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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 SNPscan™ 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.

1. 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

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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-1β 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-1β, tumor development is slow, which suggests the crucial role of IL-1β in carcinogenesis [14,15].

IL1β 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 *1L1B* 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 *1L1B* 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.

2. Materials and methods

2.1. 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.

2.2. 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 SNPscan™ 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%.

2.3. 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 vs. ≥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].

3. Results

3.1. 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%.

3.2. 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).

3.3. 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.

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