***IL1B* gene polymorphisms, age and the risk of non-small cell lung cancer in a Chinese population**

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Abstract

Background/objectives: *IL1B* rs12621220G/A (-3893), rs1143623G/C (-1464), rs16944T/C (-511) and rs1143627C/T (-31) were previously reported to be associated with non-small cell lung cancer (NSCLC) and formed a specific haplotype (GGCT) which was associated with increased *IL1B* gene expression and increased risk of NSCLC in European populations. Only the two SNPs of rs16944T/C (-511) and rs1143627C/T (-31) have been studied in Chinese populations, and the results were conflicting. Thus we studied the association of the above four SNPs with NSCLC in a large Chinese population.

Methods: We genotyped *IL1B* SNPs in a case–control study with 889 lung cancer cases and 1005 controls using the SNPscanTM Genotyping system. We used logistic regression to determine the association between each SNP and NSCLC estimated by ORs and their 95% confidence intervals (CIs), controlling for Potential confounders as appropriate.

Results: In subjects over age 63, significant associations were detected between NSCLC and *IL1B* SNPs. For rs12621220G>A (-3893) and rs1143623G>C (-1464), heterozygous variants, when compared with ancestral genotype, were significantly associated with decreased risk of NSCLC, with adjusted odds ratio (aOR) = 0.710 (0.516, 0.976), *P* = 0.035 and aOR = 0.643 (0.466, 0.886), *P* = 0.007, respectively. For rs16944T>C (-511) and rs1143627C>T (-31), homozygous variants were significantly associated with increased risk of NSCLC, with aOR = 1.482 (1.084, 2.025), *P* = 0.014 and aOR = 1.450 (1.055, 1.994), *P* = 0.022, respectively. Inference of the haplotype structures showed that rs12621220G/A (-3893), rs1143623G/C (-1464), rs16944T/C (-511) and rs1143627C/T (-31) formed two risk haplotypes (GGCC and ACTT) with linkage disequilibrium in all subjects, and they have significantly different frequencies between cases and controls after the permutation tests

for one hundred thousand times ($P = 0.0000E0$).

Conclusions: Our study provided evidence that *IL1B* SNPs might be implicated in the pathogenesis of NSCLC in the Chinese population.

Keywords: *IL1B* SNP NSCLC Susceptibility Chinese population

Introduction

Lung cancer is a leading cause of cancer related mortality [1-3]. Lung cancer accounts for around 1.8 million new cancer cases and 1.6 million deaths each year, which represent about 13.0% of all new cancer cases each year and 19.4% of cancer deaths [2]. It is estimated that 40% of new lung cancer cases arise in East Asia (mainly in China) [4]. Approximately 80% of patients are diagnosed with non-small cell histology [5]. To understand the genetic basis of individual variation, research of the relation between single nucleotide polymorphisms (SNPs) and the susceptibility to lung cancer is a hotspot in the field of lung cancer.

The pathogenesis of many human cancers arises from infection, chronic irritation and inflammation. Recent data have expanded the concept that inflammation is a critical component of tumor progression [6]. Chronic pathological inflammation is mediated via the presence of a persistent stimulus, such as tumor cells, and the resulting prolonged inflammatory cytokine exposure has the potential to promote tumor growth through the induction of angiogenesis, DNA damage, and other events favorable to tumor invasion and metastases [7]. A report about asthma patients and the risk of lung cancer in European populations [8], and a case-control study for lung cancer susceptibility in North African populations [9] suggest that chronic inflammation is significantly associated with the

risk of lung cancer.

Interleukin-1 (IL-1), a major mediator of inflammation, initiates and/or increases a wide variety of non-structural, functionally associated genes, which are expressed during inflammation [10]. IL1 family consists of α , β monomer, and its activity is mainly expressed by β . Many cell types including lung epithelial cells produce and secrete IL-1 β upon exposure to chemicals and other environmental agents [11, 12]. *IL-1* mRNA is highly expressed in many different cancer types such as non-small cell lung cancer (NSCLC), colorectal adenocarcinoma, and melanoma [13]. Previous studies have revealed that in the mice deficient in IL-1 β , tumor development is slow, which suggests the crucial role of IL-1 β in carcinogenesis [14, 15].

IL1 β is encoded by *IL1B* gene which is located in the *IL-1* gene cluster on chromosome 2q and several single-nucleotide polymorphisms (SNP) of the gene have been identified. Zienolddiny's study showed that *IL1B* rs1143627 (-31) TT and rs16944 (-511) CC in the promoter region increased risk of NSCLC in a small case-control Norwegian populations (case: 251, control: 272) in 2004 for the first time [16]. Subsequently, they provided evidence that *IL1B* rs12621220 (-3893) GA and rs1143623 (-1464) GC in the enhancer region may also affect this risk and the four SNPs (G-3893A, G-1464C, T-511C, C-31T) formed a specific risk haplotype GGCT associated with increased *IL1B* gene expression [17]. They also showed that this haplotype have high transcriptional activity in the human lung epithelial A549 cell line in vitro [18]. A study in a Danish population showed that the carriers of the C allele of *IL1B* -31 were at higher risk of lung cancer than TT genotype [19]. However, Campa's study did not support a major role of *IL1B* -31 in lung carcinogenesis within 15 centers of six countries in central and East Europe [20]. So far, only two SNPs of *IL1B* -511 and -31 have been studied in Chinese populations, and the results about *IL1B* T-511C are inconsistent in

different studies [21, 22]. Due to the varying genetic structure in different populations, these four SNPs were genotyped in a large Chinese sample to verify the association between *IL1B* SNPs (rs12621220G/A (-3893), rs1143623G/C (-1464), rs16944T/C (-511), rs1143627C/T (-31)) and NSCLC.

Materials and methods

Study population

The present study is comprised of 1894 individuals: 889 eligible cases (patients with histologically confirmed NSCLC) enrolled from Taizhou, Jiangsu province and the city of Shanghai, ~~China~~ when were newly diagnosed with lung cancer; 1005 cancer-free individuals recruited from healthy populations of the corresponding community during the same period. The enrolling criteria of the above cases included a histological diagnosis of lung cancer, no history of malignant tumor in other organs, no previous chemical therapy or medical treatment, and no history of radiation exposure. The recruitment criteria of controls included no history of cancer and any other pulmonary diseases. All subjects were ethnically Chinese Han and came from East China, including Shanghai, Taizhou (Jiangsu province), and surrounding regions. This study was approved by the School of Life Sciences, Fudan University, China.

DNA extraction and genotyping

DNA was extracted from peripheral blood samples drawn from study participants using the DP319 Genomic DNA Extraction Kit (Tiangen Corporation, China) according to the manufacturer's

protocol. SNPs were genotyped using the SNPscanTM Genotyping system (Tianhao Corporation, China) as previously described [23, 24]. A random 5% of the samples were repeated to validate genotyping procedures and the concordance rate of repeated samples was 100%.

Statistical analysis

Determination of differences in demographic variables, smoking status, pack-years, family history of cancer and grouped genotypic frequencies between the cases and control subjects were evaluated using the χ^2 test or Student *t* test. Smoking status is classified as current smokers, former smokers (those who quit smoking for >1 year prior to diagnosis or enrollment) and non-smokers (<100 cigarettes in their lifetime) [25]. To check for genotyping error, we examined departure from Hardy–Weinberg equilibrium (HWE) in controls, using a χ^2 test. We used logistic regression to determine the association between each SNP and NSCLC estimated by ORs and their 95% confidence intervals (CIs), controlling for age, gender, pack-years, smoking status and family history of cancer as appropriate. Generally, homozygote of the ancestral allele (dbSNP, NCBI) was set as the reference group. For stratified analyses, we use 63 years old, which was the median in controls (< 63 versus $\geq$ 63 years old) and gender. Statistical analyses were performed using SPSS Software V.16.0 (SPSS, Chicago, Illinois, USA). All reported P-values are two sided with $P < 0.05$ considered as significant.

The pairwise LD between *IL1B* SNPs and haplotype frequencies were estimated with Haploview as described by Barrett [26].

Results

Population characteristics

Demographic characteristics of the study subjects are shown in Table 1. The cases and controls were similar in terms of age, age stratification, gender distribution and family history of cancer (direct relatives). There was a significantly larger proportion of smokers in cases than in controls. Among smokers, the patients had consumed more cigarettes measured in pack-years over a lifetime than controls ($P < 0.001$). Adenocarcinoma (ADC), Squamous Cell Carcinoma (SCC) and Adenosquamous Carcinoma represented 48.9, 36.6 and 1.9% of cases, and 12.6% were mixed, not otherwise specified, and of uncertain classification. There were 20.4% early stage (stages I and II), 71.9% advanced stage (stages III and IV) lung cancers, and no-classified proportion was 7.8%.

IL1B SNPs information

Genotypes were obtained for $> 96\%$ of case-control populations for the four SNPs (Table 2); the SNPs were in Hardy–Weinberg equilibrium in controls ($P > 0.05$). Hence, there was no evidence of any systematic bias in genotyping. Minor allele frequencies (MAF) in controls were 39.6% for rs12621220G/A (-3893), 40.1% for rs1143623G/C (-1464), 47.0% for rs16944T/C (-511) and 47.8% for rs1143627C/T (-31). The MAF of the four SNPs in our samples were similar to those in the Han Chinese in Beijing, China (HapMap database).

Association of the individual SNPs with risk of NSCLC

The distributions of the ancestral homozygous type, heterozygous and homozygous variants in cases and controls for each SNP are shown in Table 3. There was no overall association between each SNP and non-small cell lung cancer risk. After adjusting for age, gender, smoking status, pack-years and

family history of cancer, the associations were still insignificant.

Stratified analyses with age, gender, and histological subtypes

IL1B SNPs showed significant associations for NSCLC risk within the strata of age but not gender (Table 4 and Supplementary Tables S1). In the age ≥ 63 stratum: For *IL1B* rs12621220G/A (-3893) and rs1143623G/C (-1464) with the ancestral homozygous type as reference, heterozygous but not homozygous variants showed significantly decreased risk of NSCLC, with aOR = 0.71 (0.52-0.98), $P = 0.04$ and aOR = 0.64 (0.47-0.89), $P = 0.01$, respectively; For *IL1B* rs16944T/C (-511), CC was a risk factor versus TT+TC with aOR = 1.48 (1.08, 2.03) and $P = 0.01$; For *IL1B* rs1143627C/T (-31), compared with the CC+CT genotype, TT homozygous variants conferred a significant deleterious effect for cases over 63 years old, aOR = 1.45 (1.06, 1.99), $P = 0.02$. In age < 63 stratum: only rs12621220G/A (-3893) showed significant association with NSCLC, in which compared with the GG genotype, GA was a risk factor, with aOR = 1.40 (1.02-1.91) and $P = 0.04$.

We also performed stratified analyses by other clinical information, such as histological subtypes. After stratifying by Adenocarcinoma (ADC) and Squamous Cell Carcinoma (SCC), there was no overall association found between each SNP and non-small cell lung cancer risk (Supplementary Tables S2).

As to the effects of smoking on the susceptibility of NSCLC to the SNPs, although there are more non-smokers among controls than cases and controls have smoked less pack-years than cases, no association was found between each of the four SNP and NSCLC risk either in smokers or non-smokers, or in stratified analysis by pack-years. However, it was very interesting that *IL1B* rs12621220G>A (-3893) and rs1143623G>C (-1464) have weak protective effects in former smoking

adenocarcinoma patients (OR = 0.43, 95% CI = 0.21-0.87, P = 0.02 for rs12621220G>A; OR = 0.49, 95% CI = 0.24-1.00, P = 0.050 rs1143623G>C), which was reported in our previous study [27].

Two risk haplotypes associated with NSCLC

We estimated the pairwise LDs and constructed haplotype among the four SNPs: *IL1B* rs12621220G/A (-3893), rs1143623G/C (-1464), rs16944T/C (-511) and rs1143627C/T (-31). The LD among the *IL1B* SNPs was similar in cases and controls from the D' and R² values (Figure 1A, B). The haplotypes frequencies were different in cases and controls (Table 5). Two haplotypes (GGCC and ACTT) were distributed differently between cases and controls: the GGCC haplotype was present in 0.031 of cases compared with 0.006 of controls, with $P = 1.244 \times 10^{-8}$; the ACTT haplotype was present 0.026 and 0.003, respectively, $P = 9.0869 \times 10^{-10}$. The differences of haplotype frequencies between the cases and controls were determined by using the permutations test. When assessed with 100,000 permutations, the P-value was highly significant ($P < 0.001$). Upon further stratification by cell type, we found that the association between the GGCC haplotype and increased risk of ADC and the association between the ACTT haplotype and increased risk of SCC were even more significant (Supplementary Tables S3, S4).

Discussion

The pro-inflammatory cytokine IL1 β was important in initiating and balancing the level of inflammatory response. IL1 β can be generated by lung epithelial cells [28]. IL1 β plays an important role in various lung diseases such as chronic obstructive pulmonary disease (COPD) and lung cancer

[22, 28-31]. In previous studies, it has been revealed that *IL1B* rs12621220G/A (-3893), rs1143623G/C (-1464), rs16944T/C (-511) and rs1143627C/T (-31) formed a risk haplotype (GGCT) associated with NSCLC in European populations [17]. Among these SNPs, only the last two SNPs have been studied in Chinese populations and the results are not consistent [21, 22, 32].

In current study, we have investigated the above SNPs in the *IL1B* gene and determined whether these SNPs affect NSCLC risk in a large sample of Chinese population. We found statistically significant association of NSCLC with *IL1B* in subjects over age 63. Overall, *IL1B* rs12621220G/A (-3893) has weak correlation with NSCLC only in age strata. *IL1B* rs1143623G/C (-1464) has statistically significant effects on NSCLC, which was consistent with Zienolddiny's studies in Norway population [17]. The rs1143623 CC genotype has previously been found associated with an increased level of Cholesterol (CHOL) and triglyceride (TG) concentrations in the populations with age > 65 years old [33], showing the modification of age on the effect of rs1143623 CC, which was also confirmed in our study. In addition, we confirmed the associations of rs16944 (-511) CC and rs1143627 (-31) TT with the increasing risk of NSCLC in elderly subjects (≥ 63) as in Zienolddiny's study [29]. Furthermore, some studies have demonstrated the risks in gastric cancer and breast cancer by (-31) C allele [34-37], and association with NSCLC in rs16944 (-511) C and rs1143627 (-31) T alleles [38], however, the role of rs16944T/C (-511) and rs1143627C/T (-31) SNPs is far from being clear.

The haplotype analyses showed that the SNPs formed two risk haplotypes (GGCC and ACTT) whose frequencies were significantly higher in cases than in controls. The risk haplotype GGC from -3893 G, -1464 G and -511 C was in agreement with that in Zienolddiny's report, while -31C, as a part of this haplotype, was inconsistent with Zienolddiny's finding [17]. The C allele of rs1143627C/T (-31)

can disrupt the TATA box which was believed to affect *IL1B* transcription. In addition, the rs1143627 (-31) T-variant of the promoter showed higher expression ($p < 0.001$) than the C-variant in A549 cells [39]. Electrophoretic mobility shift assays then revealed differential binding of proteins to a promoter fragment containing the SNP, and the C-allele created a new binding site for the transcription factor Yin Yang 1 (YY1) [40]. YY1 can regulate transcription either as an activator or repressor, depending on which cofactor is recruited [41]. Another risk haplotype found in the current study was ACTT, which was absent in European populations. In addition, *IL1B* SNPs have different MAF between CHB and European populations (CEU) (Table 2). Furthermore, as reported by Zienolddiny's group, each allele of *IL1B* might have one or more binding transcription factors for each SNP, and different allele has variant binding capacity of the same transcription factor (Supplementary Tables S5) [17].

In conclusion, the current study showed an association between *IL1B* SNPs and risk of NSCLC among age above 63 in a large Chinese population and two risk haplotypes were also identified to be associated with lung cancer in the Chinese population for the first time. In our future study, many more patients would be included to find the combined effects of age and smoking behavior on the susceptibility of NSCLC to the SNPs together with multiple testing analysis, and the functional analysis of the positive SNPs and haplotypes would also be performed further in the Chinese population.

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A

B

Cases

R^2	rs1143627	rs16944	rs1143623	rs12621220
D'				
rs1143627		0.746	0.562	0.268
rs16944	0.87		0.733	0.435
rs1143623	0.865	0.986		0.596
rs12621220	0.601	0.761	0.772	

Controls

R^2	rs1143627	rs16944	rs1143623	rs12621220
D'				
rs1143627		0.957	0.723	0.424
rs16944	0.992		0.731	0.444
rs1143623	0.989	0.982		0.587
rs12621220	0.765	0.773	0.774	

Figure 1. The LD results in cases and controls. (A) The plot of LD in cases (left panel) and controls (right panel) and the deeper color represents the greater value (scaling from 0–1). (B) the estimated D' and r^2 values in cases (upper panel) and controls (lower panel).

Table 1
Descriptive characteristics of study population cases and controls.

Parameter		Case (%) n = 889	Control (%) n = 1005	P-value
Age	Mean ± SD	62.30 ± 10.79	62.19 ± 10.75	0.823 ^a
Age strata	< 63	446 (50.2)	499 (49.7)	0.822 ^b
	≥ 63	443 (49.8)	506 (50.3)	
Gender	Male	639 (71.9)	698 (69.5)	0.248 ^b
	Female	250 (28.1)	307 (30.5)	
Family history of cancer (direct)	No	579 (65.1)	687 (68.4)	0.136 ^b
	Yes	310 (34.9)	318 (31.6)	
Smoking status	Non-smokers	254 (29.3)	503 (50.0)	2.080×10 ⁻⁴⁴ ^b
	Former smokers	288 (33.2)	81 (8.1)	
	Current smokers	326 (37.6)	421 (41.9)	
Pack-years (among smokers)	Mean ± SD	42.92 ± 26.65	27.23 ± 17.34	< 0.001 ^a
Histological details	Adenocarcinoma	435 (48.9)		
	Squamous cell carcinoma	325 (36.6)		
	not classified otherwise	129 (14.5)		
TNM stage	I and II	181 (20.4)		
	III and IV	639 (71.9)		
	not classified otherwise	69 (7.8)		

^a Student *t* test

^b χ^2 test

Table 2
SNPs information and genotyping results.

SNP ID	Allele	Position	MAF			Genotyping success rate	P (HWE)
			control	HapMap-CHB	HapMap-CEU		
rs12621220 (-3893)	G>A	113598255	0.396	0.405	0.302	99.79	0.769
rs1143623 (-1464)	G>C	113595829	0.401	0.386	0.328	99.68	0.793
rs16944 (-511)	T>C	113594867	0.470	0.477	0.345	99.89	0.423
rs1143627 (-31)	C>T	113594387	0.478	0.467	0.367	96.00	0.721

Table 3*IL1B* gene polymorphism associations with NSCLC

SNP	Cases N (%)	Controls N (%)	P-value	Crude OR (95% CI)	P-value ^a	aOR ^a (95% CI)
rs12621220	886	1004				
GG	316 (35.7)	368 (36.7)	--	1.00	--	1.00
GA	427 (48.2)	476 (47.7)	0.67	1.05 (0.86-1.28)	0.74	0.96 (0.77-1.20)
AA	143 (16.1)	160 (15.9)	0.77	1.04 (0.79-1.37)	0.95	1.01 (0.75-1.37)
GA+AA vs. GG	--	--	0.66	1.04 (0.87-1.26)	0.81	0.97 (0.79-1.20)
rs1143623	883	1005				
GG	320 (36.2)	362 (36.0)	--	1.00	--	1.00
GC	413 (46.8)	479 (47.7)	0.81	0.98 (0.80-1.19)	0.32	0.90 (0.72-1.12)
CC	150 (17.0)	164 (16.3)	0.80	1.04 (0.79-1.35)	0.96	0.99 (0.74-1.34)
GC+CC vs. GG	--	--	0.92	0.99 (0.82-1.20)	0.43	0.92 (0.75-1.13)
rs16944	887	1005				
TT	205 (23.1)	228 (22.7)	--	1.00	--	1.00
TC	430 (48.5)	488 (48.6)	0.86	0.98 (0.78-1.23)	0.71	0.95 (0.74-1.23)
CC	252 (28.4)	289 (28.8)	0.81	0.97 (0.75-1.25)	0.75	1.05 (0.79-1.38)
CC vs. TT+ TC	--	--	0.87	0.98 (0.81-1.20)	0.49	1.08 (0.87-1.35)
rs1143627	841	977				
CC	194 (23.1)	226 (23.1)	--	1.00	--	1.00
CT	413 (49.1)	482 (49.3)	0.99	1.00 (0.79-1.26)	0.85	0.98 (0.75-1.26)
TT	234 (27.8)	269 (27.5)	0.92	1.01 (0.78-1.31)	0.54	1.09 (0.82-1.46)
TT vs. CC+CT	--	--	0.81	1.02 (0.83-1.25)	0.36	1.11 (0.89-1.39)

^a Adjusting for age, gender, smoking status, pack-years and family history of cancer.

Table 4*IL1B* gene polymorphism associations with NSCLC in age strata.

Age	SNP	Cases N (%)	Controls N (%)	P-value	Crude OR (95% CI)	P-value ^a	aOR ^a (95% CI)
≥ 63	rs12621220G>A	442	506				
	GG	165 (37.3)	183 (36.2)	--	1.00	--	1.00
	GA	209 (47.3)	252 (49.8)	0.56	0.92 (0.70-1.22)	0.04	0.71 (0.52-0.98)
	AA	68 (15.4)	71 (14.0)	0.76	1.06 (0.72-1.57)	0.66	1.10 (0.71-1.73)
	GA+AA vs. GG	--	--	0.71	0.95 (0.73-1.24)	0.81	0.96 (0.67-1.37)
	rs1143623G>C	440	506				
	GG	170 (38.6)	182 (36.0)	--	1.00	--	1.00
	GC	195 (44.3)	247 (48.8)	0.24	0.85 (0.64-1.12)	0.01	0.64 (0.47-0.89)
	CC	75 (17.0)	77 (15.2)	0.83	1.04 (0.71-1.53)	0.76	1.07 (0.69-1.65)
	GC+CC vs. GG	--	--	0.397	0.90 (0.69-1.16)	0.04	0.74 (0.55-0.99)
	rs16944T>C	442	506				
	TT	96 (21.7)	110 (21.7)	--	1.00	--	1.00
	TC	207 (46.8)	253 (50.0)	0.70	0.94 (0.67-1.30)	0.24	0.79 (0.54-1.16)
	CC	139 (31.4)	143 (28.3)	0.56	1.11 (0.78-1.60)	0.26	1.26 (0.84-1.90)
	CC vs. TT+ TC	--	--	0.28	1.17 (0.88-1.54)	0.01	1.48 (1.08-2.03)
	rs1143627C>T	424	502				
	CC	95 (22.4)	113 (22.5)	--	1.00	--	1.00
	CT	201 (47.4)	250 (49.8)	0.79	0.96 (0.69-1.33)	0.27	0.81 (0.55-1.18)
	TT	128 (30.2)	139 (27.7)	0.62	1.10 (0.76-1.58)	0.28	1.25 (0.83-1.89)
	TT vs. CC+CT	--	--	0.40	1.13 (0.85-1.50)	0.02	1.45 (1.06-1.99)
< 63	rs12621220G>A	444	498				
	GG	151 (34.0)	185 (37.1)	--	1.00	--	1.00
	GA	218 (49.1)	224 (45.0)	0.23	1.19 (0.90-1.59)	0.04	1.40 (1.02-1.91)
	AA	75 (16.9)	89 (17.9)	0.87	1.03 (0.71-1.50)	0.98	1.01 (0.66-1.53)
	GA+AA vs. GG	--	--	0.71	0.95 (0.73-1.24)	0.81	0.96 (0.67-1.37)
	rs1143623G>C	443	499				
	GG	150 (33.9)	180 (36.1)	--	1.00	--	1.00
	GC	218 (49.2)	232 (46.5)	0.41	1.13 (0.85-1.50)	0.10	1.30 (0.95-1.79)
	CC	75 (16.9)	87 (17.4)	0.86	1.03 (0.71-1.51)	0.87	0.97 (0.63-1.47)
	GC+CC vs. GG	--	--	0.48	1.10 (0.84-1.44)	0.22	1.21 (0.90-1.62)
	rs16944T>C	445	499				
	TT	109 (24.5)	118 (23.6)	--	1.00	--	1.00
	TC	223 (50.1)	235 (47.1)	0.87	1.03 (0.75-1.41)	0.48	1.13 (0.80-1.61)
	CC	113 (25.4)	146 (29.3)	0.33	0.84 (0.59-1.20)	0.28	0.81 (0.54-1.20)
	CC vs. TT+ TC	--	--	0.18	0.82 (0.62-1.10)	0.07	0.74 (0.54-1.02)
	rs1143627C>T	417	475				
	CC	99 (23.7)	113 (23.8)	--	1.00	--	1.00
	CT	212 (50.8)	232 (48.8)	0.80	1.04 (0.75-1.45)	0.45	1.15 (0.80-1.65)
	TT	106 (25.4)	130 (27.4)	0.71	0.93 (0.64-1.35)	0.56	0.89 (0.59-1.34)
	TT vs. CC+CT	--	--	0.51	0.91 (0.67-1.22)	0.20	0.81 (0.58-1.12)

^a Adjusting for gender, smoking status, pack-years and family history of cancer.

Table 5
Haplotype frequencies in cases and controls.

rs12621220	rs1143623	rs16944	rs1143627	Cases	Controls	χ^2	P	Permutation
G>A	G>C	T>C	C>T	(%)	(%)			P-value
G	G	C	T	0.443	0.474	3.555	0.0594	0.3475
A	C	T	C	0.322	0.339	1.264	0.2609	0.8867
G	G	T	C	0.063	0.065	0.062	0.8035	1.0000
G	C	T	C	0.053	0.056	0.153	0.6954	0.9999
A	G	C	T	0.049	0.047	0.051	0.8213	1.0000
G	G	C	C	0.031	0.006	32.417	1.244×10⁻⁸	0.0000E0
A	C	T	T	0.026	0.003	37.512	9.0869×10⁻¹⁰	0.0000E0