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RESEARCH ARTICLE

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Association of heme oxygenase-1 single nucleotide polymorphisms with susceptibility to tuberculosis in Chinese Han population

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Abstract

Background: Tuberculosis (TB) is an infectious disease, caused by mycobacterium tuberculosis infection, which is associated with oxidative stress and the induction of host antioxidants to counteract this response. The heme oxygenase-1 (HO-1) single nucleotide polymorphisms have been reported to be associated with many critical diseases. Our purpose was to investigate the association of HO-1 single nucleotide polymorphisms with the susceptibility to tuberculosis in Chinese Han population.

Methods: A case-control study was performed on Chinese Han population, and a group of 638 TB patients was compared to 610 healthy controls. Three single nucleotide polymorphisms (SNPs) including rs2071746, rs5995098, and rs8140669 were genotyped using the MassARRAY platform. The genotype frequency was compared between TB patients and healthy controls. The association between the three genetic models of the three SNPs and TB risk was further investigated.

Results: The results showed that, in the case of additive model, there was significant difference of the genotype frequencies of SNP rs8140669 between the TB patients and control groups (P = .038). In the case of dominant model, the genotype frequencies of SNP rs8140669 may have difference between the two cohorts (P = .051), while the allele frequency and genotype distribution for other two SNPs showed no significant difference between the two groups (P > .05).

Conclusion: HO-1 polymorphism was associated with TB susceptibility in Chinese Han population.

KEYWORDS

Chinese Han population, heme oxygenase-1, single nucleotide polymorphisms, Tuberculosis

ICMJE Statement: All authors meet the ICMJE authorship criteria.

1 | INTRODUCTION

Tuberculosis (TB), caused by infection of mycobacterium tuberculosis, is a major public health challenge worldwide. Although the treatment seems to be effective, there is an increasing trend in the number of TB patients in developing countries. This situation is caused by the HIV/AIDS pandemic; moreover, failure to control the spread of tuberculosis is another important reason.[1,2] The patients with pulmonary TB expel the mycobacterium tuberculosis (MTB) into the air by sneezing, coughing, and talking. In general, 10% of people who infected with MTB will develop the TB disease; moreover, due to host immune system, 90% cases are asymptomatic and non-infectious.[3] By 2010, China has the second-largest number of incident cases in the world, and TB remains the main leading reason of deaths from infectious diseases.[4] As is known to all, TB is caused by the infection of MTB, but the genetic is also involved in the pathogenesis of TB. The association between genetic polymorphisms with the susceptibility to TB has been reported in a large number of researches. Interleukin 18 receptor 1 gene (IL18R1), the CISH gene, the NOD-like receptor (NOD2), and the cytotoxic T lymphocyte antigen-4 (CTLA4) have been reported to be associated with the susceptibility to TB.[4-7] However, the relationship between the HO-1 single nucleotide polymorphisms and susceptibility to tuberculosis has not yet been studied in Chinese Han population.

Heme oxygenase-1 (HO-1) is a major antioxidant, which is highly expressed in lung tissue. As the rate-limiting enzyme, it degrades free heme into carbon monoxide (CO), ferrous iron, and bilirubin.[8] It has been reported that the HO-1 plays a critical role in diverse diseases, including human malaria, neonatal jaundice, cardiovascular disease, atherosclerotic stroke, and so on.[9-12] Lung is a major target of various inflammatory, oxidative, carcinogenic, or infectious stressors, which can result in a range of lung diseases. As expected, the HO-1 is a crucial defense mechanism during acute and chronic lung diseases.[13] Therefore, we hypothesized that HO-1 may play an important role in the susceptibility to tuberculosis in Chinese Han population. We performed this case-control study to examine whether the HO-1 is a causative or protective factor in TB.

2 | MATERIALS AND METHODS

2.1 | Patients and healthy controls

This is a case-control study, and a group of 638 TB patients was compared to 610 healthy controls. All subjects were Chinese Han ethnicity, recruited from The West China Hospital of Sichuan University, located in Chengdu, the southwest of China. The pulmonary TB patients and extrapulmonary TB patients were all included in our study. All patients were recruited from the Respiratory Medicine, TB Ward. All TB patients included were confirmed according to the following criteria: (a) for the Han population; (b) clinically diagnosed by two independent experienced respiratory physicians according to standard criteria recommended by National Health and Family Planning Commission, China (WS288-2008); and (c) without evidence of immunodeficiency diseases and other lung problems. All healthy controls were from the Physical examination center. The healthy controls had no any abnormal symptoms or clinical findings on examination, and we excluded the subjects that had a history of TB or lung diseases. Healthy individuals with evidence of immunodeficiency diseases and other lung problems were also excluded. Between the patients and healthy controls, there was no statistically significant difference, in either gender or age. The study received institutional review board approval.

2.2 | DNA extraction

The peripheral blood samples (2-3ml) of all participants were collected in 5.0 mL EDTA tubes and stored at −80°C. DNA was extracted from the peripheral blood samples using the genomic DNA purification kit (Axygen Scientific Inc-USA) according to the manufacturer's instructions. The DNA was stored at −80°C until before genotyping.

2.3 | Genetic analysis

The HO-1, including three SNPs (rs2071746, rs5995098, and rs8140669) in our study, was genotyped by the Sequenom MassARRAY iPLEX platform (Sequenom) and the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry technologies. The experiment consists of an initial polymerase chain reaction (PCR), the single-base extension probes, and PCR primers were designed using the SpectroDESIGNER software (Sequenom).

2.4 | Statistical analysis

Data analyses were performed using SPSS 17.0 (IBM), which is a powerful, versatile data mining workbench that helps to build accurate predictive models quickly and intuitively, without programming. The chi-square test was used to assess the Hardy-Weinberg equilibrium (HWE) of each group. As for the major demographic characteristics, mean ± standard deviation (SD) was used to describe the age; chi-squared tests were used to examine the differences between the TB patients and the healthy controls, such as gender and smoking status; *t* tests were conducted to examine the age. The relationship between the frequency of alleles and genotypes and the TB was estimated by calculating the odds ratios (OR) and 95% confidence intervals (95% CI) by chi-squared tests. The association of the genetic model with the subjects was estimated by single-variable logistic regression analysis and using the multi-variable logistic regression analysis to adjustment the age, gender, and smoking status. Fisher's exact test was used to estimate the differences in the frequency of genotypes and alleles between the two groups. The *P* values <.05 were considered to be statistically significant.

TABLE 1 Demographic characteristics of patients and healthy controls

Demographic Variables	TB(N = 638)	Controls(N = 610)	Chi-sq or *t*	*P*
Gender				
Male	328 (51.41%)	304 (49.84%)	Chi-sq = 0.31	.578
Female	310 (48.59%)	306 (50.16%)		
Smoking status				
Non-smoker	442 (69.28%)	466 (76.39%)	Chi-sq = 7.96	.004
Smoker*	196 (30.72%)	144 (23.61%)		
Age				
Mean ± SD	36.81 ± 15.75	37.12 ± 15.66	*t* = 0.34	.734

Abbreviations: N, number of subjects; TB, tuberculosis.

*The percentage of smoking subjects among patients was higher than healthy controls (*P* = .004).

TABLE 2 The distribution of allele and genotype frequencies in TB patients and healthy controls for the three SNPs

SNP	Allele or genotype	Cases (N = 638)	Controls (N = 610)	*OR* (95% CI)	*P*
rs2071746	T	668 (55.30%)	606 (52.33%)	0.887 (0.755,1.043)	.148
	A	540 (44.70%)	552 (47.67%)		
	TT	184 (30.46%)	162 (28.42%)		
	TA	300 (49.67%)	282 (49.47%)	0.937 (0.717,1.223)	.631
	AA	120 (19.87%)	126 (22.11%)	0.838 (0.604,1.163)	.292
rs5995098	C	624 (51.49%)	607 (53.25%)	1.073 (0.913,1.262)	.393
	G	588 (48.51%)	533 (46.75%)		
	CC	166 (27.39%)	169 (29.65%)		
	CG	292 (48.18%)	269 (47.19%)	1.105 (0.843,1.449)	.469
	GG	148 (24.42%)	132 (23.16%)	1.141 (0.831,1.568)	.414
rs8140669	T	1155 (94.67%)	1099 (96.23%)	1.438 (0.970,2.133)	.069
	A	65 (5.33%)	43 (7.53%)		
	TT	547 (89.67%)	528 (92.47%)		
	TA	61 (10.00%)	43 (3.77%)	1.369 (0.910,2.059)	.131
	AA	2 (0.33%)	0 (0.00%)	524 008.7 (0.000, NA)	.980

3 | RESULTS

3.1 | Demographic characteristics

The demographic characteristics of the patients and healthy controls were listed in Table 1. A total of 638 TB patients (328 males and 310 females; aged 14-86 years, mean age 36.81 ± 15.75 years) were compared to a total of 610 healthy controls (304 males and 306 females; aged 14-89 years, mean age 37.12 ± 15.66 years). As expected, no significant differences were observed between the two groups in age and gender ($P > .05$), while we found the percentage of smoking subjects among patients was significantly higher than healthy controls ($P = .004$). The genotype distribution of the TB group was in accordance with Hardy-Weinberg equilibrium ($x^2 = 5.425$, $P = .379$), while the genotype distribution of the control group was in accordance with Hardy-Weinberg equilibrium ($x^2 = 3.579$, $P = .281$), which indicated that the samples selected in this study were representative of the population.

3.2 | Distribution of alleles and genotypes frequencies

The distribution of alleles and genotypes frequencies in TB patients and healthy controls for the three SNPs was listed in Table 2. It was approved that there were no significant differences of the alleles and genotypes frequency distribution for the three SNPs between the two groups ($P > .05$).

3.3 | The association between different genetic models and TB risk

The association between the three genetic models of the three SNPs and TB risk was shown in Table 3. Logistic regression analysis with all SNPs using three models adjusted for the age, gender, and smoking status was performed. In the case of additive model, the genotype frequencies of SNP rs8140669 were significantly different between

TABLE 3 Logistic regression analyses for the association of the three genetic models of the three SNPs with TB risk

Genetic model	SNP	Cases (N = 638)	Controls (N = 610)	A		B	
				OR (95% CI)	*P*	*OR* (95% CI)	*P*
rs2071746							
H1	TT	184 (30.46%)	162 (28.42%)	0.918 (0.780,1.079)	.299	0.921 (0.782,1.086)	.328
	TA	300 (49.67%)	282 (49.47%)				
	AA	120 (19.87%)	126 (22.11%)				
H2	TT	184 (30.46%)	162 (28.42%)	0.906 (0.705,1.165)	.443	0.903 (0.701,1.164)	.432
	TA + AA	420 (69.54%)	408 (71.58%)				
H3	TT + TA	484 (80.13%)	444 (77.89%)	0.874 (0.659,1.157)	.346	0.888 (0.668,1.181)	.415
	AA	120 (19.87%)	126 (22.11%)				
rs5995098							
H1	CC	166 (27.39%)	169 (29.65%)	1.070 (0.913,1.254)	.403	1.064 (0.906,1.249)	.449
	CG	292 (48.18%)	269 (47.19%)				
	GG	148 (24.42%)	132 (23.16%)				
H2	CC	166 (27.39%)	169 (29.65%)	1.117 (0.867,1.439)	.392	1.093 (0.846,1.412)	.497
	CG + GG	440 (72.61%)	401 (70.35%)				
H3	CC + CG	458 (75.58%)	438 (76.84%)	1.072 (0.819,1.403)	.611	1.081 (0.824,1.418)	.574
	GG	148 (24.42%)	132 (23.16%)				
rs8140669*							
H1	TT	547 (89.67%)	528 (92.47%)	1.444 (0.971,2.146)	.069	1.531 (1.022,2.292)	.038
	TA	61 (10.00%)	43 (7.53%)				
	AA	2 (0.33%)	0 (0.00%)				
H2	TT	547 (89.67%)	528 (92.47%)	1.414 (0.943,2.122)	.094	1.506 (0.997,2.275)	.051
	TA + AA	63 (10.33%)	43 (7.53%)				
H3	TT + TA	608 (99.67%)	571 (100.00%)	–	–	–	–
	AA	2 (0.33%)	0 (0.00%)				

Abbreviations: A, single-variable logistic regression analysis; B, multi-variable logistic regression analysis after adjustment for gender, age, and smoking status; H1, additive model; H2, dominant model; H3, recessive model.

*The genotype frequencies of SNP rs8140669 had significant difference between the TB patients and control groups (*P* = .038).

the TB patients and control groups (*P* = .038; OR = 1.531; 95% CI, 1.022-2.292). In the case of dominant model, the genotype frequencies of SNP rs8140669 may be different between the two cohorts (*P* = .051; OR = 1.506; 95% CI, 0.997-2.275), while the allele frequency and genotype distribution for other two SNPs showed no significant difference between the two groups (*P* > .05).

4 | DISCUSSION

Tuberculosis (TB) is a contagious and chronic disease which can threaten human health seriously. According to the World Health Organization (WHO) reports, China has been the second-highest burden of TB in the world.[14] Previous studies confirmed that host genetic variants played an important role in TB infection and progress, and several candidate genes had been identified.[15,16] However, the association between HO-1 polymorphism and TB risk is still poorly understood. In this study, we investigated the relationship of HO-1 polymorphism and TB risk.

HO-1 is an intracellular enzyme expressed in many cell types and tissues, including the lung tissues. Previous studies have shown that HO-1 levels can be used to distinguish active from latent or treated pulmonary tuberculosis,[17] and the elevated plasma levels of HO-1 may be a potential markers of pathogenesis in TB.[18] Many researches have also been reported that the genetic factors undoubtedly contributed to the susceptibility to tuberculosis. Therefore, we assumed that the HO-1 gene polymorphism may be a genetic factor for susceptibility to TB among Chinese Han population.

HO-1 is a potent antioxidant enzyme associated with cytoprotection in a number of different disease settings. It was reported that the majority of patients with active TB express either high HO-1 and low MMP-1 or vice versa.[19] It was previously shown that plasma levels of HO-1, a major antioxidant highly expressed in the lungs, can accurately distinguish active from latent TB cases or uninfected controls in both adult and pediatric populations in South India.[7,20] These studies indicated that HO-1 can serve as an important biomarker of TB disease. The most extensively studied HO-1 gene variant is

the dinucleotide repeat polymorphism, [GT]n, within the proximal promoter, at approximately −200 base pairs.[21] It is a good candidate for a functional polymorphism, since it may modulate the transcriptional activity of the gene. However, its clinical significance remains unclear. The length of the dinucleotide repeat ranges from [GT]10 to [GT]40 and thus represents a continuum of alleles.[21] A major obstacle in comparing studies showed that genotype definitions were inconsistent. [GT]25 has often been used as a cutoff, with dinucleotide repeat lengths ≤25 classified as Short (S) alleles and repeat lengths ≥26 classified as Long (L) alleles. However, the S allele cutoff can vary from [GT]23 to [GT]30, and certain studies introduce a Middle (M) allele class, the definition of which is also variable. In addition, most of the analyses to date are limited to regional patient populations, with studies of pulmonary disease involving predominantly Asians and cardiovascular studies involving whites.

Our study evaluated for the first time the association between the 3 SNPs in the HO-1 genes and susceptibility to tuberculosis in Chinese Han population. The results revealed that there were no significant differences of the alleles and genotypes frequency distribution for the three SNPs between the two groups, but the SNP rs8140669 may be closely associated with susceptibility to the TB, in the case of dominant model.

There were also some limitations in our study. The relative small sample size and the low frequency of some variant genotype would affect the final analysis. Further study with larger number of participants is needed to confirm our findings.

In conclusion, this is the first study of the association between the 3 SNPs in the HO-1 gene and susceptibility to TB in the southwestern Chinese Han population. The data in the present study show that the SNP rs8140669 in HO-1 gene may contribute to the genetic susceptibility to TB in Chinese Han population.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by The West China Hospital of Sichuan University (Chengdu, China). The patients and their families were informed and signed an informed consent.

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