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Original Article

The single nucleotide polymorphisms in TNF-α promoter are associated with susceptibility and clinical features of pulmonary tuberculosis in Chinese Uygurs

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Abstract: Objective: Tuberculosis (TB) is a serious infectious disease, particularly in Chinese Uygurs. The tumor necrosis factor alpha (TNF-α) that is involved in the interactions between the pathogens and host immune systems is highly related to TB susceptibility and diversity in different ethnic groups. The aim of this study is to investigate the relationship of TNF-α polymorphisms with pulmonary TB susceptibility in Chinese Uygurs. Methods: This case-control study included 306 patients and 280 healthy controls. The characteristics of these participants were recorded and analyzed. Five single nucleotide polymorphisms (SNPs) in TNF-α were genotyped by polymerase chain reaction-restriction fragment length polymorphism. Logistic regression was used to assess the association of the polymorphisms with TB susceptibility. Results: Individuals with BMI<18.5 kg/m^2 or smoking were susceptible to TB. The frequencies of SNP rs1800630 in the pulmonary TB patients were significantly different from those in healthy controls. In addition, the TNF-α SNP rs1799964 (OR=4.037, 95% CI 2.403-6.784) was associated with drug-resistance of TB. However, the polymorphisms of TNF-α were not related to conversion of sputum culture. Conclusion: SNPs in TNF-α promoter are associated with susceptibility and drug-resistance of pulmonary TB in Chinese Uygurs. These TNF-α polymorphisms may be considered as risk factors for active pulmonary TB.

Keywords: Tumor necrosis factor alpha (TNF-α), tuberculosis, polymorphism, susceptibility, single nucleotide polymorphisms (SNPs), drug-resistance

Introduction

It is known that the tuberculosis (TB), an infectious disease caused by Mycobacterium tuberculosis (M. tuberculosis), is one of the major public health epidemics in the world. China has the third highest TB burden in the world following India and Indonesia, accounting for 10% of the global total cases in 2014 [1]. Additionally, the Xinjiang Uygur Autonomous Region in the northwestern of China has one of the highest rates of the incidence and mortality of TB across China [2-4]. It has been reported that TB incidence has been increasing in developed countries [5]. One third of the world's population is infected with M. tuberculosis, and about 5-10% of them developed to the clinical stage of TB [6]. Many host genetic factors have been found to play significant roles in the susceptibility of TB [7, 8].

TNF-α is a proinflammatory pleiotropic cytokine that is mainly produced by macrophages and monocytes [9]. This cytokine plays important roles in many pathogenesis, including sepsis, malignant tumor, heart failure and chronic inflammatory diseases [10-13]. TNF-α gene belongs to the class I region of the major histocompatibility complex that is located on human chromosome 6p21.3 [14]. The single nucleo-

Table 1. Primers used for RFLP to detecting TNF-α gene polymorphisms

SNP Site	Forward primer Reverse primer	PCR fragment size (bp)	Annealing temperature (°C)	Restriction enzyme, incubation temperature (°C)	RFLP fragment size (bp)
rs361525-238	5'-AAACAGACCACAGA CCTGGTC-3' 5'-CTCACACTCCCCATCCTCCCGGATC-3'	155	63	Bam H1, 37	130+25
rs1800629-308	5'-GAGGCAATAGGTTTTGAGGGCCAT-3' 5'-GGGACACACAAGC ATCAAG-3'	147	58	Nco I, 37	122+25
rs1799724-857	5'-GGCTCTGAGGAAT GGGTTAC-3' 5'-CCTCTACATGGCC CTGTCTAC-3'	128	55	Mae II, 65	107+21
rs1800630-863	5'-GGCTCTGAGGAAT GGGTTAC-3' 5'-CTACATGGCCCTGTCTTCGTTACG-3'	125	55	Mae II, 65	101+24
rs1799964-1031	5'-TATGTGATGGACTCACCAGGT-3' 5'-CCTCTACATGGCCCTGTCTT-3'	264	55	Bpi I, 37	68+183+13

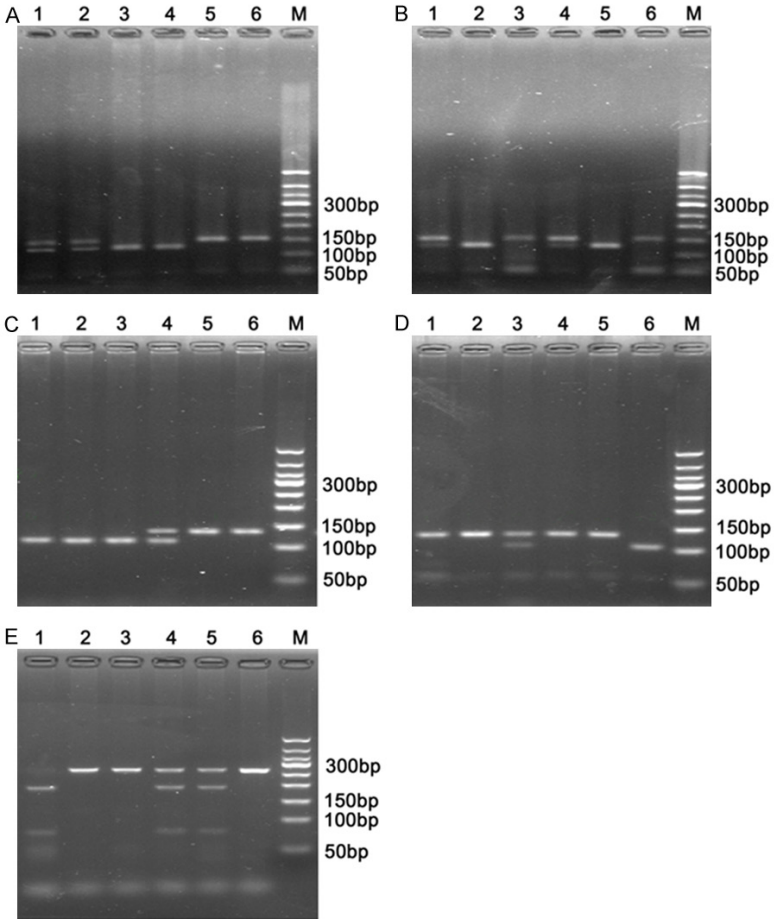

Figure 1. Gel confirmation of TNF-α gene promoter polymorphisms. PCR products were digested with restriction enzymes and separated on 3% polyacrylamide gels. A: rs361525 (-238): 1 & 2, GA; 3 & 4, GG; 5 & 6, AA; B: rs1800629 (-308): 1 & 4, GA; 2 & 5, GG; 3 & 6, AA; C: rs1799724 (-857): 1-3 CC; 4 CT; 5 & 6 TT; D: rs1800630 (-863): 1, 2, 4 & 5, CC; 3, CA; 6, AA; E: rs1799964 (-1031): 1, CC; 2, 3 & 6 TT; 4 & 5 TC. M, marker.

tide polymorphisms (SNPs) in the promoter of TNF-α have been identified, and they are thought to affect the production of TNF-α [15]. However, reports on the role of TNF-α polymorphisms in TB are not consistent till now. A meta-analysis of 18 case-control studies reported that rs1800629 G>A and rs361525 G>A polymorphisms of TNF-α increased the risk of pulmonary TB (PTB) susceptibility regardless of the ethnicity and HIV statue [16]. The rs361525 G>A was significantly associated with PTB in Asian population, while rs1800629 G>A increased the risk of PTB in African population [16]. Another meta-analysis, however, indicated that rs1800629 polymorphism was not associated with the risk of TB since the significant risk for rs1800629 A allele was found among Asians but not the Caucasians [17]. Another meta-analysis showed that there were no correlations between the rs1800629 A/G or rs361525 A/G polymorphisms and PTB susceptibility [18]. In contrast, the rs1799724 T>C polymorphism was associated with PTB susceptibility in Asian population [18].

The Chinese Uygur, one of the minority ethnic groups, has higher incidence of TB than the

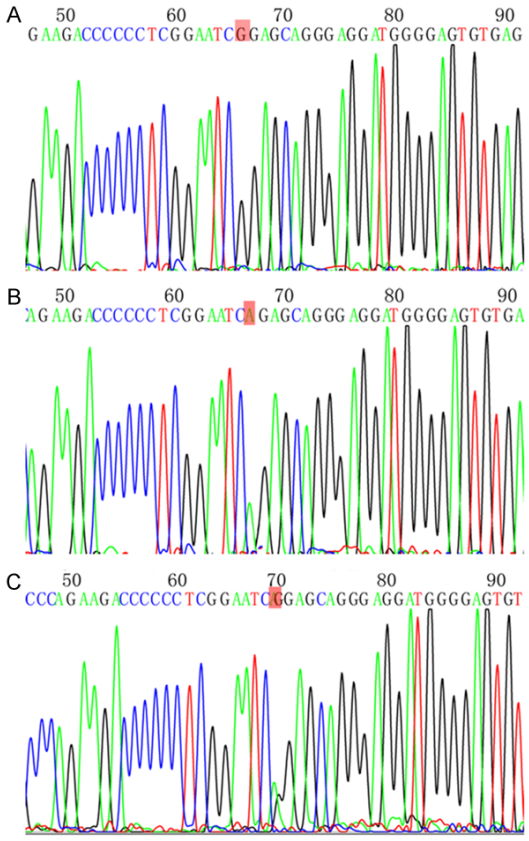

Figure 2. Sequencing confirmation of TNF-α gene promoter polymorphisms rs361525 (-238). PCR products were forward sequenced. A: GG; B: AA; C: AG.

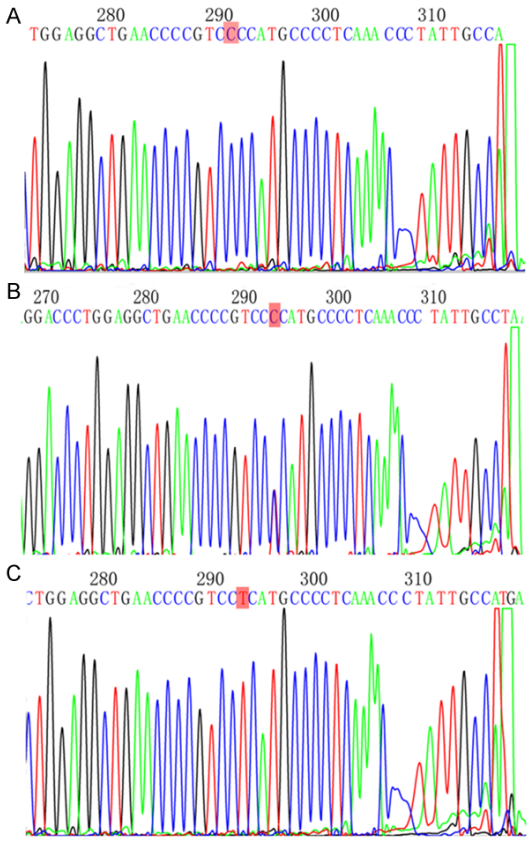

Figure 3. Sequencing confirmation of TNF-α gene promoter polymorphisms rs1800629 (-308). PCR products were reverse sequenced. A: CC; B: CT; C: TT.

Chinese Han and other Chinese minorities in Xinjiang province [3], suggesting that this group of people may be more susceptible to TB. Here, we evaluated the frequency of the five TNF-α promoter polymorphisms (rs1799964, rs1800630, rs1799724, rs1800629 and rs361525) in Uygur PTB patients and healthy controls to uncover the relationship between TNF-α gene promoter polymorphisms and PTB in this population.

Materials and methods

Subjects

A total of 306 randomly selected Uygur patients with active PTB were recruited at Kashgar Prefecture Chest Hospital (Xinjiang, China) and Chest Hospital of Xinjiang Uygur Autonomous Region, from January 2014 to August 2015. Written informed consent was obtained from the patients. This study was approved by the Research Ethics Committee in the First Affiliated Hospital of Xinjiang Medical University (Xinjiang, China).

All patients with PTB were diagnosed based on the following methods: sputum smear or culture, clinical-radiological examination and histological diagnosis. Patients with comorbidities such as lung carcinoma, asthma, bronchiectasis, diabetes mellitus and other immunosuppressive conditions were excluded. The 280 age- and sex-matched healthy controls were recruited during physical examination at the People's Hospital of Xinjiang Uygur Autonomous Region during the same time period as the TB patients were collected. The controls had no history of TB diseases. All participants were confirmed as HIV negative.

Extraction of the genomic DNA and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP)

A total of 1 mL of venous blood samples from each participant was collected into Ethylene

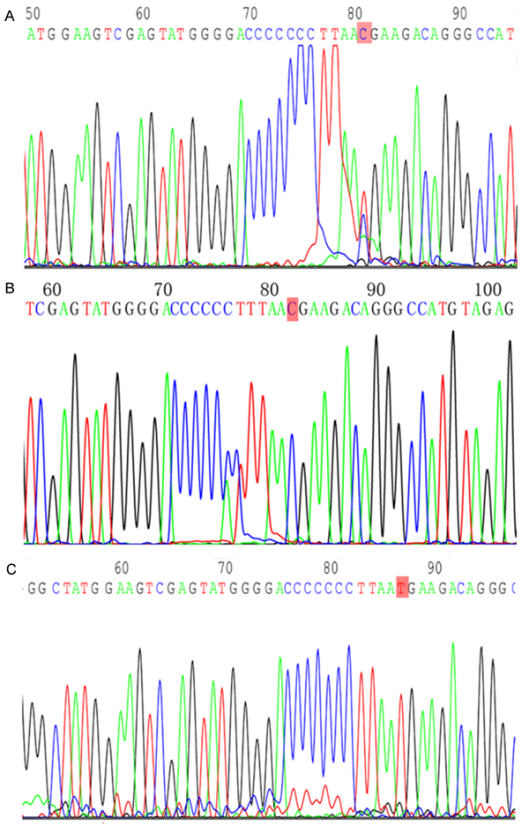

Figure 4. Sequencing confirmation of TNF-α gene promoter polymorphisms rs1799724 (-857). PCR products were forward sequenced. A: CT; B: CC; C: TT.

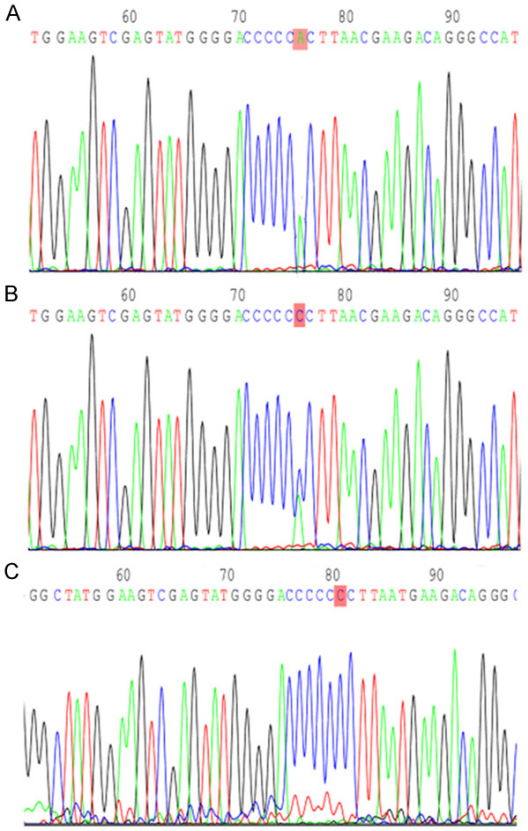

Figure 5. Sequencing confirmation of TNF-α gene promoter polymorphisms rs1800630 (-863). PCR products were forward sequenced. A: AA; B: CA; C: CC.

Diamine Tetra acetic Acid (EDTA) glass tubes and were frozen at -20°C. Total genomic DNA was extracted from peripheral blood with the Whole Blood DNA Extraction Kit (Tiangen Biotech, Co., Ltd, Beijing, China) according to the manufacturer's instructions. PCR was performed to investigate the TNF-α gene promoter polymorphisms by specific primers [19]. The primer sequences were shown in **Table 1**. PCR products were digested with restriction enzymes (Thermo Fisher Scientific, MA, USA) and separated on 3% polyacrylamide gels (**Figure 1**). Direct sequencing of 10% of the PCR products of all subjects was used to validate the PCR-RFLP results (**Figures 2**-**6**).

Statistical analysis

The SPSS 19.0 was used for all statistical analyses. The genotype and the allele frequencies for polymorphisms were calculated based on the observed genotype counts. All the polymorphisms were tested for Hardy-Weinberg equilibrium. Associations between TNF-α polymorphisms and TB were estimated using adjusted odds ratios (AORs) and 95% CIs obtained from logistic regression models. The *p* value less than 0.05 was considered as significant difference.

Results

Characteristics of the subjects

This study included 306 PTB cases and 280 controls (age and sex matched). The clinical characteristics of included participants were shown in **Table 2**. For the PTB patients, 56.5% were males and 43.5% were females with the average age of 48.82±17.27 years. For the controls, 55.7% were males and 44.3% were females with the average age of 47.69±13.37 years. Among those 306 PTB patients, there were 217 drug-sensitive patients and 89 drug-resistant ones. In the 89 drug resistant PTB patients, there were 8 multidrug-resistant (MDR) TB. As shown in **Table 2**, there was no

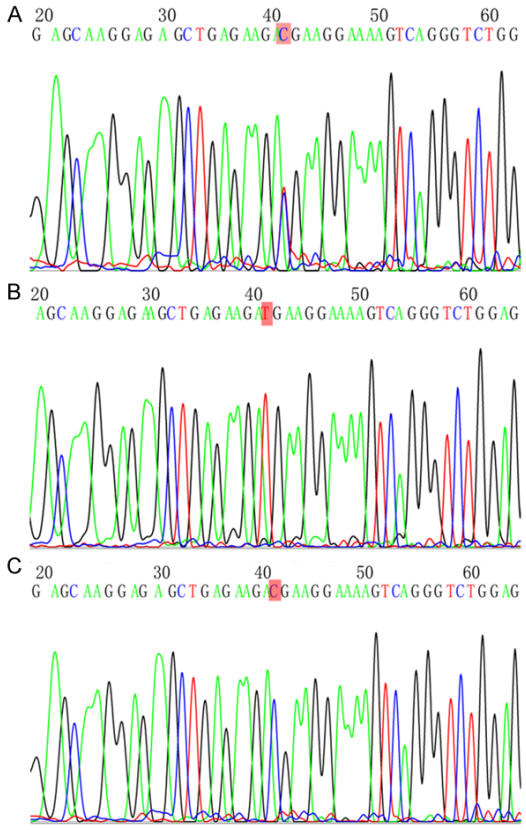

Figure 6. Sequencing confirmation of TNF-α gene promoter polymorphisms rs1799964 (-1031). PCR products were forward sequenced. A: CT; B: TT; C: CC.

significant difference in age, gender, alcohol intake, presence of TB history of household or education background between PTB patients and controls. However, BMI and smoking showed significant difference between these two groups (P<0.05), demonstrating that thin individuals (BMI<18.5) (OR=3.012, 95% CI 2.151-4.218) and smokers (OR=1.888, 95% CI 1.357-2.626) are more likely to suffer from PTB in Chinese Uygurs.

Association of the TNF-α genotype and allele frequencies with PTB

To assess the role of SNPs of TNF-α in susceptibility of PTB in Chinese Uygurs, 5 SNPs (rs1799964, rs1800630, rs1799724, rs1800629 and rs361525) in the promotor of TNF-α were studied (**Figures 1**-**6**). Genotype frequencies for 5 SNPs were in Hardy-Weinberg Equilibrium in both case and control groups (P>0.05) (**Table 3**). We found that the TNF-α rs1800629 allele was in high frequency of mutation in Uygurs, namely, G/A accounted for 37.9% in the health controls and 42.3% in PTB cases. The SNP rs1800630 (dominant model, OR=2.612, 95% CI 1.811-5.012; recessive model, OR=4.696, 95% CI 1.020-21.621; additive model, OR=2.410, 95% CI 1.577-3.683) showed significant association with PTB susceptibility (**Table 4**). Compared with CC genotype, the AA and CG genotype may increase the risk of PTB (AA vs CC, OR=5.660, 95% CI 1.226-26.124; CA vs CC, OR=2.502, 95% CI 1.635-3.829) (**Table 3**). Importantly, the -863 A allele (OR=2.525, 95% CI 1.729-3.687) showed significant difference between the cases and the controls (P<0.05) (**Table 3**). In contrast, there was no association between other SNPs and the susceptibility to PTB in Chinese Uygurs.

Association of TNF-α haplotypes with PTB

To acquire more comprehensive information about the association between TNF-α and PTB, we then calculated the haplotype frequencies. By analyzing TNF-α haplotypes in PTB cases and healthy controls, we found that 12 haplotypes were significantly associated with PTB (**Table 5**). Among these haplotypes, C-C-C-G-G (OR=0.281, 95% CI 0.111-0.714) was a protective factor for PTB, while other 11 haplotypes were risk factors.

Association of TNF-α polymorphisms with clinical outcomes of PTB

Next we investigated the relationship between SNPs and drug-resistant or conversion of sputum culture. In conversion of sputum, two-month treatment was used as a cutoff to divide the patients. The SNP rs1799964 (TC+CC vs TT, OR=4.037, 95% CI 2.403-6.784) showed significant association with drug resistance (**Table 6**). However, there were no significant association between the polymorphisms of TNF-α and conversion of sputum culture (**Table 6**).

Discussion

The TNF-α is an essential cytokine for the granuloma formation, and mice deficient in TNF-α fail to form organized granuloma, resulting in widespread dissemination of M. tuberculosis infection and rapid death of the infected animals [20]. The SNPs in the TNF-α have been linked to an increased risk of TB [14]. However,

Table 2. Basic characteristics of the participants in the study

Characteristics	Controls (N=280)	PTB Cases (N=306)	OR (95% CI)
Age, year (Mean ± SD)	48.82±17.27	47.69±13.37	
Gender (Male:Female)	156:124	173:133	
BMI (<18.5:≥18.5)	105:175	197:109*	3.012 (2.151-4.218)
Smoking status (Smoker:No-smoker)	133:147	193:113*	1.888 (1.357-2.626)
Alcohol intake status (Drinker:No-drinker)	129:151	161:145	1.300 (0.939-1.799)
Presence of TB history of household (Presence:Absence)	78:212	95:211	1.224 (0.858-1.745)
Education background (Junior high school or below:Senior high school or above)	152:128	145:161	0.758 (0.548-1.050)

PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval; SD, standard deviation; BMI, body measure index. *P<0.05, compared with control.

Table 3. Genotype and allele frequency distributions of TNF-α polymorphisms between controls and cases

dbSNP ID	Genotype/Allele	Controls n (%)	PTB cases n (%)	OR (95% CI)
rs1799964-1031 T>C	TT	180 (64.3)	192 (62.7)	1
	TC	84 (30.0)	102 (33.3)	1.138 (0.800-1.621)
	CC	16 (5.7)	12 (4.0)	0.703 (0.324-1.527)
	T	444 (79.3)	486 (79.4)	1
	C	116 (20.7)	126 (20.6)	0.992 (0.748-1.317)
HWE-P		0.147	0.734	
rs1800630-863 C>A	CC	240 (85.7)	212 (69.3)	1
	CA	38 (13.6)	84 (27.4)*	2.502 (1.635-3.829)
	AA	2 (0.7)	10 (3.3)*	5.660 (1.226-26.124)
	C	518 (92.5)	508 (83.0)	1
	A	42 (7.5)	104 (17.0)*	2.525 (1.729-3.687)
HWE-P		0.714	0.637	
rs1799724-857 C>T	CC	186 (66.4)	202 (66.0)	1
	CT	88 (31.4)	94 (30.7)	0.984 (0.691-1.399)
	TT	6 (2.1)	10 (3.3)	1.535 (0.547-4.305)
	C	460 (82.1)	498 (81.4)	1
	T	100 (17.9)	114 (18.6)	1.053 (0.782-1.417)
HWE-P		0.233	0.816	
rs1800629-308 G>A	GG	166 (59.3)	160 (52.3)	1
	GA	106 (37.9)	130 (42.3)	1.272 (0.909-1.781)
	AA	8 (2.8)	16 (5.4)	2.075 (0.864-4.983)
	G	438 (78.2)	450 (73.5)	1
	A	122 (21.8)	162 (26.5)	1.292 (0.987-1.692)
HWE-P		0.636	0.110	
rs361525-238 G>A	GG	266 (95.0)	283 (92.5)	1
	GA	13 (4.6)	22 (7.2)	1.591 (0.785-3.222)
	AA	1 (0.4)	1 (0.3)	0.940 (0.058-15.103)
	G	545 (97.3)	588 (96.1)	1
	A	15 (2.7)	24 (3.9)	1.483 (0.770-2.857)
HWE-P		0.670	0.422	

PTB, pulmonary tuberculosis; OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg Equilibrium. *P<0.05, compared with control.

the association of TNF-α polymorphisms with TB may differ among different ethnicities [16-18, 21]. Therefore, we performed this case-control study to investigate the associations

Table 4. Association of TNF-α SNP genotypes with PTB under different genotype models

dbSNP ID	Allele	Genetic Model	OR (95% CI)	ORc (95% CI)	ORa (95% CI)
rs1799964-1031	T>C	Dominant	1.168 (0.815-1.674)	1.215 (0.901-1.883)	1.321 (0.971-1.955)
		Recessive	0.673 (0.313-1.450)	0.714 (0.362-1.510)	0.831 (0.380-1.613)
		Additive	1.167 (0.823-1.654)	1.215 (1.011-1.883)	1.241 (1.053-2.001)
rs1800630-863	C>A	*Dominant	2.660 (1.759-4.023)	2.744 (1.907-4.663)	2.612 (1.811-5.012)
		*Recessive	4.696 (1.020-21.621)	4.965 (0.917-21.962)	4.811 (0.689-22.080)
		*Additive	2.410 (1.577-3.683)	2.496 (1.526-3.795)	2.512 (1.610-3.910)
rs1799724-857	C>T	Dominant	1.019 (0.723-1.435)	1.081 (0.715-1.480)	1.240 (0.700-1.681)
		Recessive	1.543 (0.553-4.302)	1.625 (0.521-3.981)	1.782 (0.590-4.122)
		Additive	0.967 (0.682-1.373)	1.121 (0.651-1.692)	1.181 (0.711-1.765)
rs1800629-308	G>A	Dominant	1.329 (0.958-1.843)	1.392 (1.045-2.001)	1.481 (1.082-2.110)
		Recessive	1.462 (0.614-3.479)	1.501 (0.735-3.711)	1.558 (0.762-3.802)
		Additive	1.212 (0.871-1.689)	1.290 (0.995-1.823)	1.693 (1.020-2.055)
rs361525-238	G>A	Dominant	1.544 (0.778-3.064)	1.580 (0.844-3.290)	1.871 (0.911-3.392)
		Recessive	0.915 (0.057-14.694)	0.931 (0.122-14.800)	1.071 (0.155-14.940)
		Additive	1.591 (0.786-3.222)	1.645 (0.882-3.351)	1.670 (0.944-3.410)

OR, odds ratio; CI, confidence interval. OR for comparison of genetic models by conditioned logistic regression analysis. ORc=OR value after Bonferroni correction. ORa=OR value adjusted for BMI and smoking. *SNP rs1800630 showed significant association with PTB susceptibility.

Table 5. Association between haplotypes frequencies of TNF-α and PTB susceptibility

	Haplotype	Control (%)	Case (%)	OR (95% CI)
TNF-α -1031/-863/-857/-308/-238	T-C-C-G-G	42.3	34.3	1
	T-C-T-G-G	7.3	9.1	1.573 (0.837-2.874)
	T-C-C-A-G	16.1	24.5	1.873 (1.190-2.948)*
	T-C-T-A-G	10.4	7.7	0.930 (0.510-1.697)
	C-C-C-A-G	4.7	1.7	0.432 (0.149-1.253)
	C-C-C-G-G	8.4	2.0	0.281 (0.111-0.714)*
	C-A-C-G-G	6.2	13.8	2.776 (1.491-5.170)*
TNF-α -1031/-863/-857/-308	T-C-C-G	43.2	36.5	1
	T-C-C-A	16.5	25.8	1.855 (1.189-2.896)*
	C-A-C-G	6.2	14.1	2.733 (1.474-5.067)*
TNF-α -1031/-863/-308/-238	T-C-G-G	48.7	43.6	1
	C-A-G-G	6.6	13.4	2.329 (1.274-4.259)*
TNF-α -863/-857/-308/-238	C-C-G-G	50.5	36.4	1
	A-C-G-G	13.5	7.9	2.387 (1.342-4.237)*
	C-C-A-G	21.0	26.5	1.757 (1.157-2.667)*
TNF-α -1031/-863/-308	T-C-G	49.8	45.6	1
	C-A-G	6.6	13.7	2.469 (1.342-4.545)*
TNF-α -863/-857/-308	C-C-G	52.4	38.4	1
	A-C-G	7.7	14.9	2.695 (1.520-4.762)*
TNF-α -863/-238	C-G	89.4	79.8	1
	A-G	7.7	14.6	2.123 (1.238-3.636)*
TNF-α -863/-308	C-G	60.0	49.2	1
	A-G	7.8	14.6	2.278 (1.307-3.968)*

*Haplotypes significantly associated with PTB.

between TNF-α gene promotor polymorphisms and PTB in Chinese Uygurs. We found a significant difference in the genotype frequencies of the rs1800630 C>A polymorphisms between

Table 6. Association of TNF-α polymorphisms and drug-sensitivity or sputum culture conversion in PTB patients

dbSNP ID	Genotype	Drug-sensitivity (N=306)		OR (95% CI)	Months of treatment for negative sputum culture (N=306)		OR (95% CI)
		Drug-sensitive N=217 n (%)	Drug-resistant N=89 n (%)		≤2 months N=239 n (%)	>2 months N=67 n (%)	
rs1799964-1031	TT	157 (72.4)	35 (36.8)	1	155 (64.9)	37 (55.2)	1
	TC+CC	60 (27.6)	54 (63.2)	4.037 (2.403-6.784)	84 (35.1)	30 (44.8)	1.496 (0.863-2.593)
rs1800630-863	CC	157 (72.4)	55 (61.8)	1	167 (69.9)	45 (67.2)	1
	CA+AA	60 (27.6)	34 (38.2)	1.618 (0.961-2.723)	72 (30.1)	22 (32.8)	1.134 (0.635-2.025)
rs1799724-857	CC	149 (68.7)	53 (59.6)	1	155 (64.9)	37 (55.2)	1
	CT+TT	68 (31.3)	36 (40.4)	1.488 (0.893-2.482)	74 (35.1)	30 (44.8)	1.698 (0.974-2.960)
rs1800629-308	GG	119 (54.8)	41 (46.1)	1	121 (50.6)	29 (43.3)	1
	GA+AA	98 (45.2)	48 (53.9)	1.422 (0.866-2.333)	108 (49.4)	38 (56.7)	1.468 (0.848-2.541)
rs361525-238	GG	203 (93.5)	80 (89.9)	1	223 (93.3)	59 (88.1)	1
	GA+AA	14 (6.5)	9 (10.1)	1.631 (0.679-3.919)	16 (6.7)	8 (11.9)	1.654 (0.650-4.205)

OR, odds ratio; CI, confidence interval.

PTB cases and healthy controls. However, no significant differences between cases and controls were found with other SNPs. This result was inconsistent with the previous meta-analyses [16-18], which may be resulted from an insufficient number of PTB cases and controls in our study. Another reason for this may be that we analyzed Uygur ethnic population rather than Han population in China.

A number of studies [16-18, 21] have investigated the association between the TNF-α rs1800629 (-308 G/A) polymorphism and the susceptibility to TB in different populations. In this study, the TNF-α rs1800629 was in highly frequent mutation regions in Uygurs. Namely, G/A accounted for 37.9% in the health control, and 42.3% in PTB cases, which is consistent with previous report [22]. However, the rs1800629 A variant was not related to an increased risk of the Uygur TB. The PTB patients with the CA and AA genotypes at the rs1800630 locus were correlated with the risk for PTB with ORs (95% CI) of 2.502 (1.635-3.829) and 5.660 (1.226-26.124) compared the patients with the GG genotype. This result is consistent with the report in Chinese people [23].

All haplotypes containing the TNF-α rs1800630 A allele were significantly correlated with PTB, suggesting it may play a role in PTB development. The OR of PTB for the TNF-α haplotype C-C-C-G-G was 0.281, while the OR for haplotype C-A-C-G-G, in which the rs1800630 allele changed from C to A, was 9.900. This result supports the hypothesis that the rs1800630 A allele is associated with the risk of PTB, although the PTB OR for haplotype C-C-C-G-G was statistically significant. Among the 306 clinical isolates of M. tuberculosis, 217 (70.9%) were drug-sensitive, 89 (29.1%) were resistant to at least one drug and 8 (2.6%) were multi-drug-resistant (MDR). To date, variants in the HLA-DRB1, -DQB1 [24] and SLC11A1 [25] have been shown to be associated with M. tuberculosis drug sensitivity. Interestingly, we observed that rs1799964 was significantly associated with drug-resistance, whereas it was not related to the susceptibility to PTB.

We also investigated the risk factors of PTB by collecting the basic characteristics of both patients and controls. It was reported that differences in BMI could be recognized in healthy populations of different ethnicity [26-28]. In this study, it was revealed that individuals with BMI<18.5 kg/m^2 were more likely to suffer from PTB in Chinese Uygurs, which was consistent with the reports of Jiang DB et al. [29] and Semunigus T et al. [30]. Finally, smoking is significantly associated with increased risks of TB infection and diseases [31, 32]. Patra J et al. [33] revealed that the risks of active TB were significantly associated with smoking in women. Consistently, we found that patients who smoke had a higher risk of PTB in Chinese Uygurs. However, we didn't analyze whether the risk of PTB was related to gender. In contrast, Jiang DB et al. [29] reported opposite outcomes that smoking didn't increase the risk of TB. We

speculated that the inconsistence may result from the difference in subject population. Participants in Jiang DB's study were collected only in Kashgar Prefecture while the subjects were from both Kashgar Prefecture and Urumqi, Xinjiang in our study.

Taken together, our study for the first time showed the association of the five TNF-α polymorphisms, including their alleles and haplotypes, with clinical features and risk of PTB in Chinese Uygurs. Future studies with sufficient case numbers will be needed to further confirm the role of TNF-α polymorphisms in the susceptibility and prognosis of PTB.

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Disclosure of conflict of interest

None.

Authors' contribution

J. D. and F. L. designed the study. X. H. and G. W. did sequencing and data analysis. X. Z. extracted DNA from blood samples. X. H., Y. Z. and X. Z. collected data and blood samples. Q. L. and F. Z. drafted the manuscript, performed experiments and data analysis. J. D. also contributed to manuscript writing. All authors read and approved the final manuscript.

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