Letter to the Editor

Interleukin 8 Gene Polymorphisms Are Not Associated with Tuberculosis Susceptibility in the Chinese Population*

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Interleukin 8 (IL8) is an important chemokine that elicits host immune response against tuberculosis (TB). However, whether there is an association between *IL8* gene polymorphism and TB susceptibility in the Chinese population is unknown. *IL8* gene was amplified and sequenced to search for nucleotide polymorphisms among the Chinese population. Four single nucleotide polymorphisms (SNPs) were identified, selected, and analyzed in a cohort of 438 patients with TB and 536 healthy controls. Allelic, genotypic, and haplotypic analysis demonstrated that the distribution of the four *IL8* SNPs between patients with TB and healthy controls was not significantly different (*P*>0.05). The four *IL8* SNPs detected in this study were not associated with TB susceptibility in the Chinese population. Secretion of IL8 by peripheral blood cells was greatly stimulated upon exposure to *Mycobacterium tuberculosis* whole cell extract, but such enhanced secretion was not associated with the *IL8* rs4073 alleles.

Tuberculosis (TB) is a worldwide threat that passively infects one-third of the world population and kills approximately two million people each year. As one of the most important cytokine, IL8 is essential to control *Mycobacterium tuberculosis* (*M. tuberculosis*) infection in humans. However, whether there is an association between *IL8* gene polymorphism and TB susceptibility is not clear, especially in the Chinese population, presenting a high TB burden.

Ma et al. reported that rs4073 polymorphism in the *IL8* promoter region was associated with TB susceptibility in two ethnic groups in the United States[1]. In contrast, Cooke et al. reported that this polymorphism was not a true factor affecting TB susceptibility in Gambia[2]. Debates[3] on conflicting results raise the question whether there is an association between *IL8* gene polymorphism and TB susceptibility, especially in countries with high TB burden. In this study, we evaluated the association between *IL8* genetic polymorphisms and TB susceptibility in a large Chinese population. The effect of *IL8* rs4073 polymorphism on IL8 expression level was also determined.

To identify single nucleotide polymorphisms (SNPs) among a Chinese population, *IL8* gene (including the promoter region, but excluding most of the first intron) was sequenced using 60 DNA samples from 30 TB patients and 30 healthy controls. Five genetic polymorphisms, rs56090111, rs4073, rs2227541, rs2227543, and rs188973626, were detected (Table 1). Two of the polymorphisms were located in the promoter region and the other three were located in the introns. No SNP was detected in exons. Further analysis showed that two SNPs rs2227541 and rs2227543 were in perfect linkage disequilibrium (LD) (Table 1). Thus, rs2227541 SNP was not used for further genotyping.

The genotypic distributions in patients with TB and controls were checked separately for Hardy-Weinberg equilibrium (HWE) test. No deviation was observed with all 4 polymorphism sites (*P*>0.05). The frequencies of the four SNPs

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(rs56090111, rs4073, rs2227543, and rs188973626) in 438 patients with TB and 536 healthy controls were evaluated. No statistically significant allelic, genotypic, or haplotypic difference was observed between the patients and the control groups (Table 2). These results suggest that the four polymorphisms are not associated with TB risk or resistance in the Chinese population.

Table 1. *IL-8* Gene Polymorphisms Identified by Sequencing

SNP rs Number	Allele (frequency)	Genotype (frequency)
rs56090111	- (0.05) / TTAA (0.95)	-- (0.00) / -TTAA (0.10) / TTAA TTAA (0.90)
rs4073	A (0.48) / T (0.52)	AA (0.22) / AT (0.53) / TT (0.25)
rs2227541[a]	- (0.48) / A (0.52)	-- (0.20) / -A (0.57) / AA (0.23)
rs2227543[a]	C (0.52) / T (0.48)	CC (0.23) / CT (0.57) / TT (0.20)
rs188973626	A (0.01) / G (0.99)	AA (0.00) / AG (0.02) / GG (0.98)

Note. [a]These two SNPs presented perfect LD.

Table 2. *IL-8* Gene Allele, Genotype, and Haplotype Association with TB

Item		TB (%)	Control (%)	χ^2	*P*	OR (95% CI)
Allele						
rs56090111	TTAA	850 (97.0)	1046 (97.6)	0.546	0.460	0.813 (0.468-1.410)
	-	26 (3.0)	26 (2.4)			
rs4073	A	369 (42.1)	436 (40.7)	0.419	0.517	1.062 (0.886-1.273)
	T	507 (57.9)	636 (59.3)			
rs2227543	C	523 (59.7)	647 (60.4)	0.085	0.770	0.973 (0.811-1.168)
	T	353 (40.3)	425 (39.6)			
rs188973626	A	4 (0.5)	2 (0.2)	1.145	0.285	2.454 (0.448-13.431)
	G	872 (99.5)	1070 (99.8)			
Genotype						
rs56090111	TTAA TTAA	413 (94.3)	510 (95.1)	1.428	0.490	
	-TTAA	24 (5.5)	26 (4.9)			
	--	1(0.2)	0 (0.0)			
rs4073	AA	75 (17.1)	88 (16.4)	0.522	0.770	
	AT	219 (50.0)	260 (48.5)			
	TT	144 (32.9)	188 (35.1)			
rs2227543	CC	148 (33.8)	192 (35.8)	0.736	0.692	
	TC	227 (51.8)	263 (49.1)			
	TT	63 (14.4)	81 (15.1)			
rs188973626	AA	0 (0.0)	0 (0.0)	1.149	0.284	
	AG	4 (0.9)	2 (0.4)			
	GG	434 (99.1)	534 (99.6)			
Haplotype[*]						
	TTAA/A/C/G	24.47 (2.8)	17.02 (1.6)	-	-	-
	TTAA/A/T/A	0.11 (0.0)	1.94 (0.2)	-	-	-
	TTAA/A/T/G	318.43 (36.4)	391.69 (36.5)	0.176	0.675	1.041 (0.862-1.258)
	TTAA/T/C/A	3.89 (0.4)	0.06 (0.0)	-	-	-
	TTAA/T/C/G	491.72 (56.1)	629.84 (58.8)	0.176	0.675	0.960 (0.795-1.160)
	TTAA/T/T/G	11.37 (1.3)	5.45 (0.5)	-	-	-
	-/A/C/G	2.90 (0.3)	0.07 (0.0)	-	-	-
	-/A/T/G	23.08 (2.6)	25.28 (2.4)	-	-	-
	-/T/T/G	0.00 (0.0)	0.65 (0.0)	-	-	-
	-/T/C/G	0.02 (0.0)	0.00 (0.0)	-	-	-

Note. [*]Frequencies lower than 3% both in TB and control groups were not evaluated by χ^2 test.

Previous studies reported that AA genotype of the IL8 rs4073 SNP in the White and African population in the USA was a risk factor for TB[1]. However, in the African population in Gambia, the same SNP was not associated with TB susceptibility[2]. In this study, we tested the distribution of *IL8* gene polymorphisms, including the rs4073 polymorphism, in a large population in China. No statistically significant difference in genetic polymorphism frequencies between patients with TB and healthy controls was observed. The possible explanation for these different results may be due to the fact that the subjects studied were of different ethnics and geographic locations. Two polymorphisms, rs2227538 and rs2227542, are reported to have minor frequency allele 0.18 and 0.1, respectively, in the African American population according to the NCBI SNP database. However, these two polymorphisms were not detected among DNA samples in the Chinese population evaluated in this study. This result indicate that these two polymorphisms might be of low frequency or do not exist in the Chinese population. The rs4073 polymorphism frequency is also quite divergent in different ethnic groups. The minor frequency allele for rs4073 is 0.27, 0.4, and 0.4 in the African American, European, and Asian population, respectively, according to the NCBI SNP database. Thus, association of *IL8* gene polymorphism and TB susceptibility in different geographic regions should be studied independently.

Biological study is another important evidence for confirming the reliable influence of genetic polymorphisms on disease susceptibility. The rs4073 SNP is one of the most studied *IL8* gene polymorphisms for disease susceptibility. However, whether this polymorphism influences IL8 expression in patients with TB is unknown. To answer this question, we detected IL8 concentration in patients with TB and healthy controls with different rs4073 genotypes. Peripheral blood samples from 30 patients with TB and 30 healthy controls (10 patients presenting each of AA, AT, and TT genotype for both groups) were collected. Each blood sample was treated with whole cell extracts of *M. tuberculosis* (WCE) and phosphate-buffered saline (PBS) for 24 h and IL8 concentration in the supernatant was determined by ELISA.

No significant difference in IL8 secretion levels was observed among the three genotypes either treated with PBS or WCE (P>0.05) in either the control (Figure 1A) or patient group (Figure 1B). However, the IL8 expression level was significantly elevated when blood samples were treated with WCE compared to PBS both in healthy controls (P=2.5E-10) and patients with TB (P=1.5E-10) (Figure 1C). Blood samples from patients with TB treated with WCE secreted much higher IL8 levels than those from healthy controls (P=7.9E-07), but there was no difference in PBS-treated samples (P>0.05) (Figure 1C). These results indicate that the IL8 expression level is associated with *M. tuberculosis* antigen stimulation and active disease status, but not with rs4073 polymorphism.

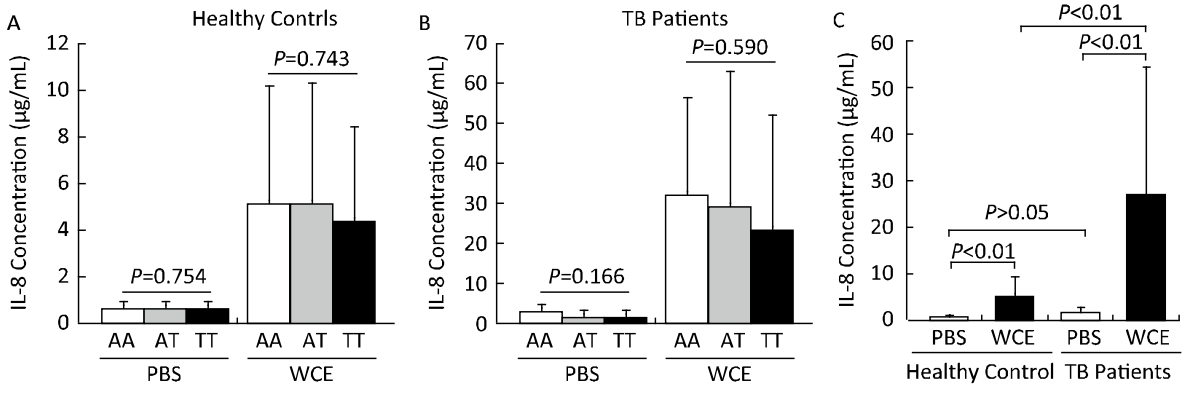

Figure 1. Detection of IL8 secreted by blood cells from patients and healthy controls with different rs4073 genotypes and under *M. tuberculosis* antigen stimulation. (A) IL8 concentration measured in healthy controls (each genotype for ten people). (B) IL8 concentration measured in patients with TB (each genotype for ten people). WCE means blood treated with whole cell extract of *M. tuberculosis*, PBS means blood treated with PBS as control. (C) IL8 concentration secreted by blood cells from healthy controls (n=30) and patients with TB (n=30).

The rs4073 SNP is one of the most studied *IL8* gene polymorphism. Previous studies indicated distinct influences of IL8 expression in several diseases. The AA and AT genotypes have been reported to be significantly associated with higher mucosal IL8 levels in gastric cancer patients from Japan[4]. The mean and median fecal IL8 levels in American patients with *Clostridium difficile* diarrhea are significantly higher for the AA genotype than that for the AT and TT genotypes[5]. In contrast, in Korean patients with idiopathic pulmonary fibrosis, IL8 protein concentration in bronchial alveolar lavage (BAL) fluid was significantly higher in subjects with rs4073 TT genotype than in subjects with AA genotype[6]. The rs4073T promoter also presents a much higher activity than the rs4073A promoter in MKN45, SC-M1 gastric carcinoma cells, and 293T cells[6-7]. These complicated results suggest that IL8 genetic polymorphism may play distinct roles in different diseases. In the present study, IL8 expression levels in blood samples from patients with TB or healthy controls with three rs4073 genotypes (AA, AT, and TT) were not significantly different (Figure 1A). IL8 expression significantly increased when samples were treated with *M. tuberculosis* antigens, especially in samples from patients with TB (Figure 1C). These results are consistent with previous reports indicating that IL8 expression is related to active disease status and severity[8-10].

In conclusion, four independent polymorphisms in the *IL8* gene detected among the Chinese population are not associated with TB susceptibility. IL8 protein secretion levels in the blood are not associated with the *IL8* rs4073 polymorphism, but correlated to *M. tuberculosis* antigen stimulation and active TB disease status.

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