Association of TNF-α-238G/A and 308 G/A Gene Polymorphisms with Pulmonary Tuberculosis among Patients with Coal Worker's Pneumoconiosis

HONG-MIN FAN[*,#], ZHUO WANG[#], FU-MIN FENG[#], KONG-LAI ZHANG[*,1], JU-XIANG YUAN[1,#], HONG SUI[#], HONG-YAN QIU[#], LI-HUA LIU[#], XIAO-JUAN DENG[#], AND JING-XUE REN[#]

[*]*School of Basic Medicine, Peking Union Medical College, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005, China;* [#]*Department of Preventive Medicine, North China Coal Medical College, Tangshan 063000, Hebei, China*

Objectives Tumor necrosis factor-α (TNF-α) may play an important role in host's immune response to *mycobacterium tuberculosis* (*M. tuberculosis*) infection. This study was to investigate the association of TNF-α gene polymorphism with pulmonary tuberculosis (TB) among patients with coal worker's pneumoconiosis (CWP). **Methods** A case-control study was conducted in 113 patients with confirmed CWP complicated with pulmonary TB and 113 non-TB controls with CWP. They were matched in gender, age, job, and stage of pneumoconiosis. All participants were interviewed with questionnaires and their blood specimens were collected for genetic determination with informed consent. The TNF-α gene polymorphism was determined with polymerase chain reaction of restriction fragment length polymorphism (PCR-RFLP). Frequency of genotypes was assessed for Hardy-Weinberg equilibrium by chi-square test or Fisher's exact probability. Factors influencing the association of individual susceptibility with pulmonary TB were evaluated with logistic regression analysis. Gene-environment interaction was evaluated by a multiplicative model with combined OR. All data were analyzed using SAS version 8.2 software. **Results** No significant difference in frequency of the TNF-α-308 genotype was found between CWP complicated with pulmonary TB and non-TB controls (χ^2=5.44, P=0.07). But difference in frequency of the TNF-α-308 A allele was identified between them (χ^2=5.14, P=0.02). No significant difference in frequencies of the TNF-α-238 genotype and allele (P=0.23 and P=0.09, respectively) was found between cases and controls either, with combined (GG and AA) OR of 3.96 (95% confidence interval of 1.30~12.09) at the -308 locus of the TNF-α gene, as compared to combination of the TNF-α-238 GG and TNF-α-308 GG genotypes. Multivariate-adjusted odds ratio of the TNF-α-238 GG and TNF-α-308 GA genotypes was 1.98 (95% CI of 1.06~3.71) for risk for pulmonary TB in patients with CWP. There was a synergic interaction between the TNF-α-308 GG genotype and body mass index (OR=4.92), as well as an interaction between the TNF-α-308 GG genotype and history of BCG immunization or history of TB exposure. And, the interaction of the TNF-α-238 GG genotype and history of BCG immunization or TB exposure with risk for pulmonary TB in them was also indicated. **Conclusions** TNF-α-308 A allele is associated with an elevated risk for pulmonary TB, whereas TNF-α-238 A allele was otherwise.

Key words: Coal worker's pneumoconiosis (CWP); Pulmonary tuberculosis (TB); Susceptibility; Polymorphism; Tumor necrosis factor (TNF), α-308, α-238; Polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP); Interaction

INTRODUCTION

Tuberculosis (TB) caused by infection of *mycobacterium tuberculosis* (*M. tuberculosis*) remains a common disease and leading cause of death, which kills about three million people annually and has infected one-third of the population worldwide[1]. Global reemergence of TB is due to pandemic of acquired immune deficiency syndrome (AIDS) and development of multidrug resistant strains of *M. tuberculosis*[2]. Pneumoconiosis is an occupational fibrotic lung disease resulting from inhalation of microscopic crystalline silica particles and a variety of dust of other categories[3]. Although pneumoconiosis and TB are two independent diseases, patients with pneumoconiosis are at risk for contracting pulmonary TB and patients with pulmonary TB will be prone to pneumoconiosis if they are exposed to dust. There are many known factors inducing pulmonary TB, such as poverty,

[1]Correspondence should be addressed to: Prof. Kong-Lai ZHANG, School of Basic Medicine, Peking Union Medical College, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005, China, Tel: 86-10-65296973, Fax: 86-10-65225752, E-mail: konglai_zhang@yahoo.com.cn; or Dr. Ju-Xiang YUAN, Department of Preventive Medicine, North China Coal Medical University, Tangshan 063000, Hebei, China, Tel: 86-315-3725719, Fax: 86-315-3725312, E-mail: nunuzh@126.com

Biographical note of the first author: Hong-min FAN, born in 1970, associate professor, graduated from Peking Union medical University in 2007, has been engaging in the research on occupational epidemiology and infectious epidemiology since 1997. The field on pneumoconiosis is her major research field.

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gender, alcohol drinking, malnutrition, diabetes, infection with human immunodeficiency virus (HIV), and so on[4-7]. Stage of patients with pneumoconiosis is one of the main factors that influence the development of pulmonary TB in them[8-9].

Host genetic factors play an important role in changing function of the single nucleotide polymorphisms (SNPs) of the gene regulating cytokine excreted by pulmonary alveolar macrophage (AM)[10], which encodes tumor necrosis factor (TNF) and resides in the central part (class III region) of major histocompatibility complex (MHC) surrounded by a large number of other immunological genes. TNF is an important cytokine for host defense against a variety of pathogenic microbes and its over-expression may lead to worsening of illness during its recovery, which is mainly regulated by the promoter region of this gene. In terms of capacity for cytokine production, an individual has a major genetic component to regulate it and there exists striking difference among different individuals. Many allelic polymorphisms of the TNF-α gene have been described in reports from several studies, including eleven in the promoter region at locuses 1031 (T/C), -863 (C/A), -857 (C/T), -308 (G/A), -238 (G/A), -1196 (C/T), -1125 (G/C), -572 (A/C), -316 (G/A), -163 (G/A), and -70 (G/A). Moreover, many studies have shown that the TNF-α promoter polymorphism plays a significant role in its transcriptional activity[10-11].

Now, a few studies have reported that susceptibility to *M tuberculosis* infection might be associated with gene polymorphism of human leukocyte antigen (HLA), TNF, interleukin-1 (IL-1), and NRAMP 1[9]. However, results of these studies varied in different populations, which might be attributed to ethnic difference of the study populations and complex mechanism of *M. tuberculosis* infection. In order to further explore more host factors influencing occurrence of *M. tuberculosis* infection, the polymorphisms of the TNF-α gene at the promoter region were studied, which had been ascribed to the polymorphism within the regulatory regions of signal sequences of the cytokine genes. TNF-α is one of the earliest cytokines implicated in pathogenesis of lung fibrosis. Furthermore, the TNF-α gene polymorphism has been found to be significantly associated with an increased risk for pulmonary fibrosis. Given that genetic variation may potentially alter inflammation and fibrosis in lung, the objective of this case-control study is to explore the relationship between TNF-α gene polymorphisms and *M. tuberculosis* infection in patients with coal worker's pneumoconiosis (CWP) and to clarify whether the polymorphism in the TNF-α promoter region is associated with *M. tuberculosis* infection. Therefore, two locuses of the TNF-α gene, TNF-α-238 and TNF-α-308, are selected to analyze frequency of their genotypes and alleles and explore the association of the TNF-α gene polymorphism with *M. tuberculosis* infection, as well as interaction between TNF-α gene polymorphism and environmental factors for pulmonary TB in patients with CWP.

MATERIALS AND METHODS

Selection of Cases and Controls

This study was reviewed and approved by the Review Board of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and of the North China Coal Medical College, respectively.

Patients with CWP were selected from Tangshan Coal Mine affiliated to Kailuan Corporation, Ltd. in Tangshan, Hebei province, China and their diagnoses were established by an expert group consisting of qualified physicians according to the national diagnostic criteria for pneumoconiosis during September 1, 2006 and January 31, 2007.

A total of 113 cases of CWP complicated with confirmed pulmonary TB diagnosed by positive culture of *M. tuberculosis* from sputum (majority of cases) or bronchial aspirate or washing (minor cases), with or without positive acid-fast sputum smear. All positive cultures were also confirmed by a nucleic acid-based assay. Bacteriological examinations were completed at microbiology laboratory of Kailuan Coal Mine Hospital at Tangshan, Hebei. Another 113 unrelated CWP patients without pulmonary TB who were matched in gender, age, job, and stage of pneumoconiosis were selected from the same mine as controls. Difference in age and length of employment exposed to dust between cases and controls were less than five years and two and a half years, respectively.

Each participant was face-to-face interviewed with a questionnaire to obtain individual information, and venous blood specimen (5 ml) was collected from each of them and stored in anti-clotted tube by 2% EDTA for genetic analysis with a written informed consent.

Genetic Analysis

DNA was extracted from blood corpuscle by a modified salting-out method and was purified by hydroxybenzene-chloroform extraction. Specific primers were designed according to the sequence and mutant heat fragment of the TNF-α 239 and TNF-α-308 genes. The inner primer of the TNF-α -238 contains forward primer 5′-AGAAGACCCCCCTCGGAACC-3′ and reverse

primer 5′-ATCTGGAGGAAGCGG TAGTG-3′, and its outer premier contains forward primer 5′-AAAGAAATGGAG GCAATAGG-3′ and reverse primer 5′-CATCTGGAGGAAGCGGTA-3′. The inner primer of the TNF-α-308 gene contains forward primer 5′-AGGCAATAGGTTTTGAGGGCCAT-3′ and reverse primer 5′-ACACTCCCCATCCTCCCT GCT-3′, and its outer premier contains forward primer 5′-AAAGAAATGGAGGCAAT AGG-3′ and reverse primer 5′-CATCTGGAG GAAGCGGTA -3′ (by Huamei Biotec Co, Beijing, China).

Standard 20-μl polymerase chain reactions (PCRs) contained approximately 50 ng DNA, 2.0 μl of 10×buffer and 25 mmol/L $MgCl_2$, 1.0 μl of 4 mmol/L DNTPs and 10 μmol/L in each primer and 0.6 U of Taq polymerase (by Huamei Biotec Co, Beijing, China) and 1.5 μl of 5% formaldehyde. Amplification was carried out in thermal cycler T-1 (by Biometro in USA). For outer primers, the amplification conditions used were as follows: Denaturation initiated at 94 ℃ for 5 min and was followed by 5 cycles of denaturation at 94 ℃ for 5 min, annealing at 49 ℃ for 1 min, extension at 72 ℃ for 1 min, and 25 cycles of denaturation at 94 ℃ for 30 s, annealing at 49 ℃ for 30 s, extension at 72 ℃ for 40 s with final extension at 72 ℃ for 1 min. For inner primers, amplification conditions used were as follows: Denaturation initiated at 94 ℃ for 5 min and was followed by 5 cycles of denaturation at 94 ℃ for 5 min, annealing at 60 ℃ for 1 min, extension at 72 ℃ for 1 min, and 25 cycles of denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 30 s, extension at 72 ℃ for 40 s with final extension at 72 ℃ for 1 min. PCR products were stored at 20 ℃ for RFLP analysis. TNF-α-238 PCR products (20 ng each) were digested using the restriction endonuclease Msp I (Beijing SBS Genetech Co., Ltd.) for 24 h at 37 ℃ with reaction buffer supplied by the manufacturer and analyzed on 3.0% agarose gel because of small size of DNA products. Presence of MspI site was indicated by cleavage of the amplified product into three fragments of 152 bp, or 133 bp, or 19 bp. PCR products of the TNF-α-308 (20 ng each) were digested using restriction endonuclease Nco I (by SBS Genetech Co., Ltd., Beijing) for 24 h at 37 ℃ with reaction buffer supplied by the manufacturer and analyzed on 3.0% agarose gel because of small size of DNA products. Presence of Nco I site was indicated by cleavage of the amplified product into three fragments of 117 bp, or 97 bp, or 20 bp.

Statistical Analysis

Frequency of genotypes was assessed for Hardy-Weinberg equilibrium by chi-square test or Fisher's exact probability if frequency in the cells of 2 by 2 tables was too small. The same test method was used to evaluate the correlation between illness (CWP-TB versus CWP only) and the TNF -α-238 or TNF-α-308 genotypes or alleles. Odds ratio (OR) with 95% confidence interval (95% CI) was calculated to show strength of correlation. Factors influencing the correlation were evaluated with logistic regression analysis. Gene-environment interaction was evaluated by a multiplicative model with combined OR as in the following formula: $OR_{int}=OR_{eg}/(OR_g.OR_e)$, where OR_{int} indicates OR for interaction, OR_{eg} indicates OR for both gene and environmental factors combined, OR_g. indicates OR for gene, and OR_e indicates OR for environmental factors, with OR_{int} being greater than unity as synergic interaction, OR_{int} being equal to unity as no interaction, and OR_{int} being less than unity as antagonistic interaction. All data were analyzed using SAS version 8.2 software (by SAS Institute Inc, Cary, NC, USA).

RESULTS

Demographic Characteristics of the Participants

All 113 patients with CWP-TB and 113 controls (only CWP without TB) were males of Han ethnic, with mean age of 71.1 (ranging 54.8~83.8) years for those with CWP-TB and 70.4 (ranging 52.9-86.7) years for those with CWP only, and mean length of employment exposed to dust of 27.8 (ranging 10-46) years and 27.5 (ranging 9-44) years, respectively. There was no significant difference in age and job between cases and controls ($P>0.05$) and 42.5% of them in tunneling, 41.6% in coal mining and 15.9% as assistant, respectively. No difference in stage of CWP between cases and controls was found, 89 (78.8%) at stage I, 22 (19.5%) at stage II and 2 (1.8%) at stage III. There was no significant difference in length of employment exposed to dust and age initiating exposure to dust between cases and controls with 21.8 plus/minus 4.5 (S.D.) years and 21.8 plus/minus 4.4 (S.D.) years, respectively, ($t=0.96$, $P>0.05$). No significant difference was found either in length of exposure to dust between cases and controls ($\chi^2=0.50$, $P=0.92$) (Table 1).

Association of TNF-α gene Polymorphisms with Susceptibility to TB

Frequencies of the allele and genotypes of the TNF-α gene tested were summarized in Table 2, and their allele frequencies reached Hardy-Weinberg equilibrium.

TABLE 1

Ages Initiating Exposure to Dust and Length of Exposure in Patients with CWP-TB and CWP only

Patient Groups	No. of Patients	Age (yrs.) Initiating Exposure to Dust		Length of Exposure to Dust (yrs)		
		Mean	SD	Before 1950	1950	1958-1975
CWP-TB						
	113	21.8	4.5	14	66	33
Only CWP						
	113	21.8	4.4	13	71	29
		t=0.22, P>0.05		χ^2=0.50, P =0.92		

TABLE 2

TNF-α Gene Polymorphism in Promoter Region among Cases and Controls

Locus	Cases (%) (n=113)	Controls (%) (n=113)	OR (95% CI)	χ^2	*P-value*
-238					
Genotype					
GG	99 (87.6)	105 (92.9)	1.00		
GA	12 (10.6)	8 (7.1)	1.59 (0.62~4.06)	0.96	0.33
AA	2 (1.8)	0	5.30 (0.25~117.80)		0.24*
GA/AA	14 (12.4)	8 (7.1)	1.86 (0.75~4.62)	1.81	0.19
Allele					
G	210 (92.9)	218 (96.5)		5.14	0.02
A	16 (7.1)	8 (3.5)			
-308					
Genotype					
GG	60 (53.1)	77 (68.2)	1.00		
GA	46 (40.7)	32 (28.3)	1.84 (1.05~3.24)	4.58	0.03
AA	7 (6.2)	4 (3.5)	2.25 (6.23~8.03)		0.22*
GA/AA	53 (46.9)	36 (31.8)	1.89 (1.10~3.25)	5.33	0.02
Allele					
G	16673.5			2.82	0.09
A	60	26.6			

Note. *Fisher's exact probability.

Frequencies of the TNF-α genotype varied in patients with CWP-TB and those with CWP only. No significant difference was found in frequency of the TNF-α genotype at -308 (GA) and -238 (GA) locuses between patients with CWP-TB and those with CWP only. Frequency of the allele A of TNF-α-308 locus was significantly higher in patients with CWP-TB than that in those with CWP only (χ^2=5.14, P=0.02). However, frequency of the allele A of the TNF-α-238 locus did not show any significant difference between cases and controls (χ^2=2.82, P=0.09), as shown in Table 2.

Results of univariate analysis showed that, as compared to patients with the GG genotype, patients of CWP-TB with the GA and AA genotypes at the -238 locus were associated with their risk for pulmonary TB, with ORs (95% CI) of 1.59 (0.62~4.06) and 5.30 (0.25~117.8), respectively because of small sample size. As compared to those with the GG genotype, patients of CWP-TB with the GA and AA genotypes at the -308 locus demonstrated significantly higher risk for pulmonary TB, with ORs (95% CI) of 1.84 (1.05~3.24) and 2.25 (6.23~8.03), respectively, as shown in Table 2.

Multivariate Logistic Regression Analysis of the Relationship between the TNF-α Gene Polymorphism in the Promoter Region and Risk for TB in Patients with CWP

Based on univariate analysis, multivariate

logistic regression analysis was used to estimate the association of the TNF-α gene polymorphism in the promoter region with the risk for pulmonary TB in patients with CWP, adjusted for age, body mass index (BMI), and history of bacillus Calmette-Guerin (BCG) immunization. The TNF-α-308 GA or AA genotypes was an independent risk factor for pulmonary TB in patients with CWP (OR=3.96, 95% CI 1.30-12.09), but no significant correlation between the genotypes of TNF-α-238 GA/AA and risk for pulmonary TB was found (OR=4.29, 95% CI 0.34-54.17), as shown in Table 3.

Association of the TNF-α-238(G/A) and TNF-α-308(G/A) Genotypes with Risk for Pulmonary TB in Patients with CWP

Compared to those with a combination of the TNF-α-238 GG and TNF-α-308 GG genotypes, patients of CWP with a combination of the TNF-α-238 GG and TNF-α-308 GA genotypes were more susceptible to pulmonary TB, with OR (95% CI) of 1.98 (1.06~3.71) adjusted for age, body mass index (BMI), history of BCG immunization, as shown in Table 4.

Interaction of the TNF-α Gene Polymorphism and Environmental Factors with Susceptibility to Pulmonary TB

As compared to those without history of exposure to TB and the TNF-α-238 GG genotype, interaction of the TNF-α-238 GA or AA genotype and history of exposure to TB with susceptibility to pulmonary TB in patients with CWP could not be estimated because of too small size of study samples. As compared to those without history of exposure to TB and the TNF-α-308 GG genotype, patients of CWP with both history of exposure to TB and the TNF-α-308 GA or AA genotype were more susceptible to pulmonary TB, with an OR of 3.89 (95% CI 1.43-10.55) adjusted for age, BMI, history of BCG immunization, and an OR for interaction of 3.38 (P=0.012) for both history of exposure to TB and the gene variation combined with a multiplicative model, as shown in Table 5.

TABLE 3

Results of Logistic Regression Analysis of Risk for Pulmonary TB in Patients of CWP with TNF-α gene Polymorphism

Locus / Genotype	$\hat{\beta}$	$SE_{(\hat{\beta})}$	Wald χ^2	P-value	OR[a] (95% CI)
-238					
GA	1.4574	1.2933	1.27	0.26	4.29 (0.34~54.17)
AA	-	-	-	-	-
GA or AA	1.4574	1.2933	1.27	0.26	4.29 (0.34~54.17)
-308					
GA	1.2793	0.5899	4.70	0.03	3.59 (1.13~11.42)
AA	1.6231	0.8492	3.66	0.06	5.07 (0.96~26.76)
GA or AA	1.3760	0.5694	5.84	0.02	3.96 (1.30~12.09)

TABLE 4

Association of Combination of TNF-α-238 (G/A) and TNF-α-308 (G/A) Genotypes with Risk for Pulmonary TB

-238 Locus	-308 Locus	No. of Patients with CWP-TB	No. of Patients with CWP only	OR (95% CI)	OR[a] (95% CI)
GG	GG	50	72	1.00	1.00
GG	GA	43	29	2.13 (1.18~3.86)	1.98 (1.06~3.71)
GG	AA	6	4	2.16 (0.58~8.05)	2.51 (0.62~10.14)
GA	GG	8	5	2.30 (0.71~7.45)	1.74 (0.52~5.19)
GA	GA	3	3	1.44 (0.28~7.43)	1.06 (0.20~5.77)
GA	AA	1	0	—	—
AA	GG	2	0	—	—
AA	GA	0	0	—	—
AA	AA	0	0	—	—

Note. [a] adjusted for age, history of BCG immunization, history of exposure to TB and length of employment exposed to dust.

TABLE 5

Interaction between History of Exposure to TB and TNF-α-238 or TNF-α-308 Gene Polymorphism

Locus	Genotype	History of Exposure to TB	No. of Patients with CWP-TB	No. of Patients with CWP only	OR[a] (95% CI)	*P*-value[c]
-238						
	GG	no	76	92	1.00	
	GG	yes	23	13	2.07 (0.93-4.42)	
	GA or AA	no	9	8	1.13 (0.41-3.14)	
	GA or AA	yes	5	0	—	
						0.010
-308						
	GG	no	48	60	1.00	
	GG	yes	12	17	0.84 (0.36-2.41)	
	GA or AA	no	34	30	1.37 (0.74-2.73)	
	Ga or AA	yes	19	6	3.89 (1.43-10.55)	
					OR_{int}=3.38[b]	0.012

Note. [a]adjusted for age, BMI, history of BCG immunization and length of employment exposed to dust; [b]OR for interaction; [c]likelihood test *P*-value.

As compared to those with a combination of BMI and the TNF-α-238 GG genotype, no interaction of the TNF-α-238 GA or AA genotype and BMI with the risk for pulmonary TB in patients with CWP was found. As compared to those with a combination of BMI and the TNF-α-308 GG genotype, interaction of BMI and the TNF-α-308 GA or AA genotype with risk for pulmonary TB in patients with CWP was found, with an OR of 5.46 (95% CI 1.48~20.81) adjusted for age and history of exposure to TB and an OR for interaction of 4.92 (P=0.002) for both gene variation and BMI combined with a multiplicative model, as shown in Table 6.

As compared to those without history of BCG immunization and the TNF-α-238 GG genotype, patients of CWP with both the TNF-α-238 GA or AA genotype and history of BCG immunization were more susceptible to pulmonary TB, with an OR of 3.89 (95% CI 1.23~12.15). As compared to those without history of BCG immunization and the TNF-α-308 GG genotype, patients of CWP with both history of BCG immunization and the TNF-α-308 GA or AA genotype were more susceptible to pulmonary TB, with an OR of 2.53 (95% CI 0.66~9.87) adjusted for age, BMI, history exposed to TB, and an OR for interaction of 2.09 (P=0.0063) for both history of BCG immunization and gene variation combined with a multiplicative model, as shown in Table 7.

TABLE 6

Interaction between BMI and TNF-α-238 and TNF-α-308 Gene Polymorphism

Locus	Genotype	BMI	No. of Patients with CWP-TB	No. of patients with CWP only	OR[a] (95% CI)	*P*-value[c]
-238						
	GG	⩾20	88	98	1.00	
	GG	<20	11	7	1.71 (0.63~4.68)	
	GA or AA	⩾20	13	8	1.79 (0.68~4.52)	
	GA or AA	<20	1	0	—	
						0.336
-308						
	GG	⩾20	51	63	1.00	
	GG	<20	9	14	0.76 (0.31~1.95)	
	GA or AA	⩾20	39	33	1.46 (0.79~2.64)	
	GA or AA	<20	14	3	5.46 (1.48~20.81)	
					OR_{int}=4.92[b]	0.002

Note. [a]adjusted for age, history of BCG immunization, history exposed to TB and length of employment exposed to dust; [b]OR for interaction; [c]likelihood test *P*-value.

TABLE 7

Interaction between History of BCG Immunization and TNF-α-238 and TNF-α-308 Gene Polymorphism

Locus	Genotype	History of BCG ImmuniZation	No. of Patients with CWP-TB	No. of Patients with CWP only	OR[a] (95% CI)	*P*-value[c]
-238						
	GG	yes	10	24	1.00	
	GG	no	89	81	2.63 (1.16~5.84)	
	Ga or AA	yes	1	0	—	
	GA or AA	no	13	8	3.89 (1.23~12.15)	
						0.031
-308						
	GG	yes	4	6	1.00	
	GG	no	56	71	1.07 (0.29~4.01)	
	GA or AA	yes	7	9	1.13 (0.23~5.80)	
	GA or AA	no	46	27	2.53(0.66~9.87)	
					OR_{int}=2.09[b]	0.0063

Note. [a] adjusted for age, BMI, history exposed to TB and length of employment exposed to dust; [b]OR for interaction; [c]likelihood test *P*-value.

DISCUSSIONS

Pneumoconiosis continues to be a common occupational disease, although it is preventable by dust control measures. Identification of new risk factors for pneumoconiosis other than known environmental factors would improve identification of patients at risk and could help determine effective prophylactic intervention. Cytokine may represent one such kind of risk factors. Despite enormous controversies, polymorphism of some cytokine genes seems to correlate with the cytokine genotype in vitro[12].

Studies with animal models have suggested that TNF-α plays a significant role in silica-induced lung damage[13-15]. Major role of TNF-α in pulmonary fibrosis is supported by evidence obtained from TNF-α deficient mouse, which are resistant to silica-induced fibrosis[16-17]. In humans, local release of TNF-α has been shown to coincide with pathogenesis of the disease[18]. TNF-α is an essential cytokine for granuloma formation, and mice deficient in TNF-α fail to form organized granuloma, resulting in widespread dissemination of *M. tuberculosis* infection and rapid death of infected animals[19]. Additionally, data of a recent study have revealed that TB could be reactivated by blocking effect of TNF-α[20]. In the promoter region of the TNF-α gene, functional polymorphism at the locus -308 has been described[21]. In vitro, the allele leads to increased expression of the TNF-α gene[22-23]. However, conflicting results have been reported regarding influence of genetic variation on expression of the TNF-α gene[21]. Association of the TNF-α-308 polymorphism with silicosis has been revealed in some literatures[9, 24]. However, no association or linkage of pulmonary TB with the TNF-α gene polymorphisms has been demonstrated in most studies[25-27]. For example, two studies in India and Cambodia showed that polymorphism of the TNF-α and TNF-β genes was not significantly associated with pulmonary TB[28-29]. But, the Indian study revealed association of linkage of the TNF-α, TNF-β and other genes with the development of pulmonary TB. Ji Chunmei, *et al*. reported that persons carrying the TNF-β AA genotype were more susceptible to pulmonary TB, but no obvious association of the TNF-α-238 genotype with TB was found[30]. Data from our study also supported this conclusion and showed no significant association of the TNF-α-238 genotype with pulmonary TB. Furthermore, the TNF-α-238 GA or AA genotypes, as compared to the TNF-α-238 CC genotype, was not associated with pulmonary TB in patients with CWP, adjusted for age, BMI, history of exposure to pulmonary TB and length of employment exposed to dust. Patients of CWP with the TNF-α-308 GA or AA genotype were associated with their risk for pulmonary TB, with OR (95% CI) of 3.96 (95% CI 1.30~12.09), as compared to those with the GG genotype, which is not consistent with the results of previous studies and should be studied further. Compared to those with a combination of the TNF-α-238 GG and TNF-α-308 GG genotypes, patients of CWP with a combination of the TNF-α-238 GG and TNF-α-308 GA genotypes were more susceptible to pulmonary TB, with OR (95% CI) of 1.98 (95% CI 1.06~3.71, *P*=0.0118).

Case-control design was used in this study to

analyze gene-environment interaction with risk for pulmonary TB in patients with CWP, which revealed a synergic interaction of the TNF-α-308 GG genotype and body mass index (OR=4.92), as well as an interaction of the TNF-α-308 GG genotype and history of BCG immunization or history exposed to TB. And, interaction of the TNF-α-238 GG genotype and history of BCG immunization or history exposed to TB with risk for pulmonary TB in them was also indicated.

Mechanism of interaction between susceptible gene for pulmonary TB and environmental factors has been studied only for a short time, so their molecular and biologic nature could not be fully elucidated in this study with limited sample size and insufficient power.

CONCLUSIONS

TNF-α-308 A allele is associated with an elevated risk for pulmonary TB, while TNF-α-238 A allele is not so. Interaction was found between the TNF-α-238 GG or TNF-α-308 GG genotype and history of BCG immunization, or history exposed to tuberculosis, or BMI on risk for pulmonary TB.

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