LUNG CANCER IN NEVER-SMOKERS

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SUMMARY

Although smoking is the main cause of lung cancer, lung cancer among never-smokers is not uncommon and is the 7th leading cause of cancer deaths worldwide. There is recent evidence that lung cancer in never-smokers may be a distinct clinical entity from smoking-related lung cancer. Lung cancer incidence in Singaporean Chinese women is relatively high given the low prevalence of smoking, and etiologic studies conducted in this population would be particularly useful in identifying risk factors for never-smoker lung cancer.

This thesis examines three potential etiologic pathways which we hypothesise to play a role in never-smoker lung cancer, based on mechanistic considerations and existing epidemiologic studies – chronic inflammation resulting in uncontrolled cell proliferation, direct damage arising from exposure to carcinogens, and pro-carcinogenic estrogen pathway signaling in lung tissue. The factors that we investigated included chronic airway inflammation and genetic polymorphisms in the inflammatory pathway (examining 6 polymorphisms in 5 genes), previous Non-Steroidal Anti-Inflammatory Drug (NSAID) use, exposure to domestic inhalants, dietary factors, and reproductive factors and polymorphisms in the estrogen signaling pathways (examining 5 polymorphisms in 4 genes).

Data from two hospital-based case-control studies (conducted from 1996 to 1998 and from 2005 to 2008) of Chinese female incident lung cancer patients, and controls frequency-matched by age and date of admission, were used. A standardized questionnaire was administered on all participants by trained interviewers, and, where permitted, blood and saliva samples were obtained. In total, 702 cases and 1578 controls were recruited, of whom 433 cases (61.7%) and 1375 controls (87.1%) were never-smokers.

Tuberculosis was positively associated with lung cancer, although this association was not statistically significant. There was no effect of asthma, atopy or chronic productive cough

individually, but the presence of one or more of these conditions was positively associated with lung cancer in individuals possessing specific genotypes in the IL1-ß-31T/C and IL1RN genes. The IL6-634 G allele was positively associated with lung cancer. A positive association with lung cancer was observed with the number of polymorphism sites where at least 1 "risk" allele was present [the "risk" alleles being evaluated were IL1-ß-31T/C (T allele), IL1RN (*2 allele), and IL6-634C/G (G allele)] among those with asthma, cough or atopy, but not in those without. Regular use of aspirin, an NSAID, was inversely associated with lung cancer, and this inverse association was strongest for those starting use 1 to 5 years prior to admission, and those whose duration of use was 12 to 60 months. These data together suggest that inflammation and inflammatory processes are important in never-smoker lung carcinogenesis.

Inhalants in the domestic environment contain compounds that may cause direct genetic damage at the cellular level, and can also provoke chronic inflammation. Contrary to our expectation, a positive relationship with daily exposure to incense or mosquito coils and to cooking fumes was observed only among smokers, with no relationship seen among never-smokers. These interactions were statistically significant. Our data suggest that smokers are more susceptible to the risk-enhancing effects of other inhalants. We postulate that these results may in part be explained by the chronic airway inflammation induced by chronic smoking.

Our study of diet focusing on meat consumption showed that meat and, in particular, fish consumption was inversely associated with lung cancer in never-smokers, but null effects were seen for processed meats and dietary heterocyclic amines. The inverse association of fish consumption with lung cancer could be due to omega-3 fatty acids (a prominent nutrient found in fish) and their effects, among others, in reducing inflammation in the local tissue milieu.

Our study of reproductive factors found that parity and menstrual cycle length were inversely associated with lung cancer, while age at first birth, age at menopause and reproductive period were positively associated. The COMT rs4680 A allele was positively associated with lung cancer, but null effects were seen with other polymorphisms. These data suggest that high circulating estrogen levels over the lifetime are associated with lung cancer risk, especially in never-smokers, and that this effect could be at least partially mediated through direct catechol estrogen damage to genetic material.

Collectively, the research studies in this thesis implicate both the inflammatory and estrogen pathways in lung carcinogenesis. Perhaps the most significant aspect of this set of findings is that chronic inflammation may play a bigger role in never-smoker lung cancer than has been previously recognized. Further research is needed to confirm these epidemiologic findings, and to delineate the precise role played by inflammation and reproductive factors in the etiology of never-smoker lung cancer.

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CHAPTER 1 LUNG CANCER: AN INTRODUCTION

Lung Cancer

A hundred years ago, lung cancer was a rare medical condition. The celebrated American surgeon Alton Ochsner noted in an article in 1973 that he remembered as a student in 1910 being asked to witness an autopsy of a patient with lung cancer because it was an unusual case. Indeed, he saw his next lung cancer case only 17 years later, in 1927. Yet in that same year, he saw eight cases within six months (1). This early cluster of lung cancer heralded a major epidemiologic shift in lung cancer incidence worldwide: today, eighty years later, lung cancer is the leading cause of cancer mortality in the world, killing an estimated 1.3 million people every year, nearly half a million more a year than from stomach cancer, the next greatest cause of cancer deaths worldwide (2).

The histology of lung cancer is fairly heterogeneous, suggesting that lung cancer may be a "collection of diseases" rather than a single disease. The main division used in clinical practice is between small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). This differentiation has prognostic and treatment significance, as SCLCs have different biological and molecular characteristics and appear to be more chemo-and radio-sensitive (3,4). Among NSCLCs, tumours are broadly classified into adenocarcinomas, squamous cell carcinomas, and large cell carcinomas. Rare tumours including carcinoid tumours are also found. Tumours tend to be heterogeneous, and tumours with mixed features have been recognized – for example: adenosquamous carcinomas (having malignant cells of both adenocarcinoma and squamous cell carcinoma histology), and combined small cell carcinoma (tumours with cells of both the small cell and the non-small cell histology) (5). The majority of tumours are NSCLC, with SCLC comprising only about 10-30% in most reported series (6). Among NSCLC,

squamous cell carcinomas were the most common in the mid to late 20th century (7), but adenocarcinomas have increased in overall incidence and now comprise the majority of tumours in most countries.

Lung cancer remains highly lethal with very low 5-year survival of about 10-20% even in the countries with the best treatment for the condition (8). Survival for both SCLC and NSCLC are equally dismal, without significant differences in survival by histologic classification. Screening for lung cancer is still investigational. Screening X-rays do not appear to be effective, although a large trial is ongoing and results from this trial will only be reported in 2015 at the earliest (9). In recent years, attention has focused on the utility of spiral Computerised Tomography screening. There is some evidence that CT screening may be useful in high-risk groups (10) and definitive trials are underway. Nevertheless, the universally dismal survival rates for lung cancer even for relatively early stage disease suggest that the best way to reduce the public health burden of lung cancers is through prevention. Identification of risk factors for lung cancer and translating this epidemiologic knowledge into public health practice to control these risk factors remain the most effective methods to reduce the burden of lung cancer.

This understanding of the importance of prevention has led to intensive investigations into risk factors for lung cancer. However, few risk (or protective) factors have been identified with certainty. These include smoking, environmental tobacco exposure (ETS) and some occupational toxins. High consumption of fruits and vegetables also appear to be protective.

The most well-known risk factor, and perhaps the most prevalent worldwide, is that of tobacco smoke. The relationship between smoking and lung cancer was gradually recognized, first by individual physicians in the 1930s and 1940s, then in 2 sets of

landmark studies by Doll and Hill in the United Kingdom and Hammond and Wynder in the United States (11). Since then, this relationship has been definitively established as the main cause of lung cancer through a wide variety of case control and cohort studies throughout the world. Relative risk estimates for cigarette smoking on lung cancer risk range from 20-40 (12), and both intensity in terms of number of cigarettes smoked and duration of smoking appear to influence risk (13). Biochemical and cellular biologic evidence supports this association. Tobacco smoke is a complex mixture of organic compounds, many of which (such as nitrosamines including NNK, polycyclic aromatic hydrocarbons, acroleins and aryl amines) are clearly carcinogenic in both *in vitro* and *in vivo* models (14-16). This carcinogenicity is mediated through a variety of biological pathways, including direct genotoxic damage to DNA, activation of growth and proliferation pathways and suppression of apoptosis, and chronic inflammation with subsequent cellular proliferative response to inflammatory injury (14-16).

Environmental tobacco smoke (ETS) exposure is now known to increase risk among never-smokers who work and live with cigarette-smokers. Relative risk estimates for ETS exposure is much lower than those for smoking, of about 1.2-1.3 in cohort studies (17-19). In some countries, because of the widespread prevalence of smoking in enclosed environments and hence exposure to ETS, this small relative risk can translate to exceedingly large population attributable risks. In the US alone, estimates suggest that 3000 to 5000 lung cancer deaths annually may be due to ETS exposure (20).

Occupational causes of lung cancer have also been identified, including radon (21), asbestos (22), arsenic (22, 23), silica (24), cadmium (25), nickel (26), and chromium exposure (26) in miners, construction workers, and heavy industry factory workers. The mechanisms of action are varied. Radon decays into radon progeny, which then emits

alpha particles that cause direct genotoxic damage to respiratory epithelial cells (21). Asbestos and silica deposit within the lungs and induce inflammation and cellular proliferation (22-24). Cadmium, nickel and chromium may both directly, and indirectly, through the generation of reactive oxygen species, damage DNA (25, 26). Coal smoke emission is also well-established as a risk factor, both in occupational and domestic settings (27), with robust supportive evidence derived from both *in vitro* and *in vivo* mechanistic and human epidemiologic studies (27).

Multiple studies have suggested that fruit and vegetable consumption are protective against lung cancer (28). A relatively recent review of the current evidence by the World Cancer Research Fund and the American Institute for Cancer Research concluded that carotenoid-containing foods are likely protective, and non-starchy vegetables, selenium-containing foods and quercetin (a flavonoid compound)-containing foods might also be protective, based on limited evidence (29). A local study has highlighted the protective effect of cruciferous vegetables, in particular in smokers (30); the putative protective compounds appear to be isothiocyanates.

Other risk factors have also been explored. Using the model of smoking-related lung carcinogenesis, where the effect of smoking is mediated through both direct carcinogenic damage and chronic inflammation, investigators have also considered the role of other possible carcinogens, such as cooking fumes (27, 31, 32), smoke emissions from incense (33), mosquito coils (34) and wood smoke (27,35). Meat-derived carcinogens as a result of cooking or food-processing methods have also been studied (36, 37). Other investigators have considered the role of chronic inflammation and immune system dysfunction by studying the risks imposed by pre-existing chronic lung disease (38, 39), chronic infection by the bacterium *chlamydiae pneumonia* (40), the

Human Papilloma Virus (41) and the Human Immunodeficiency Virus (42), and atopic conditions such as allergic rhinitis, atopic dermatitis, and allergies (43, 44).

Because of the relatively higher proportion of women among never-smoker lung cancer cases, and of some data (although disputed) suggesting higher incidence of lung cancer in never-smoking women compared to men (45), the role of estrogen-related factors and reproductive history has also been examined (46, 47).

The evidence for these putative risk factors has not been consistent, and interpretation of the results has been complicated by methodological limitations and differences in study populations.   Recent evidence for some of these factors is examined and evaluated later in the relevant chapters.

Variation in personal risk of a disease outcome may be due to both differing levels of exposure to environmental factors, with subsequent epigenetic effects on gene expression, and to genetic variation among individuals. It is likely that genetic susceptibility in lung cancer, similar to genetic susceptibility seen for other complex non-communicable diseases, is a result of the combined effect of many genes, each of which exerts only a small effect. It is also probable that genetic and environmental risks interact such that it is the joint effect that is important rather than the separate individual effects (48).

There is indeed some evidence both that genetic susceptibility is important (although the risk estimates are low), and that joint effects may be more important than individual effects separately. Genome-Wide Association Studies have identified a susceptibility locus at position 15q25 (49-51), mapping to a gene for the nicotinic acetylcholine. While two of the studies were conducted solely in smokers (49, 50), the third showed that the

locus conferred risks in both never smokers and smokers (51). Two additional loci at 6p21.33 (mapping to the BAT3-MSH5 gene locus) and 5p15.33 (the TeRT-CLPTM1L locus) were identified subsequently (52, 53). In 2010, an additional locus at 13q31.3, mapping to the GPC5 gene was found to associate with lung cancer in never smokers (54). More recently, additional risk loci at 3q28, 13q12.12 and 22q12.2 were also identified in a study conducted on Han Chinese subjects (55).

Other studies have used a candidate gene approach to identify genetic susceptibility loci. These have focused on genes involved in the metabolism of products of cigarette smoke, including both Phase 1 (CYP1A1) and Phase 2 (GST and UDP) enzymes. Phase 1 enzymes (cytochrome P450s such as CYP1A1) activate carcinogens in tobacco smoke such as PAH, while Phase 2 enzymes (such as glutathione- S transferases ([GSTs] and UDP Glucuronosyltransferases [UGTs]) detoxify phase 1 products into soluble metabolites which are then excreted (56, 57). A pooled analysis identified a polymorphism in exon 7 of the CYP1A1 gene as risk conferring in lung cancer (58). Other studies have identified the GSTM1 null genotype as risk-conferring, particularly among smokers and those with strong ETS exposure (30, 59, 60), providing an example of the importance of the joint effect of genes and environmental factors in conferring risk in diseases.

While smoking is the leading cause of lung cancer by virtue both of its high prevalence and its large causal effect, and smoking control is a key pillar in lung cancer prevention worldwide, never-smoker lung cancer is nonetheless not uncommon (45). The relative paucity of well-established common risk factors for lung cancer other than smoking and ETS suggests that additional investigations using well-designed studies are necessary. Further, in order to remove a potential masking effect of smoking, studies that are

conducted in sufficiently large numbers of never-smokers would be particularly valuable. Moreover, as I will discuss in Chapter 2, there is growing evidence that lung cancer in never-smokers is biologically different from that in smokers. This further emphasizes the importance of studying never-smoker lung cancer as a separate disease entity.

Format of thesis

The aims of this research thesis are to identify genetic and environmental risk factors for lung cancer in Chinese women, focusing specifically on never-smokers, through the analysis of data obtained from the Gene and Environment in Lung Cancer (GEL) studies. The GEL studies are two hospital-based case-control studies conducted in Singapore between 1996-1998, and 2005-2008. This thesis examines the separate and joint effects of environmental and genetic risk factors in two broad areas – inflammation and inflammatory pathways, and reproductive factors and estrogen pathways. In addition, it will examine two other possible risk factors for never-smoker lung cancer: exposure to domestic inhalants, and dietary factors. This thesis hence comprises five studies, including two for the research area on inflammation.

The first three chapters of this thesis explain the rationale for conducting the GEL studies. In this chapter, I have noted that few risk factors have been established for lung cancer, and that additional investigations into risk factors are important, as prevention of lung cancer remains the most appropriate method of control at this time. In Chapter 2, evidence for lung cancer in never-smokers as a distinct entity from smoking-related lung cancer is presented. The chapter will make the case that it is necessary to consider never-smokers separately from smokers, rather than opting for the usual practice of adjusting for smoking. Further, understanding risk factors in never-smokers is particularly important in populations with low prevalence of smoking, such as the

Singaporean Chinese women. In Chapter 3, local lung cancer data from the Singapore Cancer Registry is presented. I will argue that the ethnic variation in lung cancer rates do not appear to be explained by differences in smoking prevalence rates alone. Further, Chinese women have a disproportionately high lung cancer rate despite low smoking prevalence, suggesting that this group would be a useful study population to elucidate risk factors for never-smoking lung cancer. Results from an Age-Period-Cohort analyses of this group (Chinese women) are presented, which suggest that the relevant risk factors for Chinese women include those factors whose prevalence changed substantially during the 1970s and 1980s. These may include factors such as diet, exposure to chronic inflammation, exposure to inhalants in the domestic environments, and female reproduction. These potential factors are examined in detail in the remaining 6 chapters, which form the body of this thesis.

In Chapter 4, the design of the GEL studies and general methodology including the statistical methods used are described in detail. Chapters 5 to 9 present findings and the discussion of the association of these potential factors to lung cancer. In each of the 5 chapters, an introduction considers the current state of knowledge with regard to the factor of interest. Additional methodological issues specific to that analysis are discussed. Results are then presented, and a detailed discussion follows, during which possible limitations in interpretation of findings are also mentioned. In each case, the analysis is performed either exclusively in never-smokers, or else compares the effects of the risk factor under investigation between smokers and never-smokers.

In Chapter 5, the role of previous inflammatory events in the lung and airways and gene polymorphisms in the inflammation pathways is examined. Chapter 6 looks at the effect of chronic Non-Steroidal Anti-Inflammatory Drug (NSAID) use. Chapter 7 presents

findings of an analysis of the effect of female reproductive factors and gene polymorphisms in the estrogen metabolism pathways. Chapter 8 explores exposure to inhalants in the domestic environment, and notes that the effect of inhalants may be in part mediated through inflammation. Chapter 9 studies the effect of meat consumption on lung cancer, and raises the possibility that the protective effect of fish consumption observed could be due to omega-3 fatty acids and their anti-inflammatory activity. Collectively, this series of studies described in Chapters 5 to 9 constitute an epidemiologic investigation into risk factors for never-smoker lung cancer, focusing on 2 key areas – the role of inflammation, and the role of female reproductive factors. Finally, Chapter 10 summarises the key conclusions arising from this thesis.

CHAPTER 2 LUNG CANCER IN NEVER-SMOKERS: EVIDENCE FOR A DISTINCT CLINICAL ENTITY FROM SMOKING –RELATED LUNG CANCER

Several different lines of recent evidence suggest that lung cancer in never-smokers is a distinct entity from smoking-related lung cancer.

Epidemiologic evidence

Worldwide, never-smokers represent only about 10% of all lung cancer patients in men, but from 15% to 75% of lung cancer patients in women, with higher proportions in Asia (45, 61, 62). The relatively small proportion in males is due largely to the extremely high numbers of smoking-related lung cancer in this group. Never-smoker lung cancer is not an uncommon entity. If separated out from smoking-related lung cancers, it would still be the 7th most common cause of cancer death worldwide, on par with the global mortality of cervical cancer, pancreatic cancer and prostatic cancer (45). It has a substantial reported global mortality of about 15-20 per 100000 annually (45).

Various descriptive studies have pointed out that never-smokers with lung cancer are more likely to be females (although the lung cancer incidence in never-smokers appears similar in both males and females) (45, 61, 62), and the most common tumour histology among never-smokers is adenocarcinomas (45). In Asian countries, never-smoker cases are on average younger than smokers (63), and the stage at diagnosis tends to be more advanced among never-smokers than smokers (63). Despite that, never-smokers have better overall survival, after adjustment for known prognostic factors such as pre-morbid status and stage (63).

Evidence from cancer biology

Molecular differences in lung cancer tissue between smokers and never-smokers have been observed. The frequency of TP53 mutation frequency is higher among smokers. There is also evidence that among TP53 mutations, the proportion of G:C to T:A transversions occur more frequently in smoking–related cancers (64).

EGFR and KRAS mutations tend to occur in lung adenocarcinomas (30% and 20% of all lung adenocarcinomas, respectively), but both are rare in other histologic subtypes (65). The mutations in EGFR are mostly point mutations and deletion in the tyrosine kinase domain, leading to a constitutively active and ligand-independent receptor state (66, 67). Tumours with these "activating" mutations are dependent on continued EGFR signaling for proliferation and survival (61, 67). Activating mutations in EGFR appear to occur more frequently among never-smokers (45% vs 7%), women, and in patients of east Asian ethnicity (61, 63, 66, 67).

KRAS mutations mostly occur on codons 12, 13 and 61, and affect GTP-ase activity of the molecule (68). Mutations result in a constitutively active KRAS molecule, which then induces downstream signaling molecules RAF1 and MAPK. Axctivated MAPK phosphorylates and activates transcription factors (eg c-Jun, c-Myc and c-Fos) that induce cell proliferation (68). Evidence for the association between KRAS mutations and smoking status is inconsistent. A study using data from an Asian population reported that KRAS mutations were associated with ever smoking, males and poor histologic differentiation (69). Two smaller studies in populations of Caucasian origin did not find a significant association, although there was a trend towards lower KRAS mutation frequencies in never-smokers (66, 67). A larger study in an American population of mostly whites also failed to show an association between KRAS mutations and smoking

status, although the transversion mutations were strongly associated with ever smokers (68). It is not yet clear if these differences in findings represent true differences between Asian and European populations.

Differences in HER2 mutations have also been reported; HER2 mutations are reported in 2% of non-small cell lung cancers, and all involved deletions in exon 20 (70). HER2 mutations, EGFR mutations and KRAS mutations were mutually exclusive (70). Similar to EGFR mutations, HER2 mutations appear to be more common in females and never-smokers (70).

Recently, a group of Chinese researchers reported that an EML4-ALK fusion gene was detected in the tumours of 12 of a cohort of 103 lung cancer patients (71). This fusion protein was associated with never-smokers, younger age of onset, and adenocarcinomas without EGFR or KRAS mutations, and was also correlated with ALK protein expression but not EML-5. ALK inhibits apoptosis and promotes growth and proliferation through activation downstream of the PI3K/Akt and the MAPK pathways.

Gene expression microarray profile studies suggest that the gene expression profile in lung tumours of smokers differed from those of never-smokers (72). Although there were similarities in gene expression in the two groups, it was possible to differentiate the two groups based on expression profiles. Notably, one study found that four times as many genes changed expression in never-smokers compared to smokers. This was interpreted to suggest that adenocarcinomas in never-smokers developed locally, while smoking -related lung cancer developed in a field of genetically altered tissue (73).

Evidence from clinical trials

EGRF- Tyrosine Kinase inhibitors such as erlotinib and gefitinib were first used in the early 2000s to treat patients with advanced non-small cell lung cancer (61, 74). Re-analysis of data from several large clinical trials suggested that EGFR TKIs benefited never-smokers more than ever-smokers, and this was primarily due to the higher proportion of tumours with activating EGFR mutations among never-smokers (61). A phase III trial conducted in Asia of EGFR-TK inhibitors as first-line treatment compared to the standard of carboplatin/paclitaxel in never or light smokers showed better progression-free survival but no improvement in overall survival (75, 76), but the authors noted that about two-thirds of patients with EGFR mutation-positive tumours and assigned to standard therapy crossed over to TKIs, and this high cross-over rate could have confounded the overall survival results (76).

Summary

The available data therefore suggests that never-smoker lung cancer is a distinct biological entity from smoking–related lung cancer. Never-smoker lung cancer has a different epidemiologic profile in terms of patient age and survival. Gene expression patterns are different, and the main genes that drive cell proliferation in the tumours also differ (EGFR mutations in never-smoker lung cancers, KRAS and TP53 in smoking-related lung cancer). Consequently, tumour response to a class of anticancer agents, the EGFR-TK inhibitors, also differed by smoking status.

The carcinogenic processes in smoking –related and never-smoker lung cancers may be different as well, with different environmental and genetic risk factors. It is important at this juncture to determine if risk factors indeed do differ for never-smokers, and if so, to

establish the etiologic factors in never-smoker lung cancer. Study designs for etiologic research would need to consider smoking–related lung cancer and never-smoker lung cancer separately, instead of continuing with the current practice of treating lung cancer as a homogenous group and adjusting for smoking behavior in the analysis. Unfortunately, because of the much higher smoking prevalence in many populations, most studies of lung cancer, especially cohort studies, have not been able to accrue sufficient numbers of never-smokers to achieve sufficient power to investigate risk factors in this group separately. Singapore is one of a few populations around the world where sufficient numbers of never-smoker lung cancer can be accrued with relative ease, because of the extremely low smoking prevalence and unexpectedly high lung cancer incidence rates among its women, as I will discuss in the next chapter.

CHAPTER 3   LUNG CANCER IN SINGAPORE

I) Incidence

<u>Introduction</u>

Lung cancer was the cancer with the highest incidence in men and the third highest incidence in women (excluding skin cancers) in Singapore for the years 2003 to 2007 (77).

In this chapter, I explore epidemiologic trends for lung cancer in Singapore and look at ethnic and gender differences in these trends. These data offer clues to the possible risk factors that may be important in lung carcinogenesis in Singapore.

<u>Methods</u>

For this analysis, aggregate data for cancer numbers and estimated person-years were obtained from the Singapore Cancer Registry, by 5-year age bands, 5-year periods and 5–year birth cohort groups, and stratified by ethnicity. Information about incident cancers comes from notifications made by medical professionals, pathology reports, hospital records, and death certificates. To improve the comprehensiveness of notifications, the Registry checks all pathology reports, death reports and records of public sector hospitals and matches these against notifications. The Registry believes that cancer registration is essentially complete, since all cases diagnosed histologically, and all cases with mention of cancer in public hospital discharge forms and death certificates are included. Prior to 2002, notifications were verified through review of pathology results. After 2002, additional data from medical records were used. From 2007, cancer notifications have been made compulsory under the National Registry of Diseases Act (78).   Person-year estimates from the Cancer Registry were derived from mid-year

population estimates made by the Department of Statistics Singapore from 1968 through to 2007. Data were truncated below 30 years of age, as numbers of cases were small in those age-groups. For comparison between population groups, a Standard Population was created using the World Standard Population, but with truncation at 30 years of age.

Results

Figure 3.1 shows the Age-Standardised Rates for men and women for the period 1968-2007. (Because the Standard Population used is the World Standard Population from ages 30 to 79, the ASRs differ from those calculated by the Singapore Cancer Registry, although the trends observed are similar. Comparisons with other estimates should be made with care because of the different standard population used.) The rates show a clear gender-specific difference in lung cancer rates, with much higher rates in men than women. In addition, the rates in men show rapidly rising rates that appear to have peaked in the early 1980s, before showing a gradual decline. The rates for females have not changed so substantially, but they also show a peak in the early 1980s, with a very gradual decline subsequently.

In Figure 3.1, there is a plateau and decline in lung cancer incidence in men. Conversely, women experience a slower decline in lung cancer incidence. There is also substantial ethnic variation in lung cancer incidence. Lung cancer incidence is highest amongst the Chinese in both males and females, with significantly lower rates in Malays, and, particularly, in Indians (Figures 3.2 and 3.3).

In order to interpret the trends in lung cancer observed, smoking prevalence rates need to be considered. Table 3.1 shows the smoking prevalence rates reported in various

Figure 3.1 Lung cancer incidence in Singaporean men and women, 1968-2007

Age-Standardised Lung Cancer Incidence 1968-2007, by gender
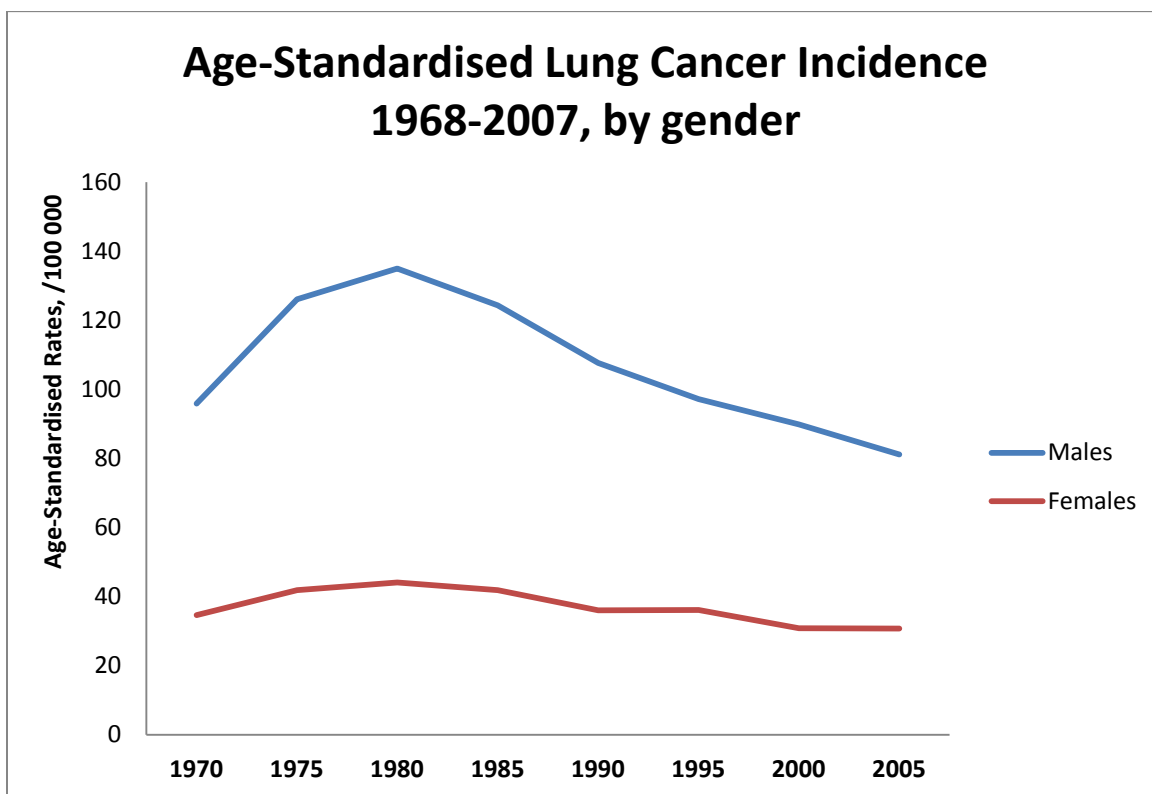
Rates/100,000	1970	1975	1980	1985	1990	1995	2000	2005
Males	95.91	126.04	134.93	124.35	107.60	97.17	89.83	81.16
Females	34.63	41.87	44.08	41.90	36.03	36.13	30.89	30.73

* Standardised to World Standard Population age 30-79

Figure 3.2 Lung cancer incidence in Singaporean Chinese, Malay and Indian men, 1968-2007

Age-standardised Lung Cancer Incidence 1968-2007, by ethnic group
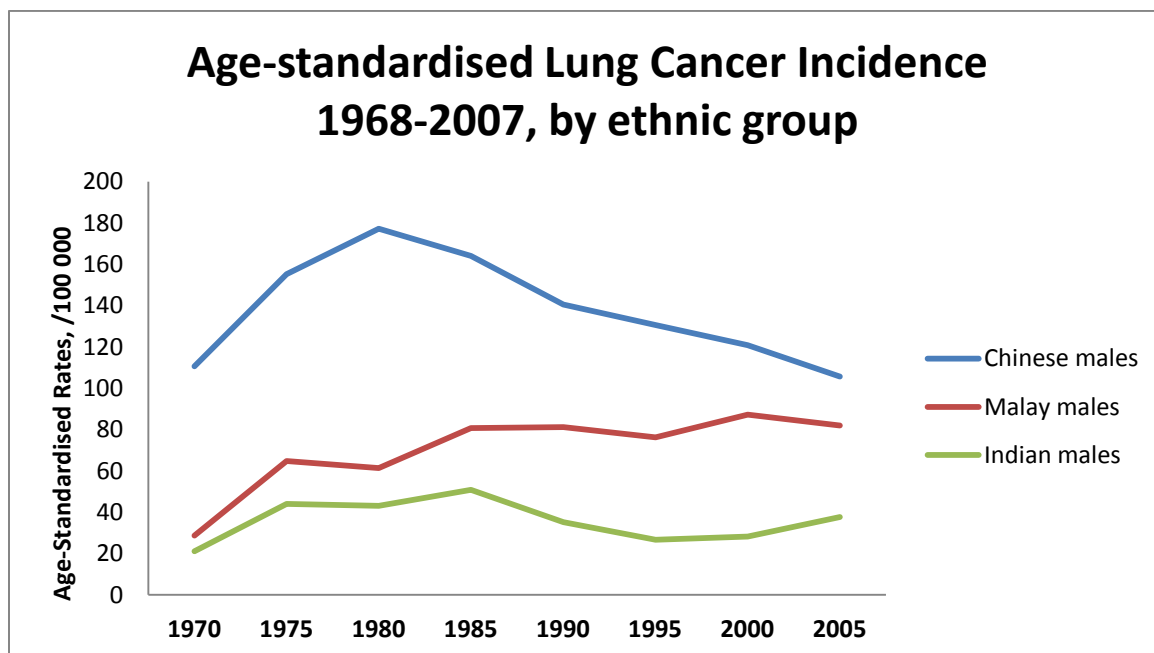
Rates/100,000	1970	1975	1980	1985	1990	1995	2000	2005
Chinese	110.58	155.27	177.17	163.98	140.52	130.64	120.81	105.65
Malays	28.66	64.77	61.42	80.73	81.19	76.17	87.21	81.97
Indians	21.07	44.05	43.14	50.91	35.16	26.67	28.29	37.67

* Standardised to World Standard Population age 30-79

Figure 3.3 Lung cancer incidence in Singaporean Chinese, Malay and Indian women, 1968-2007

Age-standardised Lung Cancer Incidence 1968-2007, by ethnic group
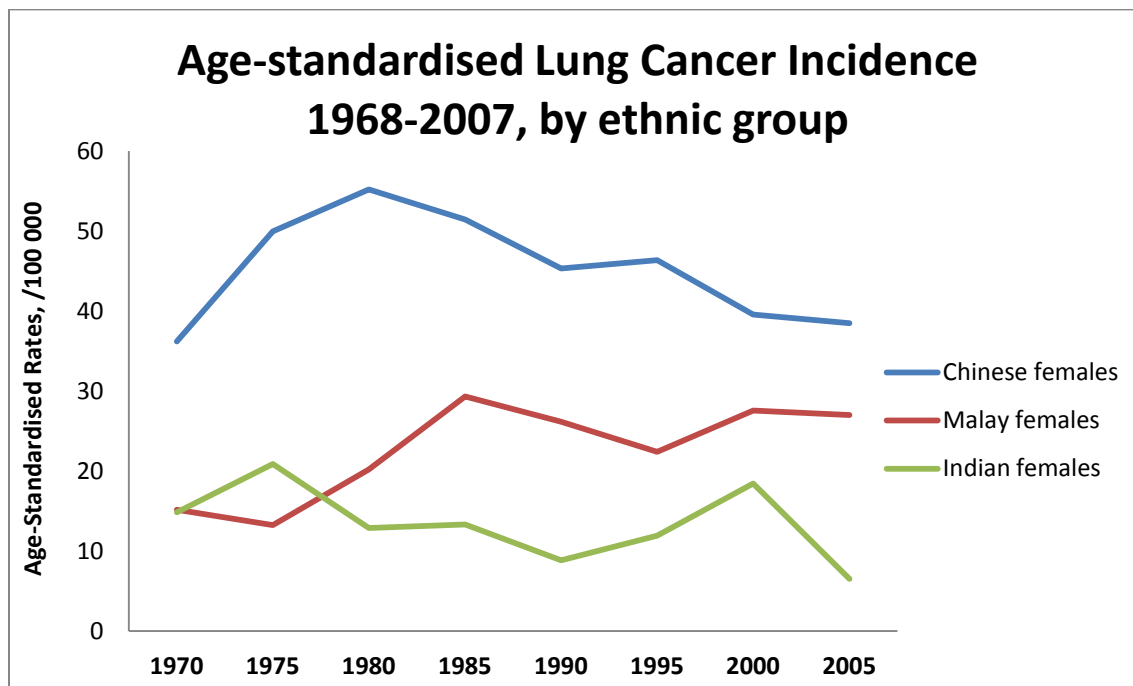
Rates/100, 000	1970	1975	1980	1985	1990	1995	2000	2005
Chinese	36.23	49.97	55.23	51.44	45.35	46.39	39.58	38.48
Malays	15.18	13.27	20.23	29.32	26.16	22.43	27.57	27.00
Indians	14.87	20.89	12.91	13.35	8.85	11.92	18.45	6.53

* Standardised to World Standard Population age 30-79

Table 3.1 Estimated smoking prevalence (current smokers) in Singaporean men and women

Yr of estimate	1974 (79)	1977 (80)	1979 (81)	1984 (80)	1987 (82)	1992 (83)	1998 (84)	2001 (85)	2004 (86)	2007 (87)	2010 (88)
Males	51.4	42	44.3	34.9	25.3	33.2	27.1	24.3	21.8	23.7	24.7
Females	8.3	4.5	6.3	3.4	2.0	3.0	3.2	3.6	3.5	3.7	4.2

* Estimates not directly standardised

Table 3.2 Estimated smoking prevalence (% current smokers) in Singaporean men and women, by ethnicity.

Year		1979 (81)	1984 (82)	1987 (82)	1992 (83)	1998 (84)	2001 (85)	2004 (86)	2007 (87)	2010 (88)
All	**Chinese**	21.5	18.1	12.8	16.5	13.8		11.7		12.8
	Malays	28.3	24.4	18.7	30.8	23.4		18.6		26.5
	Indians	24.0	17.6	13.1	15.3	16.4		12.1		10.1
Males	**Chinese**		32.9	23.4		24.0	22.5	20.5	21.2	22.2
	Malays		46.3	36.7		43.3	39.5	29.9	41.3	45.5
	Indians		31.8	23.8		29.9	18.2	22.4	20.6	17.3
Females	**Chinese**		3.6	2.3		3.2	3.7	3.1	3.6	3.8
	Malays		2.1	0.9		3.4	4.9	7.4	5.5	8.1
	Indians		0.8	0.8		0.9	0.3	1.4	1.5	2.3

community-based studies (79-88). The studies conducted in 1992, 1998, 2001, 2004, 2007 and 2010 were nationally representative surveys by the Ministry of Health, while earlier studies were community-based studies that were not representative. Caution must be taken when interpreting these findings, since some results were not taken from nationally representative populations, the definition of a smoker differed between studies, and no standardization for age was performed. Further, smoking prevalence data prior to the 1970s were not available.

Nevertheless, the results are striking in demonstrating a significant gender difference in smoking prevalence. The smoking prevalence in women never reached 10% in any of the studies, and was consistently lower than 5% in studies of the last 20 years. The smoking prevalence in men was about 50% in the 1970s, and appears to have declined since. Recent surveys suggest that between a quarter and a fifth of men are current smokers. These results suggest that smoking may largely account for the significantly higher lung cancer incidence in men compared to women, and the decline in lung cancer incidence in men in recent years may be a result of the decline in smoking prevalence from the late 80s onwards.

The attributable risk of smoking is likely to be lower for women, given the traditionally low rates of smoking, and indeed data suggest that only about half of Chinese women with lung cancer had ever smoked (89). Nevertheless, recent data suggest an increasing prevalence of smoking among young women (for example, smoking prevalence among 18-19 year old females was 3.7% in 1998 (84), 6.6% in 2004 (86) and 7.3% in 2010 (88) from the National Health Survey, which used similar sampling techniques and a similar questionnaire between the 2 surveys, and 9.1% in 2007 from the National Health Surveillance Survey (87), which had a different design and sampling from the National

Health Surveys.) If this rising trend reflects a true increase in smoking prevalence in young women, and persists, it is likely that the very gradual decline in lung cancer incidence rates in women will reverse itself in the next 20-25 years.

Smoking prevalence rates have been consistently highest in the Malays, with lower rates in the Chinese and Indians, particularly among men (81-88), see Table 3.2. While it is possible that differences in smoking behavior in terms of frequency and duration of smoking, and in biological susceptibility to cigarette smoke could explain why smoking prevalence rates do not appear to correlate with lung cancer incidence among the different ethnic groups, it is more probable that other factors account for these differences. Notably, the lung cancer incidence rates among Chinese women seem disproportionately high compared to other groups given the relatively low rates of smoking. Nevertheless, the lung cancer incidence in both Malay males and Malay females are rising, while those for Chinese males and to a lesser extent, Chinese females are dropping. This may be a consequence of the higher smoking prevalence in the Malay community observed since the 90s. If current trends persist over the next 15-20 years, incidence rates in Malays will overtake those of the Chinese for both males and females.

In 2003-2007, the most common histologic type was adenocarcinoma, comprising 43% of all cases with histology. 18% were squamous cell carcinomas, and about 10% were small cell carcinomas (77). Other registries have also reported rising incidence rates for adenocarcinomas (90, 91), and this is consistent too with the reported increase in the proportion of lung adenocarcinomas among all lung cancers (92). The data are also consistent with Seow *et al*'s review of epidemiologic trends in 1998 (89), which noted a three-fold increase in the incidence rate of adenocarcinomas among Chinese women,

with adenocarcinomas being the most common histological type in 1988-1992, comprising 51.3% of all histologically-confirmed cancers.

The relatively high lung cancer incidence seen in Singaporean Chinese women are consistent with rates reported in Chinese women in other communities around the world, in particular from findings reported in the US which have highlighted relatively high lung cancer incidence in women of Asian, in particular Chinese, ethnicity compared to other ethnic groups (93). These results highlight the need for further detailed study of lung cancer risk factors in this high-risk group, the results of which may suggest appropriate preventive and public health measures, and also elucidate etiologic mechanisms for never-smoker lung cancer, a disease entity that is difficult to study epidemiologically because of the preponderance of smokers among lung cancer cases in most populations around the world.

II) Mortality and survival

Lung cancer had the highest mortality rate in men and the second highest in women of all cancers in 2003-2007 (77). What drives the high mortality is not simply the high incidence, but also poor survival associated with lung cancer. Improvements in lung cancer treatment have resulted in at-best survival benefits measured in months rather than years, and the 5 year survival rate remains dismal.

Relative survival data from the Singapore Cancer Registry suggest that 5-year Age-Standardised Relative Survival (ASRS) has increased from about 4% in the 70s to about 10% in the late 90s and early 2000s in men, and from about 6% in the 70s to about 14% in the early 2000s in women (77). This very marginal improvement in survival reflects the very high case-fatality and the lack of substantial improvements in lung cancer

treatment. In recent years, attention has focused on the use of Tyrosine Kinase Inhibitors (TKIs) such as erlotinib and gefitinib. Although use of these TKIs have not been shown to improve survival overall, their use appears advantageous in a subgroup of EGFR mutation-positive cancers, with some improvements in survival. These EGFR mutation-positive cancers appear to be more frequent among never-smokers, Asians, and females (as discussed in Chapter 1). Although these TKIs do not appear to be a magic bullet offering a definitive breakthrough in lung cancer treatment, if early results hold out, more widespread use of TKIs in EGFR mutation positive cancers may result in improvements in relative survival, particularly among women, for whom such cancers comprise a larger proportion.

Lung cancer in Chinese women

<u>Introduction</u>

The previous section highlighted the relatively high rates of lung cancer despite consistently low prevalence of smoking among Chinese women. To understand the epidemiologic trends in this population group better, this section makes a more detailed examination of the lung cancer incidence trends in Chinese women, and includes Age-Period-Cohort (APC) modeling to allow an assessment of the relative contribution of period and cohort effects in lung cancer incidence trends among Chinese women.

<u>Methods</u>

The APC model is a Poisson regression model, where the number of events in any age group, period and birth cohort is modeled as a Poisson random variable (94). This estimate provides a quantitative method of assessing trends over time, and of evaluating the relative importance of secular changes in risk (period effects that affect all age

groups equally, for example changes in the way lung cancer is detected and diagnosed, through screening practices) from effects that vary from generation to generation (birth cohort effects affecting age groups unequally, for example changes in lifestyle and behavioural factors, such as smoking, for example).

The linear relationship between age at diagnosis, birth cohort and period of cancer diagnosis does not allow simultaneous estimation of all three effects unless further assumptions are made to the model. Different assumptions imposed can result in widely varying parameter estimates. For this brief analysis therefore, the full Age-Period-Cohort model was not used. Instead, three models were compared: a model with age as the only explanatory variable, one with age and period as explanatory variables, and a third with age and cohort as explanatory variables. To compare between these models, the goodness of fit of the models was determined using the deviance statistic; a non-significant value indicates a good fit. To compare between models, the Akaike Information Criterion (AIC) was used; smaller AIC values indicate better fit. Where appropriate, Incidence Rate Ratios (IRRs) obtained from the APC models are used to summarize the effects of age, period or cohorts.

<u>Results</u>

Figure 3.4 shows the age-specific incidence rates by time period of diagnosis, and Figure 3.5 shows the age-specific incidence rates by birth cohort. Age-specific rates from the age-group 50-54 and older are presented; rates for younger age-groups are very low. The data show a strong effect of age, with much higher age-specific rates in older age-groups than younger ones. Age-specific rates are lower in later periods (Figure 3.4). This is seen for all age-groups, but is particularly prominent in older age-groups. Figure 3.4 suggests a decrease in age-specific rates with younger cohorts. This

decrease by cohort is particularly prominent for incidence rates in older age-groups (age 60-64 and above) and is not seen in younger age-groups.

Table 3.3 shows overall results of the APC analysis. Table 3.4 reports the IRRs for age and cohort from the model with age and cohort as explanatory variables. Table 3.3 suggests that models with age alone and age and period did not fit the data well. In contrast, the age and cohort model had a good fit. The Akaike Information Criterion test similarly shows that the model containing age and cohort was superior to both a model containing age alone, and one containing age and period.

<u>Discussion</u>

The results therefore suggest that in Chinese females, differences in lifestyle factors between different cohorts of women (resulting in the strong cohort effect observed) may be important in explaining secular changes in lung cancer incidence rates. The IRR for lung cancer increases with increasing age, consistent with Figures 3.4 and 3.5. Lung cancer incidence is extremely low in those younger than 45, and rates increase rapidly beyond that. The IRRs for cohort effects suggest that, compared to the 1933-1937 cohort, older cohorts were at higher risks for lung cancer. However, the decrease in risks appears to have stabilized, and younger cohorts do not appear to be at significantly lower risks of lung cancer. In other words, the highest risks for lung cancer occur among birth cohorts who entered adulthood before or during the Second World War, while lower risks were seen in cohorts that entered adulthood in the post-war period, and who entered their 40s during the 70s and 80s in Singapore.

Figure 3.4 Age-specific lung cancer incidence by period of diagnosis in Singaporean Chinese women

Age-Specific Lung Cancer Incidence, by period of diagnosis
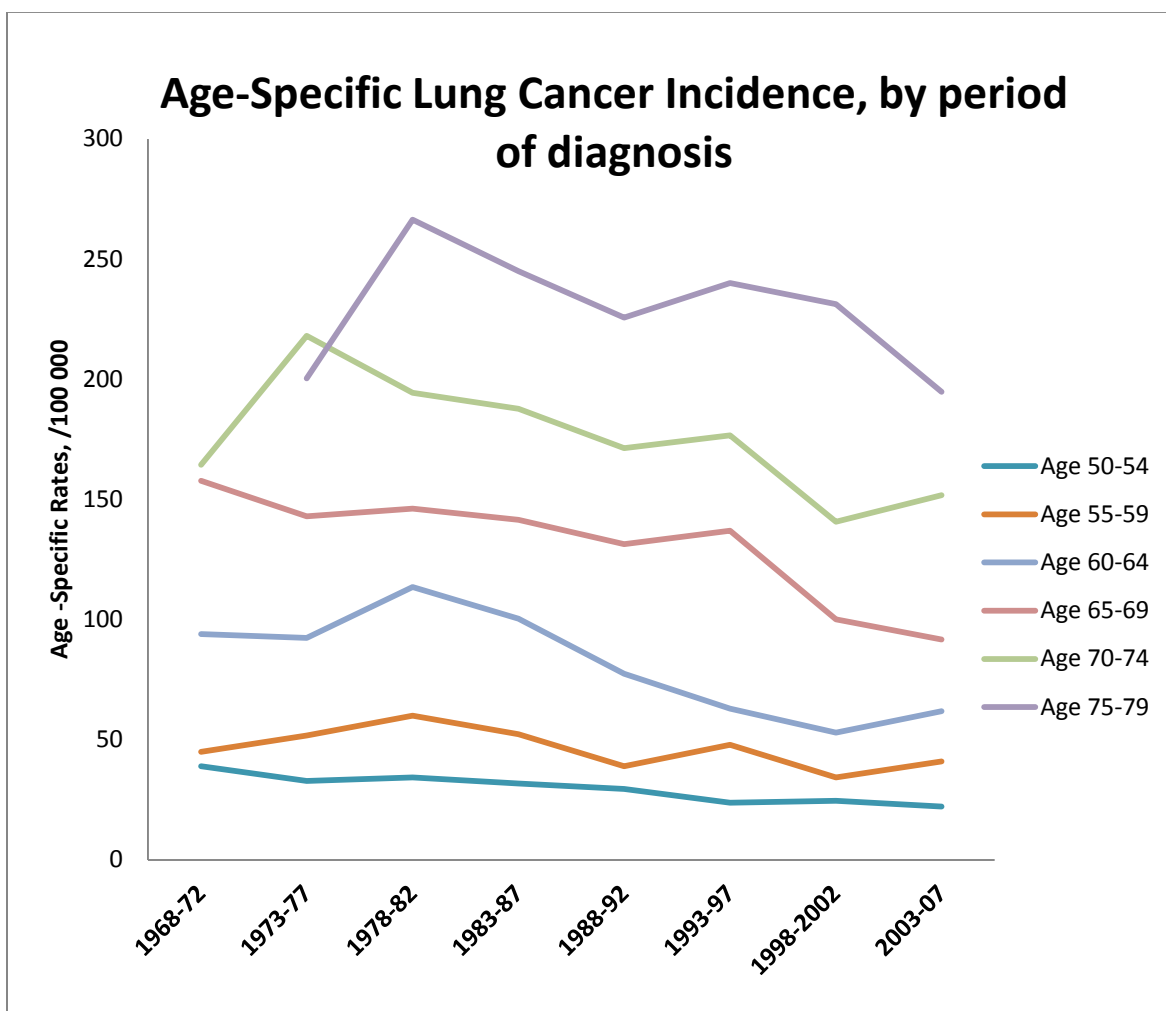
Figure 3.5 Age-specific lung cancer incidence by birth cohort in Singaporean Chinese women

Age-Specific Lung Cancer Incidence, by birth cohort
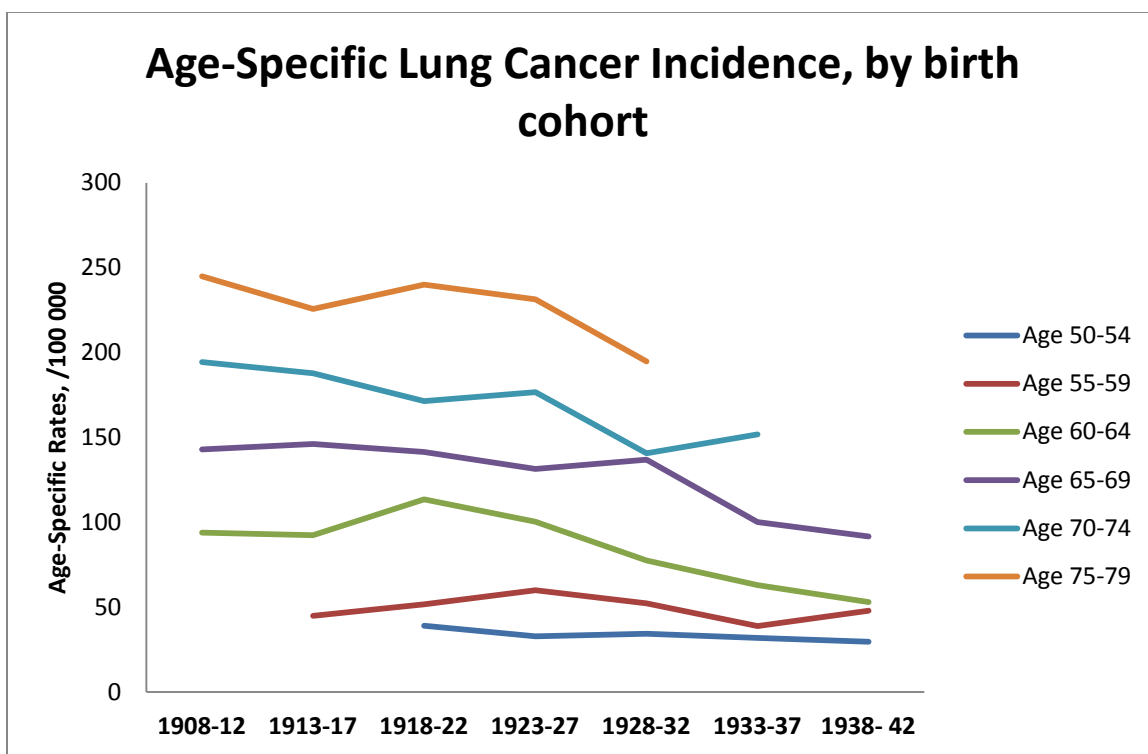
Table 3.3 Age-Period-Cohort modeling of the lung cancer incidence in Singaporean Chinese women from 1968-2007

Model	Deviance statistic	Degrees of freedom	*P*	AIC
Age	343.2	70	<0.001	824.3
Age and period	165.5	69	<0.001	718.1
Age and cohort	61.3	54	0.23	572.5

Table 3.4 Incidence rate ratio estimates of lung cancer for age-groups and cohorts of Singaporean Chinese females from APC model of age and cohort

Age- group	Incidence rate ratios (95%confidence intervals)	Birth Cohort	Incidence rate ratios (95%confidence intervals)
30-34	0.06 (0.05-0.09)	1898-1902	1.17 (0.98-1.40)
35-39	0.13 (0.10-0.16)	1903-1907	**1.54 (1.35-1.75)**
40-44	0.28 (0.23-0.33)	1908-1912	**1.38 (1.24-1.55)**
45-49	0.54 (0.47-0.62)	1913-1917	**1.31 (1.18-1.44)**
50-54	1.0	1918-1922	**1.36 (1.23-1.50)**
55-59	1.52 (1.36-1.71)	1923-1927	**1.30 (1.18-1.44)**
60-64	2.48 (2.22-2.77)	1928-1932	1.14 (1.04-1.26)
65-69	3.74 (3.35-4.17)	1933-1937	1.0
70-74	4.86 (4.35-5.43)	1938-1942	0.90 (0.81-1.05)
75-79	6.17 (5.50-6.92)	1943-1947	**0.84 (0.74-0.96)**
		1948-1952	0.92 (0.80-1.05)
		1953-1957	0.85 (0.73-1.00)
		1958-1962	0.85 (0.69-1.04)
		1963-1967	0.81 (0.60-1.08)
		1968-1972	0.90 (0.59-1.37)

Assuming that the induction period between exposure and cancer incidence is on average about 20-25 years (the induction period for cigarette smoke on lung cancer is believed to be at least 20 years and likely longer; this period will vary for other exposures), and since lung cancer incidence increases sharply after the age of 50, the relevant age of exposure to factors conferring lung cancer risks in these women is in their 30s to 40s. If these assumptions are true, then the sources of exposure that conferred a higher risk of lung cancer for Chinese women would have been common in the post-World War Two years, and then became less common, with the critical period during which the prevalence of these exposures shifted being the 1970s and 1980s.

This in turn suggests that environmental factors that might have changed substantially in the 1970s and 1980s should be investigated as possible risk factors for lung cancer in Chinese women. It is possible that changes in smoking practice may explain some of this cohort effect. The national survey conducted in Singapore in 1984 suggests that smoking was more prevalent among older women than younger ones (82), with a prevalence of about 8-9% among those aged 50-59, and 10-12% in women above the age of 60. Although these data were of all Singaporean women, Chinese women would have represented about three-quarters of the women studied and the results therefore likely reflected the prevalence in Chinese women. In contrast, in 2010, the smoking prevalence rates in Chinese women 50 to 59 years and older than 60 years were both 1.4% (88). These data offer some indirect evidence that smoking practice may have been more common among women entering adulthood in the post-war period, with a lower prevalence of smoking in women entering adulthood in the 70s and 80s, thus accounting for the lower cancer rates in Chinese women that have been observed.

However, smoking alone probably does not explain all of the effect seen. The available smoking prevalence data, although incomplete, suggest that while smoking prevalence might have been higher in Chinese women entering adulthood before or during World War compared to women entering adulthood in the post-war period, these prevalence rates (of about 9-12%) were still substantially lower (on the order of three-to-four fold lower) than those seen in men, and would not fully explain the relatively high lung cancer incidence unless we assume that women have a particularly high susceptibility to the carcinogenic effects of cigarettes. Further, Seow *et al* (89) reported that even as early as the 70s, only about half of Chinese female patients with lung cancers in Singapore had smoked. Our data from the GEL studies show that only 60% of the women with lung cancer that we recruited had smoked (see Chapter 4). These data suggest that factors other than smoking have a high attributable risk in this group of women. Finally, the late 60s to early 80s were a period of great social change in Singapore, marked by strong economic development and major lifestyle changes for the population as Singapore underwent industrialization and modernization. It is possible therefore that other environmental factors whose prevalence changed during the socio-economic transition in Singapore could account for some of the cohort effect observed.

The population of Singapore experienced major changes in living conditions in the late 1960s and 70s. The incidence rates of infectious lung diseases such as tuberculosis and pneumonia declined as Singapore became more developed (95). Cooking practices including change in stove type and fuel type also altered substantially in the 70s and 80s. Cooking practices prior to the 1960s, where a larger proportion of people lived in cramped crowded urban conditions or in kampongs, may differ substantially from cooking practices of the 70s and 80s, when Singaporeans were moved into public high-rise housing with better ventilation and modern fuel systems (96). Dietary practices

would have changed as well, as people started eating proportionately more meat and less vegetable as they became more affluent (97). Reproductive factors could also be investigated, since the economic transitions of the 70s and 80s in Singapore were also accompanied by very marked changes in reproductive behavior among Singaporeans – including delay in child-birth, decline in parity, and earlier menarche (98) (although the decline in lung cancer rates is seen starting from birth cohorts who entered their reproductive years prior to the 1970s and 1980s and who would not have experienced some of the changes in reproductive behavior seen during this period such as declines in parity.)

Genetic factors are not likely to explain the cohort effects seen, since the time frame is too short for changes in the prevalence of genetic risk factors to account for changes in lung cancer rates. However, it is possible that changes in the environment could have affected the phenotypic expression of specific genetic risk factors. A genetic factor that increases risk only in the presence of a specific environmental exposure may confer risk over and above the effect of the exposure alone. However, in the absence of the environmental exposure, the genetic factor may not increase risk. This form of gene-environment interaction will amplify the effects of "environmental" exposures.

Summary

Lung cancer is the most common cancer in men and the third most in women. There is strong variation in lung cancer incidence rates in different ethnic groups, and these cannot be explained by smoking alone. In particular, the rates in Chinese women appear disproportionately high despite low smoking prevalence. A detailed examination of lung cancer incidence in Chinese women suggests that both age and cohort effects are important explanatory variables. Older birth cohorts appear to be at higher risk of lung

cancers compared to the cohort born in 1933-1937, although younger cohorts did not appear to show further decreases in risk. Cohorts entering adulthood after World War Two, and who entered their 40s after the 1970s and 1980s were at lower risk of lung cancer compared to cohorts who came of age earlier. This suggests that relevant risk factors that should be investigated for this group of women include environmental factors in which the prevalence changed substantially during the 70s and 80s. While changes in smoking behavior may partly explain the cohort effects seen, the relatively low overall smoking prevalence in Chinese women, the high proportion of never-smokers among Chinese female lung cancer cases, and the great social and economic transitions that the population of Singapore underwent in the 70s and 80s suggest that it would be prudent to investigate other possible risk factors for lung cancer in this group of women.

The Genes and Environment in Lung Cancer (GEL) studies were designed and implemented in Singapore in order to study risk factors for lung cancer in Chinese women, focusing on potentially relevant factors such as diet, exposure to inhalants in the domestic environment, reproductive history, history of chronic inflammatory and atopic conditions of the respiratory system, and genetic factors such as polymorphisms in the somatic cell-line. These studies will be discussed in greater detail in the next chapter.

CHAPTER 4 THE GENE AND ENVIRONMENT IN LUNG CANCER (GEL) STUDIES: DESIGN AND METHODS

Data from 2 case-control studies (Gene and Environment in Lung cancer studies [GEL]) conducted in Singapore were used for this thesis.

The Genes and Environment in Lung Cancer (GEL) studies

The Genes and Environment in Lung Cancer (GEL) studies are two hospital-based case-control studies conducted in 1996-1998 and 2005-2008 from the five major public sector hospitals in Singapore. These studies were designed to investigate possible risk factors associated with lung cancer in Chinese women, a population group marked by low smoking prevalence and relatively high lung cancer rates, suggesting susceptibility to never-smoker lung cancer. These factors include previous history of inflammatory disease, anti-inflammatory drug use, exposure to inhalants other than smoking or ETS in the domestic environment, meat and meat constituents, and reproductive factors. The study aimed to interrogate two pathways: the inflammatory pathway and associated inflammatory gene polymorphisms, and the estrogen pathway and associated estrogen pathway gene polymorphisms.

Both studies used similar study designs and questionnaires. Eligible cases were Chinese females with incident primary carcinoma of the lung (all histological types) identified within 3 months of diagnosis. 787 eligible lung cancer patients were identified in the five hospitals, of whom 702 (89.2%) agreed to participate. The response rate for cases was 95.0% in the first study, and 84.6% in the second. Histological or cytological reports were reviewed and confirmed the diagnosis of primary lung carcinoma in 673 cases; 29 cases were confirmed on the basis of radiological investigations, in which

metastatic cancer to the lung from other sites was deemed to be unlikely on clinical grounds.

Controls were selected from Chinese female patients admitted to the same hospitals and frequency matched for age (within ten years) and date of admission. Patients admitted for a diagnosis and treatment of cancer or chronic respiratory disease were excluded, and no more than 10% of controls were recruited within a single diagnostic category.

The response rate among controls was 91% (96.9% in the 1st study and 85.4% in the 2nd), and data from a total of 1578 controls were available for analysis. Control patients were admitted for a wide range of conditions: 27% had diseases of skin, bones, joints and connective tissue, 11% were admitted for gastrointestinal or hepatobiliary system complaints, 14% were admitted for acute trauma, 8% were admitted for neurological or psychiatric conditions, 12% had diseases of the cardiovascular system.

Both cases and controls gave written, informed consent for the interview and the tracing of their medical records. As the studies aimed to investigate genetic susceptibility, and the possible interaction of these genetic risk loci with environmental factors, blood samples were also obtained where consent was given. The study was approved by the Institutional Review Board of the National University of Singapore and participating healthcare institutions. In total, 702 cases and 1578 controls were recruited in the 2 studies. 433 cases (61.7%) and 1375 controls (87.1%) in the study were never-smokers, defined as individuals who had not smoke at least 1 cigarette a day for a year. Of these, 298 cases and 718 controls provided blood samples, and 88 cases and 168 controls provided saliva samples.

There were minimal differences between cases and controls that provided blood samples and those who did not (data not shown). The exception to this was in Environmental Tobacco Smoke (ETS) exposure amongst controls: 52% of those who provided blood specimens reported ETS exposure compared to 44% in the study population as a whole ($P<0.05$).

A structured questionnaire was administered in-person by trained interviewers. Interviewers were not blinded to case or control status, but possible observer bias was monitored by recording and reviewing at random a sample of interviews conducted. The structured questionnaire elicited information of participants' demographic characteristics, occupational history, smoking history, family history of cancer, personal medical history (self-reported history of tuberculosis, chronic productive cough, asthma, allergic rhinitis and atopic eczema), diet (including intake of fruit and vegetable), childhood living conditions, reproductive history, exogenous hormone use and use of other medications, and indoor environmental exposures such as passive tobacco exposure, cooking, stove and oil use, and exposure to kitchen fumes and inhalants such as incense and mosquito coils. The questionnaire used is enclosed as Appendix A.

Genomic DNA was extracted from the buffy coat of 5 ml of whole blood samples using the FlexiGene DNA kit (Qiagen Inc.), in accordance with the manufacturer's protocol. Saliva DNA was extracted from 2 ml of saliva with the Oragene DNA Self-Collection Kit (DNA Genotek Inc., Ontario, Canada) and DNA concentration measured with Quantifiler Human DNA Quantification kit (Applied Biosystems, Life Technologies Inc, California, USA).

In general, the selection of sequence polymorphisms for genotyping was based on (a) their location in the promoter, untranslated region (UTR), or exons of the gene, or

published evidence showing possible effects of the polymorphism on the level and activity of the gene products; (b) a minor allele frequency $\geq$ 5% in Chinese population from the NCBI database; (c) associations with lung cancer reported previously by other researchers.

All Single Nucleotide Polymorphisms (SNP) were genotyped using a high-throughput genotyping platform base on a 5' nuclease allelic discrimination assay in a 96-well format on the ABI StepPlusOne real-time Polymerase Chain Reaction (PCR) System (Applied Biosystems). The Taqman universal PCR master mix and predesigned SNP genotyping assay mix containing PCR primers and probes were purchased from ABI. To ensure the accuracy of genotyping results, three positive controls and two negative controls were included in each 96-well plate, and 10% of DNA samples were genotyped in duplicate for each polymorphism.

<u>Statistical analysis</u>

Based on biologic considerations, as well as results from reverse stepwise analyses, the following variables were used for adjustment: age at diagnosis, country of origin, housing type, number of years in school, environmental tobacco exposure at home, history of cancer in a first degree relative, mean intake of fruit and of vegetable (in servings/week), and smoking status (current, ex-smokers, defined as smokers who have not smoked in the last 30 days, and never-smokers) as well as a study set variable (in analyses where data from both studies were combined) to indicate which case-control study the participant belonged to. Among smokers, smoking duration (in years), the logarithm of the average number of cigarettes smoked per day, and the time (in years) an individual stopped smoking prior to diagnosis (set at 0 for current smokers) were independent risk factors (not shown). Logistic regression models using combinations of these three

variables showed that smoking duration alone adequately explained the variation in risk attributable to smoking, and this was added as an adjustment variable for smokers.

Unconditional logistic regression was used, and odds ratios (ORs) and 95% confidence intervals (CIs) calculated. Tests for linear trend were performed by entering the factor of interest into the logistic regression model as an ordinal variable, where each successively higher category in that factor was assigned a higher numeric value in the variable. The likelihood ratio test was used to test for multiplicative interaction between two variables of interest by constructing a multiplicative interaction term of the 2 variables of interest, and then comparing two regression models, one without interaction term (null model), and the other with the interaction term (alternative model). No adjustments for multiple comparisons were made for tests of main effects of variables of interest. Adjustment for multiple comparisons was made for all tests of statistical interactions using the method of Benjamini and Hochberg (99), in which the P value is adjusted by taking into account the False Discovery Rate (ie the number of null hypotheses erroneously rejected) based on the number of statistical comparisons made. The χ^2 test was used to test for Hardy-Weinberg equilibrium by looking for a statistically-significant difference between the expected and observed values of the genotype counts. All P values calculated are two-tailed, and significance was set at $P< 0.05$. We used STATA statistical software, version SE 10.1 (StataCorp LP, Texas, USA) and R statistical package version 2.13.0 (R Development Core Team, Austria, Vienna) for data analyses. All SNPs studied were in Hardy-Weinberg equilibrium in the control population.

Table 4.1 summarises key baseline variables between cases and controls in the two case control studies. Cases were significantly younger and also consumed less fruit and

vegetables. They were more likely to have been born in China, live in private housing, smoke, report daily environmental tobacco exposure, and have a positive family history of lung cancer in a first-degree relative. Among smokers, cases reported longer smoking duration. Half of the cases (51.7%) had lung adenocarcinomas, and the proportion of adenocarcinomas was higher among never-smokers (62.6%) than smokers (34.2%). The proportion of adenocarcinomas also appears slightly higher than the proportions reported by the Singapore Cancer Registry (43% adenocarcinomas, 18% squamous cell carcinomas, 10% small cell carcinomas) (77). This may reflect the higher proportion of adenocarcinomas in women, especially amongst never-smokers.

Mitigating limitations of the case-control design

This section discusses the general limitations of the case-control design and the steps that have been taken to mitigate these limitations. Issues about the effect of the design and conduct of this study on the interpretation of specific research findings are discussed in the relevant sections in subsequent chapters.

Study design issues associated with case-control studies are well-known (100, 101). These include problems of information bias, reverse causation, selection bias, and random misclassification due to problems with recall.

Because outcomes are known prior to obtaining information about exposure, it is possible that systematic misclassification (information obtained from cases differ systematically from that obtained from controls that do not reflect true differences in exposure) can occur. This could be due to interviewer bias or recall bias among participants. Reverse causation can result when the disease process give rise to differences in the reporting of exposure. This can occur even though participants are

asked to recall exposures in the past if the development of the condition spans over a long period of time. Selection bias can occur when cases and controls are not chosen from the same underlying sample population. For example, this may happen when controls could not have become cases if they had developed the disease because they would have attended a different hospital and not have come to the attention of researchers. Hospital-based studies can run a higher risk of selection bias, as hospital controls may differ systematically from the population from which they are drawn. (The effect of this potential selection bias on the interpretation of findings with regard to Non-Steroidal Anti-Inflammatory Drug Use is discussed in Chapter 7.) Random misclassification can also occur as a result of inaccuracy of recall of events in the distant past by both cases and controls, resulting in biases of odds ratios estimates towards the null. The GEL studies tried to minimize these design limitations in a few ways. These are summarized in Table 4.2. The hospital-based design was felt to be preferable to a population-based design for two reasons: it was not possible to obtain a population list from which to sample, and it would be difficult to obtain high response rates from a population-based study. To minimize the risk of selection bias, controls were chosen from a wide variety of hospital departments and diagnoses groups, thereby minimizing the bias that may result from any unusual characteristics of a particular group of patients. Each specific admission diagnosis represented less than 5% of all controls. Controls were chosen from the same hospitals as the cases. This increased the likelihood that cases and controls were being drawn from the same study population, since it is likely that the controls would have attended the same hospitals if they had developed the outcome of interest (ie lung cancer). For exposures that are known *a priori* to associate with a specific group of diagnoses, controls with that group of diagnoses were excluded from analyses, in accordance with the proposal

Table 4.1 Baseline characteristics of female Chinese lung cancer patients and controls, Singapore, 1996-1998, 2005-2008

	All			Never-smokers			Ever-smokers		
	Cases (n=702)	Controls (n=1578)	*P*	Cases (n=433)	Controls (n=1375)	*P*	Cases (n=269)	Controls (n=203)	*P*
Age in yrs			**<0.001**			0.42			**0.001**
mean +/-SD	65.9+/-11.8	64.1+/-12.3		63.0+/-12.5	63.6+/-12.2		70.5+/-8.9	67.3+/-12.6	
Average weekly fruit consumption (servings)			**<0.001**			**<0.001**			0.11
mean +/-SD	6.8+/-8.5	9.0+/-8.6		7.5+/-7.1	9.3+/-8.5		5.7+/-10.2	7.2+/-8.8	
Average weekly vegetable consumption (servings)			**<0.001**			**<0.001**			0.08
mean +/-SD	21.4+/-19.3	25.6+/-21.3		22.3+/-19.4	25.9+/-21.3		20.0+/-19.0	23.3+/121.5	
Years of education			0.06			**0.02**			**0.02**
mean +/-SD	3.6+/-4.5	4.0+/-4.4		4.8+/-4.9	4.2+/-4.5		1.7+/-2.9	2.4+/-3.7	

	n	%	n	%	*P*	n	%	n	%	*P*	n	%	n	%	*P*
Dialect Group					0.03					0.008					0.01
Hokkien	290	41.7	673	43.5		151	35.2	577	42.8		139	52.1	96	48.5	
Teochew	157	22.6	307	19.9		109	25.4	265	19.7		48	18.0	42	21.2	
Cantonese	151	21.7	286	18.5		85	19.8	252	18.7		66	24.7	34	17.2	
Hainanese	40	5.8	89	5.8		38	8.9	80	5.9		2	0.8	9	4.6	
Hakka	38	5.5	138	8.9		31	7.2	125	9.3		7	2.6	13	6.6	
Others	20	2.9	53	3.4		15	3.5	49	3.6		5	1.9	4	2.0	
Country of Birth					0.001					0.012					0.59
Singapore	442	63.0	1031	65.3		274	63.3	900	65.5		168	62.5	131	64.5	
Malaysia	92	13.1	271	17.2		57	13.2	239	17.4		35	13.0	32	15.8	
China	145	20.7	234	14.8		85	19.6	198	14.4		60	22.3	36	17.7	
Other	23	3.3	42	2.7		17	3.9	38	2.8		6	2.2	4	2.0	
Current housing					0.019					0.001					0.17
1-3 room public	254	36.4	613	39.0		145	33.7	517	37.8		109	40.8	96	47.5	

flat															
4 room or larger public flat	332	47.6	775	49.3		202	47.0	682	49.8		130	48.7	93	46.0	
Private apt or house	111	15.9	183	11.7		83	19.3	170	12.4		28	10.5	13	6.4	
Marital status					**0.03**					0.81					0.57
Currently or previously married	654	93.2	1467	93.0		403	93.1	1275	92.7		251	93.3	192	94.6	
Never married	48	6.8	111	7.0		30	6.9	100	7.3		18	6.7	11	5.4	
Ever worked outside the home					**0.017**					0.56					**0.01**
Yes	513	73.1	1224	77.7		330	76.2	1065	77.6		183	68.0	159	78.3	
No	189	26.9	352	22.3		103	23.8	308	22.4		86	32.0	44	21.7	
Smoking status					**<0.001**										0.45
Never smoker[a]	433	61.7	1375	87.1											
Ex-smoker[b]	143	20.4	115	7.3							143	53.2	115	56.7	
Current smoker	126	18.0	88	5.6							126	46.8	88	43.3	
Smoking duration (years)[c]															**<0.001**
Mean +/- SD											43.7+/-17.8		33.4+/-20.3		
Average no of sticks smoked															0.06
Mean +/- SD											11.5+/-11.3		9.6+/-10.9		
Years since stopped smoking[d]															**<0.001**
Mean +/- SD											7.0+/-12.8		12.8+/-17.8		
Environmental Tobacco Smoke exposure at home					**0.008**					0.30					0.75
< Daily	334	48.1	848	54.1		226	53.1	764	55.9		108	40.2	84	41.6	
Every day	361	51.9	720	45.9		200	46.9	602	44.1		161	59.9	118	58.4	
Family history of cancer[e]					**0.003**					**<0.001**					0.45
No	531	75.6	1286	81.5		309	71.4	1112	80.9		222	82.5	174	85.7	
Yes, other sites	126	18.0	229	14.5		88	20.3	208	15.1		38	14.1	21	10.3	
Yes, lung cancer	45	6.4	63	4.0		36	8.3	55	4.0		9	3.4	8	3.9	
Histologic type															

Adenocarcinomas[f]	363	51.7				271	62.6				92	34.2			
Squamous cell carcinomas[g]	84	12				25	5.8				59	21.9			
Small cell carcinomas[h]	38	5.4				5	1.2				33	12.3			
Other histology[i]	188	26.8				115	26.6				73	27.1			
No histology/cytology	29	4.1				17	3.9				12	4.5			

Abbreviation: SD, standard deviation.

[a] Defined as have never smoked at least 1 cigarette a day for a year

[b] Have not smoked any cigarette in the 30 days prior to admission

[c] Defined as the difference between the age at which the participant stopped smoking (taken as age at diagnosis for participants who are currently smoking), and that at which she started.

[d] Defined as the difference between age at diagnosis and age at which participant stopped smoking; set as 0 for current smokers.

[e] First degree relatives

[f] Histology codes (ICD-O-3) 8140/3 – adenocarcinoma NOS, 8260/3 – papillary adenocarcinoma, NOS, 8480/3 – mucinous adenocarcinomas

[g] Histology code (ICD-O-3) 8070/3 squamous cell carcinoma, NOS

[h] Histology code (ICD O-3) 8041/3 small cell carcinoma, NOS

[i] Other histology codes, the most common being- (ICD-O-3) 8012/3 large cell carcinoma, NOS, 8046/3 non-small cell carcinoma, NOS, 8250/3 bronchio-alveolar carcinoma

by Wacholder et al. (102). As Wacholder noted, controls with a previous history of a disease associated with the exposure need not be excluded unless cases were also similarly excluded. Failure to also exclude cases introduces a selection bias. In this study, we have not excluded cases or controls with a previous history of a disease associated with an exposure of interest, since our aim in participant selection was not in obtaining cases and controls that were "healthy", simply that the exposure distribution in controls should be similar to that in the source population (See Chapter 7 for a discussion of this issue in relation to the study on NSAID use and lung cancer risk). In this study, high response rates above 85% were obtained for both cases and controls, suggesting that response bias is unlikely. Controls were also frequency-matched for age, thus minimizing selection bias in exposure levels that could occur due to age differences. We further restricted both cases and controls to Chinese females. This homogeneity in gender and ethnicity increases the power of the study and reduces the risk of population stratification (a type of selection bias) arising from differences in ethnicity. This also permitted exploration of risk factors for lung cancer in a population group with relatively high rates of lung cancer despite consistently low prevalence rates of smoking.

To reduce information bias, research nurses who performed interviews were trained, and a structured questionnaire was used to standardize the way in which information was collected. Nurses interviewed both cases and controls, and proxy interviews were not used. Interviews were recorded, and a small proportion (about 10%) of these recordings was played back to check on that the interviews were performed appropriately and completely, and that both cases and controls were interviewed in the same way.

Table 4.2 Limitations of hospital-based case-control study design, and mitigation of these limitations in the Genes and Environment in Lung Cancer studies

Limitations	Mitigation
Information bias	
<u>Recall bias</u> Cases report exposures differently from controls because knowledge of outcomes affect recall and reporting	• Participants not informed of specific hypotheses under investigation • Questions pertaining to exposures for these hypotheses embedded in an extensive questionnaire • No use of proxy interviews • Recall bias unlikely with genetic polymorphisms
<u>Interviewer bias</u> Interviewer knows the disease outcome status of their interviewees and probes cases and controls differently about exposures	• Research nurses were carefully trained • A structured questionnaire was used to standardize data collection • Interviews were recorded and a portion reviewed for quality checks
Selection bias	
Controls not picked from same study population as cases; controls could not become cases if they had developed the disease	• High response rates achieved • Controls, although hospital-based, were selected from a wide variety of departments and with different admitting diagnoses. Each diagnosis represented less than 5% of the total, number of controls • Controls picked from same hospitals as cases • Controls frequency-matched for age • Cases and controls restricted by gender and ethnicity (Chinese females) • Controls further restricted to exclude admission diagnoses that *a priori* are known to be associated with the exposure of interest
Reverse causation	
Disease causes changes in level of exposure	• Some exposures ascertained for the time point of 25 years prior to admission or diagnosis

	- Reverse causation not possible with genetic polymorphisms - Some exposures of interest (eg reproductive factors) occur either early in life-course or through life-cycle, and are not expected to be subject to reverse causation
Confounding	
Known risk factors for lung cancer may also be associated with the factors under investigation	- Data collected for these known risk factors, such as smoking, environmental tobacco smoke exposure, family history of lung cancer, and fruit and vegetable intake - Adjustment was performed in multivariate analyses to take these known risk factors into account

Participants were not informed about the specific hypotheses (especially regarding reproductive and inflammatory factors) of this study, other than that this was a study looking at risk factors for lung cancer. The questionnaire used was extensive, and questions about these groups of hypotheses were embedded within the questionnaire. The hypotheses are also not common knowledge among members of the public as they have not been widely discussed in the mass media. It is unlikely therefore that recall bias would have played a prominent role.

To minimize reverse causation, for some exposures such as cooking or fumes, we asked participants about exposure 25 years prior to admission or diagnoses, although this could increase random misclassification. Most reproductive and inflammatory factors are not likely to be subject to reverse causation since they occur early in the life course or throughout the life-cycle. We used biological samples to study polymorphisms in the somatic genome, and these are also not subject to reverse causation. Further advantages of studying genetic polymorphisms is that "Mendelian randomization" or random assortment of these polymorphisms reduces confounding, and reduces information bias in studies of gene-environment interaction since participants would not be aware of their genetic polymorphism status (103).

We collected a broad range of data, including factors that are known to be associated with lung cancer risk, such as smoking practice, fruit and vegetable consumption, and family history. This allowed us to include these variables for adjustment, thereby removing the effect of differential distribution of these variables between cases and controls.

CHAPTER 5   STUDY 1 - POLYMORPHISMS IN INFLAMMATORY PATHWAY GENES, HOST FACTORS AND LUNG CANCER

Introduction

Inflammation is implicated in the pathogenesis of other cancers such as hepatocellular carcinoma (104), and disruptions in inflammatory signaling due to chronic inflammation of altered immune response may be a common pathway in carcinogenesis (105). One of the etiologic mechanisms by which tobacco use confers risk in lung cancer may be through its effects on the immune response in the lung and the resultant excess of pro-inflammatory molecules in the lung tissue milieu (106). Previous studies implicate a past history of chronic inflammatory lung disease such as tuberculosis (107, 108), chronic bronchitis and emphysema (38, 39, 109), pulmonary fibrosis (110), and chronic rhinosinusitis (111), as risk factors for increased lung cancer risk in never-smokers. Asthma, allergic rhinitis and atopic dermatitis/eczema are related conditions that appear to be manifestations of an underlying systemic atopic disorder characterized by acute and chronic inflammation in target organs (lower respiratory tract, upper respiratory tract and skin, respectively). While asthma has been reported previously to be associated with an increase in lung cancer risk (112-114), the effect of atopy is uncertain, with reports of null or inverse associations (43, 44, 115). Other studies have identified genetic polymorphisms in key molecules in the inflammatory pathway, such as the interleukins (in particular interleukin 1-ß [IL1-ß) (116-121) and interleukin 6 [IL6]) (122, 123), the interleukin receptor antagonist (interleukin 1-ß receptor antagonist [IL1RN]) (124, 125), cyclo-oxygenase 2 (COX2) (126, 127), and Peroxisome Proliferator-Activated Receptor-γ (PPAR-γ) (128) as risk factors.

It is likely that the role of inflammation in lung cancer is mediated through interplay between host susceptibility (as reflected through polymorphisms in key inflammatory genes) and environmental exposures that either cause inflammatory insult to the lung, or confer protection in the lung from these insults. Therefore, a rational approach to identifying and quantifying lung cancer risks should consider both gene and environment factors in tandem. Such analyses may offer insights into the major biological pathways that drive carcinogenesis in lung cancer tissue. By identifying population groups at high risk of lung cancer, these findings may also have public health implications.

In Study 1, we hypothesise that previous inflammatory medical conditions (chronic lung disease, chronic cough and atopy) increase lung cancer risk in never-smokers, and predict that these risks would be modulated by polymorphisms in inflammatory genes that have been identified as risk factors in previous studies, in line with the concept of interplay between host genetic and acquired environmental factors.

Methods

For this analysis, data from never-smokers in both GEL studies were used. In total 433 cases (61.7%) and 1375 controls (87.1%) in the study were never-smokers, defined as individuals who had not smoke at least 1 cigarette a day for a year. Of these, 298 cases and 718 controls provided blood samples, for which genetic analyses could be performed.

Personal medical history of tuberculosis, asthma, allergic rhinitis, atopic eczema and prolonged chronic cough were obtained from the questionnaires. Six polymorphisms in 5 inflammatory genes [-31 *C/T* (rs 1143627) and -511 *C/T* (rs16944) in the IL1-ß gene, -634 *C/G* (rs1800796) in the IL6 gene, 8473 *C/T* (rs5275) in the COX2 gene, Pro[12]Ala in

exon 2 (rs1801282) of the PPAR- γ gene, and the 86 base-pair Variable Number of Tandem Repeats (VNTR) polymorphism in intron 2 of the IL-1ß receptor antagonist gene (IL-1RN)] were genotyped in 298 never-smoking cases and 718 never-smoking controls who provided blood samples. These genes were selected because they play essential roles within the inflammatory pathway, and have previously been reported to be associated with lung cancer (116-128).

The intron 2 VNTR in the IL1RN gene was determined as previously described (124): Primers (5'- CCCCTCAGCAACACTCC -3' and 5'- GGTCAGAAGGGCAGAGA -3') flanking the 86-bp tandem repeat region were used to amplify a DNA fragment containing the polymorphic region. PCR conditions comprised an initial denaturing step at 95°C for 5 minutes, followed by 35 cycles of 95°C for 30 seconds, 56 °C for 40 seconds, and 72°C for 45 seconds, with a final extension at 72 °C for 10 minutes. The PCR products were then analysed on 2% agarose gel electrophoresis along with a 100bp DNA marker. The wild-type allele designated allele I (IL1RN*1) (129) contains four 86-bp repeats and generated a 410-bp PCR product. The minor alleles were designated allele II to allele V (129) and corresponded to 240-bp (two repeats), 325-bp (three repeats), 500-bp (five repeats), and 959-bp (six repeats) PCR products. 10% of DNA samples were also genotyped in duplicate to ensure genotyping accuracy, and the concordance rate for the duplicates was 100%. The call rate for the VNTR analysis was 99.7%. The concordance rate for duplicate analyses of the other 5 SNPs that were genotyped was 100%, and call rates ranged from 99.8% to 100%.

Results

Table 5.1 summarises the effect of past history of lung disease or atopy on risk of lung cancer. Tuberculosis (OR 1.58, 95%CI 0.95-2.62) appeared to be associated with an increased risk of lung cancer, although this was not statistically significant. Asthma (OR 1.01, 95%CI 0.66-1.56), chronic cough (OR 1.73, 95%CI 0.65-4.60) and allergic rhinitis/atopic eczema (OR 0.93, 95% CI 0.69-1.26) were not associated with an increased risk of lung cancer in our study population. The composite measure of chronic cough, asthma, or allergic rhinitis/atopic eczema was also not associated with an increased risk of lung cancer (OR 0.97, 95%CI 0.74-1.27).

When stratified by -31T/C polymorphism genotype in IL-1β, a history of chronic cough, asthma or atopy was positively associated with lung cancer only among participants with the *T/T* genotype (OR 2.24, 95%CI 1.15-4.38) but not in participants with the *T/C* or *C/C* genotype (Table 5.2). The *P* for interaction, after adjustment for multiple testing, was 0.051. Because the polymorphism at the -511 position was in tight linkage disequilibrium with this polymorphism (R^2=0.97), similar results (not shown) were obtained for the SNP at the -511 position and a history of asthma, atopy or chronic cough.

When stratified by genotype at the VNTR polymorphism in the IL1RN gene, a history of chronic cough, asthma or atopy was positively associated with lung cancer in participants with the *2 allele (OR 5.09, 95% CI 1.39-18.67), but not in those with the *1 allele. The *P* for interaction, adjusting for multiple testing, was 0.058. Compared to having the *1/*1 genotype of the IL1RN gene and no history of chronic cough, asthma or

Table 5.1 Effect of past medical history of lung disease or atopy on risk of lung cancer in Singaporean Chinese women never-smokers

Past medical history		Cases (n=433)	Controls (n=1375)	OR[1] (95% CI)	*P* value
Tuberculosis	Yes	27 (6.2)	53 (3.8)	1.58 (0.95-2.62)	0.080
	No	406 (93.8)	1322 (96.2)	1.0	
Chronic productive cough[2]	Yes	7 (1.6)	12 (0.9)	1.73 (0.65-4.60)	0.27
	No	425 (98.4)	1361 (99.1)	1.0	
Asthma	Yes	34 (7.8)	97 (7.1)	1.01 (0.66-1.56)	0.96
	No	399 (92.2)	1278 (92.9)	1.0	
Allergic rhinitis/atopic eczema	Yes	79 (18.2)	244 (17.8)	0.93 (0.69-1.26)	0.64
	No	354 (81.8)	1131 (82.2)	1.0	
Asthma or allergic rhinitis/atopic eczema	Yes	101 (23.3)	311 (22.6)	0.93 (0.70-1.22)	0.59
	No	332 (76.7)	1064 (77.4)	1.0	
Chronic productive cough, asthma, or allergic rhinitis/atopic eczema	Yes	106 (24.5)	315 (22.9)	0.97 (0.74-1.27)	0.84
	No	327 (75.5)	1060 (77.1)	1.0	

[1] Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set

[2] Three (1 case and 2 controls) participants did not respond to this question

Table 5.2 Odds ratios and 95% Confidence Intervals for the interaction between IL1β and IL1RN genotypes and a history of chronic cough/asthma/allergic eczema/atopic rhinitis on risk of lung cancer in Singaporean Chinese women never-smokers

Polymorphism	History of chronic cough, asthma, or atopic eczema/ allergic rhinitis	Cases (n=298)	Controls (n=718)	OR[1] (95%CI)	P values	Stratified analyses, by genotype OR[1] (95%CI)	P values
IL1β -31T/C							
C/C	No	49 (16.4)	119 (16.6)	1.0		1.0	
	Yes	16 (5.4)	43 (6.0)	0.60 (0.29-1.23)	0.16	0.58 (0.27-1.27)	0.17
T/C	No	121 (40.6)	292 (40.8)	0.90 (0.59-1.38)	0.64	1.0	
	Yes	31 (10.4)	66 (9.2)	0.80 (0.44-1.43)	0.44	0.87 (0.51-1.49)	0.62
T/T	No	51 (17.1)	158 (22.1)	0.62 (0.38-1.01)	0.053	1.0	

	Yes	30 (10.1)	38 (5.3)	1.44 (0.76-2.72)	0.26	**2.24 (1.15-4.38)**	**0.018**
		LR test for interaction *P* value[2]= 0.051 (Unadjusted *P* = **0.011**)					
IL-1RN							
*1/*1	No	187 (63.6)	486 (68.5)	1.0		1.0	
	Yes	64 (21.8)	135 (19.0)	0.93 (0.64-1.36)	0.70	0.93 (0.63-1.35)	0.70
*1/*2 or *2/*2	No	31 (10.5)	78 (11.0)	1.00 (0.62-1.62)	0.98	1.0	
	Yes	12 (4.1)	11 (1.6)	**2.96 (1.23-7.12)**	**0.015**	**5.09 (1.39-18.67)**	**0.014**
		LR test for interaction *P* value[2]= 0.058 (Unadjusted *P* = **0.029**)					

[1] Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set

[2] Likelihood Ratio test for interaction p value adjusted for multiple testing using the method of Benjamini and Hochberg (1995)[45]

atopy, the presence of at least 1 *2 allele and a positive history of chronic cough, asthma or atopy, but not either factor alone, was positively associated with lung cancer (OR 2.96, 95% CI 1.23-7.12). There was no modification of the effect of chronic cough, asthma or atopy by the other 3 genotypes studied (data not presented).

Of the 6 sequence variations studied, the -634 *C/G* polymorphism in IL- 6 exhibited a main effect (Table 5.3). Using a co-dominant model, compared to the *C/C* genotype, the *C/G* genotype was positively associated with lung cancer (OR 1.51, 95% CI 1.11-2.05). The *G/G* genotype was not associated with lung cancer (OR 1.00, 95%CI 0.49-2.05), although there were relatively small numbers in this group. Using a dominant model, the presence of the G allele was positively associated with lung cancer (OR 1.44, 95%CI 1.07-1.94) compared to not having a G allele. This effect was not modulated by a history of tuberculosis, asthma, chronic cough or atopy.

Table 5.4 shows the additive effects of possessing 1 or more "risk" alleles at the 3 gene polymorphism sites for which an association with lung cancer was found in our study (the T allele at IL-1β -31T/C SNP site, the G allele at IL6-634C/G CNP site and *2 allele at IL1RN 86bp VNTR site). Compared to those without any alleles at these 3 sites, those persons having "risk" alleles at 1 site had an OR of 1.20, those with alleles at 2 sites, an OR of 1.57, and those with alleles at all 3 sites, an OR of 1.89. Although none of the individual ORs were statistically significant, the *P* value for trend was 0.026. The additive effect was seen only in those with a positive history of chronic cough, asthma or atopy (ORs 2.87, 6.76 for those with "risk" alleles at 1 or 2 and 3 sites respectively, *P* for trend 0.001) but not in those without (ORs 0.98, 1.14 for those with 'risk alleles at 1 and 2 or 3 sites respectively, *P* for trend 0.47), and this interaction was significant at *P*=0.035.

Table 5.3 Effect of polymorphisms in 6 inflammatory pathway genes on the risk of lung cancer in Singaporean Chinese women never-smokers

Inflammatory gene	Genotype	Cases (n=298)	Controls (n=718)	OR[1] (95%CI)	P value	OR(95%CI)[2]
IL1β	-31 T/C (rs 1143627)[3]					
	T/T	81 (27.2)	196 (27.4)	1.0		
	C/T	152 (51.0)	358 (50.0)	1.14 (0.80-1.61)	0.47	
	C/C	65 (21.8)	162 (22.6)	1.13 (0.74-1.71)	0.57	
	-511 C/T (rs16944)[3]					
	C/C	83 (27.9)	200 (27.9)	1.0		
	C/T	155 (52.0)	359 (50.1)	1.14 (0.81-1.60)	0.46	
	T/T	61 (20.1)	157 (21.9)	1.07 (0.70-1.63)	0.75	
IL6	-634 C/G (rs1800796)					
	C/C	163 (54.7)	449 (62.5)	1.0		1.0
	C/G	123 (41.3)	231 (32.2)	**1.51 (1.11-2.05)**	**0.008**	**1.44 (1.07-1.94)**
	G/G	12 (4.0)	38 (5.3)	1.00 (0.49-2.05)	0.99	*P* value= **0.015**
PPAR -γ	Pro[12]Ala (rs1801282)					
	C/C	274 (92.0)	653 (91.0)	1.0		
	C/G	23 (7.7)	64 (8.9)	1.04 (0.62-1.76)	0.87	
	G/G	1 (0.3)	1 (0.1)			
COX-2	-8973 T/C (rs5275)[3]					
	T/T	182 (61.3)	462 (64.4)	1.0		
	T/C	100 (33.7)	228 (31.8)	1.20 (0.88-1.64)	0.25	

	C/C	15 (5.0)	28 (3.9)	1.32 (0.66-2.64)	0.44
IL1-RN	86bp VNTR in intron 2[3,4]				
	*1/*1	251(85.4)	621 (87.5)	1.0	
	*1/*2	40 (13.6)	89 (12.5)	1.26 (0.83-1.92)	0.28
	*2/*2	3 (1.0)	0 (0)		

[1] Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set

[2] Adjusted odds ratios, comparing participants with a genotype of C/G or G/G at IL6 -634C/G versus those with a C/C genotype

[3] Excludes samples with no-calls - 2 samples for IL1β -31 T/C (rs 1143627) and -511 C/T (rs16944), 1 sample for COX2 -8973 T/C (rs5275) and 3 samples for 86bp VNTR in intron 2 in IL1RN

[4] Further excludes 7 participants with *1/*4 and 2 participants with *1/*5 genotypes

Note: IL1β -31 T/C and IL1β-511C/T are in linkage disequilibrium, R^2=0.97

Table 5.4 Additive effect of "risk" alleles at 3 gene polymorphism sites [IL1β-31TC (T allele), IL1RN 86bp VNTR (*2 allele), and IL6-634CG (G allele)] on lung cancer risk

Number of sites with at least 1 allele[1,2]	All			No history of chronic cough, asthma or allergic rhinitis/atopic eczema			History of chronic cough, asthma or allergic rhinitis/atopic eczema		
	Cases/ Controls	Odds Ratios (95% CI)[3]	*P* value	Cases/ controls	Odds Ratios (95%CI)	*P* value	Cases/ controls	Odds Ratios (95%CI)	*P* value
0	25/91	1.0		21/68	1.0		4/23	1.0	
1	143 /362	1.20 (0.71-2.01)	0.50	105/279	0.98 (0.55-1.75)	0.99	38/83	2.87 (0.71-11.53)	0.14
2	117/240	1.57 (0.93-2.67)	0.093	86/201	1.14 (0.64-2.05)	0.66	31/39	**6.76 (1.68-27.13)**	**0.007**
3	11/22	1.89 (0.75-4.75)	0.18	8/20			3/2		
		P for trend **0.026**			*P* for trend 0.47			*P* for trend **0.001**	
				P value for interaction **0.035**					

[1] Excludes 5 samples with no-calls in at least 1 of the 3 polymorphisms sites

[2] Counts were made for each individual based on the number of polymorphism sites in which there was at least 1 "risk" allele: [IL1β-31TC (T allele), IL1RN 86bp VNTR (*2 allele), and IL6-634CG (G allele)]

[3] Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set

Table 5.5 shows the Likelihood Ratio test *P* values for the interaction between the 5 SNPs with, separately, a history of tuberculosis, history of chronic cough, history of asthma, and history of atopic eczema/allergic rhinitis. As IL1β511C/T and IL1β31T/C are in strong linkage disequilibrium, only the results for IL1β31T/C are shown. The *P* values for interaction for history of asthma with IL1β31T/C and IL1RN, and for history of allergic rhinitis/atopic eczema with IL1RN are significant.

Table 5.6 shows the results for the joint effect of the composite variable of chronic cough, asthma and allergic rhinitis/atopic eczema and the remaining 3 SNPs (IL6-634C/G, PPAR Pro[12]Ala and COX2 8973T/C). No statistical interactions were observed for the composite variable of chronic cough, asthma and allergic rhinitis/atopic eczema.

Table 5.7 shows the odds ratios and 95% confidence intervals for the joint effect of the 2 SNPs of interest (ie IL1β31T/C and IL1RN) with, separately, a history of chronic cough, asthma and allergic rhinitis/atopic eczema. The joint effects for each of these 3 variables are similar for both polymorphisms, and support our use of a composite variable comprising these 3 variables of chronic cough, asthma and allergic rhinitis/atopic eczema.

Discussion

Our results suggest that among inflammatory conditions of the lung, a history of tuberculosis (but not asthma, allergic rhinitis and atopic eczema and chronic cough, individually or in combination) may be associated with an increased risk of lung cancer, although the odds ratios did not reach statistical significance. A positive association of chronic cough, asthma or atopy with lung cancer risk was evident in the presence of the T/T genotype in the IL1ß and the *2 allele in the IL1RN genes. We also demonstrated an

Table 5.5 Likelihood Ratio *P* values for interaction[1,2] between 5 genetic polymorphisms and history of tuberculosis, asthma, chronic cough, allergic rhinitis/atopic eczema

	Genetic polymorphisms				
Host factor	**IL1 31 TC**	**IL1 RN**	**IL6634CG**	**PPAR -γ Pro[12]Ala**	**COX28973T/C**
History of tuberculosis	0.79	0.64	0.38	No result[3]	0.51
History of asthma	**0.032**	**0.013**	0.62	No result[3]	0.26
History of chronic cough	0.99	0.77	0.32	No result[3]	0.62
History of allergic rhinitis/atopic eczema	0.081	**0.045**	0.49	0.37	0.47

[1] The Likelihood Ratio (LR) Test was used to test for interactions

[2] Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work, and study set

[3] No results were possible in these cases because of small numbers in one of the groups, resulting in some cells with null values during the logistic regression with the interaction term.

Table 5.6 Odds Ratios and 95% Confidence Intervals of the interaction between PPAR, COX and IL6 genotypes and history of chronic cough/asthma/allergic eczema/atopic rhinitis on risk of lung cancer in Singaporean Chinese women never-smokers

Polymorphism	History of chronic cough, asthma, or atopic eczema/ allergic rhinitis	Cases (n=298)	Controls (n=718)	OR[1] (95%CI)	P value	Stratified analyses, by genotype OR[1] (95%CI)	P value
IL6	**-634 C/G (rs1800796)**						
C/C	No	122 (40.9)	354 (49.3)	1.0		1.0	
	Yes	41 (13.8)	95 (13.2)	0.99 (0.62-1.56)	0.96	1.00 (0.62-1.61)	0.99
C/G or G/G	No	99 (33.2)	217 (30.2)	1.38 (0.99-1.93)	0.059	1.00	
	Yes	36 (12.1)	52 (7.2)	1.66 (0.99-2.78)	0.054	1.16 (0.68-2.00)	0.59
		LR test for interaction[2] *P*= 0.86 (unadjusted *P* = 0.58)					
COX2	**-8973 T/C (rs5275)**						
T/T	No	133 (44.8)	367 (51.1)	1.0		1.0	

	Yes	49 (16.5)	95 (13.2)	1.03 (0.67-1.60)	0.89	1.02 (0.65-1.60)	0.94
T/C or *C/C*	No	87 (29.3)	204 (28.4)	1.18 (0.84-1.66)	0.33	1.0	
	Yes	28 (9.4)	52 (7.2)	1.36 (0.80-2.33)	0.26	1.13 (0.64-2.01)	0.67
		LR test for interaction[2] *P*=0.86 (unadjusted *P* = 0.76)					
PPAR -γ	**Pro[12]Ala (rs1801282)**						
CC	No	203 (68.1)	517 (72.0)	1.0		1.0	
	Yes	71 (23.8)	136 (18.9)	1.06 (0.74-1.52)	0.76	1.05 (0.73-1.51)	0.78
C/G or *G/G*	No	18 (6.0)	54 (7.5)	1.02 (0.57-1.83)	0.94	1.00	
	Yes	6 (2.0)	11 (1.5)	1.22 (0.39-3.75)	0.73	0.86 (0.19-3.84)	0.84
		LR test for interaction[2] *P*=0.86 (Unadjusted *P* = 0.86)					

[1] Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work, and study set

[2] P value adjusted for multiple testing using the method of Benjamini and Hochberg (1995) [99].

Note: IL1β-511C/T polymorphism is in tight linkage disequilibrium with Il1β31T/C (R^2=0.97), and hence the interaction of this polymorphism with chronic cough, asthma or allergic rhinitis/atopic eczema was not performed separately

Table 5.7 Odds ratios and 95% Confidence Intervals for the joint effect of IL1β31T/C and Il1RN genotypes and history of chronic cough, history of asthma, and history of allergic rhinitis/atopic eczema

		IL1β31T/C									IL1RN					
		C/C			T/C			T/T			*1/*1			*1/*2 or *2/*2		
		Cases/ Controls	OR*	P	Cases/ Controls	OR*	P	Cases/ Controls	OR*	P	Cases/ Controls	OR*	P	Cases/ Controls	OR*	P
History of chronic cough	Yes	1/1	2.52 (0.14-46.63)	0.51	1/1	2.18 (0.12-40.92)	0.60	1/2	1.81 (0.16-20.72)	0.64	2/3	1.60 (0.24-10.50)	0.63	1/1	3.26 (0.20-53.88)	0.41
	No	63/161	1.11 (0.73-1.69)	0.62	151/356	1.15 (0.81-1.62)	0.45	80/194	1.0		249/617	1.0		41/88	1.22 (0.79-1.86)	0.37
History of asthma	Yes	12/12	1.00 (0.29-3.34)	0.99	9/26	0.79 (0.34-1.86)	0.59	5/13	**2.93 (1.18-7.29)**	**0.02**	20/49	0.83 (0.46-1.50)	0.53	6/2	**7.71 (1.46-40.64)**	**0.016**
	No	69/184	1.28 (0.83-1.99)	0.27	143/332	1.32 (0.91-1.90)	0.14	60/149	1.0		231/572	1.0		37/87	1.09 (0.70-1.70)	0.70
History of allergic	Yes	21/27	0.93 (0.43-1.98)	0.85	24/51	1.21 (0.65-2.24)	0.55	13/33	**2.05 (1.01-4.19)**	0.048	47/100	0.91 (0.59-1.38)	0.65	10/10	**2.90 (1.14-7.35)**	**0.025**

rhinitis/ atopic eczema	*No*	60/169	1.41 (0.88-2.26)	0.15	128/307	1.32 (0.89-1.94)	0.16	52/129	1.0		244/521	1.0		33/79	1.04 (0.65-1.66)	0.87

* Adjusted for age, history of cancer in 1st degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work, and study set

independent effect of IL6-634 C to G polymorphism in conferring risk. We found increasing odds ratios for lung cancer with increasing number of polymorphism sites where there was at least 1 "risk" allele in those with a history of chronic cough, asthma and atopy, but not in those without such a history. Taken collectively, these data support the hypothesis that inflammation plays a role in lung carcinogenesis among never-smokers.

In our study, the number of cases with a history of tuberculosis was relatively small, and the study was inadequately powered to detect a true association of this magnitude. Our finding of an increased, although non-significant, risk is consistent with other studies that have also reported increased risks associated with tuberculosis (130-132). Asthma was associated with a 1.8 fold increased odds of lung cancer in a meta-analysis of 5 case-control studies that studied the association in never-smokers (114), and other cohort studies support this finding (112, 113). On the other hand, studies of the relationship of systemic atopic conditions such as food allergies, allergic rhinitis or atopic eczema with lung cancer have mainly reported null or negative associations (43, 44, 115). Some authors (111) have proposed that local lung effects such as mucosal inflammation are the reason for the increased risk seen in asthma, rather than the shift of T lymphocyte response to a Th2-dominated activity in the hyperreactive state of the immune system, which is found also in systemic atopic conditions. If so, mild and infrequent asthmatic attacks and the use of local medications such as inhaled cortico-steroids may mitigate the risk association and may explain our null findings. Chronic cough has been identified as an independent risk factor for lung cancer (133, 134), especially among smokers. In our population of never-smokers, chronic productive cough are likely due to either undiagnosed asthma or to Chronic Obstructive Pulmonary Disease (COPD). Asthma is a

known risk factor for COPD in non-smokers (135), and eczema, allergic rhinitis and asthma are known to occur in the same patient in sequence, an effect known as the atopic march (136). We believe therefore that the cluster of symptoms or diagnoses of chronic cough, asthma, allergic rhinitis and atopic eczema are related and point to persons with an underlying atopic phenotype and a predisposition to chronic inflammation in the lungs.

We observed a main effect with the IL6-634 polymorphism in our study population. There is biological plausibility for the role of the IL6-634 G allele in increasing lung cancer risk. The -634 SNP is in the promoter region of the IL6 gene, and *in vitro* studies have indicated that the G allele is associated with an increased production and secretion of IL-6 by peripheral blood mononuclear cells (137). Our group had previously reported (122), using data from the first case-control study conducted between 1996-1998, that although a history of asthma or atopy and the G allele of IL6-634 were not associated with lung cancer on their own, the combined effect of the G allele and a history of asthma or atopy resulted in an odds ratio of 3.1 (95% confidence intervals 1.2 – 8.3) compared to the group with the *C/C* genotype and no history of asthma. We did not find any other studies investigating the IL6-634 SNP with lung cancer. Other groups have primarily investigated another SNP in the IL6 gene –IL6-174G/C (rs1800795), and most of these studies have reported null findings with this SNP (118-120, 138).

Our study implicates the C allele in IL1β511C/T and/or the T allele in at IL1β 31T/C and the *2 allele of IL1RN as alleles that confer risk to lung cancer in the presence of a background of atopy (allergic rhinitis or atopic eczema), chronic cough or asthma. The -31T/C polymorphism is a TATA-box polymorphism; the C allele disrupts this box and reduces binding and induction; hence suggesting that the T allele may be pro-

inflammatory (139). Zienolddiny *et al* previously reported an increased risk of lung cancer with the T allele at IL1β 31T/C (116), and Wu *et al* also reported that the T allele was associated with increased risk in a Chinese population (140), although other groups have reported null effects (118, 120), and results from one other study implicated, in contrast, the C allele of -31T/C as the risk allele (121). These studies were conducted in study populations of smokers or mixed populations with high proportions of smokers (ranging from 84-96%). Results for C3954T (rs1143634), the other SNP in the IL1β gene that has been commonly studied, have been similarly inconsistent, with reports both of an increased risk associated with the T allele (120, 141), and null effects (142). The inconsistencies in results could have been due to the different ethnic populations that were studied, as well as to the different distribution of relevant host factors such as exposure to environmental pollutants and pre-existing health conditions such as asthma.

The 86-bp VNTR polymorphism of the IL1RN gene contains potential regulatory protein binding sites (129), and likely has functional significance in the regulation of IL1-Ra production. In opposition to our findings, Hu *et al* reported reduced risks of lung cancer with the *2 allele in ethnic Chinese (124), but the study population in that report was predominantly (70%) male and smokers (60% of cases and 48% of controls), with relatively fewer adenocarcinomas among the lung cancer cases (38% of cancers). Further epidemiologic studies to delineate the main effect and possible interactions of IL1RN alleles are needed, as are functional studies to clearly describe the effect of the *2 allele in biological systems.

Our analysis of the summed effect of alleles at these 3 polymorphism sites suggests that there is an additive effect in lung cancer risk with increasing number of polymorphism sites where there was at least 1 allele present among those with a history of chronic

cough, asthma and atopy, but not among those without. Although this analysis was based on relatively small numbers of cases and controls with chronic cough, asthma and atopy, this finding, if replicated, would suggest that the effects of inflammatory gene polymorphisms are important only in the presence of relevant host factors such as previous medical history.

The gene-environment interactions observed in our study suggest that failure to take into account environmental and personal risk factors may explain the inconsistency of results obtained thus far with studies looking at the association of inflammation with lung cancer risk. Rothman & Greenland (100) conceptualised a causal pie where combinations of risk factors explain the occurrence of non-communicable diseases with multiple etiologies such as cancer. Most of these factors are neither necessary nor sufficient in themselves to cause illness, and it is the combination of factors that determine the risk to any individual. Some risk factors (for example smoking) may have such strong biological effects that, regardless of the underlying host genetic susceptibility or the presence of other risk factors, these factors invariably confer risk. Other factors may have weaker effects, and the risk associated with these factors may manifest only in hosts with underlying predisposition. Applying this concept to the role of inflammation in lung carcinogenesis, and in the light of our findings, the development of inflammatory pathway perturbations that result in lung carcinogenesis may depend on both the presence of 'environmental' risks that predispose to inflammatory pathway disruptions such as personal medical history, as well as on underlying host genetic susceptibility to such perturbations.

This study represents, to our knowledge, the first study of inflammatory genotypes and lung cancer in a large group of never-smokers. This feature has allowed us to

investigate weak associations in this subgroup, which may be overshadowed by smoking–related effects in other populations.

Previous studies of genetic polymorphisms have used study populations of smokers, or, when mixed populations where smokers comprised a heavy majority. Despite the inconsistencies in findings, the evidence overall appears to suggest that both a medical history of lung or inflammatory conditions and genetic variation in the inflammatory gene pathways are associated with lung cancer risk in smokers. This study adds to this body of knowledge by suggesting that similar associations are seen in never-smokers.

On the other hand, the retrospective nature of this study and the use of hospital controls may complicate the interpretation of the results. We excluded patients admitted for cancers or chronic respiratory conditions. Even if there were a selection bias in our study (with enrichment of persons with chronic diseases in the control group), the direction of this bias would have resulted in an underestimate of the true risk. We depended on participant reports of their medical history, and there may have been reporting bias with cases being more likely to report a positive medical history than controls. Because of the similarity of symptoms, some cases might have been misdiagnosed as asthma prior to the diagnosis of lung cancer being made. As we had not asked the age of onset of their pre-existing medical condition, we were not able to exclude reports of medical conditions of recent onset that could have been misdiagnosed lung cancer. However, we do not believe that this was a major source of bias because diagnostic chest imaging is readily available to family practitioners in Singapore, and this would have correctly identified lung cancer as the cause of their symptoms for most patients.

Further, these results, especially with regard to those showing gene-environment interactions, should be considered to be exploratory in nature. Only a subset of participants provided blood samples. Our analysis suggested that there were minimal differences between cases and controls that provided blood samples and those who did not (data not shown). The exception to this was in Environmental Tobacco Smoke (ETS) exposure amongst controls: 52% of those who provided blood specimens reported ETS exposure compared to 44% in the study population as a whole (P<0.05). ETS exposure has been linked to asthma, and the higher proportion of asthma among controls providing blood samples may have resulted in an attenuation of the actual effect of the composite variables of cough, asthma and atopy in analyses stratified by genotype. Mechanistic or biological effect is plausible for these interactions, but the Likelihood Ratio tests for interaction, although significant without adjustment for multiple testing at P<0.05, gave borderline significant p values after adjustment, and confirmation of these findings in other well-designed studies of lung cancer in never-smokers is needed.

In summary, these results suggest that acquired inflammatory medical conditions and inherited polymorphisms of genes in the inflammatory response pathway may interact to confer risk in lung carcinogenesis among never-smokers. The finding that the cluster of conditions of chronic cough, asthma and atopy was associated with lung cancer only in the presence of pro-inflammatory genotypes linked to the IL-1 cytokine emphasizes the need to consider host genetic susceptibility when investigating putative environmental or acquired risk factors in etiologic studies.

This study has been published (Lim WY, et al. Carcinogenesis 2011;32(4):522-9, attached as Appendix B to this thesis).

CHAPTER 6   STUDY 2 – NON-STEROIDAL ANTI-INFLAMMATORY DRUG (NSAID) USE AND LUNG CANCER

Introduction

Aspirin and non-aspirin Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) are widely-used analgesic and anti-inflammatory agents. In recent years, low-dose aspirin have also been prescribed as prophylaxis against ischaemic cardiac and neurological events. The biological effect of aspirin and NSAIDs appears to be mediated mainly through the inhibition of cyclooxygenase (COX) enzyme. COX converts arachidonic acid to prostaglandin G and H2, which are further converted to a variety of eicosanoids with biological activity (143). Activities of these eicosanoids include anti-apoptosis, promotion of angiogenesis, and stimulation of estrogen synthesis (144, 145). There are 2 isoforms of COX: COX-1, which is constitutively expressed, and COX-2, which is inducible (143). While aspirin and earlier NSAIDs are non-selective, a new class of COX-2 specific inhibitors such as celecoxib was developed and marketed in the early 2000s. Aspirin and other NSAIDs differ from each other both in terms of relative affinity for the 2 isoforms of COX, and in their mechanism of inhibition of COX. Notably, aspirin causes irreversible inhibition through covalent bonding of the COX molecule while most other NSAIDs exhibit competitive reversible inhibition (145).

In vitro and animal studies support a protective effect of aspirin and non-aspirin Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) on cancer risk. Initial studies focused on colorectal cancer, where increased cyclo-oxygenase-2 (COX-2) expression in colorectal tumour tissue was observed, in comparison with non-tumour tissue (146). In mice studies, COX-2 null $APC^{\Delta716}$ (a mouse model where truncation of the Adenomatous

Polyposis Coli gene induces intestinal polyposis) mice showed decreased intestinal polyp formation compared to COX-2 positive mice; polyp formation was reduced in COX-2 positive mice fed a novel COX-2 inhibitor (147). Epidemiologic evidence also supports the role of aspirin in protecting against colorectal cancer, with risk reduction estimates of 20-50% (148, 149).

There is growing evidence that aspirin and non-aspirin NSAIDs may also play a protective role in lung cancer. COX-2 enzymes are constitutively expressed in lung neoplastic tissue at higher levels than in non-tumour tissue (150). Animal studies show that these drugs protect against experimentally induced lung cancer (151). Clinical evidence in human patients also suggests that COX-2 expression is associated with poorer lung cancer survival (152). Further, experimental studies suggest that COX-2 regulate the activity and expression of Epithelial Growth Factor Receptor (EGFR), and is itself regulated by EGFR in complex ways that appear to depend on cell-type specificity and cell environment (66, 153). This interaction could be important in the light of recent findings that EGFR mutations are common in lung adenocarcinomas among non-smokers, women, and Asians (66). The role of inflammation (and the effect of anti-inflammatory agents such as NSAIDs) in lung carcinogenesis may therefore be subject to effect modification by factors such as ethnicity, gender and smoking status.

Epidemiologic studies of the role of NSAIDs and aspirin on lung cancer risk have been suggestive but not conclusive (154-179), with both protective and null effects being reported. Three meta-analyses have been performed on the available data, with contrasting results: one (158) reported a non-statistically significant reduced pooled OR for non-aspirin NSAID, but no effect for aspirin, a second (174) reported significant reductions in risk with regular use of aspirin and NSAIDS, but cautioned that the

association may not be causal, while the third (175) reported a borderline significant reduction in risk, with significant differences in results of pooled analysis of case-control studies (significant protective effect) compared to cohort studies (null effect).

In Study 2, we hypothesise that regular aspirin and non-aspirin NSAID use decreases lung cancer risk, and that smoking status and lung cancer tumour type modify this association.

Methods

Data for this analysis were taken from the second case-control study, comprising 399 cases and 815 controls. In accordance with Wacholder *et al*'s proposal (102), controls whose admitting diagnoses were linked to NSAID use (patients with either disease conditions whose management involve use of long-term NSAIDs, or whose admission were for conditions directly related to NSAID use) were excluded from analysis. Of the 815 controls, 85 were admitted for a diagnosis of chest pain, acute myocardial infarction, or heart failure, 11 were admitted for a stroke or a transient ischaemic attack, and 73 were admitted for management of musculoskeletal pain, including knee, hip and back pain. A further 5 were admitted for conditions directly related to NSAID use (2 persons with ulcers due to NSAID use, 1 person with migraine and rebound headache secondary to analgesia use, 1 person with non-ulcer dyspepsia secondary to NSAID use, and 1 person with nephropathy secondary to NSAID use). These were excluded for this analysis, leaving 641 controls for this analysis.

History of regular use (defined as use at least twice a week for a period of a month or more) of aspirin, non- aspirin NSAID and COX-2 selective NSAID, paracetamol, steroid

cream and steroid pills were obtained from the questionnaire. For each, the age at which use was started and the duration of use (in months) were also elicited.

Results

Comparing long-term aspirin users to non-users in cases and controls, aspirin users were older than non-users in both cases and controls, and also had fewer years of education than non-users. Among cases, aspirin users were less likely to report a positive family history of lung cancer. (Table 6.1)

Table 6.2 summarises the overall results of self-reported regular intake of 6 medication groups on lung cancer risk. Previous regular intake of aspirin was associated with a statistically significant lower risk of lung cancer in both never-smokers (OR 0.50, 95%CI 0.31-0.81) and smokers (OR 0.38, 95%CI 0.16-0.93 in smokers). No associations were seen with non-aspirin NSAID, paracetamol and COX-2 inhibitors, although the actual numbers reporting regular use were very small. No significant associations were seen with regular use of steroid pills or cream.

Table 6.3 summarises the association between onset of aspirin use and duration of use on lung cancer risk in non-smokers. The numbers of ever-smoker users in our study were too small for meaningful interpretation. The lowest risks of lung cancer occurred in non-smokers who started using aspirin between 1-5 years before admission/diagnosis, and whose duration of aspirin use was between 12 and 60 months (OR 0.35, 95%CI 0.14-1.85, and OR 0.37, 95% CI 0.16-0.85 respectively). Duration of use and onset of use appeared to be correlated (data not shown), so it was not possible to study the separate effects of duration or onset of use adjusting for the other variable.

Restricting cases to non-smokers with lung adenocarcinomas (n=165), an OR of 0.43, 95% CI 0.24-0.78 was obtained for the association between aspirin use and lung cancer. A pattern similar to that in Table 6.3 was observed when we investigated the onset of aspirin use and the duration of use in this subgroup, with lowest ORs achieved in persons starting use 1-5 years prior to admission, and in those who had used aspirin for 12-60 months. (Data not shown)

To assess the impact of controls with specific diagnoses on the estimation of the effect of aspirin use on lung cancer risk, we systematically excluded groups of controls based on their admission diagnoses (in broad groupings of accidents and injuries, orthopaedic conditions, gastrointestinal conditions, respiratory conditions, neurological conditions, cardiac conditions, renal conditions. endocrine conditions, infectious diseases, and others). No significant variation in the OR estimates were seen, with adjusted ORs in the range of 0.49-0.55 for never-smokers, and 0.28-0.39 for smokers (Table 6.4).

The inverse association of aspirin was present even when participants who used aspirin within 1 year of diagnosis of lung cancer (in cases), or admission to hospital (for controls) were excluded, with ORs and 95%CI for never-smokers and smokers of 0.44 (0.24-0.81) and 0.40 (0.15-1.04) respectively. Finally, exclusion of 32 cases of lung cancer diagnosed on radiological grounds did not materially affect the results observed, with ORs of 0.49 (95%CI 0.30-0.81) for never-smokers and 0.33 (95%CI 0.13-0.84) for smokers.

Table 6.1. Baseline Characteristics of long-term users and never-users of aspirin

	Cases			Controls		
	Use of aspirin (n=40)	No aspirin use (n=348)	*P*	Use of aspirin (n=129)	No aspirin use (n=505)	*P*
Age at diagnosis			<0.001			<0.001
mean +/-SD	73.33 +/- 9.45	65.93 +/- 11.63		63.87 +/- 12.09	69.45 +/-8.83	
Fruit consumption			0.74			0.42
mean +/-SD	7.68+/- 8.36	7.16 +/- 9.50		8.94 +/-7.51	9.66 +/-9.38	
Vegetable consumption			0.28			0.92
mean +/-SD	15.89 +/- 11.06	19.32 +/- 19.59		23.07 +/- 21.64	22.88 +/-19.98	
	n (%)	n (%)		n (%)	n (%)	
Current housing			0.28			0.34
1-3 room public flat	11 (28.2)	112 (32.6)		45 (35.2)	165 (32.9)	
4 room or larger public flat	24 (61.5)	169 (49.1)		71 (55.5)	265 (52.8)	
Private apt or house	4 (10.3)	63 (18.3)		12 (9.4)	72 (14.3)	
Years of Education			0.087			0.006
Nil	19 (47.5)	139 (40.1)		64 (49.6)	179 (35.5)	
≤6 years	15 (37.5)	98 (28.2)		40 (31.0)	171 (33.9)	
7 years or more	6 (15.0)	110 (31.7)		25 (19.4)	155 (30.7)	
Environmental Tobacco Smoke exposure at home			0.62			0.67
< daily	15 (38.5)	145 (42.7)		59 (46.1)	219 (44.0)	
Every day	24 (61.5)	195 (57.4)		69 (53.9)	279 (56.0)	
Family history of cancer[1]			0.009			0.674
No	36 (90.0)	223 (66.4)		99 (78.0)	370 (74.2)	
Yes, other sites	3 (7.5)	77 (22.9)		21 (16.5)	98 (19.6)	
Yes, lung cancer	1 (2.5)	36 (10.7)		7 (5.5)	31 (6.2)	

Abbreviation: SD, standard deviation.
[1] First degree relatives

Table 6.2 Association between use of a variety of medications and lung cancer in Chinese lung cancer patients and their controls

		Non-smokers N=820			Smokers N=220		
		Cases N=258	Controls N=562	OR (95%CI)[1]	Cases N=141	Controls N=79	OR (95%CI)[2]
Regular use of aspirin	No	227 (89.7)	443 (79.5)	1.0	122 (89.7)	63 (80.8)	1.0
	Yes	26 (10.3)	114 (20.5)	**0.50 (0.31-0.81)**	14 (10.3)	15 (19.2)	**0.38 (0.16-0.93)**
Regular use of non-aspirin NSAID	No	244 (96.4)	542 (97.3)	1.0	134 (97.8)	74 (96.1)	1.0
	Yes	9 (3.6)	15 (2.7)	1.72 (0.71-4.17)	3 (2.2)	3 (3.9)	0.57 (0.10-3.12)
Regular use of COX-2 inhibitor	No	252 (99.6)	553 (99.3)	1.0	137 (100)	77 (100)	-
	Yes	1 (0.4)	1 (0.7)	0.56 (0.06-5.24)	0	0	-
Regular use of panadol	No	243 (95.7)	531 (95.5)	1.0	128 (92.8)	75 (96.2)	1.0
	Yes	11 (4.3)	25 (4.5)	1.11 (0.53-2.35)	10 (7.2)	3 (3.8)	2.58 (0.62-10.78)
Regular use of steroid creams	No	246 (97.2)	537 (97.1)	1.0	133 (97.1)	77 (98.7)	1.0
	Yes	7 (2.8)	16 (2.9)	0.84 (0.33-2.13)	4 (2.9)	1 (1.3)	2.84 (0.29-28.27)
Regular use of steroid pills	No	245 (96.8)	540 (97.0)	1.0	135 (98.5)	75 (97.4)	1.0
	Yes	8 (3.2)	17 (3.0)	1.35 (0.54-3.38)	2 (1.5)	2 (2.6)	1.13 (0.14-9.15)

[1] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings), years of education, housing type, and history of cancer in 1st degree relative
[2] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings) years of education, housing type, history of cancer in 1st degree relative and duration (in years) of smoking
[3] Regular use refers to use of at least two times a week for at least a month or more

Table 6.3 Association between onset and length of aspirin use and lung cancer in Chinese female lung cancer patients and their controls, restricted to never-smokers

	Non-smokers		
	Cases N=258	Controls N=562	ORs (95%CI)[1]
Onset of aspirin use			
No use	227 (89.7)	443 (79.5)	1.0
Start using <1yr from admission	11 (4.4)	39 (7.0)	0.61 (0.30-1.23)
Start using 1-<3 yrs from admission	4 (1.6)	27 (4.9)	0.34 (0.11-1.00)
Start using 3-<5 years from admission	2 (0.8)	12 (2.1)	0.37 (0.08-1.74)
Start using 5 years and more from admission	9 (3.6)	36 (6.5)	0.54 (0.25-1.17)
Duration of aspirin use			
No use	227 (89.7)	443 (79.5)	1.0
Duration of use 12 month or less	11 (4.4)	40 (7.2)	0.61 (0.30-1.24)
Duration of use 12-<36 months	5 (2.0)	33 (5.9)	**0.33 (0.13-0.87)**
Duration of use 36-<60 months	2 (0.8)	10 (1.8)	0.51 (0.11-2.41)
Duration of use 60 months or more	8 (3.2)	31 (5.6)	0.54 (0.24-1.22)

[1] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings), years of education, housing type, and history of cancer in 1st degree relative

Table 6.4 Aspirin use and lung cancer risk – Sensitivity analyses

Model	Diagnoses group excluded (numbers excluded)	Never-smokers[1]		Smokers[2]	
		No of controls in model	OR (95%CI)	No of controls in model	OR (95%CI)
1	None	711	0.52 (0.33-0.83)	103	0.36 (0.16-0.83)
2	Associated with NSAID use as described in manuscript (174)	561	0.50 (0.31-0.81)	79	0.38 (0.16-0.93)
3	Accidents and injuries (70)	654	0.51 (0.32-0.81)	90	0.28 (0.12-0.66)
4	Orthopaedic conditions (183)	540	0.49 (0.31-0.80)	91	0.33 (0.14-0.77)
5	Gastrointestinal conditions (86)	635	0.53 (0.33-0.84)	93	0.36 (0.16-0.82)
6	Respiratory conditions (61)	660	0.53 (0.33-0.85)	93	0.33 (0.14-0.77)
7	Neurological conditions (77)	647	0.53 (0.33-0.85)	90	0.39 (0.17-0.93)
8	Cardiac conditions (131)	601	0.57 (0.35-0.91)	82	0.43 (0.18-1.04)
9	Renal conditions (60)	660	0.52 (0.32-0.82)	94	0.33 (0.14-0.76)
10	Endocrine conditions (36)	678	0.53 (0.33-0.85)	100	0.38 (0.17-0.89)
11	Infectious diseases and fevers (54)	664	0.52 (0.32-0.83)	96	0.42 (0.18-0.99)
12	Other miscellaneous conditions (56)	660	0.55 (0.35-0.88)	98	0.39 (0.17-0.90)
13	Diagnoses not associated with NSAID use (ie excluded controls in model 2) (640)	150	0.74 (0.40-1.39)	24	0.20 (0.05-0.82)

[1] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings), years of education, housing type, and history of cancer in 1st degree relative
[2] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings) years of education, housing type, history of cancer in 1st degree relative and duration (in years) of smoking

Discussion

These results suggest that regular aspirin consumption may be associated with a reduced risk of cancer in both never-smokers and ever-smokers. Among never-smokers, the inverse association is most prominent among those who started using aspirin more than a year from diagnosis or admission, but less than 3 years from admission or diagnosis, and those for whom duration of use of aspirin was between 12 and 36 months. Non-aspirin NSAIDs, and paracetamol and other forms of acetaminophen use were not associated with lung cancer, though the numbers of regular users in each group were small. The risk estimates were not significantly different in the subgroup of non-smokers with histologically-confirmed adenocarcinomas, compared to the overall group of cases.

A protective effect of aspirin and other NSAIDs is biologically plausible – COX2 is an inducible enzyme with pro-inflammatory and pro-carcinogenic properties, and *in vitro* and animal studies lend support to the role of COX2 in carcinogenesis (143-151). However, reported results of aspirin use and lung cancer risk are conflicting, with both protective (154-162) and null effects (163-173) being reported. Three clinical trials focusing on chemoprevention with aspirin reported non-significant inverse effects (176-178), and a recent pooled analysis of individual-level data from 7 clinical trials of daily aspirin reported an inverse effect on lung cancer death, with a protective latent period of about 5 years. The authors also reported a greater inverse effect against adenocarcinomas (179). The reasons for the inconsistency in findings include small numbers of lung cancer cases, failure to distinguish between aspirin and other NSAIDs, and inadequate control of smoking status (as have been pointed out by some of the authors). Three meta-analyses have also reported widely contrasting results (158, 174,

175) – the differences in results appear to be due to the inclusion of different studies in the meta-analyses and to the use of different definitions of exposure (aspirin and NSAIDs examined separately or in combination).

Further, the effect of aspirin and other NSAIDs may be modified by gender, and this could also explain the heterogeneity in results. The estrogen and prostaglandin pathways are known to interact, and estradiol increases COX-2 production in some cell types (180). The effect of COX-2 inhibition by aspirin and NSAIDs may thus be stronger in women. The available epidemiologic evidence is not consistent either, with reports of stronger inverse effects in men (161), stronger inverse effects in women (163, 164), and similar effects in both genders (158, 159). A possible explanation may be inadequate or residual confounding by smoking in populations where there are strong gender differences in smoking rates.

Among the studies that distinguished between aspirin and non-aspirin NSAID use, some reported stronger inverse effects of aspirin than non-aspirin NSAID (160), while others reported that the effect of non-aspirin NSAIDs was stronger (157, 158, 162). Residual confounding by smoking (because aspirin but not non-aspirin NSAIDs use may be higher in smokers as anti-thrombotic treatment for cardiovascular disease risk) may shift effects towards the null and explain the smaller effect of aspirin compared to non-aspirin NSAIDs detected in some studies (158). In contrast, our study found an inverse association of aspirin even in non-smokers, but no association in a much smaller group of regular users of non-aspirin NSAID.

Our study contributes to the growing literature on the role of NSAIDs and aspirin on lung cancer by studying the effect of aspirin and non-aspirin NSAIDs in a large group of Asian

female non-smokers, for which the effect of aspirin and non-aspirin NSAIDs have not been well-characterised. Our study also distinguished between aspirin and non-aspirin NSAID, and examined the duration and onset of use in relation to a diagnosis of lung cancer.

There are a number of limitations in this study. Aspirin and NSAID use in Singapore are commonly prescribed by physicians for the treatment of medical conditions rather than as health supplements. Hence, hospital controls may have a higher-than-representative prevalence of aspirin and NSAID use. This would bias our estimates away from the null. We have tried to minimize this by recruiting controls from a variety of admitting diagnoses, and, in line with the theory of control selection proposed by Wacholder (1996) (259), have also excluded controls whose admission diagnoses are likely to be associated (both positively and negatively) with the exposures of interest. We excluded individuals admitted for chest pain, acute myocardial infarction, or heart failure, stroke or transient ischaemic attack, and for management of musculoskeletal pain, including knee, hip and back pain, as NSAIDs and aspirin are commonly used in the management of these conditions. In addition, we also excluded individuals who were admitted for complications arising from NSAID use. We also evaluated the impact of including controls from particular diagnoses groups on the OR estimates for aspirin use and lung cancer risk, by systematically excluding controls based on their diagnoses group and testing the effect of aspirin. These analyses suggest that the results we obtained were fairly robust, and were not likely due to high aspirin use in any particular group of controls.

We relied on self-reports of medication use, and under- or over- reporting of medication use is possible. However, as this was a hospital-based study and interviews were

conducted in the hospital, such misreporting should affect cases and controls equally. We were unable to obtain information about the dosage and frequency of use of these medications in our participants, and we did not distinguish between low-dose or normal dose aspirin, thus limiting our ability to define more precisely the effect of aspirin.

Because of the relatively small size of our study and the low frequency of use of some medications such as steroid pills, some estimates reported in this study are imprecise, with wide confidence intervals. OR estimates presented in Table 6.2 for steroid pills and COX-2 inhibitors, and in Table 6.3 showing the effect of duration of aspirin use and date of onset of aspirin use in relation to lung cancer risk should be viewed as preliminary in nature. Additional well-designed studies conducted in similar populations of never-smokers are necessary to confirm our results.

Conclusion

In summary, our data suggest that regular aspirin consumption may confer protection against lung cancer in Chinese women. This finding is consistent with the well-characterised inhibitory effect of aspirin on cyclooxygenase 2, which has pro-inflammatory and pro-carcinogenic properties.

This study has been published (Lim WY, et al., Lung Cancer 2012; Apr 3, epub ahead of print, attached as Appendix C to this thesis).

CHAPTER 7 STUDY 3 - FEMALE REPRODUCTIVE FACTORS, GENE POLYMORPHISMS IN THE ESTROGEN METABOLISM PATHWAY AND LUNG CANCER

Introduction

Epidemiologic studies conducted in the 1980s implicated reproductive factors and female hormonal factors in lung carcinogenesis (108, 181-183), and this hypothesis gained momentum with evidence of the potential biological and pathological roles of estrogen in lung development and carcinogenesis. Estrogen receptors (ER), predominantly the ERβ isoform, are found in both normal lung and carcinomatous tissue (184), and ER signaling pathways are active in lung cancer cells (185), increasing cell proliferation and promoting angiogenesis. This pathway also interacts with the Epidermal Growth Factor Receptor (EGFR) pathway (186), which is believed to drive carcinogenesis in a subset of lung cancers (lung adenocarcinomas among predominantly female never-smokers) (61). *In vitro* and mouse model studies suggest that estrogens increase tumour growth, and ER antagonists significantly inhibit growth in Non-Small Cell Lung Cancer (NSCLC) cell lines (187, 188). These findings have spurred renewed interest in the possible role of female hormonal factors in lung cancer, and, including the earlier studies, at least 29 studies (46, 47, 108, 181-183, 189-211) have investigated the association of various factors such as parity (46, 47, 108, 182, 189-192, 194-200, 202-203, 211), menstrual history (46, 108, 181-183, 190, 191, 194), age at menopause (46, 47, 108, 181-183, 189, 191-194, 196, 198, 200, 203, 211), age at menarche (46, 47, 108, 182, 183, 189, 191-194, 197-200, 203, 211), estrogen replacement therapy use (46, 47, 182, 189, 196, 197, 199, 200, 203-209), and oral contraceptive use (47, 108, 189, 196, 199-201, 203, 204, 210, 211).

Findings from these studies are inconsistent, suggesting that the effects of female reproductive factors are not strong and therefore difficult to detect, or that reproductive factors interact with other host genetic or environmental factors in complex ways. The evidence that have emerged do not appear to conform to the well-established model for the association of reproductive factors with breast cancer (where early menarche and late menopause, nulliparity and late age at first birth consistently increase breast cancer risk), but no alternative model has yet emerged.

Understanding the estrogen metabolic pathway in relation to lung cancer may shed light on the role of estrogen in lung carcinogenesis. To date, few studies have examined the association of estrogen-metabolism gene polymorphisms with lung cancer. Two recent papers have investigated gene polymorphisms in estrogen-metabolism enzymes [CYP17(17-α hydroxylase), CYP19A1 (aromatase) and COMT (Catechol-O-Methyl Transferase)] (212) and in ER β (Estrogen Receptor β) (213), although these studies have used populations where non-smokers were a small minority.

Data obtained from the first case-control study conducted in 1995-1998 have been previously published and showed that among never-smokers, parity and menstrual cycle length were associated with a significantly reduced risk of lung cancer (191). The aims of Study 3 are to extend this analysis to a larger group of 702 cases and 1578 hospital controls so as to better delineate possible differences between smokers and never-smokers, and to investigate the association of estrogen metabolism gene polymorphisms with lung cancer in Chinese women.

Methods

In both case-control studies, the age at menarche, age at menopause, usual length of menstrual cycles, the number of children, and the age at first birth were elicited from participants via the in-person questionnaire. Among post-menopausal women, the reproductive period was calculated as the period of time between menopause and menarche. In the second case-control study, participants were also asked about the use of hormone replacement therapy and oral contraceptives – the age when use commenced and the period of use were obtained.

Five polymorphisms in four estrogen pathway genes (rs743572 in CYP17, rs10046 in CYP19A1, rs4680 in COMT, rs1256049 and rs4986938 in ERβ) were genotyped. These genes were selected because the proteins they encode are important within the estrogen pathway. CYP17 is an upstream enzyme that produces estrogen precursors, while CYP19A1 converts testosterone to estradiol. COMT is the rate-limiting enzyme in the detoxification of catechol estrogens (214). ERβ are found in lung tissue (184).

A total of 559 blood or saliva samples from cases (79.6% of all cases) and 988 blood or saliva samples from controls (62.6% of all controls) were obtained. Among 131 subjects having both blood and saliva samples, saliva DNA from 89% was successfully genotyped while all blood DNA were successfully genotyped. The genotypes from saliva DNA samples matched completely (100%) those of the corresponding blood DNA samples. Three positive controls and two negative controls were included in each 96-well plate, and 10% of DNA samples were genotyped in duplicate for each polymorphism. The concordance rate for the duplicate analyses was 100%. Call rates for the 5 SNPs studied ranged from 96.6 -97.5%.

Results

Participants from both GEL studies were used. In total, there were 702 cases and 1578 controls. A total of 559 blood or saliva samples that were obtained from cases (79.6% of all cases) and 988 blood or saliva samples from controls (62.6% of all controls) were used for genetic analyses.

High parity was associated with a lower risk of lung cancer in never-smokers (OR of 0.92, 95% CI 0.87, 0.97 for every additional child). Similar ORs, but with non-significant *P* values, were obtained in the smaller number of ever-smokers (Table 7.1). Higher age at first birth was positively associated with lung cancer in never-smokers (ORs 1.54, 2.17, 1.30 in those whose first child was born when they were 21-25, 26-30 and 31 years and above respectively, compared to those 20 years or fewer at first birth.) The effect was less clear among ever-smokers (adjusted $P_{interaction}$ 0.14) (Table 7.1).

Among never-smoking post-menopausal women, age at menopause was positively associated with lung cancer (ORs 1.37, 1.59 for age at menopause of 49-51 years and 52 years or older, compared to an age of menopause at 48 years or younger, P_{trend} 0.003), but not among ever-smoking women (ORs 0.95, 0.94, respectively, P_{trend} 0.81), adjusted $P_{interaction}$ 0.22 (Table 7.1). Similarly, a longer reproductive period was positively associated with lung cancer risk among never smokers (ORs 1.06, 1.25, 1.45 and 1.47 for reproductive periods of 31-33, 34-36, 37-39, and 40 years and more respectively, compared to those with a reproductive period of 30 years or fewer, P_{trend} 0.026) but not in ever-smokers (adjusted $P_{interaction}$ 0.34) (Table 7.1).

Long menstrual cycle lengths were inversely associated with lung cancer in never-smokers (OR 0.50, 95%CI 0.32, 0.80 for those reporting cycle lengths of more than 30

Table 7.1 Reproductive factors, exogenous reproductive hormone use and lung cancer

	All				Never-smokers				Ever-smokers			
	Cases	Controls	OR[a]	95% CI	Cases	Controls	OR[b]	95% CI	Cases	Controls	OR[c]	95% CI
Number of children												
0	87 (12.4)	176 (11.2)	1.0		54 (12.5)	153 (11.2)	1.0		33 (12.3)	23 (11.3)	1.0	
1-2	187 (26.7)	383 (24.3)	1.07	0.77, 1.50	135 (31.3)	344 (25.1)	1.15	0.79, 1.88	52 (19.3)	39 (19.2)	0.90	0.41, 1.93
3-4	209 (29.8)	490 (31.1)	0.84	0.60, 1.18	142 (32.9)	444 (32.4)	0.88	0.59, 1.29	67 (24.9)	46 (22.7)	0.89	0.42, 1.86
5 or more	218 (31.1)	526 (33.4)	**0.61**	**0.43, 0.88**	101 (23.4)	431 (31.4)	**0.63**	**0.40, 0.98**	117 (43.5)	95 (46.8)	0.54	0.27, 1.09
*P*trend			**0.001**				**0.006**				**0.038**	
Likelihood Ratio test for interaction between smoking status and parity *P*= 0.34; (adjusted for multiple comparisons, *P*=0.34)												
As continuous variable			**0.93**	**0.90, 0.98** *P*=0.002			**0.92**	**0.87, 0.97** *P*=0.002			0.95	0.89, 1.02 *P*=0.19
Age at first birth (years)[d]												
No children	87	176	-		54	153	-		33	23		
20 or less	146 (24.3)	412 (29.9)	1.0		63 (17.0)	339 (28.2)	1.0		83 (35.8)	73 (41.0)	1.0	
21-25	264 (43.9)	538 (39.0)	**1.49**	**1.14, 1.96**	151 (40.8)	471 (39.2)	**1.54**	**1.09, 2.17**	113 (48.7)	67 (37.6)	**1.84**	**1.12, 3.01**
26-30	143 (23.8)	279 (20.2)	**1.92**	**1.37, 2.69**	121 (32.7)	255 (21.2)	**2.17**	**1.46, 3.22**	22 (9.5)	24 (13.5)	0.86	0.40, 1.85
31 or more	49 (8.1)	151 (10.9)	1.22	0.78, 1.89	35 (9.5)	137 (11.4)	1.30	0.78, 2.18	14 (6.0)	14 (7.9)	1.32	0.51, 3.42
Likelihood Ratio test for interaction between smoking status and age at first birth *P*=0.031;(adjusted for multiple comparisons, *P*=0.14)												
As continuous variable			1.01	0.98, 1.03 *P*=0.58			1.01	0.98, 1.04 *P*=0.63			1.00	0.95, 1.06 *P*=0.97

Reproductive Period (years)											
Pre-menopausal	45	129	-		37	118	-		8	11	-	
30 or less	126 (21.7)	290 (22.7)	1.0		61 (18.1)	246 (22.3)	1.0		65 (26.8)	44 (25.1)	1.0	
31-33	97 (16.7)	230 (18.0)	1.00	0.71, 1.41	52 (15.4)	197 (17.9)	1.06	0.69, 1.63	45 (18.5)	33 (18.9)	0.89	0.47, 1.69
34-36	159 (27.4)	327 (25.6)	1.21	0.89, 1.65	85 (25.2)	282 (25.6)	1.25	0.85, 1.84	74 (30.5)	45 (25.7)	1.07	0.60, 1.91
37-39	117 (20.1)	256 (20.0)	1.22	0.87, 1.70	82 (24.3)	223 (20.2)	1.45	0.97, 2.16	35 (14.4)	33 (18.9)	0.73	0.37, 1.41
40 and more	82 (14.1)	174 (13.6)	1.26	0.87, 1.82	58 (17.2)	154 (14.0)	1.47	0.95, 2.24	24 (9.9)	20 (11.4)	0.86	0.40, 1.85
P_{trend}			0.11				**0.026**				0.54	
Likelihood Ratio test for interaction between smoking status and reproductive period P=0.28;(adjusted for multiple comparisons, P=0.34)												
As continuous variable			1.02	1.00, 1.04 P=0.13			1.02	1.00, 1.05 P=0.071			1.00	0.97, 1.04 P=0.89
Age at menarche												
12 or younger	106 (15.8)	274 (17.7)	1.0		77 (18.6)	244 (18.1)	1.0		29 (11.3)	30 (15.2)	1.0	
13-15 years	354 (52.7)	789 (50.9)	1.20	0.90. 1.60	234 (56.4)	694 (51.4)	1.24	0.90, 1.71	120 (46.7)	95 (48.0)	0.92	0.47, 1.82
16 years or older	212 (31.6)	486 (31.4)	1.04	0.75, 1.45	104 (25.1)	413 (30.6)	0.98	0.67, 1.45	108 (42.0)	73 (36.9)	1.11	0.54, 2.28
P_{trend}			0.92				0.73				0.56	
As continuous variable			1.02	0.97, 1.08 P=0.38			1.01	0.95, 1.07 P=0.74			1.08	0.98, 1.20 P=0.13
Age at menopause												

Pre-menopausal	45	129	-		37	118	-		8	11	-	
48 years of age or younger	218 (36.6)	514 (39.8)	1.0		109 (31.4)	439 (39.3)	1.0		109 (44.0)	75 (42.4)	1.0	
49-51 years of age	200 (33.6)	422 (32.6)	1.20	0.94, 1.55	118 (34.0)	363 (32.5)	**1.37**	**1.01, 1.86**	82 (33.1)	59 (33.3)	0.95	0.59, 1.54
52 years of age or older	177 (29.8)	357 (27.6)	1.34	1.03, 1.74	120 (34.6)	314 (28.1)	**1.59**	**1.16, 2.17**	57 (23.0)	43 (24.3)	0.94	0.55, 1.60
P_{trend}			0.027				**0.003**					0.81
Likelihood Ratio test for interaction between smoking status and age at menopause P=0.11;(adjusted for multiple comparisons, P=0.22)												
As continuous variable			1.02	1.00, 1.04 P=0.053			**1.03**	**1.00, 1.05** P=0.036			1.01	0.97, 1.05 P=0.58
Menstrual cycle length												
30 days or less	638 (93.6)	1389 (89.7)	1.0		399 (94.1)	1210 (89.6)	1.0		239 (92.6)	179 (90.4)	1.0	
More than 30 days	44 (6.5)	159 (10.3)	**0.61**	**0.42, 0.88**	25 (5.9)	140 (10.4)	**0.50**	**0.32, 0.80**	19 (7.4)	19 (9.6)	0.73	0.35, 1.49
Likelihood Ratio test for interaction between smoking status and menstrual cycle length P=0.22;(adjusted for multiple comparisons, P=0.33)												
Exogenous reproductive hormone use												
Never used	297 (74.4)	578 (70.9)	1.0		181 (70.2)	505 (70.9)	1.0		116 (82.3)	73 (70.9)	1.0	
Used for 5 years or less	77 (19.3)	182 (22.3)	0.82	0.59, 1.15	58 (22.5)	162 (22.8)	0.91	0.63, 1.31	19 (13.5)	20 (19.4)	0.54	0.24, 1.21
Used for more than 5 years	25 (6.3)	55 (6.8)	0.94	0.55, 1.62	19 (7.4)	45 (6.3)	1.23	0.68, 2.23	6 (4.3)	10 (9.7)	0.45	0.13, 1.55
P_{trend}			0.43				0.84					0.086

[a]Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), smoking status (current, ex- or never smoker), environmental tobacco exposure at home and a dummy variable for the study set

[b]Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), environmental tobacco exposure at home and a dummy variable for the study set

[c]Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), smoking duration (in years), environmental tobacco exposure at home and a dummy variable for the study set

[d] Additionally adjusted for number of children

days, compared to those reporting lengths of 30 days or fewer) but not in ever-smokers (adjusted $P_{interaction}$ 0.33). There were relatively few women who reported cycle lengths longer than 30 days. There was no association between hormone use and lung cancer, although the prevalence of hormone use was low. There was also no association between age at menarche and lung cancer (Table 7.1).

Of the five SNPs studied, only the SNP in the COMT gene was associated with lung cancer (Table 7.2). The A allele at rs4680 was associated with lung cancer in never-smokers (OR 1.46, 95%CI 1.12, 1.90 for *G/A* or *A/A* genotypes, compared to *G/G* genotype) but not in ever-smokers (OR 0.77, 95%CI 0.47, 1.28), adjusted $P_{interaction}$ 0.14.

In analyses stratified by age, the positive association of longer reproductive periods in never-smokers was seen only among those older than 65 years, but not in the younger age-group (adjusted $P_{interaction}$ 0.22). The inverse association of increasing parity also appeared to be stronger in the older age-group than the younger, although this interaction was not statistically significant (adjusted $P_{interaction}$ 0.95) (Table 7.3). The associations of age at menopause, age at first birth, menstrual cycle length and the rs4680 polymorphism on COMT with lung cancer were not modified by age (data not shown). Odds ratio estimates for never-smoker cases restricted to those with a confirmed histology of adenocarcinomas were similar to those obtained for all never-smokers (data not shown). Further adjustment with self-reports of exposure to cooking fumes shifted odds ratio estimates of all reproductive factors and SNPs on lung cancer risk by less than 5% in either direction.

Table 7.2 Polymorphisms in the estrogen pathway and lung cancer

	All				Never-smokers				Smokers			
	Cases	Controls	OR[a]	95%CI	Cases	Controls	OR[b]	95%CI	Cases	Controls	OR[c]	95%CI
CYP17 rs743572												
G/G	177 (42.4)	346 (35.9)	1.0		102 (29.7)	299 (35.3)	1.0		75 (37.0)	47 (40.2)	1.0	
G/A	268 (49.1)	456 (47.4)	1.18	0.91, 1.53	176 (51.3)	401 (47.4)	1.25	0.93, 1.69	92 (45.3)	55 (47.0)	1.18	0.68, 2.06
A/A	101 (18.5)	161 (16.7)	1.31	0.93, 1.83	65 (19.0)	146 (17.3)	1.30	0.89, 1.92	36 (17.7)	15 (12.8)	1.30	0.59, 2.86
G/A or A/A			1.21	0.95, 1.55			1.26	0.95, 1.68			1.21	0.72, 2.04
CYP19A1 rs10046												
A/A	145 (26.7)	248 (25.9)	1.0		90 (26.5)	222 (26.4)	1.0		55 (2.0)	26 (22.2)	1.0	
A/G	268 (49.3)	487 (50.8)	0.92	0.69, 1.21	171 (50.3)	424 (50.4)	0.99	0.72, 1.36	97 (47.6)	63 (53.9)	0.80	0.43, 1.51
G/G	131 (24.1)	223 (23.3)	1.04	0.75, 1.44	79 (23.2)	195 (23.2)	1.06	0.73, 1.54	52 (25.5)	28 (23.9)	1.02	0.48, 2.16
A/G or A/A			0.95	0.73, 1.24			1.01	0.75, 1.36			0.86	0.47, 1.57
COMT rs4680												
G/G	284 (52.3)	549 (56.9)	1.0		167 (49.1)	488 (57.6)	1.0		117 (57.6)	61 (51.7)	1.0	
G/A	220 (40.5)	353 (36.6)	1.26	0.99, 1.60	148 (43.5)	303 (35.8)	**1.49**	**1.13, 1.97**	72 (35.5)	50 (42.4)	0.70	0.42, 1.20
A/A	39 (7.2)	63 (6.5)	1.33	0.85, 2.08	25 (7.4)	56 (6.6)	1.30	0.77, 2.19	14 (6.9)	7 (5.9)	1.28	0.46, 3.55
G/A or A/A			**1.27**	**1.01, 1.60**			**1.46**	**1.12, 1.90**			0.77	0.47. 1.28
Likelihood Ratio test for interaction between smoking status and COMT rs4680 polymorphism *P*=0.047; (adjusted for multiple comparisons, *P*=0.14)												
ERβ rs1256049												
G/G	206 (38.0)	371 (38.9)	1.0		135 (39.7)	320 (38.2)	1.0		71 (35.2)	51 (44.0)	1.0	

G/A	251 (46.3)	447 (46.9)	1.00	0.78, 1.28	152 (44.7)	397 (47.4)	0.89	0.67, 1.19	99 (49.0)	50 (43.1)	1.43	0.83, 2.47
A/A	85 (15.7)	135 (14.2)	1.02	0.72, 1.45	53 (15.6)	120 (14.3)	0.98	0.66, 1.46	32 (15.8)	15 (12.9)	1.02	0.46, 2.26
G/A or *A/A*			1.00	0.79, 1.27			0.91	0.70, 1.20			1.33	0.79, 2.22
ERβ rs4986938											
G/G	446 (82.0)	807 (83.7)	1.0		277 (81.5)	704 (83.2)	1.0		169 (82.8)	103 (87.3)	1.0	
G/A	95 (17.5)	148 (15.4)	1.17	0.87, 1.59	61 (17.9)	134 (15.8)	1.08	0.77, 1.52	34 (16.7)	14 (11.9)	1.36	0.66, 2.78
A/A	3 (0.6)	9 (0.9)			2 (0.6)	8 (1.0)			1 (0.5)	1 (0.9)		

[a]Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), smoking status (current, ex- or never smoker), environmental tobacco exposure at home and a dummy variable for the study set

[b]Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), environmental tobacco exposure at home and a dummy variable for the study set

[c]Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), smoking duration (in years), environmental tobacco exposure at home and a dummy variable for the study set

Table 7.3 Selected reproductive factors and lung cancer, by age-group

	All				Never-smokers			
	Age 65 or lower		Age >65		Age 65 or lower		Age >65	
Cases/Controls (Nos)	311/801		391/777		243/725		190/650	
	OR[a]	95%CI	OR[a]	95%CI	OR[b]	95%CI	OR[b]	95%CI
Reproductive period (years)								
30 or less					1.0		1.0	
31-33					0.60	0.31, 1.18	1.77	0.98, 3.19
34-36					0.73	0.42, 1.28	**2.20**	**1.26, 3.83**
37-39					0.94	0.54, 1.64	**2.24**	**1.24, 4.04**
40 and more					0.82	0.44, 1.54	**2.66**	**1.41, 5.00**
P_{trend}					0.99		**0.001**	
As continuous variable					0.99	0.96, 1.03, P=0.77	**1.05**	**1.01, 1.09** P=0.005
					Likelihood ratio test for interaction **P=0.036** (adjusted for multiple comparisons P=0.22)			

Number of children								
0	1.0		1.0					
1-2	1.25	0.82, 1.90	0.84	0.46, 1.51				
3-4	0.94	0.60, 1.46	0.78	0.45, 1.34				
5 or more	0.76	0.42, 1.38	**0.50**	**0.30, 0.84**				
P_{trend}	0.22		**0.001**					
As continuous variable	0.96	0.88, 1.04 P=0.32	**0.92**	**0.88, 0.97 P=0.002**				
	Likelihood ratio test for interaction P=0.65 (adjusted for multiple comparisons P=0.95)							

[a] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancers, history of lung cancer), smoking status, environmental tobacco exposure at home and a dummy variable for the study set

[b] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first degree relatives (no history, history of other cancer, history of lung cancer)

Discussion

In this ethnically homogenous population with a high prevalence of never-smokers, later age at menopause, longer reproductive period, later age at first birth, short menstrual cycle lengths and low parity are associated with lung cancer, particularly among never-smokers. Low parity is associated with higher blood estrogen levels (215). Shorter menstrual cycles suggest an overall increase in the period of unopposed estrogen exposure, and late menopause and long reproductive periods imply more reproductive cycles over the lifetime and hence longer periods of estrogen exposure in total. Our results are therefore consistent with an association between higher levels of endogenous estrogen exposure over the lifetime and risk of lung cancer.

An association with reproductive history was previously observed in our first study involving a smaller group of participants (191), where high parity and long menstrual cycles were protective. This current analysis additionally finds an increased risk associated with later ages at first birth, later age at menopause, and longer reproductive period. A recently-published Singapore cohort study (189) reported similar inverse associations for parity among Chinese women never-smokers; although it did not find a significant association with age at menopause (189). While we are not able to fully explain this particular difference, we feel that, taken as a whole, data from this population suggest a role for estrogens in lung carcinogenesis among never-smoking women.

Other published studies have reported results similar to ours- namely an inverse association with higher parity (189, 191, 200, 202, 203), and positive association with higher age at menopause (108, 181, 182). A cohort study (47) did not find a main effect with menarche nor menopause, but reported that, compared to women with late age of

menarche (>15 yrs) and early age of menopause (<51 years), women with either early menarche or late menopause had a higher risk of lung cancer, and proposed that the length of exposure to estrogen may partly account for the increased risk.

Yet other studies, however, have reported null findings (46, 47, 108, 182, 192, 194, 196-198, 211) or increased risk (195, 199) with regard to parity, reduced risk with later age at menopause (192, 193, 200, 211), and inverse associations with longer reproductive period (200).

The inconsistency in findings across the various studies may be due to differences in the study populations. Studies conducted on smokers or populations containing a high proportion of smokers might not have been able to detect a weaker effect of estrogens. There is growing evidence that smoking-related lung cancer and never-smoker lung cancer are etiologically distinct. It is plausible that the effect of estrogens may specifically affect never-smoker lung cancer, given that *EGFR* mutations appear to be key drivers of this disease (61), and the estrogen pathway interacts at the cellular level with the EGFR signaling pathway (186). A recent Japanese case-control study noted that the increased risk associated with longer reproductive periods was seen only in EGFR mutation positive cancers (198).

The effect of estrogen may be modified by other biological factors. For example, Paulus *et al* (202) proposed that the different age distributions of women in the studies may explain the inconsistency of results with regard to parity, and their own data suggest that the effect of parity was seen only in late-onset lung cancer (using a cut-off of 50 years), thus suggesting that high parity may protect against post-menopausal lung cancer, but not pre-menopausal lung cancer. We also find that both longer reproductive periods and

parity had more convincing effects in older women, although the possible reasons for this are yet to be elucidated.

We did not find an association with key polymorphisms in the estrogen metabolism pathway, other than the rs4680 polymorphism in the COMT gene, where the A allele was associated with lung cancer in never-smokers. The oxidative metabolism of 17-β estradiol and estrone to catechol estrogens is postulated to be a risk factor in carcinogenesis: catechol estrogens are oxidized to quinines that can form DNA adducts and produce DNA and lipid-damaging reactive oxygen species (214). COMT methylates (thus eliminating) catechol estrogens, and is the key rate-limiting enzyme in that pathway (214). The A allele at rs4680 (codon 158) results in a point mutation, substituting the amino acid methionine for valine (216). Val/Met and Met/Met genotypes appear to have lowered enzymatic activity, with consequently increased cellular levels of catechol estrogens.

Two other case-control studies have investigated the effect of polymorphisms at this site on lung cancer risk with similar results. The Met/Met or Val/Met genotypes were also positively associated with lung cancer in a population of smokers (217). Cote *et al* (212), found that the A allele had no independent effect in a group of predominantly smokers, but lung cancer was increased among those with the A allele and a GSTM1 null genotype compared to those with the *G/G* genotype and a GSTM1 non-null genotype. In that same study, an independent effect of rs743572 (in CYP17) on lung cancer was not seen, although a positive association as seen with the C allele in combination with risk alleles in GSTM1 and GSTT1. The authors did not find a relationship with CYP19A1 and lung cancer, although the polymorphism they studied (the tetranucleotide tandem repeat in intron 4) was different from the one in this paper. Paulus *et al* (213), examining four

ERβ SNPs (the same two in our study, and two others), did not find an association of individual SNPs nor haplotypes with lung cancer risk, consistent with our results.

We had anticipated that polymorphisms in CYP19A1 and CYP17 would be important if the effect of reproductive factors is mediated through estrogen levels in target tissue (such as the lungs). Current evidence, including ours, however, is more consistent with a risk-conferring effect of catechol estrogens on lung carcinogenesis. There have been few studies looking at estrogen pathway polymorphisms in lung carcinogenesis, and further work is necessary to determine if this hypothesis is true. Inclusion of more SNPs for which functional studies indicate an effect on enzyme activity or estrogen levels, or a systematic interrogation of polymorphisms in the CYP19A1, CYP 17 and COMT genes for association with lung cancer may be useful in this regard.

We noticed suggestive interactions between smoking status, and the COMT SNP and age at first birth, and between age and reproductive period among never-smokers. However, our study is not adequately powered to detect interactions, and the interactions were no longer significant after adjustment for multiple testing. Further research using larger study populations are needed to confirm these observations.

Homogeneity with regard to gender and ethnicity and the large number of never-smokers which allows detailed investigation into risk factors pertinent to this group are particularly important in this analysis, in the light of recent understanding that smoking and non-smoking lung cancers may be different diseases. To reduce bias introduced by use of hospital controls, we chose controls from a wide variety of admitting diagnoses, and gynaecological diseases as the admitting diagnoses were very rare among our controls (about 0.4% of controls).

Our study suggests that reproductive factors are involved in lung carcinogenesis, particularly among never-smokers. However, the specific functional role of estrogens in lung cancer is still not known, and while our data suggests a role for catechol estrogens, the mechanisms involved are likely to be complex. Given the relationship between the estrogen and the EGFR pathways, and the significance of the latter in never-smoker lung cancer, it is clear that further studies, both mechanistic and epidemiologic, are needed in this area.

This study has been published (Lim WY, et al., American Journal of Epidemiology 2012; Mar 15;175(6):492-503, attached as Appendix D to this thesis).

CHAPTER 8 STUDY 4 - EXPOSURE TO INDOOR INHALANTS AND LUNG CANCER

Introduction

Fumes and airborne particulates in the indoor environment are potential risk factors for lung cancer: examples are exposure to cooking oil fumes, cooking and heating fuels (household coal & wood combustion), incense & mosquito coils and indoor radon (31, 32, 218, 219).

Exposure to cooking fumes may potentially play a role in the occurrence of lung cancer. Cooking oil fumes are known to contain at least two carcinogenic compounds, benzo[a]pyrene (BaP) and 2, 4-decadienal (2, 4-DDE), which induce lung cell survival and proliferation via the NF-κB pathway (220, 221). Cumulative exposure to cooking (frequency and duration) by means of frying (stir-frying, frying and deep-frying) increased the risk of lung cancer among female non-smokers in Hong Kong (32). Women nonsmokers were at higher risk for lung cancer if they were exposed to cooking oil fumes emitted at high temperatures and the risks were higher when the fumes were not reduced by an extractor (31).

The combustion by-products from heating and cooking are also sources of indoor air pollution. The use of coal for heating has been implicated in the high incidence of lung cancer among residents of Xuanwei, China (222), and the International Agency for Research in Cancer considers coal fuel use a Class 1 carcinogen (27). Recent results from a pooled analysis of 4181 cases and 5125 controls (223) found an increased risk with wood smoke exposure OR 1.21, 95%CI 1.06-1.38 among individuals from Europe and North America who reported predominant use of wood fuels in the house. No effect of wood use was seen in never-smokers (OR 1.01, 95%CI 0.74-1.37), although wood

use was risk-conferring in ever-smokers (OR 1.22, 95%CI 1.05-1.42). Traditional heating and cooking fuels (coal & wood) produce a variety of indoor pollutants, including respirable particles, heavy metals, polycyclic aromatic hydrocarbons, carbon monoxide, carbon dioxide, nitrogen dioxide, sulphur dioxide, and formaldehyde (219).

Incense burning, a traditional practice in Chinese households, is also powerful producer of particulate matter; and incense smoke contains carcinogens such as polycyclic aromatic hydrocarbons (PAHs), carbonyls, and benzene (30, 224, 225). Incense smoke condensates have mutagenic and genotoxic activities, and the genotoxicity of certain incense smoke condensates in mammalian cells has been shown to be higher than that of tobacco smoke condensate (226, 227). The potential impact of incense on health has also been studied outside the home (228, 229). A large prospective cohort study in Singapore reported an association between long-term incense use and the development of squamous cell carcinomas of the respiratory tract, particularly among women (33).

Mosquito coils are frequently burned indoors in Asia and to a limited extent in other parts of the world, including the United States (230). The major ingredients of the mosquito coils are pyrethrins and plant-based materials, such as wood powder, coconut shell powder, joss powder, binders, dyes, oxidants, and other additives to allow for controlled smoldering (34, 231). The combustion of the remaining materials generates large amounts of submicrometer particles and gaseous pollutants. These submicrometer particles may reach the lower respiratory tract and be coated with a wide range of organic compounds such as polycyclic aromatic hydrocarbons (PAHs). A study of mosquito coil smoke and lung cancer in Taiwan between 2002 and 2004 showed that lung cancer risk among smokers with the highest exposure to mosquito coil smoke could be as high as 14-fold, compared with non-smokers without this exposure (34).

In Study 4, we investigate if inhalant exposure from these sources is associated with lung cancer. We also predict that the impact of these compounds is modified by tobacco smoke exposure, and hypothesize that the effect of domestic inhalants is greater in never-smokers than in smokers where the effect may be masked by the more significant impact of cigarette smoking.

Methods

Participants were asked to recall exposures to domestic inhalants at 25 years prior to the diagnosis of lung cancer (for cases) or the current admission (for controls). They were asked about regular household cooking and the frequency which they cooked, the use of wood stove and charcoal stove, the use of joss sticks and scented coil/ powder, and the use of mosquito coils in their homes. In total, data from 702 cases and 1578 controls were analysed.

Results

Table 8.1 presents the effect of exposure to incense or mosquito coils, as well as cooking, on lung cancer separately for smokers and nonsmokers. A statistically significant positive relationship was observed only among smokers, and there was no association observed among lifetime nonsmokers. Among smokers, daily cooking was positively associated with lung cancer (adjusted OR 1.61, 95% CI 1.01-2.56), Also, among smokers, daily exposure to incense or mosquito coils was also positively associated with lung cancer (OR 1.53, 95% CI 0.97-2.41), after adjustment for potential confounders, although this result just missed the traditionally accepted level of significance of $P<0.05$. Daily use of charcoal or wood stove did not result in an association of lung cancer in either smokers or nonsmokers.

Table 8.1 Adjusted ORs and 95% CIs for lung cancer by cooking, incense or mosquito coil use, and charcoal and wood stove use, by smoking status

Exposure Factor	Current or ex-smokers		Nonsmokers	
	No. of cases/controls	OR(95% CI)[1]	No. of cases/controls	OR(95% CI)[2]
Cooking frequency				
< Daily	58/73	1.00	145/385	1.00
Daily	210/130	**1.61 (1.01-2.56)**	282/972	0.89 (0.68-1.16)
Use of incense or mosquito coils				
< Daily	62/65	1.00	169/488	1.00
Daily	207/138	1.53 (0.97-2.41)	265/887	0.90 (0.71-1.14)
Use of charcoal stove				
< Daily	239/180	1.00	406/1236	1.00
Daily	30/21	1.08 (0.55-2.12)	26/129	0.67 (0.43-1.05)
Use of wood stove				
< Daily	215/167	1.00	387/1177	1.00
Daily	54/33	1.25 (0.74-2.12)	45/193	0.81 (0.56-1.17)

[1] Adjusted for age (years), education(years), housing type, environmental tobacco exposure (daily vs less than daily exposure), history of cancer in the first degree relative, duration of smoking (in yrs), intake of fruit and vegetable (servings/week) and a dummy variable indicating the study to which the participant belonged, by logistic regression analysis.

[2] Adjusted for age (years), education(years), housing type, environmental tobacco exposure (daily vs less than daily exposure), history of cancer in the first degree relative, intake of fruit and vegetable (servings/week) and a dummy variable indicating the study to which the participant belonged, by logistic regression analysis.

There was a statistically significant interaction between smoking and exposure to incense or mosquito coils (*P*=0.016) or frequency of cooking (*P*<0.001), respectively, after adjustment for potential confounders (Table 8.2). Relative to nonsmokers without daily incense or mosquito coils exposure, smokers without exposure had a two and a half-fold increase in risk (OR 2.80, 95% CI 1.86-4.21) and those with daily exposure had a four-fold (OR 4.61, 95% CI 3.41-6.24) increase in risk. The same pattern of risk was observed for daily cooking exposure (OR 2.31, 95% CI 1.52-3.51; OR 4.50, 95% CI 3.21-6.30), and for wood stove use, although the latter was not statistically significant (*P*=0.061). There was no interaction between smoking and daily use of charcoal (*P*= 0.128).

Discussion

We evaluated the effects of cooking and exposure to burning of incense and mosquito coils on lung cancer risk among Singapore Chinese women, and their modification by tobacco smoking exposure. We observed strong interactions between exposure to these sources, and smoking on lung cancer risk. The results indicate that tobacco exposure is not only an important risk factor for development of lung cancer, but smokers are also more susceptible to the risk-enhancing effect of these inhalants on lung cancer.

A possible explanation for our findings is the presence of a chronic inflammatory state in the airways induced by smoking. Tobacco smoke carcinogens are known to activate pro-inflammatory responses through the action of pro-oxidative chemicals, leading to the release of cytokines, production of reactive oxygen species (ROS) and ultimately to DNA damage (232, 233). A chronic inflammatory process in the lung could also lead directly to DNA damage, enhance the effects of other carcinogenic exposures and stimulate cell proliferation and growth (234). It was found that burning incense generated high

Table 8.2 Combined effects of indoor inhalants and tobacco smoke exposure on lung cancer risk

Exposure group	Smoking status	N	OR (95% CI) [1]
Cooking frequency			
< Daily	Nonsmokers	530	1.00
Daily	Nonsmokers	1254	0.83(0.64-1.08)
< Daily	Current or ex-smokers	131	**2.31(1.52-3.51)**
Daily	Current or ex-smokers	340	**4.50(3.21-6.30)**
$P_{interaction}{}^2$ **< 0.001**			
Use of incense or mosquito coils			
< Daily	Nonsmokers	657	1.00
Daily	Nonsmokers	1152	0.91(0.72-1.16)
< Daily	Current or ex-smokers	127	**2.80(1.86-4.21)**
Daily	Current or ex-smokers	345	**4.61(3.41-6.24)**
$P_{interaction}{}^2$ **= 0.016**			
Use of charcoal stove			
< Daily	Nonsmokers	1642	1.00
Daily	Nonsmokers	155	0.67(0.43-1.04)
< Daily	Current or ex-smokers	419	**4.08(3.21-5.18)**
Daily	Current or ex-smokers	51	**4.88(2.68-8.91)**
$P_{interaction}{}^2$ = 0.128			
Use of wood stove			
< Daily	Nonsmokers	1564	1.00
Daily	Nonsmokers	238	0.78(0.55-1.13)
< Daily	Current or ex-smokers	382	**3.95(3.08-5.07)**
Daily	Current or ex-smokers	87	**5.48(3.42-8.79)**
$P_{interaction}{}^2$ =0.061			

[1] Adjusted for age (years), education(years), housing type, environmental tobacco exposure (daily vs less than daily exposure), history of cancer in the first degree relative, intake of fruit and vegetable (servings/week) and a dummy variable indicating the study to which the participant belonged, by logistic regression analysis.

[2] *P* for the Likelihood Ratio test for interaction between smoking and cooking, incense or mosquito coils, charcoal stove and wood stove.

concentrations of reactive oxygen species (ROS) in the particulate gas phase of the emissions, and might damage DNA and other biomolecules when inhaled (235).

Our findings that risk among nonsmokers is not significantly increased by these exposures are at variance with other studies which have reported a positive association in this group. The odds ratio for female nonsmokers cooking three meals/day compared with those cooking one meal/day was 3.4 (95%CI, 1.6-7.0) in a study conducted in Taiwan (31). In the study among women in Taiwan, higher frequency of mosquito coil smoke use enhanced risk of lung cancer in both smokers and nonsmokers, although the interaction with cigarette smoke was, as in the current study, synergistic (34). Differences in cooking practices, use of fume extractors, type and intensity of use of mosquito coils, or simply in the average amount of time spent at home may contribute to the difference in findings between studies, even within Chinese populations. The proportion of women who had never been employed outside the home in our study was only 22% among controls, suggesting, overall, exposure to air pollutants in the domestic environment may be less substantial than in more traditional societies.

Contrary to previous reports, we did not find an association between use of charcoal or wood stove and lung cancer risk, among both smokers and nonsmokers. Local residents infrequently use traditional fuels (charcoal or wood), and usually use modern fuels (gas, kerosene or electricity) for cooking in Singapore, and the low frequency of use may be the chief explanation for our findings. Recall and reporting bias, if present, would shift the association towards the null, as they would likely affect both cases and controls to the same extent.

We had hypothesized that cooking and exposure to domestic inhalants such as burning of mosquito coils and incense would be risk conferring for lung cancer, and that these

effects would be stronger in never-smokers than in smokers, in whom the stronger effect of smoking might mask the effects due to these exposures. In contrast, the interaction between exposure to these sources and smoking on lung cancer risk was different to that expected, in which these exposures were associated with lung cancer only in the presence of smoking, and did not have an effect on never-smokers. Our study suggests that tobacco exposure is not only an important risk factor for development of lung cancer, but that smokers are more susceptible to the risk-enhancing effects of other inhalants. A possible mechanism consistent with recent findings is the presence of a chronic inflammatory state in the airways induced by smoking (14). The interaction observed is supportive of a model in which host susceptibility acts in concert with the exposures of interest to promote lung carcinogenesis. On the other hand, we did not find any evidence that these specific exposures contribute to increased risk of lung cancer among nonsmokers. As these are fairly common exposures in the indoor environment, it is important that smokers are aware of the significant additional risk afforded by these exposures. Further research is needed to establish more definitively the level of risk from these ubiquitous compounds in the domestic environment.

This research has been published (Tang L, et al. *Environmental Health Perspectives* 2010 Sep;118(9):1257-60, attached as Appendix E to this thesis).

CHAPTER 9 STUDY 5 – MEAT CONSUMPTION AND LUNG CANCER

Introduction

Dietary factors are likely to influence risk in never-smokers (28). While current evidence suggests that fruits and vegetables protect against the development of lung cancer, the role of meat and meat components such as saturated fats and cholesterol is less well-established (236-245). Some studies have found a protective effect (243) while others have found no effect (240-242, 245) or an increased risk (239, 244). The majority of studies have been conducted in European populations, where red meat comprised a large proportion of total meat consumed. The effect of meat on lung cancer is complicated for a few reasons: red and white meat may vary in their effects. Cooking methods may also be important. Well-done red meat has been associated with an increased risk of lung cancer (36), a finding which has been attributed to the presence of heterocyclic amines (HCAs), which are produced when meats are cooked at high temperatures (246). HCAs are mutagenic *in vitro* (247), and have been shown to produce tumors in rodents (248), and to increase risk in population-based studies (37, 249, 250).

Few studies have included sufficient numbers of never-smokers to be able to identify small effects of meat and meat components in this group and most results have been reported in studies where cases were primarily smokers. It is hence not known whether effects differ substantially between smokers and non-smokers.

Dietary patterns in Singapore differ substantially from those in western countries (251). Among the Chinese, fish represents a high proportion of meat consumed, and chicken and pork are other commonly-consumed meats. In contrast, beef and other red meats

like mutton and lamb are rarely consumed. Cooking methods in the Chinese population are also distinct from those in the west, with boiling, steaming, stewing and wok-frying being common methods of meat cooking, while grilling and deep-frying are much less common. As a consequence, meat cooking methods among the Chinese population in Singapore appear to generate much lower levels of dietary HCA compared to common cooking methods in the West (252).The relationship of meat consumption to lung cancer risk among the Chinese may therefore differ from that found in Western populations, and is not well-delineated in the literature.

In Study 5, we explore the effect of self-reported fresh and processed meat, and evaluate HCA exposure on lung cancer risk.

Methods

Data for this analysis were obtained from the second case-control study, comprising 399 cases and 815 controls.

Information on usual dietary intake of commonly available fruit (17 items), vegetables (21 items), soy products (10 items) and meats (18 items) was obtained via a semi-quantitative food frequency questionnaire. Consumption of the 6 common meat groups (fish, chicken, pork, duck, squid & prawns and beef) was elicited. For each meat type, participants indicated their consumption frequency (number of times per week) and portion size. Portion size was elicited as multiples of a standard serving specific to each food item. A standard serving was defined and weights of portion sizes calculated from a pilot study among a group of middle-aged and older Chinese women. Pictures of food portions were used to aid participants. Food frequency and usual portion size were

multiplied to define the number of standard servings per week. Consumption of each food item was then categorized in tertiles (based on the distribution among controls).

The questionnaire also elicited information on cooking methods for nine selected meat-cooking method combinations (e.g. pan-fried fish, pan fried chicken, roasted pork) and on the consumption of processed meats (bacon, ham, luncheon meat, sausages and Chinese sausages). The concentrations of 5 different heterocyclic amines (HCAs) had been previously determined in 25 commonly consumed and available meat items in Singapore (252). Five food items were found to have relatively high heterocyclic amine concentrations and were commonly consumed in our population (pan-fried fish, deep-fried chicken, pan-fried chicken, grilled/roasted pork and pan-fried pork). We used the estimates of HCA concentration for each food item obtained from our previous study (252) to derive an estimate for the usual weekly intake of HCAs (in nanogrammes) in our participants. We investigated the effects of 3 HCAs – PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine), MeIQx (2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 4,8-DiMeIQx (2-amino-3,4,8-trimethlimidazo[4,5-f]quinoxaline, as well as total HCA consumption.

Results

Table 9.1 describes the association between dietary factors and lung cancer. High consumption of fruits was inversely associated with lung cancer, with an odds ratio of 0.38 (P_{trend}< .001) in the highest tertile compared to the lowest. Consumption of vegetables was also inversely associated, with an odds ratio of 0.57 (P_{trend} = .005) in the highest tertile compared to the lowest, as were cruciferous vegetables (ORs 1.19, 0.65 in 2nd and 3rd tertiles, P_{trend} = .035).

Overall, meat consumption was inversely associated with lung cancer in this study population, with an odds ratio of 0.84 and 0.57 in the 2nd and 3rd tertiles respectively compared to the 1st tertile (P_{trend}= .001). Among controls, fish comprised 50% of the meat consumed by servings, pork 19% and chicken 24%. Duck, prawns and squid were not commonly consumed (with median consumption of only 1 serving a week). Beef was very rarely consumed, with about 80% of the study population reporting no consumption (data not shown).

We observed a strong inverse relationship between fish consumption and risk of lung cancer in non-smokers (odds ratio, 0.75, 0.45 for 2nd and 3rd tertiles of fish consumption respectively, P_{trend} < .001). We further examined the effect of fish by quintiles of intake. The inverse association was seen only in the 4th and 5th quintiles of fish consumption (Figure 9.1). These two quintiles were equivalent to consumption at 7.5-15 servings of fish per week, and more than 15 servings per week, respectively. Chicken and pork consumption had no significant effect on risk of lung cancer.

Overall, processed meats were not associated with lung cancer risk (ORs 0.97, 0.76, P_{trend} 0.17). Of the various constituents comprising processed meats, bacon increased lung cancer risk (OR 1.51, 95% CI 1.07-2.15, compared to participants who do not consume bacon). Ham, luncheon meat, sausages and Chinese sausages were not associated with lung cancer (Table 9.2). None of the 3 HCAs examined (PhIP, MelQx and DiMelQx) was associated with lung cancer risk, nor was total HCA (Table 9.3).

Table 9.1 Fruit, vegetable and meat consumption and risk of lung cancer in Chinese women, Singapore 2005-8.

Servings/ week	Cases (n=258)	Controls (n= 712)	Odds ratios (95%Confidence Intervals)[1]
Fruits[2]			
1st tertile (<3.88)	97 (37.6)	226 (31.7)	1.0
2nd tertile (>3.87-11.02)	108 (41.9)	246 (34.6)	0.86 (0.61,1.21)
3rd tertile (>11.02)	53 (20.5)	240 (33.7)	**0.38 (0.25,0.58)**
P_{trend}			**<0.001**
Vegetables[3]			
1st tertile (<11.88)	103 (39.9)	233 (32.7)	1.0
2nd tertile (>11.87-24.00)	88 (34.1)	236 (33.2)	0.77 (0.54,1.10)
3rd tertile (>24.00)	67 (26.0)	243 (34.1)	**0.57 (0.40,0.83)**
P_{trend}			**0.003**
Cruciferous vegetables[4]			
1st tertile (<5.61)	83 (32.2)	232 (32.6)	1.0
2nd tertile (>5.60-12.00)	110 (42.6)	241 (33.9)	1.19 (0.84,1.69)
3rd tertile (>12.00)	65 (25.2)	239 (33.6)	**0.65 (0.44,0.96)**
P_{trend}			**0.035**

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[1] Adjusted for age, history of cancer in 1st degree relative, country of origin , dwelling type, years of education, and fruit & vegetable intake (only for meat variables)

[2] Summed weekly consumption of: banana; papaya; apple; orange or mandarin orange; pineapple; watermelon; mango; starfruit; jackfruit; plum; cantaloupe/rock melon; dried prunes; fresh fruit juice; canned peaches; pear; Chinese pear; grape

[3] Summed weekly consumption of: wong-nga-pak; pak choy (Chinese cabbage); kai lan (Chinese kale); head cabbage; cauliflower; kai choy; choy sum; sai yong choy (watercress); broccoli; kang kong (water convolvulus); por choy (spinach); sang choy (Chinese lettuce); tomatoes; french beans; long beans; snow peas; ladies' fingers (okra); red carrot and sweet potato

[4] Cruciferous vegetables – wong-nga-pak; pak choy (Chinese cabbage); kai lan (Chinese kale); head cabbage; cauliflower; kai choy; choy sum; sai yong choy (watercress); broccoli

Total meats[5]			
1st tertile (<9.70)	103 (40.1)	232 (32.9)	1.0
2nd tertile (9.70-19.60)	93 (36.2)	238 (33.7)	0.84 (0.59,1.20)
3rd tertile (>19.60)	61 (23.7)	236 (33.4)	**0.57 (0.39,0.86)**
P_{trend}			**0.007**
Fish			
1st tertile (<2.51)	112 (43.4)	231 (32.4)	1.0
2nd tertile (>2.50-10.00)	100 (38.8)	265 (37.2)	0.75 (0.53,1.05)
3rd tertile (>10.00)	46 (17.8)	216 (30.3)	**0.45 (0.29,0.68)**
P_{trend}			**<0.001**
Chicken			
1st tertile (<1.01)	125 (48.5)	358 (50.3)	1.0
2nd tertile (>1.00-2.50)	49 (19.0)	132 (18.5)	1.03 (0.68,1.54)
3rd tertile (>2.50)	84 (32.6)	222(31.2)	0.99 (0.69,1.42)
P_{trend}			0.96
Pork			
1st tertile (<1.01)	106 (41.1)	323 (45.5)	1.0
2nd tertile (>1.00-2.50)	68 (26.4)	176 (24.8)	1.09 (0.75,1.58)
3rd tertile (>2.50)	84 (32.6)	211 (29.7)	1.15 (0.81,1.63)
P_{trend}			0.43

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[5] Summed intakes of fish, chicken, pork, duck, prawns & squid, and beef

Figure 9.1 Odds ratios and the 95% confidence intervals of the association of fish consumption with lung cancer among Chinese female never smokers, by quintile of consumption,
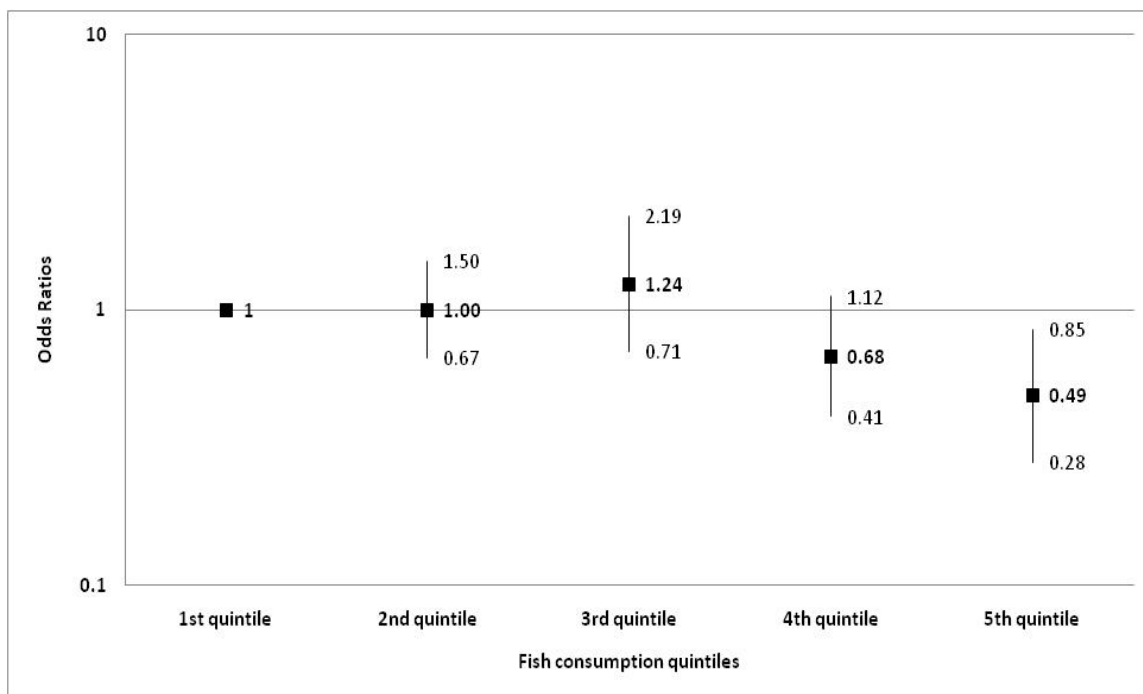
*Adjusted for age, history of cancer in first-degree relative, country of origin, dwelling type, yr of education, usual body mass index, fruit intake, and vegetable intake

Table 9.2 Consumption of processed meats and risk of lung cancer in never-smoking Chinese women

Servings/ week	Cases (n=258)	Controls (n= 712)	Odds ratios (95%Confidence Intervals)[6]
Processed meats[7]			
1st tertile (<0.30)	73 (28.6)	192 (27.2)	1.0
2nd tertile (0.30-<0.70)	100 (39.2)	263 (37.3)	0.97 (0.67,1.40)
3rd tertile (>0.69)	82 (32.2)	251 (35.6)	0.76 (0.51,1.13)
P_{trend}			0.17
Bacon[8]			
1st tertile (0)	173 (67.6)	548 (77.1)	1.0
2nd tertile (>0)	83 (32.4)	163 (22.9)	1.51 (1.07, 2.15)
Ham[9]			
1st tertile (0)	148 (59.6)	424 (59.6)	1.0
2nd tertile (>0-0.10	67 (26.0)	156 (21.9)	1.10 (0.76,1.59)
3rd tertile (>0.10)	43 (16.7)	132 (18.5)	0.80 (0.52,1.24)
P_{trend}			0.47
Luncheon Meat[10]			
1st tertile (<5.61)	101 (39.3)	306 (43.2)	1.0
2nd tertile (>5.60-12.00)	84 (32.7)	182 (25.7)	1.45 (1.01,2.09)
3rd tertile	72 (28.0)	221 (31.2)	1.05 (0.73,1.52)

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[6] Adjusted for age, history of cancer in 1st degree relative, country of origin , dwelling type, years of education, and fruit & vegetable intake
[7] Sum of serving portions consumed per week of the following 5 processed meat types: ham, bacon, luncheon meat, sausages and Chinese sausages
[8] 1 serving defined as 1 slice of bacon
[9] 1 serving defined as 1 slice of ham
[10] 1 serving defined as 1 slice of luncheon meat

(>12.00)			
P_{trend}			0.65
Sausages[11]			
1st tertile (<9.70)	147 (57.0)	436 (61.3)	1.0
2nd tertile (9.70-19.60)	34 (13.2)	77 (10.8)	1.31 (0.82,2.09)
3rd tertile (>19.60)	77 (29.8)	198 (27.9)	1.00 (0.70,1.42)
P_{trend}			0.90
Chinese sausages/waxed meat[12]			
1st tertile (<2.51)	113 (43.8)	355 (49.9)	1.0
2nd tertile (>2.50-10.00)	58 (22.5)	123 (17.3)	1.51 (1.01,2.24)
3rd tertile (>10.00)	87 (33.7)	233 (32.8)	1.13 (0.80,1.58)
P_{trend}			0.41

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[11] 1 serving defined as 0.5 sausages
[12] 1 serving defined as 2-3 slices of Chinese sausages or waxed meat.

Table 9.3 Dietary Heterocyclic Amines (HCA) and risk of lung cancer in never-smoking Chinese women

Nanogrammes/week	Cases (n=258)	Controls (n= 712)	Odds ratios (95%Confidence Intervals)[13]
Total HCA			
1st tertile (<92.32)	83 (32.4)	237 (33.6)	1.0
2nd tertile (>92.32-275.90)	98 (38.3)	238 (33.7)	1.21 (0.85-1.74)
3rd tertile (>275.90)	75 (28.3)	231 (32.7)	0.91 (0.62-1.34)
P_{trend}			0.66
PhIP			
1st tertile (<55.70)	83 (32.4)	239 (33.9)	1.0
2nd tertile (>55.70-160.47)	97 (37.9)	233 (33.0)	1.20 (0.83-1.71)
3rd tertile (>160.47)	76 (29.7)	234 (33.1)	0.89 (0.61-1.31)
P_{trend}			0.58
MeIQx			
1st tertile (<19.80)	90 (35.2)	238 (33.7)	1.0
2nd tertile (>19.79-62.90)	98 (38.3)	241 (34.1)	1.12 (0.78-1.59)
3rd tertile (>62.90)	68 (26.6)	228 (32.3)	0.78 (0.53-1.15)
P_{trend}			0.23
DiMeIQx			
1st tertile (<11.22)	92 (35.9)	238 (33.7)	1.0
2nd tertile (>11.22-34.85)	100 (39.1)	241 (34.1)	1.15 (0.81-1.63)
3rd tertile (>34.85)	64 (25.0)	228 (32.3)	0.71 (0.48-1.06)
P_{trend}			0.12

[13] Adjusted for age, history of cancer in 1st degree relative, country of origin , dwelling type, years of education, and fruit & vegetable intake

Further adjustment with self –reported exposure to meat cooking fumes did not alter the results obtained for fresh meat, processed meat and dietary HCA consumption and the association with lung cancer (data not shown).

Discussion

In this study of never-smokers, total meat intake (of mostly white meat – fish and chicken) is associated with a lower risk of lung cancer. Among meat components, fish consumption is inversely associated with lung cancer. Processed meats do not appear to be associated with lung cancer, although bacon may increase lung cancer risk among never-smokers. Dietary HCA levels arising from Chinese-style meat-cooking were not associated with lung cancer.

Recent research into the effect of meat on lung cancer risk have focused on red and processed meat. Carcinogenic compounds arising from the cooking of red meat such as HCAs (37, 244, 249, 250) and the Polycyclic Aromatic Hydrocarbon benzo(a)pyrene (244, 249) have been implicated as the mechanism conferring risk. In addition, processed meat products have also been identified as a possible risk factor for lung cancer (244, 249). Nitrosamines are formed from nitrates and nitrites added to processed meats (253), are carcinogenic (254) and may be the biological mechanism linking processed meats with lung cancer. The association of processed meats was not convincing in our study- most processed meats did not show a relationship with lung cancer, and while bacon appeared to be associated with lung cancer cases in our study, this increase was in the context of a comparison between non-consumers and ever-consumers. Consumption levels of processed meats (with the exception of Chinese

sausages) were generally low in our population. It is likely therefore that any risks associated with processed meats would be difficult to identify in our population.

We did not find a relationship with lung cancer of total HCA or any of the 3 common HCAs. This is not unexpected, as red meat consumption is relatively low in our population, and the Chinese cooking methods employed by our population do not generate significant quantities of HCAs as a by-product of cooking (251). Indeed, the consumption levels of the 3 HCAs studied in our population was about 6-8 times lower than that reported in the Missouri study (37).

Studies that have differentiated white from red meat have generally not shown any association of white meat with lung cancer (239, 240). This may be because white meat in general does not produce significant quantities of HCAs when cooked. Unusually, we show an inverse association with (mostly white) meat consumption in our population. A substitution effect of red meat by white meat intake is not a plausible explanation, given that red meat consumption is not high in our population, and no positive associations were seen with either pork or beef consumption. Confounding by socio-economic status is unlikely, since the relationship was significant in multivariate analyses, where we adjusted for SES using proxies of dwelling type and years of education. An inverse association of body mass index (BMI) with lung cancer risk, particularly in current smokers, has been proposed (255), and it is possible that meat consumption is confounded by BMI in our study, However, the relationship between meat consumption and lung cancer was not changed even after further adjustment for BMI based on "usual" weight, or weight before illness (data not shown). The most likely reason for the inverse effect of white meat is because of high fish consumption, which was the most common meat constituent consumed in this population.

The nature of the relationship between fish intake and lung cancer risk has not been clearly elucidated despite many studies looking at diet in lung cancer. Some case-control studies, especially those conducted in population with relatively low rates of fish consumption, have reported that fish consumption is positively associated with lung cancer (243, 256, 257). Other case-control studies, however, report inverse effects (239, 258, 259). In general, all these studies had a high proportion of smokers among cases, and did not look at effects separately in smokers and non-smokers. 3 case-control studies that did look specifically at never-smokers did not find an effect of fish consumption (241, 242, 260). An ecological study concluded that high fish consumption at the country level is associated with a reduction in lung cancer mortality in men, but only in countries with high levels of cigarette smoking or animal fat consumption (261). A Japanese cohort study (262) reported an inverse association of fish on incidence of lung cancer, but other studies did not find a relationship with lung cancer incidence in two cohorts in Norway (238, 263), nor with lung cancer mortality in one other cohort in Japan (264). Our data suggest that an inverse association is seen in never-smokers, and at intakes of more than 1 serving a day (Figure 9.1), and we speculate that the inconsistent findings between studies could be due to the high proportion of smokers among cases in these studies, and to differences in the range of consumption of fish in the base populations. (For example, the Galician study (243) that showed a positive relationship compared <1 time per week to >1 time per week, while the Japanese case-control study (258) showing an inverse relationship compared <1 time per week with 5 or more times per week in the highest consumption quartile.)

A protective effect of fish intake is biologically plausible. *In vitro* studies demonstrate that the omega -3 fatty acids eicosapentaenoic acid (EPA) and docosahexoic acid (DHA), both prominent constituents of fish oil, have anti-inflammatory, pro-apoptotic and anti-carcinogenic effects through their interactions with Peroxisome Proliferator-Activated Receptor-γ, nuclear factor kappa B, cyclooxygenase-2 and integrin-linked kinase (265-269). Some, but not all, epidemiologic studies investigating lung cancer risk with fish oil consumption support this (238, 270). Singapore imports almost all fish for retail consumption, of which 96-98% is consumed fresh (rather than as processed fish) (251), and relatively fatty fish such as mackerel and salmon represent a substantial proportion of fish consumed in Singapore (Agri-Food & Veterinary Authority of Singapore, unpublished data). The authors of a recent study of omega-3 content of local fish (271) estimated that consumption of about 50-60g of local fish (equivalent to about 2 serving sizes in this study) would provide about 350mg of DHA/EPA per day. It is also possible that the protective effect of fish is mediated through other vitamin or mineral constituents of fish such as selenium.

We used a semi-quantitative Food Frequency Questionnaire(FFQ), and reporting errors associated with FFQs are well-known. Such errors associated with the dietary instrument would likely be non-differential, and would bias estimates towards the null. Other types of reporting errors are also possible. We cannot exclude the possibility that dietary effects observed might have been due to dietary changes as a consequence of symptoms or disease. Because we used hospital controls, our control population's dietary intake may differ substantially from the general population because of their disease status. Further analyses excluding participants who reported that they had changed their diet recently due to illness or other factors (about 25% of controls and

31% of cases reported that they had done so) did not alter the relationship seen between meat and fish consumption and lung cancer risk in this population (data not shown). Our questionnaire did not allow us to control for total calorie intake; however, if the main findings were due to reduced overall consumption amongst cases compared to controls, we would have expected to see inverse associations across all food items. Further, adjusting for usual adult weight (as BMI) did not alter the results.

In summary, within the range of consumption prevalent in this Asian population, our findings support a protective role for white meat intake, and, specifically, fish intake, in the development of lung cancer in never-smokers.

This study has been published (Lim WY, *et al. Nutrition and Cancer* 2011; 63(6): 850-9, attached as Appendix F to this thesis).

CHAPTER 11 CONCLUSION

The research upon which this thesis is based investigated possible etiologic factors for never-smoker lung cancer. We took advantage of the relatively high incidence of lung cancer among a study population with low smoking prevalence (Singaporean Chinese females) to achieve sufficiently large numbers of never-smoker lung cancer cases. This permitted a case-control study with sufficient power to identify main effects (both environmental and genetic factors) with Odds Ratios of about 1.2-1.4. Through genotyping of biologic samples from participants, we were also able to extend the classical epidemiologic study of environmental factors by considering genetic risks and the potential interactions between the two.

In Studies 1 & 2, we added to the growing evidence in the literature that inflammation is important in lung carcinogenesis. We showed that this is true also in never-smokers, a group for which inflammatory factors, in particular genetic factors, have not been explored to any great extent. We further showed intriguing interactions between a previous history of atopic conditions and genetic polymorphisms in key genes in the inflammatory pathway. In paper 5, we found that meat consumption, and in particular of fish were inversely associated with lung cancer. Although we were unable to determine which component/s of fish this protective mechanism worked through, we highlighted the possibility that the protective effect of fish could be due to omega-3 fatty acids and their effects, among others, in reducing inflammation in the local tissue milieu through interaction with the cyclo-oxygenase pathway.

In Study 3, we explored another pathway that is currently under intense scrutiny – the estrogen pathway and reproductive factors. We provided evidence that reproductive

factors were implicated in lung carcinogenesis, especially in never-smoker lung cancer. We also postulated that the effects of reproduction were mediated through overall estrogen levels, and the effect of an estrogen metabolite – catechol estrogens.

In Study 4, we considered domestic inhalants as potential risk factors. Apart from the direct toxic effects on cellular processes and on DNA, some constituents of these inhalants are also known to be pro-inflammatory mediators. However, our hypothesis that such inhalants alone would be risk-conferring in never-smokers was not supported, and instead our data implied that a synergistic effect of inhalant exposure in the domestic environment with cigarette smoking on lung cancer might exist.

Collectively, our data identify both the inflammatory and estrogen signaling pathways to be important in lung carcinogenesis. Perhaps the most significant aspect of this set of findings is that chronic inflammation may play a bigger role in never-smoker lung cancer that has been previously recognized. This is consistent with what is known about carcinogenesis in general – inflammation is known to play a key role in the etiology of hepatocellular carcinoma and gallbladder carcinoma. Figure 10.1 summarises the etiologic mechanisms that this study has elucidated.

It is worthwhile to recall potential limitations of the case-control study design that may make data interpretation difficult, which include recall and interviewer bias, and reverse causation. The use of hospital controls may result in a control population that is not representative of the source population for some exposures of interest, such as NSAID use. Finally, the study was also underpowered to look at gene-environment interactions. We have tried to address these issues at both the implementation and analysis level, as discussed in Chapter 4.

Figure 10.1 Inflammation and estrogen as risk factors for lung cancer
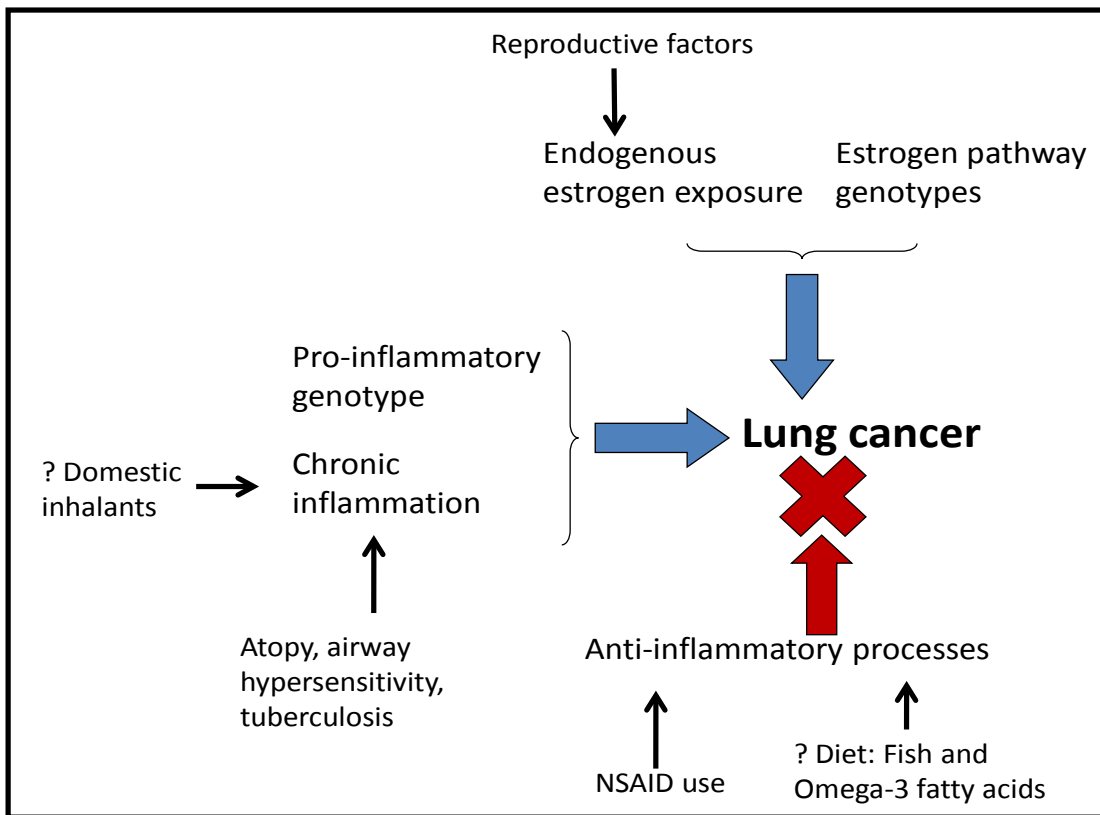
Further, in the field of observational epidemiology, where confounding and selection can be strong influences on findings, it is well-accepted by the research community that very few, if indeed, any, studies can be conclusive on their own. Instead, scientific consensus is usually reached only slowly, after evidence from the breadth of research conducted in different communities and with different study designs has been evaluated and synthesised. Further research then, will be helpful to confirm many of the observations we have made on the role of the inflammatory and estrogen pathways in lung carcinogenesis.

From an epidemiologic standpoint, further evaluation of exposures and genetic factors in both pathways in large well-designed case-control and cohort studies conducted in populations with a low prevalence of smoking are necessary, including pooling results from existing cohorts and case-control studies or performing meta-analyses of published results. If additional studies are conducted, improved precision in exposure delineation would improve the power of such studies to detect a true relationship. Examples of such improvements include a) using case-notes and medical records to obtain information about the presence of chronic lung, allergic or atopic conditions and use of NSAIDs; b) consideration of related factors such as ventilation and room partitioning, room size and amount of time spent by an individual in the presence of a fume source in the assessment of domestic inhalant exposure; and c) use of exposure biomarkers such as measures of estrogen activity in postmenopausal women, serum omega-3 fatty acid levels, and measures of fume emission products such as benzo(a)pyrene in the serum or plasma. Outcomes ascertainment should preferably be based on case-notes, medical records or reliable registry data, with or without histological ascertainment.

It is also important to consider gene-environment interaction, as our study suggests that these interactions may explain inconsistent findings in the literature. This implies that collection of biological samples for genetic analyses will be needed. It may also be useful to do a systematic evaluation of key genes in the inflammatory (such as IL10, IL1, IL6 and IL1-receptor antagonist) and estrogen (such as ER alpha and beta, CYP17 and CYP19, and COMT pathways). Such systematic evaluation should also consider possible effect modification by other exposures in the same pathway; in this regard, assessment of environmental exposures would be important. Genetic analyses should consider other types of genetic variations beyond Single Nucleotide Polymorphisms, such as Copy Number Variation, and DNA methylation patterns.

Non-epidemiologic biological research will also be useful. Such research includes genetic analyses to document all known variations in key genes of both pathways, taking into account ethnic variation, and functional assays to understand the effect of these polymorphisms. Finally, studies in *in vitro* and animal models are needed to understand the exact etiologic mechanism that underlies the role of inflammation and the mechanisms by which estrogen exerts effects in lung carcinogenesis.

Despite many advances in diagnostics and therapeutics, the survival for lung cancer remains exceedingly poor, with 5-year survival less than 10% (8). Lung cancer screening is still currently experimental, and there is as yet no consensus on its effectiveness in clinical use. For the foreseeable future, prevention will continue to be the key strategy in the public health control of lung cancer. The major advances in lung cancer control in the last half-century have been the identification of cigarette-smoking as a major preventable risk factor for lung cancer and the relatively successful public health efforts undertaken to educate the public and to effect behavior change. Much work still needs to be done in

the area of tobacco control, in particular in developing countries such as Indonesia and China, where smoking rates remain very high, and even in Singapore, which previously has had much success in reducing smoking prevalence but in recent years has seen a resurgence in smoking behavior (86-88).

Nevertheless, as lung cancer in never-smokers is not uncommon, identification of risk factors other than smoking is needed. These risk factors would unlikely have the extremely high impact that smoking has had, both because of the extremely high relative risk associated with smoking and the high prevalence of smoking. Nevertheless, further advances in lung cancer prevention will depend on the identification of such risk factors and the elucidation of the mechanisms through which these factors work to influence risk. Once such risk factors have been accurately identified, and the feasibility of reducing risk by removing these factors proven, public health practitioners can then use this knowledge to design and test lung cancer prevention strategies, thereby translating scientific findings into concrete measures aimed at influencing human behavior.

The research contained in this thesis should therefore be seen in this context. Although it is premature at this point to consider applying the results contained in this thesis in public health policy, this research nevertheless constitutes a small, but hopefully, significant and original contribution to etiologic research, and will in time form a part of the scientific pillars upon which public health practitioners can build a sound and effective prevention strategy for lung cancer in never-smokers.

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National University of Singapore
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Genes and Environment in Lung Cancer (GEL) Study
Questionnaire

Serial number ☐☐ / ☐☐☐☐

Status ☐ 1 = Case, 2 = Control, 3 = Unconfirmed case

Hospital ☐ 1 =SGH, 2=NUH, 3=TTSH, 4=CGH   Ward/ Bed/ Clinic _____

(Enter hospital at which diagnosis was made)

If control, number of refusals between last and current interview ☐

Date of interview ☐☐ / ☐☐ / ☐☐ *Hospital* ☐ *(if different from above)*

Date of diagnosis ☐☐ / ☐☐ / ☐☐ *If control, fill in date of admission*

Date of birth ☐☐ / ☐☐ / ☐☐

Age at diagnosis (in completed years) ☐☐ *if control, fill in age at admission*

Interviewer: start here

What is your (your father's) dialect group? ☐
1. Hokkien
2. Teochew
3. Cantonese
4. Hainanese
5. Hakka
6. Others _____

In which country were you born? ☐
1. Singapore
2. Malaysia *
3. PR China *
(specify city/province_____)
4. Other* _____

* In which year did you first move to Singapore? ☐☐☐☐

In your childhood (<15 years old), did you live.....? ☐
1. In a rural area (away from a town or city)
2. In an urban area (town or city)

In your early adulthood (15-30 years old), did you live.....? ☐
1. In a rural area (away from a town or city)
2. In an urban area (town or city)

During childhood (<15 years old), was your living area separate from your cooking area?

[ ]

1. Yes, separate living/sleeping and cooking areas
2. No, within the same living space

When you were a child, how many people were living in your household?

[ ]

Enter total number of adults and children sharing the same dwelling as the respondent (if living in an institution, enter '99')

When you were a child, how many people shared the same bedroom as you?

[ ]

Enter total number of other adults and children sharing the same bedroom as the respondent

When you were a child, how many people living in your household smoked any form of tobacco (cigarettes/pipes/ home-made cigarettes or cigars) on a daily basis?

[ ]
[ ]

Enter number of daily smokers in the house
If more than 0: Did this include
1. One parent
2. Both your parents
3. Neither of your parents

What type of dwelling do you <u>currently</u> live in?

[ ]

1. HDB/Other Govt 1-3 room flat (incl. shophouse, attap/zinc-roofed dwelling)
2. HDB 4-room flat

3. HDB 5-room flat or larger
4. HDB executive flat
5. Private apt or condominium
6. Terrace/semi-detached/bungalow
7. Other (state:_____)

Please tell me the street name and postal code of all residences you have lived in for more than 3 years since 1960 :

		Postcode	Type[*]	Level[**]	Traffic[***]	Years
R1	_____	[ ][ ][ ][ ]	[ ]	[ ]	[ ]	[ ][ ]
R2	_____	[ ][ ][ ][ ]	[ ]	[ ]	[ ]	[ ][ ]
R3	_____	[ ][ ][ ][ ]	[ ]	[ ]	[ ]	[ ][ ]
R4	_____	[ ][ ][ ][ ]	[ ]	[ ]	[ ]	[ ][ ]
R5	_____	[ ][ ][ ][ ]	[ ]	[ ]	[ ]	[ ][ ]
R6	_____	[ ][ ][ ][ ]	[ ]	[ ]	[ ]	[ ][ ]

*Code * 1 attap or zinc-roofed dwelling*   *4 Private apt or condominium*
*   2 shophouse(2-storeyed)*   *5 Terrace/semi-detached bungalow*
*   3 SIT/HDB flat*   *6 Other (State:_____)*
*** 1=street level/landed property   2 = 2nd-5th floor   3=6th-10th floor   4=above 10th floor*
**** 1=next to main road with heavy traffic flow   2= away from main road*

How many years did you attend school, in total? [ ][ ]   *Code "16" if number of years exceeds this*

Are you married / ever been married? [ ]   *1=ever married, 2=never married*

How many children have you had? [ ][ ]   *Include all livebirths*

In terms of occupation, are/were you.... [ ]

1. Currently employed / working outside home (includes self-employed)
2. Previously working outside the home until [ ][ ] years ago
3. Never worked outside the home for a year or more

(If currently/previously working)
Please tell me all the types of work you did for three or more years since 1960 : (cont'd next page)

Occ1		Code						
Occ2		Code						
Occ3		Code						
Occ4		Code						
Occ5		Code						
Occ6		Code						

"Did you work involve any of the following?" (1=yes, 2=no)

W1.	☐	Cooking or kitchen (including hawker food)
W2.	☐	Industrial food processing involving heating or cooking of food
W3.	☐	Contact with exhaust fumes from cars and other vehicles (e.g. bus conductor, car park attendant)
W4.	☐	Textiles (production, sewing, packing)
W5.	☐	Contact with glass wool, slag wool, or other mineral fibers
W6.	☐	Processing or packing of fine grit or powder from mineral sand
W7.	☐	Metal smelting
W8.	☐	Burning of coal/wood/kerosene or oil

Age of respondent 25 years before diagnosis = ☐☐ *years. Enter this into tables at boxes marked * **below**

	Cooking	At about age ☐☐ *
	Frequency codes 0 = never (less than yearly) 1 = less than once a month 2 = once a month – less than once a week 3 = once a week – less than once a day 4 = once a day 5 = more than once a day	
C1.	How old were you when you began to cook regularly (>1/week) at home? *If never cooked regularly, enter '99'*	years old
C2.	At about the age of ____*, how often did you (personally) **cook** at home? (if 0 or 1, then skip to C12)	
C3.	How often did you **stir-fry** food (including food that is stir fried and left to simmer)?	
C4.	How often did you **stir-fry** meat (e.g. pork, beef, chicken, fish, prawns. Does not include meat as part of a vegetable dish)?	
C4b	How often did you **stir-fry** meat as part of a vegetable dish?	
C5.	How often did you **deep-fry** food?	
C6.	How often did you **deep-fry** meat?	
C7.	What type of oil did you use **most often** for frying? 1 = Corn oil 5 = Soyabean oil 2 = Peanut oil 6 = Lard 3 = Palm oil 7 = sunflower or safflower oil 4 = Blended vegetable oil (e.g.knife or cock brand)	
C8.	How often did you eat **lard** with your food?	
C9.	How often do you fry your food with oil that has been used before?	
C10.	How often did the air in your kitchen become filled with oily "smoke" during frying?	
C11.	How often did your eyes feel irritated by the fumes during frying?	
C12.	In your home, was the cooking area in a different room from the living and sleeping areas? 1 = Yes 2 = No	

		Other inhalants	At about age __ __ __ *
		How often did you use the following type of stove for cooking at home? 1 = Never (less than monthly) 2 = Less than weekly 3 = Less than daily but more than weekly 4 = Daily	
S1.		Electric stove	
S2.		Gas stove	
S3.		Kerosene stove	
S4.		Wood stove	
S5.		Charcoal stove	
S6.		How often were joss sticks, scented coil / powder burnt inside your house? 1 = Never (less than monthly) 2 = Less than daily 3 = Once daily 4 = More than once a day/throughout the day 5 = Throughout the day and night	
S7.		How often did you use mosquito coils (spiral type) in your house? 1 = Never 2 = Seldom (less than once a week) 3 = Once a week or more 4 = Daily or nightly	

* age of respondent 25 years before diagnosis / current admission

Supplementary for those who began to cook before the age of 17

	Cooking (Enter age started to cook regularly (>1/week) in boxes**)	At about age __ __ __ **
	Frequency codes 0 = never (less than yearly) 1 = less than once a month 2 = once a month – less than once a week 3 = once a week – less than once a day 4 = once a day 5 = more than once a day	
CH1.	At about the age of ____**, *how often* did you (personally) **cook** at home?	
CH2.	How often did you **stir-fry** food (including food that is stir fried and left to simmer)?	
CH3.	How often did you **deep-fry** food?	
CH4.	How often did you use **lard** for frying (including frying pork fat on its own)?	
CH5.	How often did you eat **lard** with your food?	
CH6.	How often did you fry your food with oil that had been used before?	
CH7.	What type of oil did you use **most often** for cooking? 1 = Corn oil 5 = Soyabean oil 2 = Peanut oil 6 = Lard 3 = Palm oil 4 = Blended vegetable oil (e.g.knife or cock brand)	

CH8.	Which type of stove did you use **most often** for cooking? 1 = Electric stove 2 = Gas stove 3 = Kerosene stove 4 = Wood stove 5 = Charcoal stove	

	Smoking	
S8.	Have you ever smoked? (If yes) How many cigarettes have you ever smoked in your lifetime? 1= Never smoked 2 = Yes, fewer than 10 sticks in my lifetime 3 = Yes, between 10 and 100 sticks in my lifetime 4 = Yes, 100 or more sticks in my lifetime	
S9.	Have you ever smoked a cigarette (or any other form of tobacco) at least one a day for a year? 1 = Never (skip to S14) 2 = Ever, hand-rolled cigarette ('ang hoon') 3 = Ever, manufactured cigarette 4 = Ever, other form (specify _____)	
S10.	At what age did you start smoking?	years old
S11.	Have you smoked at least one cigarette in the past 30 days (prior to admission)? 1= No (go to S12) 2 = Yes (go to S13)	
S12.	At what age did you stop smoking? (if still smoking, enter current age)	years old
S13.	On the days that you smoked, on average, how many sticks did you smoke in a day?	sticks
S14.	Did any of your household members smoke (includes spouse, parents, children or any other relative/friends living with her) (in her presence?) 1 = No or less frequently than once a week (skip to S18) 2 = Yes	
S15.	Did these persons include your 1 = husband 2 = child(ren), parents or other relatives living with you 3 = both husband and other household members	
S16.	Did those persons smoke* 1 = Most of the day, everyday 2 = Most of the day, less often than everyday 3 = Some part of the day (less than 4 hours), everyday 4 = Some part of the day, less often than everyday	
S17.	For how many years did he/she/they smoke?* 1 = Less than 5 years 2 = 5-9 years 3 = 10-14 years 4 = 15-19 years 5 = 20 or more years	
S18.	At your workplace, were you in frequent (fairly continuous during the workday), close (in the same room) contact with colleagues who smoked? 1 = No 2 = Yes 3 = Not applicable (never worked outside the home)	

Meat intake

Place a tick in the box which corresponds to how often the respondent usually eats this type of food in the last one year. In the last column, circle A (small), B (medium) or C (large) according to photograph nearest his/her usual serving size.

No	Meats	Never or less than once a year	At least once a year but less than once a month	1-3 times a month	Once a week	2-3 times a week	4-6 times a week	Once a day	2-3 times a day	Usual portion size (only if respondent consumes >1/yr)
M1	FISH									
M1a	Pan fried fish									CHOOSE ONE – Photo 1 A – small B – medium C – large
M1b	Deep fried fish									
M2	CHICKEN									
M2a	Pan fried chicken									CHOOSE ONE – Photo 3 A – small B – medium C – large
M2b	Deep fried chicken									
M3	PORK									
M3a	Pan fried pork									CHOOSE ONE – Photo 7 A – small B – medium C – large
M3b	Roasted pork									
M3c	BBQ pork									
M3d	Bacon									CHOOSE ONE A – 1 slice or less B – 2 slices C – 3 slices or more
M3e	Ham									
M3f	Luncheon meat									
M3g	Sausages									A – ½ sausage B – 1 sausage C – 2 or more sausages
M3h	Chinese sausages or waxed meat									A – 2-3 slices or less B – 5 slices or equivalent C – 7-10 slices or more
M4	DUCK									CHOOSE ONE – Photo 3 A – small B – medium C – large
M5	PRAWNS and SQUID									CHOOSE ONE – Photo 11 A – small B – medium C – large
M6	BEEF									CHOOSE ONE – Photo 12 A – small B – medium C – large

Fruit and vegetable intake

Frequency :
Enter number of times eaten per week, on average, e.g. 7 for once daily. If consumed less than once a month, code 0, includes consumption both at home and outside of the home.

Portion size :
Enter fraction of medium serving. E.g. 2 for twice, 0.5 for half

(Average over the last 3 years)

		Medium serving	Frequency	Portion size
D1.	Banana	1 large or 2 small fruit		
D2.	Papaya	1 slice		
D3.	Apple	1 fruit		
D4.	Orange or Mandarin orange	1 fruit		
D5.	Pineapple	1 slice		
D6.	Watermelon	1 slice		
D7.	Mango	½ fruit		
D8.	Starfruit	1 fruit		
D9.	Jackfruit	6 seeds		
D10.	Plum	2 fruit		
D11.	Cantaloupe / rock melon	1 slice		
D12.	Dried prunes	5 pieces		
D13.	Fresh fruit juice (type : _____)	200ml glass		
D14.	Canned peaches	½ peach or 6 slices		
D15.	Wong-nga-pak	2 ch spoons		
D16.	Pak choy (Chinese cabbage)	2 ch spoons		
D17.	Kai lan (Chinese kale)	2 ch spoons		
D17a	Head cabbage	2 ch spoons		
D17b	Cauliflower	2 ch spoons		
D18.	Kai Choy	2 ch spoons		
D19.	Choy Sum	2 ch spoons		
D20.	Kang Kong (water convolvulus)	2 ch spoons		
D21.	Sai Yong Choy (watercress)	2 ch spoons		

		Medium serving	Frequency	Portion size
		Fruit and vegetable intake		
D22.	Por Choy (spinach)	2 ch spoons		
D23.	Sang choy (Chinese lettuce)	2 ch spoons		
D24.	Tomatoes	½ local tomato		
D25.	Broccoli	2 ch spoons		
D26.	French beans	2 ch spoons		
D27.	String (long) beans	2 ch spoons		
D28.	Snow peas	2 ch spoons		
D29.	Ladies' fingers	1 pc		
D30.	Carrot (Red)	2 ch spoons		
D31.	Sweet potato	6 wedges (1 hawker serving)		
D32.	Red chilli (fresh or pounded)	1 chilli or ½ Ch spoon if pounded		
D33.	Dried red chilli	2 pcs (1 Ch spoon)		
D34.	Soy beans (yellow)	1 Ch spoon		
D35.	Soy beans (black)	1 Ch spoon		
D36.	Soybean milk	1 200 ml glass		
D37.	Tau huay (soybean curd)	1 bowl		
D38.	Tau foo (including yong tau foo)	¼ big cake or tube		
D39.	Tau kwa (including yong tau foo)	1 cake (approx 2")		
D40.	Tau pok (including yong tau foo)	2 pcs		
D41.	Foo jook	½ pc (2 Ch spoons)		
D42.	Teem jook	1 pc (2 Ch spoons)		
D43.	Fermented soy beans (tau jeo)	½ Ch spoon or 2 tsp		

Has there been any recent (within the last three years) change in your diet due to illness or any other factor? (Circle) 1 = No, 2 = Yes (Specify) _____

_____

No		Never or less than once a year	At least once a year but less than once a month	1-3 times a month	Once a week	2-3 times a week	4-6 times a week	Once a day	2-3 times a day	4-5 times a day	6 or more times a day	Usual portion size
							Tea intake					
D44	Ceylon tea or western red tea											CHOOSE ONE A - ½ cup or less B - 1 cup (120ml) C - 2 cups or more
D45	Chinese tea Black tea (eg. Pu'er)/ Oolong (eg. Ti Kuan Yin)/ White tea (eg.Shou Mei) / Flower tea (Jasmine)											CHOOSE ONE A - ½ cup or less B - 1 cup (120ml) C - 2 cups or more
D46	Chinese (Lung Ching) or Japanese Green tea											CHOOSE ONE A - ½ cup or less B - 1 cup (120ml) C - 2 cups or more

	Other Host Factors	1=No 2=Yes	If Yes 1= < 20 yrs old 2 = 20-40 yrs 3= after age 40
	For HF1 to HF5 : Have you ever had any of the following illnesses? If Yes, when was this first diagnosed?		
HF1.	Tuberculosis		
HF2.	Chronic bronchitis or emphysema		
HF3.	Asthma		
HF4.	Pneumonia (chest infection) *during childhood*		
HF5a	Allergic rhinitis		
HF5b	Allergic skin disorders / atopic eczema		
HF5.	Has anyone in your family ever had cancer? 1 = No 2 = Yes		
HF6.	If yes, was it your …..? 1 = Parent, sibling or child 2 = Husband, or husband's family 3 = Other relative (specify: _____)		
HF7.	What type of cancer was it? 1 = Lung 2 = Other (specify : _____) ICDO-2: _____ (specify : _____) ICDO-2: _____		
HF8.	How old were you when you had your first regular menstrual period?		years old
HF9.	How old were when you had your first child? (if has not had children, code '99')		years old
HF10.	How old were you when you stopped having periods? (if pre-menopausal or had hysterectomy, code "99")		years old
HF11.	How often are/were your menstrual periods? *Record cycle length in days (e.g. once in 28 days)* *If less often than monthly, record approximate number of days between cycles (e.g. 60 for 2 monthly)* *If very irregular, ask how many periods a year and divide accordingly.*		days
HF12.	Have you ever had an operation to remove your womb (hysterectomy), and at what age was this? 1 = Yes (_____years old) 2 = No 3 = unsure		
HF13.	Have you ever had an operation to remove your ovaries (oophorectomy), and at what age was this? 1 = Yes (_____years old) 2 = No 3 = unsure		
HF14.	What is your usual (average) weight as an adult ?		kg
HF15.	What is your usual (average) height as an adult ?		cm

		Exogenous Hormone Use			

HT1	Have you ever taken hormones by pill or injection for one month or longer for menopause, birth control or any other reason?
	    1 = No / Don't know
	    2 = Yes, and currently taking them
	    3 = Yes, in the past				

HT2	For how many years did you take these hormones?
	    1 = Less than one year
	    2 = 1-2 years
	    3 = 3-5 years
	    4 = 6-9 years
	    5 = 10-14 years
	    6 = 15-19 years
	    7 = 20 years or more				

HT3	How old were you when you first started taking these hormones?
	    1 = Less than 40 years old
	    2 = 40-44 years old
	    3 = 45-49 years old
	    4 = 50-54 years old
	    5 = 55-59 years old
	    6 = 60 years or older				

HT4	What types of hormone pills or injections did you use on a regular basis for a month or more?				
		Name(s) *[brand or generic]*	Reason for use *[contraception / menopause / after removal of ovaries, etc.]*	Age started use	Duration of use (in months)
	Estrogen pills	1 2			
	Estrogen injection	1 2			
	Progesterone pills	1 2			
	Progesterone injection	1			
	Combined pills	1 2			

		Other Medication Use	

OM1	Have you ever taken pain-killers (analgesics) at least 2 times a week for a month or more?
	    1 = No / Don't know
	    2 = Yes, and currently taking them
	    3 = Yes, in the past		

OM2	Have you ever been on any long-term medication (more than 6 months) for any medical condition?
	    1 = No / Don't know (go to OM3)
	    2 = Yes (fill in details on next page)		

Name(s) *[brand or generic]*	Reason for use	Age started use	Duration of use (in months)

OM3	On average, how often have you had to take a course of antibiotics for a chest infection (e.g. a cough and fever which would not resolve)?
	    1 = Less often than five times in my lifetime / not that I can remember
	    2 = Less often than once a year
	    3 = A few times in a year
	    4 = Once every two months
	    5 = Once a month or more	

OM4	As a child (<15 years) how often did you have a chest infection (e.g. a cough and fever) that required you to take medication or see a doctor?
	    1 = Very infrequently / less often than once a year
	    2 = A few times in a year
	    3 = Once every two or three months
	    4 = Once a month or more	

OM5 — Did you use any of the following medications on a regular basis (at least twice a week for a month or more)?

	Ever a regular user 1=yes, 2=no	If yes, reason for use	Age started use	Duration of use (in months)
Aspirin (including Disprin and other brand names)				
Panadol, Biogesic, Panadeine or other forms of acetaminophen				
Voltaren, Synflex, Cataflam, Ponstan, Oruvail or other non-aspirin NSAID				
Celecoxib or other COX-2 inhibitor				
Prednisolone or other steroid pill				
Betamethasone, prednisolone or other steroid cream				

In case we need to clarify some of this information, could I have your contact number?   _____

For interviewer only: Assessment of interview data: 1   Good patient cooperative
                                                       2   Fair, patient quite cooperative
                                                       3   Poor

Serial number ☐☐ / ☐☐☐☐

Medical Record Abstract Form

If case :

Smoker: Current-smoker / Ex-smoker / Never-smoker

Name of attending physician / oncologist / radiotherapist : _____

Outcome : ☐ 1 = diagnosis confirmed   Date : ☐☐ / ☐☐ / ☐☐
 2 = other cancer (specify) : _____
 3 = other diagnosis (specify) : _____   Multiple ☐   1 = Yes 2 = No

Previous cancer: Yes/No. If yes, Site : _____ ICDO-2 _____ Yr of diagnosis: _____

If confirmed case :

Basis of diagnosis ☐ 1 = histology   Histology code ☐☐☐☐☐
 2 = cytology
 3 = radiology   Biopsy number : _____
 4 = clinical

Other remarks :

 Stage : I / II / III / IV or

On treatment at time of interview / specimen collection: No/Yes* (if so, describe   T_____ N_____ M_____
and give date started) :

If control:

Type of ward / clinic* : Medical (__) / orthopaedic / eye / ENT / surgical, *or*

Name of attending doctor : _____

Diagnosis on discharge: _____

History of cancer: Yes (Site: _____ year: _____) / No

Diabetes : Yes / No   Renal failure : Yes / No

Hypertension : Yes / No (remarks : _____)

Ischaemic heart disease : Yes / No (remarks : _____)

Chronic lung disease : Yes / No

Smoker: Current-smoker / Ex-smoker / Never-smoker / Not stated

Other remarks :

Follow-up on patient's status (cases only)

Treatment modalities received (*delete as appropriate):

Surgical resection: No / Yes * (date: _____) Chemotherapy: No / Yes* (date: _____)

Radiation therapy: No / Yes* (date: _____) Other: No / Yes* (specify: _____)

Date ☐☐ / ☐☐ / ☐☐ (1 year from interview)   1 alive, no change in diagnosis
 2 alive, diagnosis
Reviewed by _____   3 dead, lung cancer
 4 dead, diagnosis _____

Date ☐☐ / ☐☐ / ☐☐ (End of study)   1 alive, no change in diagnosis
 2 alive, diagnosis
Reviewed by _____   3 dead, lung cancer
 4 dead, diagnosis _____

Date of death ☐☐ / ☐☐ / ☐☐

Polymorphisms in inflammatory pathway genes, host factors and lung cancer risk in Chinese female never-smokers

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Inflammation appears to be important in lung carcinogenesis among smokers, but its role among never-smokers is not well established. We hypothesized that inflammatory medical conditions and gene polymorphisms interact to increase lung cancer risk in never-smokers. We interviewed 433 Singaporean female never-smoker lung cancer patients and 1375 hospital controls, and evaluated six polymorphisms in the interleukin 1-β, interleukin 6 (IL6), cyclooxygenase-2, peroxisome proliferator-activated receptor-γ and interleukin 1-β receptor antagonist (IL1RN) genes. Tuberculosis was associated with a non-significant elevated risk of lung cancer [odds ratio (OR) 1.58, 95% confidence interval (CI) 0.95–2.62]. There was no effect of asthma, atopy or chronic productive cough individually. However, the presence of one or more of these conditions (asthma, cough or atopy) increased risk (OR 2.24, 95% CI 1.15–4.38) in individuals possessing the T/T genotype at interleukin 1-β -31T/C, but not in those possessing the C/T (OR 0.87, 95% CI 0.51–1.57) or C/C genotypes (OR 0.58, 95% CI 0.27–1.27), and in individuals having the *2 variable number of tandem repeat allele of IL1RN [OR 5.09 (1.39–18.67)], but not in those without (OR 0.93, 95% CI 0.63–1.35). The IL6-634 G allele increased the risk of lung cancer (OR 1.44, 95% CI 1.07–1.94). Lung cancer risk also increased with the number of polymorphism sites where at least 1 'risk' allele was present [interleukin 1-β -31T/C (T allele), IL1RN (*2 allele) and IL6-634C/G (G allele)] among those with asthma, cough or atopy (P_{trend} 0.001) but not in those without (P_{trend} 0.47). Our results suggest that the effect of inflammatory medical conditions on lung cancer in never-smokers is modulated by host genetic susceptibility and will need to be confirmed in other studies conducted in similar populations.

Introduction

Tobacco use has been identified as the major risk factor for lung cancer, and over the past several decades, lung cancer incidence and mortality rates have paralleled tobacco use (1,2). However, there is substantial variation in the incidence of lung cancer that cannot be accounted for by tobacco use alone. In particular, the incidence of lung cancer in Chinese women in various populations around the world is much higher than expected given their relatively low smoking prevalence

Abbreviations: CI, confidence interval; IL6, interleukin 6; IL1-β, interleukin 1-β; IL1RN, IL1-β receptor antagonist; OR, odds ratio; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; VNTR, variable number of tandem repeat.

(3). Recent evidence suggests that lung cancer in never-smokers may be a different clinical entity from smoking-related lung cancer: its median age of onset is about a decade earlier than that of smoking-related lung cancer and it appears to have a better prognosis (4), incidence rates may be higher in women than men (5), and the cancer histology is more probably to be adenocarcinoma than other types (6). Mutations in the epithelial growth factor receptor are also more common in lung cancer in never-smokers than in smoking-related cancer (7).

Other risk factors such as environmental tobacco exposure (8), indoor air pollution from coal-burning heaters (9) or stoves (10), household radon exposure (11) and diet (12) have all been reported to contribute to lung cancer development in never-smokers, but the major etiologic pathways for this disease remain poorly understood. Recent research has focused on identifying a unifying hypothesis that could account for the risks seen in these disparate exposures, and the role of inflammation has been proposed as a possible candidate.

Inflammation is implicated in the pathogenesis of other cancers such as hepatocellular carcinoma (13), and disruptions in inflammatory signaling due to chronic inflammation of altered immune response may be a common pathway in carcinogenesis (14). One of the etiologic mechanisms by which tobacco use confers risk in lung cancer may be through its effects on the immune response in the lung and the resultant excess of proinflammatory molecules in the lung tissue milieu (15). Previous studies implicate a past history of chronic inflammatory lung disease such as tuberculosis (16,17), chronic bronchitis and emphysema (18), pulmonary fibrosis (19) and chronic rhinosinusitis (20), as risk factors for increased lung cancer risk in never-smokers. Asthma, allergic rhinitis and atopic dermatitis/eczema are related conditions that appear to be manifestations of an underlying systemic atopic disorder characterized by acute and chronic inflammation in target organs (lower respiratory tract, upper respiratory tract and skin, respectively). Although asthma has been reported previously to be associated with an increase in lung cancer risk (21–23), the effect of atopy is uncertain, with reports of null or protective effects (24–26). Other studies have identified genetic polymorphisms in key molecules in the inflammatory pathway, such as the interleukins [in particular interleukin 1-β (27–32) and interleukin 6 (IL6)] (33,34), the interleukin receptor antagonist (interleukin 1-β receptor antagonist (IL1RN)] (35,36), cyclooxygenase 2 (37,38) and peroxisome proliferator-activated receptor-γ (39) as risk factors.

It is probably that the role of inflammation in lung cancer is mediated through interplay between host susceptibility (as reflected through polymorphisms in key inflammatory genes) and environmental exposures that either cause inflammatory insult to the lung or confer protection in the lung from these insults. Therefore, a rational approach to identifying and quantifying lung cancer risks should consider both gene and environment factors in tandem. Such analyses may offer insights into the major biological pathways that drive carcinogenesis in lung cancer tissue. By identifying population groups at high risk of lung cancer, these findings may also have public health implications.

We hypothesize that previous inflammatory medical conditions (chronic lung disease, chronic cough and atopy) increase lung cancer risk in never-smokers and predict that these risks would be modulated by polymorphisms in inflammatory genes that have previously been identified as risk factors, in line with the concept of interplay between host genetic and acquired environmental factors.

Lung cancer is the second most common cancer in Singapore and the leading cause of cancer death (40). The incidence rate of lung cancer among Singaporean Chinese women is unexpectedly high (1,40) for the historically low rates of smoking in this group (41), and a significant proportion of lung cancers occur amongst never-smokers. We examined the association of six inflammatory gene polymorphisms and self-reported history of previous medical

conditions with lung cancer in a hospital-based case–control study of Chinese women.

Materials and methods

Participants were recruited in two hospital-based case–control studies in 1996–1998 (42) and 2005–2008 (43) from the five major public sector hospitals in Singapore. Both studies used similar study designs and questionnaires. Eligible cases were Chinese females with incident primary carcinoma of the lung (all histological types) identified within 3 months of diagnosis. Seven hundred and eighty-seven eligible lung cancer patients were identified in the five hospitals, of whom 702 (89.2%) agreed to participate. The response rate for cases was 95.0% in the first study and 84.6% in the second. Histological or cytological reports were reviewed and confirmed the diagnosis of primary lung carcinoma in 673 cases; 29 cases were confirmed on the basis of radiological investigations, in which metastatic cancer to the lung from other sites was deemed to be unlikely on clinical grounds.

Controls were selected from Chinese female patients admitted to the same hospitals and frequency matched for age (within 10 years) and date of admission. Patients admitted for a diagnosis and treatment of cancer or chronic respiratory disease were excluded, and ≤10% of controls were recruited within a single diagnostic category.

The response rate among controls was 91% (96.9% in the first study and 85.4% in the second), and data from a total of 1578 controls were available for analysis. Control patients were admitted for a wide range of conditions: 27% had diseases of skin, bones, joints and connective tissue, 11% were admitted for gastrointestinal or hepatobiliary system complaints, 14% were admitted for acute trauma, 8% were admitted for neurological or psychiatric conditions and 12% had diseases of the cardiovascular system.

Both cases and controls gave written, informed consent for the interview and the tracing of their medical records. Where consent was given, blood samples were also obtained. The study was approved by the Institutional Review Board of the National University of Singapore and participating healthcare institutions. In total, 702 cases and 1578 controls were recruited in the two studies, of which 433 cases (61.7%) and 1375 controls (87.1%) were never-smokers, defined as individuals who had not smoked at least one cigarette a day for a year. Of these, 298 cases and 718 controls provided blood samples.

A structured questionnaire was administered in-person by trained interviewers. Interviewers were not blinded to case or control status, but possible observer bias was monitored by recording and reviewing at random a sample of interviews conducted. The structured questionnaire elicited information of participants' demographic characteristics, occupational history, smoking history, family history of cancer, personal medical history (self-reported history of tuberculosis, chronic productive cough, asthma, allergic rhinitis and atopic eczema), diet (including intake of fruit and vegetable), childhood living conditions, reproductive history, exogenous hormone use and use of other medications and indoor environmental exposures such as passive tobacco exposure and exposure to kitchen fumes and inhalants such as incense and mosquito coils.

For cases and controls who provided blood samples, genomic DNA was extracted from the buffy coat of 5 ml of whole blood samples using the FlexiGene DNA kit (QIAGEN, Germantown, Maryland), in accordance with the manufacturer's protocol. Six polymorphisms in five inflammatory genes [−31 C/T (rs 1143627) and −511 C/T (rs16944) in the interleukin 1-β gene, −634 C/G (rs1800796) in the IL6 gene, 8473 C/T (rs5275) in the cyclooxygenase 2 gene, Pro12Ala in exon 2 (rs1801282) of the peroxisome proliferator-activated receptor-γ gene and the 86 base pair variable number of tandem repeats (VNTR) polymorphism in intron 2 of the IL1RNgene] were genotyped. The selection of sequence polymorphisms for genotyping was based on (i) their location in the promoter, untranslated region, or exons of the gene, or published evidence showing possible effects of the polymorphism on the level and activity of the gene products; (ii) a minor allele frequency ≥ 5% in the Chinese population from the NCBI database and (iii) associations with lung cancer reported previously by other researchers.

All single nucleotide polymorphisms (SNPs) were genotyped using a high-throughput genotyping platform base on a 5′ nuclease allelic discrimination assay in a 96-well format on the ABI StepPlusOne real-time polymerase chain reaction (PCR) system (Applied Biosystems, Carlsbad, California). The Taqman universal PCR master mix and predesigned SNP-genotyping assay mix containing PCR primers and probes were purchased from ABI. To ensure the accuracy of genotyping results, three positive controls and two negative controls were included in each 96-well plate, and 10% of DNA samples were genotyped in duplicate for each polymorphism. The concordance rate for the duplicate analyses was 100%. Call rates for the five SNPs studied ranged from 99.8 to 100%.

The intron 2 VNTR in the IL1RN gene was determined as described previously (35): primers (5′-CCCCTCAGCAACACTCC-3′ and 5′-GGTCA-

GAAGGGCAGAGA-3′) flanking the 86 bp tandem repeat region were used to amplify a DNA fragment containing the polymorphic region. PCR conditions comprised an initial denaturing step at 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 56°C for 40 s and 72°C for 45 s, with a final extension at 72°C for 10 min. The PCR products were then analyzed on 2% agarose gel electrophoresis along with a 100bp DNA marker. The wild-type allele designated allele I (IL1RN*1) (44) contains four 86 bp repeats and generated a 410 bp PCR product. The minor alleles were designated allele II to allele V (43) and corresponded to 240 bp (two repeats), 325 bp (three repeats), 500 bp (five repeats) and 959 bp (six repeats) PCR products. 10% of DNA samples were also genotyped in duplicate to ensure genotyping accuracy, and the concordance rate for the duplicates was 100%. The call rate was 99.7% for this analysis. All SNPs studied were in Hardy–Weinberg equilibrium in the control population.

Based on biologic considerations, as well as results from reverse stepwise analyses, we decided on a set of 11 variables for adjustment: age at diagnosis, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work, history of cancer in a first degree relative, mean intake of fruit and of vegetable (in servings/week) as well as a study set variable to indicate which case–control study the participant belonged to.

Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using unconditional logistic regression. We used STATA statistical software, version SE 10.1 (StataCorp LP, College Station, TX) for data analyses. All P values were calculated using two-tailed statistical tests, and the criterion for significance was set at $P < 0.05$. We adjusted for multiple testing using the method proposed by Benjamini et al.(45) to control the false discovery rate in our analyses of gene–environment interaction.

Results

Table I gives a summary of relevant characteristics of the lung cases and their controls in this study. The dialect group, birthplace, education level (in years of school) and current housing type of the participant, as well as a history of cancer in a first-degree relative, and her intake of fruit and vegetable were all significantly different between cases and controls in bivariate analyses. Two-thirds (63%, 271 cases) of cancers were adenocarcinomas.

Table II summarizes the effect of past history of lung disease or atopy on risk of lung cancer. Tuberculosis (OR 1.58, 95% CI 0.95–2.62) appeared to be associated with an increased risk of lung cancer although this was not statistically significant. Asthma (OR 1.01, 95% CI 0.66–1.56), chronic cough (OR 1.73, 95% CI 0.65–4.60) and allergic rhinitis/atopic eczema (OR 0.93, 95% CI 0.69–1.26) were not associated with an increased risk of lung cancer in our study population. The composite measure of chronic cough, asthma or allergic rhinitis/atopic eczema was also not associated with an increased risk of lung cancer (OR 0.97, 95%CI 0.74–1.27).

When stratified by −31T/C polymorphism genotype in IL-1β, a history of chronic cough, asthma or atopy increased risk only among participants with the T/T genotype (OR 2.24, 95% CI 1.15–4.38) but not in participants with the T/C or C/C genotype (Table III). The P for interaction, after adjustment for multiple testing, was 0.051. Because the polymorphism at the −511 position was in tight linkage disequilibrium with this polymorphism ($R^2 = 0.97$), similar results (data not shown) were obtained for the SNP at the −511 position and a history of asthma, atopy or chronic cough.

When stratified by genotype at the VNTR polymorphism in the IL1RN gene, a history of chronic cough, asthma or atopy increased the risk of lung cancer only in participants with the *2 allele (OR 5.09, 95% CI 1.39–18.67), but not in those with the *1 allele. The P value for interaction, after adjustment for multiple testing, was 0.058. Compared with having the *1/*1 genotype of the IL1RN gene and no history of chronic cough, asthma or atopy, the presence of at least 1 *2 allele and a positive history of chronic cough, asthma or atopy, but not either factor alone, was associated with an increased risk of lung cancer (OR 2.96, 95% CI 1.23–7.12). There was no modification of the effect of chronic cough, asthma or atopy by the other three genotypes studied.

Of the six sequence variations studied, the −634 C/G polymorphism in IL6 exhibited a main effect (Table IV). Using a codominant model, compared with the C/C genotype, the C/G genotype was associated with an increased risk of lung cancer (OR 1.51, 95% CI 1.11–2.05).

Table I. Sociodemographic characteristics of lung cancer cases and controls among Singaporean Chinese women never-smokers

	Cases [n, (%)] N = 433	Controls [n, (%)] N = 1375	P value[b]
Age in years[a] (mean, SD)	63.0 ± 12.5	63.6 ± 12.2	0.42
Dialect group[c]			0.008
Hokkien	151 (35.2)	577 (42.8)	
Teochew	109 (25.4)	265 (19.7)	
Cantonese	85 (19.8)	252 (18.7)	
Hainanese	38 (8.9)	80 (5.9)	
Hakka	31 (7.2)	125 (9.3)	
Other	15 (3.5)	49 (3.6)	
Birthplace			0.012
Singapore	274 (63.3)	900 (65.5)	
Malaysia	57 (13.2)	239 (17.4)	
China	85 (19.6)	198 (14.4)	
Other	17 (3.9)	38 (2.8)	
Education(year)			0.041
Nil	169 (39.0)	561 (40.8)	
≤6 years	119 (27.5)	435 (31.6)	
7 years or more	145 (33.5)	379 (27.6)	
Dwelling[c]			0.001
1–3 room flat	145 (33.7)	517 (37.8)	
4 room or larger flat	202 (47.0)	682 (49.8)	
Private apartment or house	83 (19.3)	170 (12.4)	
Marital status			0.809
Ever married	403 (93.1)	1275 (92.7)	
Never married	30 (6.9)	100 (7.3)	
Occupational status[c]			0.406
Currently employed outside home	128 (29.6)	374 (27.2)	
Ever employed outside home	202 (46.7)	691 (50.3)	
Never employed outside home	103 (23.8)	308 (22.4)	
Environmental tobacco smoke exposure at home[c]			0.297
< Daily	226 (53.1)	764 (55.9)	
Daily, <20 years	39 (9.2)	117 (8.6)	
Daily, 20 or more years	161 (37.8)	485 (35.5)	
Environmental tobacco smoke exposure at work[c]			0.061
No exposure/never worked outside the home	320 (74.8)	1084 (79.1)	
Exposed to smoking coworkers	108 (25.2)	287 (20.9)	
Family history of cancer[d]			<0.001
No	309 (71.4)	1112 (80.9)	
Yes, other sites	88 (20.3)	208 (15.1)	
Yes, lung cancer	36 (8.3)	55 (4.0)	
Intake of fruit, (mean, SD) (servings/week)	7.5 ± 7.1	9.3 ± 8.5	<0.001
Intake of vegetable, (mean, SD) (servings/week)	22.3 ± 19.4	25.9 ± 21.3	0.002
Histologic type			
Adenocarcinomas[e]	271 (62.6)		
Squamous cell carcinomas[f]	25 (5.8)		
Small cell carcinomas[g]	5 (1.2)		
Other histology[h]	115 (26.6)		
No histology/cytology	17 (3.9)		

[a]Refers to age at diagnosis (cases) and age at interview (controls)
[b]Pearson chi-square test for categorical variables and one way anova for continuous variables
[c]Numbers do not add up to N=433 for cases and N=1375 for controls because of missing responses to some of these variables: 31 missing for dialect group, 9 missing for dwelling type, 2 missing for occupational status, 16 missing for environmental tobacco exposure at home, and 9 missing for environmental tobacco exposure at work
[d]First-degree relative
[e]Histology codes (ICD-O-3) 8140/3—adenocarcinoma NOS, 8260/3—papillary adenocarcinoma, NOS, 8480/3—mucinous adenocarcinomas
[f]Histology code (ICD-O-3) 8070/3 squamous cell carcinoma, NOS
[g]Histology code (ICD O-3) 8041/3 small cell carcinoma, NOS
[h]Other histology codes, the most common being-(ICD-O-3) 8012/3 large cell carcinoma, NOS, 8046/3 non-small cell carcinoma, NOS, 8250/3 bronchioalveolar carcinoma

The G/G genotype was not associated with an increased risk (OR 1.00, 95% CI 0.49–2.05) although there were relatively small numbers in this group. Using a dominant model, the presence of the G allele was associated with an increased risk of lung cancer (OR 1.44, 95% CI 1.07–1.94) compared with having no G allele. This effect was not modulated by a history of tuberculosis, asthma, chronic cough or atopy.

Table V shows the additive effects of possessing one or more 'risk' alleles at the three gene polymorphism sites for which an association with lung cancer was found in our study (the T allele at IL-1β-31T/C

SNP site, the G allele at IL6-634C/G CNP site and *2 allele at IL1RN 86bp VNTR site). Compared with those without any alleles at these three sites, those persons having risk alleles at one site had an OR of 1.20, those with alleles at two sites, an OR of 1.57, and those with alleles at all three sites, an OR of 1.89. Although none of the individual ORs were statistically significant, the P value for trend was 0.026. The additive effect was seen only in those with a positive history of chronic cough, asthma or atopy (ORs 2.87, 6.76 for those with risk alleles at one and two or three sites, respectively, P for trend 0.001) but not in those without (ORs 0.98, 1.14 for those with risk alleles at

Table II. Effect of past medical history of lung disease or atopy on risk of lung cancer in Singaporean Chinese women never-smokers

Past medical history		Cases (n = 433)	Controls (n = 1375)	OR[a] (95% CI)	P value
Tuberculosis	Yes	27 (6.2)	53 (3.8)	1.58 (0.95–2.62)	0.080
	No	406 (93.8)	1322 (96.2)	1.0	
Chronic productive cough[b]	Yes	7 (1.6)	12 (0.9)	1.73 (0.65–4.60)	0.27
	No	425 (98.4)	1361 (99.1)	1.0	
Asthma	Yes	34 (7.8)	97 (7.1)	1.01 (0.66–1.56)	0.96
	No	399 (92.2)	1278 (92.9)	1.0	
Allergic rhinitis/atopic eczema	Yes	79 (18.2)	244 (17.8)	0.93 (0.69–1.26)	0.64
	No	354 (81.8)	1131 (82.2)	1.0	
Asthma or allergic rhinitis/atopic eczema	Yes	101 (23.3)	311 (22.6)	0.93 (0.70–1.22)	0.59
	No	332 (76.7)	1064 (77.4)	1.0	
Chronic productive cough, asthma or allergic rhinitis/atopic eczema	Yes	106 (24.5)	315 (22.9)	0.97 (0.74–1.27)	0.84
	No	327 (75.5)	1060 (77.1)	1.0	

[a]Adjusted for age, history of cancer in first degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set
[b]Three (one case and two controls) participants did not respond to this question

Table III. ORs and 95% CIs for the interaction between IL1β and IL1RN genotypes and a history of chronic cough/asthma/allergic eczema/atopic rhinitis on risk of lung cancer in Singaporean Chinese women never-smokers

Polymorphism	History of chronic cough, asthma or atopic eczema/allergic rhinitis	Cases (n = 298)	Controls (n = 718)	OR[a] (95% CI)	P values	Stratified analyses, by genotype OR[a] (95% CI)	P values
IL1β-31T/C							
C/C	No	49 (16.4)	119 (16.6)	1.0		1.0	
	Yes	16 (5.4)	43 (6.0)	0.60 (0.29–1.23)	0.16	0.58 (0.27–1.27)	0.17
T/C	No	121 (40.6)	292 (40.8)	0.90 (0.59–1.38)	0.64	1.0	
	Yes	31 (10.4)	66 (9.2)	0.80 (0.44–1.43)	0.44	0.87 (0.51–1.49)	0.62
T/T	No	51 (17.1)	158 (22.1)	0.62 (0.38–1.01)	0.053	1.0	
	Yes	30 (10.1)	38 (5.3)	1.44 (0.76–2.72)	0.26	2.24 (1.15–4.38)	0.018
LR test for interaction P value[b] = 0.051 (unadjusted P = 0.011)							
IL1RN							
*1/*1	No	187 (63.6)	486 (68.5)	1.0		1.0	
	Yes	64 (21.8)	135 (19.0)	0.93 (0.64–1.36)	0.70	0.93 (0.63–1.35)	0.70
*1/*2 or *2/*2	No	31 (10.5)	78 (11.0)	1.00 (0.62–1.62)	0.98	1.0	
	Yes	12 (4.1)	11 (1.6)	2.96 (1.23–7.12)	0.015	5.09 (1.39–18.67)	0.014
LR test for interaction P value[b] = 0.058 (unadjusted P = 0.029)							

[a]Adjusted for age, history of cancer in first-degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set
[b]Likelihood Ratio test for interaction P value adjusted for multiple testing using the method of Benjamini et al.(45)

one and two or three sites, respectively, P for trend 0.47), and this interaction was significant at P=0.035.

Supplementary Table 1, available on *Carcinogenesis* Online shows the likelihood ratio test P values for the interaction between the five SNPs with, separately, a history of tuberculosis, history of chronic cough, history of asthma and history of atopic eczema/allergic rhinitis. As IL1β511C/T and IL1β31T/C are in strong linkage disequilibrium, only the results for IL1β31T/C are shown. The P values for interaction for history of asthma with IL1β31T/C and IL1RN and for history of allergic rhinitis/atopic eczema with IL1RN are significant.

Supplementary Table 2, available on *Carcinogenesis* Online shows the results for the joint effect of the composite variable of chronic cough, asthma and allergic eczema/atopic eczema and the remaining three SNPs (IL6-634C/G, peroxisome proliferator-activated receptor Pro[12]Ala and cyclooxygenase 2 8973T/C). No statistical interactions were observed for the composite variable of chronic cough, asthma and allergic rhinitis/atopic eczema.

Supplementary Table 3, available on *Carcinogenesis* Online shows the ORs and 95% CIs for the joint effect of the two SNPs of interest (i.e. IL1β31T/C and IL1RN) with, separately, a history of chronic cough, asthma and allergic rhinitis/atopic eczema. The joint effects for each of these three variables are similar for both polymorphisms, and support our use of a composite variable comprising these three variables of chronic cough, asthma and allergic rhinitis/atopic eczema.

Discussion

Our results suggest that among inflammatory conditions of the lung, a history of tuberculosis (but not asthma, allergic rhinitis and atopic eczema and chronic cough, individually or in combination) may be associated with an increased risk of lung cancer although the ORs did not reach statistical significance. A positive association of chronic cough, asthma or atopy with lung cancer risk was evident in the presence of the T/T genotype in the IL1β and the *2 allele in the IL1RN genes. We also demonstrated an independent effect of IL6-634 C to G polymorphism in conferring risk. We found increasing ORs for lung cancer with increasing number of polymorphism sites where there was at least one 'risk' allele in those with a history of chronic cough, asthma and atopy but not in those without such a history. Taken collectively, these data support the hypothesis that inflammation plays a role in lung carcinogenesis among never-smokers.

In our study, the number of cases with a history of tuberculosis was relatively small, and the study was inadequately powered to detect a true association of this magnitude. Our finding of an increased, although non-significant, risk is consistent with other studies that have also reported increased risks associated with tuberculosis (46–48). Asthma was associated with a 1.8-fold increased risk of lung cancer in a meta-analysis of five case–control studies that studied the association in never-smokers (23), and other cohort studies support this finding (21,22). On the other hand, studies of the relationship of

Table IV. Effect of polymorphisms in six inflammatory pathway genes on the risk of lung cancer in Singaporean Chinese women never-smokers

Inflammatory gene	Genotype	Cases ($n = 298$)	Controls ($n = 718$)	OR[a] (95% CI)	P value	OR (95% CI)[b]
IL1β	−31T/C (rs 1143627)[c]					
	T/T	81 (27.2)	196 (27.4)	1.0		
	C/T	152 (51.0)	358 (50.0)	1.14 (0.80–1.61)	0.47	
	C/C	65 (21.8)	162 (22.6)	1.13 (0.74–1.71)	0.57	
	−511 C/T (rs16944)[c]					
	C/C	83 (27.9)	200 (27.9)	1.0		
	C/T	155 (52.0)	359 (50.1)	1.14 (0.81–1.60)	0.46	
	T/T	61 (20.1)	157 (21.9)	1.07 (0.70–1.63)	0.75	
IL6	−634 C/G (rs1800796)					
	C/C	163 (54.7)	449 (62.5)	1.0		1.0
	C/G	123 (41.3)	231 (32.2)	1.51 (1.11–2.05)	0.008	1.44 (1.07–1.94)
	G/G	12 (4.0)	38 (5.3)	1.00 (0.49–2.05)	0.99	P value = 0.015
PPAR-γ	Pro¹²Ala (rs1801282)					
	C/C	274 (92.0)	653 (91.0)	1.0		
	C/G	23 (7.7)	64 (8.9)	1.04 (0.62–1.76)	0.87	
	G/G	1 (0.3)	1 (0.1)			
COX-2	−8973 T/C (rs5275)[c]					
	T/T	182 (61.3)	462 (64.4)	1.0		
	T/C	100 (33.7)	228 (31.8)	1.20 (0.88–1.64)	0.25	
	C/C	15 (5.0)	28 (3.9)	1.32 (0.66–2.64)	0.44	
IL1RN	86 bp VNTR in intron 2[c,d]					
	*1/*1	251(85.4)	621 (87.5)	1.0		
	*1/*2	40 (13.6)	89 (12.5)	1.26 (0.83–1.92)	0.28	
	*2/*2	3 (1.0)	0 (0)			

IL1β -31 T/C and IL1β-511C/T are in linkage disequilibrium, $R^2 = 0.97$. COX-2, cyclooxygenase; PPAR- γ, peroxisome proliferator-activated receptor.
[a]Adjusted for age, history of cancer in first-degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study set
[b]Adjusted ORs, comparing participants with a genotype of C/G or G/G at IL6 −634C/G versus those with a C/C genotype
[c]Excludes samples with no-calls—two samples for IL1β −31 T/C (rs 1143627) and −511 C/T (rs16944), one sample for COX2 −8973 T/C (rs5275) and three samples for 86bp VNTR in intron 2 in IL1RN
[d]Further excludes seven participants with *1/*4 and two participants with *1/*5 genotypes

Table V. Additive effect of 'risk' alleles at three gene polymorphism sites [IL1β-31TC (T allele), IL1RN 86 bp VNTR (*2 allele), and IL6-634CG (G allele)] on lung cancer risk

Number of sites with at least 1 allele[a,b]	All			No history of chronic cough, asthma or allergic rhinitis/atopic eczema			History of chronic cough, asthma or allergic rhinitis/atopic eczema		
	Cases/controls N=298/718	ORs (95% CI)[c]	P value	Cases/controls	ORs (95% CI)	P value	Cases/controls	ORs (95% CI)	P value
0	25/91	1.0		21/68	1.0		4/23	1.0	
1	143/362	1.20 (0.71–2.01)	0.50	105/279	0.98 (0.55–1.75)	0.99	38/83	2.87 (0.71–11.53)	0.14
2	117/240	1.57 (0.93–2.67)	0.093	86/201	1.14 (0.64–2.05)	0.66	31/39	6.76 (1.68–27.13)	0.007
3	11/22	1.89 (0.75–4.75)	0.18	8/20			3/2		
		P for trend 0.026			P for trend 0.47			P for trend 0.001	

P value for interaction 0.035

[a]Excludes five samples with no-calls in at least one of the three polymorphism sites
[b]Counts were made for each individual based on the number of polymorphism sites in which there was at least one 'risk' allele: [IL1β-31TC (T allele), IL1RN 86bp VNTR (*2 allele) and IL6-634CG (G allele)]
[c]Adjusted for age, history of cancer in first-degree relative, fruit and vegetable consumption, country of origin, dialect group, housing type, number of years in school, environmental tobacco exposure at home, environmental tobacco exposure at work and study

systemic atopic conditions such as food allergies, allergic rhinitis or atopic eczema with lung cancer have mainly reported null or negative associations (24–26). Some authors (20) have proposed that local lung effects such as mucosal inflammation are the reason for the increased risk seen in asthma, rather than the shift of T lymphocyte response to a Th2-dominated activity in the hyperreactive state of the immune system, which is found also in systemic atopic conditions. If so, mild and infrequent asthmatic attacks and the use of local medications such as inhaled corticosteroids may mitigate the risk association and may explain our null findings. Chronic cough has been identified as an independent risk factor for lung cancer (49,50), especially among smokers. In our population of never-smokers, chronic productive cough are probably due to either undiagnosed asthma or to chronic obstructive pulmonary disease. Asthma is a known risk factor for

chronic obstructive pulmonary disease in non-smokers (51), and eczema, allergic rhinitis and asthma are known to occur in the same patient in sequence, an effect known as the atopic march (52). We believe, therefore, that the cluster of symptoms or diagnoses of chronic cough, asthma, allergic rhinitis and atopic eczema are related and point to persons with an underlying atopic phenotype and a predisposition to chronic inflammation in the lungs.

We observed a main effect with the IL6-634 polymorphism in our study population. There is biological plausibility for the role of the IL6-634 G allele in increasing lung cancer risk. The −634 SNP is in the promoter region of the IL6 gene, and *in vitro* studies have indicated that the G allele is associated with an increased production and secretion of IL-6 by peripheral blood mononuclear cells (53). Our group had previously reported (33), using data from the first

case–control study conducted between 1996–1998, that although a history of asthma or atopy and the G allele of IL6-634 did not increase risk of lung cancer on their own, the combined effect of the G allele and a history of asthma or atopy resulted in an OR of 3.1 (95% CIs 1.2–8.3) compared with the group with the C/C genotype and no history of asthma. We did not find any other studies investigating the IL6-634 SNP with lung cancer. Other groups have primarily investigated another SNP in the IL6 gene–IL6-174G/C (rs1800795), and most of these studies have reported null findings with this SNP (29–31,54).

Our study implicates the C allele in IL1β511C/T and the T allele in IL1β 31T/C and the *2 allele of IL1RN as alleles that confer risk to lung cancer in the presence of a background of atopy (allergic rhinitis or atopic eczema), chronic cough or asthma. The −31T/C polymorphism is a TATA-box polymorphism; the C allele disrupts this box and reduces binding and induction; hence suggesting that the T allele may be proinflammatory (55). Zienolddiny et al. (27) previously reported an increased risk of lung cancer with the T allele at IL1β31T/C, and Wu et al. (56) also reported that the T allele was associated with increased risk in a Chinese population, although other groups have reported null effects (29,31), and results from one other study implicated, in contrast, the C allele of −31T/C as the risk allele (32). These studies were conducted in study populations of smokers or mixed populations with high proportions of smokers (ranging from 84 to 96%). The inconsistencies in results could have been due to the different ethnic populations that were studied, as well as to the different distribution of relevant host factors such as exposure to environmental pollutants and pre-existing health conditions such as asthma. Results for C3954T (rs1143634), the other SNP in the IL1β gene that has been commonly studied, have been similarly inconsistent, with reports both of an increased risk associated with the T allele (31,57) and null effects (58).

The 86bp VNTR polymorphism of the IL1RN gene contains potential regulatory protein-binding sites (43), and probably has functional significance in the regulation of IL1-Ra production. In opposition to our findings, Hu et al. (35) reported reduced risks of lung cancer with the *2 allele in ethnic Chinese, but the study population in that report was predominantly (70%) male and smokers (60% of cases and 48% of controls), with relatively fewer adenocarcinomas among the lung cancer cases (38% of cancers). Further epidemiologic studies to delineate the main effect and possible interactions of IL1RN alleles are needed, as are functional studies to clearly describe the effect of the *2 allele in biological systems.

Our analysis of the summed effect of alleles at these three polymorphism sites suggests that there is an additive effect in lung cancer risk with increasing number of polymorphism sites where there was at least one allele present among those with a history of chronic cough, asthma and atopy, but not among those without. Although this analysis was based on relatively small numbers of cases and controls with chronic cough, asthma and atopy, this finding, if replicated, would suggest that the effects of inflammatory gene polymorphisms are important only in the presence of relevant host factors such as previous medical history.

The gene–environment interactions observed in our study suggest that failure to take into account environmental and personal risk factors may explain the inconsistency of results obtained thus far with studies looking at the association of inflammation with lung cancer risk. Rothman et al. (59) conceptualized a causal pie where combinations of risk factors explain the occurrence of non-communicable diseases with multiple etiologies such as cancer. Most of these factors are neither necessary nor sufficient in themselves to cause illness, and it is the combination of factors that determine the risk to any individual. Some risk factors (for example, smoking) may have such strong biological effects that, regardless of the underlying host genetic susceptibility or the presence of other risk factors, these factors invariably confer risk. Other factors may have weaker effects, and the risk associated with these factors may manifest only in hosts with underlying predisposition. Applying this concept to the role of inflammation in lung carcinogenesis, and in the light of our findings, the development of inflammatory pathway perturbations that result in lung carcinogenesis may depend on both the presence of 'environmental' risks that predispose to inflammatory pathway disruptions such as personal medical history as well as on underlying host genetic susceptibility to such perturbations.

Our study represents, to our knowledge, the first study of inflammatory genotypes and lung cancer in a large group of never-smokers. This feature has allowed us to investigate weak associations in this subgroup, which may be overshadowed by smoking-related effects in other populations.

Previous studies of genetic polymorphisms have used study populations of smokers or mixed populations where smokers comprised a heavy majority. Despite the inconsistencies in findings, the evidence overall appears to suggest that both a medical history of lung or inflammatory conditions and genetic variation in the inflammatory gene pathways are associated with lung cancer risk in smokers. Our study adds to this body of knowledge by suggesting that similar associations are seen in never-smokers.

On the other hand, the retrospective nature of our study and the use of hospital controls may complicate the interpretation of our results. To reduce possible selection bias, we sampled from a wide variety of hospital departments and admission symptoms. In addition, we excluded patients admitted for cancers or chronic respiratory conditions. Even if there were a selection bias in our study (with enrichment of persons with chronic diseases in the control group), the direction of this bias would have resulted in an underestimate of the true risk. We depended on participant reports of their medical history, and there may have been reporting bias with cases being more probably to report a positive medical history than controls. Because of the similarity of symptoms, some cases might have been misdiagnosed as asthma prior to the diagnosis of lung cancer being made. As we had not asked the age of onset of their pre-existing medical condition, we were not able to exclude reports of medical conditions of recent onset that could have been misdiagnosed lung cancer. However, we do not believe that this was a major source of bias because diagnostic chest imaging is readily available to family practitioners in Singapore, and this would have correctly identified lung cancer as the cause of their symptoms for most patients. We did not conduct any interviews solely with next of kin, hence eliminating possible biases resulting from proxy reports.

Furthermore, our results, especially with regard to those showing gene–environment interactions, should be considered to be exploratory in nature. Only a subset of participants provided blood samples. Our analysis suggested that there were minimal differences between cases and controls that provided blood samples and those who did not (data not shown). The exception to this was in environmental tobacco smoke exposure amongst controls: 52% of those who provided blood specimens reported environmental tobacco smoke exposure compared with 44% in the study population as a whole ($P < 0.05$). Environmental tobacco smoke exposure has been linked to asthma, and the higher proportion of asthma among controls providing blood samples may have resulted in an attenuation of the actual effect of the composite variables of cough, asthma and atopy in analyses stratified by genotype. Mechanistic or biological effect is plausible for these interactions, but the likelihood ratio tests for interaction, although significant without adjustment for multiple testing at $P < 0.05$, gave borderline significant P values after adjustment, and confirmation of these findings in other well-designed studies of lung cancer in never-smokers is needed.

In summary, our results suggest that acquired inflammatory medical conditions and inherited polymorphisms of genes in the inflammatory response pathway may interact to confer risk in lung carcinogenesis among never-smokers. Our finding that the cluster of conditions of chronic cough, asthma and atopy confer risk only in the presence of proinflammatory genotypes linked to the IL-1 cytokine emphasizes the need to consider host genetic susceptibility when investigating putative environmental or acquired risk factors in etiologic studies.

Supplementary material

Supplementary Tables 1–3 can be found at http://carcin.oxfordjournals.org/

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Aspirin and non-aspirin non-steroidal anti-inflammatory drug use and risk of lung cancer

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ABSTRACT

There is evidence that aspirin and non-aspirin non-steroidal anti-inflammatory drug (NSAID) have anti-carcinogenic properties, but their effect on lung cancer, in particular in never-smokers, is unclear. Information on past or current use of anti-inflammatory medication was obtained in 398 Chinese female primary lung cancer cases and 814 controls in a hospital-based study in Singapore. 65% of cases and 88% of controls were never-smokers. Controls were excluded if they had been admitted for conditions associated with aspirin or NSAID use ($n = 174$). Regular aspirin use (twice a week or more, for a month or more) was associated with a reduced risk of lung cancer (adjusted odds ratio [OR] 0.50, 95% confidence intervals [95%CI] 0.31–0.81 in non-smokers; OR 0.38, 95%CI 0.16–0.93 in smokers). Regular use of non-aspirin NSAID, paracetamol, steroid creams and steroid pills was uncommon and no association with lung cancer was detected. Our results suggest that aspirin consumption may reduce lung cancer risk in Asian women and are consistent with current understanding of the role of cyclooxygenase in lung carcinogenesis.

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1. Introduction

Aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) are widely prescribed analgesic and anti-inflammatory agents. Low-dose aspirin has also been prescribed as prophylaxis against ischaemic cardiac and neurological events. The biological effect of aspirin and NSAIDs appears to be mediated mainly through the inhibition of cyclooxygenase (COX) enzyme. COX converts arachidonic acid to prostaglandin G and H2, which are further converted to a variety of eicosanoids [1]. Activities of these eicosanoids include anti-apoptosis, promotion of angiogenesis, and stimulation of estrogen synthesis [2,3]. There are 2 isoforms of COX: constitutively expressed COX-1, and COX-2, which is inducible [1]. While aspirin and earlier NSAIDs are non-selective, a new class of COX-2 specific inhibitors such as celecoxib was developed in the early 2000s. Aspirin and other NSAIDs differ from each other both in terms of their relative affinity for the 2 isoforms of COX, and in their mechanism of inhibition of COX. Notably, aspirin causes irreversible inhibition through covalent bonding of the COX molecule while most other NSAIDs exhibit competitive reversible inhibition [3].

In vitro and animal studies support a protective effect of aspirin and non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) on cancer risk. Initial studies in colorectal cancer found increased cyclo-oxygenase-2 (COX-2) expression in colorectal tumour tissue compared to non-tumour tissue [4]. In mice studies, COX-2 null APC$^{\Delta 716}$ (a mouse model where truncation of the Adenomatous Polyposis Coli gene induces intestinal polyposis) mice showed decreased intestinal polyp formation compared to COX-2 positive mice; polyp formation was reduced in COX-2 positive mice fed a novel COX-2 inhibitor [5]. Human epidemiologic evidence supports the role of aspirin in protecting against colorectal cancer, with risk reduction estimates of 20–50% [6,7].

There is increasing evidence that aspirin and non-aspirin NSAIDs may also play a protective role in lung cancer. COX-2 enzymes are constitutively expressed in lung neoplastic tissue at higher levels than in non-tumour tissue [8]. Animal studies show that these drugs protect against experimentally induced lung cancer [9]. Clinical evidence in human patients also suggests that COX-2 expression is associated with poorer lung cancer survival [10]. Further, experimental studies suggest that COX-2 regulates the activity and expression of epithelial growth factor receptor (EGFR), and is

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itself regulated by EGFR in complex ways that appear to depend on cell-type specificity and cell environment [11,12]. This interaction could be important in the light of recent findings that EGFR mutations are common in lung adenocarcinomas among non-smokers, women, and Asians [12]. The role of inflammation (and the effect of anti-inflammatory agents such as NSAIDs) in lung carcinogenesis may therefore be subject to effect modification by factors such as ethnicity, gender and smoking status.

Epidemiologic studies of the role of NSAIDs and aspirin on lung cancer risk have been suggestive but not conclusive [13–38], with both protective and null effects being reported. Three meta-analyses have been performed on the available data, with contrasting results: one [17] reported a non-statistically significant reduced pooled OR for non-aspirin NSAID, but no effect for aspirin, a second [33] reported significant reductions in risk with regular use of aspirin and NSAIDS, but cautioned that the association may not be causal, while the third [34] reported a borderline significant reduction in risk, with significant differences in results of pooled analysis of case–control studies (significant protective effect) compared to cohort studies (null effect). Few studies have looked at the effect in never-smokers, and, to our knowledge, no studies have been performed in an Asian population.

Lung cancer is the second most common cancer and the leading cause of cancer death in Singapore [39]. The incidence rate of lung cancer among Singaporean Chinese women is unexpectedly high [39] for the historically low rates of smoking in this group [40], and a significant proportion of lung cancers occur among non-smokers. We investigate the possible association between regular aspirin and non-aspirin NSAID use and lung cancer, and explored whether smoking status and lung cancer tumour type modified the association, using data from a hospital-based case–control study of 398 Chinese female lung cancer patients and 814 controls conducted in 2005–2008 in the 5 major public sector hospitals in Singapore.

2. Materials and methods

A detailed account of our methodology is given elsewhere [41]. Briefly, eligible cases were Chinese women with a diagnosis of primary lung carcinoma (all histological types). Of 496 lung cancer patients identified, 473 were eligible, and 400 (84.6%) consented to the interview. Histological or cytological reports were reviewed and confirmed the diagnosis of primary lung carcinoma in 366 of these women; we excluded 2 patients who were diagnosed with metastatic cancer to the lung. 32 cases were confirmed on the basis of radiological investigations in which metastatic cancer to the lung from other sites was deemed clinically unlikely. A total of 398 confirmed lung cancer cases were included.

Controls were obtained from patients admitted to the same hospitals, frequency-matched by 10-year age groups. Exclusion criteria were the diagnosis or management of malignancy or chronic respiratory disease (excluding tuberculosis). Controls were admitted from a wide range of diagnoses and no more than 10% of controls shared the same diagnosis. 821 out of 962 patients (85.4%) agreed to participate. 6 were subsequently excluded because malignancy was diagnosed, and 1 was reclassified as a case. In total 814 controls were enrolled. Of these, 85 were admitted for chest pain, acute myocardial infarction, or heart failure, 11 for stroke or transient ischaemic attack, and 73 for musculoskeletal pain. A further 5 were admitted for conditions directly related to NSAID use (2 persons with ulcers due to NSAID use, 1 person with migraine and rebound headache secondary to analgesia use, 1 person with non-ulcer dyspepsia secondary to NSAID use, and 1 person with nephropathy secondary to NSAID use). These were excluded for this analysis, leaving 640 controls for this analysis.

Research nurses conducted face-to-face interviews of both cases and controls using a standardised questionnaire. A random sample of interviews were recorded and reviewed as part of quality checks. Next-of-kin were allowed to corroborate information provided by the subjects, but proxy interviews were not used. The study protocol was approved by the National University of Singapore Institutional Review Board and the Ethics Committees of the participating healthcare institutions.

The structured questionnaire elicited information about demographic characteristics, occupational history, smoking history, family history of cancer, personal medical history, diet (including intake of fruits and vegetables), and indoor environmental exposures. History of regular use (defined as use at least twice a week for a period of a month or more) of aspirin, non-aspirin NSAID and COX-2 selective NSAID, paracetamol, steroid cream and steroid pills was obtained. For each drug, the age at which use was started and the duration of use (in months) were also elicited. Never-smokers were defined as participants who had not smoked at least 1 cigarette a day for at least a year. Ex-smokers were defined as those who had not smoked any cigarette in the 30 days prior to the interview. Univariate analyses were performed using the chi-square test, t-test and one-way ANOVA, where appropriate. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated using unconditional logistic regression. STATA statistical software, version SE 10.1 (StataCorp LP, TX, USA) was used for data. All P value calculations are two-tailed, with significance set at $P < 0.05$.

Based on the results of reverse stepwise analyses and our own literature review, we decided on a set of 6 variables for adjustment in the multivariate analyses: age at diagnosis, housing type, educational history (in years of school), history of cancer in first degree relative, and intake of fruits and vegetables. Among smokers, smoking duration, the average number of cigarettes smoked, and the duration since an individual stopped smoking were independent risk factors (not shown). Logistic models of combinations of these 3 variables showed that the duration of smoking alone adequately explained the variation in risk attributable to smoking among smokers, and this alone was added as an adjustment variable for smokers.

3. Results

Table 1 gives a summary of relevant characteristics of the lung cases and their controls in this study, stratified by smoking status. Among never-smokers, cases and controls differed significantly in terms of average weekly fruit and vegetable consumption, housing type, educational level, and family history (in 1st degree relative) of cancer. Two-thirds (65%) of cases and 88% of controls were never-smokers. The majority of histological diagnoses (64% among never-smoker cases and 42% of smoker cases) were adenocarcinomas.

Comparing long-term aspirin users to non-users in cases and controls, aspirin users were older than non-users in both cases and controls, and also had fewer years of education than non-users. Among cases, aspirin users were less likely to report a positive family history of lung cancer (see Supplementary Table 4).

Table 2 summarises the overall results of self-reported regular intake of 6 medication groups on lung cancer risk. Previous regular intake of aspirin was associated with a statistically significant lower risk of lung cancer in both never-smokers (OR 0.50, 95%CI 0.31–0.81) and smokers (OR 0.38, 95%CI 0.16–0.93 in smokers). No associations were seen with non-aspirin NSAID, paracetamol and COX-2 inhibitors, although the numbers reporting regular use were very small. No associations were seen with regular use of steroid pills or cream.

Table 3 summarises the association between onset of aspirin use and duration of use on lung cancer risk in non-smokers. The

Table 1
Baseline characteristics of female Chinese lung cancer patients and controls, Singapore, 2005–2008.

	All			Never-smokers			Smokers		
	Cases (n = 398)	Controls (n = 640)	P	Cases (n = 257)	Controls (n = 561)	P	Cases (n = 141)	Controls (n = 79)	P
Age in years									
Mean ± SD	66.68 ± 11.63	65.02 ± 11.69	0.026	63.85 ± 12.01	64.44 ± 11.51	0.50	71.84 ± 8.85	69.14 ± 12.18	0.31
Median	(68)	(66)		(64)	(66)		(72)	(72)	
Average weekly fruit consumption (servings)									
Mean ± SD	7.40 ± 9.52	9.55 ± 9.05	<0.001	9.84 ± 9.01	7.69 ± 7.32	<0.001	6.87 ± 12.60	7.49 ± 9.10	0.70
Median	(4.85)	(7.51)		(5.76)	(7.81)		(3.39)	(5.01)	
Average weekly vegetable consumption (servings)									
Mean ± SD	19.64 ± 20.38	23.03 ± 20.34	0.009	20.37 ± 20.38	23.53 ± 20.85	0.043	18.30 ± 20.38	19.43 ± 15.85	0.67
Median	(13.76)	(17.95)		(14.50)	(18.21)		(12.51)	(15.36)	

	All			Never-smokers			Smokers		
	Cases, n (%)	Controls, n (%)	P	Cases, n (%)	Controls, n (%)	P	Cases, n (%)	Controls, n (%)	P
Dialect group			0.71			0.38			0.08
Hokkien	175 (44.1)	287 (45.0)		92 (35.9)	242 (43.2)		83 (58.9)	45 (57.7)	
Teochew	87 (21.9)	129 (20.2)		65 (25.4)	115 (20.5)		22 (15.6)	14 (18.0)	
Cantonese	74 (18.6)	103 (16.1)		47 (18.4)	94 (16.8)		27 (19.2)	9 (11.5)	
Hainanese	18 (4.5)	35 (5.5)		17 (6.6)	29 (5.2)		1 (0.7)	6 (7.7)	
Hakka	31 (7.8)	58 (9.1)		26 (10.2)	56 (10.0)		5 (3.6)	2 (2.6)	
Others	12 (3.0)	26 (4.1)		9 (3.5)	24 (4.3)		3 (2.1)	2 (2.6)	
Country of birth			0.31			0.49			0.81
Singapore	270 (67.8)	455 (71.1)		177 (68.9)	402 (71.7)		93 (66.0)	53 (67.1)	
Malaysia	60 (15.1)	102 (15.9)		38 (14.8)	87 (15.5)		22 (15.6)	15 (19.0)	
China	51 (12.8)	65 (10.2)		30 (11.7)	56 (10.0)		21 (14.9)	9 (11.4)	
Others	17 (4.3)	18 (2.8)		12 (4.7)	16 (2.9)		5 (3.6)	2 (2.5)	
Current housing			0.15			0.011			0.70
1–3 rooms public flat	126 (32.1)	213 (33.5)		76 (29.9)	181 (32.4)		50 (36.0)	32 (41.0)	
4 rooms or larger public flat	197 (50.1)	338 (53.1)		120 (47.2)	297 (53.2)		77 (55.4)	41 (52.6)	
Private apt or house	70 (17.8)	85 (13.4)		58 (22.8)	80 (14.3)		12 (8.6)	5 (6.4)	
Years of education			0.52			0.014			0.50
Nil	161 (40.6)	247 (38.6)		81 (31.5)	203 (36.2)		80 (57.1)	44 (55.7)	
≤6 years	118 (29.7)	212 (33.1)		72 (28.0)	189 (33.7)		46 (32.9)	23 (29.1)	
7 years or more	118 (29.7)	181 (28.3)		104 (40.5)	169 (30.1)		14 (10.0)	12 (15.2)	
Marital status			0.17			0.23			0.73
Currently or previously married	372 (93.5)	583 (91.1)		240 (93.4)	510 (90.9)		132 (93.6)	73 (92.4)	
Never married	26 (6.5)	57 (8.9)		17 (6.6)	51 (9.1)		9 (6.4)	6 (7.6)	
Ever worked outside the home			0.12			0.68			0.19
Yes	312 (78.4)	526 (82.3)		209 (81.3)	462 (82.5)		103 (73.1)	64 (81.0)	
No	86 (21.6)	113 (17.7)		48 (18.7)	98 (17.5)		38 (26.9)	15 (19.0)	
Smoking status			<0.001						
Current smoker	44 (11.1)	24 (3.7)							
Ex-smoker[a]	97 (24.4)	55 (8.6)							
Never smoker	257 (64.6)	561 (87.7)							
Environmental tobacco smoke exposure at home			0.34			0.64			0.91
<Daily	161 (41.4)	281 (44.5)		121 (48.6)	259 (46.8)		40 (28.6)	22 (27.9)	
Every day	228 (58.6)	351 (55.5)		128 (51.4)	294 (53.2)		100 (71.4)	57 (72.2)	
Family history of cancer[b]			0.037			0.006			0.37
No	265 (68.7)	475 (75.2)		165 (65.5)	414 (74.6)		100 (74.6)	61 (79.2)	
Yes, other sites	84 (21.8)	119 (18.8)		59 (23.4)	110 (19.8)		25 (18.7)	9 (11.7)	
Yes, lung cancer	37 (9.6)	38 (6.0)		28 (11.1)	31 (5.6)		9 (6.7)	7 (9.1)	
Histologic type									
Adenocarcinomas[c]	224 (56.3)			165 (64.2)			59 (41.8)		
Squamous cell carcinomas[d]	31 (7.8)			9 (3.5)			22 (15.6)		
Small cell carcinomas[e]	19 (4.8)			3 (1.2)			16 (11.3)		
Other histology[f]	92 (23.1)			62 (24.1)			30 (21.3)		
No histology/cytology	32 (8.0)			18 (7.0)			14 (9.9)		

Abbreviation: SD, standard deviation.

[a] Have not smoked any cigarette in the 30 days prior to admission.
[b] First degree relatives.
[c] Histology codes (ICD-O-3): 8140/3 – adenocarcinoma NOS; 8260/3 – papillary adenocarcinoma, NOS; 8480/3 – mucinous adenocarcinomas.
[d] Histology code (ICD-O-3): 8070/3 – squamous cell carcinoma, NOS.
[e] Histology code (ICD O-3): 8041/3 – small cell carcinoma, NOS.
[f] Other histology codes, the most common being (ICD-O-3): 8012/3 – large cell carcinoma, NOS; 8046/3 – non-small cell carcinoma, NOS; 8250/3 – bronchio-alveolar carcinoma.

numbers of ever-smoker users in our study were too small for meaningful interpretation. The lowest risks of lung cancer occurred in non-smokers who started using aspirin between 1 and 5 years before admission/diagnosis, and whose duration of aspirin use was between 12 and 60 months (OR 0.35, 95% CI 0.14–0.85, and OR 0.37, 95% CI 0.16–0.85, respectively). Duration of use and onset of use appeared to be correlated (data not shown), so it was not possible to study the separate effects of duration or onset of use adjusting for the other variable.

Restricting cases to non-smokers with lung adenocarcinomas (n = 165), an OR of 0.43, 95% CI 0.24–0.78 was obtained for the association between aspirin use and lung cancer. A pattern similar to that in Table 3 was observed when we investigated the onset of aspirin use and the duration of use in this subgroup, with lowest ORs

Table 2
Association between use of a variety of medications and lung cancer in Chinese lung cancer patients and their controls, Singapore, 2005–2008.

		Non-smokers N = 818			Smokers N = 220		
		Cases N = 257	Controls N = 561	OR (95%CI)[a]	Cases N = 141	Controls N = 79	OR (95%CI)[b]
Regular use of aspirin[c]	No	226 (89.7)	442 (79.5)	1.0	122 (89.7)	63 (80.8)	1.0
	Yes	26 (10.3)	114 (20.5)	0.50 (0.31–0.81)	14 (10.3)	15 (19.2)	0.38 (0.16–0.93)
Regular use of non-aspirin NSAID[c]	No	243 (96.4)	541 (97.3)	1.0	134 (97.8)	74 (96.1)	1.0
	Yes	9 (3.6)	15 (2.7)	1.72 (0.71–4.19)	3 (2.2)	3 (3.9)	0.57 (0.10–3.12)
Regular use of COX-2 inhibitor[c]	No	251 (99.6)	552 (99.3)	1.0	137 (100)	77 (100)	–
	Yes	1 (0.4)	4 (0.7)	0.56 (0.06–5.25)	0	0	–
Regular use of panadol[c]	No	243 (96.0)	530 (95.5)	1.0	128 (92.8)	75 (96.2)	1.0
	Yes	10 (4.0)	25 (4.5)	1.02 (0.47–2.21)	10 (7.2)	3 (3.8)	2.58 (0.62–10.78)
Regular use of steroid creams[c]	No	245 (97.2)	536 (97.1)	1.0	133 (97.1)	77 (98.7)	1.0
	Yes	7 (2.8)	16 (2.9)	0.84 (0.33–2.14)	4 (2.9)	1 (1.3)	2.84 (0.29–28.27)
Regular use of steroid pills[c]	No	244 (96.8)	539 (96.9)	1.0	135 (98.5)	75 (97.4)	1.0
	Yes	8 (3.2)	17 (3.1)	1.35 (0.54–3.38)	2 (1.5)	2 (2.6)	1.13 (0.14–9.15)

[a] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings), years of education, housing type, and history of cancer in 1st degree relative.

[b] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings), years of education, housing type, history of cancer in 1st degree relative and duration (in years) of smoking.

[c] Regular use refers to use of at least two times a week for at least a month or more.

achieved in persons starting use 1–5 years prior to admission, and in those who had used aspirin for 12–60 months (data not shown).

To assess the impact of controls with specific diagnoses on the estimation of the effect of aspirin use on lung cancer risk, we systematically excluded groups of controls based on their admission diagnoses (in broad groupings of accidents and injuries, orthopaedic conditions, gastrointestinal conditions, respiratory conditions, neurological conditions, cardiac conditions, renal conditions, endocrine conditions infectious diseases, and others). No significant variation in the OR estimates were seen, with adjusted ORs in the range of 0.49–0.55 for never-smokers, and 0.28–0.39 for smokers (see Supplementary Table 5).

The protective effect of aspirin was present even when participants who used aspirin within 1 year of diagnosis of lung cancer (in cases), or admission to hospital (for controls) were excluded, with ORs and 95%CI for never-smokers and smokers of 0.44 (0.24–0.81) and 0.40 (0.15–1.04), respectively. Finally, exclusion of 32 cases of lung cancer diagnosed on radiological grounds did not materially affect the results observed, with ORs of 0.49 (95%CI 0.30–0.81) for never-smokers and 0.33 (95%CI 0.13–0.84) for smokers.

4. Discussion

Our results suggest that regular aspirin consumption may be associated with a reduced risk of cancer in both never-smokers and ever-smokers. Non-aspirin NSAIDs, and paracetamol and other forms of acetaminophen use were not associated with reduced risk of lung cancer, though the numbers of regular users in each group were small. The risk estimates were not significantly different in the subgroup of non-smokers with histologically confirmed adenocarcinomas, compared to the overall group of cases.

A protective effect of aspirin and other NSAIDs is biologically plausible – COX2 is an inducible enzyme with pro-inflammatory and pro-carcinogenic properties, and in vitro and animal studies lend support to the role of COX2 in carcinogenesis [1–9]. However, reported results of aspirin use and lung cancer risk are conflicting, with both protective [13–21] and null effects [22–32] being reported. Three clinical trials focusing on chemoprevention with aspirin reported non-significant protective effects [35–37], and a recent pooled analysis of individual-level data from 7 clinical trials of daily aspirin reported a protective effect on lung cancer death, with a protective latent period of about 5 years. The authors also reported a greater protective effect against adenocarcinomas [38]. The reasons for the inconsistency in findings include small numbers of lung cancer cases, failure to distinguish between aspirin and other NSAIDs, and inadequate control of smoking status (as have been pointed out by some of the authors). The widely contrasting results from meta-analyses [17,33,34] – the differences in results appear to be due to differences in studies included, and to the use of different definitions of exposure (aspirin and NSAIDs examined separately or in combination).

Table 3
Association between onset and length of aspirin use and lung cancer in Chinese female lung cancer patients and their controls, restricted to never-smokers.

	Non-smokers		
	Cases n = 257	Controls n = 561	ORs (95%CI)[a]
Onset of aspirin use			
No use	226 (89.7)	442 (79.5)	1.0
Start using <1 year from admission	11 (4.4)	39 (7.0)	0.61 (0.30–1.23)
Start using 1–<5 years from admission	6 (2.4)	39 (7.0)	0.35 (0.14–0.85)
Start using 5 years and more from admission	9 (3.6)	36 (6.5)	0.54 (0.25–1.18)
Duration of aspirin use			
No use	226 (89.7)	442 (79.5)	1.0
Duration of use 12 month or less	11 (4.4)	40 (7.2)	0.61 (0.30–1.24)
Duration of use 12–<60 months	7 (2.8)	43 (7.7)	0.37 (0.16–0.85)
Duration of use 60 months or more	8 (3.2)	31 (5.6)	0.54 (0.24–1.22)

[a] Adjusted for age at diagnosis/admission, average weekly fruit and vegetable consumption (in servings), years of education, housing type, and history of cancer in 1st degree relative.

The effect of aspirin and other NSAIDs may be modified by gender, and this could also explain the heterogeneity in results. The estrogen and prostaglandin pathways are known to interact, and estradiol increases COX-2 production in some cell types [42]. The effect of COX-2 inhibition by aspirin and NSAIDs may thus be stronger in women. The available epidemiologic evidence is not consistent, with stronger protective effects in men [20], stronger protective effects in women [22,23], and similar effects in both genders [17,18] all being reported. A possible explanation may be residual confounding by smoking in populations where there are strong gender differences in smoking rates.

Among the studies that distinguished between aspirin and non-aspirin NSAID use, some reported stronger protective effects of aspirin than non-aspirin NSAID [19], while others reported that the effect of non-aspirin NSAIDs was stronger [16,17,21]. Residual confounding by smoking (because aspirin but not non-aspirin NSAIDs use may be higher in smokers as anti-thrombotic treatment for cardiovascular disease risk) may shift effects towards the null and explain the smaller effect of aspirin compared to non-aspirin NSAIDs detected in some studies [17]. In contrast, our study found protective effects of aspirin even in non-smokers, but no protective effect in a much smaller group of regular users of non-aspirin NSAID.

Our study contributes to the growing literature on the role of NSAIDs and aspirin on lung cancer by studying the effect of aspirin and non-aspirin NSAIDs in a large group of Asian female non-smokers, for which the effect of aspirin and non-aspirin NSAIDs has not been well characterised.

There are a number of limitations in this study. We used a hospital-based case–control design. Aspirin and NSAID use in Singapore is prescribed by physicians for the treatment of medical conditions rather than as health supplements. Hence, controls may have a higher-than-representative prevalence of aspirin and NSAID use. This would bias our estimates away from the null. We have tried to minimise this by recruiting controls from a variety of admitting diagnoses, and, in line with the theory of control selection proposed by Wacholder [43], have also excluded controls whose admission diagnoses are likely to be associated (both positively and negatively) with the exposures of interest. We excluded individuals admitted for chest pain, acute myocardial infarction, or heart failure, stroke or transient ischaemic attack, and for management of musculoskeletal pain, including knee, hip and back pain, as NSAIDs and aspirin are commonly used in the management of these conditions. In addition, we also excluded individuals who were admitted for complications arising from NSAID use. We also evaluated the impact of including controls from particular diagnoses groups on the OR estimates for aspirin use and lung cancer risk, by systematically excluding controls based on their diagnoses group and testing the effect of aspirin. These analyses suggest that the results we obtained were fairly robust, and were not likely due to high aspirin use in any particular group of controls.

We have tried to mitigate the well-known problems of recall and interviewer bias in case–control designs by training our interviewers, having them interview both cases and controls, and recording a random sample of interviews to assess quality. We do not believe recall bias to be a major source of error as the putative relationship between NSAID use and lung cancer is not well known, and the questions about NSAIDs and aspirin use were embedded in a longer questionnaire that covered a variety of exposures. We relied on self-reports of medication use, and under- or over-reporting of medication use is possible. However, as this was a hospital-based study and interviews were conducted in the hospital, such misreporting should affect cases and controls equally. We were unable to obtain information about the dosage and frequency of use of these medications in our participants, and we did not distinguish between low-dose or normal dose aspirin, thus limiting our ability to define more precisely the effect of aspirin.

Because of the relatively small size of our study and the low frequency of use of some medications such as steroid pills, some estimates reported in this study are imprecise, with wide confidence intervals. OR estimates presented in Table 2 for steroid pills and COX-2 inhibitors, and in Table 3 showing the effect of duration of aspirin use and date of onset of aspirin use in relation to lung cancer risk should be viewed as preliminary in nature. Additional well-designed studies conducted in similar populations of never-smokers are necessary to confirm our results.

The key strengths of our study are that we have restricted participants to Chinese women, a population sub-group that appears to be at unusually high risk of lung cancer [44]: this recruitment strategy increases the precision of our study by reducing the variation in genetic and environmental factors due to ethnicity that may also be associated with lung cancer. We obtained high response rates in both cases and controls, and were able to control for multiple potential confounders such as smoking, socio-economic status, and diet.

5. Conclusion

In summary, our data suggest that regular aspirin consumption may confer protection against lung cancer in Chinese women.

Conflict of interest statement

None declared.

Acknowledgement

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.lungcan.2012.03.005.

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Original Contribution

Female Reproductive Factors, Gene Polymorphisms in the Estrogen Metabolism Pathway, and Risk of Lung Cancer in Chinese Women

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The authors examined relations between reproductive factors and 5 estrogen pathway gene polymorphisms (*CYP17* rs743572, *CYP19A1* rs10046, *ERβ* rs1256049, *ERβ* rs4986938, and *COMT* rs4680) among 702 Singapore Chinese female lung cancer cases and 1,578 hospital controls, of whom 433 cases (61.7%) and 1,375 controls (87.1%) were never smokers. Parity (per child, odds ratio (OR) = 0.92, 95% confidence interval (CI): 0.87, 0.97) and menstrual cycle length (for ≥30 days vs. <30 days, OR = 0.50, 95% CI: 0.32, 0.80) were inversely associated with lung cancer in never smokers, while age at first birth (for ages 21–25, 26–30, and ≥31 years vs. ≤20 years, ORs were 1.54, 2.17, and 1.30, respectively), age at menopause (for ages 49–51 and ≥52 years vs. ≤48 years, ORs were 1.37 and 1.59; P_{trend} = 0.003), and reproductive period (for 31–33, 34–36, 37–39, and ≥40 years vs. ≤30 years, ORs were 1.06, 1.25, 1.45, and 1.47; P_{trend} = 0.026) were positively associated. Among smokers, parity was inversely associated with lung cancer, but there was no association with other reproductive factors. The *COMT* rs4680 A allele was positively associated with lung cancer in never smokers (for G/A or A/A vs. G/G, OR = 1.46, 95% CI: 1.12, 1.90) but not in ever smokers. No associations were seen with other polymorphisms. These results support a risk-enhancing role of estrogens in lung carcinogenesis among never smokers.

estrogen; etiology; lung neoplasms; reproductive history

Abbreviations: PCR, polymerase chain reaction; SNP, single nucleotide polymorphism.

Lung cancer is the leading cause of cancer death in the world (1), and smoking is the major risk factor for the disease (2). However, lung cancer among never smokers is not uncommon, and annual global mortality from lung cancer among never smokers is as high as 15–20 per 100,000 persons (3). Previous studies suggest that female never smokers may be more likely to develop lung cancer than men (4), although this is disputed (5). Women appear more likely than men to develop adenocarcinoma, especially among never smokers (6).

Epidemiologic studies conducted in the 1980s implicated reproductive factors and female hormonal factors in lung carcinogenesis (7–10), and this hypothesis gained momentum with evidence of the potential biologic and pathologic roles of estrogen in lung development and carcinogenesis. Estrogen receptors, predominantly the β isoform, are found in both normal lung and carcinomatous tissue (11), and estrogen receptor signaling pathways are active in lung cancer cells (12), increasing cell proliferation and promoting angiogenesis. This pathway also interacts with the epidermal growth factor receptor pathway (13), which is believed to drive carcinogenesis in a subset of lung cancers (lung adenocarcinomas among predominantly female never smokers) (14). In vitro and mouse model studies suggest that estrogens increase tumor growth, and estrogen receptor antagonists significantly inhibit growth in non-small-cell lung cancer cell lines (15, 16). These findings have spurred renewed interest in the possible role of female hormonal factors in lung cancer, and, including the earlier studies, at least 28 studies (7–10, 17–41) have investigated the association of lung cancer with factors such as parity (8, 9,17–19, 21–29, 31–33, 41), menstrual history (7–10, 17, 18, 21, 22), age at menopause

(7–10, 18–23, 25, 27, 29, 32, 33, 41), age at menarche (8–10, 18–23, 26–28, 29, 32, 33, 41), estrogen replacement therapy (9, 22, 23, 25, 26, 28, 29, 32–39), and oral contraceptive use (8, 22, 25, 28–30, 32–34, 4–41).

Findings from these studies are inconsistent, suggesting that the effects of female reproductive factors are not strong and therefore difficult to detect, or that reproductive factors interact with other host genetic or environmental factors in complex ways. The existing evidence does not appear to conform to the well-established model for the association of reproductive factors with breast cancer (where early menarche and late menopause, nulliparity, and late age at first birth consistently increase breast cancer risk), but no alternative model has yet emerged.

Understanding the estrogen metabolic pathway in relation to lung cancer may shed light on the role of estrogen in lung carcinogenesis. To date, few studies have examined the association of estrogen-metabolism gene polymorphisms with lung cancer. Two recent studies investigated gene polymorphisms in estrogen-metabolism enzymes (cytochrome P-450 17-α hydroxylase (17,20-lyase) (*CYP17*), cytochrome P-450 19A1 (aromatase) (*CYP19A1*), and catechol-*O*-methyl transferase (*COMT*)) (42) and in estrogen receptor β (*ERβ*) (43), although these studies used populations in which nonsmokers were a small minority.

Lung cancer is the leading cause of cancer death in Singapore (44). Despite low rates of smoking (45), the incidence of lung cancer among Singaporean Chinese women is unexpectedly high (44), with a significant proportion of cancers occurring in nonsmokers. This suggests that factors other than smoking may play an unusually important role in lung cancer in this population.

We previously reported that among never smokers, parity and menstrual cycle length were associated with a significantly reduced risk of lung cancer, based on a study of 303 cases and 765 hospital controls (18). Our aims in the current study were to extend this analysis to a larger group of 702 cases and 1,578 hospital controls so as to better delineate possible differences between smokers and never smokers and to investigate the association of estrogen metabolism gene polymorphisms with lung cancer in Chinese women.

MATERIALS AND METHODS

Data for this study were derived from 2 hospital-based case-control studies conducted in 1996–1998 and 2005–2008 among participants recruited from the 5 major public-sector hospitals in Singapore. Both studies used similar study designs and questionnaires, and methods are reported in detail elsewhere (46, 47). Eligible cases were Chinese women with incident primary carcinoma of the lung (all histologic types) who were interviewed within 3 months of diagnosis. The average interval between diagnosis and interview was 22 days, and 79% were interviewed within 1 month of diagnosis. A total of 787 eligible lung cancer patients were identified, of whom 702 (89.2%) agreed to participate. Histologic or cytologic reports confirmed the diagnosis of primary lung carcinoma in 673 cases; 29 cases were confirmed on the basis of results from radiologic investigations, where metastatic spread of cancer to the lung was deemed unlikely.

Controls were selected from Chinese female patients admitted to the same hospitals and were frequency-matched for age (within 10 years) and date of admission. Patients admitted for the diagnosis and treatment of cancer or chronic respiratory disease were excluded, and no more than 10% of controls were recruited within a single diagnostic category. Control patients were admitted for a wide range of conditions, with few patients (0.4% of controls) having been admitted for gynecologic or obstetric conditions. The response rate among controls was 91%, with a total of 1,578 controls recruited. Of the 702 cases and 1,578 controls, 433 cases (61.7%) and 1,375 controls (87.1%) were never smokers, defined as never having smoked at least 1 cigarette per day for a year.

Approval for this study was obtained from the institutional review boards of the National University of Singapore and participating health-care institutions. Written consent was obtained from all participants. Trained interviewers administered face-to-face a structured questionnaire which elicited a detailed reproductive history, including parity, age at the birth of the first child, usual menstrual cycle length, age at menarche, and, where relevant, age at menopause. Among postmenopausal women, the reproductive period was measured as the time period between menarche and menopause. Information on exogenous reproductive hormone use (as hormone replacement therapy or oral contraception) was elicited only in the second case-control study. Data on participants' demographic characteristics, occupational history, smoking history, family history of cancer, personal medical history, diet, medication use, and indoor environmental exposures were also obtained.

Blood and/or saliva samples were obtained where consent was given. A total of 559 samples from cases (79.6% of all cases) and 988 samples from controls (62.6% of all controls) were obtained. Genomic DNA was extracted from the buffy coat of 5 mL of whole blood samples using the FlexiGene DNA kit (Qiagen Inc., Hilden, Germany). Saliva DNA was extracted from 2 mL of saliva with the Oragene DNA Self-Collection Kit (DNA Genotek Inc., Kanata, Ontario, Canada), and DNA concentration was measured with the Quantifiler Human DNA Quantification kit (Applied Biosystems (Life Technologies), Foster City, California).

Five polymorphisms in 4 estrogen pathway genes (rs743572 in *CYP17*, rs10046 in *CYP19A1*, rs4680 in *COMT*, and rs1256049 and rs4986938 in *ERβ*) were genotyped. We selected these genes because the proteins they encode are influential in the estrogen pathway. Cytochrome P-450 17-α hydroxylase is an upstream enzyme that produces estrogen precursors, while cytochrome P-450 19A1 converts testosterone to estradiol. Catechol-*O*-methyl transferase is the rate-limiting enzyme in the detoxification of catechol estrogens (48). Estrogen receptor β is found in lung tissue. Polymorphisms within these genes were selected for their potential role in affecting the activity of the enzymes, as demonstrated by their location in the gene (promoter regions, the untranslated region, or exons) and by functional and epidemiologic studies. Single nucleotide polymorphisms (SNPs) chosen had to have a minor allele frequency of ≥5% in Chinese populations.

All SNPs were genotyped on a high-throughput genotyping platform base using a 5′-nuclease allelic discrimination

assay in a 96-well format, with the ABI StepPlusOne Real-Time PCR System (Applied Biosystems). TaqMan universal polymerase chain reaction (PCR) master mix and predesigned SNP genotyping assay mix containing PCR primers and probes were purchased from Applied Biosystems. Among 131 subjects who had both blood and saliva samples, saliva DNA was successfully genotyped from 89%, while all blood DNA samples were successfully genotyped. The genotypes from saliva DNA samples matched completely (100%) those of the corresponding blood DNA samples. Three positive controls and 2 negative controls were included in each 96-well plate, and 10% of DNA samples were genotyped in duplicate for each polymorphism. The concordance rate for the duplicate analyses was 100%. Call rates for the 5 SNPs studied ranged from 96.6% to 97.5%.

A set of 10 adjustment variables, chosen on the basis of biologic considerations and reverse stepwise analyses of the data set, were used: age at diagnosis, country of birth, housing type, number of years of education, smoking status, secondhand smoke exposure, history of cancer in a first-degree relative, mean intake of fruits and vegetables (servings/week), and a variable to indicate which case-control study the participant belonged to. Among current and former smokers, duration of smoking (in years), the logarithm of the average number of cigarettes smoked per day, and time (in years) since quitting smoking prior to diagnosis (set at 0 for current smokers) were independent risk factors (not shown). Logistic regression models using combinations of these 3 variables showed that smoking duration alone adequately explained the variation in risk attributable to smoking, and this was added as an adjustment variable for smokers.

Unconditional logistic regression was used, and odds ratios and 95% confidence intervals were calculated. Tests for linear trend were performed by entering the factor of interest into the logistic regression model as an ordinal variable, where each successively higher category in that factor was assigned a higher numeric value in the variable. The likelihood ratio test was used to test for interaction between factors. Adjustment for multiple comparisons was made for all tests of statistical interaction using the method of Benjamini and Hochberg (49). All P values calculated were 2-tailed, and significance was set at $P < 0.05$. We used Stata statistical software, version SE 10.1 (StataCorp LP, College Station, Texas), for data analyses. All SNPs studied were in Hardy-Weinberg equilibrium in the control population.

RESULTS

Cases were significantly younger than controls and consumed less fruit and vegetables. They were more likely to have been born in China, to live in private housing, to smoke, to report daily secondhand smoke exposure, and to have a positive family history of lung cancer in a first-degree relative. Among smokers, cases reported a longer duration of smoking (Table 1). Half of the cases (51.7%) had lung adenocarcinoma, and the proportion of adenocarcinomas was higher among never smokers (62.6%) than among smokers (34.2%).

High parity was associated with a lower risk of lung cancer in never smokers (for every additional child, odds ratio (OR) = 0.92, 95% confidence interval (CI): 0.87, 0.97).

Similar odds ratios, but with nonsignificant P values, were obtained in the smaller number of ever smokers (Table 2). Higher age at first birth was associated with an increased risk of lung cancer in never smokers (among those whose first child had been born when they were aged 21–25, 26–30, and ≥31 years, odds ratios were 1.54, 2.17, and 1.30, respectively, compared with those aged ≤20 years at the first birth). The effect was less clear among ever smokers (adjusted $P_{interaction} = 0.14$) (Table 2).

Age at menopause was associated with lung cancer among postmenopausal women who had never smoked (for ages at menopause of 49–51 years and ≥52 years compared with ≤48 years, odds ratios were 1.37 and 1.59, respectively; $P_{trend} = 0.003$) but not among those who had ever smoked (odds ratios were 0.95 were 0.94, respectively; $P_{trend} = 0.81$); the adjusted P value for interaction was 0.22) (Table 2). Similarly, a longer reproductive period was associated with lung cancer risk among never smokers (for reproductive periods of 31–33, 34–36, 37–39, and ≥40 years compared with ≤30 years, odds ratios were 1.06, 1.25, 1.45, and 1.47, respectively; $P_{trend} = 0.026$) but not among ever smokers (adjusted $P_{interaction} = 0.34$) (Table 2).

Long menstrual cycle lengths were protective in never smokers (for reported cycle lengths of >30 days vs. ≤30 days, OR = 0.50, 95% CI: 0.32, 0.80) but not in ever smokers (adjusted $P_{interaction} = 0.33$). There were relatively few women who reported cycle lengths longer than 30 days. There was no association between hormone use and lung cancer, although the prevalence of hormone use was low. There was also no association between age at menarche and lung cancer (Table 2).

Of the 5 SNPs studied, only the SNP in the *COMT* gene was associated with lung cancer (Table 3). The A allele at rs4680 was associated with lung cancer in never smokers (for the G/A or A/A genotype compared with the G/G genotype, OR = 1.46, 95% CI: 1.12, 1.90) but not in ever smokers (OR = 0.77, 95% CI: 0.47, 1.28; adjusted $P_{interaction} = 0.14$).

In analyses stratified by age, the risk-conferring effect of longer reproductive periods in never smokers was seen only among women older than 65 years, not in the younger age group (adjusted $P_{interaction} = 0.22$). The protective effect of increasing parity also appeared to be stronger in the older age group than in the younger age group, although this interaction was not statistically significant (adjusted $P_{interaction} = 0.95$) (Table 4). The effects of age at menopause, age at first birth, menstrual cycle length, and the *COMT* rs4680 polymorphism were not modified by age (data not shown). Odds ratio estimates for never-smoker cases restricted to persons with a confirmed histology of adenocarcinoma were similar to those obtained for all never smokers (data not shown). Further adjustment for self-reported exposure to cooking fumes shifted odds ratio estimates for all reproductive factors and SNPs by less than 5% in either direction.

DISCUSSION

In this ethnically homogenous population with a high prevalence of never smoking, later age at menopause, longer reproductive period, later age at first birth, short menstrual cycle length, and low parity were associated with lung cancer,

Table 1. Baseline Characteristics of Female Chinese Lung Cancer Patients and Controls, Singapore, 1996–1998 and 2005–2008

	Total							Never Smokers							Ever Smokers						
	Cases (n = 702)			Controls (n = 1,578)			P Value	Cases (n = 433)			Controls (n = 1,375)			P Value	Cases (n = 269)			Controls (n = 203)			P Value
	Mean (SD)	No.	%	Mean (SD)	No.	%		Mean (SD)	No.	%	Mean (SD)	No.	%		Mean (SD)	No.	%	Mean (SD)	No.	%	
Age, years	65.9 (11.8)			64.1 (12.3)			<0.001	63.0 (12.5)			63.6 (12.2)			0.42	70.5 (8.9)			67.3 (12.6)			0.001
Fruit consumption, servings/week	6.8 (8.5)			9.0 (8.6)			<0.001	7.5 (7.1)			9.3 (8.5)			<0.001	5.7 (10.2)			7.2 (8.8)			0.11
Vegetable consumption, servings/week	21.4 (19.3)			25.6 (21.3)			<0.001	22.3 (19.4)			25.9 (21.3)			<0.001	20.0 (19.0)			23.3 (21.5)			0.08
Years of education	3.6 (4.5)			4.0 (4.4)			0.06	4.8 (4.9)			4.2 (4.5)			0.02	1.7 (2.9)			2.4 (3.7)			0.02
Dialect							0.03							0.008							0.01
Hokkien		290	41.7		673	43.5			151	35.2		577	42.8			139	52.1		96	48.5	
Teochew		157	22.6		307	19.9			109	25.4		265	19.7			48	18.0		42	21.2	
Cantonese		151	21.7		286	18.5			85	19.8		252	18.7			66	24.7		34	17.2	
Hainanese		40	5.8		89	5.8			38	8.9		80	5.9			2	0.8		9	4.6	
Hakka		38	5.5		138	8.9			31	7.2		125	9.3			7	2.6		13	6.6	
Other		20	2.9		53	3.4			15	3.5		49	3.6			5	1.9		4	2.0	
Country of birth							0.001							0.012							0.59
Singapore		442	63.0		1,031	65.3			274	63.3		900	65.5			168	62.5		131	64.5	
Malaysia		92	13.1		271	17.2			57	13.2		239	17.4			35	13.0		32	15.8	
China		145	20.7		234	14.8			85	19.6		198	14.4			60	22.3		36	17.7	
Other		23	3.3		42	2.7			17	3.9		38	2.8			6	2.2		4	2.0	
Current housing status							0.019							0.001							0.17
Public apartment, 1–3 rooms		254	36.4		613	39.0			145	33.7		517	37.8			109	40.8		96	47.5	
Public apartment, ≥4 rooms		332	47.6		775	49.3			202	47.0		682	49.8			130	48.7		93	46.0	
Private apartment or house		111	15.9		183	11.7			83	19.3		170	12.4			28	10.5		13	6.4	
Marital status							0.03							0.81							0.57
Currently or previously married		654	93.2		1,467	93.0			403	93.1		1,275	92.7			251	93.3		192	94.6	
Never married		48	6.8		111	7.0			30	6.9		100	7.3			18	6.7		11	5.4	
Ever working outside the home							0.017							0.56							0.01
Yes		513	73.1		1,224	77.7			330	76.2		1,065	77.6			183	68.0		159	78.3	
No		189	26.9		352	22.3			103	23.8		308	22.4			86	32.0		44	21.7	
Smoking status							<0.001														0.45
Never smoker[a]		433	61.7		1,375	87.1																
Ex-smoker[b]		143	20.4		115	7.3											143	53.2		115	56.7	
Current smoker		126	18.0		88	5.6											126	46.8		88	43.3	
Duration of smoking, years[c]															43.7 (17.8)			33.4 (20.3)			<0.001
No. of cigarettes smoked per day															11.5 (11.3)			9.6 (10.9)			0.06

	n	%	n	%	P	n	%	n	%	P	n	%	n	%	P
Years since stopping smoking[d]											7.0 (12.8)		12.8 (17.8)		<0.001
Secondhand smoke exposure at home					0.008					0.30					0.75
Less than daily	334	48.1	848	54.1		226	53.1	764	55.9		108	40.2	84	41.6	
Every day	361	51.9	720	45.9		200	46.9	602	44.1		161	59.9	118	58.4	
Family history of cancer[e]					0.003					<0.001					0.45
No	531	75.6	1,286	81.5		309	71.4	1,112	80.9		222	82.5	174	85.7	
Yes, lung cancer	45	6.4	63	4.0		36	8.3	55	4.0		9	3.4	8	3.9	
Yes, at other sites	126	18.0	229	14.5		88	20.3	208	15.1		38	14.1	21	10.3	
Histologic type															
Adenocarcinoma[f]	363	51.7				271	62.6				92	34.2			
Squamous cell carcinoma[g]	84	12				25	5.8				59	21.9			
Small cell carcinoma[h]	38	5.4				5	1.2				33	12.3			
Other[i]	188	26.8				115	26.6				73	27.1			
No histologic or cytologic data	29	4.1				17	3.9				12	4.5			

Abbreviations: ICD-O-3, *International Classification of Diseases for Oncology*, Third Edition; NOS, not otherwise specified; SD, standard deviation.

[a] Defined as having never smoked at least 1 cigarette per day for a year.

[b] Having not smoked any cigarettes during the 30 days prior to admission.

[c] Defined as the difference between the age at which the participant stopped smoking (taken as the age at diagnosis or admission for participants who were currently smoking) and the age at which she started.

[d] Defined as the difference between age at diagnosis and age at which the participant stopped smoking; set as 0 for current smokers.

[e] First-degree relatives.

[f] Histology codes (ICD-O-3) 8140/3 (adenocarcinoma, NOS), 8260/3 (papillary adenocarcinoma, NOS), and 8480/3 (mucinous adenocarcinoma).

[g] Histology code (ICD-O-3) 8070/3 (squamous cell carcinoma, NOS).

[h] Histology code (ICD O-3) 8041/3 (small cell carcinoma, NOS).

[i] Other histology codes (ICD-O-3), the most common being 8012/3 (large cell carcinoma, NOS), 8046/3 (non-small-cell carcinoma, NOS), and 8250/3 (bronchioalveolar carcinoma).

Table 2. Odds Ratios for Lung Cancer According to Reproductive Factors and Use of Exogenous Reproductive Hormones, Singapore, 1996–1998 and 2005–2008

| | Total | | | | | | Never Smokers | | | | | | Ever Smokers | | | | | | Test for Interaction[a] | |
| | Cases | | Controls | | OR[b] | 95% CI | Cases | | Controls | | OR[c] | 95% CI | Cases | | Controls | | OR[d] | 95% CI | P Value | Adjusted P Value[e] |
	No.	%	No.	%			No.	%	No.	%			No.	%	No.	%				
Parity (no. of children)																			0.34	0.34
0	87	12.4	176	11.2	1		54	12.5	153	11.2	1		33	12.3	23	11.3	1			
1–2	187	26.7	383	24.3	1.07	0.77, 1.50	135	31.3	344	25.1	1.15	0.79, 1.88	52	19.3	39	19.2	0.90	0.41, 1.93		
3–4	209	29.8	490	31.1	0.84	0.60, 1.18	142	32.9	444	32.4	0.88	0.59, 1.29	67	24.9	46	22.7	0.89	0.42, 1.86		
≥5	218	31.1	526	33.4	0.61	0.43, 0.88	101	23.4	431	31.4	0.63	0.40, 0.98	117	43.5	95	46.8	0.54	0.27, 1.09		
P_{trend}						0.001						0.006						0.038		
As a continuous variable					0.93	0.90, 0.98					0.92	0.87, 0.97					0.95	0.89, 1.02		
P value						0.002						0.002						0.19		
Age at first birth, years[f]																			0.031	0.14
No children	87		176				54		153				33		23					
≤20	146	24.3	412	29.9	1		63	17.0	339	28.2	1		83	35.8	73	41.0	1			
21–25	264	43.9	538	39.0	1.49	1.14, 1.96	151	40.8	471	39.2	1.54	1.09, 2.17	113	48.7	67	37.6	1.84	1.12, 3.01		
26–30	143	23.8	279	20.2	1.92	1.37, 2.69	121	32.7	255	21.2	2.17	1.46, 3.22	22	9.5	24	13.5	0.86	0.40, 1.85		
≥31	49	8.1	151	10.9	1.22	0.78, 1.89	35	9.5	137	11.4	1.30	0.78, 2.18	14	6.0	14	7.9	1.32	0.51, 3.42		
As a continuous variable					1.01	0.98, 1.03					1.01	0.98, 1.04					1.00	0.95, 1.06		
P value						0.58						0.63						0.97		
Reproductive period, years																			0.28	0.34
Premenopausal	45		129				37		118				8		11					
≤30	126	21.7	290	22.7	1		61	18.1	246	22.3	1		65	26.8	44	25.1	1			
31–33	97	16.7	230	18.0	1.00	0.71, 1.41	52	15.4	197	17.9	1.06	0.69, 1.63	45	18.5	33	18.9	0.89	0.47, 1.69		
34–36	159	27.4	327	25.6	1.21	0.89, 1.65	85	25.2	282	25.6	1.25	0.85, 1.84	74	30.5	45	25.7	1.07	0.60, 1.91		
37–39	117	20.1	256	20.0	1.22	0.87, 1.70	82	24.3	223	20.2	1.45	0.97, 2.16	35	14.4	33	18.9	0.73	0.37, 1.41		
≥40	82	14.1	174	13.6	1.26	0.87, 1.82	58	17.2	154	14.0	1.47	0.95, 2.24	24	9.9	20	11.4	0.86	0.40, 1.85		
P_{trend}						0.11						0.026						0.54		
As a continuous variable					1.02	1.00, 1.04					1.02	1.00, 1.05					1.00	0.97, 1.04		
P value						0.13						0.071						0.89		

	Cases, No.	%	Controls, No.	%	OR	95% CI	Cases, No.	%	Controls, No.	%	OR	95% CI	Cases, No.	%	Controls, No.	%	OR	95% CI		
Age at menarche, years																					
≤12	106	15.8	274	17.7	1		77	18.6	244	18.1	1		29	11.3	30	15.2	1			
13–15	354	52.7	789	50.9	1.20	0.90, 1.60	234	56.4	694	51.4	1.24	0.90, 1.71	120	46.7	95	48.0	0.92	0.47, 1.82		
≥16	212	31.6	486	31.4	1.04	0.75, 1.45	104	25.1	413	30.6	0.98	0.67, 1.45	108	42.0	73	36.9	1.11	0.54, 2.28		
P_{trend}					0.92						0.73						0.56			
As a continuous variable					1.02	0.97, 1.08					1.01	0.95, 1.07					1.08	0.98, 1.20		
P value					0.38						0.74						0.13			
Age at menopause, years																				0.11	0.22
Premenopausal	45		129				37		118				8		11					
≤48	218	36.6	514	39.8	1		109	31.4	439	39.3	1		109	44.0	75	42.4	1			
49–51	200	33.6	422	32.6	1.20	0.94, 1.55	118	34.0	363	32.5	1.37	1.01, 1.86	82	33.1	59	33.3	0.95	0.59, 1.54		
≥52	177	29.8	357	27.6	1.34	1.03, 1.74	120	34.6	314	28.1	1.59	1.16, 2.17	57	23.0	43	24.3	0.94	0.55, 1.60		
P_{trend}					0.027						0.003						0.81			
As a continuous variable					1.02	1.00, 1.04					1.03	1.00, 1.05					1.01	0.97, 1.05		
P value					0.053						0.036						0.58			
Menstrual cycle length, days																				0.22	0.33
≤30	638	93.6	1,389	89.7	1		399	94.1	1,210	89.6	1		239	92.6	179	90.4	1			
>30	44	6.5	159	10.3	0.61	0.42, 0.88	25	5.9	140	10.4	0.50	0.32, 0.80	19	7.4	19	9.6	0.73	0.35, 1.49		
Exogenous reproductive hormone use																					
Never used	297	74.4	578	70.9	1		181	70.2	505	70.9	1		116	82.3	73	70.9	1			
Used for ≤5 years	77	19.3	182	22.3	0.82	0.59, 1.15	58	22.5	162	22.8	0.91	0.63, 1.31	19	13.5	20	19.4	0.54	0.24, 1.21		
Used for >5 years	25	6.3	55	6.8	0.94	0.55, 1.62	19	7.4	45	6.3	1.23	0.68, 2.23	6	4.3	10	9.7	0.45	0.13, 1.55		
P_{trend}					0.43						0.84						0.086			

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Likelihood ratio test for interaction with smoking status.

[b] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), smoking status (current, ex-, or never smoker), secondhand smoke exposure at home, and a dummy variable for the study set.

[c] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), secondhand smoke exposure at home, and a dummy variable for the study set.

[d] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), duration of smoking (in years), secondhand smoke exposure at home, and a dummy variable for the study set.

[e] Adjusted for multiple comparisons.

[f] Additionally adjusted for number of children.

Table 3. Odds Ratios for Lung Cancer According to Polymorphisms in the Estrogen Pathway, Singapore, 1996–1998 and 2005–2008

Polymorphism	Total						Never Smokers						Ever Smokers					
	Cases		Controls		OR[a]	95% CI	Cases		Controls		OR[b]	95% CI	Cases		Controls		OR[c]	95% CI
	No.	%	No.	%			No.	%	No.	%			No.	%	No.	%		
CYP17 rs743572																		
G/G	177	42.4	346	35.9	1		102	29.7	299	35.3	1		75	37.0	47	40.2	1	
G/A	268	49.1	456	47.4	1.18	0.91, 1.53	176	51.3	401	47.4	1.25	0.93, 1.69	92	45.3	55	47.0	1.18	0.68, 2.06
A/A	101	18.5	161	16.7	1.31	0.93, 1.83	65	19.0	146	17.3	1.30	0.89, 1.92	36	17.7	15	12.8	1.30	0.59, 2.86
G/A or A/A					1.21	0.95, 1.55					1.26	0.95, 1.68					1.21	0.72, 2.04
CYP19A1 rs10046																		
A/A	145	26.7	248	25.9	1		90	26.5	222	26.4	1		55	2.0	26	22.2	1	
A/G	268	49.3	487	50.8	0.92	0.69, 1.21	171	50.3	424	50.4	0.99	0.72, 1.36	97	47.6	63	53.9	0.80	0.43, 1.51
G/G	131	24.1	223	23.3	1.04	0.75, 1.44	79	23.2	195	23.2	1.06	0.73, 1.54	52	25.5	28	23.9	1.02	0.48, 2.16
A/G or A/A					0.95	0.73, 1.24					1.01	0.75, 1.36					0.86	0.47, 1.57
COMT rs4680[d]																		
G/G	284	52.3	549	56.9	1		167	49.1	488	57.6	1		117	57.6	61	51.7	1	
G/A	220	40.5	353	36.6	1.26	0.99, 1.60	148	43.5	303	35.8	1.49	1.13, 1.97	72	35.5	50	42.4	0.70	0.42, 1.20
A/A	39	7.2	63	6.5	1.33	0.85, 2.08	25	7.4	56	6.6	1.30	0.77, 2.19	14	6.9	7	5.9	1.28	0.46, 3.55
G/A or A/A					1.27	1.01, 1.60					1.46	1.12, 1.90					0.77	0.47, 1.28
ERβ rs1256049																		
G/G	206	38.0	371	38.9	1		135	39.7	320	38.2	1		71	35.2	51	44.0	1	
G/A	251	46.3	447	46.9	1.00	0.78, 1.28	152	44.7	397	47.4	0.89	0.67, 1.19	99	49.0	50	43.1	1.43	0.83, 2.47
A/A	85	15.7	135	14.2	1.02	0.72, 1.45	53	15.6	120	14.3	0.98	0.66, 1.46	32	15.8	15	12.9	1.02	0.46, 2.26
G/A or A/A					1.00	0.79, 1.27					0.91	0.70, 1.20					1.33	0.79, 2.22
ERβ rs4986938																		
G/G	446	82.0	807	83.7	1		277	81.5	704	83.2	1		169	82.8	103	87.3	1	
G/A	95	17.5	148	15.4	1.17	0.87, 1.59	61	17.9	134	15.8	1.08	0.77, 1.52	34	16.7	14	11.9	1.36	0.66, 2.78
A/A	3	0.6	9	0.9			2	0.6	8	1.0			1	0.5	1	0.9		

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), smoking status (current, ex-, or never smoker), secondhand smoke exposure at home, and a dummy variable for the study set.

[b] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), secondhand smoke exposure at home, and a dummy variable for the study set.

[c] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), duration of smoking (in years), secondhand smoke exposure at home, and a dummy variable for the study set.

[d] Likelihood ratio test for interaction between smoking status and the *COMT* rs4680 polymorphism: $P = 0.047$ (adjusted for multiple comparisons, $P = 0.14$).

particularly among never smokers. Low parity is associated with higher blood estrogen levels (50). Shorter menstrual cycles suggest an overall increase in the period of unopposed estrogen exposure, and late menopause and long reproductive periods imply more reproductive cycles over the lifetime and hence longer periods of estrogen exposure in total. Our results are therefore consistent with an association between higher levels of endogenous estrogen exposure over the lifetime and risk of lung cancer.

An association with reproductive history was previously observed in our first study involving a smaller group of participants (18), where high parity and long menstrual cycles were protective. In the current analysis, we additionally found an increased risk associated with later age at first birth, later age at menopause, and longer reproductive period. In a recently published Singapore cohort study, Seow et al. (32)

reported similar protective effects for parity among female Chinese never smokers, although they did not find a significant association with age at menopause. While we are not able to fully explain this particular difference, we feel that, taken as a whole, data from this population suggest a role for estrogens in lung carcinogenesis among never-smoking women.

In other published studies, investigators have reported results similar to ours—namely, a protective effect of higher parity (18, 29, 31–33) and increased risk with higher age at menopause (7–9). In a cohort study, Liu et al. (23) did not find a main effect with menarche or menopause, but they reported that, compared with women with a late age at menarche (>15 years) and an early age at menopause (<51 years), women with either early menarche or late menopause had a higher risk of lung cancer. They proposed that the length of

Table 4. Odds Ratios for Lung Cancer According to Selected Reproductive Factors, by Age Group, Singapore, 1996–1998 and 2005–2008

	Total				Never Smokers			
	Age ≤65 Years (311 Cases, 801 Controls)		Age >65 Years (391 Cases, 777 Controls)		Age ≤65 Years (243 Cases, 725 Controls)		Age >65 Years (190 Cases, 650 Controls)	
	OR[a]	95% CI	OR[a]	95% CI	OR[b]	95% CI	OR[b]	95% CI
Reproductive period, years[c]								
≤30					1		1	
31–33					0.60	0.31, 1.18	1.77	0.98, 3.19
34–36					0.73	0.42, 1.28	2.20	1.26, 3.83
37–39					0.94	0.54, 1.64	2.24	1.24, 4.04
≥40					0.82	0.44, 1.54	2.66	1.41, 5.00
P_{trend}					0.99		0.001	
As a continuous variable					0.99	0.96, 1.03	1.05	1.01, 1.09
P value					0.77		0.005	
Parity (no. of children)[d]								
0	1		1					
1–2	1.25	0.82, 1.90	0.84	0.46, 1.51				
3–4	0.94	0.60, 1.46	0.78	0.45, 1.34				
≥5	0.76	0.42, 1.38	0.50	0.30, 0.84				
P_{trend}	0.22		0.001					
As a continuous variable	0.96	0.88, 1.04	0.92	0.88, 0.97				
P value	0.32		0.002					

Abbreviations: CI, confidence interval; OR, odds ratio.

[a] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), smoking status, secondhand smoke exposure at home, and a dummy variable for the study set.

[b] Adjusted for age at diagnosis, fruit and vegetable consumption (in servings/week), country of origin, dwelling type, years of education, history of cancer in first-degree relatives (no history, history of lung cancer, history of other cancers), secondhand smoke exposure at home, and a dummy variable for the study set.

[c] Likelihood ratio test for interaction: $P = 0.036$ (adjusted for multiple comparisons: $P = 0.22$).

[d] Likelihood ratio test for interaction: $P = 0.65$ (adjusted for multiple comparisons: $P = 0.95$).

exposure to estrogen may partly account for the increased risk (23). Yet other investigators, however, have reported null findings (8, 9, 19, 21–23, 25–27, 41) or increased risk (24, 28) with regard to parity, reduced risk with later age at menopause (19, 20, 29, 41), and reduced risk with a longer reproductive period (29).

The inconsistency in findings across the various studies may be due to differences in the study populations. Studies conducted in smokers or in populations containing a high proportion of smokers might not have been able to detect a weaker effect of estrogens. There is growing evidence that smoking-related lung cancer and never-smoker lung cancer are etiologically distinct. It is plausible that the effect of estrogens may apply specifically to lung cancer among never smokers, given that mutations in the epidermal growth factor receptor gene (*EGFR*) appear to be key drivers of this disease (14) and the estrogen pathway interacts at the cellular level with the epidermal growth factor receptor signaling pathway (13). In a recent Japanese case-control study, Matsuo et al. (27) noted that the increased risk associated with longer

reproductive periods was seen only in *EGFR* mutation-positive cancers.

The effect of estrogen may be modified by other biologic factors. For example, Paulus et al. (31) proposed that the different age distributions of women in the studies may explain the inconsistency of results with regard to parity, and their own data suggested that the effect of parity was seen only in late-onset lung cancer (using a cutoff of 50 years), thus suggesting that high parity may protect against postmenopausal lung cancer but not premenopausal lung cancer. We also found that both longer reproductive periods and parity had more convincing effects in older women, although the possible reasons for this are yet to be elucidated.

We did not find an association with key polymorphisms in the estrogen metabolism pathway, other than the rs4680 polymorphism in the *COMT* gene, where the A allele was associated with lung cancer in never smokers. The oxidative metabolism of 17β estradiol and estrone to catechol estrogens is postulated to be a risk factor in carcinogenesis: Catechol estrogens are oxidized to quinines that can form

DNA adducts and produce DNA- and lipid-damaging reactive oxygen species (48). Catechol-*O*-methyl transferase methylates (thus eliminating) catechol estrogens and is the key rate-limiting enzyme in that pathway (48). The A allele at rs4680 (codon 158) results in a point mutation, substituting the amino acid methionine for valine (51). Val/Met and Met/Met genotypes appear to have lowered enzymatic activity, with consequently increased cellular levels of catechol estrogens.

Two other case-control studies have investigated the effect of polymorphisms at this site on lung cancer risk, with similar results. The Met/Met and Val/Met genotypes were also associated with an increased risk of lung cancer in a population of smokers (52). Cote et al. (42) found that the A allele had no independent effect in a group of subjects who were predominantly smokers, but lung cancer risk was increased among those with the A allele and a glutathione *S*-transferase M1 (*GSTM1*) null genotype in comparison with those with the G/G genotype and a *GSTM1* nonnull genotype. In that same study, an independent effect of rs743572 (in *CYP17*) on lung cancer risk was not seen, although the C allele in combination with risk alleles in *GSTM1* and *GSTT1* increased risk (42). The authors did not find a relation between *CYP19A1* and lung cancer, although the polymorphism they studied (the tetranucleotide tandem repeat in intron 4) was different from the one studied here. Paulus et al. (43), examining 4 *ERβ* SNPs (the same 2 as in our study, and 2 others), did not find an association of individual SNPs or haplotypes with lung cancer risk, which is consistent with our results.

We had anticipated that polymorphisms in *CYP19A1* and *CYP17* would be important if the effect of reproductive factors is mediated through estrogen levels in target tissue (such as the lungs). However, current evidence, including ours, is more consistent with a risk-conferring effect of catechol estrogens on lung carcinogenesis. Few studies have examined estrogen pathway polymorphisms in lung carcinogenesis, and further work is necessary to determine whether this hypothesis is true. Inclusion of more SNPs for which functional studies indicate an effect on enzyme activity or estrogen levels, or a systematic interrogation of polymorphisms in the *CYP19A1*, *CYP17*, and *COMT* genes for associations with lung cancer, may be useful in this regard.

We noticed suggestive interactions between smoking status and the *COMT* SNP and age at first birth, and between age and reproductive period among never smokers. However, our study did not have adequate statistical power to detect interactions, and the interactions were no longer significant after adjustment for multiple testing. Further research using larger study populations is needed to confirm these observations.

The strengths of our study include homogeneity with regard to gender and ethnicity and the large number of never smokers, which allowed detailed investigation of risk factors pertinent to this group. This is particularly important in the light of recent understanding that smoking lung cancer and nonsmoking lung cancer may be different diseases. Methodological limitations associated with the case-control study design are well-known, and we attempted to address these limitations in a variety of ways. Reporting bias is unlikely to have been a major problem, as the relation between reproductive factors and lung cancer has rarely been reported in popular media, and participants were asked about reproductive factors in the context of a long questionnaire investigating a variety of factors. We assessed interviewer bias by recording and reviewing a random subset of interviews. To reduce bias introduced by the use of hospital controls, we chose controls from a wide variety of admitting diagnoses, and gynecologic diseases as the admitting diagnoses were very rare among our controls (about 0.4% of controls).

Our study suggests that reproductive factors are involved in lung carcinogenesis, particularly among never smokers. However, the specific functional role of estrogens in lung cancer is still not known, and while our data suggest a role for catechol estrogens, the mechanisms involved are likely to be complex. Given the relation between the estrogen and epidermal growth factor receptor pathways and the significance of the latter in lung cancer among never smokers, it is clear that further studies, both mechanistic and epidemiologic, are needed in this area.

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Lung Cancer in Chinese Women: Evidence for an Interaction between Tobacco Smoking and Exposure to Inhalants in the Indoor Environment

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BACKGROUND: Epidemiologic data suggest that Chinese women have a high incidence of lung cancer in relation to their smoking prevalence. In addition to active tobacco smoke exposure, other sources of fumes and airborne particles in the indoor environment, such as cooking and burning of incense and mosquito coils, have been considered potential risk factors for lung cancer.

OBJECTIVES: We used a case–control study to explore effects of inhalants from combustion sources common in the domestic environment on lung cancer and their modification by active tobacco smoking.

METHODS: We analyzed 703 primary lung cancer cases and 1,578 controls. Data on demographic background and relevant exposures were obtained by face-to-face interviews in the hospital.

RESULTS: We observed a positive relationship with daily exposure to incense or mosquito coils and to cooking fumes only among smokers, and no association among lifetime nonsmokers. Interactions between smoking and frequency of cooking, or exposure to incense or mosquito coils were statistically significant and consistent with synergistic effects on lung cancer. The odds ratio (OR) comparing smokers without daily incense or mosquito coil exposure with nonsmokers without daily exposure was 2.80 [95% confidence interval (CI), 1.86–4.21], whereas the OR comparing smokers with daily exposure to the same referent group was 4.61 (95% CI, 3.41–6.24). In contrast, daily exposure to incense or mosquito coils was not associated with lung cancer among nonsmokers (OR = 0.91; 95% CI, 0.72–1.16). We observed the same pattern of associations for smokers without (OR = 2.31; 95% CI, 1.52–3.51) and with (OR = 4.50; 95% CI, 3.21–6.30) daily cooking exposure compared with nonsmokers, with no evidence of an association with daily cooking exposure among nonsmokers.

CONCLUSION: Our results suggest that active tobacco smoking not only is an important risk factor for development of lung cancer, but also may cause smokers to be more susceptible to the risk-enhancing effects of other inhalants.

KEY WORDS: Chinese, combustion sources, females, inhalants, interaction, lung cancer, tobacco smoking. *Environ Health Perspect* 118:1257–1260 (2010). doi:10.1289/ehp.0901587 [Online 14 May 2010]

Lung cancer accounts for a substantial proportion of cancer incidence and mortality throughout the world (Parkin et al. 2005). In addition to tobacco smoke exposure (both active and secondhand), fumes and airborne particulates in the indoor environment have been considered as potential risk factors for lung cancer; examples include exposure to cooking oil fumes, cooking and heating fuels (household coal and wood combustion), incense and mosquito coils, and indoor radon (Ko et al. 2000; Wang et al. 2002; Yu et al. 2006; Zhang and Smith 2007).

Exposure to cooking fumes may potentially play a role in the occurrence of lung cancer. Cooking oil fumes are known to contain at least two carcinogenic compounds, benzo[*a*]pyrene and 2,4-decadienal, which induce lung cell survival and proliferation via the nuclear factor-κB pathway (Hung et al. 2005, 2007). Cumulative exposure to cooking (frequency and duration) by means of frying (stir-frying, frying, and deep-frying) was positively associated with the risk of lung cancer among female nonsmokers in Hong Kong

(Yu et al. 2006). Women nonsmokers were at higher risk for lung cancer if they were exposed to cooking oil fumes emitted at high temperatures, and the risks were higher when the fumes were not reduced by an extractor (Ko et al. 2000).

The combustion by-products from heating and cooking are also sources of indoor air pollution. In Canada, a case–control study of lung cancer in 1996–2001 reported that, among women, the odds ratio (OR) for those exposed to both traditional heating and cooking sources (coal and wood) was 2.5 [95% confidence interval (CI), 1.5–3.6] relative to women not exposed to either source (Ramanakumar et al. 2007). Traditional heating and cooking fuels (coal and wood) produce a variety of indoor pollutants, including respirable particles, heavy metals, polycyclic aromatic hydrocarbons (PAHs), carbon monoxide, carbon dioxide, nitrogen dioxide, sulfur dioxide, and formaldehyde (Zhang and Smith 2007). The use of coal for heating has been implicated in the high incidence of lung cancer among residents of Xuanwei, China (Lan et al. 2002).

Incense burning, a traditional practice in Chinese households, is also powerful producer of particulate matter, and incense smoke contains carcinogens such as PAHs, carbonyls, and benzene (Lin and Tang 1994; Lofroth et al. 1991). Incense smoke condensates have mutagenic and genotoxic activities, and the genotoxicity of certain incense smoke condensates in mammalian cells has been shown to be higher than that of tobacco smoke condensate (Chen and Lee 1996; Rasmussen 1987). The potential impact of incense on health has also been studied outside the home (Chiang and Liao 2006; Chiang et al. 2009). A large prospective cohort study in Singapore reported an association between long-term incense use and the development of squamous cell carcinomas of the respiratory tract, particularly among women (Friborg et al. 2008).

Mosquito coils are frequently burned indoors in Asia and to a limited extent in other parts of the world, including the United States (World Health Organization 1998). The major ingredients of the mosquito coils are pyrethrins and plant-based materials, such as wood powder, coconut shell powder, and joss powder, as well as binders, dyes, oxidants, and other additives to allow for controlled smoldering (Chen et al. 2008; Krieger et al. 2003). The combustion of these materials generates large amounts of submicrometer particles and gaseous pollutants. These submicrometer particles may reach the lower respiratory tract and could be coated with a wide range of organic compounds, such as PAHs. A study of mosquito coil smoke and lung cancer in Taiwan between 2002 and 2004 showed that lung cancer risk among smokers with the highest exposure to mosquito coil smoke was 14 times higher than nonsmokers without this exposure (Chen et al. 2008).

In this study, we used the case–control study design to investigate whether inhalant

exposure from these sources plays a significant role in enhancing risk of lung cancer among Singapore Chinese women, a population with a large proportion of nonsmokers. We also wished to explore whether the impact of these compounds is modified by active tobacco smoke exposure.

Materials and Methods

Participants were cases and controls who were recruited for two hospital-based case–control studies during 1996–1998 and 2005–2008, from the five major public hospitals in Singapore. Both studies used similar methods and questionnaires. Eligible cases were Chinese females with newly diagnosed primary carcinoma of the lung. The average time between diagnosis and interview was 22 days (79.4% were interviewed within 1 month of diagnosis). A total of 703 lung cancer patients (89.2% of those identified as eligible) agreed to participate. Histologic or cytologic reports were reviewed and confirmed the diagnosis of primary lung carcinoma in 674 cases; 29 cases were confirmed on the basis of radiologic investigations, in which metastatic cancer to the lung from other sites was deemed to be unlikely on clinical grounds. Controls

were selected from Chinese female patients, frequency matched for age (within 5 years), hospital admitted to, and date of admission (within 1 month). Patients admitted for a diagnosis and treatment of cancer or chronic respiratory disease were excluded, and no more than 10% of controls were recruited within a single diagnostic category.

The response rate among controls was 90.6%, and data from a total of 1,578 controls were available for analysis. Control patients were admitted for a wide range of conditions: 27% had diseases of skin, bones, joints, and connective tissue; 11% were admitted for gastrointestinal or hepatobiliary system complaints; 14% were admitted for acute trauma; 8% were admitted for neurological or psychiatric conditions; and 12% had diseases of the cardiovascular system.

Both eligible cases and controls gave written, informed consent for the interview and the tracing of their medical records, and the study was approved by the Institutional Review Board of the National University of Singapore and the participating health care institutions.

All subjects were interviewed in person by trained interviewers, using a structured questionnaire. Interviewers were not blinded to case

or control status, but we recorded and reviewed at random a sample of interviews conducted to ensure standardization of the data collection processes. The structured questionnaire covered demographic characteristics, occupational history, active smoking history, family history of cancer, personal medical history (e.g., history of tuberculosis), dietary intake of fruits and vegetables, and indoor environmental exposures (including secondhand smoking exposure, cooking exposure, and exposure to incense and mosquito coil burning).

The participant's smoking history included the number of cigarettes smoked daily and the total duration of smoking. A regular smoker was defined as one who smoked at least one cigarette per day for ≥ 1 year. Ex-smokers were smokers who had stopped smoking for ≥ 30 days at the time of interview. Questions on secondhand smoke exposure included "Did any of your household members smoke (including spouse, parents, children, or any other relative/friend living with you) in your presence more than once a week?" Family history of cancer was defined as the presence of any cancer within first-degree relatives. Information on intake of fruits and vegetables was collected using a semiquantitative food frequency questionnaire that elicited the usual weekly number of servings of 17 fruit and 21 vegetable items over the 3 years before admission. The time period for inhalant exposure was set at 25 years before admission, and all questions asked participants to recall exposures 25 years before age of diagnosis of lung cancer (or age at admission for controls). For cooking exposure, participants were asked about the frequency with which they personally cooked at home (with six categories of response, ranging from "never" to "more than once a day"), the cooking methods used, and the age at which they began to do this regularly. The frequency of incense/mosquito coil burning (i.e., less than daily, once daily, more than once a day/throughout the day, throughout the day and night) was also ascertained. In each case, the question was asked (e.g., "How often were joss sticks, scented coil/powder burnt inside your house?"), and the respondent asked to select the most appropriate frequency category. For the purpose of the analysis, exposures were categorized as less than daily ("< daily") and once or more every day ("daily").

ORs and their 95% CIs were calculated for risk of lung cancer for smokers and nonsmokers separately using unconditional logistic regression adjusting for age (years), education (years), housing type, secondhand smoke exposure (daily vs. less than daily exposure), history of cancer in the first-degree relative, duration of smoking (in years; for ex-smokers and current smokers), fruit and vegetable intake (servings/week), and study set (1996–1998 or 2005–2008 study). These adjustment variables

Table 1. Sociodemographic characteristics of lung cancer cases and controls, Singapore Chinese women [n (%)].

Characteristic	Cases ($n = 703$)	Controls ($n = 1,578$)	p-Value[a]
Age [years (mean ± SD)][b]	65.9 ± 11.9	64.1 ± 12.3	0.001
Birthplace			0.001
Singapore	443 (63.0)	1031 (65.3)	
Malaysia	92 (13.1)	271 (17.2)	
China	145 (20.6)	234 (14.8)	
Other	23 (3.3)	42 (2.7)	
Education (years)			0.037
None	342 (48.7)	678 (43.0)	
≤ 6	193 (27.5)	491 (31.1)	
≥ 7	167 (23.8)	409 (25.9)	
Dwelling			0.020
Flat, 1–3 rooms	255 (36.5)	613 (39.0)	
Flat, ≥ 4 rooms	332 (47.6)	775 (49.3)	
Private apartment or house	111 (15.9)	183 (11.7)	
Marital status			0.956
Ever married	654 (93.0)	1,467 (93.0)	
Never married	49 (7.0)	111 (7.0)	
Occupational status			0.018
Currently employed outside home	154 (21.9)	415 (26.3)	
Ever employed outside home	360 (51.2)	809 (51.3)	
Never employed outside home	189 (26.9)	352 (22.3)	
Smoking history			< 0.001
Nonsmoker	434 (61.7)	1,375 (87.1)	
Ex-smoker[c]	143 (20.3)	115 (7.3)	
Current smoker	126 (17.9)	88 (5.6)	
Secondhand smoke exposure at home			0.009
< Daily	335 (48.1)	848 (54.1)	
Daily	361 (51.9)	720 (45.9)	
Family history of cancer[d]			0.001
No	532 (75.7)	1,286 (81.5)	
Yes	171 (24.3)	292 (18.5)	
Servings/week of fruit (mean ± SD)	6.8 ± 8.5	9.0 ± 8.6	< 0.001
Servings/week of vegetables (mean ± SD)	21.4 ± 19.3	25.6 ± 21.3	< 0.001

[a]Pearson chi-square test for categorical variables and t-test for continuous variables. [b]Age at diagnosis (cases) and age at interview (controls). [c]Had not smoked any cigarette in the 30 days before admission. [d]First-degree relative with history of cancer of any site.

were modeled with age, fruit consumption, and vegetable consumption as continuous variables, and all other variables as categorical ones, with the respective categories, as shown in Table 1. Among smokers, intensity of smoking was highly correlated with duration, and further adjustment for the former did not affect the ORs, so it was excluded in the final statistical model. We used STATA statistical software (version SE 10.1; StataCorp LP, College Station, TX, USA) for data analyses. All p-values were calculated using two-tailed statistical tests, and the criterion for significance was set at $p < 0.05$. Interactions were assessed using the likelihood ratio test to estimate p-values; in each test for interaction, models that included the interaction term were compared with those that did not.

Results

We analyzed data from 703 cases with primary lung cancer and 1,578 controls. Data on exposures of interest and potential confounders were available for almost all participants, with the highest proportion of missing data for exposures being 1.1% (for the cooking variable). Table 1 describes sociodemographic characteristics of the cases and controls. Cases were significantly more likely to be current smokers (17.9% vs. 5.6%) or ever smokers (20.3% vs. 7.3%; ex-smokers: age-adjusted OR = 3.85; 95% CI, 2.93–5.01; current smokers: age-adjusted OR = 4.49; 95% CI, 3.34–6.02). They were also more likely to have been exposed to secondhand smoke at home daily (51.9% vs. 45.9% for controls). Cases had a higher proportion of family history of cancer than did controls (24.3% vs. 18.5%). The mean weekly number of servings of fruits and vegetables was lower among cases than among controls (6.8 and 21.4 vs. 9.0 and 25.6, respectively).

Associations between lung cancer and exposure to incense or mosquito coils, and with exposure to daily cooking, were strongly dependent on smoking status; Table 2 presents these results separately for smokers and nonsmokers. We observed a statistically significant positive relationship only among smokers and observed no association among lifetime nonsmokers. Among smokers, women who cooked daily had a higher risk than those who cooked less than daily (adjusted OR = 1.61; 95% CI, 1.01–2.56). Also, smokers with exposure to incense or mosquito coils daily were more likely to have lung cancer than those with less frequent exposure (OR = 1.53; 95% CI, 0.97–2.41) after adjustment for potential confounders. Daily use of charcoal or wood stove was not associated with lung cancer in either smokers or nonsmokers.

We found a statistically significant interaction between smoking and exposure to incense or mosquito coils ($p = 0.016$) or frequency of cooking ($p < 0.001$), respectively,

after adjustment for potential confounders (Table 3). The OR comparing smokers without daily incense or mosquito coils exposure with nonsmokers without daily exposure was 2.80 (95% CI, 1.86–4.21), whereas the OR comparing smokers with daily exposure with the same referent group was 4.61 (95% CI, 3.41–6.24). In contrast, daily exposure to incense or mosquito coils was not associated with lung cancer among nonsmokers (OR = 0.91; 95% CI, 0.72–1.16). We observed the same pattern of associations for smokers without (OR = 2.31; 95% CI, 1.52–3.51) and with daily cooking exposure (OR = 4.50; 95% CI, 3.21–6.30) compared with nonsmokers, with no evidence of an association with daily cooking exposure among nonsmokers. We observed the same pattern for wood stove use, although the interaction was not statistically significant ($p = 0.061$). We found no interaction between smoking and daily use of charcoal ($p = 0.128$).

Discussion

We examined the effects of cooking and exposure to burning of incense and mosquito coils on lung cancer risk among Singapore Chinese women, and their modification by active tobacco smoking exposure. We observed strong interactions between exposure to these sources and smoking on lung cancer risk. The results indicate that active tobacco smoking not only is an important risk factor for development of lung cancer, but also may cause smokers to be more susceptible than nonsmokers to adverse effects of these inhalants on lung cancer as well.

A possible explanation for our findings is the presence of a chronic inflammatory state in the airways induced by smoking. Tobacco smoke carcinogens are known to activate proinflammatory responses through the action of prooxidative chemicals, leading to the release of cytokines, production of

Table 2. Adjusted ORs[a] and 95% CIs for lung cancer by cooking, incense or mosquito coil use, and charcoal and wood stove use, by smoking status.

Exposure factor	Current or ex-smokers		Nonsmokers	
	Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
Cooking frequency				
< Daily	58/73	1.00	145/385	1.00
Daily	210/130	1.61 (1.01–2.56)	282/972	0.89 (0.68–1.16)
Use of incense or mosquito coils				
< Daily	62/65	1.00	169/488	1.00
Daily	207/138	1.53 (0.97–2.41)	265/887	0.90 (0.71–1.14)
Use of charcoal stove				
< Daily	239/180	1.00	406/1,236	1.00
Daily	30/21	1.08 (0.55–2.12)	26/129	0.67 (0.43–1.05)
Use of wood stove				
< Daily	215/167	1.00	387/1,177	1.00
Daily	54/33	1.25 (0.74–2.12)	45/193	0.81 (0.56–1.17)

[a]Adjusted as described in "Materials and Methods."

Table 3. Combined effect estimates for lung cancer in association with indoor inhalants and smoking.

Exposure group	Smoking status	n	OR (95% CI)[a]
Cooking frequency			
< Daily	Nonsmokers	530	1.00
Daily	Nonsmokers	1,254	0.83 (0.64–1.08)
< Daily	Current or ex-smokers	131	2.31 (1.52–3.51)
Daily	Current or ex-smokers	340	4.50 (3.21–6.30)
p-Value (interaction)[b] < 0.001			
Use of incense or mosquito coils			
< Daily	Nonsmokers	657	1.00
Daily	Nonsmokers	1,152	0.91 (0.72–1.16)
< Daily	Current or ex-smokers	127	2.80 (1.86–4.21)
Daily	Current or ex-smokers	345	4.61 (3.41–6.24)
p-Value (interaction)[b] = 0.016			
Use of charcoal stove			
< Daily	Nonsmokers	1,642	1.00
Daily	Nonsmokers	155	0.67 (0.43–1.04)
< Daily	Current or ex-smokers	419	4.08 (3.21–5.18)
Daily	Current or ex-smokers	51	4.88 (2.68–8.91)
p-Value (interaction)[b] = 0.128			
Use of wood stove			
< Daily	Nonsmokers	1,564	1.00
Daily	Nonsmokers	238	0.78 (0.55–1.13)
< Daily	Current or ex-smokers	382	3.95 (3.08–5.07)
Daily	Current or ex-smokers	87	5.48 (3.42–8.79)
p-Value (interaction)[b] = 0.061			

[a]Adjusted as described in "Materials and Methods." [b]p-Value for the likelihood ratio test for interaction between smoking and cooking, incense or mosquito coils, charcoal stove, and wood stove.

reactive oxygen species (ROS), and ultimately DNA damage (Azad et al. 2008; Hecht 2008). A chronic inflammatory process in the lung could also lead directly to DNA damage, enhance the effects of other carcinogenic exposures, and stimulate cell proliferation and growth (Ohshima and Bartsch 1994). Burning incense generates high concentrations of ROS in the particulate gas phase of the emissions, and might damage DNA and other biomolecules when inhaled (Szeto et al. 2009).

Our findings that these exposures are not associated with risk among nonsmokers are at variance with other studies that reported positive associations in nonsmokers. The OR for female nonsmokers cooking three meals/day compared with those cooking one meal/day was 3.4 (95% CI, 1.6–7.0) in a study conducted in Taiwan (Ko et al. 2000). In the study among women in Taiwan, higher frequency of mosquito coil smoke use was positively associated with lung cancer in both smokers and nonsmokers, although the interaction with cigarette smoke was synergistic (Chen et al. 2008), as in the present study. Differences in cooking practices, use of fume extractors, type and intensity of use of mosquito coils, or simply in the average amount of time spent at home may contribute to the difference in findings among studies, even within Chinese populations. The proportion of women who had never been employed outside the home in our study was only 22% among controls, suggesting that overall exposure to air pollutants in the domestic environment may be less substantial in our population than in more traditional societies.

Contrary to previous reports, we did not find a significant association between use of charcoal or wood stoves and lung cancer risk, among either smokers or nonsmokers. In Singapore, local residents infrequently use traditional fuels (charcoal or wood) and usually use modern fuels (gas, kerosene, or electricity) for cooking, and the low frequency of use may be the chief explanation for our findings. We also recognize that there are limitations to the data presented. Because the study is retrospective, recall and reporting biases by subjects are inevitable concerns. We believe that these biases are not likely to be differential, because we did not make our hypothesis known to our participants, and the possible association between inhalant exposure and lung cancer is not widely known among the public. These errors, if present, would probably shift the association toward the null, because they would likely affect both cases and controls to the same extent.

Conclusions

Our study suggests that active tobacco smoking not only is an important risk factor for development of lung cancer, but also may cause smokers to be more susceptible to the risk-enhancing effects of exposure to cooking and burning of incense and mosquito coils. A possible mechanism consistent with recent findings is the presence of a chronic inflammatory state in the airways induced by smoking. The interaction observed supports a model in which host susceptibility acts in concert with the exposures of interest to promote lung carcinogenesis. On the other hand, we found no evidence that these specific exposures contribute to increased risk of lung cancer among nonsmokers. Because cooking and burning of incense and mosquito coils are fairly common exposures in the indoor environment, it is important that smokers be aware of the significant additional risk afforded by these exposures. Although our results suggest a weaker effect, if any, among nonsmokers, further research is needed to establish more definitively the level of risk from these ubiquitous compounds in the domestic environment.

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Meat Consumption and Risk of Lung Cancer Among Never-Smoking Women

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The relationship between diet and lung cancer, apart from the protective effect of fruit and vegetables, is poorly understood. Reports on the role of dietary components such as meat are inconsistent, and few studies include sufficient numbers of nonsmokers. We examined the relationship between meat consumption and never-smoking lung cancer in a hospital-based case–control study of Singapore Chinese women, a population with low smoking prevalence. Three hundred and ninety-nine cases and 815 controls were recruited, of whom 258 cases and 712 controls were never smokers. A standardized questionnaire (which included a food frequency questionnaire module) was administered by trained interviewers. Among these never smokers, fruit and vegetable intake were inversely associated with lung cancer risk. Seventy-two percent of meat consumed was white meat (chicken or fish). Meat consumption overall was inversely associated with lung cancer [adjusted odds ratio (OR), 0.88, 0.59 for second, third tertiles, P_{trend} = .012]. An inverse relationship between fish consumption and lung cancer (adjusted OR, 0.81, 0.47 for 2nd, 3rd tertiles, P_{trend} < .001) was observed. No association was seen between consumption of processed meats and lung cancer, nor between dietary heterocyclic amines and lung cancer. Our data suggest that fish consumption may be protective against lung cancer in never smokers.

INTRODUCTION

Lung cancer is the leading cause of cancer death worldwide (1). Tobacco consumption is the major risk factor for lung cancer (2). However, lung cancer in nonsmokers would be the seventh most common cancer worldwide if it were ranked separately (3). Lung cancer incidence in Chinese women in various populations around the world is much higher than expected given

their relatively low smoking prevalence (4). Investigations into explanations for this increased risk have focused on factors such as environmental tobacco exposure (5), indoor air pollution from use of cooking stoves and coal burners (3), occupational exposure to carcinogens (6), household radon exposure (7), fumes arising from wok-frying of meats (3), and immune system dysregulation as reflected by previous medical conditions such as asthma and atopy (3). It is, however, still not clear what the key etiologic pathways are for this disease in never smokers.

Dietary factors are likely to influence risk in never smokers (8). While current evidence suggests that fruits and vegetables protect against the development of lung cancer, the role of meat and meat components such as saturated fats and cholesterol is less well established (9–18). Some studies have found an inverse effect (16), whereas others have found no effect (13–15,18) or an increased risk (12,17). The majority of studies have been conducted in European populations, in which red meat comprised a large proportion of total meat consumed. The effect of meat on lung cancer is complicated for a few reasons: red and white meat may vary in their effects. Cooking methods may also be important. Well-done red meat has been associated with an increased risk of lung cancer (19), a finding that has been attributed to the presence of heterocyclic amines (HCAs), which are produced when meats are cooked at high temperatures (20). HCAs are mutagenic in vitro (21), and have been shown to produce tumors in rodents (22) and to increase risk in population-based studies (23–25).

Few studies have included sufficient numbers of never smokers to be able to identify small effects of meat and meat components in this group and most results have been reported in studies in which cases were primarily smokers. It is hence not known whether effects differ substantially between smokers and nonsmokers.

Dietary patterns in Singapore, an Asian city-state with a resident population of 4 million persons mostly of Chinese,

Malay, and Indian ancestry, differ substantially from those in Western countries (26). Among the Chinese, fish represents a high proportion of meat consumed, and chicken and pork are other commonly consumed meats. In contrast, beef and other red meats such as mutton and lamb are rarely consumed. Cooking methods in the Chinese population are also distinct from those in the West, with boiling, steaming, stewing, and wok-frying being common methods of meat cooking while grilling and deep-frying are much less common. As a consequence, meat-cooking methods among the Chinese population in Singapore appear to generate much lower levels of dietary HCAs compared to common cooking methods in the West (27). The relationship of meat consumption to lung cancer risk among the Chinese may therefore differ from that found in Western populations.

Lung cancer is the leading cause of cancer death in Singapore, and it is the most common cancer in men and the third most common cancer in women (28). Although the smoking prevalence in Chinese women in Singapore is low, at about 3% (29), the incidence rate of lung cancer is comparable to other communities with much higher incidence of smoking (1,28), and a significant proportion of lung cancers occur among non-smokers. We thus have a unique opportunity to investigate risk factors specific to non-smoking-related lung cancer in an Asian Chinese population. Differences in the type and variety of meat consumed between our population and others may also offer insights into risks associated with meat intake. In a hospital-based case–control study of Chinese women, we examined the association of fresh and processed meat consumption with lung cancer risk in never smokers.

MATERIALS AND METHODS

We conducted a hospital-based case–control study from February 2005 to January 2008 in the 5 major public-sector hospitals in Singapore. Eligible cases were Chinese women with a diagnosis of primary lung carcinoma (all histological types). The methodology has been previously described (30).

A total of 497 lung cancer patients were identified in the 5 hospitals. We excluded 14 cases that did not satisfy the eligibility criteria, and 9 others that were not contactable for a variety of reasons (e.g., some were too ill), leaving 474 women. Of these, 401 (84.6%) consented to the interview. Histological or cytological reports were reviewed and confirmed the diagnosis of primary lung carcinoma in 372 of these women; we excluded 2 patients who were subsequently confirmed to have metastatic cancer to the lung. Twenty-nine cases were confirmed on the basis of radiological investigations, in which metastatic cancer to the lung from other sites was deemed to be unlikely on clinical grounds. In total, we had 399 confirmed lung cancer cases in this study. Two hundred and fifty-eight never smokers (65%) were used for this analysis.

We selected controls from among Chinese female patients admitted to the same hospitals, frequency-matched by 10-yr age groups and admitted or seen in the hospital within 30 days of the date of diagnosis of the corresponding case. Exclusion criteria were the diagnosis or management of malignancy or chronic respiratory disease (excluding tuberculosis). No more than 10% of controls were recruited within a single diagnostic category.

Of a total of 962 eligible controls identified, 821 (85.3%) consented to be interviewed. One was reclassified as a case, while 6 were subsequently excluded because their admission was due to a malignancy, leaving 815 participants as controls. Control patients had a wide range of conditions: 23% had diseases of skin, bones, joints, and connective tissue; 11% were admitted for gastrointestinal or hepatobiliary system complaints; 10% were admitted for acute trauma; 8% each were admitted for respiratory tract diseases and for neurological or psychiatric conditions; 16% had diseases of the cardiovascular system; 7% each had renal or urinary problems; and generalized infections such as dengue and viral fever. Seven hundred and twelve never smokers (87%) were included for this analysis.

All subjects (cases and controls) were interviewed using a standardized questionnaire by trained research nurses interviewing both cases and controls. Training and supervision was conducted by the same investigator throughout. Interviewers were not blind to case or control status, but interviews were recorded, and a random sample was checked. None of the interviews were conducted solely with the next-of-kin, although relatives, if present, were allowed to give information that was corroborated by the subject. The study protocol was approved by the National University of Singapore Institutional Review Board and the Ethics Committees of the participating healthcare institutions.

Our questionnaire elicited history of personal and passive tobacco exposure, cooking, exposure to kitchen fumes and inhalants such as incense and mosquito coils, family history of cancer, childhood living conditions, occupational history, past history of lung diseases and allergies, menstrual and reproductive history, exogenous hormone use, and use of other medications.

Information on usual dietary intake of commonly available fruit (17 items), vegetables (21 items), soy products (10 items), and meats (18 items) was obtained via a semiquantitative FFQ. Consumption of the 6 common meat groups (fish, chicken, pork, duck, squid and prawns, and beef) was elicited. For each meat type, participants indicated their consumption frequency (number of times per wk) and portion size. Portion size was elicited as multiples of a standard serving specific to each food item. A standard serving was defined and weights of portion sizes calculated from a pilot study among a group of middle-aged and older Chinese women. Pictures of food portions were used to aid participants. Food frequency and usual portion size were multiplied to define the number of standard servings per wk. Consumption of each food item was then categorized in tertiles (based on the distribution among controls).

The questionnaire also elicited information on cooking methods for 9 selected meat-cooking method combinations (e.g., pan-fried fish, pan-fried chicken, roasted pork) and on the consumption of processed meats (bacon, ham, luncheon meat,

sausages, and Chinese sausages). The concentrations of 5 different HCAs had been previously determined in 25 commonly consumed and available meat items in Singapore (27). Five food items were found to have relatively high HCA concentrations and were commonly consumed in our population (pan-fried fish, deep-fried chicken, pan-fried chicken, grilled/roasted pork, and pan-fried pork). We used the estimates of HCA concentration for each food item obtained from our previous study (27) to derive an estimate for the usual weekly intake of HCAs (in nanograms) in our participants. We investigated the effects of 3 HCAs—PhIP [2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine], MeIQx [2-amino-3,8-dimethylimidazo(4,5-f)quinoxaline], 4,8-DiMeIQx [2-amino-3,4,8-trimethlimidazo(4,5-f)quinoxaline]—as well as total HCA consumption.

Data analyses were performed using Stata v. 10 (StataCorp 2007; College Station, TX) software. Unconditional logistic regression with the case–control status taken as the dependent variable was performed, with adjustment for age, family history of cancer, smoking status, country of origin, housing type, and years of education [as proxies for socioeconomic status (SES)], usual body mass index (BMI) in adulthood (defined as usual body weight prior to current illness, in kilograms, divided by the square of the height, in meters), and total fruit and total vegetable consumption (measured in standard servings/wk, as a continuous variable). The inclusion of variables for adjustment in the multiple logistic regression model was based on biologic considerations as well as stepwise backward regression. Tests for linear trend were performed by treating the 3 tertiles for each food group as a continuous variable.

RESULTS

Table 1 gives a summary of relevant characteristics among the 258 cases and 712 controls who were never smokers. The median age was 63.5 yr among cases, and 66 yr among controls. Usual BMI, years of education, and housing type differed significantly between cases and controls in bivariate analysis. One-third (34.8%) of cases had a first-degree relative with a history of cancer, compared to 23.6% in controls ($P \leq .001$).

Table 2 describes the association between dietary factors and lung cancer. High consumption of fruits was inversely associated with lung cancer, with an odds ratio (OR) of 0.39 ($P_{trend} < .001$) in the highest tertile compared to the lowest. An inverse association was also seen with consumption of vegetables, with an OR of 0.57 ($P_{trend} = .003$) in the highest tertile compared to the lowest, and with cruciferous vegetables (ORs = 1.25, 0.66 in second and third tertiles, respectively, $P_{trend} = .046$).

Overall, an inverse association with meat consumption was seen in this study population, with an OR of 0.88 and 0.59 in the second and third tertiles respectively, compared to the first tertile ($P_{trend} = .012$). Among controls, fish comprised 50% of the meat consumed by servings, pork 19%, and chicken 24%. Duck, prawns, and squid were not commonly consumed (with median consumption of only 1 serving a wk). Beef was very rarely

consumed, with about 80% of the study population reporting no consumption (data not shown).

We observed a strong inverse relationship between fish consumption and risk of lung cancer in nonsmokers (ORs = 0.81, 0.47 for 2nd and 3rd tertiles of fish consumption, respectively, $P_{trend} < .001$). We further examined the effect of fish by quintiles of intake. The inverse association was seen only in the fourth and fifth quintiles of fish consumption (Fig. 1). These 2 quintiles were equivalent to consumption at 7.5–15 servings of fish per wk, and more than 15 servings per wk, respectively. Chicken and pork consumption had no significant effect on risk of lung cancer.

Overall, processed meats were not associated with lung cancer risk (ORs = 1.04, 0.83, $P_{trend} = 0.37$). Of the various constituents comprising processed meats, bacon increased lung cancer risk (OR = 1.51, 95% CI 1.06–2.17, compared to participants who do not consume bacon). Ham, luncheon meat, sausages, and Chinese sausages were not associated with lung cancer (Table 3). None of the 3 HCAs examined (PhIP, MeIQx, and 4,8-DiMeIQx) was associated with lung cancer risk nor was total HCA (Table 4).

Further adjustment with self-reported exposure to meat cooking fumes did not alter the results obtained for fresh meat, processed meat, and dietary HCA consumption and the association with lung cancer (data not shown).

DISCUSSION

In this study of never smokers, total meat intake (of mostly white meat—fish and chicken) is associated with a lower risk of lung cancer. Among meat components, fish consumption is associated with a reduced risk of lung cancer. Processed meats do not appear to be associated with lung cancer risk, although bacon may increase lung cancer risk among never smokers. Dietary HCAs arising from Chinese-style meat-cooking did not increase lung cancer risk.

Recent research into the effect of meat on lung cancer risk have focused on red and processed meat. Carcinogenic compounds arising from the cooking of red meat such as HCAs (17,23–25) and the polycyclic aromatic hydrocarbon benzo(a)pyrene (17,24) have been implicated as the mechanism conferring risk. In addition, processed meat products have also been identified as a possible risk factor for lung cancer (17,24). Nitrosamines are formed from nitrates and nitrites added to processed meats (31), are carcinogenic (32), and may be the biological mechanism linking processed meats with lung cancer. The association of processed meats was not convincing in our study; most processed meats did not show a relationship with lung cancer and, although bacon appeared to be associated with lung cancer cases in our study, this increase was in the context of a comparison between nonconsumers and ever-consumers. Consumption levels of processed meats (with the exception of Chinese sausages) were generally low in our population. It is likely therefore that any risks associated with processed meats would be difficult to identify in our population.

TABLE 1
Baseline characteristics of never-smoking female Chinese lung cancer patients and controls

Baseline Characteristic	Cases (n = 258)		Controls (n = 712)		P
Age (yr)					
Mean +/− SD	63.80 +/−12.02		64.86 +/−11.63		.22
Median	63.5		66		
Usual body mass index[a]					
Mean +/− SD	22.45+/−3.81		24.21 +/−4.88		<0.01
Median	(23.37)		(22.07)		
	n	%	n	%	
Dialect group					.41
Hokkien	92	35.8	303	42.7	
Teochew	65	25.3	145	20.4	
Cantonese	48	18.7	123	17.3	
Hainanese	17	6.6	40	5.6	
Hakka	26	10.1	69	9.7	
Others	9	3.5	30	4.2	
Country of birth					.69
Singapore	178	69.8	497	69.8	
Malaysia	38	14.7	117	16.4	
China	30	11.6	74	10.4	
Other	12	4.7	24	3.4	
Education					.01
Nil	81	31.4	266	37.4	
6 yr or less	121	47.5	233	32.7	
7 yr or more	58	22.8	213	29.9	
Current housing					.01
1–3 room public flat	76	29.8	234	33.1	
4 room or larger public flat	121	47.5	373	52.8	
Private apt or house	58	22.8	99	14.0	
Marital status					.42
Currently or previously married	241	93.4	654	91.9	
Never married	17	6.6	58	8.2	
Ever worked outside the home					.87
Yes	210	81.4	582	81.9	
No	48	18.6	129	18.1	
Environmental tobacco smoke exposure at home					.43
Less than daily	122	48.8	321	45.9	
Every day	128	51.2	378	54.1	
Family history of cancer[b]					<.001
No	165	65.2	537	76.4	
Yes, other cancers	60	23.7	131	18.6	
Yes, lung cancer	28	11.1	35	5.0	
Exposure to cooking fumes[c]					0.093
Cooked <1/mo	59	23.2	147	20.9	
Cooked at least 1/mo; kitchen filled with fumes <1/wk	156	61.4	405	57.5	
Cooked at least 1/mo; kitchen filled with fumes 1/wk or more	39	15.4	153	21.7	

[a]Calculated as usual body weight (in kilograms) divided by the square of the height (in meters).

[b]First-degree relative with history of cancer of any site.

[c]Exposure to cooking fumes when frying, estimated at 25 yr before diagnosis or admission to hospital.

TABLE 2
Fruit, vegetable, and meat consumption and risk of lung cancer in Chinese women, Singapore 2005–2008

Servings/wk	Cases (n = 258)	Controls (n = 712)	Odds Ratios (95% Confidence Intervals)[a]
Fruits[b]			
1st tertile (<3.88)	97 (37.6)	226 (31.7)	1.0
2nd tertile (>3.87–11.02)	108 (41.9)	246 (34.6)	0.86 (0.60, 1.22)
3rd tertile (>11.02)	53 (20.5)	240 (33.7)	0.39 (0.26, 0.60)
P_{trend}			<.001
Vegetables[c]			
1st tertile (<11.88)	103 (39.9)	233 (32.7)	1.0
2nd tertile (>11.87–24.00)	88 (34.1)	236 (33.2)	0.81 (0.56, 1.16)
3rd tertile (>24.00)	67 (26.0)	243 (34.1)	0.57 (0.39, 0.83)
P_{trend}			.003
Cruciferous vegetables[d]			
1st tertile (<5.61)	83 (32.2)	232 (32.6)	1.0
2nd tertile (>5.60–12.00)	110 (42.6)	241 (33.9)	1.25 (0.88, 1.79)
3rd tertile (>12.00)	65 (25.2)	239 (33.6)	0.66 (0.44, 0.98)
P_{trend}			.046
Total meats[d]			
1st tertile (<9.70)	103 (40.1)	232 (32.9)	1.0
2nd tertile (9.70–19.60)	93 (36.2)	238 (33.7)	0.88 (0.61, 1.26)
3rd tertile (>19.60)	61 (23.7)	236 (33.4)	0.59 (0.39, 0.89)
P_{trend}			.012
Fish			
1st tertile (<2.51)	112 (43.4)	231 (32.4)	1.0
2nd tertile (>2.50–10.00)	100 (38.8)	265 (37.2)	0.81 (0.57, 1.14)
3rd tertile (>10.00)	46 (17.8)	216 (30.3)	0.47 (0.31, 0.73)
P_{trend}			.001
Chicken			
1st tertile (<1.01)	125 (48.5)	358 (50.3)	1.0
2nd tertile (>1.00–2.50)	49 (19.0)	132 (18.5)	1.08 (0.72, 1.63)
3rd tertile (>2.50)	84 (32.6)	222 (31.2)	1.00 (0.69, 1.45)
P_{trend}			.95
Pork			
1st tertile (<1.01)	106 (41.1)	323 (45.5)	1.0
2nd tertile (>1.00–2.50)	68 (26.4)	176 (24.8)	1.09 (0.75, 1.60)
3rd tertile (>2.50)	84 (32.6)	211 (29.7)	1.15 (0.80, 1.64)
P_{trend}			.44

[a]Adjusted for age, history of cancer in first-degree relative, country of origin, dwelling type, yr of education, usual body mass index, and fruit and vegetable intake (only for meat variables).

[b]Summed weekly consumption of banana; papaya; apple; orange or mandarin orange; pineapple; watermelon; mango; starfruit; jackfruit; plum; cantaloupe/rock melon; dried prunes; fresh fruit juice; canned peaches; pear; Chinese pear; and grapes.

[c]Summed weekly consumption of wong-nga-pak; pak choy (Chinese cabbage); kai lan (Chinese kale); head cabbage; cauliflower; kai choy; choy sum; sai yong choy (watercress); broccoli; kang kong (water convolvulus); por choy (spinach); sang choy (Chinese lettuce); tomatoes; French beans; long beans; snow peas; ladies' fingers (okra); red carrot; and sweet potato.

[d]Summed intakes of fish, chicken, pork, duck, prawns and squid, and beef.

We did not find a relationship with lung cancer of total HCA or any of the 3 common HCAs. This is not unexpected, as red meat consumption is relatively low in our population, and the Chinese cooking methods employed by our population do not generate significant quantities of HCAs as a by-product of cooking (26). Indeed, the consumption levels of the 3 HCAs studied in our population was about 6–8 times lower than that reported in the Missouri study (23).

Studies that have differentiated white from red meat have generally not shown any association of white meat with lung
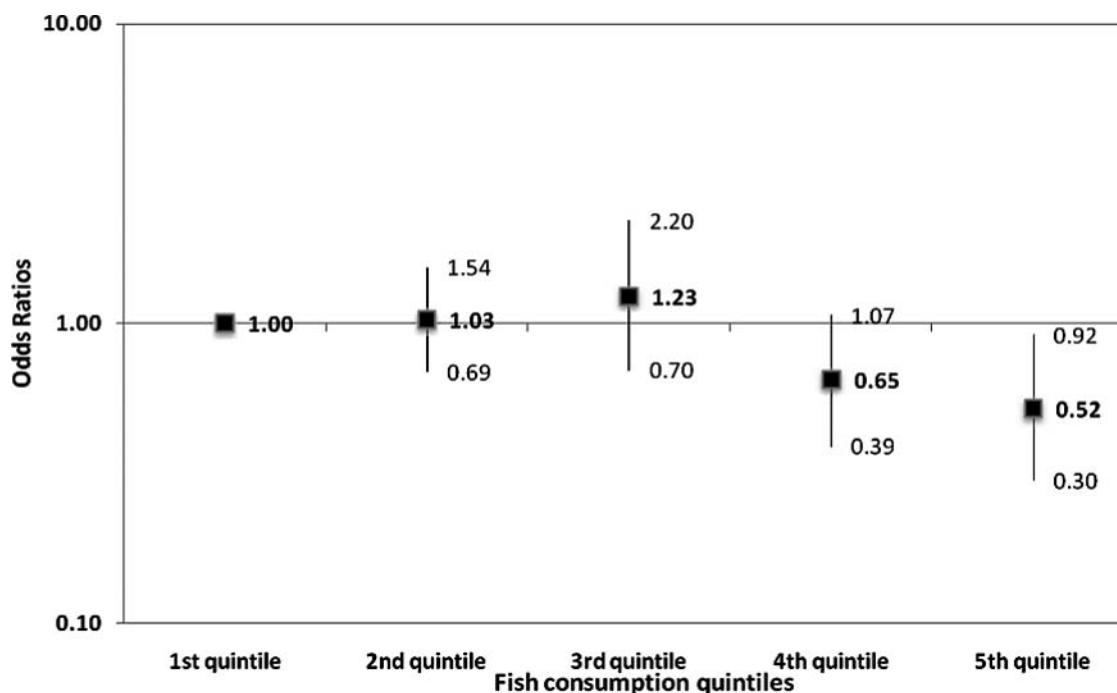
FIG. 1. Odds ratios and the 95% confidence intervals of the association of fish consumption with lung cancer among Chinese female never smokers, by quintile of consumption, adjusted for age, history of cancer in first-degree relative, country of origin, dwelling type, yr of education, usual body mass index, fruit intake, and vegetable intake.

cancer (12,13). This may be because white meat in general does not produce significant quantities of HCAs when cooked.

In contrast to most other studies that have reported either a direct or no association between meat consumption and lung cancer, our study suggests an inverse association. This association is completely driven by fish consumption, because fish is the main source of meat in this population and the only type of meat to have a significant inverse association with lung cancer risk. The reasons for this are not clear. A substitution effect of red meat by white meat (i.e., fish) intake is possible, although no increased risks were seen with either pork or beef consumption. Confounding by socio-economic status (SES) is unlikely, since the relationship was significant in multivariate analyses, after adjustment for SES using proxies of dwelling type and years of education. An inverse association of BMI with lung cancer risk, particularly in current smokers, has been proposed (33), and it is possible that meat consumption is confounded by BMI in our study. However, we adjusted for BMI based on usual body weight before the development of illness in our analyses, and residual confounding is unlikely to explain these findings. Perhaps meat consumption may simply reflect better overall nutritional status, with adequate intakes of trace minerals and vitamins conferring protection.

Of the various meat types, the most striking relationship observed in our study was the inverse association of fish intake with lung cancer. The nature of the relationship between fish intake and lung cancer risk has not been clearly elucidated despite many studies looking at diet in lung cancer. Some case–control

studies, especially those conducted in population with relatively low rates of fish consumption, have reported that fish consumption is positively associated with lung cancer (16,34,35). Other case–control studies, however, report inverse effects (12,36,37). In general, all these studies had a high proportion of smokers among cases and did not look at effects separately in smokers and nonsmokers. Three case–control studies that did look specifically at never smokers did not find an effect of fish consumption (14,15,38). An ecological study concluded that high fish consumption at the country level is associated with a reduction in lung cancer mortality in men, but only in countries with high levels of cigarette smoking or animal fat consumption (39). A Japanese cohort study (40) reported an inverse effect of fish intake on incidence of lung cancer, but no association of fish consumption with lung cancer incidence was found in two cohorts (11,41) in Norway, nor with lung cancer mortality in one other cohort in Japan (42). Our data suggest that an inverse association is seen in never smokers and at intakes of more than 1 serving a day (Fig. 1), and we speculate that the inconsistent findings between studies could be due to the high proportion of smokers among cases in these studies and to differences in the range of consumption of fish in the base populations. [For example, the Galician study (16) that showed a positive relationship compared <1 time per wk to >1 time per wk, while the Japanese case–control study (36) showing an inverse relationship compared <1 time per wk with 5 or more times per wk in the highest consumption quartile.]

A protective effect of fish intake is biologically plausible. In vitro studies demonstrate that the omega-3 fatty acids

TABLE 3
Consumption of processed meats and risk of lung cancer in never-smoking Chinese women

Servings/wk	Cases (n = 258)	Controls (n = 712)	Odds Ratios (95% Confidence Intervals)[a]
Processed meats[b]			
1st tertile (<0.30)	73 (28.6)	192 (27.2)	1.0
2nd tertile (0.30–<0.70)	100 (39.2)	263 (37.3)	1.04 (0.71, 1.51)
3rd tertile (>0.69)	82 (32.2)	251 (35.6)	0.83 (0.55, 1.25)
P_{trend}			.37
Bacon[c]			
1st tertile (0)	173 (67.6)	548 (77.1)	1.0
2nd tertile (>0)	83 (32.4)	163 (22.9)	1.51 (1.06, 2.16)
Ham[d]			
1st tertile (0)	148 (59.6)	424 (59.6)	1.0
2nd tertile (>0–0.10)	67 (26.0)	156 (21.9)	1.12 (0.77, 1.62)
3rd tertile (>0.10)	43 (16.7)	132 (18.5)	0.81 (0.52, 1.26)
P_{trend}			.52
Luncheon Meat[e]			
1st tertile (<5.61)	101 (39.3)	306 (43.2)	1.0
2nd tertile (>5.60–12.00)	84 (32.7)	182 (25.7)	1.53 (1.06, 2.22)
3rd tertile (>12.00)	72 (28.0)	221 (31.2)	1.20 (0.82, 1.74)
P_{trend}			.25
Sausages[f]			
1st tertile (<9.70)	147 (57.0)	436 (61.3)	1.0
2nd tertile (9.70–19.60)	34 (13.2)	77 (10.8)	1.20 (0.74, 1.93)
3rd tertile (>19.60)	77 (29.8)	198 (27.9)	1.00 (0.69, 1.43)
P_{trend}			.93
Chinese sausages/waxed meat[g]			
1st tertile (<2.51)	113 (43.8)	355 (49.9)	1.0
2nd tertile (>2.50–10.00)	58 (22.5)	123 (17.3)	1.54 (1.03, 2.31)
3rd tertile (>10.00)	87 (33.7)	233 (32.8)	1.23 (0.87, 1.74)
P_{trend}			.19

[a]Adjusted for age, history of cancer in first-degree relative, country of origin, dwelling type, yr of education, usual body mass index, and fruit and vegetable intake.

[b]Sum of serving portions consumed per wk of the following 5 processed meat types: ham, bacon, luncheon meat, sausages, and Chinese sausages.

[c]1 serving defined as 1 slice of bacon.

[d]1 serving defined as 1 slice of ham.

[e]1 serving defined as 1 slice of luncheon meat.

[f]1 serving defined as 0.5 sausages.

[g]1 serving defined as 2–3 slices of Chinese sausages or waxed meat.

eicosapentaenoic acid (EPA) and docosahexoic acid (DHA), both prominent constituents of fish oil, have antiinflammatory, proapoptotic, and anticarcinogenic effects through their interactions with Peroxisome Proliferator-Activated Receptor-γ, nuclear factor kappa B, cyclooxygenase-2, and integrin-linked kinase (43–47). Some, but not all, epidemiologic studies investigating lung cancer risk with fish oil consumption support this (11,48). Singapore imports almost all fish for retail consumption, of which 96–98% is consumed fresh (rather than as processed fish) (26), and relatively fatty fish such as mackerel and salmon represent a substantial proportion of fish consumed

in Singapore (Agri-Food and Veterinary Authority of Singapore, unpublished data). The authors of a recent study of omega-3 content of local fish (49) estimated that consumption of about 50–60 g of local fish (equivalent to about 2 serving sizes in this study) would provide about 350 mg of DHA/EPA per day. It is also possible that the protective effect of fish is mediated through other vitamin or mineral constituents of fish. Lastly, the inverse association seen may be due to substitution of fish for red meat.

A key strength of our study is the homogeneity of the study population in relation to ethnicity and gender, and the large

TABLE 4
Dietary heterocyclic amines (HCA) and risk of lung cancer in never-smoking Chinese women

Nanograms/wk	Cases (n = 258)	Controls (n = 712)	Odds Ratios (95% Confidence Intervals)[a]
Total HCA			
1st tertile (<92.32)	83 (32.4)	237 (33.6)	1.0
2nd tertile (>92.32–275.90)	98 (38.3)	238 (33.7)	1.31 (0.91–1.89)
3rd tertile (>275.90)	75 (28.3)	231 (32.7)	0.98 (0.66–1.45)
P_{trend}			0.96
PhIP			
1st tertile (<55.70)	83 (32.4)	239 (33.9)	1.0
2nd tertile (>55.70–160.47)	97 (37.9)	233 (33.0)	1.28 (0.89–1.85)
3rd tertile (>160.47)	76 (29.7)	234 (33.1)	0.98 (0.66–1.46)
P_{trend}			0.98
MelQx			
1st tertile (<19.80)	90 (35.2)	238 (33.7)	1.0
2nd tertile (>19.79–62.90)	98 (38.3)	241 (34.1)	1.16 (0.81–1.66)
3rd tertile (>62.90)	68 (26.6)	228 (32.3)	0.81 (0.55–1.20)
P_{trend}			0.34
4,8-DiMelQx			
1st tertile (<11.22)	92 (35.9)	238 (33.7)	1.0
2nd tertile (>11.22–34.85)	100 (39.1)	241 (34.1)	1.20 (0.84–1.71)
3rd tertile (>34.85)	64 (25.0)	228 (32.3)	0.76 (0.51–1.13)
P_{trend}			0.22

[a]Adjusted for age, history of cancer in first-degree relative, country of origin, dwelling type, yr of education, usual body mass index, and fruit and vegetable intake.

number of nonsmokers in our study population. These features allow us to elucidate weaker associations in this important subgroup, which may be overshadowed by smoking-related effects in other populations. At the same time, we are mindful of limitations of these data that are inherent to the retrospective nature of the study. We used a semiquantitative FFQ, and reporting errors associated with FFQs are well known. Such errors associated with the dietary instrument would likely be nondifferential and would bias estimates toward the null. Other types of reporting errors are also possible. We cannot exclude the possibility that dietary effects observed might have been due to dietary changes as a consequence of symptoms or disease. Because we used hospital controls, our control population's dietary intake may differ substantially from the general population because of their disease status. However, we used controls who were admitted to a wide variety of hospital departments and with different admitting diagnoses. In addition, excluding participants who reported that they had changed their diet recently due to illness or other factors (about 25% of controls and 31% of cases reported that they had done so) did not alter the relationship seen between meat and fish consumption and lung cancer risk in this population (data not shown). Our questionnaire did not allow us to control for total calorie intake; however, we adjusted for usual BMI in our analyses. Moreover, if the main findings were

due to reduced overall consumption amongst cases compared to controls, we would have expected to see inverse associations across all food items.

In summary, within the range of consumption prevalent in this Asian population, our findings support a protective role for white meat intake and, specifically, fish intake in the development of lung cancer in never smokers.

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