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Association between Toll-like receptor 9 gene polymorphisms and risk of bacterial meningitis in a Chinese population

X.H. Wang, H.P. Shi and F.J. Li

Tuberculosis Hospital of Shanxi Province, Xi'an, China

Corresponding author: H.P. Shi
E-mail: haipingshii@163.com

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ABSTRACT. We determined whether two common single nucleotide polymorphisms (SNPs) in the Toll-like receptor 9 gene (*TLR9*) (*TLR9+2848* rs352140 and *TLR9-1237* rs5743836) influenced susceptibility to bacterial meningitis in a Chinese population. The study comprised 126 patients with bacterial meningitis and 252 control subjects, all of whom were recruited from the Tuberculosis Hospital of Shanxi Province. Genotyping of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 was performed by polymerase chain reaction coupled with restriction fragment length polymorphism. Using logistic regression analysis, we found that individuals with the AA genotype were associated with an increased risk of bacterial meningitis compared with those with the GG genotype (OR = 0.43, 95%CI = 0.19-0.95; P = 0.03). In a recessive model, the AA genotype was correlated with an elevated risk of bacterial meningitis compared with the GG+GA genotype (OR = 0.49, 95%CI = 0.22-0.99; P = 0.04). However, no significant differences were observed in the association between the *TLR9-1237* rs5743836 polymorphism and the risk of bacterial meningitis in the codominant, dominant, or recessive models. In conclusion, the results of our

study suggest an association between the *TLR9+2848* polymorphism and a reduced risk of bacterial meningitis in the codominant and recessive models.

Key words: *TLR9+2848*; *TLR9-1237*; Polymorphism; Bacterial meningitis

INTRODUCTION

Bacterial meningitis is a serious infectious disease of the central nervous system, and is associated with high mortality. Although advanced antimicrobial and anti-inflammatory treatments have been developed for bacterial meningitis, the treatment outcome for the condition is poor, and it is estimated that the mortality rate is 4-10% (Edmond et al., 2010). Approximately 20% of patients with bacterial meningitis have neurological sequelae, such as hearing loss, focal neurological deficits, and cognitive impairment (Weisfelt et al., 2006a; Hoogman et al., 2007; Brouwer et al., 2010). It is well known that bacterial meningitis is mainly caused by *Streptococcus pneumonia* and *Neisseria meningitidis*. Several factors are associated with susceptibility to infection with pneumococci and meningococci, such as passive smoking and an immunocompromised state (Weisfelt et al., 2006b; Heckenberg et al., 2008). However, not all patients with similar risk factors become infected with pneumococci or meningococci, which suggests that molecular factors may play an important role in susceptibility to those bacteria.

Recent epidemiologic studies have shown that genetic polymorphisms in the activation pathway proteins C2 (classical pathway), factor D (alternative pathway), and properdin (alternative pathway) are associated with the development of pneumococcal and meningococcal diseases (Jönsson et al., 2005; Sprong et al., 2006; Ricklin et al., 2010). Toll-like receptors (TLRs) constitute a major family of pathogen recognition receptors that are expressed in various cells inside the central nervous system, such as microglia and astrocytes, and recognize pathogen-associated molecular patterns. Previous studies have reported that *ILR4+896* and *TLR9+2848* are associated with susceptibility to bacterial meningitis (Allen et al., 2003; Sanders et al., 2011). In this study, we determined whether two common single-nucleotide polymorphisms (SNPs) of the Toll-like receptor 9 gene (*TLR9*) (*TLR9+2848* rs352140 and *TLR9-1237* rs5743836) could influence susceptibility to bacterial meningitis in a Chinese population.

MATERIAL AND METHODS

Patients

This study comprised 126 bacterial meningitis patients recruited from the Tuberculosis Hospital of Shanxi Province. All the patients were diagnosed by positive bacterial culture of the cerebrospinal fluid, or detection of the pathogen in the cerebrospinal fluid by Gram staining plus clinical signs, such as acute onset, fever, or meningeal irritation. Patients who had hospital-acquired bacterial meningitis were excluded from our study.

A total of 252 control subjects were selected from patients who came to our hospital for regular health check-ups during the same period, and the control subjects had no history of bacterial meningitis. The control subjects were age- and gender-matched with the bacterial meningitis patients (2:1). The clinical characteristics of the bacterial meningitis patients and the control subjects were selected from medical records. All the bacterial meningitis patients and control subjects voluntarily participated in the study and gave their informed consent. The

ethical committee of the Tuberculosis Hospital of Shanxi Province approved the study protocols.

DNA extraction and SNP genotyping

Peripheral blood (5-10 mL) was drawn from the participants for DNA extraction, and the blood samples were stored at -20°C until required. Genomic DNA was isolated from the peripheral blood samples using a TIANamp Blood DNA Kit (Tiangen, Beijing, China). Genotyping of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 was performed by polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). The primers used for *TLR9+2848* rs352140 were 5'-CCGCTGTGCAGGTGCTAGAC-3' (forward) and 5'-CCAAAGGGCTGGCTGTTGTA-3' (reverse); the primers used for *TLR9-1237* rs5743836 were 5'-GGCCTTGGGATGTGCTGTT-3' (forward) and 5'-GGTGACATGGGAGCAGAGACA-3' (reverse). The following conditions were used for PCR amplification: an initial denaturation step of 15 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, 72°C for 30 s, and a final extension step of 72°C for 10 min. The restriction enzymes for *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 were *Bst*UI and *Bst*NI, respectively. The PCR products were confirmed using 2% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

Statistical analysis

The differences in demographic and clinical characteristics between patients with bacterial meningitis and control subjects were compared by the chi-square test and the *t*-test. The Fisher's exact test was used to determine whether the frequencies of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 departed from the Hardy-Weinberg equilibrium. The association between *TLR9*+2848 rs352140 and *TLR9-1237* rs5743836 polymorphisms and risk of bacterial meningitis was investigated using conditional logistic regression analysis, and the results are expressed by odds ratios (OR) and 95% confidence intervals (95% CIs). The homozygotes of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 were taken as reference groups. All statistical analyses were conducted using the SPSS 20.0 package (SPSS Inc., Chicago, IL, USA). Two-sided P values of < 0.05 were considered statistically significant.

RESULTS

The demographic and clinical characteristics of the bacterial meningitis patients and the control subjects are shown in Table 1. Because of matching by gender and age, no significant differences were found between the bacterial meningitis patients and the control subjects in

Table 1. Baseline characteristics of included subjects.

Variables	Patients	%	Controls	%	Chi-square or *t*-test	P value
Mean age, years	56.32 ± 15.70		55.74 ± 14.94		0.35	0.36
<55	58	46.03	133	52.78	1.53	0.22
≥55	68	53.97	119	47.22		
Gender						
Female	60	47.22	120	47.62	0.00	1.00
Male	66	52.78	132	52.38		
Smoking habit						
No	92	73.41	192	76.19	0.45	0.50
Yes	34	26.59	60	23.81		

terms of gender and age (P > 0.05). Moreover, no significant differences were found between the bacterial meningitis patients and the control subjects in smoking status ($c^2 = 0.45$, P = 0.50).

The Fisher's exact test confirmed that the genotype distributions of *TLR9+2848* rs352140 (P = 0.94) and TLR9-*1237* rs5743836 (P = 0.35) were in agreement with the Hardy-Weinberg equilibrium in the control group (Table 2). The chi-square test revealed no significant differences in the genotype distributions of *TLR9+2848* rs352140 ($c^2 = 5.01$, P = 0.08) and *TLR9-1237* rs5743836 ($c^2 = 0.89$, P = 0.64) between the bacterial meningitis patients and the control subjects. The minor allele frequencies of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 in the controls were in line with those in the database.

Using logistic regression analysis, we found that individuals with the AA genotype were associated with an increased risk of bacterial meningitis compared with those with the GG genotype (OR = 0.43, 95%CI = 0.19-0.95; P = 0.03) (Table 3). In a recessive model, the AA genotype was correlated with an elevated risk of bacterial meningitis compared with the GG+GA genotype (OR = 0.49, 95%CI = 0.22-0.99; P = 0.04). However, no significant differences were observed in the association of the *TLR9-1237* rs5743836 polymorphism with the risk of bacterial meningitis in the codominant, dominant, or recessive models.

Table 2. Distributions of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 single-nucleotide polymorphisms (SNPs).

SNP	Patients	%	Controls	%	P for HWE	Chi-square value	P value	Minor allele frequency	
								In database	In controls
TLR9+2848 rs352140									
GG	55	43.65	89	35.32					
GA	60	47.62	122	48.41					
AA	11	8.73	41	16.27	0.94	5.01	0.08	0.4155	0.4048
TLR9-1237 rs5743836									
TT	91	72.22	173	68.65					
TC	32	25.40	69	27.38					
CC	3	2.38	10	3.97	0.35	0.89	0.64	0.1725	0.1766

HWE = Hardy-Weinberg equilibrium.

Table 3. Association between *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 single-nucleotide polymorphisms (SNPs) and risk of bacterial meningitis.

SNP	Patients	%	Controls	%	OR (95%CI)[1]	P value
TLR9+2848 rs352140						
Codominant						
GG	55	43.65	89	35.32	1.0 (Ref.)	-
GA	60	47.62	122	48.41	0.80 (0.49-1.29)	0.33
AA	11	8.73	41	16.27	0.43 (0.19-0.95)	0.03
Dominant						
GG	55	43.65	89	35.32	1.0 (Ref.)	-
GA+AA	71	56.35	163	64.68	0.70 (0.45-1.12)	0.12
Recessive						
GG+GA	115	91.27	211	83.73	1.0 (Ref.)	-
AA	11	8.73	41	16.27	0.49 (0.22-0.99)	0.04
TLR9-1237 rs5743836						
Codominant						
TT	91	72.22	173	68.65	1.0 (Ref.)	-
TC	32	25.40	69	27.38	0.88 (0.52-1.47)	0.61
CC	3	2.38	10	3.97	0.57 (0.10-2.29)	0.40
Dominant						
TT	91	72.22	173	68.65	1.0 (Ref.)	-
TC+CC	35	27.78	79	31.35	0.84 (0.51-1.38)	0.48
Recessive						
TT+TC	123	97.62	242	96.03	1.0 (Ref.)	-
CC	3	2.38	10	3.97	0.59 (0.10-2.35)	0.42

DISCUSSION

In this study, we investigated the impact of *TLR9+2848* rs352140 and *TLR9-1237* rs5743836 polymorphisms on the development of bacterial meningitis in a sample of the Chinese population. Our results revealed a significant association between the *TLR9+2848* rs352140 polymorphism and susceptibility to bacterial meningitis, and the AA genotype of *TLR9+2848* rs352140 reduced the occurrence of bacterial meningitis compared with the GG genotype. However, no significant association was found between the *TLR9-1237* rs5743836 polymorphism and the development of bacterial meningitis.

TLR9 is a well-known intracellular pathogen recognition receptor, and the gene that encodes it plays an important role in identifying unmethylated cytosine-phosphate-guanine movement in viral DNA (Sanders et al., 2011). The authors of a previous study reported that the *TLR9* gene causes an intracellular receptor signal cascade, and thus evokes nuclear transcription factors resulting in the production of pro- and anti-inflammatory cytokines (Hemmi et al., 2000). The authors of previous studies have reported that *TLR9* gene polymorphisms are associated with the development of several inflammatory diseases (Bharti et al., 2014; Berenson et al., 2015; Trejo-de la O et al., 2015; Wen et al., 2015; Wujcicka et al., 2015). Bharti et al. (2014) conducted a study in a Chinese population and assessed the association between *TLR9* polymorphisms and pulmonary tuberculosis; they found that the *TLR9* rs187084 polymorphism was associated with reduced risk of pulmonary tuberculosis. Trejo-de la O et al. (2015) conducted a study in a Mexican population and revealed that the A allele of *TLR9+2848* was associated with increased risk of duodenal ulcers, and modified the expression of inflammatory cytokines in the gastric mucosa. Wen et al. (2015) reported that *TLR9+2848* had a correlation with systemic lupus erythematosus in a Chinese population. Berenson et al. (2015) indicated that *TLR9-1237* expression was associated with the development and severity of chronic obstructive pulmonary disease. The studies mentioned above suggest that *TLR9* gene polymorphisms are associated with inflammatory diseases.

The authors of only three studies have reported an association between *TLR9* gene polymorphisms and the development of bacterial meningitis (Sanders et al., 2011; Sanders et al., 2012; van Well et al., 2012). The association between *TLR9* gene polymorphisms and susceptibility to meningococcal meningitis was investigated in a study by van Well et al. (2012); they found that the *TLR9-1237* gene polymorphism increased the risk of hearing loss in patients with bacterial meningitis. Sanders et al. (2012) revealed that the *TLR9-1237* gene polymorphism plays a protective role in the development and severity of meningococcal meningitis. Sanders et al. (2011) reported an association between the *TLR9+2848* polymorphism and a reduced risk of bacterial meningitis in children. In this study, we found that the AA genotype of *TLR9+2848* rs352140 was associated with a reduced risk of bacterial meningitis compared with the GG genotype, which is in line with previous studies. Further studies with large sample sizes are required to confirm our findings.

In conclusion, the results of our study suggest an association between the *TLR9+2848* polymorphism and a reduced risk of bacterial meningitis in the codominant and recessive models. Future studies using larger sample sizes may help in elucidating the impact of *TLR9* gene polymorphisms on the development of bacterial meningitis.

Conflicts of interest

The authors declare no conflict of interest.

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