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MCP-1 gene polymorphisms in North Chinese patients with pulmonary tuberculosis

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ABSTRACT. Pulmonary tuberculosis (PTB) remains one of the most important infectious diseases worldwide. Several studies have suggested that genetic factors may affect the susceptibility to PTB, but the specific genes involved have not been fully characterized. The gene for monocyte chemoattractant protein 1 (*MCP-1*) has been linked to an increased risk of tuberculosis in some Mexican and Korean populations. To explore the role of the *MCP-1* gene in the susceptibility to PTB in a North Chinese population, we evaluated the association between *MCP-1* -2518A/G gene polymorphisms and the risk for tuberculosis. Polymerase chain reaction amplification of genomic DNA followed by restriction fragment length polymorphism analysis was used. There was a significant increase in the frequency of the GG genotype of *MCP-1* -2518 in 136 patients with PTB compared to that in 152 healthy controls ($P = 0.008$, $\chi^2 = 7.133$, odds ratio = 1.96). Similarly, the frequencies of the A/G alleles in the 2 groups differed; the frequency of allele G was higher in patients with PTB ($P = 0.011$, $\chi^2 = 6.428$, odds ratio = 1.536). In conclusion, the -2518A/G polymorphism in the *MCP-1* gene

was found to be associated with an increased susceptibility to PTB in a North Chinese population.

Key words: Genetic polymorphism; Pulmonary tuberculosis; Monocyte chemoattractant protein 1

INTRODUCTION

Tuberculosis (TB) is a serious health problem with an estimated 8-10 million new cases per year worldwide, resulting in 1-2 million deaths every year (Elbam, 2008). The population in China faces a high burden of TB. TB is a chronic infectious disease that is mainly caused by *Mycobacterium tuberculosis*. It is estimated that approximately one-third of the world's population is infected with *M. tuberculosis* (World Health Organization, 2006), but only 5-10% of those infected will develop a clinical disease, indicating the existence of host factors regulating disease expression (Bellamy, 1998; Frieden et al., 2003). In addition, twin studies have shown an increased concordance rate among monozygotic compared to dizygotic twins, indicating the importance of host genetic factors in the development of TB (Leandro et al., 2009). In fact, a large number of single-nucleotide polymorphisms in various genes have reported to be associated with the development of TB, such as the polymorphisms of the HLA haplotype, vitamin D_3 receptor, and SCL11A1, among others (Bellamy, 2003; Fernando and Britton, 2006). Thus, identifying host genetic factors for susceptibility to TB would greatly aid the global control of this disease.

Monocyte chemoattractant protein 1 (MCP-1) is an important chemokine and was the third chemokine to be purified to homogeneity after platelet factor 4 and interleukin-8 (Matsushima et al., 1989). MCP-1 has 76 amino acid residues and its gene is located on 17q11.2-q12 (Rollins et al., 1991). This chromosomal region has been linked to the susceptibility to TB and contains genes that encode for several chemokines that may contribute to immunity against TB (Jamieson et al., 2004). It has been demonstrated that *MCP-1* is related to pulmonary tuberculosis (PTB) in Mexicans and Koreans (Flores-Villanueva et al., 2005). However, the genetic associations between *MCP-1* -2518A/G polymorphisms and PTB in North China have not been reported.

In the study, we examined the *MCP-1* -2518A/G polymorphisms in 136 unrelated patients with PTB and 152 unrelated healthy controls of Han nationality in North China using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP). The aim of the study was to investigate the role of *MCP-1* -2518A/G polymorphisms in the genetic susceptibility to PTB.

MATERIAL AND METHODS

Subjects

The study used a case-control design to compare healthy controls and patients with PTB. The study enrolled 136 unrelated patients with PTB of Han nationality from North China. All patients were registered in the Respiratory Department of the Beijing Tuberculosis Research Institute between October 2009 and February 2011. Patients clinically and radiologically diagnosed with PTB and confirmed by sputum smear and culture for *Mycobacterium*

TB were included. All patients were human immunodeficiency virus-negative, and none presented with other infections, diseases, or immunosuppressive conditions. A total of 152 controls were unrelated healthy people of Han nationality from North China who had no history of TB and no evidence of prior TB noted on chest radiograph. All subjects gave informed consent for the study.

MCP-1 genotyping

Genomic DNA from patients and controls was extracted from peripheral blood leukocytes using standard methods (Davis et al., 1980; Miller et al., 1988). The *MCP-1*-2518 A/G polymorphism was genotyped by PCR-RFLP. The PCR system contained 2 U *Taq* DNA polymerase, 5 μL 10X PCR buffer, 5 μL loading dye, 5 μL stabilizer, 2 μL DNA, 2 μL of each forward and reverse primers, and 30 μL pure water. PCR primer sequences were: forward primer 5'-TTCTCTTCTACGGGATCTGGG-3', reverse primer 5'-GTCTCTCCTGGCTTAGT CAT-3'. PCRs were performed under the following conditions: 1 cycle of denaturation at 95°C for 3 min; followed by 35 cycles at 94°C for 40 s, 59°C for 40 s, and 74°C for 40 s; and a final extension step at 72°C for 4 min. PCR products (10 μL) were digested for 16 h by *Pvu*II in a final volume of 20 μL that contained 10X enzyme buffer. The resulting fragments were separated by electrophoresis on 3% ethidium bromide-stained agarose gel and were visualized under ultraviolet light. The PCR products were 466-bp fragments, which contained a unique *Pvu*II restriction site. This site was intact if G was at position -2518. *Pvu*II digests the 466-bp DNA segment from G/G homozygous individuals into 327- and 139-bp fragments. The DNA segment from G/A heterozygous individuals yielded 466-, 327-, and 139-bp fragments. PCR products from A/A homozygous individuals were not cut with *Pvu*II, yielding only one 466-bp fragment.

Statistical analysis

Allele and genotype frequencies were calculated by direct counting. Hardy-Weinberg equilibrium was assessed using the χ^2 test. Differences in the frequencies of alleles or genotypes between the different groups were estimated using the χ^2 test or the Fisher exact test. A 2-sided $P < 0.05$ was considered to be statistically significant, and odds ratios (ORs) with 95% confidence intervals (CIs) were also calculated for comparisons showing significant P values. All statistical analyses were conducted using SPSS version 17.0 program (SPSS, Inc.; Chicago, IL, USA).

RESULTS

Demographics of the subjects

The study participants included 136 patients with PTB and 152 healthy controls. Their baseline characteristics are summarized in Table 1.

Genotypes and alleles of *MCP-1* in patients with PTB and controls

The distributions of the AA, AG, and GG genotypes of *MCP-1* -2518 did not deviate

from Hardy-Weinberg equilibrium (P > 0.05) in patients with PTB and controls. There was a significant increase in the frequency of the GG genotype in patients with PTB compared to controls (P < 0.05, χ^2 = 7.133, OR = 1.96). The frequency of the AA and AG genotypes of *MCP-1* -2518 did not differ between patients with PTB and controls (P > 0.05). The frequency of the A/G alleles in the 2 groups was also different; the frequency of allele G was higher in patients with PTB (P < 0.05, χ^2 = 6.428, OR = 1.536) (Table 2).

Table 1. Demographic and clinical characteristics of subjects.

	PTB N = 136	Control N = 152
Age (years) median (range)	36 (18-56)	30 (21-50)
Gender (M/F)	61/75	68/84
Pulmonary tuberculosis	136	
Culture proven	66 (48.5%)	
Pathological diagnosed	70 (51.5%)	
Drug sensitive	88 (64.7%)	
Drug resistant	48 (35.3%)	
Initial treatment	93 (68.4%)	
Retreatment	43 (31.6%)	

Table 2. Alleles and genotypes of *MCP-1* in patients with pulmonary tuberculosis (PTB) and controls.

	PTB	Control	χ^2	P	OR (95%CI)
AA	16.9 (23/136)	23.1 (35/152)	1.669	0.196	
GG	41.2 (56/136)	26.3 (40/152)	7.133	0.008	1.96 (1.192-3.222)
AG	41.9 (57/136)	50.6 (77/152)	2.207	0.137	
A	37.9 (103/272)	48.3 (147/304)	6.428	0.011	0.651 (0.467-0.908)
G	62.1 (169/272)	51.7 (157/304)	6.428	0.011	1.536 (1.102-2.142)

Genotypes and alleles of *MCP-1* in patients with PTB

The frequency of the AA, AG, and GG genotypes and the A and G alleles of the *MCP-1* -2518 gene did not differ between drug-sensitive patients with PTB and drug-resistant patients (P > 0.05) (Table 3). The frequency of the AA, AG, and GG genotypes and the A and G alleles of the *MCP-1* -2518 gene did not differ between initial treatment patients with PTB and retreatment patients (P > 0.05) (Table 4).

Table 3. Alleles and genotypes of *MCP-1* in drug sensitive and drug resistant patients with pulmonary tuberculosis.

	AA	GG	AG	A	G
Drug sensitive	18.2 (16/88)	38.6 (34/88)	43.2 (38/88)	39.8 (70/176)	60.2 (106/176)
Drug resistant	14.6 (7/48)	45.8 (22/48)	39.6 (19/48)	34.4 (33/96)	65.6 (63/96)
χ^2	0.286	0.593	0.165	0.769	0.769
P	0.495	0.415	0.684	0.38	0.38

Table 4. Alleles and genotypes of *MCP-1* in initial treatment and retreatment patients with pulmonary tuberculosis.

	AA	GG	AG	A	G
Initial treatment	18.3 (17/93)	39.8 (37/93)	41.9 (39/93)	33.9 (63/186)	66.1 (123/186)
Retreatment	13.9 (6/43)	44.2 (19/43)	41.9 (18/43)	34.9 (30/86)	65.1 (56/86)
χ^2	0.392	0.235	0.000	0.027	0.027
P	0.531	0.628	0.993	0.87	0.87

DISCUSSION

It is well known that approximately one-third of the world's population is infected with *M. tuberculosis*. However, 90% of infected individuals remain healthy, indicating the effectiveness of the different immune mechanisms in the resistance against this *Mycobacterium*. Although both acquired and innate mechanisms contribute to the killing of *M. tuberculosis*, the precise mechanisms that confer resistance against this infection are not fully understood. Nevertheless, it seems evident that different genes are involved in the susceptibility to *Mycobacterium* TB infection (Kramnik et al., 2000; Delgado et al., 2002). The functional significance of the MCP-1 chemokine in attracting monocytes to the site of infectious lesions and its presumed role in the pathogenesis of tuberculosis and the granulomatous response suggests that variations in the *MCP-1* gene participate in the susceptibility to or protection against tuberculosis. In the study, we explored the association between the -2518A/G polymorphisms in the *MCP-1* gene and PTB in a Han population from North China.

We found a significant increase in the frequency of the GG genotype in patients with PTB compared to in controls (P = 0.008, OR = 1.96, 95%CI = 1.192-3.222). Similarly, the frequency of the A/G alleles in the 2 groups also differed; the frequency of allele G was higher in patients with PTB (P = 0.011, OR = 1.536, 95%CI = 1.102-2.142). In contrast, the frequency of the AA, AG, and GG genotypes and the A and G alleles of the *MCP-1* -2518 gene did not differ between drug-sensitive patients with PTB and drug-resistant patients (P > 0.05). The frequency of the AA, AG, and GG genotypes and the A and G alleles of the *MCP-1* -2518 gene did not differ between initial treatment patients with PTB and retreatment patients (P > 0.05).

Several studies have reported the association between *MCP-1*-2518A/G polymorphisms and tuberculosis risk, but the results have been inconsistent in different populations. The results of the study are consistent with those of previous reports regarding the association between *MCP-1* -2518A/G polymorphisms and tuberculosis in Mexicans, Koreans, Peruvians, Tunisians, and Zambians (Flores-Villanueva et al., 2005; Buijtels et al., 2008; Ganachari et al., 2010; Ben-Selma et al., 2011). Others have reported different results, such as in South African Coloreds, Indians, and Ghanaians (Alagarasu et al., 2009; Moller et al., 2009; Thye et al., 2009). Different genetic backgrounds may account for these differences. In addition, PTB is a complex disease that results from the infection with *Mycobacterium* TB and genetic susceptibility. These differences in genetic susceptibility may also result from individual status or other factors (Zhang et al., 2010). Thus, further studies are needed to assess the effect of additional interactions in different ethnicities and to validate our results. The molecular mechanism of the association between the *MCP-1* gene and PTB may be that the MCP-1 is a potent chemotactic factor for monocytes (Flores-Villanueva et al., 2005; Ben-Selma et al., 2011), which plays critical roles in the recruitment of macrophages and T lymphocytes for controlling the dissemination of *Mycobacterium* TB (Xu et al., 2009). The -2518A/G polymorphism in the *MCP-1* regulatory region influences the levels of *MCP-1* expression in response to inflammatory stimuli. The G allele of *MCP-1* may be the allele associated with a higher *MCP-1* expression in certain inflammatory conditions (Tumgor et al., 2008).

In conclusion, we examined the association between -2518A/G polymorphisms in the *MCP-1* gene and PTB. Our results suggest that the -2518A/G polymorphisms in the *MCP-1* gene are associated with an increased susceptibility to PTB in our North Chinese population. However, because of the relatively small number of patients and the possibility of racial differences, further investigation using a larger sample size is necessary to confirm the present findings.

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