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Original Article

MIF, TGF-β1, IFN-γ and NRAMP1 gene polymorphisms in relation to the clinicopathological profile of spinal tuberculosis in Chinese Han population

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Abstract: MIF, TGF-β1, IFN-γ and NRAMP1 gene polymorphisms play an important role in pathogenesis of immune diseases. However, the relationship between the MIF, TGF-β1, IFN-γ and NRAMP1 gene polymorphism and their correlation with the clinicopathological profile in spinal tuberculosis are still unknown. We undertook this present study to investigate these gene polymorphisms and their relationships between the clinicopathological profile and spinal tuberculosis in Chinese Han population. The genotypes of MIF (rs755622 G/C), TGF-β1 (rs1800469 T/C, rs4803455 A/C), IFN-γ (rs2069718 C/T) and NRAMP1 (rs17235416 del/TGTG) genes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in 110 spinal tuberculosis patients and 110 healthy controls. The C allele in rs755622 and rs4803455 were both significantly more common in spinal TB patients than healthy controls. The C allele and CC, CT genotype in rs1800469, as well as the C allele and AC, CC genotype in rs4803455 were both significantly higher in patients with CRP level exceeding 10 mg/L and ESR level exceeding 20 mm/h group. The other parameters were not significantly different. The present study suggests that MIF, TGF-β1 genes may affect susceptibility to spinal TB and increase the risk of developing the disease and shows that TGF-β1 polymorphism at rs1800469 and rs4803455 may be associated with the degree of inflammation in spinal TB.

Keywords: MIF, TGF-β1, INF-γ, NRAMP1, polymorphisms, spinal tuberculosis

Introduction

In developing countries, tuberculosis remains one of the most common diseases, and it is considered that Mycobacterium tuberculosis has infected about one third of the world's population [1]. However about 20% of the infected people will develop extra pulmonary tuberculosis, and spinal tuberculosis is the most serious form of extra pulmonary tuberculosis [2]. According to the World Health Organization (WHO), spinal tuberculosis is associated with great disability and high morbidity rate [3]. The reason why some patients infected with Mycobacterium tuberculosis has a higher risk of developing spinal tuberculosis and others limit the disease is still unknown, but the host susceptibility genes may play an important role [4, 5].

Cytokines produced by infected macrophages and T lymphocytes may play an important role in the development and incidence of tuberculosis [6]. The Mycobacterium tuberculosis infection consists of two distinct T cell factor model. T helper 1 (Th1) cytokines are associated with resistance to infection, such as IFN-γ, and Th2 cytokines are associated with progressive disease [7]. TGF-β is another T cell regulatory cytokine which are mainly produced by the Th3 cells that may lead to fibrosis or cavity formation in the pathogenesis of tuberculosis [8]. The cytokines MIF play an important role in the regulation of the Th1/Th2 balance in host's inflammatory reaction and immune responses [9], which can inhibit the migration of macrophage and promote aggregation of inflammation or infection [10]. Animal experiments revealed that rats Nramp1 gene has a certain effect in initiation and progression of murine tuberculosis. The NRAMP1 is the human homologue of the mouse Nramp1 gene, and according to some studies the polymorphism may be associated with the

Table 1. Primer sequences, restriction enzymes used and restriction digestion patterns for genotyping of MIF, TGF-β1, IFN-γ and NRAMP1 polymorphisms

Polymorphisms	Primer sequence (5'-3')	PCR Product size	Restriction enzymes used	Recognition sites	Genotype	Product size After restriction
MIF rs755622 (G/C)	F: CTGACTTCTCGGACACCACT	352 bp	AluI	GGCGCACCGCTCCAAGCTGTTCTCCACTTGG	GG	352 bp
	R: AAGGGTAAGGGGCCATCTTC				GC	198+154/352 bp
					CC	198+154 bp
TGF-β1 rs1800469 (C/T)	F: TGGAGTGCTGAGGGACTCTG	489 bp	Bsu36I	CTGACCCTTCCATCCCTCAGGTGTCCTGTTG	CC	360+129 bp
	R: AGGCGGAGAAGGCTTAATCC				CT	360+129/48 bp9
					TT	489 bp
TGF-β1 rs4803455 (A/C)	F: GCTGCAAACATTCTGGGGTT	98 bp	Bse3DI	CAGTAACTTAGAAGTCATTGCTAATGATTCC	AA	98 bp
	R: CCAGCCGGAATCATTAGCAA				AC	74+24/98 bp
					CC	74+24 bp
IFN-γ rs2069718 (C/T)	F: CAAGAGGAAGGTAAATGATC	274 bp	Bse8I	GTAAATGATCCACATCTTATGAAGCATCATC	CC	253+21 bp
	R: ACACCAAATCCAAAACGAGTG				CT	253+21/274 bp
					TT	274 bp
NRAMP1 rs17235416 (Del/TGTG)	F: GCATCTCCCCAATTCATGGT	240/244 bp	FokI	GCCTGCTGGA(TGTG)GAGGGGGCGC	Del/Del	240 bp
	R: AACTGTCCCACTCTATCCTG				TGTG/Del	33+211/240 bp
					TGTG/TGTG	33+211 bp

F: Forward; R: Reverse; bp: base pairs. Primer sequences were designed by Sangon Biotech.; Del: Del is missing the TGTG; rs17235416: When TGTG exists, the product is 244 bp, can be enzyme digestion, when TGTG is missing, product cannot be enzyme digestion, and the product is 240 bp.

susceptibility to human tuberculosis [11]. Thus, MIF, IFN γ, TGF-β1 and NRAMP1 play a crucial role in the pathogenesis of tuberculosis.

Previous studies have reported some association between MIF, IFN-γ, TGF-β1, NRAMP1 gene polymorphisms and the pulmonary TB [12-15]. However, there are no research data on the correlation between MIF, IFN-γ, TGF-β1, NRAMP1 gene polymorphisms and spinal TB in Chinese population, and more precisely its relation to the clinicopathological profile of spinal tuberculosis.

Therefore, the aim of this work was to elucidate whether there is any association between the MIF, IFN-γ, TGF-β1, NRAMP1 gene polymorphisms and their susceptibility to spinal tuberculosis. In addition, this study also analyzed the relationship between the MIF, IFN-γ, TGF-β1 and NRAMP1 gene polymorphisms with the clinicopathological profiles of spinal tuberculosis in Chinese population.

Materials and methods

Study population

The written informed consent was obtained from patients or their relatives. This study was approved by the ethics committee of Guangxi province (China). This study included 110 Han patients who are living in Guangxi Province, China. They were diagnosed with spinal TB and underwent surgery in the First Affiliated Hospital of Guangxi Medical University from 1st Jan 2010 to 30th December 2014. These spinal TB patients were presented with the typical symptoms such as moderate fever, weakness, back pain and paraparesis, and all of them were assigned into the spinal TB group. Diagnosis of spinal TB was made by performing a hematological examination, histopathological investigation, imaging methods such as radiography, computed tomography (CT) or magnetic resonance imaging (MRI), and exclusion of other diseases such as acquired immune deficiency syndrome (AIDS), tumors, pulmonary TB and ankylosing spondylitis. The control group included 110 Han healthy subjects and subjects with pulmonary TB, spinal TB, and other extrapulmonary tuberculosis were excluded by imaging. The following data were collected for the two groups: gender, age, duration of symptoms, pain intensity (visual analog scale score, VAS), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR).

Genotyping assay

Genomic DNA was isolated from blood samples by using TRIzol® (Life Technologies, USA) according to the manufacturers' instructions and the extracted genomic DNA was stored at -20°C until further use. DNA concentrations and qualities were measured by Nanodrop2000 micro-volume spectrophotometer (Thermo Scientific, USA) using absorbance measurements. The primer sequences and results are shown in **Table 1**, and they were designed by using the UPL Assay Design Centre web service. Restriction enzymes (New England Biolabs, Inc, Ipswich, USA) used and the restriction digestion patterns for different alleles are given in **Table 1**. DNA was amplified in the PCR machine through the PCR thermal cycling with the following conditions: initial denaturation at 95°C for 10 min, followed by 30 amplification cycles of 95°C for 30 s, annealing temperature (given separately) and extension at 60°C for 30 s, and 72°C for 1 min, in the end a final extension at 72°C for 10 min, annealing at 4°C. PCR products were digested with the respective restriction enzymes of optimum temperature and time, and then the fragments were separated in 2% agarose gel containing 0.5 mg/ml ethidium bromide by electrophoresis at 120 V and visualized under UV light.

Statistical analysis

Statistical analysis was performed by SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). The general conditions of the two groups were compared by t tests and Chi-square test. The deviation of polymorphism from Hardy-Weinberg equilibrium was examined by Chi-square test through comparing with observed and expected genotype frequencies in the two groups. Chi-square test was used to compare the difference between the genotype and allele frequencies of MIF, TGF-β1, IFN γ and NRAMP1 in two groups. Odds ratio (OR) and corresponding 95% confidence intervals (95% CI) were calculated by multiple logistic regression using case/control status as the dependent variable. $P<0.05$ (two-tailed) was considered statistically significant. Also, significant probability values obtained were corrected for multiple testing (Bonferroni correction).

Table 2. MIF, TGF-β1, IFN-γ and NRAMP1 polymorphisms distribution in Spinal TB group and Control group

Allele genotype	Spinal TB (%) (n=110)	Control (%) (n=110)	*P* value	Odds ratio (95% CI)
rs755622 (G/C)				
C	66 (30.0)	44 (20.0)		1
G	154 (70.0)	176 (80.0)	<0.01	1.47 (1.22-3.89)
CC	13 (11.8)	6 (5.5)		1
GG	57 (51.8)	72 (65.5)	0.04	2.37 (1.02-7.90)
GC	40 (36.4)	32 (29.0)	0.52	1.87 (0.87-6.73)
rs1800469 (T/C)				
T	116 (52.7)	107 (48.6)		1
C	104 (47.3)	113 (51.4)	0.44	1.78 (1.35-2.72)
TC	48 (43.6)	47 (42.7)		1
TT	34 (30.9)	30 (27.3)	0.48	1.32 (0.89-4.03)
CC	28 (25.5)	33 (30)	0.76	0.44 (0.15-2.49)
rs4803455 (A/C)				
C	156 (70.9)	131 (59.5)		1
A	64 (29.1)	89 (40.5)	<0.01	1.35 (1.08-2.86)
CC	54 (49.1)	39 (35.5)		1
AA	8 (7.3)	18 (16.4)	0.02	3.36 (1.02-7.98)
AC	48 (43.6)	53 (48.1)	0.12	1.33 (0.64-2.64)
rs2069718 (C/T)				
C	32 (14.5)	22 (10.0)		1
T	188 (85.5)	198 (90.0)	0.25	1.21 (0.78-4.32)
CT	32 (29.1)	20 (18.2)		1
CC	0 (0)	1 (0.9)	0.21	0.55 (0.13-1.58)
TT	78 (70.9)	89 (80.9)	0.09	1.26 (0.98-2.44)
rs17235416 (del/TGTG)				
TGTGdel	38 (17.3)	19 (8.6)		1
TGTG	182 (82.7)	201 (91.4)	0.02	2.39 (1.65-4.21)
TGTG/del	30 (27.3)	17 (15.5)		1
TGTGdel/del	4 (3.6)	1 (0.9)	0.82	0.44 (0.08-4.53)
TGTG/TGTG	76 (69.1)	92 (83.6)	0.04	2.34 (1.22-6.85)

Results

MIF, TGF-β1, IFN-γ and NRAMP1 polymorphisms in spinal TB patients and healthy controls

We evaluated the frequencies of MIF, TGF-β1, IFN-γ and NRAMP1 polymorphisms in Spinal TB group and Control group (**Table 2**). All the genotype frequency distributions were in agreement with Hardy-Weinberg equilibrium (P<0.05). The CC genotype in rs755622 (OR=2.37, 95% CI=1.02-7.90, P=0.04) and CC genotype in rs4803455 (OR=3.36, 95% CI=1.02-7.98, P=0.02) were both significantly higher in spinal TB than in controls. The TGTGdel allele carrier (OR=2.39, 95% CI=1.65-4.21, P=0.02) and TGTG/del genotype (OR=2.34, 95% CI=1.22-6.85, P=0.04) in rs17235416 were significantly more common in spinal TB patients than those of healthy controls. Statistical significant differences for both polymorphisms, however, were lost after Bonferroni correction of the *p* values. The frequency of rs755622 C allele (OR=1.47, 95% CI=1.22-3.89, P<0.01) and rs4803455 C allele (OR=1.35, 95% CI=1.08-2.86, P<0.01) were significantly higher in spinal TB patients when compared with those of healthy controls. Statistical significant difference was still kept after Bonferroni correction of the *p* values. The allele and genotype frequencies of rs1800469 and rs2069718 polymorphisms did not differ significantly.

Association between MIF, TGF-β1, IFN-γ and NRAMP1 polymorphisms and clinical parameters in spinal TB patients.

We further evaluated the associations of stratification analysis of MIF, TGF-β1, IFN γ and NRAMP1 polymorphisms with clinicopathological factors in spinal TB group. The results of stratification analysis with parameters of age, gender, level of ID herniation, pain intensity (VAS), duration of symptoms, CRP, ESR, smoking habits are shown in **Table 3**. The CT, CC genotype (OR=16.54, 95% CI=5.46-87.29, P<0.01 and OR=6.88, 95% CI=2.14-26.76, P<0.01) and C allele carrier (OR=4.89, 95% CI=1.64-9.28, P<0.01) in rs1800469 frequency were both significantly higher in patients with CRP level exceeding 10 mg/L. The CT, CC genotype (OR=13.14, 95% CI=2.98-59.60, p<0.01 and OR=24.26, 95% CI=5.28-78.12, P<0.01) and C allele carrier (OR=7.35, 95% CI=4.05-27.64,

Table 3. Stratification analysis of MIF, TGF-β1, IFN-γ and NRAMP1polymorphisms in spinal TB group

Clinical characteristics						Odds ratio (95% CI)/*p* value		
rs755622 (G/C)	G	C	GG	GC	CC	G vs. C	GG vs. GC	GG vs. CC
Sex								
Male	96	40	36	24	8	1.26 (0.49-1.57)	1.65 (0.80-3.26)	1.29 (0.48-4.35)
Female	58	26	21	16	5	0.63	0.85	0.87
Age (year)								
≤30	19	9	7	5	2	0.68 (0.32-2.65)	0.75 (0.16-3.75)	0.69 (0.33-4.48)
>30	135	57	50	35	11	0.73	0.89	0.71
Level of ID herniation								
C2-T8	48	20	18	12	4	1.01 (0.18-2.75)	1.35 (0.643.24)	1.31 (0.68-4.78)
T9-S1	106	46	39	28	9	0.79	0.71	0.82
Pain intensity (VAS)								
0-5	60	26	22	16	5	0.74 (0.61-1.97)	0.89 (0.37-3.97)	0.97 (0.17-2.98)
6-10	94	40	35	24	8	0.74	0.67	0.82
Duration of symptoms								
≤3 Months	68	30	25	18	6	1.02 (0.27-2.31)	1.01 (0.68-2.87)	0.99 (0.49-4.16)
>3 Months	84	36	32	22	7	0.91	0.88	0.81
CRP (mg/L)								
≤10	51	19	20	11	4	1.37 (0.91-2.14)	1.71 (0.42-3.69)	1.04 (0.55-4.79)
>10	103	47	37	29	9	0.47	0.35	0.81
ESR (mm/h)								
≤20	30	12	11	8	2	1.24 (0.68-2.56)	0.91 (0.25-2.98)	1.02 (0.37-6.99)
>20	124	54	46	32	11	0.91	0.98	0.84
Smoking habits								
Smokers	67	29	25	17	6	1.02 (0.67-2.46)	1.24 (0.31-3.51)	0.97 (0.48-3.75)
Non-smokers	87	37	32	23	7	0.89	0.71	0.69
rs1800469 (T/C)	T	C	TT	TC	CC	T vs. C	TT vs. TC	TT vs. CC
Sex								
Male	72	64	21	30	17	1.04 (0.37-2.77)	0.82 (0.31-2.74)	1.135 (0.12-4.56)
Female	34	40	13	18	11	0.17	0.76	0.86
Age (year)								
≤30	14	14	4	6	4	0.78 (0.31-1.27)	0.81 (0.47-4.32)	0.690 (0.43-3.89)
>30	102	90	30	42	24	0.61	0.89	0.64
Level of location								
C2-T8	37	31	11	15	8	1.42 (0.45-3.68)	1.34 (0.19-3.12)	1.37 (0.23-3.98)
T9-S1	79	73	23	33	20	0.57	0.79	0.68
Pain intensity(VAS)								
0-5	45	41	13	19	11	0.91 (0.32-1.89)	0.83 (0.17-2.86)	0.81 (0.26-3.27)
6-10	71	63	21	29	17	0.91	0.88	0.96
Duration of symptoms								
≤3 Months	51	47	15	21	13	0.91 (0.25-1.98)	1.14 (0.61-2.77)	0.88 (0.47-2.96)
>3 Months	65	57	19	27	15	0.75	0.89	0.80
CRP (mg/L)								
≤10	52	16	24	4	6	4.89 (1.64-9.28)	16.54 (5.46-87.29)	6.88 (2.14-26.76)
>10	64	88	10	44	22	<0.01	<0.01	<0.01
ESR (mm/h)								
≤20	37	5	17	3	1	7.35 (4.05-27.64)	13.14 (2.98-59.60)	24.26 (5.28-78.12)
>20	79	99	17	45	27	<0.01	<0.01	<0.01

Smoking habits								
Smokers	51	45	15	21	12	1.08 (0.57-1.93)	1.21 (0.21-2.98)	1.14 (0.39-3.64)
Non-smokers	65	59	19	27	16	0.87	0.86	0.79
rs4803455 (A/C)	A	C	AA	AC	CC	A vs. C	AA vs. AC	AA vs. CC
Sex								
Male	40	96	5	30	33	1.11 (0.35-1.97)	1.02 (0.11-4.79)	1.27 (0.36-5.89)
Female	24	60	3	18	21	0.82	0.97	0.99
Age (year)								
≤30	8	20	1	6	7	0.82 (0.49-2.78)	1.08 (0.34-9.97)	0.81 (0.19-9.87)
>30	56	136	7	42	47	0.87	0.95	0.91
Level of ID herniation								
C2-T8	25	47	3	19	14	1.21 (0.56-3.24)	0.84 (0.17-4.68)	1.29 (0.95-8.67)
T9-S1	39	109	5	29	40	0.28	0.97	0.37
Pain intensity (VAS)								
0-5	24	58	3	18	20	1.31 (0.24-2.97)	1.60 (0.87-5.64)	1.22 (0.44-4.97)
6-10	40	98	5	30	34	0.85	0.97	0.91
Duration of symptoms								
≤3 Months	28	70	4	20	25	0.84 (0.35-1.97)	1.30 (0.91-8.64)	1.41 (0.32-6.12)
>3 Months	36	86	4	28	29	0.66	0.88	0.92
CRP (mg/L)								
≤10	31	37	7	17	10	3.51 (1.92-7.88)	11.64 (3.77-67.850)	13.60 (2.34-78.82)
>10	33	119	1	31	44	<0.01	<0.01	<0.01
ESR (mm/h)								
≤20	20	22	6	8	7	3.44 (1.08-6.87)	14.23 (2.67-77.17)	16.45 (6.68-78.34)
>20	44	134	2	40	47	<0.01	<0.01	<0.01
Smoking habits								
Smokers	28	68	4	20	24	1.08 (0.76-2.64)	1.67 (0.43-68.97)	2.01 (0.94-6.79)
Non-smokers	36	88	4	28	30	0.87	0.54	0.72
rs2069718 (C/T)	C	T	CC	CT	TT	C vs. T	TT vs. TC	TT vs. CC
Sex								
Male	20	116	0	20	48	1.45 (0.97-2.82)	1.12 (0.65-2.76)	NS
Female	12	72	0	12	30	0.84	0.87	NS
Age (year)								
≤30	4	24	0	4	10	0.72 (0.61-3.74)	1.44 (0.71-2.45)	NS
>30	28	164	0	28	68	0.91	0.90	NS
Level of ID herniation								
C2-T8	10	58	0	10	24	1.64 (0.47-3.39)	1.28 (0.34-2.89)	NS
T9-S1	22	130	0	22	54	0.94	0.91	NS
Pain intensity (VAS)								
0-5	13	73	0	13	30	1.33 (0.27-2.71)	0.97 (0.17-2.74)	NS
6-10	19	115	0	19	48	0.76	0.81	NS
Duration of symptoms								
≤3 Months	14	84	0	14	35	0.81 (0.34-2.79)	1.34 (0.41-3.71)	NS
>3 Months	18	104	0	18	43	0.90	0.94	NS
CRP (mg/L)								
≤10	10	60	0	10	25	0.95 (0.21-2.97)	1.27 (0.36-2.95)	NS
>10	22	128	0	22	53	0.95	0.94	NS
ESR (mm/h)								
≤20	6	36	0	6	15	1.35 (0.47-2.74)	1.01 (0.43-2.28)	NS
>20	26	152	0	26	63	0.94	0.95	NS

Smoking habits								
Smokers	14	82	0	14	34	1.11 (0.24-2.87)	0.89 (0.13-2.97)	NS
Non-smokers	18	106	0	18	44	0.97	0.96	NS
rs17235416 (-/TGTG)	+	-	++	+-	--	+ versus -	++ versus +-	++ versus --
Sex								
Male	113	23	47	19	2	1.12 (0.52-2.41)	0.98 (0.41-2.32)	1.39 (0.14-14.01)
Female	69	15	29	11	2	0.75	0.91	0.62
Age (year)								
≤30	22	6	9	4	1	0.86 (0.36-2.05)	0.90 (0.31-2.62)	0.68 (0.07-7.01)
>30	160	32	67	26	3	0.76	0.85	0.64
Level of ID herniation								
C2-T8	57	11	24	9	1	1.12 (0.55-2.31)	1.06 (0.45-2.48)	1.21 (0.29-17.22)
T9-S1	125	27	52	21	3	0.76	0.84	0.74
Pain intensity (VAS)								
0-5	72	14	30	12	1	1.02 (0.61-2.82)	0.82 (0.36-2.27)	1.46 (0.25-17.61)
6-10	110	24	46	18	3	0.70	0.92	0.59
Duration of symptoms								
≤3 Months	81	17	34	13	2	0.89 (0.24-2.63)	1.02 (0.36-2.79)	0.92 (0.33-6.51)
>3 Months	101	21	42	17	2	0.91	0.87	0.79
CRP (mg/L)								
≤10	58	12	24	10	1	1.03 (0.24-3.29)	0.89 (0.45-2.94)	1.28 (0.23-12.61)
>10	124	26	52	20	3	0.90	0.89	0.70
ESR (mm/h)								
≤20	34	8	14	6	1	0.81 (0.26-2.71)	0.87 (0.13-2.94)	0.59 (0.17-7.21)
>20	148	30	62	24	3	0.70	0.80	0.71
Smoking habits								
Smokers	79	17	33	13	2	0.91 (0.31-1.82)	1.04 (0.23-2.67)	0.69 (0.21-6.32)
Non-smokers	103	21	43	17	2	0.81	0.98	0.86

P<0.01) in rs1800469 frequency were both significantly higher in patients with ESR level exceeding 20 mm/h. The frequency of AC, CC genotype (OR=11.64, 95% CI=3.77-67.85, P<0.01 and OR=13.60, 95% CI=2.34-78.82, P<0.01) and C allele (OR=3.51, 95% CI=7.92-7.88, P<0.01) in rs4803455 were both significantly higher in patients with CRP level exceeding 10 mg/L. The AC, CC genotype in rs4803455 (OR=14.23, 95% CI=2.67-77.17, P<0.01 and OR=16.45, 95% CI=6.68-78.34, P<0.01) and the C allele carrier in rs4803455 frequency (OR=3.44, 95% CI=1.08-6.87, P<0.01) were significantly higher in patients with ESR level exceeding 20 mm/h. Other parameters were not significantly different.

Discussion

In this study, we investigated the significance of the relationship between MIF, IFN-γ, TGF-β1, NRAMP1 gene polymorphisms and their susceptibility to TB and evaluated the associations of stratification analysis of their polymorphisms with clinical pathological factors in spinal TB group.

The degree of ESR was associated with TB illness and progressive weight loss, so the determination of ESR can be correlated with the progress of the disease and its prognosis after therapy.CRP is an inflammatory marker in acute trauma and infection, and the level of CRP increased dramatically. Liu et al. have reported that the Kawasaki disease patients with CC genotype in rs223895 had a significantly higher ESR than other genotypes [16]. Other researchers have reported that the ESR and CRP had no significant difference regarding genotypes or allelic frequency between patients with rheumatoid arthritis and healthy controls [17]. However, Liu et al. reported that the CRP levels, and the ESR exhibited multiple correlations with the transcript levels of several inter-

leukins (IL's) in spinal tuberculosis patients [18]. So we speculated that the association of single nucleotide polymorphisms in several candidate genes under study with the ESR and CRP levels may be associated with the degree of inflammation in spinal TB.

MIF is known as a T-cell-derived cytokine primarily appearing in the inflammatory response against pathogens [19]. It can inhibit the random migration of macrophages and the growth of pathogenic Mycobacterium tuberculosis in vitro study [20]. In fact, the C allele and (GC+CC) genotype of rs755622 were reported to be associated with TB susceptibility in the Cambodian population and Chinese population [21, 22]. Similarly, according to our data, increase in the frequency of MIF rs755622 allele C suggested is association with the progression of spinal TB.

TGF-β1 is a potent immunosuppressive cytokine which can regulate growth and differentiation of many cell types and is mainly produced by activated macrophages in response to tissue damage [23, 24]. It can modulate T cell function and also can inhibit macrophage activation and lymphocyte proliferation [25]. Moreover, TGF-β can reduce the ability of macrophage to contain the tubercle bacilli and deactivate macrophages [26, 27]. It was reported that, rs1800469 allele have no association with susceptibility to spinal TB in Chinese iron miners and Chinese people [28, 29], which was consistent with our findings. But our study showed that the C allele and CT, CC genotype may be associated with the degree of inflammatory action in spinal TB. To date, there are no report on association between rs4803455 and TB. In our study, we noticed that the C allele of rs4803455 was associated with the susceptibility to spinal TB and our study also showed that the C allele and AC, CC genotype may be associated with the degree of inflammation in spinal TB.

Interferon gamma (IFN-γ) is mainly produced by T helper 1 cells and plays an important role during the early non-specific phase of host defense through the cell-mediated immune response. The IFN-γ can control M. tuberculosis infection by activated macrophages [30]. Previous studies have shown that the rs2069718 C allele was found to be associated with tuberculosis in the female subgroup in the Chinese pediatric population of North China [14] and genotype TT was associated with an increased risk of TB in Han Taiwanese population [31]. Contrarily, in our study no difference was shown in the rs2069718 polymorphism between spinal TB and healthy controls.

NRAMP1 protein has the characteristics of membrane transporters and is located in late resting macrophage cell, after the pathogen of macrophage cell is transferred to the phagosome membrane [32]. NRAMP1 protein plays an important role in the macrophage cell membrane cation channels through inhibiting the uptake of Fe2+ and Mn2+, and eventually be digested [33, 34]. Nugraha et al. reported that the rs17235416 was found to be associated with tuberculosis in Indonesia population [35], but another studies had shown that there was no association with the risk of TB in the Thais and the Greek population [13, 36]. However, in our study on rs17235416 polymorphism, no difference was shown in the rs17235416 polymorphism between spinal TB and healthy controls.

There were many limitations in this study. First of all, due to the rare incidence of tuberculosis of the spine, this study is relatively limited with a small number of patients. As we carried out the analysis with a small patient group, the data may not represent a patient's actual susceptibility and may have a lower or higher susceptibility. Second, the cases were collected from the same hospital, which may reduce the validity of the results. A further study with a large patient sample may be necessary in Chinese population to provide more justification.

Our results demonstrated for the first time that the polymorphisms in MIF, TGF-β1 and NRAMP1 gene may affect susceptibility to spinal TB and increased risk of developing the disease in the Chinese population and further investigations showed that the polymorphism in rs1800469 and rs4803455may be associated with the degree of inflammation in spinal TB. Advances in our understanding of spinal TB genetics in Chinese population may be valuable markers to predict the risk for the development of spinal TB and to enhance efforts to control this disease.

Disclosure of conflict of interest

None.

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