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Association of CD14 G(-1145)A and C(-159)T polymorphisms with reduced risk for tuberculosis in a Chinese Han population

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ABSTRACT. Although the role of CD14 in mediating signals from Toll-like receptors to recognize *Mycobacterium tuberculosis* is known, how polymorphisms in this gene affect the susceptibility to develop tuberculosis are still not clear. We examined whether single nucleotide polymorphisms at positions -1145 and -159 in the promoter region of the CD14 gene are associated with tuberculosis in a Chinese Han population in a case-control study of 432 Chinese patients with tuberculosis and 404 ethnically matched healthy controls. Genotyping was performed to identify polymorphisms of the CD14 gene by PCR-DNA sequencing. Both the frequency of allele T in the C(-159)T polymorphism (odds ratio (OR) = 1.4; 95% confidence interval (95%CI) = 1.148-1.708) and allele G in the G(-1145)A polymorphism (OR = 1.512; 95%CI = 1.236-1.849) were significantly more frequent in cases than in controls. The

frequencies of genotypes CC and CT in the C(-159)T polymorphism, as well as the frequencies of genotypes AA and AG, were lower in cases than in controls. Based on our results, we conclude that G(-1145)A and C(-159)T polymorphisms of CD14 are associated with decreased risk for the development of tuberculosis in the Chinese Han population.

Key words: CD14; SNPs; Tuberculosis; Chinese Han

INTRODUCTION

Tuberculosis (TB) is a serious disease and is the leading cause of deaths among all infectious diseases around the world, especially in Asia and Africa. Although one-third of the world's population is thought to be infected with *Mycobacterium tuberculosis*, only 5-15% of people develop active TB disease during their lifetime (Rosman and Oner-Eyupoglu, 1998). Some evidence suggests that certain genetic factors may be involved in innate immunity and play important roles in susceptibility to TB (Sugawara et al., 2003a,b). Single nucleotide polymorphisms (SNPs) in genes encoding Toll-like receptors (TLRs) (Yim et al., 2006; Ferwerda et al., 2007; Davila et al., 2008), Toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) (Nejentsev et al., 2008), interferon gamma (Ding et al., 2008), and vitamin D3 receptor (Ding et al., 2007) have all been considered to account for the susceptibility to TB.

CD14 is considered a key pattern recognition receptor protein in the innate immune system (Triantafilou and Triantafilou, 2002). When anchored by ligands to bacterial antigens, such as the lipopolysaccharide binding protein-lipopolysaccharide complex, CD14 interacts with TLRs to induce nuclear factor κ-B signaling and transcription of pro- and anti-inflammatory cytokines (Härtel et al., 2008). CD14 is constitutively expressed primarily on the surface of monocytes, macrophages, and neutrophils as membrane CD14 (mCD14) (Ulevitch and Tobias, 1995). A soluble form of CD14 (sCD14) is abundant in serum and is derived from both secretion of CD14 and enzymatically cleaved glycosyl-phosphatidylinositol-anchored mCD14 (Zhang et al., 2008). Increased levels of CD14 have been reported in TB patients (Lawn et al., 2000; Rosas-Taraco et al., 2007).

Two SNPs (G-1145A and T-159C) have been identified within the CD14 promoter in a Chinese Han population. Both these SNPs significantly reduced transcriptional activity of the CD14 promoter and were associated with low CD14 expression. Thus, it was concluded that the CD14/-1145 and -159 polymorphisms result in variants that may function in a synergistic fashion (Gu et al., 2008). Although, CD14 plays a central role in innate immunity through recognition of *Mycobacterium tuberculosis*, little is known about its polymorphisms and their possible relationship to the development of TB. The present study, therefore, aimed at investigating G(-1145)A and C(-159)T polymorphisms in CD14, and their association with TB in the Chinese Han population.

MATERIAL AND METHODS

Patients and controls

Four hundred and thirty-two unrelated patients diagnosed with TB (confirmed by clinical, radiological, and bacteriological investigations) were enrolled in the TB group. All TB pa-

tients were undergoing standard TB treatment at the TB clinic of the Sixth Hospital of Shaoxing and Hangzhou Red Cross Hospital between October 2005 and October 2009. Patients were excluded if they tested positive for human immunodeficiency virus, or were taking immunosuppressive agents. The control group comprised 404 healthy, unrelated blood donors with no history of TB or other immune diseases. All control subjects were from the same ethnic (Han) population and geographical origin, and were living in the same region as the patients with TB (Southeast China). This study was approved by the Ethics Committees of the Faculty of Medicine (Zhejiang University, China), and informed consent was obtained from all subjects before blood sampling.

Genotype identification

CD14 G(-1145)A and C(-159)T polymorphisms were determined by polymerase chain reaction (PCR), followed by direct sequencing. Genomic DNA was extracted from blood samples with the salting-out method (Rousseau et al., 1994).

Detection of the CD14 G(-1145)A polymorphism

The PCR included forward 5'-GGCAACAGAGCCAAACTCAG-3' and reverse 5'-GCCCTCCCCTCAGTACAATC-3' primers. PCR was performed by denaturing at 94°C for 3 min, followed by 34 cycles at 94°C for 50 s, 58°C for 30 s, and 72°C for 50 s, and a final extension at 72°C for 5 min. The amplified products were purified and then identified by scanning with the ABI 3100 sequencer (Applied Biosystems, Carlsbad, CA, USA).

Detection of the CD14 C(-159)A polymorphism

The PCR included forward 5'-GTGCCAACAGATGAGGTTCAC-3' and reverse 5'-GCCTCTGACAGTTTATGTAATC-3' primers. PCR was performed using denaturing at 94°C for 3 min, followed by 34 cycles at 94°C for 50 s, 56°C for 30 s, and 72°C for 50 s, and a final extension of 72°C for 5 min. The amplified products were purified and then identified by scanning with the ABI 3100 sequencer (Applied Biosystems).

Statistical analysis

Analysis of polymorphic loci was performed using the Mutation Explorer/Mutation Surveyor, Version 2.2 (Manaster et al., 2005). The comparisons of age and gender between the disease and control groups were done by the chi-square test and the *t*-test using SPSS 16.0. The genotype frequencies in the control group were tested for Hardy-Weinberg equilibrium. The differences between genotype and allele frequencies between the case and control groups were determined using the chi-square test. A P value of <0.05 was considered to be statistically significant. The online SNP analysis software (http://bioinfo.iconcologia.net/SNPstats) was used to analyze the association of CD14 polymorphisms with TB (Liu et al., 2009).

RESULTS

The characteristics of TB patients and healthy control subjects are shown in Table 1.

The average age was 38.5 ± 16.7 years (range = 18-70 years) in TB patients and 36.7 ± 11.2 years (range = 20-62 years) in the control group. Females constituted 42.1% of the TB group, and 39.4% of the control group. Mean age, gender, or body mass index did not significantly differ between TB patients and controls (P > 0.05).

Table 1. Characteristics of healthy controls and tuberculosis (TB) patients.

	TB group (N = 432)	Control group (N = 404)	P
Age [years, range (mean ± SD)]	18-70 (38.5 ± 16.7)	20-62 (36.7 ± 11.2)	0.470
Gender: female (%)	182 (42.1)	159 (39.4)	0.415
Body mass index (mean ± SD)	21.1 ± 3.2	23.1 ± 4.1	0.093
Pulmonary TB	408	ND	/
Extrapulmonary TB	24	ND	/
Tuberculin skin test (>10 mm) (%)	375 (86.8)	ND	/
Presence of TB history of relatives (%)	43 (10.0)	30 (7.4)	0.196
BCG vaccination (%)	210 (48.2)	214 (53.0)	0.208

ND = not determined. Extrapulmonary TB includes lymphadenitis, pleural, bone and renal tuberculosis.

Sequence analysis of CD14 G(-1145)A and C(-159)T polymorphisms were undertaken in 432 TB patients and 404 controls. The genotypes for SNPs were in Hardy-Weinberg equilibrium in the control group (P > 0.05). The CD14 promoter region was amplified and sequenced by PCR in disease and control groups. PCR fragments and DNA sequencing of CD14 G(-1145)A and C(-159)T are shown in Figures 1 and 2, respectively. CD14 G(-1145)A and C(-159)T polymorphisms were significantly associated with TB.

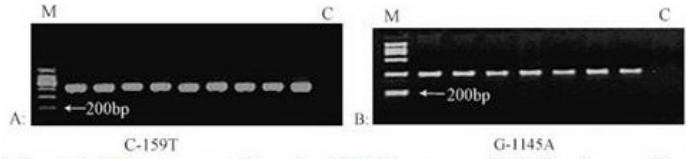

Figure 1. Agarose gel (1%) showing PCR fragments of CD14 polymorphisms. *Lane M* = 100-bp marker; *lane C* = control. **A.** PCR fragments of CD14-159. **B.** PCR fragments of CD14-1145.

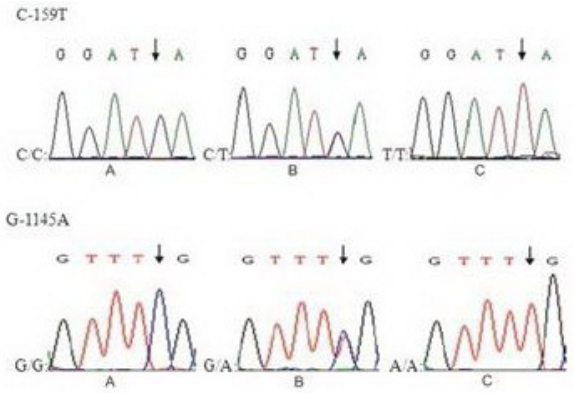

Figure 2. DNA sequences of the CD14 promoter of G(-1145)A and C(-159)T. C-159T: **A.** genotype C/C; **B.** genotype C/T; **C.** genotype T/T. G-1145A: **A.** genotype G/G; **B.** genotype G/A; **C.** genotype A/A.

The frequency of the T allele of the C(-159)T polymorphism was higher in cases than in controls (63.53 *vs* 55.44%, respectively), and was significantly associated with TB (OR = 1.4; 95%CI = 1.148-1.708; P = 0.001; Table 2). The frequencies of genotypes CC and CT of the C(-159)T polymorphism were lower in cases than controls (Table 3). Similarly, the frequency of the G allele of the G(-1145)A polymorphism was higher in cases than controls (64.00 *vs* 53.13%, respectively), and was also significantly associated with TB (OR = 1.512; 95%CI = 1.236-1.849; P < 0.001) (Table 2). The frequencies of genotypes AA and AG of the G(-1145)A polymorphism were lower in cases than controls (Table 3).

Table 2. Allele frequencies for the CD14 SNPs in cases and controls.

		Allele frequency		Statistics	
		Patients [N (%)]	Controls [N (%)]	P	OR (95%CI)
C-159T	T	521 (63.53%)	448 (55.44%)	0.001	1.4
	C	299 (36.47%)	360 (44.56%)		(1.148-1.708)
HWE (P)			0.39		
G-1145A	G	553 (64.00%)	390 (53.13%)	<0.0001	1.512
	A	311 (46.00%)	344 (46.87%)		(1.236-1.849)
HWE (P)			0.36		

OR = odds ratio; 95%CI = 95% confidence interval; HWE = Hardy-Weinberg equilibrium.

Table 3. Genotypic frequencies of SNPs in the CD14 gene in cases and controls.

SNPs	Genotype	Patients	Controls	P value	OR	95%CI
C-159T	T/T	186 (45.37%)	120 (29.70%)		1	
	C/T	149 (36.34%)	208 (51.49%)	<0.0001	0.46	0.34-0.63
	C/C	75 (18.29%)	76 (18.81%)		0.63	0.42-0.93
Total		410	404			
G-1145A	G/G	186 (43.06%)	108 (29.43%)		1	
	A/G	181 (41.90%)	174 (47.41%)	<0.0001	0.60	0.44-0.83
	A/A	65 (15.05%)	85 (23.16%)		0.44	0.29-0.65
Total		432	367			

OR = odds ratio; 95%CI = 95% confidence interval.

By comparing cases and controls, we found that CT and CC genotypes of the C(-159)T polymorphism were associated with decreased risk of TB, with an OR = 0.46 and 0.63, respectively (95%CI = 0.34-0.63 and 0.42-0.93; P < 0.0001). In addition, GA and AA genotypes of the G(-1145)A polymorphism were also protective against the disease, with an OR = 0.60 and 0.44, respectively (95%CI = 0.44-0.83 and 0.20-0.65; P < 0.0001; Table 3).

DISCUSSION

We performed a case-control study to investigate the association between CD14 G(-1145)A and C(-159)T polymorphisms and susceptibility to TB. To our knowledge, the present study is the first to demonstrate a possible association between the CD14 G(-1145)A polymorphism and TB. Our results suggested that G(-1145)A and C(-159)T polymorphisms in the CD14 may be new risk factors for TB. We found that in the G(-1145)A polymorphism, the frequency of the G allele or the GG genotype in the healthy control subjects was similar to what has been reported in other populations such as Americans (Vercelli et al., 2001),

Singaporeans (Liang et al., 2006) and Chinese (Gu et al., 2008). Therefore, the CD14-1145 G allele may represent a common allele in different populations, leading to similarities between disease associations and SNPs within different populations. Evaluation of the CD14 C(-159)T polymorphism showed that the TT genotype may be a risk factor for the development of TB. Similar results have been observed in a Mexican population (Rosas-Taraco et al., 2007). More studies are needed in other populations to confirm these results.

Gu et al. (2008) reported that both SNPs (G-1145A and T-159C) can significantly affect the transcriptional activity of the CD14 promoter, which may influence CD14 expression. Therefore, the CD14 polymorphism may be a genetic factor responsible for individual differences in the expression of CD14 and inflammatory response to TB. However, these authors found that the levels of mCD14/sCD14 in TB patients with the CD14-1145 GG genotype did not differ compared to other genotypes. Similar results were reported by Rosas-Taraco et al. (2007) in a study of the CD14 C(-159)T polymorphism and development of pulmonary TB; although the CD14-159 TT genotype appeared to be a risk factor for TB, no differences were found between CD14-159 genotypes and the levels of either mCD14 or sCD14. These negative results may be due to the complexity of gene-environment interactions, which play an important role in microbial exposure and influence CD14 expression in TB patients and even in controls. Multiple gene-gene interactions may also influence CD14 expression within individuals, as functional SNP linkages (for example, CD14 -1359, -1145, and -159) do exist in the CD14 gene (Vercelli et al., 2001). It is still early to reach a final conclusion that susceptibility to TB is associated with the polymorphisms within the CD14 promoter, only because change in CD14 expression is caused by gene variation. For CD14-1145 SNPs, more evidence is needed to prove whether the GG genotype of the CD14-1145 can influence the expression of CD14.

Increasing evidence suggests that CD14 signaling is part of host response to intracellular bacterial pathogens such as *Mycobacterium tuberculosis*, and increased sCD14 levels were documented in sera and bronchoalveolar lavage fluid of patients with active TB (Hoheisel et al., 1995; Juffermans et al., 1998). However, the effect of CD14 expression levels on the development of TB is still poorly understood. It has been speculated that an upregulation of the CD14 expression by *Mycobacterium tuberculosis* may contribute to the immune pathogenesis of TB by facilitating interactions with mannosylated lipoarabinomannan. This can lead to increased transforming growth factor (TGF)-β production and suppression of immune response (Shams et al., 2003). It has also been suggested that CD14 plays a crucial role in regulating IgE responses by promoting Th1 differentiation and suppressing Th2-dependent IgE responses (Vercelli et al., 2001; Kang et al., 2006). More study is required to further investigate the association between the CD14 polymorphisms and TB. Our results suggest that CD14 G(-1145)A and C(-159)T polymorphisms in the CD14 promoter could be new risk factors for TB in the Chinese Han population.

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