Carcinogenesis vol.32 no.4 pp.522–529, 2011
doi:10.1093/carcin/bgr006
Advance Access publication January 20, 2011

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

Wei-Yen Lim*, Ying Chen, Safiyya Mohamed Ali, Khoon Leong Chuah[1], Philip Eng[2], Swan Swan Leong[3], Elaine Lim[4], Tow Keang Lim[5], Alan WK Ng[4], Wee Teng Poh[6], Augustine Tee[7], Ming Teh[5], Agus Salim and Adeline Seow

Department of Epidemiology & Public Health National University of Singapore, Singapore, Singapore S(117597), [1]Department of Pathology, Tan Tock Seng Hospital, Singapore, Singapore S(308433), [2]Mount Elizabeth Hospital, Singapore, Singapore S(228510), [3]Department of Medical Oncology, National Cancer Centre, Singapore, Singapore S(169610), [4]Department of Respiratory & Critical Care Medicine, Tan Tock Seng Hospital, Singapore, Singapore S(308433), [5]Department of Pathology, National University Hospital, Singapore, Singapore, S(119074), [6]Department of Laboratory Medicine, Changi General Hospital, Singapore, Singapore S(529889) and [7]Division of Respiratory Medicine, Changi General Hospital, Singapore, Singapore S(529889)

*To whom correspondence should be addressed. Tel: +6567738956; Fax: +6564789913;
Email: wei-yen_lim@nuhs.edu.sg

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 (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

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.

© The Author 2011. Published by Oxford University Press. All rights reserved. For Permissions, please email: journals.permissions@oup.com

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

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, Pro^{12}Ala 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).

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

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

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

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 rhinitis/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

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

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	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)[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

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

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.

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

Supplementary material

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

Funding

National Medical Research Council Singapore (NMRC/0897/2004 and NMRC/1075/2006).

Conflict of Interest Statement: None declared.

References

1. Boyle,P. *et al*. (2008) *World cancer report 2008. International Agency for Research in Cancer*. IARC Press, Lyon.
2. World Health Organization. (2008) *WHO report on the global tobacco epidemic, 2008: The Mpower package*. World Health Organisation, Geneva, Switzerland.
3. Koo,L.C. *et al*. (1990) Worldwide epidemiological patterns of lung cancer in nonsmokers. *Int. J. Epidemiol.* , **19**, S14–S23.
4. Toh,C.K. *et al*. (2006) Never-smokers with lung cancer: epidemiologic evidence of a distinct disease entity. *J. Clin. Oncol.*, **24**, 2245–2251.
5. Wakelee,H.A. *et al*. (2007) Lung cancer incidence in never smokers. *J. Clin. Oncol.*, **25**, 472–478.
6. Subramanian,J. *et al*. (2007) Lung cancer in never smokers: a review. *J. Clin. Oncol.*, **25**, 561–570.
7. Shigematsu,H. *et al*. (2005) Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancer. *J. Natl Cancer Inst.*, **97**, 339–346.
8. Brennan,P. *et al*. (2004) Secondhand smoke exposure in adulthood and risk of lung cancer among never smokers: a pooled analysis of two large studies. *Int. J. Cancer.*, **109**, 125–131.
9. Ko,Y.C. *et al*. (2000) Chinese food cooking and lung cancer in women nonsmokers. *Am. J. Epidemiol.*, **151**, 140–147.
10. Kleinerman,R.A. *et al*. (2002) Lung cancer and indoor exposure to coal and biomass in rural China. *J. Occup. Environ. Med.*, **44**, 338–344.
11. Darby,S. *et al*. (2005) Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. *BMJ.*, **330**, 223.
12. Vainio,H. *et al*. (2006) Fruits and vegetables in cancer prevention. *Nutr. Cancer.*, **54**, 111–142.
13. Seitz,H.K. *et al*. (2006) Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol. Chem.*, **387**, 349–360.
14. Balkwill,F. *et al*. (2001) Inflammation and cancer: back to Virchow? *Lancet.*, **357**, 539–545.
15. Martey,C.A. *et al*. (2004) Cigarette smoke induces cyclooxygenase-2 and microsomal prostaglandin E2 synthase in human lung fibroblasts: implications for lung inflammation and cancer. *Am. J. Physiol. Lung Cell. Mol. Physiol.*, **287**, L981–L91.
16. Brenner,A.V. *et al*. (2001) Previous pulmonary diseases and risk of lung cancer in Gansu Province, China. *Int. J. Epidemiol.*, **30**, 118–124.
17. Wu-Williams,A.H. *et al*. (1990) Lung cancer among women in north-east China. *Br. J. Cancer.*, **62**, 982–987.
18. Mayne,S.T. *et al*. (1999) Previous lung disease and risk of lung cancer among men and women nonsmokers. *Am. J. Epidemiol.*, **149**, 13–20.
19. Hubbard,R. *et al*. (2000) Lung cancer and cryptogenic fibrosing alveolitis: a population based cohort study. *Am. J. Respir. Crit. Care Med.*, **161**, 5–8.
20. Koh,W.P. *et al*. (2008) Chronic rhinosinusitis and risk of lung cancer in the Singapore Chinese Health Study. *Int. J. Cancer.*, **123**, 1398–1402.
21. Brown,D.W. *et al*. (2005) Asthma and risk of death from lung cancer: NHANES II Mortality Study. *J. Asthma.*, **42**, 597–600.
22. Boffetta,P. *et al*. (2002) Lung cancer risk in a population-based cohort of patients hospitalized for asthma in Sweden. *Eur. Respir. J.*, **19**, 127–133.
23. Santillan,A.A. *et al*. (2003) A meta-analysis of asthma and lung cancer (United States). *Cancer Causes Control.*, **14**, 327–334.
24. Talbot-Smith,A. *et al*. (2003) Allergy, atopy and cancer:A prospective study of the 1981 Busselton Cohort. *Am. J. Epidemiol.*, **157**, 606–612.
25. Wang,H. *et al*. (2006) Atopic diseases, immunoglobulin E and risk of cancer of the prostate, breast, lung and colorectum. *Int. J. Cancer.*, **119**, 675–701.
26. Castaing,M. *et al*. (2005) Is the risk of lung cancer reduced among eczema patients? *Am. J. Epidemiol.*, **162**, 542–547.
27. Zienolddiny,S. *et al*. (2004) Polymorphisms of the interleukin-1 beta gene are associated with increased risk of non-small cell lung cancer. *Int. J. Cancer.*, **109**, 353–356.
28. Asada,M. *et al*. (2006) Interleukin-1 beta gene polymorphisms associated with risk of lung cancer in Japanese. *Lung Cancer.*, **54**, 261–263.
29. Campa,D. *et al*. (2005) Lack of association between polymorphisms in inflammatory genes and lung cancer risk. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 538–539.
30. Vogel,U. *et al*. (2008) Polymorphisms in genes involved in the inflammatory response and interaction with NSAID use or smoking in relation to lung cancer risk in a prospective study. *Mutat. Res.*, **639**, 89–100.
31. Engels,E.A. *et al*. (2007) Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. *Cancer Res.*, **67**, 6520–6527.
32. Lee,K.M. *et al*. (2007) Polymorphisms in immunoregulatory genes, smoky coal exposure and lung cancer risk in Xuan Wei, China. *Carcinogenesis.*, **28**, 1437–1441.
33. Seow,A. *et al*. (2006) Joint effect of asthma/atopy and an IL-6 gene polymorphism on lung cancer risk among lifetime non-smoking Chinese women. *Carcinogenesis.*, **27**, 1240–1244.
34. Van Dyke,A.L. *et al*. (2009) Cytokine and cytokine receptor single-nucleotide polymorphisms predict risk for non-small cell lung cancer among women. *Cancer Epidemiol. Biomarkers Prev.*, **18**, 1829–1840.
35. Hu,Z. *et al*. (2006) Allele 2 of the interleukin-1 receptor antagonist gene (IL1RN*2) is associated with a decreased risk of primary lung cancer. *Cancer Lett.*, **236**, 269–275.
36. Lind,H. *et al*. (2005) Interleukin 1 receptor antagonist gene polymorphism and risk of lung cancer: a possible interaction with polymorphisms in the interleukin1 beta gene. *Lung Cancer.*, **50**, 285–290.
37. Hu,Z. *et al*. (2005) A common polymorphism in the 3'UTR of cyclooxygenase 2/prostaglandin synthase 2 gene and risk of lung cancer in a Chinese population. *Lung Cancer.*, **48**, 11–17.
38. Park,J.M. *et al*. (2006) Relationship between cyclooxygenase 8473 T>C polymorphism and the risk of lung cancer: a case control study. *BMC Cancer.*, **6**, 70.
39. Chen,D. *et al*. (2008) Genetic variants in peroxisome proliferator-activated receptor γ gene are associated with risk of lung cancer in a Chinese population. *Carcinogenesis.*, **29**, 342–350.
40. Seow,A. *et al*. (2004) *Trends in Cancer Incidence in Singapore 1968–2002. Singapore Cancer Registry, Report No. 6.* Singapore Cancer Registry, Singapore, Singapore.
41. Epidemiology & Disease Control Division. (2005) *National Health Survey 2004*. Ministry of Health, Singapore, Singapore.
42. Seow,A. *et al*. (2000) Fumes from meat cooking and lung cancer risk in Chinese women. *Cancer Epidemiol. Biomarkers Prev.*, **9**, 1215–1221.
43. Tang,L. *et al*. (2010) Lung cancer in Chinese women: evidence for an interaction between tobacco smoking and exposure to inhalants in the indoor environment. *Environ. Health Perspectives.*, **118**, 1257–1260.
44. Tarlow,J.K. *et al*. (1993) Polymorphism in human IL-1 receptor antagonist gene intron 2 is caused by variable numbers of an 86-bp tandem repeat. *Hum. Genet.*, **91**, 403–404.
45. Benjamini,Y. *et al*. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Statist. Soc. Ser. B.*, **57**, 289–300.
46. Alavanja,M.C. *et al*. (1992) Preexisting lung disease and lung cancer among nonsmoking women. *Am. J. Epidemiol.*, **136**, 623–632.
47. Liang,H.Y. *et al*. (2009) Facts and fiction of the relationship between preexisting tuberculosis and lung cancer risk: a systematic review. *Int. J. Cancer.*, **125**, 2936–2944.
48. Wang,X.R. *et al*. (2009) Previous pulmonary disease and family cancer history increase the risk of lung cancer among Hong Kong women. *Cancer Causes Control.*, **20**, 757–763.
49. Kubík,A.K. *et al*. (2002) Lung cancer risk among Czech women: a case-control study. *Prev. Med.*, **34**, 436–444.
50. Islam,S.S. *et al*. (1994) Declining FEV1 and chronic productive cough in cigarette smokers: a 25-year prospective study of lung cancer incidence in Tecumseh, Michigan. *Cancer Epidemiol. Biomarkers Prev.*, **3**, 289–298.
51. Salvi,S.S. *et al*. (2009) Chronic obstructive pulmonary disease in non-smokers. *Lancet.*, **374**, 733–743.
52. Ker,J. *et al*. (2009) The atopic march: what's the evidence? *Ann. Allergy Asthma Immunol.*, **103**, 282–289.
53. Kitamura,A. *et al*. (2002) Interleukin-6 polymorphism (-634C/G) in the promoter region and the progression of diabetic nephropathy in Type 2 diabetes. *Diabet. Med.*, **19**, 1000–1005.
54. Colakogullari,M. *et al*. (2008) The involvement of IL-10, IL-6, IFN-, TNF- and TGF- gene polymorphisms among Turkish lung cancer patients. *Cell Biochem. Funct.*, **26**, 283–290.

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013

55. El-Omar,E.M. *et al*. (2000) Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature.*, **404**, 398–402.
56. Wu,K.S. *et al*. (2010) Influence of interleukin-1 beta genetic polymorphism, smoking and alcohol drinking on the risk of non-small cell lung cancer. *Clin. Chim. Acta.*, **411**, 1441–1446.
57. Kiyohara,C. *et al*. (2010) IL1β rs1143634 polymorphism, cigarette smoking, alcohol use and lung cancer risk in a Japanese population. *J. Thorac. Oncol.*, **5**, 299–304.
58. Ter-Minassian,M. *et al*. (2008) Apoptosis gene polymorphisms, age, smoking and the risk of non-small cell lung cancer. *Carcinogenesis.*, **29**, 2147–2152.
59. Rothman,K.J. *et al*. (2008) *Modern Epidemiology.* Lippincott Williams & Wilkins, Philadelphia, PA.

Received July 27, 2010; revised December 21, 2010; accepted January 15, 2011

Downloaded from http://carcin.oxfordjournals.org/ by guest on February 19, 2013