can be distinguished from normal tissue. [120] They also can have aberrant action with no alteration in expression levels as some studies have seen that SNPs in miRNA target sites affect interaction with mRNA.[121-123] They are also seen to play a role in metastasis [124, 125], angiogenesis[126-129] , and via interaction with p53. [130-135]

The study of SNPs in genes encoding in miRNA have shown that polymorphisms exist at low levels for the most part. However, their target sites have been shown to display variation. [136, 137] Studies focusing on miRNA involved with Barrett's esophagus and adenocarcinoma have been reported and 11 miRNAs were reported to have association with different stages of the disease. Expression profiles of miRNA have been performed in squamous cell esophageal carcinoma from cases in Chaoshan Arian in China. Several reports have found miR-21 to be upregulated miRNA and miR-203 to be downregulated in cancer. Both miRNA-143 and miRNA-145 were found to be downregulated in the study of squamous cell cancer and were also correlated with tumor death. These miRNAs have also been found to be downregulated in various cancer cells including colorectal, lung, and nosopharyngeal cancers.

Some miRNAs have been implicated in the Wnt pathway specifically as either up or down regulators. miRNA-449, when inhibited, upregulates Wnt signaling. miRNA-315 has recently been reported to act on the the Wnt pathway by targeting axin and notum, negative regulators in the pathway. miRNA-8 inhibits by several ways including the inhibition of TCF protein expression. [138] Ji et al found an association of microRNA-181 expression with Wnt/B-Catenin activation both in vivo and in vitro. The activation of the pathway induces the expression of 4 mature miR-181s.[139] Conversely the inhibition of signal reduces the levels of miR-181s. miRNA-200a has been found to directly target ß-catenin mRNA to inhibit translation and block Wnt signaling. Downregulation of miRNA-200a and the upregulation of ß-catenin was observed in human meningioma samples. [140]

Evidence is quickly emerging on the role of miRNAs in carcinogenesis.[141] The association in prognosis with miR21 and miR-375 has been demonstrated in separate investigations. [142] MiRNA

203 was observed to halt cell proliferation in squamous cell esophageal cancer suggesting that it may be a candidate for a therapeutic agent. [143] MiR-29c has been observed to cause cell cycle arrest and inhibit the proliferation of esophageal cancer cells in vitro whereas miR-106b may enhance proliferation of these cells [144, 145] Additionally, there have been publications suggesting the interaction of miRNAs with other genes that regulate cell proliferation. One study observed that p53 regulated miR-34 as well as miR-215 suggesting that microRNAs work in concert with other genes in carcinogenesis.[146] MiR-10b was found to be over expressed in esophageal cancer tissues when compared to adjacent normal tissue. [147]

1.5 Gaps in Literature

Esophageal cancer is a complex disease with a multi-factorial causes and remains largely understudied. To date, there have not been any epidemiologic studies researching genetic susceptibility markers of the Wnt pathway in esophageal cancer. Preclinical data have suggested that proteins including Wnt proteins, axin, GSD, disheveled, frizzled, and Beta-catenin in the Wnt pathway play a role in carcinogenesis and that alterations in the genetic code could affect the actions of these proteins. Recently, there have been preclinical data to suggest that a minor subset of progenitor cells underlying the squamous epithelium of the esophagus is responsible for the regeneration and maintenance of the esophagus suggesting involvement of stem cell pathways.[148] While mutations in this pathway have been observed in other cancers and disease indications, they have not been observed for esophageal cancer. Moreover, little is known about miRNAs involved in this pathway or their role in esophageal carcinogenesis. For those miRNAs involved, no previous studies have examined either SNPs in the genes encoding these miRNAs or their target sites. This

study may potentially add novel knowledge on the role of the stem cell pathway in the esophageal carcinogenesis.

1.6 Rationale for study

Esophageal cancer is among the most common cancers in China and the rest of the Asian Pacific Rim and is among the cancers with the poorest survival rates in both developing and developed parts of the world. Though tobacco smoking and alcohol drinking are known risk factors, the genetic components involved in this multi-factorial causal pathway are still not very well understood. Recently, researchers have demonstrated that the epithelium of the esophagus is generated and maintained by a small proportion of underlying cells that divide into proliferating cells and meanwhile, little remains known about the stem cell biology of the esophageal epithelium[149]. This study attempts to illustrate what effects, if any, polymorphisms in the stem cell pathway may have on esophageal cancer may be useful in understanding genetic involvement and possible gene-environmental interactions and may be used in the risk assessment and the early detection of esophageal cancer as well as the identification of high-risk individuals for intervention with the aim of reducing the incidence and mortality of esophageal cancers. To date, no known study has investigated the Wnt pathway and its associated miRNAs in a large epidemiological study of esophageal cancer in a Chinese population.

CHAPTER 2.

Study Methodology

2.1 Study Objectives

This study takes advantage of the already collected epidemiological data and biological specimens in a large population-based case-control study of esophageal cancer. Our objective is to observe associations between genetic markers and esophageal cancer as well as any potential gene-environmental interactions. To date, no other study of esophageal cancer has been as large in a Chinese population.

2.2 Specific aims and hypotheses

Hypothesis 1

Esophageal cancer has been previously identified as a major public health problem in certain areas of the world including China. Though some risk factors have been identified, there may be other risk and protective factors yet to be identified.

Specific Aim 1

To assess the independent associations of other possible risk or protective factors including passive smoking exposure and garlic consumption on esophageal cancer and investigate whether these factors modify the associations of known risk factors such as tobacco smoking and alcohol consumption as well as potential genetic factors on the development of esophageal cancer in a high-risk Chinese population.

Hypothesis 2

Esophageal cancer has been previously identified as an aggressive cancer partially associated with physical injury to the esophagus. The Wnt pathway is a critical stem cell pathway and is responsible for regeneration and regulation of tissue homeostasis. Genes associated with the stem cell pathway including ß-catenin , axin, frizzled, disheveled, and wnt proteins, may contain markers of susceptibility for esophageal cancer.

Specific Aim 2

To assess the independent associations of cancer stem cell-related genes on the susceptibility of esophageal cancer and investigate whether single nucleotide polymorphisms (SNPs) in genes of ß-catenin , axin, frizzled, disheveled, and wnt proteins modify the associations of known risk factors such as tobacco smoking and alcohol consumption on the development of esophageal cancer in a high-risk Chinese population.

Hypothesis 3

The Wnt pathway has been observed to be associated with several microRNAs (miRNA) which regulate gene expression in carcinogenesis. SNPs of genes in miRNAs or their target sites may be associated with susceptibility of esophageal cancer.

Specific Aim 3

To evaluate the independent associations of SNPs of miRNA-181, miRNA-200, miRNA-449, miRNA-503, and miRNA-589 involved with stem cell pathway on the development of esophageal cancer and investigate whether polymorphisms modify the associations of known risk factors such

as tobacco smoking and alcohol drinking on the development of esophageal cancer in a high-risk Chinese population.

Hypothesis 4

Esophageal cancer is a rare event where the causes are multi-factorial and involve the coordination of numerous genetic molecular events. The SNPs examined may interact with each other, resulting in a modification of their associations on esophageal carcinogenesis.

Specific Aim 4

To explore the gene-gene interactions between the SNPs in the stem cell pathway and the related mi-RNAs pathways on the susceptibility of esophageal cancer in a high-risk Chinese population.

2.3 Background

This project employs a population-based case control study design. Epidemiologic data and patient specimens had been previously collected. With this project, we will assay genetic susceptibility markers to evaluate the independent associations as well as interactions between genetic and environmental factors.

The study recruited 1789 incident cases of primary esophageal cancer and 4,966 population controls from Taixing, Dafeng, and Ganyu in Jiangsu province. Epidemiologic data has been collected from patients with esophageal cancer as well as controls by interviewers. Blood specimens were drawn from all subjects, stored in a local laboratory in China, and then transferred to the Specimen Bank at the University of California, Los Angeles Jonsson Comprehensive Cancer Center. DNA extraction for the detection of polymorphisms for the subjects occurred at the Molecular Epidemiology

Laboratory at UCLA which contains the appropriate equipment to store samples and perform molecular assays. Genotyping was performed at UCLA Genotype and Sequencing Core, with a customized Fluidigm Dynamic 96.96 Array™ Assay (Fluidigm, South San Francisco, CA).

Using SNPs of interest we evaluated the association between potential genetic susceptibility markers of cancer stem cell pathways including miRNAs and esophageal cancer in this high-risk population. Only SNPs with a minor allele frequency of 5% or greater in the Han Chinese population were selected. Additionally, SNPs were observed for potential gene-gene and gene-environment interactions.

2.4 Study Population

2.4.1 Dafeng and Ganyu

The study took place in Dafeng, Ganyu, and Taixing which are located in Jiangsu province located in the southern-east part of China. Dafeng and Ganyu counties are rural areas in northern Jiangsu with combined population of approximately 1.8 million residents in total. Both areas have increased incidence of esophageal cancer though Ganyu has a lower age-standardized mortality (24 per 100,000) compared to Dafeng (36 per 100,0000) from 1996-2002.

Potential cases were identified from regional cancer registries that formed in the late 1990s. Eligible cases were all patients who were diagnosed with esophageal cancer from with a pathologically or clinically confirmed diagnosis of primary esophageal cancer. The cases were coded with the International Classification of Diseases, 10^{th} revision (ICD-10, code C15.0 to C15.9) All regional hospitals were required to report esophageal cancer cases to the local Center for Disease Control and Prevention (CDC.) We intended to interview all incident patients with primary esophageal

cancer during the study period who consented to be interviewed and met the following inclusion criteria:

1-patients must be newly diagnosed

2-aged 25-70 years

3-have no previous history of any cancer diagnosis

4-have lived in the study area for 5 years or more

5- be in stable medical condition as determined by their physician.

In Dafeng, 46% of cases were histologically confirmed; 30% of cases were histologically confirmed in Ganyu.

For the recruitment of cases, a list of newly diagnosed esophageal cancer cases from all hospitals in the area was obtained from the local tumor registry. Following IRB approval of Jiangsu Provincial Health Department, the interviewers located the patients, explained the study and received written informed consent. Epidemiologic data were obtained by face-to-face interviews using standardized questionnaires. Non-fasting 5ml blood samples were collected at the time of interview. Detailed information on the pathologic diagnosis was obtained by consultation with the pathologist or medical records.

Between 2003-2007, recruitment rates of 68% and 75% registered patients were observed in Dafeng and Ganyu, respectively. All eligible cases who agreed to sign an informed consent to participate in the study were interviewed. All patients who consented were interviewed and had a blood sample drawn.

Eligible controls were healthy and cancer-free individuals from the general population of Taixing, Dafeng, and Ganyu. The controls were frequency-matched with cases on age (within 5 years) and gender. We intended to interview all randomly selected eligible controls during the study period that gave consent and met the following inclusion criteria:

1-in stable medical condition

2-able to answer questions reliably

3-have lived in the area for at least 5 years or more.

The controls were randomly selected from a residential list. Following the selected list, the interviewer located the controls, explained the study, interviewed them at their home, and collected approximately 5 ml of blood sample. If the potential control refused participation, another control was identified in the same fashion.

During the recruitment period, the response rate was approximately 70% with a total of 1,018 healthy population controls for this study. All controls recruited completed interviews and had blood samples drawn.

2.4.2 Study Population—Taixing

This population-based case-control study took place in Taixing City in Jiangsu Province. Study participants aged 20 years or older must have lived in Taixing for over 10 years to participate. The study took place for 6 months between 6/1/2000-12/30/2000 and included all newly diagnosed cases of esophageal, stomach and liver cancer. For the purposes of this study, only esophageal

cancer cases were included in our analysis. Cases were confirmed pathologically or clinically and reported to the Taixing Tumor Registry. A total of 218 esophageal cancer cases and 464 population-based controls were included in this study. Controls were randomly selected from a list of residents, frequency-matched with cases on gender and age as well as residential block. Recruitment rates for cases and controls were 67.0% and 89.4%, respectively. All study participants signed written informed consent with IRB approval by Fudan University School of Public Health.

2.5 Epidemiologic Data Collection

In-person interviews were conducted on all cases and controls in this study using a standard questionnaire. Once written informed consent had been obtained, interviews were administered and a physical examination was performed. All of our interviewers were trained and interview sessions were monitored by supervisors. Cases were interviewed in the home or the hospital; controls were interviewed at home. On average, interviews lasted 1 hour for each subject.

Epidemiologic data were obtained by in-person interviews using the same standardized questionnaire which included information on: (1) demographic characteristics, (2) lifestyle habits inclusive of active and passive exposure to cigarette smoking and alcohol drinking, (3) family history of cancer, (4) dietary habits, (5) occupational exposures and (6) other environmental exposures. The physical examination took place at the time of interview. Various anthropometric measurements were taken including body mass index (BMI—kg/m2.) The Chinese national standard for defining BMI quartiles is as follows: underweight: <18.5, normal: 18.5-23.9, overweight: 24-27.9, obesity: >=28. All questionnaire data has been double data entered into an Epidata 3.0 database. Appropriate security systems are in place to protect confidentiality and prevent unauthorized access.

Study personnel collected 5 ml of blood from cases and controls at the time of interview. All samples were transported to the local laboratory refrigerator (4^0C) immediately thereafter. DNA was extracted and sent to the Specimen Bank at the University of California, Los Angeles Jonsson Comprehensive Cancer Center with appropriate packaging.

2.6 SNP Selection

The SNPs reported in Tables 1-5 were screened for minor allele frequencies of at least 5% in Han Chinese. No SNPs were found to be in linkage disequilibrium at our accepted r^2 threshold of 0.80 and thus no SNPs were excluded from our analysis based solely on this test. Additionally, SNPs with supporting publications on their potential associations in cancer were explored. Eleven SNPs were identified for possible analysis. Once the genotyping had been performed, 2 failed to produce laboratory results for this study. From the list of 9 SNPs, the following exclusion criteria were used in our analysis: 1-minor allele frequencies of >5% in the controls, 2-SNPs that did not follow Hardy-Weinberg equilibrium (HWE) among controls and 3-genotyping call rate of over 80%. Three additional SNPs were excluded because their HWE p-value exceeded the Bonferroni-adjusted p-value cut-off. The SNPs for this project were part of a larger project including 96 SNPs so we used the Bonferroni-adjusted cut-point of 0.05/96.

2.7 Laboratory Analysis

DNA was extracted using a modified phenol-chloroform protocol. A 1 ml blood clot was transferred to a 2 ml centrifuge tube with 1 ml PBS and centrifuged at 16,000g for 15 minutes at room temperature. After discarding the supernatant, the pellet was resuspended in 250µl 10% SDS containing 5µl freshly thawed proteinase K and incubated for 2 hours at 55°C. Using equal volume of Tris-saturated phenol chloroform isoamylalcohol solution DNA was extracted from precipitate of

two volumes of ice-cold absolute ethanol in a freezer at lowest temperature available for about 15 minutes. The DNA was resuspended in 300μl of storage buffer and stored at 4°C. DNA purity and concentration was assessed by spectrophotometric measurement of absorbance at 260/280 nm.

Aliquots from DNA samples of the study population were placed onto 96-well plates for genotyping using the Fluidigm system. Genotyping was performed at UCLA Genotype and Sequencing Core, with a customized Fluidigm Dynamic 96.96 Array™ Assay (Fluidigm, South San Francisco, CA). The assays are based on allele-specific PCR SNP detection chemistry with the reliability of Dynamic Array™ integrated fluidic circuits (IFCs). The SNP type Assay employs tagged, allele specific PCR primers and a common reverse primer. A universal probe set is used in every reaction, producing uniform fluorescence and Fluidigm provides locus-specific primer sequences that allow one to confirm target locations.

For quality control, one positive control (consisting of DNA samples purchased from the Coriell Repository) as well as one negative control (reagent mix with no DNA) was included in each reaction plate. Replicate DNA aliquots, which comprised approximately 1% of the samples, were distributed throughout the reaction plates. Laboratory staff was blinded to all identifiers and research information about the samples. Call rates were above 85% for all SNPs. Duplicate samples were run at random for quality assurance with 100% concordance.

2.8 Statistical Analysis

Statistical analyses were performed using SAS v9.1.3 (Cary, NC). A chi-square test was used to test for deviations from HWE. Unconditional logistic regression was employed to estimate crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CI). Adjusted proportional

attributable fractions were calculated using methods from Briuzzi et al while adjusting for the same confounding factors for adjusted ORs.[150]

Stratified analysis on smoking status included those who were ever or never smokers by self-report on the questionnaire. Stratified analysis on drinking status was performed on those that self-reported as never or occasionally drinking compared to often and daily drinkers. When considering the combination of drinking and smoking in additional stratification, never and occasional drinkers who never smoked were considered one stratum. Likewise, ever smokers who drank often or daily were in the other strata.

Individuals who reported any ETS at home or work or both were considered as exposed to ETS and these reported non-ETS exposure at both home and work were considered non-exposure ETS. In the analysis of dose-response relationship, we assigned scores of 0 for non-exposure to ETS, 1 for light, 2 for moderate, and 3 for heavy for each of the 2 sources (work and home) in the separate analysis of exposure at either work or home. When both exposures at home and work were combined, we added up their exposures together with a combined possible score of 6. For the combined analysis, scores of 1-2 were considered light, 3-4 was considered moderate, and 5-6 was considered strong exposure. Dose-response relationships were evaluated when treating the degree of ETS exposures (0, 1, 2, and 3) as a continuous variable in logistic regression model.

The following variables were identified for this study and included in statistical models for this study: age (continuous), gender (female=2 and male=1; except when stratified by gender), income 10 years ago (continuous), body mass index (BMI, continuous), education (illiterate=1, primary school=2, and middle school and above=3), family history of esophageal cancer in the first-degree relative (no=0, yes=1), study site (Dafeng=1, Ganyu=2, Taixing=3), and alcohol consumption (categories

of never=0, occasional=1, often=2, everyday=3).

2.8.1 Statistical model

The following variables were included in our model along with their correspondent values in the logistic regression model:

Dependent Variables

The dependent variable in this study is:

Esophageal cancer [yes/no]

Independent Variables

The independent variables in this study are:

- Tobacco use
    - Ever/never
    - Continuous (packyears)
- Alcohol use
    - Ever/never
    - Never/Occasional/Frequent/Daily
- Wnt pathway and associated miRNA SNPs (log additive, recessive, and dominant models)

Covariates

The potential confounders in this study are:

- Age
    - Continuous (years)

    - 10 year categories
- gender (male/female)
- body mass index
    - continuous
    - <18.5kg/m^2 18.5-24,24-28,>28
- family history of cancer (first degree relative)
- education level (illiterate, primary school, secondary school)
- exposure to environmental tobacco smoke at home (yes/no)
- exposure to environmental tobacco smoke at work (yes/no)
- income (per person in household/per month)
    - continuous
    - <1000, 1000-1500, >1500-2000, >2000

The potential confounding by the above variables is based on prior knowledge of their roles as independent risk factors for esophageal cancer. Additionally in this study, exposure to environmental tobacco smoke was found to be associated with esophageal cancer so it was added to the list of potential confounders.

Variables listed above will be used in the logistic regression models used to assess the specific aims for this study:

$$\text{Logit}\,R(X) = \alpha + \sum_{i=1}^{n} \beta_i X_i + \sum_{j=1}^{m} \gamma_j X_p X_q$$

where R(X) is the binary outcome esophageal cancer diagnosis and α is the y-axis intercept that represents the value of the log odds when all other covariates (Xi) are 0. β_i is the coefficient for each

variable in the model and represents the log odds ratio for a one-unit change for that variable when all other variables remain constant. Likewise, γ_j is the coefficient for product terms X_p and X_q which allows for evaluation of interaction on the multiplicative scale. Interaction will also be examined using stratified analysis of gene and environment variables.

In order to study the joint association, a joint odds ratio was calculated

SNP 1	SNP 2	Case	Control
-	-	A	B
+	-	C	D
_	+	E	F
+	+	G	H

$$OR_{joint} = G*B/A*H$$

Since this study analyzed independent associations of SNPs in various strata, issues of multiple comparisons could have potentially arisen.

2.8.2 Power analysis

Using POWER Version 3.0[151, 152], the power for detecting the independent association of an exposure in the entire population was over 99.9% for an OR 1.5 and 2.0. For an OR of 1.2 the power reached above 90% for a prevalence of 0.40. For our exposures under investigation for the entire study population, passive smoking and garlic intake, the exposure prevalence was roughly half the study population.(Supplemental Table S2.1) The power for detecting an independent association of one SNP was calculated for prevalence of the allele from 0.25 to 0.50 since the lowest MAF for

all SNPs was 0.249. For those subjects with genotyping data available, power reached above 90% for a prevalence of 0.40 to detect an OR of 1.2—the power went down to 83.3% for prevalence of 25%. For ORs of 1.5 and 2.0, the power was above 99.9 with a 2-tailed significance level of 0.05. Values are shown in Supplemental Table S2.2. For combined effects of 2 SNPS, the power was estimated between 66.1% and 72.2% for an OR of 1.2 and over 99% for ORs of 1.5 and 2.0 (Supplemental Table S2.3). These estimates were based under the assumption of a univariate analysis and it is likely that the power may decrease with a multivariate analysis.

2.8.3 Missing data

Missing data in this study was assessed for all variables. For variables in our model, all but 2 had less than 2% missing. For these variables with very little missing, complete case analysis was performed. Twenty percent of subjects in Dafeng/Ganyu and 10% of subjects in Taixing had missing smoking pack years. For smoking pack years data, we imputed data using the median pack years of the controls for each area. Also, subjects in Taixing had missing data for BMI. Likewise, we imputed BMI by using the median BMI from the controls in Taixing.

Genotyping data was available in limited proportion of the subjects. To assess the potential selection bias, distribution of variables was compared between those with complete data and those without complete data.

2.8.4 Genetic susceptibility

For the analysis of SNPs, the main exposures were the selected genetic polymorphisms in the genes of interest in the Wnt pathway as well as the associated miRNA. The association between genetic polymorphisms and esophageal cancer were presented using adjusted ORs with 95% confidence

intervals. ORs were used to estimate rate ratios, and 95% confidence intervals will be included to reflect the precision of the OR estimates. In addition to the variables of primary interest, models included potential confounders including age, gender, tobacco smoking, alcohol drinking, variables related to socioeconomic status such as income earned and education. The inclusion of confounders was based upon prior knowledge and confounding within our dataset. The selection of covariates was based on prior knowledge and statistical association between the variables and outcome in the dataset. All previously established covariates were found to be associated in our study with p-values less than 0.05.

2.8.5 Gene-gene and gene-environment interactions

Interactions between genes and between gene and environment in risk for esophageal cancer were calculated in this study. Environmental factors of interest in interactions include alcohol consumption, active and passive tobacco smoking, as well as the combination of the 2 variables. Gene-environment interactions were assessed by looking at stratified analyses of smokers and non-smokers, alcohol drinkers and non-drinkers, and non-drinkers/non-smokers versus drinkers/smokers. For interaction odds rations, crude estimates of effect were compared to adjusted odds ratios where product terms were added into the logistic model. Gene-gene interactions between various genetic polymorphisms are also of interest and were calculated. Gene-gene interactions were assessed using a joint associations model which allows the observation of multiplicative effects.

CHAPTER 3.

RESULTS

Our analysis included 1,789 cases of esophageal cancer and 4,966 controls (Table 3.1). Fifty two percent of the cases came from Ganyu, a region of higher incidence rates for esophageal cancer. A large proportion of cases and controls self-reported as illiterate (56.5% and 48.0%). Additionally, the majority of cases and controls were ever smokers; 69.9% of cases and 57.9% of controls. Mean pack-years were high among cases and controls—22.6 (SD: 24.5) pack-years for cases and 16.9 (SD: 22.3) pack-years in controls.

The cases and controls differed on many of the demographic variables with associated p-values less than 0.05 including age, county, income, BMI, education, and family history of esophageal cancer. Initially, the study matched cases and controls on age and gender though this match was broken during the course of the study. As a result, age differed among cases and controls. There was a higher proportion of controls aged under 50 years old (12.1% versus 5.8% of cases). Conversely, there were more cases in the 60-70 (35.2% versus 31.4%) and 70-80 (28.8% versus 27.5%) age groups than controls which was expected given the association of age and esophageal cancer. Gender differed between cases and controls as well though the p-value was slightly above 0.05 (0.067) with a slightly higher percentage of males in the cases (76.7% versus 74.6%). Males have a higher risk by 2-4 times worldwide so without the entirety of the study matching on gender, we expect there to be a difference.

Socioeconomic status has long been found to be associated with esophageal cancer and so the

respective variables in this study including income and education differed between cases and controls. In assessing income reported 10 years ago, more cases comprised the lowest group of under 1000 Yuans per person/month than controls (37.5% versus 27.1%). Likewise, a higher percentage of cases self-reported as illiterate (56.5% versus 48.0%). Without matching on geographical location, the majority of cases came from Ganyu (52.0%), an area of higher risk for esophageal cancer, while the majority of controls came from Dafeng (51.0%). A higher percentage of cases reported a family history of esophageal cancer in a first degree relative (23.6% versus 20.2%) which is expected based on previous reports of family history. BMI was also differed with cases having a higher proportion of BMI under 18.5. Since esophageal cancer affects a patient's ability to eat, weight loss or lower weight can be expected among cases in this case-control study.

Cases and controls also differed in lifestyle habits of tobacco smoking and alcohol drinking. A higher percentage of cases were considered ever smokers (69.9% versus 57.9%). With respect to alcohol drinking, often and daily drinking was observed more in the cases than the controls; (often drinking: 18.3% versus 14.2% and daily: 29.2% versus 24.0% of the controls). The observed higher proportion of smokers and frequent drinkers among cases is expected given the association of esophageal cancer with smoking and drinking.

As expected, many of the smoking and drinking variables were associated with esophageal cancer (Tables 3.2 and 3.3). Adjusted odds ratios for former and current smokers were found to be 2.00 (95% CI: 1.62-2.56) and 1.44 (1.23-1.69), respectively. The age of smoking was found to be associated with those starting before 20 to have an adjusted OR of 1.41 (95% CI: 1.15-1.74). Those beginning smoking between 20-30 and 30-40 had an adjusted OR of 1.58 (95% CI:1.33-1.87) and 1.56 (95% CI: 1.23-1.97). Likewise, years of smoking was seen to have an association with the

strongest effect estimate for those smoking between 30-40 years (aOR=1.89, 95% CI: 1.55-2.29). The association for those smoking over 40 years was weaker yet still significant (aOR=1.47, 95% CI: 1.23-1.75) likely due to competing risks for those smoking for that duration. For those who smoke daily, the adjusted odds ratios increased with the amount smoked. An adjusted OR of 3.67 (95% CI: 2.55-5.28) was seen for the over 40 times daily group. Pack years of smoking appeared to have a dose-response relationship with esophageal cancer in our study. Beginning at 0-10 pack years, the adjusted OR was found to be 1.29 (95%CI: 0.97-1.71) compared to the never smokers in this study. The effect estimates increased with every group (10-20, 20-30, 30-40) with the highest category of 40 pack years and over found to have an association of 1.87 (95%CI; 1.53-2.28). We also observed increasing adjusted ORs with increasing alcohol drinking. Often and daily drinkers had adjusted ORs of 1.48 (95%CI; 1.21-1.80) and 1.50 (95%CI: 1.26-1.79), respectively. Consumption of alcohol in grams in the 90s and the previous year had an association with esophageal cancer (aOR=1.59, 95%CI: 1.31-1.93 and aOR=1.78, 95%CI: 1.48-2.15, respectively).

3.1 Passive smoking (Tables 3.4-3.8)

Associations of environmental tobacco smoking and esophageal cancer among the whole study population with both smokers and non-smokers combined are presented in Table 3.5. Exposure to environmental tobacco smoking was higher in cases for both at home as work. Almost half of the cases (46.8%) reported exposure to environmental tobacco smoking at home and 22.3% reported exposure at work. On the other hand, 38.5% of controls were exposed at home and 17.1% at work. For ETS exposure at home (Table 3.4), the adjusted OR for exposure to environmental tobacco smoking was found to be 1.21 (95% CI: 1.06-1.38). When we analyzed 4 levels of exposure to environmental tobacco smoking (none, slight, medium, and heavy), heavy exposure was associated with an adjusted OR of 1.45 (95% CI: 1.14-1.80) with a p-value for trend found to be 0.006. When stratified by gender, adjusted ORs for women were higher than those in men (OR=1.29, 95% CI:

1.01-1.65 versus 1.16 95% CI: 0.99-1.36). Subsequently, heavy exposure to environmental tobacco smoking at home was 1.65 (95% CI: 1.12-2.41) in women with a p-value for trend of 0.024 which was stronger than in men with an adjusted OR of 1.33 (95% CI: 1.00-1.78). For exposure to ETS at work (Table 3.5), those with ETS exposure had an adjusted OR of 1.36 (95% CI: 1.15-1.60) when both men and women combined. The association was similar in men (OR=1.40, 95% CI: 1.17-1.68) and attenuated in women (OR=1.19 95% CI: 0.79-1.79.) The association was strengthened as level of exposure reported at work increased. Overall, heavy exposure to environmental tobacco smoking at work resulted in an OR of 2.33 (95% CI: 1.54-3.51) with a p for trend <0.0001. A similar association was observed for men (OR=2.11 95% CI: 1.34-3.32, p<0.0001); numbers of cases and controls for women among the various levels of exposure were relatively small and produced no apparent trend.

We analyzed environmental tobacco smoking at home and work among only non-smokers in order to avoid potential residual confounding effects by active smoking (Table 3.6 and 3.7.) Overall, exposure to environmental tobacco smoking at home among non-smokers was associated with esophageal cancer for men and women combined (OR=1.23 95% CI: 0.99-1.53). Similarly, exposure to environmental tobacco smoking at work was associated with the disease (OR=1.38 95% CI: 0.99-1.91). The OR in men was similar (OR=1.41, 95% CI: 0.90-2.21) to that of women (OR=1.33, 95% CI: 0.82-2.16.) When we analyzed non-smokers for 4 levels of exposure (none, slight, medium/heavy), exposure to ETS at work produced a trend when men and women were conbimed (p=0.008) with an adjusted OR for medium/heavy exposure of 2.03 (95% CI: 1.27-3.26). Similarly, a trend was observed in men (p=0.018) and an adjusted OR for medium/heavy exposure was found to be 2.55 (95% CI: 1.40-4.64.). No clear trend was observed among women for ETS exposure at work because of small numbers of female non-smoking cases and controls.

We performed analyses of environmental tobacco smoking when exposures at both home and work were combined (Table 3.8). Among the overall population with both smokers and non-smokers, ETS exposure (Yes vs. No) was positively associated with esophageal cancer with an adjusted OR of 1.29 (95% CI: 1.13-1.46). . Among nonsmokers, the association was similar with an adjusted OR of 1.29 (95% CI: 1.04-1.59.). The population attributable fractions were 12.8% (95% CI: 5.62%-19.7%) and 11.4% (95% CI: 0.90%-21.7%) for the overall population and non-smokers, respectively. For both smokers and non-smokers combined, individuals exposed to environmental tobacco smoking at home and at work were associated with an adjusted OR of 1.74 (95% CI: 1.42-2.13) compared to those that were not exposed at either home or work. Even if the exposure came from one source (home or work) there was still an association observed (ORadj=1.18; 95% CI: 1.03-1.35). The association appeared to be similar among nonsmokers with an adjusted OR of 1.78 (95% CI: 1.19-2.68) when exposure occurred at both sources and 1.22 (95% CI: 0.98-1.52) when the exposure came from any one source. The association appeared to strengthen with the combined (work and home) level of exposure when analyzed for various degrees of exposure (none, slight, medium, heavy). Overall, the OR for heavy exposure at home and work was found to be 2.55 (95% CI: 1.66-3.92, p for trend<0.0001.) Among nonsmokers, the association for Medium/Heavy was 1.55 (95% CI: 1.09-2.15) with a p for trend of 0.016.

3.2 Garlic consumption (Tables 3.9-3.13)

We analyzed the association of frequency of garlic intake and its association with esophageal cancer. For this analysis we used data obtained from the question "how often do you eat raw garlic?" as well as the food frequency question about garlic intake. With either variable, there appeared to be an inverse association—for those with often intake of raw garlic or a frequency of 2 or more times per

week—an adjusted OR of 0.67 (0.53-0.85) and an adjusted OR of 0.76 (0.63-0.90), respectively. The association is similar in men (Table 3.9) with adjusted ORs of 0.67 (0.51-0.88) and 0.77 (0.62-0.95), respectively. The association is also similar in women though the confidence interval is widened likely due to the smaller numbers of women in this study. When we performed a stratified analysis on never and ever smokers the results were similar (Table 3.10). The adjusted OR for those who consumed garlic more than 2 times per week was found to be 0.70 (0.52-0.94) in never smokers and 0.83 (0.68-1.02).The association was similar in male never and ever smokers but not apparent in females never and ever smokers. Similarly, garlic intake had an inverse association with esophageal cancer risk for those who were unexposed and exposed to environmental tobacco smoke (Table 3.12) with adjusted ORs of those who eat garlic more than 2 times a week of 0.79 (0.50-1.03) and 0.52 (0.31-0.87), respectively.

3.3 Wnt pathway polymorphisms (Tables 3.14-3.18)

We analyzed 5 SNPs of the wnt pathway and its association with esophageal cancer and found no significant associations (Table 3.14). After adjusting for age, education, smoking exposure, alcohol intake, study site, income, and family history, rs3729629 and rs4730775 (WNT2) had weak inverse associations of (CC+C/T vs CC aOR: 0.89, 95% CI: 0.75-1.06 and T/T+C/T vs C/C aOR: 0.89 95% CI: .75-1.07). Similarly, rs4835761 (WNT8A) had an inverse association with an aOR of 0.88 (95% CI: 0.74-1.06, G/G+A/G vs A/A). The association for Wnt2 (rs3729629 and rs4730775) varied by smoking status; never smokers had a negative association with esophageal cancer for rs3729629 with an aOR of 0.62 (95% CI: 0.37 -1.04, C/C+G/C vs G/G) while ever smokers had an aOR of 0.94 (95% CI: 0.67-1.31, C/C+G/C vs G/G) in Table 3.15. For rs4730775 (Wnt2), never smokers had a negative association with esophageal cancer with an aOR of 0.72 (95% CI: 0.53-0.98; T/T+C/T vs. C/C). When stratified by alcohol (Table 3.16), never and infrequent

drinkers had inverse of associations with rs3729629 and rs4730775 (WNT2) (CC+C/G vs G/G aOR: 0.76, 95% CI: 0.60-0.96 and T/T+C/T vs C/C aOR: 0.79 95% CI: .62-1.00, respectively) while there was no association for frequent and daily drinkers (CC+C/G vs G/G aOR: 1.05, 95% CI: 0.80-1.36 and T/T+C/T vs C/C aOR: 1.03 95% CI: 0.79-1.34, respectively). Similarly, rs4835761 (WNT8A) had an inverse association with an aOR of 0.82 (95% CI: 0.64-1.05, G/G+A/G vs A/A) in never and infrequent drinkers while there was no association observed in frequent and daily drinkers (aOR 0.97 95% CI: 0.74-1.28, G/G+A/G vs A/A. For rs222851 (DVL2) we observed a potential weak association for never and infrequent drinkers with an aOR of 1.19 (95% CI: 0.93-1.52, G/G+A/G vs A/A) and no association in frequent and daily drinkers aOR of 0.88 (95% CI: 0.74-1.06, G/G+A/G vs A/A).

We also examined those who drank and smoke versus those neither drank nor smoke (Table 3.17). For non-drinkers and non-smokers, there was an inverse association observed with the rs2241802 (FZD3) with an adjusted OR of 0.68 (95% CI: 0.46-1.00; G/A+A/A vs. G/G) while there was no association observed in the drinkers/smokers group (aOR=1.00, 95% CI: 0.82-1.49; G/A+A/A vs. G/G). A similar pattern was observed for Wnt2 with inverse associations observed in the non-smoking/non-drinking category for rs3729629 and rs4730775 (aOR: 0.54, 95% CI: 0.37-0.78; CC+C/G vs G/G and aOR: 0.67 95% CI: 0.46-0.98 T/T+C/T vs C/C, respectively). No association was observed in the drinkers/smokers group for rs3729629 and rs4730775 Wnt 2.

3.4 The association miRNA 589 polymorphism and esophageal cancer

We did not observe an association of rs2953 miRNA589 (CTNNB1 binding site, Table 3.18) with esophageal cancer with an adjusted OR of 0.95 (95% CI: 0.80-1.13; G/T+G/G vs. T/T). When stratified by smoking status (Table 3.19), we observed a potential inverse association among smokers with an adjusted OR of 0.84 (95% CI: 0.69-1.03; G/T+G/G vs. T/T) while there was no

association observed among never smokers (aOR=1.13, 95% CI: 0.84-1.51; G/T+G/G vs. T/T). We stratified by alcohol drinking frequency (Table 3.20) and there was a potential weak inverse association for frequent and daily drinkers with an adjusted OR of 0.88 (95% CI: 0.71-1.08 G/T+G/G vs. T/T) while there was no association observed for never and infrequent drinkers (aOR=1.01, 95% CI 0.79-1.27 G/T+G/G vs. T/T). No apparent association was observed for the group of smokers and drinkers with an adjusted odds ratio of 0.81 (95% CI: 0.56-1.16, Table 3.21).

3.5 Interactions between Wnt pathway polymorphisms and associated miRNA polymorphisms and the association of esophageal cancer

We analyzed the joint effects of a miRNA-related SNP and a Wnt pathway SNP. Combined effects with rs2953 miRNA589 and other Wnt pathway SNPs (Rs2241802 FZD3, rs3729629 WNT2, rs4835761 WNT8A, and rs222851 DVL2) did not appear to have an association when including the entire study population. However, when performing a stratified analysis of smokers and non-smokers (Table 3.23) the aROR for rs2953 miRNA589 and rs3729629 Wnt2 demonstrated an inverse association in smokers (aROR=0.58, 95% CI=0.35-0.96; G/G+A/G vs A/A for rs2953 and C/C+G/C vsG/G for rs3729629). This association remained when we stratified on both drinking and smoking status (Table 3.25). For ever smokers and frequent/daily drinkers, the aROR was 0.48 (95% CI: 0.26-0.88 G/G+A/G vs A/A for rs2953 and C/C+G/C vsG/G for rs3729629).

CHAPTER 4.

DISCUSSION

4.1 Passive smoking

ETS exposure has been found associated with esophageal cancer in this case-control study with adjusted ORs of 1.29 (95% CI: 1.13-1.46) when both smokers and non-smokers combined and 1.29 (95% CI: 1.04-1.59 among nonsmokers. The population attributable fractions (PAFs) were 12.8% (5.63%-19.7%) and 11.4% (0.90%-21.7%) for overall population and nonsmokers, respectively. Dose-response relationships between ETS exposure and esophageal cancer were observed for both overall population as well as non-smokers. Though there has been not much attention paid to the effects of ETS on esophageal cancer, ETS-lung cancer relationship among non-smokers has been studied extensively.[26] In a meta-analysis by Hackshaw et al, a combined OR of 1.23 (95%CI: 1.13-1.34) for both men and women with 39 published studies. All studies in this meta-analysis examined non-smokers with spouses who smoked[153]. Our results of ETS-esophageal cancer among non-smokers are consistent with the findings of ETS-lung cancer meta-analysis with the point estimates of ETS and esophageal cancer among Chinese population of 1.29 (95%CI: 1.04-1.59), indicating similar biological mechanisms for both lung cancer and esophageal cancer.

Few papers have been published on ETS and esophageal cancer. One case-control study of 107 cases of squamous cell esophageal cancer in Huaian, Jiangsu Province of China by Wang et al reported an unadjusted OR of 2.04 (95% CI: 1.14-3.70) for ETS-esophageal cancer, however, the association disappeared in multivariate analysis when adjusting for potential confounding factors. [18] Another case-control study in China by Sun et al of 250 squamous cell esophageal cancer cases found environmental tobacco smoking to be associated with an adjusted OR of 2.42 (95% CI: 1.36-

4.32), but active smoking was not adjusted in the multivariate analysis.[17] Both studies of Chinese populations had methodological issues, relatively small sample sizes and insufficient power to further perform analysis among non-smokers. A study conducted in the U.S. assessed the effects of environmental tobacco smoking on esophageal adenocarcinoma and found an OR of 1.49 (95% CI: 0.65-3.40) among nonsmokers with environmental tobacco smoking exposure based on only 22 non-smoking cases.[154] To the best of our knowledge, this is a case-control study with the largest sample size that observed positive associations between ETS and esophageal cancer in a Chinese population. In addition, there has been no previous study on the effects of environmental tobacco smoking and esophageal cancer in non-smokers in Chinese population.

Involuntary smoking (or environmental tobacco smoking) has been defined as a group one carcinogen with sufficient evidence for lung cancer.[155] However, the evidence for ETS associated with other smoking related cancers are very limited. As documented by IARC, there are at least 69 known carcinogens identified in ETS, including polycyclic aromatic hydrocarbons and tobacco-specific N-nitrosamines which can cause direct damage to tissues including the esophagus. [155] Traditionally, ETS has been observed to cause genetic mutations of the epithelium of target tissues.[156, 157] Exposure to ETS among non-smokers has been observed to increase 4-ABP-hemoglobin adducts.[158] More recently, its carcinogenic properties have been enhanced by its ability to induce premature aging of mitochondria by oxidative mitochondrial metabolism.[159]

Given the biological plausibility and the evidence presented on lung cancer as well as the large sample size of the current study, the association observed between ETS and esophageal cancer in this study is not likely to be by chance along. In this study, we have carefully adjusted potential confounding factors when we analyzed smokers and non-smokers together, especially active

smoking and alcohol drinking, two major risk factors for esophageal cancer in Chinese population. In addition, we have conducted our analyses on the relationship only among non-smokers in order to reduce potential residual confounding effect of active smoking. It is a general belief that active smoking might have a strong confounding effect on the relationship between ETS and the disease, and any analysis of ETS-disease relationship should only be conducted among non-smokers because simply adjusting for active smoking in the overall population including both smokers and non-smokers, residual confounding effects by active smoking might still exist. In our results of combined analyses presented in Table 4, we did not observed any difference in terms of point estimate of ETS-disease between overall population with both smokers and non-smokers and non-smokers only, indicating that the residual confounding effect by active smoking is minimum when active smoking is adjusted among overall population.

Because of the retrospective nature of case-control study design, our observation has been largely relied on retrospective self-report of ETS. It is therefore subject to differential recall among cases and controls which could result in an over-estimate of an association between ETS and esophageal cancer. However, since ETS was not a known risk factor for esophageal cancer in the Chinese population and, in addition, self-reported ETS exposure has been suggested to be valid, the potential differential recall bias might be minimum.[160] Although this is a population-based case-control study, there might still be potential selection bias because the disease is deadly and patients at an advanced stage might die before we approach them for inclusion in the study, which was reflected by relatively lower response rate of 67-75%. Considering that smoking might also be related to stages of cancer, the observed association might be underestimated.

A population-based case-control study in a high risk population with a large sample size is a major strength. The epidemiologic data were collected systematically with a comprehensive questionnaire and a quality control procedure in place. The study evaluated ETS-esophageal cancer among both overall population and non-smokers with consideration of adjustment for potential confounding factors.

Our study found an association among non-smokers (OR=1.38, 95% CI 0.99-1.91) with similar associations observed in men and women. We observed a dose-response relationship among increasing level of exposure with an OR for heavy exposure at 2.03 (95% CI: 1.27-3.26.) This strong association suggests that one potentially effective prevention strategy for a high risk area would be to prohibit smoking in the workplace.

4.2 Garlic Consumption

In our study, garlic consumption was found to be inversely associated with esophageal cancer risk with an adjusted OR of 0.67 (95% CI: 0.53-0.85) for those in the entire cohort that self-reported often consumption of raw garlic. This inverse association was seen across strata of drinking status and smoking status with an adjusted OR of 0.67 (95% CI: 0.48-0.96) among the group of frequent drinkers who were ever smokers. Garlic has been thought to have some anticarcinogenic properties though it has not been widely studied for esophageal cancer. Wargovich et al demonstrated that diallyl sulfide (DAS), the principal thioether of garlic, could inhibit tumor formation in the esophagus of rats. [161] Raw and cooked garlic has also been found to have reproducible inverse associations in stomach and colorectal cancers. [162] Recently raw garlic consumption was found to be a protective factor for lung cancer among a Chinese population. [163]For esophageal cancer, other studies have demonstrated an inverse association with allium vegetables that include raw garlic consumption but have not looked at the factor alone.[164]

4.3 Wnt Pathway

When evaluating susceptibility of genetic markers, differences among strata of major risk factors such as tobacco smoking and alcohol drinking for esophageal cancer are expected. In this study, we found that the association for Wnt2 (rs4730775 and rs3729629) varied by tobacco smoking and alcohol drinking status with weak associations present. Never smokers had an inverse association with esophageal cancer with an aOR of 0.72 (95% CI: 0.53-0.98; T/T+C/T vs. C/C). Similar yet not significant results were seen for rs3729629. However, we did not find an association in the entire study population for Wnt2 rs4730775 (OR= 0.89, 95% CI: 0.75-1.07 T/T+C/T vs C/C) or for smokers (OR= 0.98, 95% CI: 0.80-1.20 T/T+C/T vs C/C). When stratified by alcohol status, never and infrequent drinkers had inverse of associations with rs4730775 (Wnt2) (T/T+C/T vs C/C aOR: 0.79 95% CI: .62-1.00) while there was no association for frequent and daily drinkers (T/T+C/T vs C/C aOR: 1.03 95% CI: 0.79-1.34). Likewise, there was inverse association of Wnt2 in the non-smoking/non-drinking category for rs4730775 (aOR: 0.67 95% CI: 0.46-0.98 T/T+C/T vs C/C) and rs3729629 (aOR: 0.54, 95% CI: 0.37-0.78) while no association was observed in the frequent drinkers/smokers group (aOR:0.99, 95% CI: 0.74-1.31 and aOR:1.09, 95% CI: 0.82-1.44).

For the 2 SNPs encoding Wnt2 ligands, the inverse association was seen in the group without the high risk lifestyle habits. Wnt2 is thought to activate the Wnt pathway by binding to the receptor and initiating the cascade of events along the pathway. Though the exact mechanism remains unknown, upregulation of Wnt2 by estrogen has been previously seen to increase growth of a breast cancer cell line[165] whereas the gene does not appear to be amplified in gastric cell cancer in one study.[166] Other studies have suggested that the Wnt/β-catenin pathway is activated in gastric cancers.[167] Another study showed differential expression of the Wnt2 gene with higher levels among colorectal cancer cases than normal colon.[168] With respect to esophageal cancer, one

study found Wnt2 to promote growth of an esophageal cancer cell line.[169]. At the protein level, a study of non-small cell lung cancer tissues observed overexpression of Wnt2 protein when compared to normal tissues.[170] Since our study measured association of SNPs and esophageal cancer, further studies would be necessary to investigate the potential effects of the genotypes in the dominant model to determine how these SNPs could reduce risk of esophageal cancer. Ideally, our study would have benefited by available corresponding esophageal cancer tissues to determine the effects of these genotypes on protein expression.

Limited evidence of Wnt8A potential role in carcinogenesis currently exists. In our study, rs4835761 (Wnt8A) was found to have a potential inverse association with an aOR of 0.82 (95% CI: 0.66-1.01, G/G+A/G vs A/A) in ever smokers while there was no association observed in never smokers (aOR 0.89 95% CI: 0.66-1.21, G/G+A/G vs A/A). Among other strata, it became difficult to assess what associations, if any, rs4835761 had with esophageal cancer. In frequent drinkers, there may be a potential negative association (aOR=0.77, 95% CI: 0.55-1.09, G/G+A/G vs A/A). Limited evidence exists for the manner in which Wnt8A effects carcinogenesis though it is thought to regulate the Wnt pathway in some capacity.[171] Like the Wnt2 SNPs in our study, Wnt8A genotypes in the dominant model are associated with an inverse effect though in the strata of higher risk with respect to esophageal cancer suggesting that these alternate genotypes may provide some protective effect to the disease and that their effects are modified by environmental factors. Additional studies would be needed to assess whether this inverse association can be replicated in these genes. Future studies into the mechanism of these genotypes would enhance our findings in this study as well.

Though we understand disheveled and frizzled to be a part of the Wnt pathway, little has been

published about DVL2 and FZD3. DVL2 has been previously found to be overexpressed in colorectal cancer but there have been no investigation on the effects of SNPs in the DVL2 genes. [82]While we did not see any significant associations for the study population overall, there were some potential weak associations in strata of non-smokers and non-drinkers with an inverse association observed for rs2241802 (FZD3) (aOR: 0.68 (95% CI: 0.46-1.00; G/A+A/A vs. G/G) while there was no association observed in the drinkers/smokers group (aOR=1.00, 95% CI: 0.82-1.49; G/A+A/A vs. G/G). For rs222851 (DVL2) we observed a potential weak association for never and infrequent drinkers with an aOR of 1.19 (95% CI: 0.93-1.52, G/G+A/G vs A/A) and no association in frequent and daily drinkers aOR of 0.88 (95% CI: 0.74-1.06, G/G+A/G vs A/A).

4.4 microRNA

Though emerging evidence on the role microRNAs play in carcinogenesis continues to surface[172-174], we did not observe an overall association of rs2953 miRNA589 (CTNNB1 binding site) with esophageal cancer with an adjusted OR of 0.95 (95% CI: 0.80-1.13; G/T+G/G vs. T/T). Guo et al first observed microRNA expression profiles in esophageal cancer in 2008. In their study, they were able to distinguish normal tissue from tumor tissue based on expression profiles and found various microRNAs either with increased or decreased expression.[175] Feber et al performed another expression profile analysis of squamous cell carcinoma and adenocarcinomas of the esophagus and also found differential expression.[176] These studies examined expression profiles and not polymorphisms of binding sites. Additionally, neither reported any data on microRNA-589. Furthermore, there are not any supporting functional studies of microRNA binding sites for β-catenin which would add to the knowledge of polymorphisms in microRNA binding sites and there effects on gene regulation, protein expression and carcinogenesis.

The association of microRNA-589 appeared to be modified by smoking status where we observed a potential inverse association among smokers with an adjusted OR of 0.84 (95% CI: 0.69-1.03; G/T+G/G vs. T/T) while there was no association observed among never smokers. While the effect is not significant, a potential for association exists. When assessing the combined effects of Wnt2, the aROR for rs2953 miRNA589 and rs3729629 Wnt2 combined demonstrated a potential inverse association in smokers (aROR=0.58, 95% CI=0.35-0.96; G/G+A/G vs A/A for rs2953 and C/C+G/C vsG/G for rs3729629). This association remained when we stratified on both drinking and smoking status. For ever smokers and frequent/daily drinkers, the aROR was 0.48 (95% CI: 0.26-0.88 G/G+A/G vs A/A for rs2953 and C/C+G/C vsG/G for rs3729629). However, when assessing Wnt2 alone, the inverse association was seen in the never smokers strata suggesting a possibility that there may be gene-environment interactions as well as other gene-gene interactions.

Our analysis of combined effects with rs2953 miRNA589 and other Wnt pathway SNPs (Rs2241802 FZD3, rs3729629 WNT2, rs4835761 WNT8A, and rs222851 DVL2) did not appear to have an association when including the entire study population. Though there are over ten thousand articles describing the interactions of B-catenin with other genes as potential mechanism in carcinogenesis, none have observed the combined effects of SNPs in the microRNA binding site with SNPs of other genes in the Wnt pathway. [177-182]

4.5 Strengths and Limitations

This population based case-control study obtained was able to obtain a substantial number of esophageal cancer cases which is favorable to the power of the analysis. To our knowledge, this study represents the largest sample size for esophageal cancer to date. Our study had enough power to determine the effect of passive smoking on esophageal cancer in never smokers. Susceptibility

markers generally have lower point estimates making sample size a strength for this study. Additionally, the study population is homogeneous with respect to race which will reduce the need for population stratification on ethnicity when analyzing genetic data.

Healthy controls were randomly selected from the same base population from which the cases arose, attempting to remove a source of selection bias for control selection. Additionally, cases and controls differed in this study with respect to demographic variables and lifestyle habits. With respect to the differences in age and other socio-economic, these differences could tend to bias our effects and overestimate our associations. Though we adjusted for these variables in the analysis to prevent confounding, we cannot confirm that no residual confounding exists. Selection bias could remain an issue as esophageal has poor survival rates. This study might select cases with higher than average survival which could dilute the strength of association toward the null or under-represent the genetic markers of more aggressive esophageal cancer. Limitations to this study also include the retrospective nature of case-control study design which data was collected after diagnosis of cases. Potential recall bias and other information bias may exist about lifestyle factors. The differential recall of information in this retrospective study could cause overestimation or underestimation of effects but in this study would likely overestimate effects of known risk factors.

Missing data could also have been an issue for our study though for the variable pack years of smoking, we compared the complete case analysis with the imputed data and obtained nearly the same result. A substantial portion of subjects did not have biological specimens that could be evaluated so if the subset of population with genotyping differed from those that did not, our results could be subject to selection bias. Though we did not see many positive results, the issue of multiple comparisons could have produced spurious results in different stratum.

4.6 Conclusion

In conclusion, the observed associations between ETS and esophageal cancer suggest environmental tobacco smoke may play an important role in the esophageal carcinogenesis. The association is clear for the study population overall, with a dose-response relationship observed. Additionally, the similar point estimates identified in non-smokers suggest that the observed effect among the overall population was not affected by residual confounding from active smoking. Since China remains a large consumer of tobacco products with an estimated 50% of males smoking, intervention programs have been underway to prevent tobacco-related diseases including cancer[183]. Ideally, one would reduce smoking overall but in light of these findings, it may be worthwhile to direct focus of programs to the work environment as well as at home. Our estimates for population attributable fractions also suggest that effective intervention of ETS at work as well as at home may potentially reduce esophageal cancer rates in China as well as other high risk areas in the world.

The observed associations between genetic markers of the Wnt pathway and esophageal cancer are relatively weak with some indications of potential gene-environmental interaction. Further study on tumor samples could prove useful in determining the pathway's role in carcinogenesis. Our findings also suggest that garlic consumption has a protective effect for esophageal cancer. The protective effect is maintained across multiple strata and as well as the apparent dose-response relationship. In high risk areas, garlic consumption may be a way to reduce risk. Furthermore, its action could be studied further to help elucidate the steps in carcinogenesis that can be potentially inhibited.

FIGURES AND TABLES

Supplemental Table S1.1 Wnt proteins and potential SNPs

Wnt Protein	Chromosome Location	Protein Amino Acid length	SNPs
Wnt1	12q13	370	rs17123475 rs17123478
Wnt2	7q31.3	360	rs17132543 rs6948009 rs4730775 rs4727847 rs3779548 rs3779547 rs3729629 rs2228946 rs2024233 rs1051751 rs733154 rs39315 rs39312
Wnt2B/13	1p13	372	rs3790606 rs2273368
Wnt3	17q21	333	rs104894653 rs10514911 rs3851781 rs199525 rs199501 rs199498 rs111769 rs70602
Wnt3A	1q42	352	rs3121310 rs3094912 rs752107 rs708114
Wnt4	1p36.23-p35.1	351 (precursor)	rs121908653 rs121908650 rs121908652 rs121908651
Wnt5A	3p21-p14	381	rs7622120 rs566926
Wnt5B	12p13.3	359	rs2270031
Wnt6	2q35	365 (precursor)	
Wnt7A	3p25	349	rs104893835 rs104893832
Wnt7B	22q13	349	

Wnt8A	5q31	351	rs4835761 rs2040862
Wnt8B	10q24	328	rs3793771
Wnt9A (previously Wnt14)	1q42	365	
Wnt9B (previously Wnt15)	17q21	357	rs2165846 rs1530364
Wnt10A	2q35	417	rs121908122 rs121908123 rs121908121 rs121908120 rs121908119 rs121908118
Wnt10B	12q13	389 (precursor)	rs34201045 rs121918349
Wnt11	11q13	354	rs4944092 rs1568507 rs1533767 rs1533763 rs689095 rs596339
Wnt16	7q31	365	

Supplemental Table S1.2 List of Frizzeled genes and potential SNPs

Gene	Location	Protein Amino Acid	SNPs
FZD1	7q21	647	rs3750145 rs2232163 rs2232158 rs2232157 rs2232156 rs2232152 rs2232151
FZD2	17q21.1	641	
FZD3	8p21	666	rs2323019 rs2241802 rs960914 rs880481 rs352203
FZD4	11q14.2	537	rs80358308
FZD5	2q33.3	585	
FZD6	8q22.3-23.1	706	rs3758096
FZD7	2q33	574	rs13034206 rs2280509
FZD8	10p11.21	694	
FZD9 (previously FZD3)	7q11.23	591	
SMOH	7q32.3	787	rs121918347 rs121918348

Supplemental Table S1.3 List of Dishevelled proteins and potential SNPs

Dishevelled Protein	Chromosome location	SNPs
DVL1	1p36	
DVL2	17q21	rs118204014 rs222851
DVL3	3q27	
DVL1L1	22q	

Supplemental Table S1.4 GSK3B SNP

GSK3ß Protein	Chromosome location	SNPs
GSK3ß	3q13.3	rs6438552

Supplemental Table S1.5 Axin1 SNP

Axin1 Protein	Chromosome location	SNPs
Axin1	16p13.3	rs1981492

Supplemental Table S1.6 Beta Catenin mutations in human cancer by Polakis[65]:

tissue	freq.	S29	Y30	L31	D32	S33	G34	I35	H36	S37	G38	A39	T40	T41	T42	A43	P44	S45	L46	S47	G48	K49	Δ	reference
colorectal	9/202						1							3				5					0	Samowitz 1999
colorectal	2/92													1				1						Kitaeva 1997
colorectal-w/o APC mutation	7/58																						7	Iwao 1998
colorectal-w/o APC mutation	13/27					2	1							3				5					1	Sparks 1998
colorectal HNPCC	12/28				2		2			1				2				5						Miyaki 1999
colorectal w/ MSI	13/53																	6						Mirabelli-primdahl 1999
colorectal w/o MSI	0/27																							Mirabelli-primdahl 1999
desmoid, sporadic	1/1													1									0	Shitoh 1999
desmoid, sporadic	22/42													10				13						Tejpar 1999
endometrial w/ MSI	3/9				2	1																		Mirabelli-primdahl 1999
endometrial w/o MSI	10/28				3	1	2			3				1										Mirabelli-primdahl 1999
gastric, intestinal-type	7/26	2			5																			Park 1999
gastric, diffuse-type	0/17																							Park 1999
hepatocellular w/HCV	9/22				3	1				3				1				2						Huang 1999
hepatocellular	12/35				1	1	2	1	1				1	2				2		1			2	Van Nhieu 1999
hepatocellular	6/26				2		1			1								1					1	de la Coste 1998
hepatocellular	14/75				5	1	1							1				4					2	Miyoshi 1998
hepatocellular	21/119				3	3	1	1		2				4				8					2	Legois 1999
hepatoblastoma, sporadic	8/9						2			1								1					4	Jeng 2000
hepatoblastoma, sporadic	27/52				2		3			1				5									16	Koch 1999
hepatoblastoma	12/18				2		1							1				1					7	Wei 2000
kidney, Wilm's tumor	6/40													1				2					3	Koesters 1999
medulloblastoma, sporaic	3/67					2				1														Zurawel 1998
melanoma	1/65																	1						Garcia-Rostan 1999
ovarian, endometriod	7/13				3	1				2				1									0	Gamallo 1999
ovarian, endometriod	3/11									2				1										Palacios 1998
ovarian, endometriod	10/83					2	2			6														Wright 1999
pancreatic tumors	0/111																							Geddes 1999
pilomatricoma	12/16				2	4	3			2				1										Chan 1999
prostate cancer	5/104				1	2								1				1						Voeller 1998
thyroid, anaplastic	19/31					1			1	3	1		8	2	1	1	4	2	1	2		9	8	Garcia-rostan 1999
uterine endometrium	10/76					1				2				4				3						Fukuchi 1998

Supplemental Table S1.7 TCF-LEF genes and potential SNPs

Gene	Location	SNPs
TCF1 (TCF7)	5q31.1	rs137853246 rs137853243 rs137853242 rs137853241 rs137853238 rs137853247 rs137853245 rs137853244 rs137853240 rs137853239 rs137853237 rs137853236 rs1169305 rs1169288
TCF3 (TCF7L1)	2p11.2	rs6754757 rs6732834 rs6709476
TCF4 (TCF7L2)	10q25.3	rs121909121 rs12255372 rs11196205 rs4506565 rs290487 rs121909123 rs121909122 rs121909120 rs9960767 rs11196218 rs7903146 rs7901695
LEF1 (Substitution for TCF2)	4q23-25	

Supplemental Table S1.8 miRNAs related to the Wnt pathway and their SNPs

miRNA	Target	Action on Wnt signaling	SNPs
miRNA-181b,c, d	PIAS	Potential positive regulator	rs1050057
miRNA-200a	PPAR gamma	Potential negative regulator	rs3774923
miRNA-449	Wnt2b	Potential positive regulator	rs2273368
	Axin2		rs10438779
miRNA-503	TCF4 (TCF7L2)		rs1056877
miRNA-589	CTNNB1	Potential positive/negative regulator	rs4135388 rs2953 rs4135243 rs4135387

Supplemental Table S2.1 Power analysis for detecting an independent association of an exposure among the entire population.

Prevalence	*OR=1.2*	*OR=1.5*	*OR=2.0*
0.25	83.3%	>99.9%	>99.9%
0.30	87.1%	>99.9%	>99.9%
0.35	89.2%	>99.9%	>99.9%
0.40	90.4%	>99.9%	>99.9%
0.45	91.0%	>99.9%	>99.9%
0.50	91.0%	>99.9%	>99.9%

Supplemental Table S2.2 Power analysis for detecting the independent association of 1 SNP

Prevalence	*OR=1.2*	*OR=1.5*	*OR=2.0*
0.25	83.3%	>99.9%	>99.9%
0.30	87.1%	>99.9%	>99.9%
0.35	89.2%	>99.9%	>99.9%
0.40	90.4%	>99.9%	>99.9%
0.45	91.0%	>99.9%	>99.9%
0.50	91.0%	>99.9%	>99.9%

Supplemental Table S2.3 Power analysis for detecting the combined ORs of 2 SNPs

Prevalence	*OR=1.2*	*OR=1.5*	*OR=2.0*
0.25	66.1%	99.7%	99.5%
0.30	69.7%	99.8%	99.5%
0.35	72.0%	99.8%	99.4%
0.40	73.0%	99.8%	99.3%
0.45	73.0%	99.7%	98.9%
0.50	72.2%	99.5%	98.4%

Supplemental Table S2.4 Final list of SNPs

SNP	Gene	Genotyping Rate	MAF in controls	HWE in controls
rs2953	CTNNB1	92.9%	0.259	0.115
rs3729629	WNT2	92.7%	0.327	0.430
rs4730775	WNT2	90.9%	0.249	0.202
rs4835761	WNT8A	90.4%	0.423	0.112
rs2241802	FZD3	90.5%	0.435	0.002
rs222851	DVL2	90.2%	0.379	0.051

Table 3.1: Demographic data among cases and controls

	Cases	Controls	p-value
	N=1789	N=4966	
Gender			
Male	1373 (76.7%)	3703 (74.6%)	0.067
Female	414 (23.3%)	1259 (25.4%)	
Age			
<50	104 (5.8%)	601 (12.1%)	<0.0001
50-60	425 (23.8%)	1128 (22.7%)	
60-70	629 (35.2%)	1559 (31.4%)	
70-80	516 (28.8%)	1364 (27.5%)	
>80	115 (6.4%)	314 (6.3%)	
County			
Dafeng	639 (35.7%)	2532 (51.0%)	<0.0001
Ganyu	930 (52.0%)	2018 (40.6%	
Taixing	220 (12.3%)	415 (8.4%)	
Income 10 years ago (Yuans, per person/per month)			
<1000	670 (37.5%)	1348 (27.1%)	<0.0001
1000-1500	321 (17.9%)	825 (16.6%)	
1500-2500	414 (23.1%)	1221 (24.6%)	
>2500	344 (19.2%)	1493 (30.1%)	
BMI (kg/m^2)			
<18.5	266 (14.9%)	327 (6.6%)	<0.0001
18.5-24.0	1201 (67.1%)	3178 (64.0%)	
24.0-28.0	242 (13.5%)	1188 (23.9%)	
>28.0	66 (3.7%)	263 (5.3%)	
Education			
illiteracy	1011 (56.5%)	2383 (48.0%)	<0.0001
primary school	555 (31.0%)	1538 (31.0%)	
middle school and above	217 (12.1%)	1041 (21.0%)	
Family History of EC	423 (23.6%)	1002 (20.2%)	0.0021

Table 1 (cont.)

Smoking			
Never	530 (29.6%)	2089 (42.1%)	<0.0001
Ever	1250 (69.9%)	2876 (57.9%)	
Mean Pack-years (SD)	22.6 (24.5)	16.9 (22.3)	
Alcohol Drinking			
Never	620 (34.7%)	2148 (43.3%)	<0.0001
Occasional	307 (17.2%)	918 (18.5%)	
Often	328 (18.3%)	704 (14.2%)	
Daily	523 (29.2%)	1193 (24.0%)	

Table 3.2 Association of smoking and esophageal cancer in the study population

Smoking Status	Cases	Controls	OR_{unadj}	95%CI	OR_{adj}[2]	95% CI
Never	434	1869	1.00		1.00	
Former	248	532	2.01	1.67-2.41	**2.00**	**1.62-2.56**
Current	783	1922	1.75	1.54-2.01	**1.44**	**1.23-1.69**
Age of smoking						
Never smoker	434	1869	1.00		1.00	
After 40	56	160	**1.51**	**1.09-2.08**	1.32	0.94-1.84
30-40	156	352	**1.91**	**1.54-2.37**	**1.56**	**1.23-1.97**
20-30	575	1315	**1.88**	**1.63-2.17**	**1.58**	**1.33-1.87**
Before 20	360	865	**1.79**	**1.53-2.11**	**1.41**	**1.15-1.74**
Years of smoking						
Never smoker	434	1869	1.00		1.00	
under 20 years	111	303	**1.58**	**1.24-2.01**	1.24	0.92-1.67
20-30	150	418	**1.55**	**1.25-1.91**	**1.31**	**1.02-1.68**
30-40	315	639	**2.12**	**1.79-2.52**	**1.89**	**1.55-2.29**
over 40 years	561	1284	**1.88**	**1.63-2.17**	**1.47**	**1.23-1.75**
Daily amount of smoking						
Never smoker	434	1869	1.00		1.00	
0-5	41	132	1.34	0.93-1.93	1.10	0.75-1.61
5-10	74	221	**1.44**	**1.09-1.91**	1.21	0.89-1.65
10-20	239	627	**1.64**	**1.37-1.97**	**1.55**	**1.27-1.90**
20-40	437	1080	**1.74**	**1.50-2.03**	**1.69**	**1.41-2.02**
>40	69	87	**3.42**	**2.45-4.76**	**3.67**	**2.55-5.28**
Pack years						
Never smoker	434	1869	1.00		1.00	
0-10	85	273	**1.34**	**1.03-1.75**	1.29	0.97-1.71
10-20	119	363	**1.41**	**1.12-1.78**	**1.33**	**1.03-1.70**
20-30	154	394	**1.68**	**1.36-2.08**	**1.55**	**1.22-1.96**
30-40	175	387	**1.95**	**1.58-2.40**	**1.82**	**1.44-2.30**
>=40	322	708	**1.96**	**1.66-2.32**	**1.87**	**1.53-2.28**

Table 3.3 Association of drinking and esophageal cancer in the study population

Drinking Status	Cases	Controls	OR*unadj*	95%CI	OR*adj*[1]	95% CI
Never	620	2148	1.00		1.00	
Occasionally	307	918	1.16	0.99-1.36	**1.25**	**1.03-1.51**
Often	328	704	**1.61**	**1.38-1.89**	**1.48**	**1.21-1.80**
Daily	523	1193	**1.52**	**1.33-1.74**	**1.50**	**1.26-1.79**
Age drinking began						
Never	620	2148	1.00		1.00	
After 40	155	494	1.09	0.89-1.33	1.11	0.88-1.40
30-40	279	591	**1.64**	**1.38-1.94**	**1.60**	**1.30-1.97**
20-30	600	1398	**1.49**	**1.30-1.70**	**1.53**	**1.29-1.82**
Before 20	113	300	**1.31**	**1.03-1.65**	**1.40**	**1.06-1.86**
Weekly intake 1 year ago						
Never	620	2148	1.00		1.00	
under 250g	125	417	1.04	0.83-1.29	1.22	0.94-1.58
250-<500	198	598	1.15	0.95-1.38	1.25	0.99-1.58
>=500	445	1088	**1.42**	**1.23-1.63**	**1.59**	**1.31-1.93**
Weekly intake in the 90s						
Never	620	2148	1.00		1.00	
under 250g	127	388	1.13	0.91-1.41	1.29	0.99-1.68
250-<500	204	588	**1.20**	**1.00-1.44**	**1.38**	**1.10-1.73**
>=500	573	1214	**1.64**	**1.43-1.87**	**1.78**	**1.48-2.15**

[1] adjusted on age, gender, income, BMI, education, family history of EC, cigarette smoking

Table 3.4 Association of environmental tobacco smoke exposure at home and esophageal cancer in the study population

ETS at home

			Both men and women			
	Cases	**Controls**	**OR$_{crude}$**	**95%CI**	**OR$_{adj}$[1]**	**95% CI**
No	933	3002	1.00		1.00	
Yes	820	1877	**1.41**	**1.26-1.57**	**1.21**	**1.06-1.38**
None	933	3002	1.00		1.00	
Slight	366	782	**1.51**	**1.30-1.74**	1.09	0.90-1.30
Medium	196	545	1.16	0.97-1.38	1.08	0.88-1.33
Heavy	153	381	**1.29**	**1.06-1.58**	**1.45**	**1.14-1.80**
				p=.0005		p=0.006
			Women			
No	175	617	1.00		1.00	
Yes	227	609	**1.31**	**1.05-1.65**	**1.29**	**1.01-1.65**
None	175	617	1.00		1.00	
Slight	63	204	1.09	0.78-1.51	1.03	0.71-1.48
Medium	61	200	1.08	0.77-1.50	1.13	0.79-1.61
Heavy	59	144	**1.45**	**1.02-2.04**	**1.65**	**1.12-2.41**
				p=.068		p=0.024
			Men			
No	758	2385	1.00		1.00	
Yes	593	1268	**1.47**	**1.30-1.67**	1.16	0.99-1.36
None	758	2385	1.00		1.00	
Slight	303	578	**1.65**	**1.40-1.94**	1.11	0.89-1.37
Medium	135	345	1.23	0.99-1.53	1.06	0.82-1.37
Heavy	94	237	1.25	0.97-1.61	**1.33**	**1.00-1.78**
				p=0.0005		p=0.075

[1] **adjusted on packyears (continuous), passive smoking from work (yes/no), age (continuous), gender (except when stratified by gender), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.5 Association of environmental tobacco smoke exposure at work and esophageal cancer in the study population

ETS at work

			Both Men and Women			
	Cases	**Controls**	**OR$_{crude}$**	**95%CI**	**OR$_{adj}$[2]**	**95% CI**
No	1341	4000	1.00		1.00	
Yes	384	823	**1.39**	**1.22-1.60**	**1.36**	**1.15-1.60**
None	1341	4000	1.00		1.00	
Slight	163	415	1.17	0.97-1.42	1.11	0.89-1.39
Medium	146	287	**1.52**	**1.23-1.87**	**1.54**	**1.22-1.96**
Heavy	52	68	**2.28**	**1.58-3.29**	**2.33**	**1.54-3.51**
				p<.0001		p<.0001
			Women			
No	353	1104	1.00			
Yes	39	111	1.10	0.75-1.61	1.19	0.79-1.79
None	353	1104	1.00		1.00	
Slight	24	56	1.34	0.82-2.19	1.31	0.78-2.20
Medium	7	38	0.58	0.26-1.30	0.68	0.29-1.58
Heavy	8	10	2.50	0.98-6.39	**2.96**	**1.06-8.23**
				p=.403		p=.231
			Men			
No	988	2896	1.00		1.00	
Yes	345	712	**1.42**	**1.23-1.65**	**1.40**	**1.17-1.68**
None	988	2896	1.00		1.00	
Slight	139	359	1.14	0.92-1.40	1.09	0.85-1.41
Medium	139	249	**1.64**	**1.31-2.04**	**1.69**	**1.31-2.18**
Heavy	44	58	**2.23**	**1.49-3.31**	**2.11**	**1.34-3.32**
				p<.0001		p<.0001

[1] adjusted on packyears (continuous), passive smoking from home (yes/no), age (continuous), gender (except when stratified by gender), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (categories: never, occasional, often, everyday)

Table 3.6 Association of environmental tobacco smoke exposure at home and esophageal cancer in non-smokers

ETS at home	Both men and women					
	Cases	Controls	OR_{crude}	95%CI	OR_{adj}[1]	95% CI
No	283	1311	1.00		1.00	
Yes	225	711	**1.47**	**1.20-1.79**	1.23	0.99-1.53
None	283	1311	1.00		1.00	
Slight	77	263	**1.36**	**1.02-1.80**	1.10	0.81-1.49
Medium/Heavy	98	367	1.24	0.96-1.60	1.22	0.92-1.62
				p=.047		p=.154
			Women			
No	137	495	1.00		1.00	
Yes	158	445	1.28	0.99-1.67	1.17	0.88-1.56
None	137	495	1.00		1.00	
Slight	50	150	1.20	0.83-1.75	1.08	0.71-1.62
Medium/Heavy	68	240	1.02	0.74-1.42	1.07	0.74-1.53
			p=.774		p=.706	
			Men			
No	146	816	1.00		1.00	
Yes	67	266	**1.41**	**1.02-1.94**	1.25	0.88-1.76
None	146	816	1.00		1.00	
Slight	27	113	1.34	0.85-2.11	1.02	0.62-1.67
Medium/Heavy	30	127	1.32	0.86-2.04	1.31	0.81-2.11
			p=.128		p=.316	

[1] passive smoking exposure from work (yes/no), age (continuous), gender (except when stratified by gender), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (categories: never, occasional, often, everyday)

Table 3.7 Association of environmental tobacco smoke exposure at work and esophageal cancer in the non-smokers

ETS at work

			Both men and women			
	Cases	**Controls**	**OR$_{crude}$**	**95%CI**	**OR$_{adj}$[1]**	**95% CI**
No	437	1783	1.00		1.00	
Yes	62	216	1.17	0.87-1.58	1.38	0.99-1.91
None	437	1783	1.00		1.00	
Slight	31	120	1.05	0.70-1.59	1.08	0.70-1.66
Medium/Heavy	29	83	1.43	0.92-2.20	**2.03**	**1.27-3.26**
			p=0.136		p=.008	
				Women		
No	259	858	1.00		1.00	
Yes	28	76	1.22	0.77-1.92	1.33	0.82-2.16
None	259	858	1.00		1.00	
Slight	19	39	1.62	0.92-2.84	1.51	0.83-2.74
Medium/Heavy	9	31	0.96	0.45-2.05	1.26	0.57-2.81
			p=.467		p=.241	
				Men		
No	178	925	1.00		1.00	
Yes	34	140	1.26	0.84-1.90	1.41	0.90-2.21
None	178	925	1.00		1.00	
Slight	12	81	0.77	0.41-1.44	0.78	0.40-1.51
Medium/Heavy	20	52	**2.00**	**1.17-3.43**	**2.55**	**1.40-4.64**
			p=.063		p=0.018	

[2]passive smoking exposure from home (yes/no), age (continuous), gender (except when stratified by gender), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (categories: never, occasional, often, everyday)

Table 3.8 Effects of environmental tobacco smoke at home and at work among the entire study population and non-smokers

	cases	controls	OR_{crude}	95% CI	OR_{adj}	95% CI
	Smokers and Non-Smokers[1]					
No passive smoking exposure	735	2518	1.00		1.00	
Any exposure to passive smoking	976	2271	**1.47**	**1.32-1.65**	**1.29**	**1.13-1.46**
PAF (95% CI) [1]					**12.8%**	**(5.62%-19.7%)**
	Nonsmokers only[2]					
No passive smoking exposure	245	1160	1.00			
Any exposure to passive smoking	245	820	**1.42**	**1.16-1.73**	**1.29**	1.04-1.59
PAF (95% CI) [2]					**11.4%**	**(0.90%-21.7%)**
	Smokers and Non-Smokers[1]					
No passive smoking exposure	735	2518	1.00		1.00	
Passive smoking at either home or work	728	1828	**1.36**	**1.21-1.54**	**1.18**	**1.03-1.35**
Passive smoking at both home and work	248	443	**1.92**	**1.61-2.29**	**1.74**	**1.42-2.13**
p_{trend}			p<.0001		p<.0001	
	Nonsmokers only[2]					
No passive smoking exposure	245	1160	1.00		1.00	
Passive smoking at either home or work	205	711	**1.37**	**1.11-1.68**	1.22	0.98-1.52
Passive smoking at both home and work	40	109	**1.74**	**1.18-2.56**	**1.78**	**1.19-2.68**
p_{trend}			p=.0003		p=.004	
	Smokers and Non-Smokers[1]					
No passive smoking exposure	735	2518	1.00		1.00	
Light	549	1361	**1.38**	**1.22-1.57**	1.14	0.97-1.32
Medium	213	522	**1.40**	**1.17-1.67**	**1.51**	**1.23-1.85**
Heavy	44	60	**2.51**	**1.69-3.74**	**2.55**	**1.66-3.92**
p_{trend}				p<.0001		p<.0001
	Nonsmokers only[2]					
No passive smoking exposure	245	1160	1.00		1.00	
Light	133	490	**1.29**	**1.02-1.63**	1.12	0.87-1.45
Medium/Heavy	60	203	**1.40**	**1.02-1.93**	**1.55**	**1.10-2.18**
p_{trend}			p=0.011		p=.018	

[1] adjusted on packyears (continuous), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing), and alcohol consumption (categories: never, occasional, often, everyday)

[2] adjusted for age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing), and alcohol consumption (categories: never, occasional, often, everyday)

Table 3.9 Association of garlic consumption and esophageal cancer in the study population

	cases	controls	OR (crude)	95% CI	ORadj[1]	95% CI
Both Males and Females						
Not often	788	2488	1.00		1.00	
Occasional	780	1843	**0.75**	**0.67-0.84**	0.89	0.76-1.04
Often	211	608	0.91	0.77-1.09	**0.67**	**0.53-0.85**
p_{trend}			0.003		0.002	
0/week	650	2077	1.00		1.00	
<2 times a week	269	583	**0.68**	**0.57-0.80**	0.85	0.68-1.07
2 or more times a week	608	1606	**0.83**	**0.73-0.94**	**0.76**	**0.63-0.90**
p_{trend}			0.003		0.002	
Males						
Not often	523	1684	1.00		1.00	
Occasional	659	1487	**0.70**	**0.61-0.80**	0.93	0.77-1.12
Often	184	516	0.87	0.72-1.06	**0.67**	**0.51-0.88**
p_{trend}			0.002		0.009	
0/week	425	1401	1.00		1.00	
<2 times a week	240	481	**0.61**	**0.50-0.74**	0.88	0.68-1.15
2 or more times a week	502	1264	**0.76**	**0.66-0.89**	**0.77**	**0.62-0.95**
p_{trend}			0.0005		0.01	
Females						
Not often	265	804	1.00		1.00	
Occasional	121	356	1.17	0.76-1.82	0.78	0.57-1.08
Often	27	92	1.07	0.82-1.40	0.62	0.36-1.07
p_{trend}			0.83		0.05	
0/week	225	676	1.00		1.00	
<2 times a week	29	102	1.17	0.76-1.82	0.69	0.41-1.17
2 or more times a week	106	342	1.07	0.82-1.40	0.74	0.53-1.04
p_{trend}			0.56		0.09	

[1] **adjusted on packyears of smoking (continuous), passive smoking from work and home (yes/no), age (continuous), gender (M/F), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.10 Association of garlic consumption and esophageal cancer in smokers and non-smokers

	Non-smokers						Smokers					
	cases	**controls**	**OR (crude)**	**95% CI**	**ORadj[1]**	**95% CI**	**cases**	**controls**	**OR (crude)**	**95% CI**	**ORadj[1]**	**95% CI**
					Both Males and Females							
Not often	270	1130	1.00		1.00		517	1349	1.00		1.00	
Occasional	204	697	**0.81**	**0.66-0.99**	0.96	0.74-1.25	573	1146	**0.77**	**0.67-0.88**	0.85	0.71-1.01
Often	54	236	1.04	0.75-1.43	0.69	0.46-1.04	156	371	0.91	0.74-1.13	**0.74**	**0.57-0.95**
p_{trend}			0.42		0.13				0.03		0.01	
0/week	229	953	1.00		1.00		420	1124	1.00			
<2 times a week	58	210	0.87	0.63-1.20	0.84	0.57-1.23	211	373	**0.66**	**0.54-0.81**	0.97	0.76-1.24
2 or more times a week	156	638	0.98	0.78-1.23	**0.70**	**0.52-0.94**	451	968	**0.80**	**0.69-0.94**	0.83	0.68-1.02
p_{trend}			0.84		0.02				0.01		0.05	

[1] **adjusted on passive smoking from work and home (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.11 Association of garlic consumption and esophageal cancer in never, occasional, often and daily drinkers

	Alcohol Consumption															
Garlic intake	Never				Occasional				Often				Daily			
	ca	co	aOR[1]	95% CI	ca	co	aOR[1]	95% CI	ca	co	aOR[1]	95% CI	ca	co	aOR[1]	95% CI
Not often	339	1198	1.00		120	431	1.00		111	286	1.00		217	573	1.00	
Occasional	218	707	0.90	0.70-1.16	156	372	1.24	0.83-1.83	166	302	0.79	0.52-1.19	237	460	0.73	0.53-1.00
Often	61	228	0.74	0.49-1.10	31	111	0.69	0.37-1.31	50	115	0.63	0.36-1.10	68	153	**0.55**	**0.35-0.86**
p_{trend}			0.14				0.62				0.10				0.01	
0/week	288	1006	1.00		91	367	1.00		88	232	1.00		182	472	1.00	
<2 /week	69	232	0.82	0.56-1.20	52	91	1.15	0.64-2.08	61	94	1.00	0.56-1.76	87	166	0.65	0.42-1.01
2+/week	170	617	**0.72**	**0.54-0.95**	111	323	0.96	0.62-1.48	136	266	0.87	0.56-1.37	190	398	**0.64**	**0.45-0.90**
p_{trend}			0.03				0.76				0.51				0.02	

[1] adjusted on packyears (continuous), passive smoking from work and home (yes/no), age (continuous), gender (M/F), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing)

Table 3.12 Association of garlic consumption and esophageal cancer stratified by gender and smoking status

						Males						
Not often	88	553	1.00		1.00		435	1131	1.00		1.00	
Occasional	105	398	**0.60**	**0.44-0.82**	0.85	0.71-1.02	551	1089	**0.76**	**0.65-0.88**	0.85	0.71-1.02
Often	30	155	0.82	0.52-1.29	**0.73**	**0.56-0.95**	153	360	0.91	0.73-1.13	**0.73**	**0.56-0.95**
p_{trend}			0.06		0.02				0.04		0.02	
0/week	77	459	1.00		1.00		348	942	1.00		1.00	
<2 times a week	31	120	0.65	0.41-1.03	1.01	0.78-1.29	209	361	**0.64**	**0.52-0.79**	1.01	0.78-1.29
2 or more times a week	73	365	0.84	0.59-1.19	0.84	0.68-1.04	428	899	**0.78**	**0.66-0.92**	0.84	0.68-1.04
p_{trend}			0.30		0.11				0.00		0.07	
						Females						
Not often	182	586	1.00		1.00		82	218	1.00		1.00	
Occasional	99	299	0.94	0.71-1.24	0.81	0.56-1.15	22	57	0.98	0.56-1.70	0.73	0.35-1.51
Often	24	81	1.05	0.65-1.70	0.64	0.36-1.15	3	11	1.38	0.38-5.07	1.00	0.20-4.89
p_{trend}			0.91		0.10				0.81		0.55	
0/week	152	494	1.00		1.00		72	182	1.00		1.00	
<2 times a week	27	90	1.03	0.64-1.64	0.76	0.44-1.32	2	12	2.37	0.52-10.9	0.35	0.07-1.89
2 or more times a week	83	273	1.01	0.75-1.37	0.74	0.50-1.10	23	69	1.19	0.69-2.05	0.63	0.31-1.24
p_{trend}			0.93		0.14				0.47		0.20	

[1] **adjusted on passive smoking from work and home (yes/no), age (continuous), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.13 Association of garlic consumption and esophageal cancer in never smokers and never drinkers and ever smokers/frequent drinkers

	Non-smokers/Non-Drinkers						Smokers/Daily Drinkers					
	ca	co	OR (crude)	95% CI	ORadj[1]	95% CI	ca	co	OR (crude)	95% CI	ORadj[1]	95% CI
not often	208	882	1.00		1.00		**282**	638	1.00		1.00	
Occasional	129	471	0.86	0.67-1.10	1.06	0.78-1.44	334	572	**0.76**	**0.62-0.92**	**0.77**	**0.60-0.98**
Often	23	141	0.94	0.91-2.30	0.57	0.33-1.01	83	178	0.95	0.71-1.27	**0.67**	**0.48-0.96**
p_{trend}			0.64		0.23				0.14		0.02	
0/week	180	743	1.00		1.00		235	513	1.00		1.00	
<2 times a week	37	150	0.98	0.66-1.46	0.90	0.56-1.42	125	208	0.76	0.58-1.00	0.78	0.56-1.08
2 or more times a week	102	425	1.01	0.77-1.32	0.83	0.59-1.17	273	483	0.81	0.65-1.00	0.78	0.60-1.01
p_{trend}			0.96		0.29				0.06		0.08	

[1] **adjusted on passive smoking from work and home (yes/no), age (continuous), gender (M/F), income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taxing)**

Table 3.14 Demographic data for cases and controls with genotyping information available

	Cases	Controls	
	N=1278	N=2849	
Gender			
Male	992 (77.6%)	2036 (71.5%)	<0.0001
Female	286 (22.4%)	813 (28.5%)	
Age			
<50	84 (6.6%)	394 (13.8%)	<0.0001
50-60	333 (26.0%)	659 (23.1%)	
60-70	434 (33.9%)	939 (33.0%)	
70-80	359 (28.0%)	711 (25.0%)	
>80	70 (5.5%)	146 (5.1%)	
County			
Dafeng	406 (31.7%)	2070 (72.7%)	<0.0001
Ganyu	654 (51.1%)	367 (12.9%)	
Taixing	220 (17.2%)	411 (14.4%)	
Income			
<1000	513 (41.1%)	758 (27.0%)	<0.0001
1000-1500	209 (16.8%)	438 (15.6%)	
1500-2500	285 (22.8%)	712 (25.3%)	
>2500	241 (19.3%)	903 (32.1%)	
BMI (kg/m2)			
<18.5	198 (15.5%)	201 (7.1%)	<0.0001
18.5-24.0	864 (67.9%)	1807 (63.5%)	
24.0-28.0	168 (13.2%)	693 (24.3%)	
>28.0	43 (3.4%)	145 (5.1%)	
Education			
illiteracy	700 (54.9%)	1131 (39.7%)	<0.0001
primary school	422 (33.1 %)	999 (35.1%)	
middle school and above	153 (12.0%)	718 (25.2%)	
Family History of EC	307 (24.0%)	973 (23.6%)	0.223

Smoking			
Never	370 (29.1%)	1211 (42.5%)	<0.0001
Ever	901 (70.9%)	1637 (57.5%)	
Mean Pack-years (SD)	22.9 (24.8)	17.7(22.7)	<0.0001
Alcohol Drinking			
Never	433 (34.1%)	1254 (44.1%)	<0.0001
Occassional	203 (16.0%)	483 (17.0%)	
Often	242 (19.1%)	374 (13.1%)	
Daily	392 (30.9%)	735 (25.8%)	

Table 3.15 Association of Wnt pathway polymorphisms and esophageal cancer among the entire study population

	Genotype	Cases	Controls	OR	95%CI	OR_{adj}[1]	95% CI
rs22418002 (FZD3)							
	G/G	373	878	1.00		1.00	
	G/A	497	1214	0.96	0.82-1.13	1.00	0.82-1.22
	A/A	224	536	0.98	0.81-1.20	1.00	0.78-1.28
				p=0.816		p=0.996	
	G/A+A/A	721	1750	0.97	0.84-1.13	1.00	0.83-1.20
	A/A			1.01	0.84-1.20	1.00	0.80-1.24
rs3729629 (WNT2)							
	G/G	553	1226	1.00		1.00	
	G/C	448	1166	**0.85**	**0.74-0.99**	0.89	0.74-1.06
	C/C	117	297	0.87	0.69-1.11	0.90	0.67-1.20
				p=0.062		p=0.237	
	C/C+G/C	565	1463	**0.86**	**0.75-0.99**	0.89	0.75-1.06
	C/C			0.94	0.75-1.18	0.95	0.72-1.26
rs4730775 (WNT2)							
	C/C	650	1511	1.00		1.00	
	C/T	373	969	0.90	0.77-1.04	0.86	0.71-1.04
	T/T	74	177	0.97	0.73-1.29	1.08	0.76-1.55
				p=0.301		p=0.490	
	T/T+C/T	447	1146	0.91	0.79-1.05	0.89	0.75-1.07
	C/C			1.01	0.77-1.34	1.15	0.81-1.63
rs4835761 (WNT8A)							
	A/A	410	893	1.00		1.00	
	A/G	477	1243	**0.84**	**0.71-0.98**	0.89	0.74-1.08
	G/G	203	491	0.9	0.74-1.10	0.86	0.67-1.10
				p=0.152		p=0.180	
	G/G+A/G	680	1734	0.85	0.74-1.00	0.88	0.74-1.06
	G/G			1.00	0.83-1.19	0.91	0.73-1.15
rs222851 (DVL2)							
	A/A	405	1041	1.00		1.00	
	A/G	505	1189	1.09	0.94-1.28	1.08	0.90-1.31
	G/G	173	399	1.11	0.90-1.38	1.08	0.82-1.40
				p=0.235		p=0.487	
	G/G+A/G	678	1588	1.10	0.95-1.27	1.08	0.90-1.29
	G/G			1.06	0.88-1.29	1.03	0.80-1.31

[1] **adjusted on packyears (continuous), passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.16 Association of Wnt pathway polymorphisms and esophageal cancer among smokers and non-smokers

		Non smokers						Smokers					
	Genotype	Cases	Controls	crude OR	95%CI	OR_{adj}[1]	95% CI	Cases	Controls	crude OR	95%CI	OR_{adj}[1]	95% CI
rs22418002 (FZD3)													
	G/G	103	365	1.00		1.00		266	513	1.00		1.00	
	G/A	144	535	0.95	0.72-1.27	0.94	0.67-1.31	350	678	1.00	0.82-1.21	1.08	0.86-1.35
	A/A	64	219	1.04	0.73-1.48	1.00	0.66-1.52	158	317	0.96	0.76-1.22	1.17	0.84-1.64
				p=0.912		p=0.949				p=0.994		p=0.955	
	G/A+A/A	208	754	0.98	0.75-1.28	0.96	0.70-1.31	508	995	0.99	0.82-1.18	1.05	0.90-1.29
	A/A			1.07	0.78-1.46	1.04	0.72-1.51			0.96	0.78-1.19	0.95	0.74-1.22
rs3729629 (WNT2)													
	G/G	157	495	1.00		1.00		391	731	1.00		1.00	
	G/C	130	517	0.79	0.61-1.03	**0.65**	**0.48-0.89**	314	649	0.91	0.75-1.09	1.00	0.81-1.24
	C/C	31	125	0.78	0.51-1.21	0.62	0.37-1.04	86	171	0.94	0.71-1.25	0.94	0.67-1.31
				p=0.093		p=0.007				p=0.404		p=0.789	
	C/C+G/C	161	642	0.79	0.50-0.89	**0.65**	**0.48-0.86**	400	820	0.91	0.77-1.08	0.99	0.81-1.21
	C/C			0.87	0.58-1.32	0.77	0.47-1.25			0.98	0.75-1.30	0.94	0.68-1.29
rs4730775 (WNT2)													
	C/C	189	630	1.00		1.00		456	881	1.00		1.00	
	C/T	100	404	0.83	0.63-1.08	**0.71**	**0.52-0.99**	269	564	0.92	0.77-1.11	0.94	0.76-1.17
	T/T	22	83	0.88	0.54-1.45	0.77	0.42-1.40	52	94	1.07	0.75-1.53	1.17	0.77-1.76
				p=0.242		p=0.064				p=0.751		p=0.889	
	T/T+C/T	122	487	0.84	0.65-1.08	**0.72**	**0.53-0.98**	321	658	0.94	0.79-1.12	0.98	0.80-1.20
	C/C			0.95	0.58-1.55	0.87	0.48-1.56			1.10	0.78-1.57	1.19	0.79-1.79

rs4835761 (WNT8A)													
	A/A	120	381	1.00		1.00		287	512	1.00		1.00	
	A/G	136	524	0.82	0.62-1.09	0.83	0.60-1.15	337	718	0.84	0.69-1.02	0.83	0.66-1.04
	G/G	58	202	0.91	0.64-1.30	1.07	0.71-1.61	143	289	0.88	0.69-1.13	0.78	0.58-1.04
				p=0.432		p=0.989				p=0.190		p=0.065	
	G/G+A/G	194	726	0.85	0.66-1.10	0.89	0.66-1.21	480	1007	0.85	0.71-1.01	0.82	0.66-1.01
	G/G			1.02	0.74-1.40	1.19	0.82-1.72			0.98	0.78-1.22	0.87	0.67-1.12
rs222851 (DVL2)													
	A/A	116	463	1.00		1.00		285	578	1.00		1.00	
	A/G	149	494	1.20	0.92-1.58	1.13	0.82-1.55	353	694	1.03	0.85-1.25	1.10	0.88-1.38
	G/G	43	154	1.11	0.75-1.65	1.15	0.72-1.84	129	245	1.07	0.83-1.38	1.01	0.75-1.38
				p=0.354		p=0.454				p=0.605		p=0.742	
	G/G+A/G	192	648	1.18	0.91-1.53	1.13	0.84-1.53	482	939	1.04	0.87-1.25	1.08	0.88-1.33
	G/G			1.01	0.70-1.45	1.08	0.70-1.66			1.05	0.83-1.33	0.96	0.73-1.26

[1] **adjusted on passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.17 Association of Wnt pathway polymorphisms and esophageal cancer among drinkers and non-drinkers

		Non-drinkers						Daily drinkers					
	Genotype	Cases	Controls	crude OR	95%CI	OR_{adj}[1]	95% CI	Cases	Controls	crude OR	95%CI	OR_{adj}[1]	95% CI
rs22418002 (FZD3)													
	G/G	139	379	1.00		1.00		111	222	1.00		1.00	
	G/A	174	536	0.89	0.68-1.15	0.92	0.67-1.25	157	316	0.78	0.74-1.34	0.98	0.67-1.42
	A/A	71	253	0.77	0.55-1.06	0.77	0.52-1.14	63	131	0.96	0.66-1.40	1.08	0.67-1.74
				p=0.105		p=0.200				p=0.851		p=0.793	
	G/A+A/A	245	789	0.85	0.67-1.08	0.87	0.65-1.16	220	447	0.81	0.63-1.06	1.01	0.71-1.43
	A/A			0.82	0.61-1.10	0.81	0.57-1.15			0.97	0.69-1.35	1.10	0.72-1.67
rs3729629 (WNT2)													
	G/G	180	532	1.00		1.00		174	326	1.00		1.00	
	G/C	175	529	0.98	0.77-1.24	0.85	0.64-1.13	121	292	0.78	0.59-1.03	0.99	0.70-1.41
	C/C	35	128	0.81	0.54-1.22	0.70	0.43-1.14	37	72	0.96	0.62-1.49	1.23	0.71-2.13
				p=0.405		p=0.109				p=0.320		p=0.605	
	C/C+G/C	210	657	0.95	0.75-1.19	0.82	0.63-1.08	158	364	0.81	0.63-1.06	1.04	0.74-1.45
	C/C			0.82	0.55-1.21	0.76	0.47-1.21			1.08	0.71-1.64	1.23	0.73-2.08
rs4730775 (WNT2)													
	C/C	227	660	1.00		1.00		203	420	1.00		1.00	
	C/T	132	428	0.90	0.70-1.15	0.77	0.58-1.04	104	225	0.96	0.72-1.27	1.17	0.82-1.67
	T/T	22	84	0.76	0.47-1.25	0.74	0.41-1.33	24	43	1.16	0.68-1.96	1.45	0.74-2.85
				p=0.204		p=0.076				p=0.861		p=0.214	
	T/T+C/T	154	512	0.88	0.69-1.11	0.77	0.58-1.01	128	268	0.99	0.76-1.29	1.21	0.86-1.69
	C/C			0.79	0.49-1.29	0.81	0.46-1.46			1.17	0.70-1.97	1.37	0.71-2.67

rs4835761 (WNT8A)													
	A/A	144	403	1.00		1.00		125	219	1.00		1.00	
	A/G	183	540	0.95	0.74-1.22	0.97	0.72-1.30	133	340	**0.69**	**0.51-0.92**	0.74	0.51-1.07
	G/G	58	212	0.77	0.54-1.08	0.74	0.49-1.11	65	121	0.94	0.65-1.37	0.86	0.54-1.38
				p=0.163		p=0.196				p=0.385		p=0.342	
	G/G+A/G	241	752	0.90	0.71-1.14	0.90	0.68-1.20	198	461	**0.75**	**0.57-0.99**	0.77	0.55-1.09
	G/G			0.79	0.58-1.08	0.75	0.51-1.10			1.16	0.83-1.63	1.02	0.67-1.56
rs222851 (DVL2)													
	A/A	135	476	1.00		1.00		125	275	1.00		1.00	
	A/G	181	520	1.23	0.95-1.58	1.28	0.95-1.73	148	300	1.09	0.81-1.45	0.94	0.65-1.34
	G/G	63	175	1.27	0.90-1.79	1.21	0.80-1.84	51	95	1.18	0.79-1.76	1.06	0.64-1.73
				p=0.105		p=0.194				p=0.393		p=0.728	
	G/G+A/G	244	695	1.24	0.97-1.57	1.27	0.96-1.68	199	395	1.11	0.85-1.45	0.97	0.69-1.35
	G/G			1.14	0.83-1.55	1.06	0.72-1.54			1.13	0.78-1.64	1.09	0.69-1.73

[1] **adjusted on packyears (continuous), passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing)**

Table 3.18: Association of Wnt pathway SNPs and EC among non-drinkers/non-smokers versus drinkers/smokers

				Non smokers and non-drinkers						Drinkers and smokers			
rs2241802 (FZD3)													
	G/G	73	220	1.00		1.00		145	237	1.00		1.00	
	G/A	78	340	**0.69**	**0.48-0.99**	**0.65**	**0.43-0.98**	183	303	0.99	0.75-1.30	1.11	0.80-1.53
	A/A	32	145	0.67	0.42-1.06	0.78	0.46-1.32	92	146	1.03	0.74-1.44	1.11	0.76-1.64
				p=0.048		p=0.181				p=888		p=0.545	
	G/A+A/A	110	485	**0.68**	**0.49-0.96**	0.68	0.46-1.00	275	449	1.00	0.78-1.29	1.11	0.82-1.49
	A/A			0.82	0.54-1.25	0.99	0.61-1.60			1.04	0.77-1.39	1.05	0.75-1.84
rs3729629 (WNT2)													
	G/G	97	298	1.00		1.00		212	340	1.00		1.00	
	G/C	75	339	0.68	0.48-0.95	0.54	0.38-0.80	168	287	0.94	0.73-1.21	1.10	0.88-1.93
	C/C	15	77	0.60	0.32-1.09	0.52	0.26-1.02	46	78	0.95	0.63-1.42	1.06	0.67-1.68
				p=0.017		p=0.003				p=0.664		p=0.638	
	C/C+G/C	90	416	0.67	0.48-0.92	0.54	0.37-0.78	214	365	0.94	0.74-1.20	1.09	0.82-1.44
	C/C			0.72	0.41-1.29	0.70	0.36-1.34			0.97	0.66-1.43	1.02	0.65-1.58
rs4730775 (WNT2)													
	C/C	117	391	1.00		1.00		256	415	1.00		1.00	
	C/T	53	254	0.70	0.49-1.00	**0.65**	**0.43-0.98**	135	244	0.90	0.69-1.17	0.92	0.68-1.24
	T/T	12	57	0.70	0.37-1.36	0.74	0.36-1.53	31	43	1.17	0.72-1.90	1.42	0.81-2.48
				0.06		0.07				p=0.932		p=0.631	
	T/T+C/T	65	311	0.70	0.50-0.98	**0.67**	**0.46-0.98**	166	287	0.94	0.73-1.20	0.99	0.74-1.31
	C/C			0.80	0.42-1.52	0.86	0.42-1.75			1.22	0.75-1.96	1.46	0.85-2.53

rs4835761 (WNT8A)													
	A/A	67	239	1.00		1.00		153	229	1.00		1.00	
	A/G	81	327	0.88	0.61-1.27	0.86	0.57-1.31	174	343	0.76	0.58-1.00	0.80	0.58-1.09
	G/G	36	131	0.98	0.62-1.55	1.03	0.62-1.73	84	119	1.06	0.75-1.49	0.99	0.66-1.48
				p=0.820		p=0.974				p=0.844		p=0.687	
	G/G+A/G	117	458	0.91	0.65-1.28	0.91	0.62-1.35	258	462	0.84	0.65-1.08	0.85	0.63-1.14
	G/G			1.05	0.70-1.59	1.12	0.71-1.78			1.24	0.91-1.69	1.12	0.78-1.62
rs222851 (DVL2)													
	A/A	70	307	1.00		1.00		158	274	1.00		1.00	
	A/G	90	305	1.29	0.91-1.84	1.38	0.93-2.06	185	306	1.05	0.80-1.37	1.05	0.77-1.43
	G/G	22	94	1.03	0.60-1.75	1.20	0.65-2.22	75	108	1.20	0.85-1.72	1.18	0.79-1.77
				p=0.505		p=0.250				p=0.333		p=0.444	
	G/G+A/G	112	399	1.23	0.88-1.72	1.35	0.92-1.97	260	414	1.09	0.85-1.40	1.08	0.81-1.44
	G/G			0.90	0.55-1.47	1.01	0.57-1.80			1.17	0.85-1.62	1.15	0.80-1.67

[1] **adjusted on passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing)**

Table 3.19 Association of miRNA589 binding site for CTNNB1 and esophageal cancer

	Cases	Controls	crude OR	95% CI	adj OR	95% CI
rs2953 (miRNA589; CTNNB1)						
T/T	632	1509	1.00		1.00	
G/T	387	1001	0.92	0.80-1.07	0.94	0.78-1.12
G/G	95	194	1.17	0.90-1.52	1.02	0.73-1.41
			p=0.850		p=0.719	
G/T+G/G	482	1195	0.96	0.84-1.11	0.95	0.80-1.13
G/G			1.21	0.93-1.56	1.04	0.76-1.44

[1] **adjusted on packyears (continuous), passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.20 Association of miRNA589 binding site for CTNNB1 and esophageal cancer among smokers and never-smokers

	Never smokers						Ever smokers					
	Cases	Controls	crude OR	95% CI	adj OR	95% CI	Cases	Controls	crude OR	95% CI	adj OR	95% CI
rs2953 (miRNA589; CTNNB1)												
T/T	173	654	1.00		1.00		454	854	1.00		1.00	
G/T	116	417	1.05	0.81-1.37	1.07	0.79-1.46	268	584	0.86	0.72-1.04	0.84	0.67-1.03
G/G	29	77	1.42	0.90-2.25	1.46	0.84-2.52	65	117	1.05	0.76-1.45	0.87	0.60-1.27
			p=0.211		p=0.243				p=0.003		p=0.144	
G/T+G/G	145	494	1.11	0.86-1.42	1.13	0.84-1.51	333	701	0.89	0.75-1.06	0.84	0.69-1.03
G/G			1.40	0.89-2.18	1.42	0.83-2.41			1.11	0.81-1.52	0.93	0.65-1.35

[1] **adjusted on passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu), and alcohol consumption (categories: never, occasional, often, everyday)**

Table 3.21 Association of miRNA589 binding site for CTNNB1 and esophageal cancer among never and infrequent drinkers versus frequent and daily drinkers

	Never and Infrequent drinkers						Frequent and Daily drinkers					
	Cases	Controls	crude OR	95% CI	adj OR	95% CI	Cases	Controls	crude OR	95% CI	adj OR	95% CI
						rs2953 (miRNA589; CTNNB1)						
T/T	318	946	1.00		1.00		308	562	1.00		1.00	
G/T	195	595	0.98	0.79-1.20	0.98	0.77-1.26	189	404	0.86	0.68-1.07	0.90	0.68-1.18
G/G	48	110	1.30	0.90-1.87	1.12	0.71-1.78	46	84	1.00	0.68-1.47	0.90	0.55-1.46
			p=0.41		p=0.80				p=0.42		p=0.45	
G/T+G/G	243	705	1.03	0.85-1.24	1.01	0.79-1.27	235	488	0.88	0.71-1.08	0.90	0.69-1.16
G/G			1.31	0.92-1.87	1.13	0.72-1.77			1.07	0.73-1.55	0.94	0.59-1.50

[1] **adjusted on packyears (continuous), passive smoking from other source (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu), hot tea drinking (yes/no)**

Table 3.22 Association of miRNA589 binding site for CTNNB1 and esophageal cancer among never smokers/non-drinkers and ever smokers/frequent drinkers

	Cases	Controls	crude OR	95% CI	adj OR	95% CI	Cases	Controls	crude OR	95% CI	adj OR	95% CI
					rs2953 (miRNA589; CTNNB1)							
T/T	101	403	1.00		1.00		244	380	1.00		1.00	
G/T	70	270	1.03	0.74-1.46	1.11	0.75-1.64	148	268	0.87	0.67-1.11	0.85	0.63-1.15
G/G	15	48	1.25	0.60-2.82	1.28	0.63-2.60	35	59	0.92	0.59-1.45	0.78	0.46-1.31
			p=0.559		p=0.441				p=0.370		p=0.206	
G/T+G/G	85	318	1.07	0.77-1.47	1.14	0.79-1.64	183	327	0.87	0.68-1.11	0.84	0.63-1.11
G/G			1.23	0.67-1.25	1.22	0.61-2.45			0.98	0.63-1.52	0.82	0.50-1.37

[1] **adjusted on passive smoking from work and home (yes/no), age (continuous), gender, income (continuous), BMI (continuous), education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing)**

Table 3.23 Combined effects of miRNA589 binding site for CTNNNB1 and esophageal cancer among the entire population

		Cases	Controls	aOR	95% CI	aROR	95% CI
rs2953 (miRNA589; CTNNB1)	rs2241802 (FZD3)						
A/A	G/G	195	453	1.00			
A/A	G/A+A/A	254	631	1.03	0.79-1.36		
G/G+A/G	G/G	122	306	0.99	0.71-1.37		
G/G+A/G	G/A+A/A	171	455	0.90	0.67-1.22	0.83	0.52-1.34
rs2953 (miRNA589; CTNNB1)	rs3729629 (WNT2)						
A/A	G/G	282	667	1.00			
A/A	C/C+G/C	236	601	0.91	0.71-1.17		
G/G+A/G	G/G	183	400	1.11	0.84-1.45		
G/G+A/G	C/C+G/C	148	432	0.78	0.58-1.04	0.76	0.51-1.14
rs2953 (miRNA589; CTNNB1)	rs4835761(WNT8A)						
A/A	A/A	215	476	1.00			
A/A	G/G+A/G	252	626	0.90	0.69-1.18		
G/G+A/G	A/A	137	306	0.94	0.68-1.30		
G/G+A/G	G/G+A/G	156	463	0.78	0.58-1.05	0.92	0.60-1.41
rs2953 (miRNA589; CTNNB1)	rs222851 (DVL2)						
A/A	A/A	194	536	1.00			
A/A	G/G+A/G	291	607	1.21	0.93-1.57		
G/G+A/G	A/A	131	356	0.83	0.66-1.23		
G/G+A/G	G/G+A/G	169	459	0.98	0.74-1.32	0.91	0.60-1.38

[1] adjusted on packyears (continuous), passive smoking from other source (yes/no), age (continuous), income (continuous), bmi (continuous), gender, education (illiterate, primary school, and middle school and above), family history of EC (any malignancy in first-degree relative), study site (Dafeng/Ganyu/Taixing), and alcohol consumption (never, occasional, often, everyday)

Table 3.24 Combined effects of microRNA589 binding site for CTNNNB1 with other SNPsand esophageal cancer among ever smokers and never smokers

		Non-Smokers				Smokers			
		Ca	**Co**	**aOR**	**95% CI**	**Ca**	**Co**	**aOR**	**95% CI**
rs2953 (micro-RNA589; CTNNB1)	**rs2241802 (FZD3)**								
A/A	**G/G**	47	186	1.00		145	267	1.00	
A/A	**G/A+A/A**	71	284	1.02	0.66-1.57	182	346	1.02	0.75-1.40
G/G+A/G	**G/G**	36	127	1.19	0.71-1.99	85	179	0.88	0.60-1.30
G/G+A/G	**G/A+A/A**	51	193	1.10	0.70-1.72	119	262	0.91	0.66-1.26
aROR					0.99 (0.48-2.06)				1.19(0.82-1.72)
rs2953 (micro-RNA589; CTNNB1)	rs3729629 (WNT2)								
A/A	**G/G**	85	265	1		195	402	1	
A/A	**C/C+G/C**	57	274	0.48	0.31-0.74	176	327	1.37	1.01-1.84
G/G+A/G	**G/G**	49	164	0.83	0.53-1.31	132	236	1.12	0.81-1.55
G/G+A/G	**C/C+G/C**	46	186	0.62	0.38-1.00	101	246	0.91	0.66-1.25
aROR					1.53 (0.77-3.03)				**0.58 (0.35-0.96)**
rs2953 (micro-RNA589; CTNNB1)	Rs4835761 (WNT8A)								
A/A	**A/A**	59	199	1		154	277	1	
A/A	**G/G+A/G**	66	273	0.84	0.55-1.28	183	352	0.92	0.68-1.25
G/G+A/G	**A/A**	41	134	1.03	0.63-1.70	96	172	0.95	0.65-1.38
G/G+A/G	**G/G+A/G**	44	182	1.01	0.65-1.55	111	281	0.77	0.56-1.06
aROR					1.27 (0.61-2.64)				0.69(0.42-1.13)
rs2953 (micro-RNA589; CTNNB1)	rs222851 (DVL2)								
A/A	**A/A**	52	239	1		140	297	1	
A/A	**G/G+A/G**	80	353	1.04	0.68-1.57	209	353	1.16	0.85-1.57
G/G+A/G	**A/A**	35	159	1.05	0.65-1.57	95	197	0.98	0.68-1.40
G/G+A/G	**G/G+A/G**	55	180	1.26	0.81-1.94	113	279	0.91	0.66-1.25
aROR					0.99 (0.48--2.06)				0.85 (0.51-1.40)

Table 3.25 Combined effects of miRNA589 binding site for CTNNNB1 with other Wnt pathway SNPs and esophageal cancer among infrequent and frequent drinkers

		Infrequent Drinkers				Frequent Drinkers			
		Ca	**Co**	**aOR**	**95% CI**	**Ca**	**Co**	**aOR**	**95% CI**
rs2953 (miRNA589; CTNNB1)	rs2241802 (FZD3)								
T/T	G/G	95	291	1		97	162	1	
T/T	G/A+A/A	129	381	1.19	0.82-1.72	123	248	0.98	0.67-1.44
G/T+G/G	G/G	61	171	1.19	0.76-1.85	60	135	0.81	0.51-1.28
G/T+G/G	G/A+A/A	86	281	0.90	0.60-1.34	84	172	0.94	0.64-1.40
aROR					0.64 (0.35-1.14)				1.21 (0.63-2.33)
rs2953 (miRNA589; CTNNB1)	rs3729629 (WNT2)								
T/T	G/G	151	401	1		128	266	1	
T/T	C/C+G/C	118	381	0.72	0.51-1.00	115	220	1.50	1.05-2.14
G/T+G/G	G/G	79	239	0.89	0.62-1.29	102	160	1.30	0.89-1.90
G/T+G/G	C/C+G/C	79	261	0.60	0.41-0.88	68	170	0.92	0.63-1.35
aROR					0.94 (0.54-1.62)				0.61 (0.33-1.11)
rs2953 (miRNA589; CTNNB1)	Rs4835761 (WNT8A)								
T/T	A/A	111	319	1		115	189	1	
T/T	G/G+A/G	193	585	0.97	0.70-1.35	176	350	0.85	0.59-1.22
G/T+G/G	A/A	100	225	1.28	0.86-1.88	72	153	0.69	0.43-1.09
G/T+G/G	G/G+A/G	139	455	0.83	0.59-1.19	151	323	0.86	0.59-1.25
aROR					0.71 (0.40-1.25)				1.26 (0.66-2.42)
rs2953 (miRNA589; CTNNB1)	rs222851 (DVL2)								
T/T	A/A	94	358	1		110	209	1	
T/T	G/G+A/G	206	545	1.59	1.11-2.28	183	324	0.85	0.57-1.27
G/T+G/G	A/A	99	263	1.00	0.64-1.56	88	198	0.82	0.52-1.28
G/T+G/G	G/G+A/G	139	424	1.13	0.76-1.69	132	278	0.87	0.57-1.33
aROR					0.71 (0.40-1.26)				1.26 (0.67-2.33)

Table 3.26 Combined effects of microRNA589 binding site for CTNNNB1 with other Wnt pathway SNPs and esophageal cancer among drinkers/smokers and non-drinkers/non-smokers

		Non-smokers/Infrequent and non-drinkers				Smokers/Drinkers			
		Ca	**Co**	**aOR**	**95% CI**	**Ca**	**Co**	**aOR**	**95% CI**
rs2953 (miRNA589; CTNNB1)	rs2241802 (FZD3)								
A/A	G/G	47	187	1		95	119	1	
A/A	G/A+A/A	70	284	0.91	0.56-1.46	112	187	0.82	0.54-1.25
G/G+A/G	G/G	36	127	1.04	0.59-1.82	53	106	0.71	0.43-1.17
G/G+A/G	G/A+A/A	51	191	0.97	0.58-1.60	77	131	0.74	0.47-1.17
aROR					1.02 (0.50-2.11)				1.25 (0.66-2.46)
rs2953 (miRNA589; CTNNB1)	rs3729629 (WNT2)								
A/A	G/G	84	265	1		116	206	1	
A/A	C/C+G/C	57	274	0.49	0.32-0.76	112	165	1.45	0.98-2.15
G/G+A/G	G/G	49	163	0.82	0.52-1.29	93	128	1.46	0.96-2.20
G/G+A/G	C/C+G/C	46	185	0.62	0.39-1.01	61	125	1.01	0.64-1.58
aROR					1.55 (0.79-3.06)				**0.48 (0.26-0.88)**
rs2953 (miRNA589; CTNNB1)	Rs4835761 (WNT8A)								
A/A	A/A	59	199	1		103	134	1	
A/A	G/G+A/G	65	273	0.75	0.47-1.20	109	185	0.85	0.57-1.28
G/G+A/G	A/A	41	134	0.88	0.51-1.51	52	96	0.74	0.45-1.21
G/G+A/G	G/G+A/G	44	180	0.82	0.49-1.36	72	159	0.62	0.40-0.96
aROR					1.08 (0.68-1.77)				0.74 (0.49-1.12)
rs2953 (miRNA589; CTNNB1)	rs222851 (DVL2)								
A/A	A/A	51	239	1		100	154	1	
A/A	G/G+A/G	80	253	1.16	0.74-1.82	122	177	0.91	0.61-1.34
G/G+A/G	A/A	35	159	0.77	0.45-1.32	60	114	0.68	0.43-1.07
G/G+A/G	G/G+A/G	55	178	1.23	0.76-1.98	75	146	0.83	0.54-1.27
aROR					1.37 (0.68-2.77)				1.35 (0.72-2.53)

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