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IL18 and *IL18R1* polymorphisms, lung CT and fibrosis: a longitudinal study in coal miners

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ABSTRACT: It has been suggested that interleukin (IL)-18 plays a role in the development of inflammatory and fibrosing lung diseases.

Associations of polymorphisms in the genes coding for IL-18 (*IL18* /G-656T, C-607A, G-137C, T113G, C127T) and its receptor (*IL18R1* /C-69T) with coal workers' pneumoconiosis (CWP) were studied in 200 miners who were examined in 1990, 1994 and 1999. Coal-dust exposure was assessed according to job history and ambient measures. The main health outcome was lung computed tomography (CT) score in 1990. Internal coherence was assessed by studying CT score in 1994, 4-yr change in CT score and CWP incidence and prevalence.

CT score in 1990 was a good predictor of radiographic grade in 1999 and, therefore, an appropriate subclinical quantitative trait. The *IL18* -137C allele was associated with lower CT score in 1990 and 1994 (1.24 *versus* 1.69 and 1.57 *versus* 2.46, respectively), slower progression of CT score between 1990 and 1994 and lower pneumoconiosis prevalence in 1999 relative to the G allele (0.33 *versus* 0.77 and 8.2 *versus* 19.6%, respectively). Smoking- or dust-adjustment, and stratification on *IL18R1* genotype and adjustment for haplotype effects did not change the conclusions.

In conclusion, the results of the present study suggest a role for *IL18* in reducing the development of this fibrosing lung disease.

KEYWORDS: Computed tomography, epidemiology, genetics, *IL18*, *IL18R1*

Interleukin (IL)-18 is a recently described lymphokine involved in neutrophil activation, reactive oxygen species (ROS) synthesis [1], pro-inflammatory cytokine production, nuclear factor (NF)-κB activation and degranulation [2]. A role for IL-18 in pulmonary inflammation has been suggested by studies in rodent models, but its importance is not clearly understood. In epidemiological genetic studies, the *IL18* C-607A single nucleotide polymorphism (SNP) was significantly associated with higher prevalence of sarcoidosis in Japanese subjects [3] but not in Dutch subjects [4]. The *IL18* A105C SNP was significantly associated with asthma [5], the G-allele of the *IL18* promoter variant (-137G/C) was associated with an increased risk of atopic asthma in the Swiss Study on Air Pollution and Lung Disease in Adults (SAPALDIA) Cohort Study [6], and the *IL18* G-656A, G-137C, T-133G, T113G and C127T SNPs were significantly associated with high immunoglobulin (Ig)E levels, specific sensitisation to common allergens and seasonal allergic rhinitis in 105 Caucasian families [7]. However, these findings have not been replicated [8].

DAVIS *et al.* [9] proposed that IL-18 plays an early role in the reiterative process of macrophage--lymphocyte interaction following silica exposure in mice, leading to chronic inflammation, tissue injury and collagen production. KITASATO *et al.* [10] reported markedly elevated levels of IL-18 in the serum and bronchoalveolar lavage of patients with idiopathic pulmonary fibrosis compared with controls. Coal workers' pneumoconiosis (CWP) is another inflammatory and fibrosing lung disease caused by chronic inhalation of particles. The overall hypothesis of the present study was that *IL18* and *IL18R1* polymorphisms contribute to the pathogenesis of CWP. To test this hypothesis, the present authors investigated the associations of *IL18* and *IL18R1* SNPs with computed tomography (CT) score, a quantitative subclinical phenotype predicting the occurrence and the evolution of the disease [11–13], and with disease prevalence. The primary health outcome for the study was the CT score at the first survey,

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when nearly all miners were active. The internal coherence of the results was tested by studying associations with CT score at the second survey and with CWP prevalence at the end of the follow-up. CT score change between 1990 and 1994 was largely considered as a measure of the activity of the disease between both surveys. The present authors also tested the effects of the interactions between polymorphisms in *IL18* and its receptor and coal-dust exposure (*i.e.* gene X-environment interaction), and between *IL18* and *IL18R1* polymorphisms (*i.e.* gene X-gene interaction) on lung CT score and disease prevalence.

METHODS

Study sample

The study design has been described elsewhere [14]. Briefly, unrelated coal miners (aged 34–50 yrs) were recruited in 1990 to be contrasted according to exposure and chest radiography. They included: 80 subjects heavily exposed to underground coal-dusts ($\geq$10 yrs at the coal face), with chest radiography classified 0/1 or 1/0 and without health alteration from any other diseases; 80 healthy subjects exposed to underground coal-dusts, with normal chest radiography classified 0/0; and 80 healthy subjects slightly exposed, with normal chest radiography. The three groups were matched for age and smoking habits [12]. Miners were re-examined in 1994 and in 1999.

The present study sample included 200 coal miners for whom genetic, environmental and health data were available from 1990 to 1999; no differences regarding genotype, exposure and health data were found between the study sample and those not included in the analyses (n=40). In 1990, 96% of the miners were active; the proportion of retired miners rose from 24 to 88% between 1994 and 1999. The appropriate ethical committee approved the study and written consent was obtained from all subjects.

Environment

Besides smoking, detailed information on high or low current exposure based on job description and cumulative exposure was recorded [15]. High current exposure refers to miners working at the coal face, mining, stope or drift advance. Low current exposure refers to those working in ventilation maintenance, pumping, haulage, shaft, stock equipment or safety. Cumulative personal exposure to dust was estimated from each person's job history and from dust measurements at various sites of the coal mine. The estimates were summations of each dust measurement ($mg \cdot m^{-3}$) for the respective time spent in each job. Estimated cumulative exposures to dust were calculated until 1999 and expressed as $mg \cdot m^{-3} \cdot yr^{-1}$.

Radiographic examination

CT scans were performed in 1990 and 1994 for all subjects, as already described elsewhere [12]. Briefly, the lungs were divided into six areas: the upper zones above the carina, the middle zones between the level of the carina and the lower pulmonary veins, and the lower zones below the level of the lower pulmonary veins. Micronodules were defined as opacities <7 mm in diameter and nodules were defined as opacities 7-20 mm in diameter. Micronodules, nodules and other abnormalities, such as emphysema and profusion of shadows, were recorded according to the criteria of the consensus meetings for CT scans established by the Society for Thorax Imagery and the Thorax Club in September 1989 and June 1990 [16], and according to the pathology standards for Coal Workers' Pneumoconiosis defined by the College of American Pathologists [17]. Profusion of abnormalities was graded 0-3 (absent, rare, intermediate, high profusion), and was estimated for each of the six lung zones (upper, middle and lower parts of both lungs), giving a total CT score of 0-18. Analyses were based on the mean CT score calculated for each individual by dividing their total score by six. The readings of the scans for the first and second examinations (1990 and 1994) were performed at the same time by two experienced radiologists blinded to individual exposure and radiographic findings. Where discrepancies occurred, a consensus was reached.

At each survey (1990, 1994 and 1999), chest radiographs taken in the yearly medical examination were interpreted by two independent and experienced physicians, according to the International Labour Office (ILO) standardised classification of radiographs of pneumoconiosis [18]. The films were presented in random order, without any information about the occupational and medical history of the subjects. For statistical analyses, the 12-point ILO profusion grades were reduced to three points: 0/- and 0/0, 0/1 and 1/0, and $\geq$1/1. Subjects with a profusion grade of <1/1 were considered as not having pneumoconiosis, those with a profusion grade of 0/- or 0/0 were considered as having normal chest radiography, and those with a profusion grade of 0/1 or 1/0 were suspected to be in the process of evolution to pneumoconiosis. Pneumoconiosis was defined by a grade $\geq$1/1.

Genotyping

IL18 -137, -607, +113, and +127 SNPs were genotyped in 2004, as previously described [19]. *IL18* -656 and *IL18R1* -69 SNPs were genotyped by allelic discrimination using TaqMan probes. Primers and TaqMan probes were designed using Primer Express v2.0 (Applied Biosystems, Foster City, CA, US). The forward and reverse primers for *IL18R1* are 5′-ttttttaaaaatctgtgtgccagaa-3′ and 5′-cagccaaagctttcaaacaaaa-3′, respectively and TaqMan probes are 5′-ttatgaaAgtttaaaaatc-6-carboxyfluorescein(Fam)-3′ and 5′-ttatgaaGgtttaaaaat-Vic-3′. The forward and reverse primers for *IL18* -656 are 5′-taggtcagtctttgctatcattcca-3′ and 5′-acactttctgcaacagaaagtaagct-3′, respectively and TaqMan probes are 5′-aattttggtaTccctctc-Fam-3′ and 5′-aattttggtaGccctct-Vic-3′. For both assays, primers, probes, and TaqMan Universal MasterMix with no AmpErase UNG (Applied Biosystems) were utilised according to the manufacturer's standard protocol in a Prism 7000 Sequence Detection System (Applied Biosystems). Genotypes were determined by manual clustering using Prism 7000 sequence detection software version 1 (Applied Biosystems).

Statistical methods

Standard statistical tests (Chi-squared or Fisher exact test when appropriate, and logistic regression for qualitative variables; ANOVA and multiple regression analysis for quantitative variables) were performed. Significance was assessed at the 5% two-sided level.

All analyses were first conducted considering each *IL18* SNP separately and the main outcome CT score in 1990, where 96%

of the miners were active. Associations of each SNP with CT score in 1994, change in CT score between 1990 and 1994, and 1999 CWP incidence and prevalence were also investigated to test the coherence of the results and the activity of the disease (change in CT score between 1990 and 1994). No *a priori* adjustment was performed. CT score was not normally distributed, but nonparametric Kruskal-Wallis and standard parametric tests (ANOVA and unpaired t-test) gave similar p-values. Analyses were conducted considering subjects heterozygous and subjects homozygous for the variant allele, and variant allele carriers, as in previous studies [3–7]. *IL18* haplotype analysis was then performed using a maximum likelihood method for haplotype-phenotype association as implemented in the Testing Haplotype Effects In Association Studies (THESIAS) program [20, 21]. The most frequent haplotype (*IL18* -607C/*IL18* -137G (CG)) was used as the reference.

Interaction between genetic polymorphisms and exposure to coal-mine dusts, or between both genetic polymorphisms (*IL18* and its receptor *IL18R1*) on health outcomes (CT score and pneumoconiosis prevalence) were statistically tested using multivariate linear or logistic regression models.

TABLE 1 Characteristics of coal miners

Age yrs	42.6±3.5
Smoking habits	
Nonsmokers	49 (24.5)
Ex-smokers	42 (21.0)
Current smokers	109 (54.5)
Pack-yrs	12.7±11.1
Current coal-dust exposure#	
No exposure (retirement)	8 (4.0)
Low exposure	95 (47.5)
High exposure	97 (48.5)
Cumulative coal-dust exposure mg·m^{-3}·yr^{-1}	52.5±39.2
Geographical origin	
France	135 (67.5)
Other European countries	62 (31.0)
North Africa	3 (1.5)
CT score	1.47±1.97
Chest radiographic grade	
0/0	134 (67.0)
0/1	45 (22.5)
1/0	21 (10.5)
CT score in 1994	2.02±2.65
Chest radiographic grade in 1994	
0/0	144 (72.0)
0/1	31 (15.5)
1/0	18 (9.0)
⩾1/1 (pneumoconiotic)	7 (3.5)
Chest radiographic grade in 1999	
0/0	134 (67.0)
0/1	17 (8.5)
1/0	21 (10.5)
⩾1/1	28 (14.0)

Data are for 1990, unless otherwise stated, and are presented as mean±SD or n (%). CT: computed tomography. #: based on job description.

RESULTS

The characteristics of the 200 miners included in the analyses are summarised in table 1. The mean age of the miners in 1990 was 43 yrs. More than half of the miners were current smokers, and 48.5% were highly exposed to coal-mine dusts in 1990. Almost 68% were born in France and only 1.5% had their geographical origin in non-European countries. Among all coal miners, the CT score increased by ~40% between 1990 and 1994, and the prevalence of pneumoconiosis rose from 3.5% to 14% between 1994 and 1999.

CT score and radiographic grade were highly associated in 1990 and in 1994. Mean±SD CT scores in 1990 were 0.71±1.19, 2.98±2.20 and 3.09±2.61 in miners with radiographic grades of 0/0, 0/1 and 1/0, respectively (trend test, $p<0.0001$). In 1994, mean±SD CT scores were 1.07 ±1.38, 3.87±2.95 and 4.39±3.60, with the seven pneumoconiotic miners (⩾1/1) having a mean±SD score of 7.43±4.20 (trend test, $p<0.0001$). CT score and cumulative coal-dust exposure (mg·m^{-3}·yr^{-1}) were highly correlated in 1990 and in 1994 ($r=0.35$; $p<0.0001$ and $r=0.29$; $p<0.0001$, respectively).

In 1999, 26 (92.9%) of the 28 pneumoconiotic miners were those heavily exposed to underground coal-dusts with chest radiographs classified 0/1 or 1/0 in 1990, two (7.1%) were those exposed to underground coal-dusts with normal chest radiographs classified 0/0 in 1990. No miner slightly exposed with normal chest radiograph in 1990 had pneumoconiosis in 1999. Figure 1 shows the relationship between radiographic grade in 1999 and CT score in 1990. At the end of the 10-yr follow-up, 46 subjects had worsened radiographic findings and 28 of them were pneumoconiotic. CT score in 1990 was significantly higher in miners whose radiographic findings had worsened in 1999, compared with those who had not (3.67±2.39 (n=46) *versus* 0.81±1.22 (n=154); $p<0.0001$), and in miners who

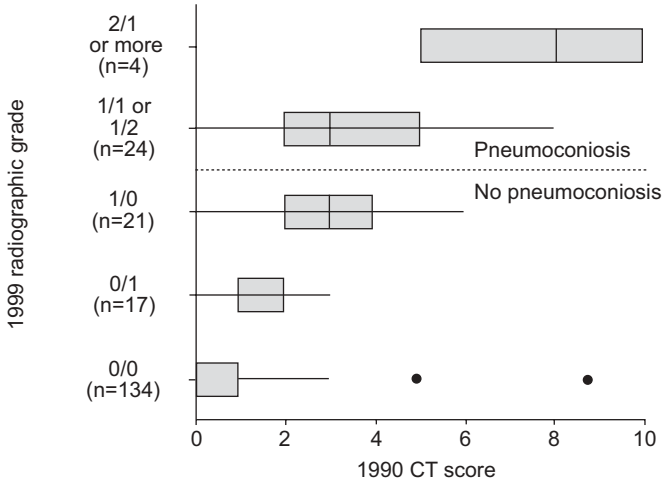

FIGURE 1. Box plots showing computed tomography (CT) score, a quantitative trait predicting the evolution to pneumoconiosis, in 1990 *versus* chest radiographic grade in 1999. Plots show the median (|), the first and third quartile (▫), the first and last decile (---) and the maximum (●) of CT score for each radiographic grade category. Mean±SD CT scores for each category are 0.72±1.19, 1.35±1.00, 2.76±1.70, 3.58±2.22 and 7.75±2.63, respectively ($p<0.0001$).

developed pneumoconiosis compared with others (4.18±2.68 (n=28) *versus* 1.03±1.41 (n=172); p<0.0001).

Genotype and allele frequencies

Minor allele frequencies were: 0.42 for *IL18* G-656A and C-607A; 0.285 for *IL18* G-137C, T+113G, and C+127T; and 0.347 for *IL18R1* C-69T. All of these fit predictions for Hardy-Weinberg equilibrium (all p>0.6). Complete linkage disequilibrium was observed between the *IL18* -656 and -607 genotypes, and between *IL18* -137, +113 and +127 genotypes. Three haplotypes were found: *IL18* -607C/*IL18* -137G (CG), 58.0%; AC, 28.5%; and AG, 13.5%. All miners homozygous for the *IL18* -137C allele were homozygous for the *IL18* -607A allele, and 63.7% of miners homozygous for the *IL18* -137G allele were homozygous for the *IL18* -607C allele (p<0.0001 for association). No differences in genotype or allele distributions were observed according to the geographical origin of the miners (data not shown).

Association of IL18 -607, IL18 -137 and IL18R1 SNPs with stage of pneumoconiosis

No significant association was found between *IL18* or *IL18R1* SNPs and CT score in 1990 (table 2). Furthermore, no significant associations were found between *IL18* C-607A or *IL18R1* C-69T genotype and CT score in 1994, change in CT score between 1990 and 1994, or pneumoconiosis incidence or prevalence.

Lower CT score in 1990, significantly lower CT score in 1994 and slower progression of CT score were found in *IL18* -137C carriers (*i.e.* miners homozygous or heterozygous for -137C). No *IL18* -137C carrier had pneumoconiosis in 1994, and the *IL18* -137C allele was significantly associated with lower disease prevalence in 1999.

No interaction was observed between SNPs and coal-dust exposure on CT score in 1990 and in 1994, change in CT score or disease prevalence in 1999 (data not shown). Analysing smoking- or dust-adjusted CT score and disease prevalence, or stratifying on *IL18R1* C-69T genotype did not change the conclusions.

Haplotype analyses

No association between *IL18* -607/-137 haplotype AC and CT score in 1990 was found (table 3). The *IL18* -607/-137 haplotype AC was associated at borderline significance with CT score in 1994, and was significantly associated with a slower progression of CT score between 1990 and 1994 and with a lower prevalence of disease in 1999. No significant association was found with haplotype AG.

DISCUSSION

The present study tested the hypothesis that polymorphisms in *IL18* and *IL18R1* contribute to the pathogenesis of CWP, an inflammatory and fibrosing lung disease. The results show significant associations of the *IL18* -137C allele with CT score in 1994, slower progression of CT score between 1990 and 1994 and lower pneumoconiosis prevalence in 1999 relative to the G allele. Adjusting for haplotype effects confirmed the results. Furthermore, analysis of smoking- or dust-adjusted CT score or disease prevalence, or stratifying on *IL18R1* genotype, did not change the conclusions.

CT is not used as a standard method to assess pneumoconiosis, although it is a sensitive tool for the evaluation of lung

TABLE 2 Association of polymorphisms in the genes coding for interleukin-18 (*IL18*) and its receptor (*IL18R1*) with stages of pneumoconiosis in coal miners

Polymorphism	Subjects n	1990 CT score	p-value#	1994 CT score	p-value#	ΔCT score 1990–1994	p-value#	ΔCWP incidence %				1999 CWP prevalence %	p-value
								1990–1994	p-value	1994–1999	p-value		
***IL18* C-607A**													
CC	65	1.48±2.26		2.31±2.96		0.83±1.58		4.6		14.5		18.5	
CA	102	1.56±1.90		1.98±2.47		0.42±1.34		2.9		10.1		12.7	
AA	33	1.18±1.57	0.6	1.61±2.60	0.5	0.42±1.41	0.2	3.0	0.9	6.3	0.4	9.1	0.4
CA or AA	135	1.47±1.82	0.4	1.89±2.49	0.4	0.42±1.35	0.09	3.0	0.7	9.2	0.3	11.8	0.2
***IL18* G-137C**													
GG	102	1.69±2.23		2.46±2.95		0.77±1.49		6.9		13.7		19.6	
GC	82	1.19±1.57		1.46±1.91		0.27±1.22		0.0		7.3		7.3	
CC	16	1.50±2.00	0.4	2.12±3.46	0.05	0.62±1.93	0.08	0.0	0.03	12.5	0.4	12.5	0.06
GC or CC	98	1.24±1.64	0.2	1.57±2.23	0.02	0.33±1.35	0.03	0.0	0.01	8.2	0.2	8.2	0.02
***IL18R1* C-69T**													
CC	86	1.66±2.17		2.29±2.71		0.63±1.32		2.3		14.3		16.3	
CT	89	1.40±1.97		1.99±2.83		0.58±1.57		5.6		8.3		13.5	
TT	25	1.04±0.98	0.7	1.24±1.48	0.2	0.20±1.32	0.5	0.0	0.5	8.0	0.4	8.0	0.6
CT or TT	114	1.32±1.81	0.7	1.82±2.60	0.2	0.50±1.52	0.3	4.4	0.7	8.3	0.2	12.3	0.4

Data are presented as mean±SD unless otherwise stated. CT: computed tomography; Δ: change in; CWP: coal workers' pneumoconiosis (radiographic grade ⩾1/1). #: Kruskal-Wallis test.

TABLE 3 Association of haplotypes of the gene coding for interleukin-18 (*IL18*) with stages of pneumoconiosis in coal miners

-607/-137 haplotype	CT score 1990		CT score 1994		ΔCT score 1994–1990		CWP prevalence 1999	
	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
CG	Reference		Reference		Reference			
AC	-0.23 (-0.68-0.22)	0.3	-0.52 (-1.06-0.01)	0.06	-0.29 (-0.58- -0.001)	0.05	0.50 (0.25-0.99)	<0.05
AG	0.18 (-0.45-0.81)	0.6	0.06 (-0.79-0.91)	0.9	-0.12 (-0.66-0.43)	0.7	1.06 (0.46-2.41)	0.9

CT: computed tomography; Δ: change in; CWP: coal workers' pneumoconiosis (radiographic grade ⩾1/1); OR: odds ratio; CI: confidence interval.

parenchyma [11]. The determination of genetic factors is greatly enhanced by considering subclinical quantitative phenotypes [22]. CT has been proposed as a screening method to distinguish normal from early pneumoconiosis [13]. In the present study, the predictive value of CT score as an appropriate subclinical quantitative phenotype was confirmed. Results with a 10-yr follow-up confirm and extend those reported in a 4-yr period [12]. As mines have been totally closed in France, it was not possible to build a replication sample. Furthermore, the use of CT in coal mining is still limited at an international level. CWP, however, remains a major occupational disease in terms of public health burden worldwide and it is important to better understand the genetic modifiers of this environmental disease.

The present study has some limitations. Not all of the *IL18* and *IL18R1* SNPs were considered, nor were other genes involved in the IL-18 pathway which might contribute, alone or in combination, to IL-18 variability and in turn affect CWP susceptibility. However, the SNPs analysed were those with functional significance that have been previously published. Furthermore, the sample size of the population studied was small. A precise assessment of the power could not be performed prior to the study; variations of CT score across the general population are not known, as it is not possible to perform CT scanning on a large scale for ethical and technical reasons. However, it was anticipated that the contrasted disease status chosen by design and the availability of such a sensitive score would increase the power to detect differences compared with classical designs based on random samples using only chest radiographic measures.

Few epidemiological genetic studies have examined the associations of the *IL18* A-607C and G-137C SNPs with chronic inflammatory and fibrosing lung diseases, and none have simultaneously investigated the role of environmental factors and polymorphism in *IL18R1*. Furthermore, none has considered CT score. KRUSE *et al.* [7] reported a significant association of the *IL18* 137C allele with high serum IgE levels, specific sensitisation to common allergens and seasonal allergic rhinitis in 105 German families. The *IL18* A-607C SNP was unrelated to these phenotypes. In populations recruited in the same areas, the *IL18* A-607C and G-137C SNPs were unrelated to bronchial asthma in 230 children compared to 270 controls [8]. Recently, a significant association of *IL18* -137G allele with increased risk for atopic asthma in the SAPALDIA Cohort Study [6] was found. TAKADA *et al.* [3] reported a significantly higher frequency of the *IL18* -607C allele in 119 Japanese sarcoidosis patients compared with 130 controls, and no association was found for the *IL18* G-137C SNP. The significant association was not replicated in a population of Dutch Caucasians, where 133 sarcoidosis patients were compared with 103 controls [4]. The inconsistency between studies may be due to differences in *IL18* -607A and -137C allelic frequencies between populations, or in asthma onset or biological pathways during disease progression. Furthermore, the pleiotropic role of IL-18 with varying effects according to the cytokine milieu (*i.e.* T-helper (Th) type 2 cytokines when considering atopic phenotypes, or Th2 and Th1 cytokines when considering asthma [23]) could also partly explain these differences.

In the present study, only the *IL18* G-137C SNP was significantly associated with pneumoconiosis phenotype and prevalence. Taking into account coal-dust exposure, which is the first cause of the disease, and stratifying on *IL18R1* genotype did not change the conclusions. Genotype and allelic frequencies were very close to those reported in previous studies in Caucasians [4, 6–8]. Haplotypes found in the present study were identical to those reported by GIEDRAITIS *et al.* [24] in a Swedish population. GIEDRAITIS *et al.* [24] found that the haplotype -656T/-607A/-137C/+113G/+127T (-607/-137 AC in the present study) was clearly associated with lower promoter activity and lower *IL18* gene expression than haplotypes CG and AG. Furthermore, in C carriers at position -137, no correlation was found between IL-18 and interferon (IFN)-γ mRNA levels, whereas a strong correlation was found in those homozygous wildtype, with GG at -137.

The results of the present study were also consistent with the study of WEI *et al.* [25], in which markedly reduced incidence and severity of collagen-induced arthritis was found in IL-18-/- mice compared with wildtype mice. This was accompanied *in vitro* by significantly reduced production of pro-inflammatory cytokines, including IFN-γ. Further, significantly reduced lung collagen was observed in IFN-γ-deficient mice exposed to silica compared with wildtype mice [9]. Pneumoconiosis is another collagen-related disease, including the activation of alveolar macrophages, ROS synthesis and the production of pro-inflammatory cytokines such as tumour necrosis factor and NF-κB. IL-18 is involved in all of these steps [1–2].

In conclusion, it was found that the *IL18* G-137C single nucleotide polymorphism was associated with lower computed tomography score, slower progression of computed

tomography score and lower pneumoconiosis prevalence. Furthermore, smoking- or dust-adjustment, stratification on *IL18R1* genotype and adjustment for haplotype effects did not change these conclusions. The results are consistent, support the biological and functional significance of *IL18* and suggest its potential role in reducing the development of this inflammatory and fibrosing lung disease. However, it is premature to consider any clinical application of the findings and replication of these findings in additional populations is warranted.

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REFERENCES

1 Cho D, Song H, Kim YM, *et al.* Endogenous interleukin-18 modulates immune escape of murine melanoma cells by regulating the expression of Fas ligand and reactive oxygen intermediates. *Cancer Res* 2000; 60: 2703–2709.
2 Gracie JA, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol* 2003; 73: 213–214.
3 Takada T, Suzuki E, Morohashi K, Gejyo F. Association of single nucleotide polymorphism in the IL-18 gene with sarcoidosis in a Japanese population. *Tissue Antigens* 2002; 60: 36–42.
4 Janssen R, Grutters JC, Ruven HJT, *et al.* No association between interleukin-18 gene polymorphisms and haplotypes in Dutch sarcoidosis patients. *Tissue Antigens* 2004; 63: 578–583.
5 Higa S, Hirano T, Mayumi M, *et al.* Association between interleukin-18 gene polymorphism 105A/C and asthma. *Clin Exp Allergy* 2003; 33: 1097–1102.
6 Imboden M, Nicod L, Nieters A, *et al.* The common G-allele of interleukin-18 single-nucleotide polymorphism is a genetic risk factor for atopic asthma. The SAPALDIA Cohort Study. *Clin Exp Allergy* 2006; 36: 211–218.
7 Kruse S, Kuehr J, Moseler M, *et al.* Polymorphisms in the IL18 gene are associated with specific sensitisation to common allergens and allergic rhinitis. *J Allergy Clin Immunol* 2003; 111: 117–122.
8 Heinzmann A, Gerhold K, Ganter K, *et al.* Association study of polymorphisms within interleukin-18 in juvenile idiopathic arthritis and bronchial asthma. *Allergy* 2004; 59: 845–849.
9 Davis GS, Holmes CE, Pfeiffer LM, Hemenway DR. Lymphocytes, lymphokines and silicosis. *J Environ Pathol Toxicol Oncol* 2001; 20: 53–65.
10 Kitasato Y, Hoshino T, Okamoto M, *et al.* Enhanced expression of interleukin-18 and its receptor in idiopathic pulmonary fibrosis. *Am J Respir Cell Mol Biol* 2004; 31: 619–625.
11 Remy-Jardin M, Remy J, Farre I, Marquette CH. Computed tomographic evaluation of silicosis and coal workers' pneumoconiosis. *Radiol Clin North Am* 1992; 30: 1155–1176.
12 Bourgkard E, Bernadac P, Chau N, Bertrand JP, Teculescu D, Pham QT. Can the evolution to pneumoconiosis be suspected in coal miners? A longitudinal study. *Am J Respir Crit Care Med* 1998; 158: 504–509.
13 Savranlar A, Altin R, Mahmutyazicioglu K, *et al.* Comparison of chest radiography and high-resolution computed tomography findings in early and low-grade coal worker's pneumoconiosis. *Eur J Radiol* 2004; 51: 175–180.
14 Nadif R, Jedlicka A, Mintz M, Bertrand JP, Kleeberger S, Kauffmann F. Effect of TNF and LTA polymorphisms on biological markers of response to oxidative stimuli in coal miners: a model of gene-environment interaction. *J Med Genet* 2003; 40: 96–103.
15 Attfield MD, Morring K. The derivation of estimated dust exposures for U.S. coal miners working before 1970. *Am Ind Hyg Assoc J* 1992; 53: 248–255.
16 Frija J. Sémantique des pneumopathies infiltrantes diffuses étudiées par la tomodensitométrie. *Rev Imag Med* 1991; 3: 355–361.
17 Kleinerman J, Green F, Harley RA, *et al.* Pathology standards for coal worker's pneumoconiosis: report of the pneumoconiosis committee of the College of American Pathologists to the National Institute for Occupational Safety and Health. *Arch Pathol Lab Med* 1979; 103: 375–432.
18 International Labour Office (ILO). Guideline for the use of ILO international classification of radiographs of pneumoconiosis. Geneva, ILO division of Occupational Safety and Health Sciences, 1980.
19 Brown RH, Hamilton RG, Mintz M, Jedlicka AE, Scott AL, Kleeberger SR. Genetic predisposition to latex allergy: role of interleukin 13 and interleukin 18. *Anesthesiology* 2005; 102: 496–502.
20 Tregouet DA. Testing Haplotype Effects In Association Studies (THESIAS), genecanvas.ecgene.net/downloads.php?cat_id=1 Date last updated: February 21, 2006. Date last accessed: June 20, 2006.
21 Tregouet DA, Barbaux S, Poirier O, *et al.* SELPLG gene polymorphisms in relation to plasma SELPLG levels and coronary artery disease. *Ann Hum Genet* 2003; 67: 504–511.
22 Schork NJ. Genetics of complex disease: approaches, problems, and solutions. *Am J Respir Crit Care Med* 1997; 156: S103–S109.
23 Nakanishi K, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 2001; 19: 423–474.
24 Giedraitis V, He B, Huang WX, Hillert J. Cloning and mutation analysis of the human IL-18 promoter: a possible role of polymorphisms in expression regulation. *J Neuroimmunol* 2001; 112: 146–152.
25 Wei XQ, Leung BP, Arthur HML, McInnes IB, Liew FY. Reduced incidence and severity of collagen-induced arthritis in mice lacking IL-18. *J Immunol* 2001; 166: 517–521.