Cytokine single-nucleotide polymorphisms and risk of non-small-cell lung cancer

Cristina Pérez-Ramírez[a,d], Ahmed Alnatsha[a,f], Marisa Cañadas-Garre[a], Eduardo Villar[b], Javier Valdivia-Bautista[c], María J. Faus-Dáder[d] and Miguel Á. Calleja-Hernández[a,e]

Objective **Lung cancer, particularly the non-small-cell lung cancer (NSCLC) subtype, is the leading cause of cancer-related death worldwide. Several functional polymorphisms in inflammatory cytokine genes, such as IL1B, IL6, IL12A, IL13 and IL16, have been associated with the risk of NSCLC. The aim of this study was to evaluate the association between ILs gene polymorphisms and the risk of developing NSCLC.**

Participants and methods **A retrospective case–control study was carried out, including 174 NSCLC cases and 298 controls of Spanish origin. *IL1B (rs1143634), IL1B (rs12621220), IL1B (rs1143623), IL1B (rs16944), IL1B (rs1143627), IL12A (rs662959), IL13 (rs1881457), IL6 (rs1800795)* and *IL16 (rs7170924)* gene polymorphisms were analysed by TaqMan.**

Results **The genotypic logistic regression model adjusted by smoking status showed that the IL1B rs1143634-TT genotype was associated with a lower risk of NSCLC ($P = 0.04312$; odds ratio = 0.226; 95% confidence interval = 0.044–0.840). No other gene polymorphisms showed an association with NSCLC in any of the models tested.**

Conclusion **In conclusion, IL1B rs1143634 was significantly associated with a higher risk of NSCLC. No influence of IL1B rs12621220, rs1143623, rs16944, rs1143627, IL12A rs662959, IL13 rs1881457 and IL16 rs7170924 on the risk of developing NSCLC was found in our study. *Pharmacogenetics and Genomics* 27:438–444**

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[a]Pharmacogenetics Unit, Pharmacy Service, [b]Pathology Service, UGC Pathological Anatomy, [c]Medical Oncology Service, Biosanitary Research Institute, University Hospital Complex of Granada, Departments of [d]Biochemistry, [e]Pharmacology, Faculty of Pharmacy, University of Granada, Granada, Spain and [f]Department of Molecular Medicine, Faculty of Medicine, University of Tübingen, Tübingen, Germany

Correspondence to Marisa Cañadas-Garre, PhD, Centre for Public Health, Queen's University of Belfast c/o Regional Genetics Centre, Level A, Tower Block, Belfast City Hospital, Lisburn Road, Belfast, BT9 7AB, UK
Tel: +44 2890 638460; fax: +44 2890 235900;
e-mail: marisacgarre@gmail.com

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Introduction

Lung cancer is the second most commonly diagnosed form of cancer, with an incidence of 14% in both sexes (after prostate cancer in men and breast cancer in women), as well as the leading cause of cancer-related death worldwide. Around 224 300 new cases and 158 000 deaths were estimated to occur in 2016 according to the most recent statistics of cancer in the USA [1].

Lung cancers are broadly classified into two types: non-small-cell lung cancers (NSCLCs) and small cell lung cancers. NSCLC (the most common lung cancer, accounting for about 80% of all cases) presents three types: squamous cell carcinoma, adenocarcinoma and large cell carcinoma [2–6]. The guidelines of the American Joint Committee on Cancer show that the majority of the patients are diagnosed at an older age (≈65 years) and in late stage (IIIB–IV) [4–6]. Therefore, the prognosis of lung cancer is very poor, with a 5-year survival rate as low as 5% for IIIB and 1% for IV stages [4–6]. Although smoking is the major cause of lung cancer (responsible from > 80% of the cases), a small fraction of nonsmokers eventually develop lung cancer, suggesting that other factors may influence lung cancer carcinogenesis. Remarkably, several studies have found that genetic alterations, such as single-nucleotide polymorphisms (SNPs), are associated with a high risk of developing lung cancer [2,7].

The innate immune system plays a prominent role in acute inflammation, activating immune cells to fight infection. However, long-standing inflammation secondary to chronic infection may lead to tumour development through prolonged inflammatory cytokine production [8,9]. Interleukins (ILs) comprise the largest group of cytokines that promote

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growth, differentiation and activation of immune cells [10]. The procarcinogenic effects of ILs exposure comprise DNA damage, enhancement of cell proliferation, inhibition of apoptosis and stimulation of angiogenesis [8,9,11]. Thus, there is an evident association between chronic inflammation, infection and carcinogenesis. Interestingly, almost 20% of all cancers have been attributed to chronic infection [12]. However, functional polymorphisms in inflammatory cytokine genes, such as IL1B, IL6, IL12A, IL13 and IL16, have been associated with the risk of NSCLC [13–19].

IL1B is a proinflammatory cytokine that mediates both acute and chronic inflammation [20]. IL1B may be secreted in lung epithelial cells and is involved in cell proliferation, differentiation and apoptosis by inducing the expression of several inflammation-related genes such as tumour necrosis factor-α and reactive oxygen species [20,21]. Thus, genetic alterations in this gene may be involved in NSCLC development [14,17]. IL6 is a pleiotropic cytokine secreted by lymphoid and nonlymphoid cells, which is involved in the inflammation response and exerts both proinflammatory and antiinflammatory effects [22]. Particularly in cancer, IL6 plays an important role in carcinogenesis, increasing cell survival, proliferation and inhibiting apoptosis [23,24]. Germline variations in IL6 have been associated with increased serum IL6 levels, which promote cancer development [25–28]. IL12A is an immunoregulatory cytokine secreted by dendritic cells, macrophages, neutrophils and human B-lymphoblastoid cells that have shown an effect on T and natural killer cells, increasing their proliferation and cytotoxicity [29,30]. Moreover, IL12A shows antiangiogenic activity that may lead to antagonization of proangiogenic signals, which may be detected during the progression of malignancies [31,32]. In particular, several studies have reported an association between genetic alterations in this gene and cancer development [33,34]. IL13 is a immunoregulatory cytokine secreted by many types of cells, such as B cells, mast cells, basophils, natural killer and dendritic cells, but especially inflammation T helper type 2 cells are the major regulators of IL13 production [35]. A connection between IL13 and the mitogen-activated protein kinase pathway has been found, increasing tumour invasion and metastasis [36]. Several gene polymorphisms in IL13 have been found to play a role in NSCLC clinical outcomes, particularly progression and recurrence [37].

IL16 is a proangiogenic cytokine produced by peripheral blood mononuclear cells that plays a key role in the inflammatory response by activating second messengers and mediators of inflammation [38,39]. High IL16 and IL12A serum levels have been associated with the risk of cancer [40–43]. Gene polymorphisms altering its production and/or activity may therefore have an influence on cancer promotion [44–46].

On the basis of the above factors, the identification of genetic alterations in ILs may be crucial to predict NSCLC development. To date, there are few studies exploring the influence of polymorphisms in ILs genes on the risk of lung cancer. The aim of this study was to evaluate the association between ILs gene polymorphisms and the risk of NSCLC.

Participants and methods

A retrospective case–control study was carried out.

Study participants

This study involved 174 NSCLC cases and 298 controls of South Spanish Caucasian origin. Cases were diagnosed histologically or cytologically as NSCLC (stages I–IV). Controls were individuals older than 18 years who had resided in the same geographic area, with a nonpersonal history of malignant neoplasm, recruited from the same hospital.

This case–control study was carried out in accordance with the declaration of Helsinki with the approval of the Ethics and Research Committee of the Sistema Sanitario Público de Andalucía Biobank. The participants signed a written informed consent form for blood/saliva sample collection and donation to the Biobank. The identification of samples was based on nonpatient identifiers and they were treated confidentially.

Sociodemographic and clinical variables

Sociodemographic data including sex, previous lung disease, smoking status and age at diagnosis were collected from clinical records. Histopathological data (tumour histology and stage) were also collected. The staging system used to classified tumours was based on the guidelines of the American Joint Committee on Cancer [4].

Genetic variables

DNA isolation

Samples were obtained from the Hospital Universitario Virgen de las Nieves Biobank, a part of the Sistema Sanitario Público de Andalucía Biobank. Blood samples (3 ml) were collected in BD Vacutainer K3E Plus Blood Collection Tubes (BD Vacutainer Systems, Plymouth, UK). Saliva samples were collected in 50 ml BD Falcon conical tubes (BD Vacutainer Systems). DNA was extracted using the QIAamp DNA Mini Kit (QiagenGmBH, Hilden, Germany) according to the manufacturer's instructions for DNA purification from blood or saliva and stored at −40°C.

Detection of gene polymorphisms

IL1B (rs1143634), *IL1B (rs12621220)*, *IL1B (rs1143623)*, *IL1B (rs16944)*, *IL1B (rs1143627)*, *IL12A (rs662959)*, *IL13 (rs1881457)*, *IL6 (rs1800795)* and *IL16 (rs7170924)* gene polymorphisms were analysed by real-time PCR using TaqMan probes (Thermo Fisher Scientific,

Waltham, Massachusetts, USA). The genotyping methodology has been described previously [47].

Statistical analysis

Descriptive analysis was carried out using R 3.0.1 [47]. Quantitative data were expressed as the mean ± SD for normally distributed variables or medians and percentiles (25 and 75) for non-normal distributed variables. The Shapiro–Wilk's test was used to assess normality.

Hardy–Weinberg equilibrium and pairwise haplotype frequencies were estimated, and Lewontin's D' and the linkage disequilibrium coefficient (r^2) were calculated. The bivariate association between NSCLC risk and polymorphisms was assessed for multiple models (genotypic, additive, allelic, dominant and recessive), using the Pearson's χ^2 and Fisher's exact test, and evaluated by odds ratio (OR) and their corresponding 95% confidence intervals (CIs). The models were defined as follows: genotypic (DD vs. Dd vs. dd), dominant (DD and Dd vs. dd), recessive (DD vs. Dd and dd), allelic (D vs. d) and additive, D being the minor allele and d being the major allele. Bonferroni's correction was used for multiple comparisons. Unconditional logistic regression models (genotypic, dominant, recessive and additive) were considered to determine the influence of potential confounding variables on the risk of lung cancer. All tests were two sided, with a significance level of P less than 0.05, and were performed using the free, open-source whole-genome association analysis toolset R 3.2.2 or PLINK [48,49].

Results

Patients' characteristics

A total of 174 NSCLC cases and 298 controls were included in the study, whose clinicopathologic characteristics are described in Table 1. There were significant differences between cases and controls in terms of age ($P < 0.001$; OR = 0.15; 95% CI = 0.09–0.24; > 60 vs. <60 years), sex ($P < 0.001$; OR = 0.43; 95% CI = 0.28–0.66; female vs. male) and smoking status ($P < 0.001$; OR = 26.35; 95% CI = 13.22–55.31; current smokers vs. nonsmokers and $P < 0.001$; OR = 3.11; 95% CI = 1.86–5.22; Former smokers vs. nonsmokers), but not in terms of previous lung disease ($P = 0.618$; OR = 0.88; 95% CI = 0.56–1.37; yes vs. no). The case group included 46 (26.44%) women and 128 (73.56%) men. The median age of the cases was 61.5 (P_{25}, P_{75}: 53, 68). The control group included 135 (45.3%) women and 163 (54.7%) men. The median age of the controls was 72.90 (P_{25}, P_{75}: 67, 80). The proportion of current smokers was higher in the case group ($P < 0.01$; Table 1). The distribution of previous lung disease was similar in both groups. The most frequent histologic type of NSCLC was adenocarcinoma (109/174; 62.64%) and most of the cases (119/174; 68.39%) presented advanced stage (IIIB–IV).

Table 1 Clinicopathologic characteristics of non-small-cell lung cancer cases and controls

	Cases [*n* (%)]	Controls [*n* (%)]	*P*-value[a]
Sex			
Female	46 (26.44)	135 (45.3)	< 0.001
Male	128 (73.56)	163 (54.7)	
Previous lung disease			
Yes	44 (25.29)	83 (27.85)	0.618
No	130 (74.71)	215 (72.15)	
Smoking status			
Current smokers	85 (48.85)	17 (6.32)	< 0.001
Former smokers	62 (35.63)	107 (39.78)	
Nonsmokers	27 (15.52)	145 (53.90)	
Age [P_{50} (P_{25}, P_{75})]	61.5 (53, 68)	72.90 (67, 80)	< 0.001[b]
< 60	80 (45.98)	33 (11.07)	< 0.001
> 60	94 (54.02)	265 (88.93)	
Histology			
Adenocarcinoma	109 (62.64)	–	–
Squamous cell carcinoma	61 (35.06)	–	
Unknown	4 (2.30)	–	
Tumour stage			
I, II or IIIA	55 (31.61)	–	–
IIIB or IV	119 (68.39)	–	

[a]*P* for χ^2-test.
[b]*P* for Wilcoxon's test.

Genotype distribution

Genotype frequencies were in agreement with the values expected under the Hardy–Weinberg equilibrium model. Linkage disequilibrium values D' and r^2 are shown in Supplementary Table S1 (Supplemental digital content 1, *http://links.lww.com/FPC/B263*). Particularly, IL1B rs1143627/IL1B rs16944 ($r^2 = 0.956741$; $D' = 1.000$) and IL1B rs1143623/IL1B rs12621220 ($r^2 = 0.976046$; $D' = 0.989$) pairs were in strong linkage disequilibrium.

Influence of gene polymorphisms on the risk of non-small-cell lung cancer

The bivariate analysis was carried out in multiple models: genotypic, additive, allelic, dominant and recessive (Supplementary Table S2, Supplemental digital content 2, *http://links.lww.com/FPC/B264*). IL1B rs1143634 was the only polymorphism associated with the risk of NSCLC (Table 2). In particular, patients carrying the C-allele were at a higher risk of NSCLC versus those with the T-allele ($P_{\text{Bonferroni-corrected}} = 0.04896$; OR = 0.628; 95% CI = 0.453–0.874).

The genotypic logistic regression model adjusted by smoking status showed that IL1B rs1143634-TT was associated with a lower risk of NSCLC ($P = 0.04312$; OR = 0.226; 95% CI = 0.044, 0.840; Table 3). No other gene polymorphisms showed an association with NSCLC in any of the models tested.

Discussion

On the basis of an increasing recognition of an aetiologic role for inflammation in lung carcinogenesis, we sought in this study to investigate the association between NSCLC and polymorphisms in inflammation-related genes, such as *IL1B (rs1143634), IL1B (rs12621220), IL1B (rs1143623),*

Table 2 Influence of the IL rs1143634 gene polymorphisms on the risk of non-small-cell lung cancer

Models	Genotype	Cases [*n* (%)]	Controls [*n* (%)]	*P*-value[a]	OR[c]	95% CI
Genotypic	CC	114 (65.5)	162 (54.4)		1.00	
	CT	57 (32.8)	117 (39.3)	0.013	0.69	0.46–1.02
	TT	3 (1.7)	19 (6.4)	0.013	0.22	0.05–0.68
Dominant	CC	114 (65.5)	162 (54.4)		1.00	
	T	60 (34.5)	136 (45.6)	0.023	0.63	0.42–0.94
Recessive	C	171 (98.3)	279 (93.6)		1.00	
	TT	3 (1.7)	19 (6.4)	0.037	0.26	0.08–0.88
Allelic	C	285 (81.9)	441 (74.0)		1.00	
	T	63 (18.1)	155 (26.0)	0.005	0.63	0.45–0.87
Additive	–	–	–	0.004[b]	0.61	0.44–0.86

CI, confidence interval; OR, odds ratio.
[a]*P*-value for χ^2-test.
[b]*P*-value for the Cochran–Armitage test for trend.
[c]Unadjusted or crude ORs.

Table 3 Influence of clinical characteristic and IL rs1143634 gene polymorphisms on the risk of non-small-cell lung cancer

	Genotypic						Dominant			Recessive		
	TT vs. CC			CT vs. CC			T vs. CC			TT vs. C		
	P-value[a]	OR[b]	95% CI	*P*-value[a]	OR[b]	95% CI	*P*-value[a]	OR[b]	95% CI	*P*-value[a]	OR[b]	95% CI
Smoking status												
Current smokers	<0.001	8.72	4.82–16.57	<0.001	8.72	4.82–16.57	<0.001	8.49	4.72–16.02	<0.001	8.83	4.88–16.75
Nonsmokers	<0.001	0.33	0.19–0.55	<0.001	0.33	0.19–0.55	<0.001	0.35	0.19–0.54	<0.001	0.33	0.19–0.55
IL1B rs1143634	0.043	0.23	0.04–0.84	0.425	0.83	0.51–1.32	0.196	0.74	0.46–1.17	0.053	0.24	0.05–0.89

CI, confidence interval; OR, odds ratio.
[a]*P*-value for an unconditional logistic regression model.
[b]Adjusted ORs.

IL1B (rs16944), *IL1B (rs1143627)*, *IL12A (rs662959)*, *IL13 (rs1881457)*, *IL6 (rs1800795)* and *IL16 (rs7170924)*. These variants were determined in 174 cases of NSCLC and 298 controls of South Spanish Caucasian origin.

The proinflammatory cytokine IL1B, which can be generated by lung epithelial cells, is involved in the regulation of the inflammatory response [50]. Overall, in our study, the T-allele at IL1B rs1143634 conferred significantly reduced NSCLC susceptibility ($P_{\text{Bonferroni-corrected}} = 0.04896$; OR = 0.628; 95% CI = 0.453–0.874), although the T-allele had been reported previously as a risk factor for lung cancer ($P = 0.001$; OR = 1.27; 95% CI = 1.10–1.47) in a large study of 1553 cases and 1730 controls of non-Hispanic Caucasian origin, matched to the cases by age, sex, ethnicity and smoking status (never, former, current), by Engels *et al.* [51]. Several differences exist between this and our cohort, which may be responsible for the different results. First, the study by Engels and colleagues included not only NSCLC patients but also 23.7% of patients with other types of lung cancer. In addition, the age was less than 60 years in 73% of the controls. Our study comprised a homogeneous cohort of NSCLC patients and 90.3% of our controls were 60 years or older, which notably decreases the possibility of developing a lung cancer during their life, and consequently, the selection bias. Although the minor allele frequency in both cohorts were very similar, the cohort of Engels and colleagues comprised non-Hispanic Caucasians, and our patients were all Spanish Caucasians. The trend of the T-allele to be a risk factor for lung cancer has been found in other studies, but the evidence is not overwhelming [52,53]. In our patients, smoking was a strong risk factor for NSCLC, even overcoming the protective effect of the TT genotype in smokers, as shown by the different OR obtained for current/nonsmokers (Table 3). Similar to our results, other studies have shown this confounding effect of smoking on NSCLC susceptibility given by IL1B rs1143634 polymorphism. A study reported that the TT genotype was associated with an increased risk of lung cancer in Caucasians, but only in former smokers ($P = 0.026$; OR = 1.74, 95% CI = 1.07–2.85) and men ($P = 0.034$; OR = 1.80, 95% CI = 1.04–3.11) [52]. However, this SNP was not associated with lung cancer in the total population (2644 cases/1619 controls) ($P = 0.26$; OR = 1.23, 95% CI = 0.86–1.75; TT vs. CC). An almost similar result was reported in 462 cases and 379 controls of Japanese origin [53]. The T-allele added a 45% of the excess risk for lung cancer in ever smokers, and patients with excessive alcohol intake and at least one T-allele had a significantly higher risk ($P < 0.01$; OR = 2.48; 95% CI = 1.36–4.54) than drinkers with appropriate intake and the CC genotype; however, the overall analysis failed to find an association of the SNP with lung cancer susceptibility in the total population ($P = 0.11$; OR = 1.45; 95% CI = 0.93–2.26) [53]. Two other studies, carried out in European Caucasian patients (363 cases/440 controls) and 8705 cases and 11562 controls

from an ethnically diverse patient population, failed to find an association of IL1B rs1143634 with lung cancer [54,55]. A meta-analysis combining these four last studies could find an association with a higher risk of lung cancer for the TT genotype of IL1B rs1143634 (OR = 0.92; 95% CI = 0.86–0.99; $I^2 = 0\%$; $P_{heterogeneity} = 0.449$; CC/CT vs. TT), but with a rather low effect and negligible added risk for this polymorphism as the OR was very close to 1 [14]. In our study, the effect was opposite (TT genotype as a protective factor for NSCLC), but more intense, and appeared to have a higher effect as the number of T-alleles increased (Table 3).

For the other IL1B polymorphisms, different studies have investigated their effect on lung cancer susceptibility. The IL1B rs12621220, rs1143623, rs16944 and rs1143627 gene polymorphisms showed significant associations for NSCLC risk in several studies [14,17]. Recently, a Chinese study including 889 cases and 1005 controls reported an association between these SNPs and the risk of NSCLC in stratified analysis by age [17]. The AG genotype for IL1B rs12621220 was associated in less than 63 age strata with an increased risk of NSCLC ($P = 0.04$; OR = 0.71; 95% CI = 0.52–0.98). However, in the more than or equal to 63 age strata, the AG genotype was associated with a decreased risk of NSCLC ($P = 0.04$; OR = 1.40; 95% CI = 1.02–1.91), which was in agreement with a Caucasian study of 363 cases and 440 controls that reported a reduced risk of NSCLC for the A-allele ($P = 0.012$; OR = 0.69; 95% CI = 0.52–0.92) [54]. The C-allele for IL1B rs1143623 was associated with a decreased risk of NSCLC in the more than or equal to 63 age strata ($P = 0.04$; OR = 0.74; 95% CI = 0.55–0.99), which is also in agreement with the Caucasian study described previously (363 cases/440 controls) ($P = 0.001$; OR = 0.63; 95% CI = 0.47–0.83) [54]. For IL1B rs16944, the CC genotype was associated with a higher risk of NSCLC ($P = 0.01$; OR = 1.48; 95% CI = 1.08–2.03) in the more than or equal to 63 age strata, which was not in agreement with a previous meta-analysis consisted of 6 studies (1279 cases/2248 controls) from European Caucasian and Asian patients that reported a decreased risk of NSCLC for the CC genotype (OR = 0.92; 95% CI = 0.86–0.99; $I^2 = 0\%$; $P_{heterogeneity} = 0.431$; CC vs. TT) [14]. The IL1B rs1143627-TT genotype was associated with a higher risk of NSCLC in the more than or equal to 63 age strata ($P = 0.02$; OR = 1.45; 95% CI = 1.06–1.99), which was in agreement with a previous meta-analysis that enroled five studies (3435 cases/4719 controls) from European Caucasian and Asian patient genotypes (OR = 1.23; 95% CI = 1.06–1.43; $I^2 = 43.9\%$; $P_{heterogeneity} = 0.129$; TT/CT vs. CC) [14]. In our study, we did not found an association between IL1B rs12621220, rs1143623, rs16944 and rs1143627 gene polymorphisms and the risk of NSCLC.

The IL6 rs1800795 gene polymorphism showed no significant association with NSCLC risk in our study as reported recently in a meta-analysis including five studies (2801 cases/3234 controls) from the Caucasian population (OR = 1.029; 95% CI = 0.957–1.106; $I^2 = 0\%$; $P_{heterogeneity} = 0.478$; C vs. G) [56]. To date, there are no studies that have evaluated the effect of *IL12A (rs662959)*, *IL13 (rs1881457)* and *IL16 (rs7170924)* on the risk of lung cancer; however, they have showed associations with multiple NSCLC endpoints, such as disease-free survival and overall survival [37,57]. In this study, we did not find an effect of IL12A rs662959, IL13 rs1881457 and IL16 rs7170924 gene polymorphisms on the risk of developing NSCLC.

The main limitation of this study is the limited size of the sample compared with other studies, particularly in the cases. This may have prevented the detection of the associations of some polymorphisms. However, despite this limited sample, and after applying Bonferroni correction to avoid false-positive associations, the effect of IL1B rs1143634 on NSCLC susceptibility remained. The strengths of our study include a very homogeneous cohort of cases, only composed by NSCLC patients diagnosed by the same team of pathologists, recruited from the same geographic area, which increases their uniformity. In addition, controls were recruited older than cases to decrease a potential selection bias.

In summary, our results suggested that the IL1B rs1143634 gene polymorphism may substantially act as a risk factor for NSCLC development. Unexpectedly, in this study the C-allele was linked to the risk of lung cancer, contrary to the result of Engels and colleagues, which was the only study with a positive association for this SNP and showed a significant association between T-allele and lung cancer risk. Therefore, our findings need to be replicated in larger studies and in different populations to resolve the differences. More studies could also contribute towards finding additional associations between other inflammation genes and the risk of NSCLC.

Conclusion

IL1B rs1143634 was significantly associated with a higher risk of NSCLC. No influence of IL1B rs12621220, rs1143623, rs16944, rs1143627, IL12A rs662959, IL13 rs1881457 and IL16 rs7170924 was found on the risk of developing NSCLC in our patients.

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Conflicts of interest

There are no conflicts of interest.

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