Published in final edited form as:

J Cancer Res Clin Oncol. 2013 February ; 139(2): 231–242. doi:10.1007/s00432-012-1314-z.

A two-SNP *IL-6* promoter haplotype is associated with increased lung cancer risk

Jun Chen,
Department of Surgery, The First Affiliated Hospital, Soochow University, 188 Shizi Street, Suzhou 215006, People's Republic of China

Reng-Yun Liu,
Soochow University Laboratory of Cancer Molecular Genetics, Medical College of Soochow University, 199 Ren'ai Road, Sino-Singapore Industrial Park, Suzhou 215123, People's Republic of China

Lixin Yang,
Department of Cardiothoracic Surgery and Pneumology, Changhai Hospital of Shanghai, The Second Military Medical University, Shanghai, People's Republic of China

Jun Zhao,
Department of Surgery, The First Affiliated Hospital, Soochow University, 188 Shizi Street, Suzhou 215006, People's Republic of China

Xueying Zhao,
State Key Laboratory of Genetic Engineering and Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, People's Republic of China

Daru Lu,
State Key Laboratory of Genetic Engineering and Key Laboratory of Contemporary Anthropology, School of Life Sciences, Fudan University, Shanghai, People's Republic of China

Nengjun Yi,
Section on Statistical Genetics, Department of Biostatistics, School of Public Health, University of Alabama at Birmingham, Birmingham, AL, USA

Baohui Han,
Department of Respiratory Disease, Shanghai Chest Hospital, Shanghai Jiaotong University, Shanghai, People's Republic of China

Xiao-Feng Chen,
Department of Surgery, Shanghai Pulmonary Hospital, Shanghai, People's Republic of China

Kui Zhang,
Section on Statistical Genetics, Department of Biostatistics, School of Public Health, University of Alabama at Birmingham, Birmingham, AL, USA

Correspondence to: Boris Pasche, bpasche@uabmc.edu; Xiangdong Li, xdlijia@yahoo.com.cn; Hong-Tao Zhang, htzhang@suda.edu.cn.

Jun Chen, Reng-Yun Liu, Lixin Yang and Jun Zhao contributed equally to this work.

Conflict of interest The authors declared no conflicts of interest.

Jun He,
Department of Surgery, The First Affiliated Hospital, Soochow University, 188 Shizi Street, Suzhou 215006, People's Republic of China

Zhe Lei,
Soochow University Laboratory of Cancer Molecular Genetics, Medical College of Soochow University, 199 Ren'ai Road, Sino-Singapore Industrial Park, Suzhou 215123, People's Republic of China

Yifeng Zhou,
Soochow University Laboratory of Cancer Molecular Genetics, Medical College of Soochow University, 199 Ren'ai Road, Sino-Singapore Industrial Park, Suzhou 215123, People's Republic of China

Boris Pasche,
Division of Hematology/Oncology, Department of Medicine and Comprehensive Cancer Center, University of Alabama at Birmingham, 1802 6th Ave South, NP 2566, Birmingham, AL 35294-3300, USA

Xiangdong Li, and
Department of Surgery, The First Affiliated Hospital, Soochow University, 188 Shizi Street, Suzhou 215006, People's Republic of China

Hong-Tao Zhang
Soochow University Laboratory of Cancer Molecular Genetics, Medical College of Soochow University, 199 Ren'ai Road, Sino-Singapore Industrial Park, Suzhou 215123, People's Republic of China

Suzhou Key Laboratory for Molecular Cancer Genetics, Suzhou, People's Republic of China

Boris Pasche: bpasche@uabmc.edu; Xiangdong Li: xdlijia@yahoo.com.cn; Hong-Tao Zhang: htzhang@suda.edu.cn

Abstract

Background—Aberrant expression of interleukin-6 (IL-6) may play an important role in lung carcinogenesis. Whether *IL-6* promoter haplotypes are associated with lung cancer risk and their functions have not yet been studied. We tested the hypothesis that single-nucleotide polymorphism (SNP) and/or haplotypes of *IL-6* promoter are associated with risk of lung cancer.

Methods—Two functional *IL-6* promoter SNPs (-6331T>C and -572C>G) were genotyped in the discovery group including 622 patients and 614 controls, and the results were replicated in an independent validation group including 615 patients and 638 controls. Luciferase reporter gene assays were conducted to examine the function of *IL-6* promoter haplotypes.

Results—None of the functional *IL-6* promoter SNPs were associated with lung cancer risk in either study. However, a two-SNP CC (-6331C and -572C) *IL-6* promoter haplotype was significantly more common among cases than among controls in both groups ($P = 0.031$ and $P = 0.035$, respectively), indicating that this haplotype is associated with increased lung cancer risk {adjusted odds ratio [OR], 1.56 [95 % confidence interval (95 % CI), 1.04–2.34] and 1.51 [95 % CI, 1.03–2.22], respectively}. Combined analysis of both studies showed a strong association of this two-SNP haplotype with increased lung cancer risk (adjusted OR, 1.53; 95 % CI, 1.16–2.03; *P*

= 0.003). Comparably, luciferase reporter assays of A549 lung cancer cell lines transfected with the CC haplotype revealed that the two-SNP haplotype had significantly higher *IL-6* transcriptional activity compared with cells transfected with the common haplotype.

Conclusions—This is the first evidence of identifying an *IL-6* promoter haplotype (CC) associated with increased risk of lung cancer.

Keywords

IL-6; Haplotype; Lung cancer; Risk; Transcriptional activity

Introduction

Accumulating evidence suggests that chronic inflammation predisposes individuals to different types of cancer (Balkwill and Mantovani 2001; Coussens and Werb 2002). Over 15 % of the worldwide incidence of cancer can be attributed to chronic infections, of which inflammation is a major component (Pisani et al. 1997). The link between inflammation and cancer is thought to occur through two pathways: inflammation can initiate or promote oncogenic transformation and genetic as well epigenetic alterations can generate an inflammatory microenvironment that further reinforces tumor progression (Mantovani et al. 2008). As a member of signal transducer and activator of transcription (STAT) family proteins, STAT3 is constitutively activated in various types of cancer (Bromberg et al. 1999) and plays an important role in inflammation-associated tumorigenesis initiated by genetic changes (Gao et al. 2007; Rebouissou et al. 2009) and environmental factors (Hodge et al. 2005; Yu et al. 2009). Among inflammatory factors, interleukin-6 (IL-6) is essentially required for STAT3 activation (Zhong et al. 1994).

IL-6 is a pleiotropic cytokine that functions in inflammatory response, cell survival, proliferation and apoptosis (Kishimoto 2005). Recent studies show that IL-6 and its major effector STAT3 play a central role in the epigenetic switch from non-transformed epithelia to cancer cells (Iliopoulos et al. 2009, 2010). Elevated expression of IL-6 via autocrine and paracrine mechanisms leading to subsequent chronic inflammation also exhibits promoting and suppressive roles in tumor development (Grivennikov and Karin 2008; Knupfer and Preiss 2007; Okamoto et al. 1997; Park et al. 2010). Common genetic variants, especially the functional polymorphisms located in the promoter region of candidate genes that may quantitatively change the gene's expression, are associated with risk of cancer and other complex diseases (Dong et al. 2008; Joosten et al. 2001). Three functional polymorphisms, including -6331T>C, -572C>G (somewhere also named -634 C>G) and -174G>C, associated with *IL-6* transcription activity have been found in the *IL-6* promoter region (Fishman et al. 1998; Nakajima et al. 1999; Smith et al. 2008). The -572C>G and -174G>C *IL-6* promoter variants were reported not to be associated with lung cancer risk (Campa et al. 2005; Engels et al. 2007; Seow et al. 2006; Van Dyke et al. 2009). Besides, the present study has shown no association between -6331T>C or -572C>G and lung cancer risk, respectively.

Given the facts that haplotypes have more power to detect associations with risk of complex diseases than single polymorphism (Johnson et al. 2001; Manolio et al. 2008) and the G to C

polymorphism at -174 of *IL-6* is extremely rare in Asian, including Chinese (Gu et al. 2008; Lim et al. 2002; Zhai et al. 2001; Pan et al. 2011), we decided to examine the association of *IL-6* haplotypes consisting of the two functional polymorphisms of *IL-6* promoter (-6331T>C and -572C>G) with risk of lung cancer and to define the functional relevance of risk haplotypes.

Materials and methods

Study subjects

A total of 1,237 patients with lung cancer and 1,252 cancer-free individuals from East China were recruited in a discovery group and an independent validation group and were used to evaluate association of *IL-6* promoter SNPs and/or haplotypes with risk of lung cancer.

In the discovery group (Table 1), consecutive cases (n = 622) with a biopsy-confirmed diagnosis of lung cancer were recruited from the First Affiliated Hospital of Soochow University (Suzhou, China) between September 2003 and May 2008. The response rate for enrollment into the study was 90 %. A peripheral blood sample was obtained from each recruited patient. During the same time period, control samples (n = 614) were randomly recruited with a response rate of 87 % from healthy individuals who were participating in health checkup examinations conducted at the same hospital. None of the individuals in the control group had any personal history of cancer at the time of blood donation.

In the independent validation group (Table 1), blood samples (n = 615) were consecutively obtained from patients with a diagnosis of lung cancer at Shanghai Chest Hospital and Shanghai Pulmonary Hospital (Shanghai, China) between October 2003 and December 2007. The response rate was 86 and 85 % from patients recruited at these two hospitals. All lung cancer cases were histologically confirmed at the hospitals where the patients were diagnosed. Blood specimens from cancer-free controls (n = 638) were randomly recruited with a response rate of 82 % from healthy individuals coming for annual checkup at the same hospitals during the same time period.

Although there were no restrictions on age, gender and histology, patients who had previous cancers and/or previous radiotherapy or chemotherapy were excluded. All biopsy samples were carefully reviewed by the Department of Pathology of each hospital to confirm the diagnosis. Patients and controls were geographically matched individuals with the same age range. Each participant (cases and controls) was interviewed, and the following demographic variables were obtained: age, gender and smoking history (Table 1). All cases and controls were genetically unrelated ethnic Han Chinese population from Shanghai, and its surrounding regions including Jiangsu and Zhejiang provinces. The study protocol was approved by the Academic Advisory Boards of Soochow University and Fudan University.

SNPs' selection

The SNP database (www.ncbi.nlm.nih.gov/snp) and Hap-Map SNP database (www.hapmap.org, HapMap phase 3 release #27) were used to explore SNPs among Han Chinese population. Eleven SNPs were obtained from a 14-kb region of *IL-6* stretching from 7 kb upstream of the transcriptional start site to 2 kb downstream of the 3′ untranslated

region. Of them, 6 SNPs were common variants (minor allele frequencies >5 %), including two (rs10499563 and rs1800796) in the promoter region, three (rs2069837, rs1524107, rs2066992) in intron 2 and one (rs2069852) in the 1-kb region downstream of the coding region (Fig. 1). Notably, after performing linkage disequilibrium (LD) analysis by the Haploview program (Barrett et al. 2005), we found that four common SNPs (rs1800796, rs1524107, rs2066992 and rs2069852) were in strong LD since Lewontin coefficient (D′) and squared correlation coefficient (r^2) among these four SNPs are above 0.8 among Chinese population. Thus, one SNP can be picked as tagging SNP, and two SNPs (rs10499563 and rs1800796) in the promoter region can tag 83.3 % (5 of 6) of common variants within IL-6 gene if we use r^2 0.80 as the threshold (Fig. 1). Therefore, we selected the two promoter SNPs, rs10499563 (-6331T>C) and rs1800796 (-572C>G), to investigate whether variants in the *IL-6* promoter are capable of affecting expression of *IL-6* and susceptibility to lung cancer.

Genotyping

Genomic DNA was extracted from blood samples with the salting-out method (Miller et al. 1988). The *IL-6* promoter region containing the -6331T>C polymorphism was amplified by polymerase chain reaction (PCR). The PCR was performed in a total volume of 25ul containing 50–100 ng genomic DNA, 1 unit Taq polymerase (Takara, Japan), 1× Taq polymerase buffer (Mg^{2+} plus), 0.2 mM of each dNTP, and 0.4uM each of forward (5′-GCCTGGTCT GGCCTGTATAA-3′) and reverse (5′-CCCAAGGACCTG TTAGTGGA-3′) primers (primer set 1). The DNA template was denatured at 95 °C for 5 min and then amplified for 30 cycles at 94 °C for 45 s, 60 °C for 40 s, and 72 °C for 45 s, followed by a terminal 5-min extension phase at 72 °C. Each 222-bp PCR product was digested with TaaI (Fermentas, Vilnius, Lithuania) at 65 °C for 16 h. The various genotypes were separated using 12 % polyacrylamide gel electrophoresis followed by visualization with ethidium bromide (EB). The 171- and 51-bp fragments were diagnostic for the C allele, and the 222-bp fragment was diagnostic for the T allele.

The *IL-6* -572C>G polymorphism was amplified using the same conditions (as genotyped for the -6331T>C variant) with the following primers (primer set 2: forward: 5′-TGGCAAAAAGGAGTCACACA-3′ and reverse: 5′-CC CAAGCCTGGATTATGAAG-3′), and the annealing temperature was 62 °C. The 162-bp products were digested with MbiI (Fermentas, Vilnius, Lithuania) at 37 °C for 16 h. The different alleles were observed after gel electrophoresis and EB staining: the 88- and 74-bp fragments were diagnostic for the G allele, and the 162-bp fragment was diagnostic for the C allele.

Confirmation of the genotype data obtained from above PCR-restriction fragment length polymorphism (PCR-RFLP) analysis was undertaken by direct sequencing, which was performed on an Applied Biosystems 3700 DNA Analyzer according to manufacturer's instructions (PE Applied Biosystems, Foster City, CA).

Construction of luciferase reporter gene plasmids

PCR primers were designed as for primer set 1 but contained recognition sites for Kpn I in the forward primer and Pst I in the reverse primer. This set of primers were used to produce

a 222-bp fragment (-6,379 to -6,158) flanking the *IL-6* -6331T>C polymorphism from genomic DNA, which was obtained from a cancer-free subject homozygous for -6331T allele. A 751-bp sequence (-635 to +116) of *IL-6* was amplified from the genomic DNA of a healthy subject homozygous for the -572G allele. The primer sequences for the 751-bp amplicon were the same as the set 2 primer but contained recognition sites for Pst I in the forward primer and Hind III in the reverse primers. The two fragments were digested with Pst I restriction enzyme (Takara, Dalian, China) and then linked with T4 DNA ligase (Takara, Dalian, China) to form a longer fragment harboring -633IT allele and -572G allele. After cutting the fragment with Kpn I and Hind III (Takara, Dalian, China), we cloned it into the pGL3-basic luciferase vector (Promega, Madison, WI, USA). The resulting plasmid was named p-TG. The p-CG reporter plasmid containing -6331C allele and -572G allele was constructed as p-TG.

The G alleles at nucleotide position -572 relative to the transcriptional start site of *IL-6* in p-TG and p-CG constructs were site-specifically mutated to C alleles using Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA, USA) for creating the plasmids p-TC and p-CC. Primers are available upon request. Before cell transfection, the sequence of each construct was confirmed by direct sequencing.

Cell lines and cell culture

Human lung cancer cells (A549) and human bronchial epithelial (HBE) cells were used to determine the transcriptional activities of *IL-6* promoter harboring different haplotypes. Two cell lines were cultured in RPMI 1640 medium with 10 % fetal calf serum (GIBCO/Invitrogen, Grand Island, NY, USA) and incubated at 37 °C in a humidified environment with 5 % CO2.

Transient transfection and luciferase reporter gene assays

Cells were transfected with 800 ng pGL3-basic constructs with different *IL-6* promoter haplotypes or 800 ng pGL3-basic empty plasmid (as a promoterless control) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), and 32 ng *Renilla* pRL-TK plasmid (Promega, Madison, WI, USA) was cotransfected as a normalizing control. Sixteen hours later, the plates were washed with PBS buffer, changed with fresh medium, and then incubated for 24 h in the presence or absence of IL-1 (R&D systems, Minneapolis, MN, USA). Finally, luciferase activity of the transfected cells was determined using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) on a TD20/20 Luminometer (Turner Designs, Sunnyvale, CA, USA). For each plasmid construct, three independent transfection experiments were carried out, and each was done in triplicate. Results are reported as relative luciferase activities, which are obtained by dividing firefly luciferase activity with *Renilla* luciferase activity.

Statistical analysis

The data are presented as mean ± standard error of mean (SEM) for continuous variables or as numbers (percentage) for categorical variables. Comparisons between groups were performed with *t* test for continuous variables or with Chi-square test for categorical

variables. Hardy-Weinberg equilibrium (HWE) analysis for genotype distribution in controls was carried out by a Pearson's goodness-of-fit Chi-square test.

We used logistic regression models to assess the effects of the genotypes and the haplotypes on risk of lung cancer. We included age, gender and smoking history as covariates in the models to adjust for possible confounding effects. For the genotype association analysis, we used the additive model to code the main-effect variables of each SNP. In our haplotype association analyses, we first inferred the haplotypes using log-linear modeling embedded within an expectation-maximization (EM) algorithm (Fallin and Schork 2000; Mander 2001) and then adopted the logistic regression model to analyze the association between each haplotype and risk of lung cancer using the most common haplotype as the reference haplotype (Lin and Zeng 2006; Marchenko et al. 2008; Spinka et al. 2005). All the statistical analyses above were implemented with STATA (STATA/SE version 10.1 for Windows; Stata Corp, College Station, Texas, USA) and GraphPad Prism (version 5.01 for Windows; GraphPad Software, Inc., San Diego, CA, USA).

Results

Characteristics of patients with lung cancer and controls

The characteristics of lung cancer cases and controls enrolled in the two groups are shown in Table 1. There was no significant difference with respect to age between cases and controls in both groups. The proportions of male subjects and smokers were higher among patients with lung cancer than controls. As expected, smoking was associated with risk of lung cancer (discovery group: OR, 3.12; 95 % CI, 2.43–1.02; $P < 0.001$; validation group: OR, 2.83; 95 % CI, 2.20–3.64; $P < 0.001$) while gender was not associated with risk (discovery group: OR, 1.02; 95 % CI, 0.77–1.33; $P = 0.902$; validation group: OR, 0.97; 95 % CI, 0.74–1.27; $P = 0.808$).

IL-6 genotypes and risk of lung cancer

To determine whether any of the two promoter variants (-6331T>C and -572C>G) can modify risk of lung cancer, we genotyped these two SNPs by PCR-RFLP in the two independent groups. Fifteen percent of samples from cases and controls were validated by direct sequencing, and all sequence results were consistent with the PCR-RFLP analyses (Figs. 2, 3). The genotyping results showed that frequencies of these two polymorphisms were in Hardy-Weinberg equilibrium in controls in both groups (discovery group: $P = 0.055$ for -6331T>C and $P = 0.873$ for -5720G; validation group: $P = 0.297$ for -6331T>C and $P = 0.990$ for -572C>G). In the discovery group, no significant difference in frequencies of genotype and allele at -6331T>C was observed between lung cancer patients and controls. Although the -572G allele frequency was significantly higher in controls than patients ($P = 0.041$), neither genotype nor allele at -572C>G was associated with lung cancer risk (Table 2). In the validation group, no significant difference in frequencies of genotype and allele at -6331T>C and -572C>G was found between patients with lung cancer and controls (Table 2).

After stratification by age, gender, smoking history and histology, we did not observe any association between the -6331T>C polymorphism and lung cancer risk in both groups (Table 3). No association between the -572C>G polymorphism and lung cancer risk was found in the validation group, albeit there was an association of this polymorphism with lung cancer risk by histology stratification in the discovery group (Table 4).

IL-6 haplotypes and risk of lung cancer

Given the fact that association studies based on haplotypes of multiple markers instead of genotypes at single markers significantly improve the power of mapping and characterizing disease-causing genes (Akey et al. 2001; Johnson et al. 2001; Manolio et al. 2008), we sought to assess whether various haplotypes consisting of the two SNPs of *IL-6* promoter (-6331T>C and -572C>G) were associated with risk of lung cancer. LD analysis found that these two common polymorphisms shared high D' value and low r^2 value in controls (discovery group: $D' = 0.847$, $r^2 = 0.361$; validation group: $D' = 0.833$, $r^2 = 0.367$), suggesting that the two polymorphisms are suitable for haplotype reconstruction.

After applying haplotype reconstruction analysis according to the genotyping data in lung cancer patients and controls, we discovered that the CC (-6331C and -572C) haplotype was significantly more common among lung cancer patients than among controls in either group ($P = 0.031$ and $P = 0.035$, respectively), indicating that this haplotype is associated with increased lung cancer risk {adjusted OR, 1.56 [95 % CI, 1.04–2.34] and 1.51 [95 % CI, 1.03–2.22], respectively} (Table 5). Furthermore, when combining the two independent groups, we found that the CC haplotype was significantly associated with increased risk of lung cancer compared with the common TC haplotype (adjusted OR, 1.53; 95 % CI, 1.16–2.03; $P = 0.003$).

Although an association of the CG (-6331C and -572G) haplotype with decreased lung cancer risk was observed marginally significant in the discovery group (adjusted OR, 0.80; 95 % CI, 0.64–1.00; $P = 0.046$), this association was not validated in the validation group (adjusted OR, 0.84; 95 % CI, 0.67–1.04; $P = 0.110$) (Table 5).

Effects of four different IL-6 promoter haplotypes on transcriptional activity

Next, we tested the hypothesis that the CC (-6331C and -572C) *IL-6* haplotype alters *IL-6* transcriptional activity. We therefore constructed luciferase reporter vectors containing four different *IL-6* promoter haplotypes (Fig. 4a) and transiently transfected them into A549 and HBE cells, respectively.

As illustrated in Fig. 4, a significant induction of luciferase activity was observed in the two cell lines in the presence of IL-1, which is consistent with previous findings (Terry et al. 2000; Woods et al. 1998). In HBE cells stimulated with IL-1, reporter gene expression driven by the -6331C allelic *IL-6* promoter was ~1.44-fold higher than that driven by the -6331T allelic counterpart (1.61 ± 0.16 versus 1.12 ± 0.05, $P = 0.043$), and reporter gene expression driven by the -572C allelic *IL-6* promoter was ~1.35-fold higher than that driven by the -572G allelic counterpart (1.12 ± 0.05 versus 0.83 ± 0.06, $P = 0.025$) (Fig. 4b). The similar findings were observed in HBE cells without stimulation of IL-1 (Fig. 4b) and in

A549 cells with or without stimulation of IL-1 (Fig. 4c). These findings demonstrated the biological significance of the two SNPs on *IL-6* promoter activity. We further evaluated the combined effects of the -6331T>C and -572C>G on transcriptional activity and found that the constructs containing the CC promoter haplotype showed significantly higher transcriptional activities than those containing the common TC haplotype (Fig. 4b, c), suggesting a significant synergic effect between -6331C and -572C alleles in the context of haplotype on increasing transcriptional activity of *IL-6*.

Discussion

This is the first study investigating the association of *IL-6* promoter haplotypes with lung cancer. In this study, we found that the CC (-6331C and -572C) *IL-6* promoter haplotype was significantly associated with a 56 % increased risk of lung cancer in the discovery group and 51 % in the validation group.

Three functional polymorphisms associated with IL-6 transcription activity have been identified in the IL-6 promoter region (Fishman et al. 1998; Nakajima et al. 1999; Smith et al. 2008). One is a -174G>C polymorphism (rs1800795), located within the core promoter of *IL-6*, which modulates transcriptional response to β-adrenergic activation of the GATA1 transcription factor in vitro. The -174C allele is significantly associated with lower levels of plasma IL-6 (Cole et al. 2010; Fishman et al. 1998). Several investigations have shown that the -174G>C polymorphism contributes to the pathogenesis of colorectal cancer (Landi et al. 2003; Slattery et al. 2009) and Kaposi sarcoma (Foster et al. 2000; Gazouli et al. 2004). However, molecular epidemiologic studies have not shown an association between this functional polymorphism and lung cancer susceptibility in the European population (Campa et al. 2004, 2005; Colakogullari et al. 2008; Engels et al. 2007; Seifart et al. 2005; Van Dyke et al. 2009). Our metaanalysis of 83 studies involving 44,735 cases and 60,747 controls did not show a significant association between the -174G>C polymorphism and cancer risk, including lung cancer (Liu et al. 2012). Of note, the -174C allele is extremely rare in Asian, including Chinese (Gu et al. 2008; Lim et al. 2002; Zhai et al. 2001; Pan et al. 2011).

Another functional polymorphism -6331T>C (rs 10499563), which is common in the Chinese population (Table 2) and located near a distal regulatory region upstream of the *IL-6* transcription start site, was reported to modulate IL-6 expression level in acute inflammation via a mechanism involving binding of the POU2F1 transcription factor (Smith et al. 2008). We did not find any association between the polymorphism and lung cancer susceptibility in this report. The third functional variant -572C>G (rs 1800796), is common in the Chinese population (Table 2) and was associated with transcriptional activity of *IL-6* promoter in human myelocytic leukemia cells (Gu et al. 2008). The present study revealed that this polymorphism was not associated with lung cancer risk, which provided support for what Seow et al. observed in lung cancer, albeit Seow et al. (2006) reported that in the presence of -572G allele, atopy and asthma were associated with lung cancer. This could be explained by the fact that many factors, such as sample size and specific clinical features, may influence the results obtained from the case-control association studies.

While investigating the two variants (rs 10499563 and rs 1800796) of *IL-6* promoter region, we also explored the role of four non-promoter common polymorphisms, including rs2069837, rs1524107, rs2066992 and rs2069852 (Fig. 1). Of the four variants, rs1524107, rs2066992 and rs2069852 share higher D' and r^2 values (>0.8) with the -572C>G polymorphism (rs 1800796) among Chinese population. Thus, the two promoter variants (-6331T>C and -572C>G) can represent all the common variants of *IL-6* except for rs2069837. Additionally, we genotyped rs2069837 and found no difference in the frequency for rs2069837 between patients with lung cancer and cancer-free controls ($P = 0.39$), suggesting that rs2069837 is not associated with susceptibility to lung cancer.

Taken together, the previous (Colakogullari et al. 2008; Gu et al. 2008; Seow et al. 2006; Van Dyke et al. 2009) and present studies suggested that none of the three functional *IL-6* promoter polymorphisms (-174G>C, -572C>G and -6331T>C) are associated with risk of lung cancer in the Chinese population. However, when performing haplotype-based analyses, we found that the CC (-6331C and -572C) *IL-6* promoter haplotype was significantly associated with increased lung cancer risk. These findings are consistent with the notion that *IL-6* haplotypes are more functionally relevant to certain diseases than single polymorphisms (Fife et al. 2005; Terry et al. 2000). More recently, Hardy and Singleton also suggested that a collection of multiple variants may confer a graded risk of disease (Hardy and Singleton 2009). We have also shown an association between *TGFBRI* haplotypes and risk of lung cancer while no single SNP was associated with risk (Lei et al. 2009).

Our luciferase assays demonstrated a significant difference in transcriptional activity between the -633 IT and -6331C alleles, and between the -572C and -572G alleles in HBE and A549 cells. Smith et al. (2008) reported higher IL-6 transcription with the T allele at -6331T>C in the *IL-6* promoter region. Kitamura et al. (2002) found the -572G allele to be associated with increased secretion and production of IL-6. Pan et al. (2011) also reported that the -572G allele was associated with higher circulating levels of IL6. Despite of different methods used in these documents and their different results from ours, the polymorphisms -6331T>C and -572C>G in the *IL-6* promoter region were shown to play an important role in regulating *IL-6* transcription activity. More importantly and intriguingly, in the present study, we found a significantly increased transcriptional activity when the -6331C and -572C alleles (CC haplotype) are simultaneously present in the *IL-6* promoter, suggesting an interplay between these two SNPs within a haplotype. Taken together, these findings are consistent with our association analysis of the haplotypes and support the notion that haplotypes have more power to detect associations with risk of complex diseases than single polymorphism (Johnson et al. 2001; Manolio et al. 2008).

In conclusion, our results suggest that the CC (-6331C and -572C) *IL-6* promoter haplotype increase *IL-6* transcriptional activity and is associated with risk of lung cancer in the Chinese population.

Acknowledgments

This work was supported in part by the grants from National Natural Science Foundation of China (81171894 and 30973425 to H.-T. Zhang), Program for New Century Excellent Talents in University (NCET-09-0165 to H.-T. Zhang), SRF for ROCS, State Education Ministry (2008890 to H.-T. Zhang), Shanghai Science and Technology

Research Program (09JC1402200 to D. Lu), Jiangsu Province's Key Provincial Talents Program (RC2011106 to J. Zhao), Jiangsu Province's Outstanding Medical Academic Leader Program (LJ201138 to J. He), Qing-Lan Project of Education Bureau of Jiangsu Province (to H.-T. Zhang), "333" Project of Jiangsu Province Government (to H.-T. Zhang), Soochow Scholar Project of Soochow University (to H.-T. Zhang), and Suzhou Key Laboratory for Molecular Cancer Genetics (SZS201209 to H.-T. Zhang).

References

Akey J, Jin L, Xiong M. Haplotypes vs single marker linkage disequilibrium tests: what do we gain? Eur J Hum Genet. 2001; 9:291–300. [PubMed: 11313774]

Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001; 357:539–545. [PubMed: 11229684]

Barrett JC, Fry B, Mailer J, et al. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]

Bromberg JF, Wrzeszczynska MH, Devgan G, et al. Stat3 as an oncogene. Cell. 1999; 98:295–303. [PubMed: 10458605]

Campa D, Zienolddiny S, Maggini V, et al. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. Carcinogenesis. 2004; 25:229–235. [PubMed: 14604894]

Campa D, Hung RJ, Mates D, et al. Lack of association between polymorphisms in inflammatory genes and lung cancer risk. Cancer Epidemiol Biomarkers Prev. 2005; 14:538–539. [PubMed: 15734985]

Colakogullari M, Ulukaya E, Yilmaztepe Oral A, et al. The involvement of IL-10, IL-6, IFN-gamma, TNF-alpha and TGF-beta gene polymorphisms among Turkish lung cancer patients. Cell Biochem Funct. 2008; 26:283–290. [PubMed: 17654453]

Cole SW, Arevalo JM, Takahashi R, et al. Computational identification of gene-social environment interaction at the human IL6 locus. Proc Natl Acad Sci USA. 2010; 107:5681–5686. [PubMed: 20176930]

Coussens LM, Werb Z. Inflammation and cancer. Nature. 2002; 420:860–867. [PubMed: 12490959]

Dong LM, Potter JD, White E, et al. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. JAMA. 2008; 299:2423–2436. [PubMed: 18505952]

Engels EA, Wu X, Gu J, et al. Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. Cancer Res. 2007; 67:6520–6527. [PubMed: 17596594]

Fallin D, Schork NJ. Accuracy of haplotype frequency estimation for biallelic loci, via the expectation-maximization algorithm for unphased diploid genotype data. Am J Hum Genet. 2000; 67:947–959. [PubMed: 10954684]

Fife MS, Ogilvie EM, Kelberman D, et al. Novel IL-6 haplotypes and disease association. Genes Immun. 2005; 6:367–370. [PubMed: 15815691]

Fishman D, Faulds G, Jeffery R, et al. The effect of novel polymorphisms in the interleukin-6 (IL-6) gene on IL-6 transcription and plasma IL-6 levels, and an association with systemic-onset juvenile chronic arthritis. J Clin Invest. 1998; 102:1369–1376. [PubMed: 9769329]

Foster CB, Lehrnbecher T, Samuels S, et al. An IL6 promoter polymorphism is associated with a lifetime risk of development of Kaposi sarcoma in men infected with human immunodeficiency virus. Blood. 2000; 96:2562–2567. [PubMed: 11001912]

Gao SP, Mark KG, Leslie K, et al. Mutations in the EGFR kinase domain mediate STAT3 activation via IL-6 production in human lung adenocarcinomas. J Clin Invest. 2007; 117:3846–3856. [PubMed: 18060032]

Gazouli M, Zavos G, Papaconstantinou I, et al. The interleukin-6-174 promoter polymorphism is associated with a risk of development of Kaposi's sarcoma in renal transplant recipients. Anticancer Res. 2004; 24:1311–1314. [PubMed: 15154666]

Grivennikov S, Karin M. Autocrine IL-6 signaling: a key event in tumorigenesis? Cancer Cell. 2008; 13:7–9. [PubMed: 18167335]

Gu W, Du DY, Huang J, et al. Identification of interleukin-6 promoter polymorphisms in the Chinese Han population and their functional significance. Crit Care Med. 2008; 36:1437–1443. [PubMed: 18434913]

Hardy J, Singleton A. Genomewide association studies and human disease. N Engl J Med. 2009; 360:1759–1768. [PubMed: 19369657]

Hodge DR, Hurt EM, Farrar WL. The role of IL-6 and STAT3 in inflammation and cancer. Eur J Cancer. 2005; 41:2502–2512. [PubMed: 16199153]

Iliopoulos D, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. Cell. 2009; 139:693–706. [PubMed: 19878981]

Iliopoulos D, Jaeger SA, Hirsch HA, et al. STAT3 Activation of miR-21 and miR-181b-1 via PTEN and CYLD are part of the epigenetic switch linking inflammation to cancer. Mol Cell. 2010; 39:493–506. [PubMed: 20797623]

Johnson GC, Esposito L, Barratt BJ, et al. Haplotype tagging for the identification of common disease genes. Nat Genet. 2001; 29:233–237. [PubMed: 11586306]

Joosten PH, Toepoel M, Mariman EC, et al. Promoter haplotype combinations of the platelet-derived growth factor alpha-receptor gene predispose to human neural tube defects. Nat Genet. 2001; 27:215–217. [PubMed: 11175793]

Kishimoto T. Interleukin-6: from basic science to medicine- 40 years in immunology. Annu Rev Immunol. 2005; 23:1–21. [PubMed: 15771564]

Kitamura A, Hasegawa G, Obayashi H, et al. Interleukin-6 polymorphism (-634C/G) in the promotor region and the progression of diabetic nephropathy in type 2 diabetes. Diabet Med. 2002; 19:1000–1005. [PubMed: 12647840]

Knupfer H, Preiss R. Significance of interleukin-6 (IL-6) in breast cancer (review). Breast Cancer Res Treat. 2007; 102:129–135. [PubMed: 16927176]

Landi S, Moreno V, Gioia-Patricola L, et al. Association of common polymorphisms in inflammatory genes interleukin (IL)6, IL8, tumor necrosis factor alpha, NFKB1, and peroxisome proliferator-activated receptor gamma with colorectal cancer. Cancer Res. 2003; 63:3560–3566. [PubMed: 12839942]

Lei Z, Liu RY, Zhao J, et al. TGFBR1 haplotypes and risk of non-small-cell lung cancer. Cancer Res. 2009; 69:7046–7052. [PubMed: 19690145]

Lim CS, Zheng S, Kim YS, et al. The -174 G to C polymorphism of interleukin-6 gene is very rare in koreans. Cytokine. 2002; 19:52–54. [PubMed: 12200114]

Lin DY, Zeng D. Likelihood-based inference on haplotype effects in genetic association studies. J Am Stat Assoc. 2006; 101:89–104.

Liu RY, Song XX, Chen P, et al. Association between IL6-174G/C and cancer: a meta-analysis of 105,482 individuals. Exp Ther Med. 2012; 3:655–664. [PubMed: 22969947]

Mander AP. Haplotype analysis in population-based association studies. Stat J. 2001; 1:58–75.

Manolio TA, Brooks LD, Collins FS. A HapMap harvest of insights into the genetics of common disease. J Clin Invest. 2008; 118:1590–1605. [PubMed: 18451988]

Mantovani A, Allavena P, Sica A, et al. Cancer-related inflammation. Nature. 2008; 454:436–444. [PubMed: 18650914]

Marchenko YV, Carroll RJ, Lin DY, et al. Semiparametric analysis of case-control genetic data in the presence of environmental factors. Stat J. 2008; 8:305–333.

Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16:1215. [PubMed: 3344216]

Nakajima T, Ota N, Yoshida H, et al. Allelic variants in the interleukin-6 gene and essential hypertension in Japanese women. Genes Immun. 1999; 1:115–119. [PubMed: 11196657]

Okamoto M, Lee C, Oyasu R. Interleukin-6 as a paracrine and autocrine growth factor in human prostatic carcinoma cells in vitro. Cancer Res. 1997; 57:141–146. [PubMed: 8988055]

Pan M, Gao SP, Jiang MH, et al. Interleukin 6 promoter polymorphisms in normal Han Chinese population: frequencies and effects on inflammatory markers. J Investig Med. 2011; 59:272–276.

Park EJ, Lee JH, Yu GY, et al. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. Cell. 2010; 140:197–208. [PubMed: 20141834]

Pisani P, Parkin DM, Munoz N, et al. Cancer and infection: estimates of the attributable fraction in 1990. Cancer Epidemiol Biomarkers Prev. 1997; 6:387–400. [PubMed: 9184771]

Rebouissou S, Amessou M, Couchy G, et al. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. Nature. 2009; 457:200–204. [PubMed: 19020503]

Seifart C, Plagens A, Dempfle A, et al. TNF-alpha, TNF-beta, IL-6, and IL-10 polymorphisms in patients with lung cancer. Dis Markers. 2005; 21:157–165. [PubMed: 16276011]

Seow A, Ng DP, Choo S, et al. Joint effect of asthma/atopy and an IL-6 gene polymorphism on lung cancer risk among lifetime non-smoking Chinese women. Carcinogenesis. 2006; 27:1240–1244. [PubMed: 16344268]

Slattery ML, Wolff RK, Herrick J, et al. Tumor markers and rectal cancer: support for an inflammation-related pathway. Int J Cancer. 2009; 125:1698–1704. [PubMed: 19452524]

Smith AJ, D'Aiuto F, Palmen J, et al. Association of serum interleukin-6 concentration with a functional IL6 -6331T>C polymorphism. Clin Chem. 2008; 54:841–850. [PubMed: 18356242]

Spinka C, Carroll RJ, Chatterjee N. Analysis of case-control studies of genetic and environmental factors with missing genetic information and haplotype-phase ambiguity. Genet Epidemiol. 2005; 29:108–127. [PubMed: 16080203]

Terry CF, Loukaci V, Green FR. Cooperative influence of genetic polymorphisms on interleukin 6 transcriptional regulation. J Biol Chem. 2000; 275:18138–18144. [PubMed: 10747905]

Van Dyke AL, Cote ML, Wenzlaff AS, et al. Cytokine and cytokine receptor single-nucleotide polymorphisms predict risk for non-small cell lung cancer among women. Cancer Epidemiol Biomarkers Prev. 2009; 18:1829–1840. [PubMed: 19505916]

Woods KV, Adler-Storthz K, Clayman GL, et al. Interleukin-1 regulates interleukin-6 secretion in human oral squamous cell carcinoma in vitro: possible influence of p53 but not human papillomavirus E6/E7. Cancer Res. 1998; 58:3142–3149. [PubMed: 9679982]

Yu H, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat Rev Cancer. 2009; 9:798–809. [PubMed: 19851315]

Zhai R, Liu G, Yang C, et al. The G to C polymorphism at -174 of the interleukin-6 gene is rare in a Southern Chinese population. Pharmacogenetics. 2001; 11:699–701. [PubMed: 11692078]

Zhong Z, Wen Z, Darnell JE Jr. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science. 1994; 264:95–98. [PubMed: 8140422]

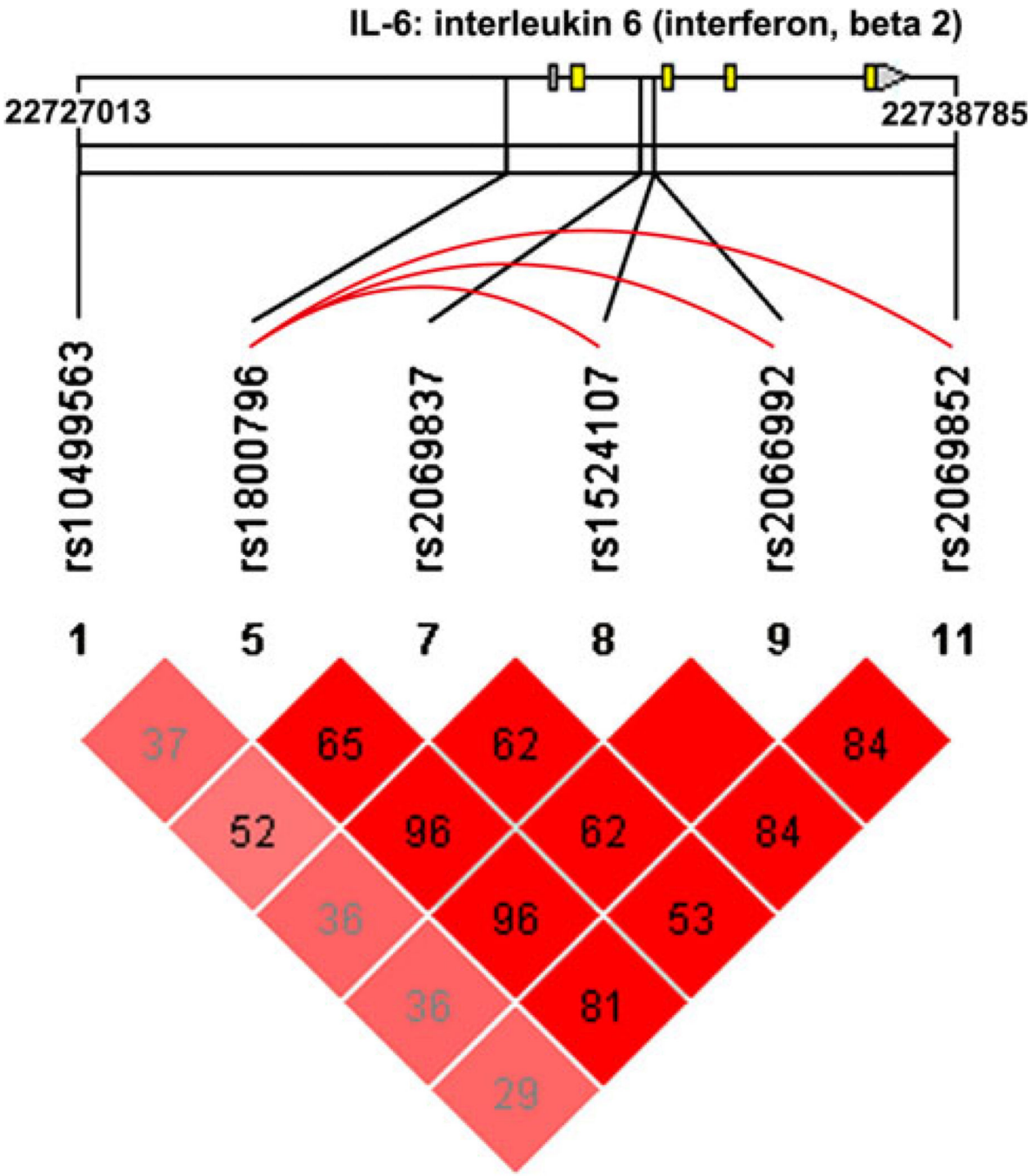

Fig. 1.

Pairwise LD between six polymorphisms of *IL-6* gene. Totally, eleven SNPs were obtained from a 14-kb region of *IL-6* from 7 kb upstream of the transcriptional start site to 2 kb downstream of the 3′ untranslated region in the Chinese population. Of them, six SNPs were common variants (minor allele frequencies >5 %), including two (rs10499563 and rs1800796) in the promoter region, three (rs2069837, rs1524107, rs2066992) in the intron 2 and one (rs2069852) in the 1 kb downstream of the coding region. Among four non-promoter variants, rs1524107, rs2066992 and rs2069852 share higher D′ and r^2 values

(>0.8) with rs1800796. Thus, the two promoter variants can represent all the common variants of *IL-6* except for rs2069837 with a threshold of 0.80 for r^2. The value in each *diamond* indicates pairwise correlation between tagging SNPs (measured as r^2) located at the *upper left* and the *upper right sides* of the *diamond*. The shading with a *red-to-white* gradient reflects higher to lower LD values (measured as D′)

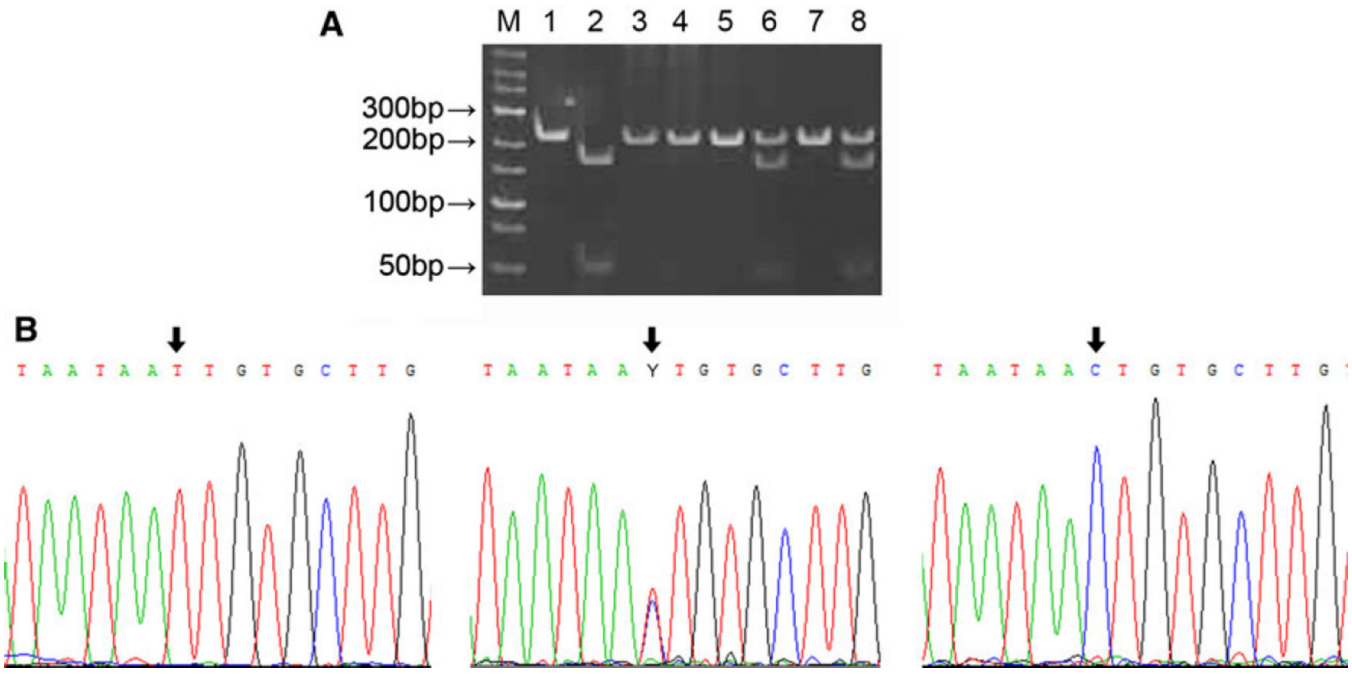

Fig. 2.

Representative analysis of the rs10499563 (-6331T>C) polymorphism. **a** PCR-RFLP analysis for the -6331T>C polymorphism. *M* DNA size marker; *lanes 1, 3,4, 5* and *7* TT genotype; *lanes 6, 8* TC genotype; *lane 2* CC genotype, **b** DNA sequencing analysis for genotypes of the -6331T>C polymorphism. The three charts represent the TT, TC and CC genotypes, respectively. The *arrows* localize the base changes at the nucleotide positions

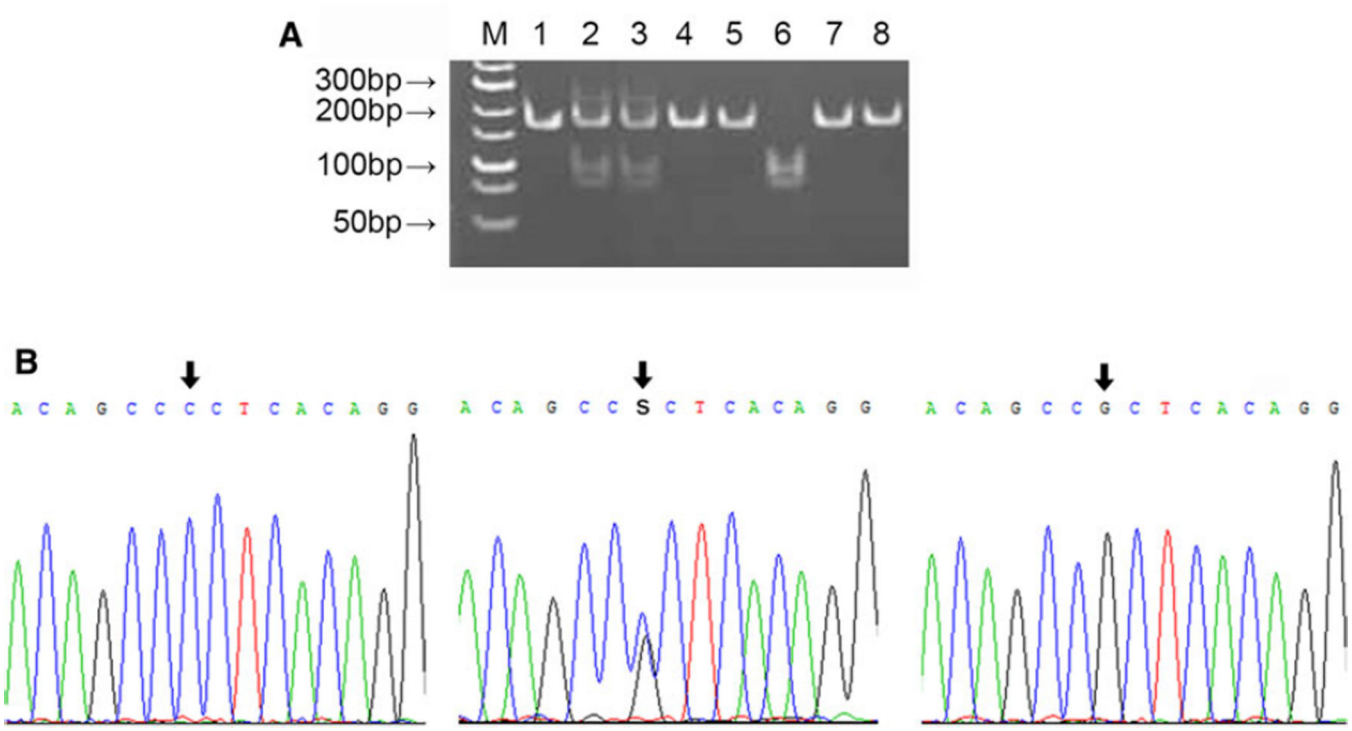

Fig. 3.

Representative analysis of the rs1800796 (-572C>G) polymorphism. **a** PCR-RFLP analysis for the -572C>G polymorphism. *M* DNA size marker; *lanes 1*, *4*, *5*, *7* and *8* CC genotype; *lanes 2*, *3* CG genotype; *lane 6* GG genotype. **b** DNA sequencing analysis for genotypes of the -572C>G polymorphism. The three charts represent the CC, CG and GG genotypes, respectively. The *arrows* localize the base changes at the nucleotide positions

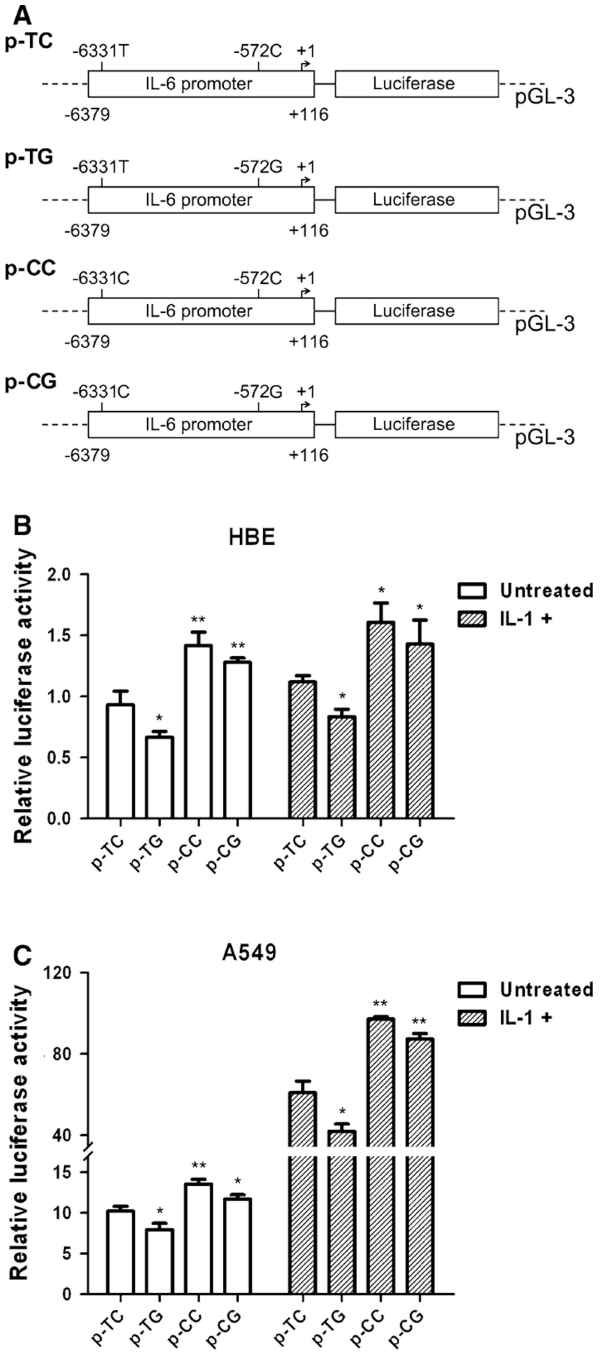

Fig. 4.

Transient reporter gene expression assays with constructs containing *IL-6* promoter haplotypes. **a** Schematic representation of luciferase reporter constructs carrying the *IL-6* promoter region. Luciferase activity profiles were assayed following transfection of the constructs into HBE (**b**) and A549 (**c**). Cells were incubated for 24 h in the absence or presence of IL-1. Firefly luciferase activity was normalized for transfection efficiency by co-transfection with a *Renilla* pRL-TK plasmid. Relative luciferase activities were calculated by dividing firefly luciferase activity with *Renilla* luciferase activity. Data shown are the

mean ± SEM from three independent transfection experiments, each done in triplicate. *P < 0.05 and **P < 0.01 compared with the p-TC construct

Table 1

Characteristics of patients with lung cancer and controls in two independent groups

	Discovery group				Validation group			
	Patients (*n* = 622), *n* (%)	**Controls (*n* = 614), *n*(%)**	***P*[a]**	**OR (95 % CI)[b]**	**Patients (*n* = 615), *n* (%)**	**Controls (*n* = 638), *n*(%)**	***P*[a]**	**OR (95 % CI)[b]**
Age (Mean ± SE)	57.6 ± 0.5	58.3 ± 0.4	0.268	1.00 (0.99–1.01)	58.1 ± 0.4	57.9 ± 0.4	0.669	1.00 (0.99–1.01)
Gender								
Female	163 (26.2)	224 (36.5)		1	167 (27.1)	231 (36.2)		1
Male	459 (73.8)	390 (63.5)	<0.001	1.02 (0.77–1.33)	448 (72.9)	407 (63.8)	0.001	0.97 (0.74–1.27)
Smoking history								
No	239 (38.4)	407 (66.3)		1	243 (39.5)	412 (64.6)		1
Yes	383 (61.6)	207 (33.7)	<0.001	3.12 (2.43–1.02)	372 (60.5)	226 (35.4)	<0.001	2.83 (2.20–3.64)
Histology[c]								
AC	293 (47.1)				302 (49.1)			
SCC	184 (29.6)				167 (27.1)			
Others	94 (15.1)				103 (16.8)			
SCLC	51 (8.2)				43 (7.0)			

[a] *P* values were determined using *t* test for age, Chi-square test for gender and smoking history

[b] OR and 95 % CI were evaluated using logistic regression

[c] *AC* adenocarcinoma, *SCC* squamous cell carcinoma, *Others* including large cell carcinoma and adeno-squamous carcinoma, *SCLC* small-cell lung cancer

Table 2

Genotype and allelic frequencies of two *IL-6* promoter polymorphisms among lung cancer cases and controls and associations with risk of lung cancer

	Discovery group				Validation group			
	Patients (*n* = 622), *n*(%)	**Controls (*n* = 614), *n* (%)**	***P*[a]**	**OR (95 % CI)[b]**	**Patients (*n* = 615), *n*(%)**	**Controls (*n* = 638), *n*(%)**	***P*[a]**	**OR (95 % CI)[b]**
IL-6 -6331T>C								
Genotype								
TT	397 (63.8)	391 (63.7)		1	392 (63.7)	394 (61.7)		1
TC	201 (32.3)	188 (30.6)		1.05 (0.82–1.36)	183 (29.8)	209 (32.8)		0.82 (0.64–1.06)
CC	24 (3.9)	35 (5.7)	0.289	0.84 (0.64–1.11)	40 (6.5)	35 (5.5)	0.44	1.10 (0.86–1.41)
Allele								
T	995 (80.0)	970 (79.0)		1	967 (78.6)	997 (78.1)		1
C	249 (20.0)	258 (21.0)	0.541	0.95 (0.78–1.17)	263 (21.4)	279 (21.9)	0.769	0.95 (0.79–1.15)
IL-6 -572C>G								
Genotype								
CC	349 (56.1)	309 (50.3)		1	333 (54.2)	321 (50.3)		1
CG	229 (36.8)	252 (41.0)		0.83 (0.65–1.06)	245 (39.8)	263 (41.2)		0.90 (0.70–1.14)
GG	44 (7.1)	53 (8.6)	0.116	0.85 (0.68–1.07)	37 (6.0)	54 (8.5)	0.164	0.82 (0.65–1.03)
Allele								
C	927 (74.5)	870 (70.8)		1	911 (74.1)	905 (70.9)		1
G	317 (25.5)	358 (29.2)	0.041	0.84 (0.70–1.01)	319 (25.9)	371 (29.1)	0.079	0.85 (0.71–1.02)

[a] *P* values were determined using Chi-square test for distribution of genotypes and alleles

[b] OR and 95 % CI were evaluated using logistic regression, adjusted for age, gender and smoking history

J Cancer Res Clin Oncol. Author manuscript; available in PMC 2015 August 13.

Table 3

Stratification analysis of the distribution of rs10499563 (-6331T>C) genotypes in lung cancer patients and controls

	Patients			Controls			P^a	OR (95 % CI)^b		
	TT, *n*(%)	TC, *n*(%)	CC, *n*(%)	TT, *n*(%)	TC, *n* (%)	CC, *n* (%)		TT	TC	CC
Discovery group										
Age (years)										
<60	212 (64.4)	104 (31.6)	13 (4.0)	215 (61.6)	111 (31.8)	23 (6.6)	0.295	1.00	0.99 (0.70–1.41)	0.79 (0.54–1.14)
≥60	185 (63.1)	97 (33.1)	11 (3.8)	176 (66.4)	77 (29.1)	12 (4.5)	0.559	1.00	1.12 (0.77–1.64)	0.96 (0.62–1.49)
Gender										
Female	112 (68.7)	46 (28.2)	5 (3.1)	144 (64.3)	63 (28.1)	17 (7.6)	0.159	1.00	0.75 (0.52–1.08)	0.62 (0.37–1.04)
Male	285 (62.1)	155 (33.8)	19 (4.1)	247 (63.3)	125 (32.1)	18 (4.6)	0.839	1.00	1.13 (0.84–1.53)	0.99 (0.70–1.40)
Smoking history										
No	154 (64.4)	74 (31.0)	11 (4.6)	256 (62.9)	127 (31.2)	24 (5.9)	0.769	1.00	0.97 (0.68–1.38)	0.87 (0.60–1.26)
Yes	243 (63.4)	127 (33.2)	13 (3.4)	135 (65.2)	61 (29.5)	11 (5.3)	0.395	1.00	1.15 (0.80–1.67)	0.81 (0.54–1.23)
Histology^c										
AC	190 (64.8)	92 (31.4)	11 (3.8)	391 (63.7)	188 (30.6)	35 (5.7)	0.458	1.00	1.02 (0.75–1.39)	0.81 (0.57–1.15)
SCC	118 (64.1)	61 (33.2)	5 (2.7)				0.246	1.00	1.10 (0.75–1.61)	0.74 (0.45–1.22)
Others	56 (59.6)	33 (35.1)	5 (5.3)				0.682	1.00	1.25 (0.77–2.02)	1.04 (0.63–1.72)
SCLC	33 (64.7)	15 (29.4)	3 (5.9)				0.984	1.00	0.95 (0.50–1.81)	1.04 (0.56–1.95)
Validation group										
Age (years)										
<60	206 (63.4)	99 (30.5)	20 (6.1)	233 (64.2)	108 (29.7)	22 (6.1)	0.976	1.00	0.95 (0.67–1.34)	1.05 (0.75–1.46)
≥60	186 (64.1)	84 (29.0)	20 (6.9)	161 (58.6)	101 (36.7)	13 (4.7)	0.108	1.00	0.66 (0.45–0.96)	1.11 (0.75–1.62)
Gender										
Female	104 (62.3)	49 (29.3)	14 (8.4)	145 (62.8)	71 (30.7)	15 (6.5)	0.763	1.00	1.01 (0.64–1.59)	1.11 (0.75–1.64)
Male	288 (64.3)	134 (29.9)	26 (5.8)	249 (61.2)	138 (33.9)	20 (4.9)	0.425	1.00	0.76 (0.56–1.02)	1.10 (0.80–1.50)
Smoking history										
No	159 (65.4)	67 (27.6)	17 (7.0)	263 (63.8)	123 (29.9)	26 (6.3)	0.801	1.00	0.90 (0.63–1.29)	1.04 (0.75–1.43)
Yes	233 (62.6)	116 (31.2)	23 (6.2)	131 (58.0)	86 (38.0)	9 (4.0)	0.154	1.00	0.76 (0.53–1.08)	1.21 (0.81–1.80)
Histology^c										

	Patients			Controls			P^a	OR (95 % CI)^b		
	TT, *n*(%)	TC, *n*(%)	CC, *n*(%)	TT, *n*(%)	TC, *n* (%)	CC, *n* (%)		TT	TC	CC
AC	195 (64.6)	87 (28.8)	20 (6.6)	394 (61.7)	209 (32.8)	35 (5.5)	0.422	1.00	0.79 (0.58–1.08)	1.09 (0.81–1.46)
SCC	107 (64.1)	51 (30.5)	9 (5.4)				0.853	1.00	0.82 (0.56–1.21)	1.02 (0.68–1.52)
Others	64 (62.1)	30 (29.1)	9 (8.8)				0.378	1.00	0.82 (0.51–1.32)	1.42 (0.94–2.14)
SCLC	26 (60.5)	15 (34.9)	2 (4.6)				0.943	1.00	0.99 (0.51–1.94)	0.97 (0.45–2.08)

[a] *P* values were determined using Chi-square test for distribution of genotypes

[b] OR and 95 % CI were evaluated using logistic regression, adjusted for age, gender and smoking history

[c] *AC* adenocarcinoma, *SCC* squamous cell carcinoma, *Others* including large cell carcinoma and adeno-squamous carcinoma, *SCLC* small-cell lung cancer

Table 4

Stratification analysis of the distribution of rs1800796 (-572C>G) genotypes in lung cancer patients and controls

	Patients			Controls			P^a	OR (95 % CI)^b		
	CC, *n* (%)	CG, *n*(%)	GG, *n* (%)	CC, *n* (%)	CG, *n*(%)	GG, *n*(%)		CC	CG	GG
Discovery group										
Age (years)										
<60	180 (54.7)	121 (36.8)	28 (8.5)	166 (47.6)	152 (43.5)	31 (8.9)	0.161	1.00	0.80(0.57–1.13)	0.92(0.68–1.24)
≥60	169 (57.7)	108 (36.8)	16 (5.5)	143 (54.0)	100 (37.7)	22 (8.3)	0.364	1.00	0.90 (0.62–1.29)	0.80(0.56–1.13)
Gender										
Female	89 (54.6)	64 (39.3)	10 (6.1)	115 (51.3)	92 (41.1)	17 (7.6)	0.758	1.00	0.91 (0.59–1.40)	0.92(0.60–1.41)
Male	260 (56.6)	165 (36.0)	34 (7.4)	194 (49.7)	160 (41.0)	36 (9.2)	0.126	1.00	0.79 (0.59–1.07)	0.83 (0.64–1.08)
Smoking history										
No	131 (54.8)	90 (37.7)	18 (7.5)	203 (49.9)	171 (42.0)	33 (8.1)	0.477	1.00	0.82(0.58–1.15)	0.92(0.68–1.25)
Yes	218 (56.9)	139 (36.3)	26 (6.8)	106 (51.2)	81 (39.1)	20 (9.7)	0.285	1.00	0.83 (0.58–1.20)	0.79 (0.58–1.08)
Histology^c										
AC	165 (56.3)	104 (35.5)	24 (8.2)	309 (50.3)	252 (41.1)	53 (8.6)	0.227	1.00	0.79 (0.58–1.07)	0.93 (0.71–1.21)
SCC	97 (52.7)	79 (42.9)	8 (4.4)				0.159	1.00	1.06(0.74–1.53)	0.65 (0.43–0.98)
Others	52 (55.3)	34 (36.2)	8 (8.5)				0.644	1.00	0.82(0.51–1.32)	0.91 (0.61–1.38)
SCLC	35 (68.6)	12 (23.5)	4 (7.8)				0.035	1.00	0.43 (0.22–0.86)	0.80(0.46–1.38)
Validation group										
Age (years)										
<60	174 (53.5)	129 (39.7)	22 (6.8)	198 (54.5)	139 (38.3)	26 (7.2)	0.925	1.00	1.13(0.81–1.57)	0.99 (0.72–1.36)
≥60	159 (54.8)	116 (40.0)	15 (5.2)	123 (44.7)	124 (45.1)	28 (10.2)	0.015	1.00	0.66 (0.46–0.95)	0.65 (0.46–0.93)
Gender										
Female	87 (52.1)	70 (41.9)	10 (6.0)	115 (49.8)	92 (39.8)	24 (10.4)	0.301	1.00	1.01 (0.66–1.55)	0.74(0.50–1.10)
Male	246 (54.9)	175 (39.1)	27 (6.0)	206 (50.6)	171 (42.0)	30 (7.4)	0.410	1.00	0.85(0.63–1.13)	0.86(0.65–1.15)
Smoking history										
No	131 (53.9)	98 (40.3)	14 (5.8)	208 (50.5)	168 (40.8)	36 (8.7)	0.345	1.00	0.93 (0.67–1.29)	0.78 (0.56–1.09)
Yes	202 (54.3)	147 (39.5)	23 (6.2)	113 (50.0)	95 (42.0)	18 (8.0)	0.505	1.00	0.87 (0.61–1.23)	0.86(0.61–1.19)
Histology^c										

	Patients			Controls			P[a]	OR (95 % CI)[b]		
	CC, *n* (%)	CG, *n*(%)	GG, *n* (%)	CC, *n* (%)	CG, *n*(%)	GG, *n*(%)		CC	CG	GG
AC	170 (56.3)	116 (38.4)	16 (5.3)	321 (50.3)	263 (41.2)	54 (8.5)	0.102	1.00	0.81 (0.61–1.09)	0.75 (0.55–1.01)
SCC	94 (56.3)	60 (35.9)	13 (7.8)				0.384	1.00	0.78(0.54–1.14)	0.91 (0.65–1.27)
Others	42 (40.8)	53 (51.5)	8 (7.8)				0.144	1.00	1.53 (0.97–2.39)	1.13 (0.74–1.71)
SCLC	27 (62.8)	16 (37.2)	0 (0.0)				0.081	1.00	0.73 (0.38–1.40)	-

[a] *P* values were determined using Chi-square test for distribution of genotypes

[b] OR and 95 % CI were evaluated using logistic regression, adjusted for age, gender and smoking history

[c] *AC* adenocarcinoma, *SCC* squamous cell carcinoma, *Others* including large cell carcinoma and adeno-squamous carcinoma, *SCLC* small-cell lung cancer

Table 5

Association of *IL-6* promoter haplotypes with risk of lung cancer

Haplotype[a]	Discovery group			Validation group		
	Cases/controls[b]	OR (95 % CI)[c]	*P*	Cases/controls[b]	OR (95 % CI)[c]	*P*
TC	68.5/67.1	1.00	-	67.7/66.8	1.00	-
TG	11.5/11.9	0.94 (0.73–1.22)	0.655	10.9/11.3	0.95 (0.73–1.23)	0.685
CC	6.0/3.8	1.56 (1.04–2.34)	0.031	6.3/4.1	1.51 (1.03–2.22)	0.035
CG	14.0/17.2	0.80 (0.64–1.00)	0.046	15.0/17.7	0.84 (0.67–1.04)	0.110

[a] Two polymorphic alleles from left to right (-6331T>C and -572C>G) were used for haplotype reconstruction

[b] Haplotype frequencies (%) of *IL-6* promoter in lung cancer cases and controls

[c] OR and 95 % CI were evaluated using logistic regression, adjusted for age, gender and smoking history