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The association between the -2518A/G polymorphism in the MCP-1 gene and the risk of pulmonary tuberculosis in Sichuan Chinese population

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Abstract. – OBJECTIVE: The -2518A/G polymorphism in the *Monocyte chemotactic protein-1 (MCP-1)* gene may play an important role in regulating immunological reactions and may be associated with pulmonary tuberculosis (PTB). However, the relationship for the populations in Sichuan province of China remains unknown. The objective of the current study was to analysis that association.

PATIENTS AND METHODS: A total of 386 PTB patients and 398 controls were recruited. The genotypes were identified using PCR-RFLP and sequencing method. Data was analyzed using SPSS 11.0 software.

RESULTS: Significant association was found between the polymorphism and the risk of PTB: AG vs. AA: OR = 1.37, 95% CI = 0.98-1.92 and *p* = 0.06; GG vs. AA: OR = 1.69, 95% CI = 1.14-2.50 and *p* = 0.009; AG+GG vs. AA: OR = 1.47, 95% CI = 1.07-2.01 and *p* = 0.02; G vs. A: OR = 1.31, 95% CI = 1.08-1.60 and *p* = 0.007.

CONCLUSIONS: The current study suggested that the 2518A/G polymorphism in the MCP-1 gene was associated with risk of PTB in population of Sichuan province in China.

Key Words:
Tuberculosis, MCP-1, Polymorphism, Chinese, Sichuan.

Introduction

Tuberculosis (TB) is the major cause of infectious-related deaths worldwide and is a major public health threaten, especially in developing countries[1-5]. Almost one-third of the world's populations have ever been infected by *M. tuberculosis*[6]. However, most of them did not develop to active TB, and only 10% would fall ill lifetime. China ranks the second among the 22 high-burden countries, with different incidence and prevalence in different provinces[6,7].

Sichuan province is located in the west part of China. There are 80.41 million people living in the region of 0.48 million square kilometers. Based on the 1990 National TB Epidemiology Survey in China, the prevalence rate of TB in Sichuan was the second, following Tibet. Moreover, there is little information about the molecular epidemiology of TB in populations of Sichuan province, so there is an urgent need for studies addressing the molecular epidemiology of TB in this area.

Mycobacterium tuberculosis (MTB) is the main cause of TB, in addition, environmental and genetic factors are considered to play important roles in determining the susceptibility to TB. Chemokines have been implicated in the pathogenesis of TB. They mediate innate and adaptive immune responses by their ability to recruit, activate, and costimulate T cells and monocytes. Monocyte chemotactic protein-1 (MCP-1) is one of the generally chemotactic to mononuclear cells. Its expression can modulate TH1 cytokine expression and down-regulate proinflammatory responses and, thus, plays important roles in the pathogenesis of TB[8-12].

Polymorphisms have become increasingly important tools for the study of the structure and history of the human genome, and they are also useful polymorphic markers to investigate genetic susceptibility to disease. The human *MCP-1*

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gene is located on 17q11.2[8,9]. An important polymorphism named -2518A/G polymorphism has been shown to be associated with risk of TB in different ethnicities[13,14]. However, the association between the polymorphism with the risk of PTB in Sichuan province remains poorly understood. The aim of the current study was to investigate the relationship between the polymorphism and the risk of PTB in Sichuan province of China.

Patients and Methods

Participants

The case group comprised patients with PTB admitted to West China hospital and the Tenth Hospital of Chengdu. The inclusion criteria were PTB as previously mentioned[15]. The control group comprised healthy Han individuals who underwent physical examinations at West China hospital and the 452nd Military Hospital, and in whom PTB, spinal TB, and other extrapulmonary TB were all excluded. Cases and controls with any of the following conditions were excluded from the study: HIV positive and known to present any autoimmune, chronic inflammatory, or any conditions of other diseases.

Genomic DNA Extraction

Two milliliters of fasting peripheral venous blood was harvested in the morning and placed in acid citrate dextrose tubes. White blood cell genomic DNA was extracted using a whole-blood genomic DNA quick extraction kit (Tiagen, Beijing, China). DNA concentrations were determined using an ultraviolet spectrophotometer. DNA was diluted to 100 ng/ml with double distilled water and stored at –70°C.

Genotyping

Standard polymerase chain reaction(PCR) was performed as previously mentioned. The forward primer and reverse primer are as followed: 5'-GCTCCGGGCCCAGTATCT-3' and 5'-ACAGGGAAGGTGAAGGGTATGA-3'. The amplified fragment was 236bp. PCR reaction conditions were initial denaturation at 95°C for 4 min, followed by 40 cycles of 94°C for 30s, 56°C for 30s, 72°C for 1 min, followed by a final extension at 72°C for 10 min and annealing at 4°C. The 236bp product was digested for 12h at 37°C with 3U of the *PvuII* restriction enzyme. Restriction fragments were separated on 3% agarose gel in a TAE buffer. The gel was stained with ethidium bromide and visualized under UV light. The products were analyzed to determine the presence of various genotypes of the various genotypes: (1) AA genotype yields only a single 236bp band; (2) GG genotype results in two bands (182bp and 54bp); (3) A/G genotype results in three bands (236bp, 184bp and 54bp). Genotypes validation was performed by sending the samples of the AA, AG and GG genotypes to be sequenced in the Invitrogen Company (Shanghai, China).

Statistical Analysis

Data were analyzed using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). The genotype frequency of the control group was calculated to confirm Hardy-Weinberg equilibrium. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the association between the *MCP-1* polymorphism and PTB risk. A $p < 0.05$ was considered statistically significant.

Results

General conditions

The characteristics of both PTB patients and controls is shown in Table I. A total of 386 PTB patients and 398 controls were enrolled. There was no statistical differences for both age and gender distributions between two groups. The ratio of males was higher than females in both groups.

Hardy-Weinberg Equilibrium Test

The distribution frequencies of the genotypes and alleles for both groups are shown in Table II. The genotype frequencies in PTB patients were 24.6%, 49.0%, 26.4% for AA, AG, GG genotype respectively, and were 32.4%, 47.0%, 20.6% for AA, AG, GG genotype respectively in control group. The allele frequencies in PTB group were 49.1% and 50.9% for A and G respectively, while were 55.9% and 44.1% in control group. The genotype distribution in the control group was consistent to the HWE ($p > 0.05$).

Table I. General condition for both PTB cases and controls.

Group	Age (years)	Male (n)	Female (n)
PTB	42 ± 8	212	174
Control	43 ± 7	216	182

Table II. Distribution of the genotypes and alleles for both PTB patients and controls.

Group	Genotypes			Allele	
	AA	AG	GG	A	G
Control	95 (24.6%)	189 (49.0%)	102 (26.4%)	379 (49.1%)	393 (50.9%)
Case	129 (32.4%)	187 (47.0%)	82 (20.6%)	445 (55.9%)	351 (44.1%)

Analysis of the -2518A/G Polymorphism in the MCP-1 Gene and the Risk of PTB

Table III shows the association between the polymorphism and the risk of PTB. Take the A allele as reference, significant association was found between the polymorphism and the risk of PTB: AG vs. AA: OR = 1.37, 95% CI = 0.98-1.92 and $\pi = 0.06$; GG vs. AA: OR = 1.69, 95% CI = 1.14-2.50 and $\pi = 0.009$; AG+GG vs. AA: OR = 1.47, 95% CI = 1.07-2.01 and $\pi = 0.02$; G vs. A: OR = 1.31, 95% CI = 1.08-1.60 and $\pi = 0.007$.

Discussion

Host genetic susceptibility, environmental factors and other factors might contribute to the pathogenesis of PTB. The MCP-1 is one important chemokine that plays important roles in the development of PTB[17,18]. The -2518A/G polymorphism in the *MCP-1* gene might contribute to the risk of PTB. There were several studies revealed the association with the risk of PTB in diversity populations; however, the clear roles in the population of Sichuan province in China have been uncovered. Thus, we performed the current case-control study to assess the associations.

A total of 386 PTB patients and 398 controls were recruited. Our results showed that the polymorphism was associated with increased risk of PTB. Taken the AA genotype as reference, individuals who carried GG genotype might have 69% increased risk of PTB, and the G allele carriers (GG+GA) might have 47% increased risk of PTB. Taken the A allele as reference, individuals who carried G genotype might have 31% increased risk of PTB. Previously, we performed a meta-analysis to assess the association between the risk of TB and -2518A/G polymorphism in the *MCP-1* gene[16]. The current results were consistent with the previous meta-analysis, which validated the association between the polymorphism and the risk of PTB.

We would like to underline that we did not measure the level of MCP-1 in the plasma. Since we just studied the most important polymorphism in the *MCP-1* gene, other polymorphisms were not analyzed. All the included populations were Han nationality, thus, the minorities were not included.

Conclusions

We demonstrated that the -2518A/G polymorphism in the *MCP-1* gene is a risk factor for PTB in Sichuan province in China. Further multicenter, large-scale genetic screening studies are needed to validate these associations.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Table III. Analysis of the -2518A/G polymorphism in the MCP-1 gene and the risk of PTB.

Genotype/Allele	PTB patients (n = 386)	Control (n = 398)	OR (95%CI)	*p* value
AA	95	129	Reference	
AG	189	187	1.37 (0.98, 1.92)	0.06
GG	102	82	1.69 (1.14, 2.50)	0.009
AG+GG	291	269	1.47 (1.07, 2.01)	0.02
A	379	445	Reference	
G	393	351	1.31 (1.08, 1.60)	0.007

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