Original Article

TNF-α and *IL-1RA* Polymorphisms and Silicosis Susceptibility in Chinese Workers Exposed to Silica Particles: A Case-Control Study

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Abstract

Objective To assess the association of *TNF-α* and *IL-1RA* SNPs with the risk of silicosis in Chinese workers exposed to silica particles.

Methods Case-control study design was used to enroll 68 silicotic patients induced by silica particles and 68 healthy workers matched for length of silica particle exposure as controls. Both cases and controls were from the same company in southwest China, and each of them was requested to complete a questionnaire. Blood samples were drawn for genomic DNA extraction from each participant. The genotyping of *TNF-α* (-238 and -308) and *IL-1RA* (+2018) was performed using polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) and SYBR green-based quantitative polymerase chain reaction (qPCR), respectively. Unconditional logistic regression model was used to estimate odds ratios (ORs) and their 95% confidential intervals (CI) for SNPs.

Results No significant differences were found between cases and controls in particles exposure length, body mass index (BMI), and status of smoking and alcohol consumption except for age (*P*=0.001) and blood type (*P*=0.042). The frequencies of *TNF-α* (-238) and *IL-1RA* (+2018) genotypes in cases were significantly different from those in controls, (*P*=0.001 and *P*=0.002, respectively), while a borderline significant difference was found in the frequencies of *TNF-α* (-308) between cases and controls (*P*=0.063). The variants of three SNPs increased the risk of silicosis in the Chinese workers exposed to silica particles. The adjusted ORs of *TNF-α* (-308), *TNF-α* (-238) and *IL-1RA* (+2018) were 2.8 (95% CI: 1.1-7.5), 20.9 (95% CI: 1.8-236.4) and 4.0 (95% CI: 1.6-10.1), respectively.

Conclusion It is suggested that cytokine polymorphisms of *TNF-α* (-238, -308) and *IL-1RA* (+2018) are associated with the risk of silicosis in the Chinese workers exposed to silica particles. Further independent studies on the interaction between SNPs and exposure to silica particles with a larger sample size are therefore warranted.

Key words: *TNF-α, IL-1RA*; Polymorphism; Silicosis; Case-control study

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INTRODUCTION

Silicosis is pneumoconiosis of lung fibrosis caused by inhalation of silica particles usually at low levels but for long periods; and it is a common occupational disease among the workers who are exposed to silica particles. The underlying mechanisms of silicosis are still unclear. However, one principal hypothesis is that the inhalation of silica particles in lung are engulfed by macrophages, thereby activating inflammatory networks and leading to the release of inflammatory cytokines into the alveolar space. The consequence of these chronic inflammatory stimuli is the occurrence of silicosis[1]. As two of the major cells in immunity response, the differentiation of B and T cells are regulated by inflammatory cytokines such as tumor necrosis factor (TNF) cytokine and interleukin-1 (IL-1)[2]. TNF-α and IL-1 have been shown to play important roles in the pathogenesis of silica particle-induced pulmonary inflammatory reactions and chronic inflammatory disease[3-5]. IL-1 receptor antagonist (IL-1RA), a naturally occurring anti-inflammatory protein that belongs to a member of the IL-1 family with a structure similar to IL-1, competitively inhibits the binding of IL-1 to IL-1 receptor, and is also involved in human inflammatory diseases[6].

Since silicosis is irreversible and incurable other than elimination and control of silica particles it is of practical significance，for the prevention of silicosis and for the reduction of related deaths and cost, to identify workers at high risk.. Occupational exposure to silica particles is a known risk factor. However, owing to the fact that not all the individuals who have a similar exposure history develop lung fibrosis[7-8] genetic factors may also contribute to the risk of the disease. Several studies have demonstrated that *TNF-*α and *IL-1RA* gene polymorphisms are related to the occurrence and development of silicosis in Caucasian and African coal miners[7,9,11-14]. Both *TNF-*α (-308) variant and *IL-1RA* (+2018) raise the risk of silicosis[12,15]. However, there are few studies investigating the associations of polymorphisms in *TNF-*α and *IL-1RA* genes with silicosis in Chinese workers who are exposed to silica particles. Additionally, although in the promoter of *TNF-*α, four specific point mutations of G to A are identified at the -163, -238, -308, and -376 sites, respectively[16-18], which may affect *TNF-*α expression[11-12], the SNPs at -238 and -308 are two points examined and are associated with the risk of silicosis in most of the studies. Thus, we hypothesize that polymorphisms in the *TNF-*α (-308, -238) promoter region and *IL-1RA* (+2018) are associated with individual susceptibility to silicosis. The purpose of this study was to determine the associations of *TNF-*α and *IL-1RA* SNPs with the risk of silicosis in Chinese workers exposed to silica particles.

METHODS

Participants

This study was approved by the Ethical Committee of the West China School of Public Health, Sichuan University. In this study, 68 silicosis cases and 68 healthy controls who were matched for the length of exposure to silica particles were recruited after excluding those who had different dust exposure status or immune system diseases. All cases and controls were steel workers from the same plant of a steel-making company located in Southwest China. Each pair of them had the matched conditions including length of exposure to silica particles (±1 year), age (±5 old years) and similar proportions of education, smoking and alcohol consumption history. Since few women were among silica particle-exposed workers in the plant to match gender, we only included men workers in the study. All cases were diagnosed using the chest radiographs, which were reviewed by independent radiologists and occupational disease committee, based on the International Labor Organization Classification[19]. All controls had no history or symptoms of disease including pulmonary disease. All the participants were provided informed consent forms and completed a detailed questionnaire (covering demographic characteristics, and occupational history) which included age, length of exposure to silica particles, education, current smoking status and alcohol consumption. The physical examinations were performed by the West China Occupational Diseases Hospital and the Public Health Constitute of Sichuan University.

TNF-α and IL-1RA Genotyping

Genomic DNA was extracted using phenol/chloroform conventional method from blood samples collected from each study subject. The quality and quantity of genomic DNA samples were determined using spectrophotometer.

To determine *TNF-*α and *IL-1RA* genotypes, polymerase chain reaction-based restriction fragment

length polymorphism (PCR-RFLP) assays were performed. The primer sequences for *TNF-α* polymorphisms are *TNF-α* (-308) sense 5'-GAGGCAATAGGTTTTGAGGGCCAT, *TNF-α* (-308) antisense 5'-CATCAAGGATACCCCTCACACT, *TNF-α* (-238) sense 5'-CCCCAAAAGAAATGGAGGCAAT, and *TNF-α* (-238) antisense 5'-CACTCCCCATCCTCCCG GATC (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, China). Hot-start PCR reactions were carried out on a thermal cycler of PE9600 PCR (America Perkin Elmer Companies, USA) in a 20 μL reaction volume, which contained 5 μ Taq polymerase (MBI Fermentas, Vilnius, Lithuania), 10 mmol of each dNTP (MBI Fermentas), 10×PCR Buffer, 25 mmol $MgCl_2$ (MBI Fermentas), the paired primers for either *TNF-α* (-308) or *TNF-α* (-238), and 5.0 μL genomic DNA. The PCR reaction conditions for *TNF-α* (-308) consisted of initial denaturing at 94 °C for 2 min, followed by adding the Taq enzyme and 35 cycles of denaturing at 94 °C for 10 s, annealing at 50 °C for 30 s and extensions at 72 °C for 40 s, with a final extension at 72 °C for 5 min. For *TNF-α* (-238), the PCR reaction conditions were the same as for *TNF-α* (-308) except annealing temperature at 47 °C for 30 s. PCR products were digested with either NcoI for *TNF-α* (-308) or BamHI for *TNF-α* (-238) at 37 °C for 5 h, and 3% agarose gel electrophoresis (Tito Enterprise CO., Ltd, USA) was run to check the status of the digested fragments. For *TNF-α* (–308), homozygous G/G had one band sizing 114 bp of restriction digested fragment of PCR products on gel, heterozygous G/A had two bands sizing 114 bp and 135 bp, respectively, and homozygous A/A had one band sizing 135bp; for *TNF-α* (-238), homozygous G/G had one band sizing 114 bp, heterozygous G/A had two bands sizing 114 bp and 131 bp, respectively, and homozygous A/A had one band sizing 131 bp.

SYBR green-based quantitative PCR (qPCR) was performed on FTC2000 system (FungLyn Biotech Co., Ltd, Shanghai city, China) to determine the *IL-1RA*(+2018) genotypes. The sequences for the primers are a pair of outer primers, IL1RAEF: 5'-GAGGAACAACCAACTAGTAG and IL1RAER: 5'-AAAGTGACGTGATGCCTA, and 3 inter primers, IL1RAT: 5'-CTGAGGAACAACCAACTAGTTGCT, IL1RAC: 5'-CTGAGGAACAACCAACTAGTTGCC' and IL1RAIR:5'-CGTGATGCCTACATACATTGACT (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd). Pre-amplification of genomic DNA was performed with outer primers in a 20 μL reaction volume and under the PCR reaction conditions of initial denaturing at 94 °C for 2 min, followed by 15 cycles of denaturing at 94 °C for 10 s, annealing at 50 °C for 30 s, and extension at 72 °C for 40 s. Then qPCR was performed in a 50 μL reaction volume, which contained 5 μ Taq polymerase (BioDev Company, China), 10 mmol dNTP (Promega Corporation), 10×PCR Buffer, 25 mmol $MgCl_2$ (TakaRa Company, Japan), 1 μL SYBR green I(Roche Company, Germany), either IL1RAT and IL1RAIR or IL1RAC and ILRAIR, and pre-amplified genomic DNA. qPCR reaction conditions consisted of initial denaturing at 94 °C for 3 min, followed by 35 cycles of denaturing at 94 °C for 30 s, annealing at 60 °C for 30 s and extension at 72 °C for 1 min.

Genotypes of *TNF-α* (-308) and *TNF-α* (-238) and *IL-1RA* (+2018) for selected samples were confirmed by sequencing method on ABI PRISMTM 377 DNA SEQUENCER (Shanghai Sangon Biological Engineering Technology & Services Co., Ltd, China). Figure 1-3 showed the representative sequencing results of ABI sequencer for the selected samples from each genotype of the SNPs. The genotyping results of both PCR-RFLP and qPCR were consistent with direct sequencing.

Statistical Analysis

Continuous variables were expressed as the mean±standard deviation (SD) and the differences were tested by generalized linear model (GLM) or wilcoxon rank sum test if appropriate. Chi-square test was used to analyze Hardy-Weinberg Equilibrium (HWE) for genotype distribution in controls and other categorical variables. Both univariate and multivariate unconditional logistic regression were conducted to estimate odds ratios (ORs) and their 95% confidence intervals (95% CI) for each polymorphism and to adjust potential confounding factors such as age, body mass index (BMI), smoking status and alcohol consumption, length of exposure to silica particles education, and blood type. All *P* values ≤0.05 were considered as statistical significance. Statistical analyses were performed using the SPSS 16.0 statistical software package.

RESULTS

Demographic Characteristics of Participants

The demographic characteristics of all participants enrolled in this study are shown in Table 1. The average age of the 136 subjects was 43.1 years

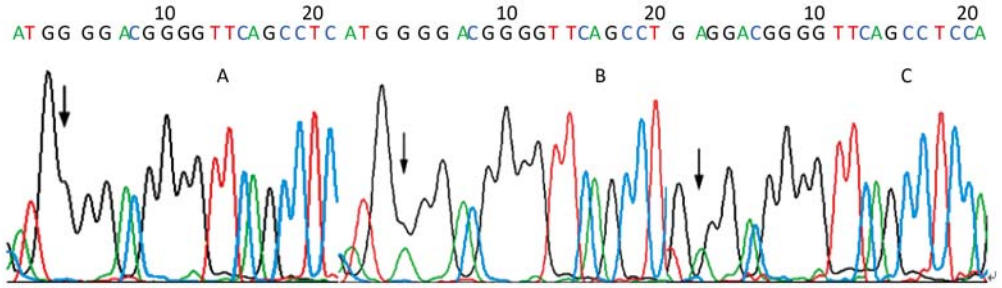

Figure 1. The representative sequences of *TNF-α* (-308) genotypes. G/G, (B) G/A, (C) A/A.

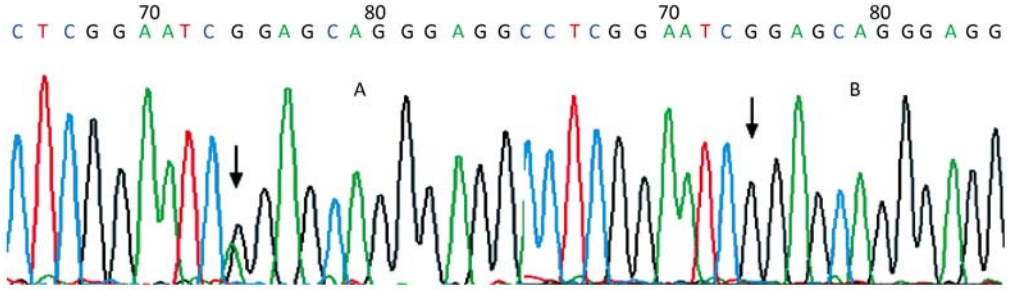

Figure 2. The representative sequences of *TNF-α* (-238) genotypes. (A) G/A, (B) G/G.

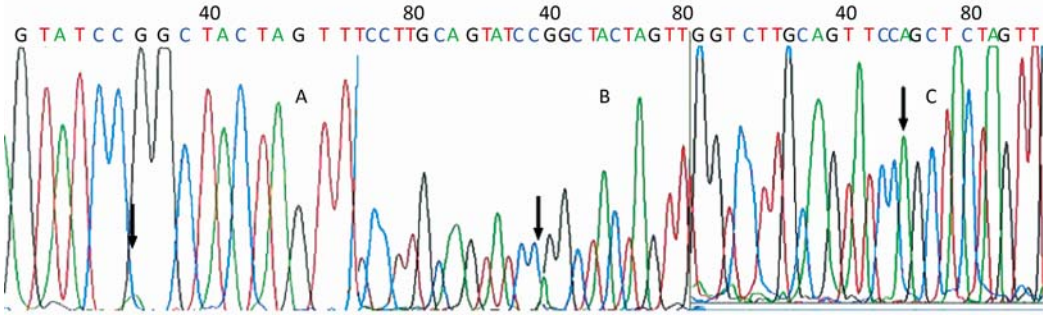

Figure 3. The representative sequences of *IL-1RA* (+2018) genotype. (A) C/C, (B) T/C, (C) T/T.

with the variation of 8.6 years. There were no significant differences in the length of exposure to silica particles, BMI, alcohol consumption, and current smoking status between cases and controls. But there was a highly significant difference in age. The distributions of blood type showed statistically significant differences between cases and controls (P=0.042).

Association of TNF-α (-308), TNF-α (-238), and IL-1RA (+2018) Genotypes with Silicosis

The distributions of *TNF-α* (-308), *TNF-α* (-238), and *IL-1RA* (+2018) genotypes were shown in Table 2. For *TNF-α* (-308) genotypes in all participants, homozygous G/G accounted for 69.12%, heterozygous G/A were 27.94% and homozygous A/A were 2.94%. For *TNF-α* (-238) genotypes, G/G accounted for 89.71%, G/A were 9.56%, and A/A were 0.73%. For *IL-1RA* (+2018) genotypes, homozygous T/T accounted for 63.97%, T/C were 32.35% and homozygous C/C were 3.68%.

The distributions of *TNF-α* (-308), *TNF-α* (-238), and *IL-1RA* (+2018) genotypes in controls had no deviation from Hardy-Weinberg equilibrium, and P values were 0.996, 0.380, and 0.944, respectively (data not shown). In additive model, we found different distributions of three SNPs between cases and controls; more cases carried mutants than controls. For

Table 1. Demographic Characteristics of the Participants

Variables	Total (*n*=136)	Groups [mean±SD/*n*(%)]		*P*-value
		Case (*n*=68)	Control (*n*=68)	
Age(years)	43.1± 8.6	45.5±8.5	40.6±8.1	0.001
Silica particle exposure length (years)	19.3 ± 7.5	19.6±6.6	18.9±8.3	0.600
BMI (kg/m^2)	22.2 ± 3.3	22.3±3.4	22.1±3.1	0.804
Education				
Illiteracy	4	3 (4.4)	1 (1.5)	
Elementary School	49	24 (35.3)	25 (36.8)	
Middle School	71	35 (51.5)	36 (52.9)	0.843
High School	10	6 (8.8)	4 (5.9)	
College and Above	2	1 (1.5)	1 (1.5)	
Blood Type				
A	48	28 (41.2)	20 (29.4)	
B	37	12 (17.6)	25 (36.8)	0.042
AB	35	17 (25)	18 (26.5)	
O	16	11 (16.2)	5 (7.4)	
Current Smoking				
Yes	94	47 (69.1)	47(69.1)	1.000
No	42	21 (30.9)	21(30.9)	
Alcohol Consumption				
Yes	89	41 (60.3)	48(70.6)	0.207
No	47	27 (39.7)	20(29.4)	

Note. BMI was calculated as weight (kg)/height(m^2).

Table 2. Genotype of *TNF-α* (-308), *TNF-α* (-238) and *IL-1RA (+2018)* in Cases and Controls

Genotype	*n* (%)	*n* (%)		*P*-value
		Case (*n*=68)	Control (*n*=68)	
***TNF-α*(-308)**				
G/G	94 (69.12)	42 (61.8)	52 (76.5)	
G/A	38 (27.94)	23 (33.8)	15 (22.1)	0.063
A/A	4 (2.94)	3 (4.4)	1 (1.5)	
G/A or A/A	42 (30.88)	26 (38.2)	16 (23.5)	0.052
***TNF-α* (-238)**				
G/G	122 (89.71)	55 (80.9)	67 (98.5)	
G/A	13 (9.56)	12 (17.6)	1 (1.5)	0.001
A/A	1 (0.73)	1 (1.5)	0 (0.0)	
G/A or A/A	14 (10.29)	13 (19.1)	1 (1.5)	<0.001
***IL-1RA* (+2018)**				
T/T	87 (63.97)	35 (51.5)	52 (76.5)	
T/C	44 (32.35)	29 (42.6)	15 (22.1)	0.002
C/C	5 (3.68)	4 (5.9)	1 (1.5)	
T/C or C/C	49 (36.03)	33 (48.5)	16 (23.5)	0.002

TNF-α (-308), 61.8%, 33.8%, and 4.4 % of cases were found to carry G/G, G/A, and A/A genotype, respectively, while in controls, the percentage of the genotypes were 76.5%, 22.1%, and 1.5%, respectively. For *TNF-α* (-238), 80.9%, 17.6%, and 1.5% of cases carried G/G, G/A, and A/A genotype, respectively, while in controls the percentage of the genotypes were 98.5%, 1.5%, and 0, respectively. For *IL-1RA* (+2018), 51.5%, 42.6%, and 5.9% of cases had T/T, T/C, and C/C genotype, respectively, while in controls the percentage of the genotypes were 76.5%, 22.1%, and 1.5%, respectively. *P* values for the differences in the distribution of *TNF-α* (-308), *TNF-α* (-238) and *IL-1RA*(+2018) between cases and controls were 0.063, 0.001, and 0.002, respectively. Similar results were obtained in dominant model, in which heterozygous and homozygous mutants were combined. The associations of *TNF-α* (-238) and *IL-1RA*(+2018) genotypes with the disease were statistically significant, and their *P* values were <0.001 and 0.002, respectively, whereas the association of *TNF-α* (-308) genotype with the disease was borderline significant (*P*=0.052).

To further examine the associations of the SNPs with the disease, we performed unconditional logistical regression models. Due to the small sample size and small frequencies of variants in both case and control groups, we combined the heterozygous and homozygous mutants into a group in comparison to homozygous wild type (aka dominant model) (Table 3). Univariate analyses showed that the mutants of *TNF-α* (-238) and *IL-1RA* (+2018) significantly increased the risk on silicosis; the ORs for both SNPs were 15.8 (95% CI: 2.0-124.9) and 3.1 (95% CI: 1.5-6.4), respectively. However, a borderline significant increase was observed in the risk of the disease for *TNF-α* (-308); the OR was 2.0 (95% CI: 1.0-4.2). To adjust the potential confounding factors, we also performed multivariate unconditional logistic regression model with the adjustment for age, BMI, smoking, alcohol consumption, length of silica particle exposure, blood type, and education background, and found that the associations of three SNPs with the disease were statistically significant. The adjusted ORs for *TNF-α* (-308), *TNF-α* (-238) and *IL-1RA* (+2018) were 2.8 (95% CI: 1.1-7.5), 20.9 (95% CI: 1.8-236.4), and 4.0 (95% CI: 1.6-10.1), respectively.

Table 3. Associations of *TNF-α* (-308), *TNF-α* (-238), and *IL-1RA (+2018)* Genotypes with Silicosis

Variables	Univariate Analysis			Multivariate Analysis		
	OR	95% CI	*P* value	Adj-OR	95% CI	*P* value
***TNF-α*(*-308*)**						
G/G	1			1		
G/A or A/A	2	1.0～4.2	0.065	2.8	1.1～7.5	0.038
***TNF-α*(*-238*)**						
G/G	1			1		
G/A or A/A	15.8	2.0～124.9	0.009	20.9	1.8～236.4	0.014
***IL-1RA(+2018*)**						
T/T	1			1		
T/C or C/C	3.1	1.5～6.4	0.003	4	1.6～10.1	0.004

DISCUSSION

In this case-control study we genotyped three SNPs in inflammatory cytokine genes of *TNF-α* and *IL-1RA*, and demonstrated the associations of these SNPs with the risk of silicosis in Chinese workers who were exposed to silica particles. We found that *TNF-α* (-238) and *IL-1RA*(+2018) were significantly associated with the increased risk of silicosis; approximately 14- and 2-fold elevated risks were observed, respectively. A borderline significantly increased risk was found for *TNF-α* (-308) mutants. However, the associations remained or turned statistically significant after the adjustment for the potential confounding factors including age, BMI, the status of smoking and alcohol drinking, length of silica particle exposure blood type and education background. Findings suggest that the Chinese workers exposed to silica particles who carry mutants of either *TNF-α* or *IL-1RA*, particularly *TNF-α* (-238) mutants, will have approximately 14- or 2-fold increased risk of silicosis in comparison to those with the wild type. Our study further supports

the notion that the mutants of *TNF-α* and *IL-1RA* can increase the risk of silicosis in the workers exposed to silica particles as well. If our results can be validated in independent studies with a larger sample size, it will be helpful to screen genotypes in recruiting the study subjects for identifying who might be at a risk to silicosis if they are exposed to silica particles.

TNF-α and IL-1 are two inflammatory cytokines that play important roles in the pathogenesis of pulmonary inflammatory diseases[20-23]. Animal models have shown that TNF-α and IL-1 are associated with silica-induced lung damage[24-26]. Population-based studies showed that patients with pneumoconiosis among coal workers had significantly increased TNF-α and IL-1[1,4,7,10]. Previous studies have shown that the human *IL-1* gene cluster polymorphisms have been associated with several chronic inflammatory diseases[5-6,12,25]. Through competitively blocking the binding of *IL-1* to IL-1 receptor, *IL-1RA* is involved in inflammatory response. Thus, the balance between IL-1 and IL-1RA plays an important role in the regulation of inflammatory processes[27]. Increased IL-1RA has been shown to protect cytokine-induced lung damage[28]. It also has been reported to be associated with systemic lupus erythematosus, ulcerative colitis, lichen sclerosis and alopecia areata[29-30], as well as fibrosing alveolitis and silicosis in coal miners[12,31]. The variant (T/C) of *IL-1RA* (+2018) is a synonymous SNP located in exon 2 of the IL-1RA gene. It has been reported that the genotype CC has significantly higher IL-1RA expression with the stimulation of LPS or endotoxin-stripped particles[41]. However, the results from another study showed that the variant might not have direct effect on the mRNA expression, but indirectly effect through its linkage with a variable-number tandem repeat (VNTR) in intron 2 of the IL-1RA gene[42]. Tarlow and his colleagues[43] demonstrated that there were three putative protein binding sites (an IFN-α silencer, an IFN-β silencer and an acute-phase response element) in the region surrounding the VNTR of IL-1RA. The presence of the variant may interfere with the binding of transcription factors to these regulatory elements, or influence the stability of RNA. Yucesoy and his colleagues[11-12] demonstrated that *IL-1RA*(+2018) raised the risk of silicosis in coal miners. In agreement with these reports, we found that *IL-1RA*(+2018) polymorphism was associated with silicosis in Chinese workers; more cases carried the *IL-RA* variant than controls (allele frequency was 27.21% in cases *vs* 12.50% in controls).

At the positions of both -238 and -308 in the promoter region of *TNF-α* gene, a G/A substitution occurs. D'Alfonso and Richiardi[17] showed that the allele A at -238 and -308 of *TNF-α* was associated with *TNF-α* expression. The allele A at -308 of *TNF-α* was found to increase the mRNA levels of *TNF-α* by affecting the binding of a transcription factor[32]. Elevated *TNF-α* can induce the proliferation and differentiation of fibroblast and transcription of collagen[38]. Animal models showed that anti-TNF-α could significantly ameliorate the development of silica-induced pulmonary fibrosis[39-40]. In our study, we found that more cases carried the variants of *TNF-α* than controls; for *TNF-α* (-308), the variant allele frequency was 21.32% in cases while 12.50% in controls; For *TNF-α* (-238), the variant allele frequency was 19.0% in cases while 2.4% in controls. Our finding suggest that *TNF-α* polymorphisms, particularly at -238, predisposed individuals to silicosis in Chinese workers is consistent with the previous reports in Caucasian. Yucesoy and his colleagues[12] reported that *TNF-α* (-308) and *TNF-α* (-238) all increased the risk of silicosis in coal miners. Whyte and his colleagues[33] also showed that the SNPs in *TNF-α* were associated with the increased risks of fibrosing alveolitis in Italians. The South African miners, who carried *TNF-α* SNPs, were found to be at a risk to silicosis[9]. Other studies demonstrated that *TNF-α* and *IL-1RA* gene polymorphisms were associated with the occurrence and development of silicosis in Caucasian and African coal miners[7,9,11-14]. Interestingly, a borderline significant association of *TNF-α* (-308) with the disease was found in our study, however, it turned statistically significant after adjustment for the potential confounding factors. This may be caused by the difference in physiological function between cases and controls which may lead to underscoring the effect of *TNF-α* (-308), given that young people usually have much stronger immunity response than the older. Additionally, other studies have shown that *TNF-α* (-308) is clearly linked to the risk of silicosis[1,7,32]. One possibility for this discrepancy is most likely due to ethnicity difference between ours and others. We also can not rule out the potential effect of sample size.

Although TNF release was shown to be stable over time[34-36], the phenotype may change after an absence of the stimulating effect of dust exposure. The underlying mechanisms are still unknown. Recently, Suzanne et al.[37] have shown that *TNF-α*

might not directly induce but indirectly induced by silica's action on macrophage; the increase of *TNF-α* after silica exposure is likely a consequence of the release of IL-1b production induced by silica. This suggests that IL-1 and TNF-α may interplay in the development of silicosis. However, since the relatively small sample size, we could not examine the interaction between *IL-1RA* and *TNF-α* polymorphisms to the susceptibility of silicosis. Additionally, due to the lack of the detailed information on exposure dose of silica particles, we were unable to evaluate the interaction between gene and the exposure dose.

CONCLUSION

In this study, we have confirmed that *TNF-α* (-238) and *IL-1RA* (+2018) variants significantly increase the risk of silicosis in silica-exposed Chinese workers , and that the increased risk associated with *TNF-α* (-308) is borderline significant. After adjustment for the potential confounding factors, all the three tested SNPs are statistically significantly associated with the risk of silicosis, suggesting that among silica-exposed Chinese workers, those carrying the variants have higher risk of silicosis than those carrying the wild type. These findings may be useful in making strategies to prevent workers occupationally exposed to silica particle from developing silicosis. If our observations can be validated in independent studies with a larger sample size, screening genetic backgrounds, although it is controversial in ethics concerns, may be useful in protecting human health in the future.

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