Iran J Public Health, Vol. 45, No.1, Jan 2016, pp.20-26

Original Article

Association of Single Nucleotide Polymorphisms in Toll-like Receptors with *Acinetobacter baumanii* Infectionin a Chinese Population

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(Received 10 Apr 2015; accepted 12 Jul 2015)

Abstract

Background: During recent years, infection of *Acinetobacter baumanii* showed a rapid growth in hospitals and community. Toll-like receptors (TLRs) are the most important pattern recognition receptors, which play a critical role during recognizing invading pathogens by the natural immune system. Our objective was to determine the associations of TLRs polymorphisms with the susceptibility to *A. baumanii* infection in a Chinese population.

Methods: We carried out a case-control study, genotyping 13 polymorphisms of TLR-2, TLR-4, TLR-5 and TLR-9 genes on 423 *A. baumanii*-infected patients and 385 exposed controls. Thirteen SNPs at the TLR-2 (rs3804099, rs7656411 and rs76112010), TLR-4 (rs1927914, rs10759932 and rs11536889), TLR-5 (rs1341987, rs1640827, rs1861172, rs2241097, rs5744174 and rs17163737) and TLR9 (rs187084) genes were analyzed. SNP genotyping was performed using an improved multiplex ligation detection reaction (iMLDR) technique.

Results: The SNP of TLR-9, rs187084, was related to *A. baumanii*-infection significantly under recessive model (G/G, to A/A + G/A, $P = 0.0064$, OR = 0.59, 95% CI = 0.40-0.86) after adjustment with age. Besides, the haplotype GCG of TLR-4 was significantly associated with *A. baumanii* infection ($P = 0.027$).

Conclusion: TLR-4 and TLR-9 may be related to the susceptibility to *A. baumanii* infection in a Chinese population.

Keywords: *Acinetobacter baumanii*, Gene polymorphisms, Toll-like receptors

Introduction

During recent years, infection of *Acinetobacter baumanii* showed a rapid growth in hospitals and community. *A. baumanii* infection mainly occurs in the lung and blood, the notable feature being that multi-drug resistant strains cause outbreaks in hospitals. Large outbreaks of *A. baumanii* infection have been reported in hospitals from USA, France, Spain, Belgium, Britain and South Korea, which brought great economic loss to the patients and hospitals (1-5). Recently, *A. baumanii* infection increases the mortality rate by 7.8~23% through cohort and case-control studies (6). In the past few years, reports about breaks of *A. baumanii* infection have also increased in China. Since the outbreak among over 20 wards in Beijing Union Medical College Hospital in 2004, similar cases have been reported in a number of cities, including Chongqing and Hangzhou (7-10).

When pathogens invade the body, the natural immune system recognizes foreign pathogens through pattern recognition receptors. So far, Toll-like receptors (TLRs), a member of the interleukin-1 receptor (IL-1R) superfamily, are regarded as the most important pattern recognition receptors. The TLR family contains 11 members, found in monocytes, macrophages and neutrophils. TLR-1, TLR-6 and TLR-10 do not recognize the ligand directly, and play their roles by forming heterodimers with other TLRs. TLR-3 recognizes some viral double-stranded RNAs. Although TLR-7 and TLR-8 were reported to interact with imidazoquinoline, the synthetic and antiviral small molecule, their natural ligands have not been identified. Currently, researches about TLR-11 are few, and lipopolysaccharide (LPS), PGN or poly (I: C) cannot stimulate NF-κB activation in TLR-11-expressing cells. Therefore, TLR-2, TLR-4, TLR-5 and TLR-9 are the most important TLR members related to the immunal protection against bacteria in the body.

More and more studies indicate that single nucleotide polymorphisms (SNPs) in TLR-2, TLR-4, TLR-5 and TLR-9 are closely related to the susceptibility to diseases in humans. TLR-2 has a molecular weight of 89.8 kDa, forms heterodimers with TLR-1 or TLR-6, and interacts with bacterial ligands such as lipopeptides, peptidoglycan and membrane fatty acid of Gram-positive bacteria (11). One variant of TLR-2 (G2477A/Arg753Gln), with an incidence of 2.7~9.4% in the population, is associated with the susceptibility to asthma, atopic dermatitis and the infection of *Mycobacterium tuberculosis, Staphylococcus aureus* and *Candida* (12, 13). Another variant of TLR-2 (C2029T/Arg677Trp) is related to the pathogenesis of *Lepromatous leprosy* in the Asian population. Furthermore, the levels of interleukin-12 (IL-12) are decreased in peoples carrying this polymorphism (14).

TLR-4, a protein with 839 amino acids and a molecular weight of 95.6kDa, can form homodimers. TLR-4 recognizes heat shock proteins and cilia of Gram-positive bacteria, envelope glycoproteins of retroviruses, heat-labile compositions of *Mycobacteria*, as well as muramic acids of *Spirochetes* (15). Furthermore, TLR-4 plays a definite and critial role during recognizing LPS of *Escherichia coli, Neisseria meningitidis, Salmonella* and *Legionella pneumophila*, as well as subsequent signal transduction (14). Two common SNPs in TLR-4, A1287G/Asp299Gly and C13174T/Thr399Ile, change its extracellular structure, slow its response to LPS and increase the incidence of septic shock (16). Besides, these SNPs are also closely related to the pathogenesis of severe respiratory syncytial virus (RSV) bronchiolitis (17). Another two common SNPs in TLR-4 (A896G and C1196T) have protective effects against the infection of *Legionella pneumophila* (18).

TLR-5 contains 858 amino acids with a molecular weight of 97.7 kDa, and can form homodimers or heterodimers with TLR-4. TLR-5 recognizes flagellin, and induces production of high levels of interleukin-8 (IL-8). One TLR-5 variant (1174C/T) is related to the susceptibility to infection of *L. pneumophila*(19). TLR-9 recognizes unmethylated CpG DNA as homodimers. It was reported that a polymorphism in the TLR9 promoter (-1237T/C) is related to the susceptibility to asthma, eczema, and Crohn's disease (14).

Despite the prevalence of *A. baumanii* infection in many hospitals and communities, the reason underlying *A. baumanii* outbreaks remains unclear. Previous studies mainly focus on the mechanism of drug resistance of bacteria, ignoring the role of the interaction between the susceptibility to infection and the baterial genotype.

In this study, we investigated the mechanism of *A. baumanii* infection from the perspective of host genetics. We screened SNPs in TLR-2, TLR-4, TLR-5 and TLR-9, and studied whether polymorphisms in TLRs were associated with the susceptibility to *A. baumanii* infection.

Materials and Methods

Study subjects and DNA isolation

The study was approved by the review board/Ethics Committees of the Chinese PLA General Hospital. Each patient understood the experimental situation, and was voluntary for this study. Cases of *A. baumanii* infection were collected in major cities of China using the national

hospital infection surveillance network. The experimental group contained 423 infected patients. Three hundred and eighty five uninfected patients from the same ward and with the same gender were selected as the controls. Besides, the ages of infected and uninfected patients from the same ward were less than 2 years. To reduce the interference of population stratification, all the patients in this study were Han Chinese. Three milliliters of EDTA-anticoagulated peripheral blood were collected from each patient. Genomic DNA was extracted using Qiagen blood mini kit (Qiagen, CA, USA), and stored at -80 °C.

SNP selection and genotyping

Thirteen SNPs at the TLR-2 (rs3804099, rs7656411 and rs76112010), TLR-4 (rs1927914, rs10759932 and rs11536889), TLR-5 (rs1341987, rs1640827, rs1861172, rs2241097, rs5744174 and rs17163737) and TLR9 (rs187084) genes were analyzed (*TA. baumaniile* 1). SNPs were selected according to their frequencies and positions, as well as their associations with infectious diseases reported previously. SNP genotyping was performed using an improved multiplex ligation detection reaction (iMLDR) technique (Genesky Biotechnologies Inc., Shanghai, China). The rate of data accuracy is over 98%. The genotyping success rates of rs3804099, rs7656411, rs76112010, rs1927914, rs10759932, rs11536889, rs1341987, rs1640827, rs1861172, rs2241097, rs5744174, rs17163737 and rs187084 were 99.5%, 99.3%, 99.5%, 99.4%, 99.5%, 99.8%, 99.8%, 99.8%, 99.8%, 99.8%, 99.8% and 99.5%, respectively.

Statistical analysis

The association of *A. baumanii* infection with gender and age were examined using the Pearson chi-square test and the *t* test, respectively. The chi-square tests were used to determine whether each polymorphism was in Hardy–Weinberg equilibrium. A multiple logistic regression model was used to investigate the individual effect of TLR-2, TLR-4, TLR-5 and TLR-9 SNPs on *A. baumanii* infection. The analysis was based on additive, recessive and dominant models, and adjusted for age. The odd ratios (ORs) with 95% confidence intervals (CIs) were presented. The statistical analysis was performed using SNPalyze V4.0 (DYNACOM, Kanagawa, Japan). LD analysis was performed using Haploview V4.2. A *P* value less than 0.05 was considered statistically significant.

Results

There were 423 *A. baumanii*-infected patients and 385 controls. We selected 13 SNPs at the TLR-2 (rs3804099, rs7656411 and rs76112010), TLR-4 (rs1927914, rs10759932 and rs11536889), TLR-5 (rs1341987, rs1640827, rs1861172, rs2241097, rs5744174 and rs17163737) and TLR9 (rs187084) genes (Table 1). The Pearson chi-square test showed that the susceptibility to *A. baumanii* infection was not associated with the gender significantly ($P = 0.98$). However, *A. baumanii*-infection increased with ages, as indicated by the *t* test ($P = 8.6 \times 10^{-6}$). Therefore, the subsequent analysis was adjusted by age.

Table 1: Characteristics of the studied SNPs at the TLR-2, TLR-4, TLR-5 and TLR-9 genes

Gene	dbSNP ID	Allele	Region	MAF	HWE P value/(Controls)	HWE *P* value/ (Cases)
TLR-2	rs3804099	T->C	exon 4	0.30	1	0.34
TLR-2	rs7656411	G->T	3' near gene	0.49	0.43	0.22
TLR-2	rs76112010	G->A	promoter	0.17	0.37	1
TLR-4	rs1927914	A->G	promoter	0.40	0.06	0.83
TLR-4	rs10759932	T->C	promoter	0.29	0.71	0.91
TLR-4	rs11536889	G->C	3'UTR	0.23	0.33	0.39
TLR-5	rs1341987	G->C	intron 3	0.25	0.046	0.23
TLR-5	rs1640827	T->C	intron 2	0.16	0.021	0.70
TLR-5	rs1861172	T->C	3'UTR	0.20	0.13	0.64
TLR-5	rs2241097	A->C	intron 6	0.18	1	0.73
TLR-5	rs5744174	A->G	exon 7	0.20	0.23	0.64
TLR-5	rs17163737	G->T	intron 6	0.26	0.08	0.09
TLR-9	rs187084	A->G	promoter	0.39	0.021	1

MAF, minor allele frequency; HWE, Hardy–Weinberg equilibrium; statistically significant values are shown in bold.

The positions and minor allele frequencies (MAFs) of the SNPs are presented in Table 1. All SNPs are in Hardy-Weinberg equilibrium (HWE), except for rs1341987 (P = 0.046 in control patients) and rs1640827 (P = 0.021 in control patients) of TLR-5 and rs187084 of TLR-9 (P = 0.021 in control patients). These two polymorphisms of TLR-5 were excluded during the subsequent analysis.

The associations of TLR2, TLR4, TLR5 and TLR9 gene polymorphisms with *A. baumanii* infection after logistic regression analysis are shown in Table 2. The SNP of TLR-9, rs187084, was related to *A. baumanii*-infection under the additive model (2A/A + G/A, to G/G, P = 0.05, OR = 0.82, 95% CI = 0.67-1.00) and recessive model (G/G, to A/A + G/A, P = 0.0064, OR = 0.59, 95% CI = 0.40-0.86) after adjustment with age. However, as mentioned above, rs187084 did not obey the HWE rule in control patients.

Table 2: Association of TLR-2, TLR-4, TLR-5 and TLR-9 gene polymorphisms with Ab infection after logistic regression analysis

Gene	dbSNP ID	Genotype	Control Number (%)	Disease Number (%)	Additive model		Dominant model		Recessive model	
					OR (95%CI)	*P*	OR (95%CI)	*P*	OR (95%CI)	*P*
TLR-2	rs3804099	T/T	205 (51.2)	181 (47)	1.06 (0.85-1.32)	0.60	1.20 (0.90-1.60)	0.20	0.78 (0.48-1.27)	0.32
		C/T	152 (38)	172 (44.7)						
		C/C	43 (10.8)	32 (8.3)						
TLR-2	rs7656411	G/G	106 (26.6)	110 (28.6)	0.94 (0.77-1.14)	0.51	0.87 (0.63-1.20)	0.39	0.96 (0.69-1.34)	0.82
		G/T	194 (48.6)	179 (46.6)						
		T/T	99 (24.8)	95 (24.7)						
TLR-2	rs76112010	G/G	279 (69.6)	265 (69)	0.99 (0.76-1.29)	0.94	1.01 (0.74-1.38)	0.94	0.83 (0.36-1.88)	0.65
		G/A	109 (27.2)	108 (28.1)						
		A/A	13 (3.2)	11 (2.9)						
TLR-4	rs1927914	A/A	144 (36.1)	130 (33.8)	1.17 (0.95-1.44)	0.15	1.12 (0.83-1.51)	0.45	1.44 (0.96-2.16)	0.078
		G/A	206 (51.6)	190 (49.4)						
		G/G	49 (12.3)	65 (16.9)						
TLR-4	rs10759932	T/T	211 (52.6)	182 (47.4)	1.24 (0.99-1.56)	0.055	1.31 (0.98-1.74)	0.065	1.34 (0.80-2.24)	0.27
		C/T	161 (40.1)	166 (43.2)						
		C/C	29 (7.2)	36 (9.4)						
TLR-4	rs11536889	G/G	242 (60.2)	230 (59.7)	1.04 (0.82-1.32)	0.76	0.98 (0.73-1.30)	0.87	1.48 (0.77-2.81)	0.23
		G/C	143 (35.6)	131 (34)						
		C/C	17 (4.2)	24 (6.2)						
TLR-5	rs1341987	G/G	221 (55)	210 (54.5)	1.06 (0.84-1.36)	0.61	1.04 (0.78-1.38)	0.8	1.32 (0.67-2.62)	0.42
		C/G	165 (41)	155 (40.3)						
		C/C	16 (4)	20 (5.2)						
TLR-5	rs1640827	T/T	271 (67.4)	271 (70.4)	0.95 (0.72-1.26)	0.73	0.90 (0.66-1.22)	0.48	2.17 (0.64-7.40)	0.20
		C/T	127 (31.6)	106 (27.5)						
		C/C	4 (1)	8 (2.1)						
TLR-5	rs1861172	T/T	252 (62.7)	243 (63.1)	1.03 (0.79-1.33)	0.84	1.01 (0.75-1.35)	0.95	1.21 (0.54-2.72)	0.64
		C/T	139 (34.6)	128 (33.2)						
		C/C	11 (2.7)	14 (3.6)						
TLR-5	rs2241097	A/A	267 (66.4)	258 (67)	0.99 (0.77-1.28)	0.95	0.99 (0.74-1.34)	0.97	0.96 (0.45-2.06)	0.92
		C/A	121 (30.1)	113 (29.4)						
		C/C	14 (3.5)	14 (3.6)						
TLR-5	rs5744174	A/A	252 (62.7)	243 (63.1)	1.02 (0.79-1.31)	0.91	1.01 (0.75-1.35)	0.96	1.10 (0.50-2.43)	0.82
		G/A	138 (34.3)	128 (33.2)						
		G/G	12 (3)	14 (3.6)						
TLR-5	rs17163737	G/G	225 (56)	208 (54)	1.05 (0.84-1.32)	0.65	1.09 (0.82-1.44)	0.57	1.00 (0.57-1.75)	0.99
		G/T	149 (37.1)	151 (39.2)						
		T/T	28 (7)	26 (6.8)						
TLR-9	rs187084	A/A	146 (36.4)	154 (40.1)	0.82 (0.67-1.00)	0.05	0.89 (0.67-1.19)	0.44	0.59 (0.40-0.86)	0.0064
		G/A	174 (43.4)	179 (46.6)						
		G/G	81 (20.2)	51 (13.3)						

OR: odd ratio; CI: confidenceinterval. Statistically significant values are shown in bold.

The haplotypes of TLR-2, TLR-4 and TLR-5 are listed in Table 3. We found a significant association of the haplotype GCG of TLR-4 with *A. baumanii*-infection ($P = 0.027$). No significant differences were detected in the distribution of TLR-2 and TLR-5 haplotypes between *A. baumanii*-infected patients and controls. Compared with the common haplotype ATG of TLR-4, the haplotye GCG contained two mutations both in the promoter region (Table 1).

Table 3: Haplotype distribution of TLR-2, TLR-4 and TLR-5 polymorphisms in Ab infected patients and controls

Haplotype[a]	Frequency[b] (%)	OR (95% CI)	*P* value
TLR-2			
GTT	31.89	1	-
GCG	29.28	1.078 (0.834-1.393)	0.565
GTG	21.53	1.014 (0.766-1.342)	0.922
ATT	16.00	0.993 (0.732-1.347)	0.963
GCT	0.62	-	-
ATG	0.50	-	-
ACG	0.19	-	-
TLR-4			
ATG	38.09	1	-
GCG	28.85	1.323 (1.032-1.694)	0.027
ATC	22.46	1.177 (0.901-1.538)	0.231
GTG	10.36	1.071 (0.755-1.520)	0.700
GCC	0.19	-	-
GTC	0.06	-	-
TLR-5			
TAAG	35.61	1	-
TAAT	25.81	1.081 (0.834-1.400)	0.555
CGAG	20.16	1.060 (0.802-1.403)	0.681
TACG	18.24	1.026 (0.770-1.368)	0.859
CAAG	0.06	-	-
TGAG	0.06	-	-
TGAT	0.06	-	-

[a]Haplotype: TLR-2 [rs76112010, rs3804099, rs7656411]; TLR-4 [rs1927914, rs10759932, rs11536889]; TLR-5 [rs1861172, rs5744174, rs2241097, rs17163737].
[b]Frequency: the haplotypefrequency in the whole population including Ab-infected patients and controls.
OR: odd ratio; CI: confidence interval. Statistically significant values are shown in bold.

Discussion

Infection is the result of interactions between the pathogen and the host. The major risk factors of *A. baumanii* infection include the severity of the underlying disease, surgical operations, a large area of trauma (especially burns), premature birth, ever living in ICU, prolonged hospitalization, contact with contaminated ward or equipment, invasive procedures (such as mechanical ventilation, indwelling tubes, etc.), and ever using antibiotics. Although these factors are statistically associated with *A. baumanii* infection, they cannot accurately predict the risk of exposure, it is difficult to explain why some patients exposed to these factors are infected, and some are not.

TLRs are the most important pattern recognition receptors, through which the natural immune system recognizes foreign pathogens. Many studies indicate that gene polymorphisms in TLRs are

closely related to the susceptibility to diseases in humans. Although TLR9 detect bacterial DNA and recognize dsDNA from viruses and the genomes of protozoa (20) , and the innate immune system appears to use TLR9 for detecting unmethylated CpG dinucleotides, which are relatively common in bacterial and viral genomes but are highly methylated and rare in vertebrate genomes (21, 22), but in this study, the SNP of TLR-9, rs187084, was significantly related to *A. baumanii* infection especially under the recessive model. However, this SNP was not in HWE in control patients. Therefore, more random and more extensive sampling may be required to confirm further this association. The haplotype GCG of TLR-4 was significantly associated with *A. baumanii* infection. Interestingly, the haplotye GCG contained two mutations both in the promoter region, compared with the common haplotype ATG. We speculate that these two SNPs in the TLR-4 promoter, rs1927914 and rs10759932, influence its transcriptional regulation.

Conclusion

One SNP of TLR-9 (rs187084) and the haplotype GCG of TLR-4 are significantly associated with *A. baumanii* infection. Our studies provide insights to the mechanism of *A. baumanii* infection from the perspective of the host.

Ethical considerations

Ethical issues (Including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

Acknowledgements

This study was supported by grants from the National Natural Science Foundation ofChina (no. 30972523 and 31140046) and the Foundation of the "Twelfth Five YearPlan" for Medical Science Development of the People's Liberation Army (nosBWS11J048). The authors declare that there is no conflict of interests.

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