

Original Article

Association of gene polymorphisms in toll-like receptors 4 with bacterial infection after orthotopic liver transplantation in Han Chinese patients

Fu Yang¹, Hao Li², Ying Pu¹, Chunguang Wang², Junwei Fan², Tonghai Xing², Zhihai Peng², Fang Fang¹, Lin Zhong²

Departments of ¹Nursing, ²General Surgery, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Shanghai 200080, China

Received January 12, 2017; Accepted February 7, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: Bacterial infections represent the most common complications after liver transplantation (LT). Toll-like receptor 4 (*TLR4*) plays a pivotal role in recognizing pathogens. The study aimed to determine the association between *TLR4* single nucleotide polymorphisms (SNPs) with susceptibility to bacterial infection within 6 months after orthotopic liver transplantation. This was a prospective cohort study of 113 consecutive LT recipients (n=44 with infections; n=69 without infection as controls) at the Shanghai Jiaotong University Affiliated First People Hospital (China) between January 2007 and January 2011. The association between *TLR4* SNPs (rs1927907, rs1927914, rs11536889, rs1927906, and rs2149356) in recipients with the susceptibility to bacterial infection after orthotopic liver transplantation within 6 months was analyzed. A total of 44 transplant recipients (38.9%) developed bacterial infections within 6 months of LT, including 19 and 25 patients with Gram-positive and negative bacteria, respectively. Pulmonary infection (n=21), cholangitis (n=9), sepsis (n=9), and other bacterial infections (n=5) were observed. Recipient *TLR4* rs1927907, rs1927914, rs11536889, and rs2149356 SNPs were associated with infections within 6 months after LT. Multivariate analysis showed that endotracheal intubation time ≥ 72 h ($P=0.040$, $OR=2.84$, 95% CI 1.05-7.70) and rs2149356 (AA vs. AC/CC, $P=0.003$, $OR=4.24$, 95% CI 1.65-10.93) were independently associated with bacterial infection within 6 months after LT. Kaplan-Meier analysis indicated that patients with the AA rs2149356 genotype could be at higher risk of developing a bacterial infection within 6 months after LT. Prolonged duration of endotracheal intubation and the *TLR4* rs2149356 SNP were independently associated with infections after LT within 6 months.

Keywords: Toll-like receptor 4, single nucleotide polymorphism, bacterial infection, liver transplantation

Introduction

Liver transplantation (LT) is an effective and cost-efficient option for the treatment of end-stage liver diseases [1]. With the development of surgical techniques and new immunosuppressive regimens, patient survival after LT has increased in the past decade [1]. However, bacterial infections are the most frequent complications post-LT as well as being the leading cause of morbidity and mortality in LT recipients [1-4]. Hence, identifying patients at high risk of developing infections is of vital importance for improving long-term prognosis after LT. Most bacterial infections occur within 8 weeks after LT. In addition, the spectrum of

infection after liver transplantation changes with time. Early infections occur in the first month after transplant, and 90% of them are nosocomial bacterial and fungal infections; mid-term infections occur within the first 1-6 months after LT, and are mainly viral infections. Late infections occur after six months, are relatively rare, and most of them are opportunistic infections [1-4].

So far, studies have suggested that clinical parameters significantly associated with infection include age, MELD scores, Child-Pugh scores, empirical antibiotic administration, large volume of blood loss and packed red cell transfusion, operation time, ICU stay, reoperation,

and acute renal insufficiency [1, 2, 5-7]. In addition, we previously showed that recovery diet, prolonged endotracheal intubation, and biliary complications were risk factors for infection [8]. Interestingly, some patients with no definitive clinical risk factors are still susceptible to infections [7, 9]. There is growing evidence that susceptibility and response to infectious diseases is, in part, inheritable. Particularly, single-nucleotide polymorphisms (SNPs) in innate immune response genes may play a key role in the development of post-LT infections [10-12].

As pattern recognition receptors, toll-like receptors (*TLRs*) play important roles in activating innate immunity and developing adaptive immunity by inducing the expression of specific cytokines and chemokines [12]. They can recognize various components of bacteria, viruses, fungi, and parasites [12]. Among the *TLR* family members, *TLR4* recognizes the lipopolysaccharides (LPS) of Gram-negative bacteria [12]. Patients with *TLR4* polymorphisms are more likely to suffer from intestinal infections, and are highly susceptible to bowel inflammation diseases like Crohn's disease and ulcerative colitis [13]. In addition, an independent association was reported between *TLR4* polymorphisms and infections in renal transplant recipients [14]. It has been shown that some polymorphisms such as Asp299Gly decreased the affinity of *TLR4* protein to LPS [15]. A study of 238 renal transplant recipients showed that *TLR4* polymorphisms (Asp299Gly and Thr399Ile) were associated with a higher risk of severe infections [16]. Other common *TLR4* polymorphisms were also associated with infections in different contexts: the rs1927914 SNP has been associated with the development of diabetic foot [17], the rs1927907 SNP has been associated with the response to tacrolimus after LT [18], the rs11536889 SNP has been associated with worst outcomes of sepsis [19], the rs1927906 SNP has been associated with susceptibility to tuberculosis [20], and the rs2149356 SNP has been associated with invasive bacterial infections [21]. Therefore, *TLR4* polymorphisms might play an important role in the susceptibility to infections after LT.

Nevertheless, the genetic basis of susceptibility to infection after LT is still poorly understood. In this context, the aim of our study was to investigate the association between *TLR4*

gene polymorphisms and bacterial infection in Han Chinese patients within 6 months after LT. Results of this study could help identifying patients at higher risk of infection after LT. More aggressive prophylactic measures could be taken, improving their prognosis.

Materials and methods

Study population

This was a prospective cohort study of 113 consecutive LT recipients from the Shanghai Jiaotong University Affiliated First Peoples Hospital (China) between July 2007 and January 2011. Inclusion criteria were: 1) aged 18 years or older; 2) compatible blood and tissue types with donors; and 3) Han Chinese. The exclusion criterion was combined liver/kidney transplantation. In patients who underwent two LT surgeries, only the data from the first LT were included for analysis.

All patients were evaluated using the United Network for Organ Sharing (UNOS) Model for End-Stage Liver Disease (MELD) scoring system [22]. Among patients with hepatocellular carcinoma, UNOS TNM stage, histological grade, Milan criteria, tumor size, and multinodular tumor frequency were assessed. All LT were performed using cadaveric livers and orthotopic LT (OLT), with end-to-end biliary anastomosis without T-tube drainage. All livers were from willing donors.

Clinical and demographic data including perioperative demographics and clinical characteristics (age, gender, Child-Pugh score, encephalopathy grade, diabetes, dialysis, use of antibiotics, MELD score, and indication for LT), operative variables (operation time, anhepatic phase, blood loss, and transfusion), and clinical events within 6 months post-transplantation (use of prednisone, rejection, duration of initial intubation, tracheotomy, biliary complication, transfusion, intensive care unit [ICU] stay, reoperation, recovery diet time, renal function, and dialysis) were collected. In our study, there were 19 females and 94 males, with a median age of 48 years. The main reasons for LT were hepatocellular carcinoma (53.1%, n=60), followed by hepatitis B-related cirrhosis (29.2%, n=33). Among these patients, bacterial infection occurred in 44 recipients (38.9%) within 6 months after LT (**Table 1**): 19 of these cases (43.2%) were due to Gram-positive bacteria

TLR4 SNPs, bacterial infection and transplantation

Table 1. Characteristics of the patients

Variables	Total n=113	Infection n=44	Control n=69	P
Pre-operation Variables				
Age (years)	48 (42, 55)	49 (42, 55)	48 (42, 54)	0.748
Gender				0.018*
Male, n (%)	94 (83.2)	32 (72.7)	62 (89.9)	
Female, n (%)	19 (16.8)	12 (27.3)	7 (10.1)	
Encephalopathy grades II-IV, n (%)	5 (4.4)	2 (4.5)	3 (4.3)	0.960
Diabetes, n (%)	8 (7.1)	2 (4.5)	6 (8.7)	0.480
Dialysis, n (%)	2 (1.8)	1 (2.3)	1 (1.4)	1.000
Pre-LT broad-spectrum antibiotics, n (%)	25 (22.1)	17 (38.6)	8 (11.6)	0.001**
MELD score	10 (8, 14)	11 (8, 14)	10 (8, 13)	0.410
Child-Pugh score	7 (5, 9)	7 (5, 9)	7 (5, 8)	0.599
Main reasons leading to LT, n (%)				0.386
Hepatocellular carcinoma	60 (53.1)	20 (45.5)	40 (58.0)	
Cirrhosis related to hepatitis B viruses	33 (29.2)	12 (27.3)	21 (30.4)	
Hepatitis C viruses and alcohol-related cirrhosis	3 (2.7)	2 (4.5)	1 (1.4)	
Autoimmune cirrhosis	4 (3.5)	2 (4.5)	2 (2.9)	
Other etiologies	13 (11.5)	8 (18.2)	5 (7.2)	
Operation variables				
Operation time (h)	6.0 (6.0, 8.0)	6.5 (6.0, 8.0)	6 (5.5, 7.0)	0.096
Anhepatic phase (min)	60.0 (55.0, 60.0)	60 (59, 60)	60 (50, 60)	0.450
Blood loss volume (l)	2.6 (1.2, 5.3)	2.8 (1.2, 6.3)	2.6 (1.3, 5.0)	0.672
Transfusion of red cells (units)	8.0 (2.0, 14.0)	9 (5.0, 14.0)	6 (0, 12.0)	0.243
Post-operation variables				
Post-LT without application of prednisone, n (%)	32 (33.3)	16 (36.3)	16 (23.2)	0.051
Acute rejection with high-dose corticosteroids or ATG therapy, n (%)	20 (17.7)	9 (20.5)	11 (15.9)	0.540
Endotracheal intubation time ≥72 (h)	22 (19.5)	13 (29.5)	9 (13.0)	0.031*
Tracheotomy, n (%)	5 (4.4)	4 (9.1)	1 (1.4)	0.074
Biliary complication, n (%)	15 (13.3)	10 (22.7)	5 (7.2)	0.018*
Transfusion of red cells (units)	0 (0, 4)	0 (0, 7.5)	0 (0, 3)	0.122
Indwelling time of ICU (h)	384.0 (264.0, 552.0)	504 (336.0, 648.0)	312 (216.0, 480.0)	<0.001***
Recovery diet time (h)	56.0 (77.0, 102.0)	77.5 (54.5, 130.25)	77.0 (56.0, 98.0)	0.089
Renal dysfunction, n (%)	7 (6.2)	4 (9.1)	3 (4.3)	0.428

Continuous variables are presented as median (Q1, Q3). Categorical variables are presented as counts and percentages. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, infection group vs. the control group. ICU, intensive care unit; LT, liver transplant; MELD: model for end-stage liver disease; ATG: anti-thymocyte globulin.

and 25 (56.8%) to Gram-negative bacteria. The most frequent bacterial infections were pneumonia (n=21, 47.7%), followed by sepsis (n=9, 20.5%), cholangitis (n=9, 20.5%), and other infections (n=5, 11.3%) [23].

The study was approved by the Ethics Committee of the First People's Hospital affiliated to Shanghai Jiaotong University (2013KY046), and performed in accordance with the Declaration of Helsinki. Each patient provided a written informed consent, as well as the legal representative of the liver donor.

Infection definitions and infectious prophylaxis protocol

The presence of an infectious episode was determined by the presence of any one of the

following criteria [14]: 1) a positive culture of a pathogenic microorganism in any sample for two consecutive tests (e.g., lung, common bile duct, blood, and others); 2) isolation of any microorganism from a sample obtained under sterile conditions; or 3) isolation of a potentially pathogenic microorganism in a sample from any location, accompanied with compatible symptoms of infection (e.g., chills, fever, hypotension, or characteristic CT/chest X-ray properties) within 6 months after LT. We included only those infectious events considered to be major bacterial infections: bloodstream infections, pneumonia, cholangitis, and other infections.

All patients received the same presurgical antibiotic prophylaxis one hour before surgery: 1) antianaerobic drug (metronidazole) + third-

generation cephalosporins or cephalosporins/enzyme inhibitors or penicillin/enzyme inhibitors (cefoperazon sodium/sulbactam sodium or piperacillin/tazobactam sodium); 2) preoperative bath of chlorhexidine; 3) oral wash of compound chlorhexidine gargle; and 4) ultrasonic atomization of ambroxol hydrochloride + terbutaline sulfate or ipratropium bromide aerosol. If secondary infections occurred, sensitive antibiotics were given according to the results of bacterial culture and susceptibility testing. If extended-spectrum beta-lactamase (ESBL) infection occurred, metronidazole + cefoperazon sodium/sulbactamsodium or piperacillin/tazobactam sodium or carbapenem antibiotics (imipenem-cilastin) were given. If Gram-positive bacterial infections (such as MRSA and MRSE), glycopeptide antibiotics (vancomycin or teicoplanin) were used. In the presence of fungal infections, echinocandins (caspofungin or micafungin)/triazoles (voriconazole or posaconazole) were used.

Immunosuppressive and rejection therapy

All LT patients received primary standard immunosuppressive therapy, including tacrolimus (FK506) or cyclosporine and low-dose prednisone. Acute rejection episodes were diagnosed by patients' clinical presentations, serum biochemical results, and liver biopsy. Rejection episodes were mainly treated with methylprednisolone and increasing FK506 blood concentrations. Persistent or steroid-resistant rejection was treated with antithymocyte globulin (ATG) (2.5 mg/kg/d for 7-10 days) in the absence of thrombocytopenia and leukopenia.

Polymorphisms and genotyping

Genomic DNA was extracted from EDTA-anti-coagulated whole blood of the recipients using the QIAamp DNA Blood mini kit (QIAGEN, Valencia, CA, USA). Genotyping of polymorphisms was performed using a Sequenom Mass ARRAY platform according to the manufacturer's protocols (Sequenom, San Diego, CA, USA) and as previously described [24]. Five TLR4 SNPs (rs1927907, rs1927914, rs11536889, rs1927906, and rs2149356) gene were genotyped.

Follow-up

The follow-up included visits at the Outpatient Department, telephone inquiries, and hospital

review. For the first 3 months, the patients were seen every weeks. For months 4-6, the patient was seen monthly. For months 7-12, the patient was seen every 2 months. The patient was seen every 2-3 months for the second year, and then lasted for years 3-5. Each visit included blood routine examination, liver and kidney function, blood drug concentration, and HBV virological indicators.

Recipients with primary malignancy disease underwent chest and abdomen CT and whole body bone scan. Immunosuppressant therapy was adjusted according to the test results.

Statistical analysis

If normally distributed, continuous data are expressed as means \pm standard deviation (SD) and were compared using independent samples *t*-test. If non-normally distributed, data are expressed as median (Q1, Q3) and were compared using the Mann-Whitney U test for independent samples. Categorical variables are expressed as absolute count and frequencies, and were compared using the chi-square test or Fisher's exact, as appropriate. The differences of allele and genotype distribution between the non-infection and infection groups were analyzed using the chi-square test or Fisher's exact test, as appropriate. Genotypes were analyzed for deviations from the Hardy-Weinberg equilibrium. SHEsis Online Version (<http://analysis.bio-x.cn/myAnalysis.php>) was used to analyze linkage disequilibrium. Risk factors for bacterial infection were evaluated using multivariate logistic regression analysis with the stepwise forward method. First, univariate analysis of all variables was performed. Variables with $P < 0.05$ were subsequently used in the multivariate analysis. Cumulative hazard for infection incidence after liver transplantation within 6 months was analyzed using the Kaplan-Meier method and the log-rank test. The Bonferroni method was used to correct for multiple comparisons when applicable. SPSS 19.0 (IBM, Armonk, NY, USA) was used for analysis. Two-tailed P -values < 0.05 were considered statistically significant.

Results

Association analyses of TLR4 polymorphisms with susceptibility to bacterial infection

Five TLR4 SNPs (rs1927907, rs1927914, rs11536889, rs1927906, and rs2149356)

TLR4 SNPs, bacterial infection and transplantation

Table 2. Single-nucleotide polymorphisms

SNP	SNP type	Major/minor allele	MAF	HWE P value
rs1927907	Intron	G/A	0.30	0.70
rs1927914	Upstream variant 2 KB	T/C	0.46	0.54
rs11536889	3'utr	G/C	0.20	0.80
rs1927906	Downstream variant 500	A/G	0.08	0.92
rs2149356	Intron	C/A	0.45	0.55

MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium.

Table 3. Genotype and allele distributions of the five single-nucleotide polymorphisms in *TLR4* between the infection and control groups

SNP	Genotype and Allele	Infection (n=44)	Control (n=69)	P value
rs1927907, n (%)	A/A	9 (22.0)	2 (3.1)	0.008**
	A/G	14 (34.1)	27 (41.5)	
	G/G	18 (43.9)	36 (55.4)	
rs1927914, n (%)	A	32 (39.0)	31 (23.8)	0.019*
	G	50 (61.0)	99 (76.2)	
rs1927906, n (%)	C/C	16 (37.2)	9 (13.0)	0.009**
	C/T	18 (41.9)	35 (50.7)	
	T/T	9 (20.9)	25 (36.2)	
	C	50 (58.1)	53 (38.4)	
rs11536889, n (%)	T	36 (41.9)	85 (61.6)	0.004**
	C/C	2 (4.7)	2 (2.9)	
	C/G	8 (18.6)	28 (41.2)	
	G/G	33 (76.7)	38 (55.9)	
	C	12 (14.0)	33 (24.3)	
rs1927906, n (%)	G	74 (86.0)	103 (75.7)	0.063
	A/A	35 (81.4)	56 (87.5)	
	A/G	7 (16.3)	8 (12.5)	
	G/G	1 (2.3)	0 (0.0)	
	A	77 (89.5)	120 (93.8)	
rs2149356, n (%)	G	9 (10.5)	8 (6.2)	0.264
	A/A	16 (37.2)	9 (13.8)	
	A/C	17 (39.5)	30 (46.2)	
	C/C	10 (23.3)	26 (40.0)	
	A	49 (57.0)	47 (36.2)	
rs2149356, n (%)	C	37 (43.0)	83 (63.8)	<0.001***
	C	37 (43.0)	83 (63.8)	

Data are shown as number (percentage) of subjects. The P value was determined using the Chi-square test or Fisher exact test, as appropriate. OR: odds ratio; CI: confidence interval. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$, infection group vs. control group.

were identified. All of the genotype distributions were consistent with the Hardy-Weinberg equilibrium ($P > 0.05$) (Table 2).

Table 3 presents the distribution of the genotypes and alleles between the two groups.

Compared with controls, the infection group had a higher frequency of the A allele of rs1927907 (39.0% vs. 23.8%, $P = 0.019$), as well as a higher frequency of the A/A genotype (22.0% vs. 3.1%, $P = 0.008$). The infection group had a higher frequency of the C allele of rs1927914 (58.1% vs. 38.4%, $P = 0.004$), as well as a higher frequency of the C/C genotype (37.2% vs. 13.0%, $P = 0.009$). The infection group had a higher frequency of the G/G genotype of rs11536889 (76.7% vs. 55.9%, $P = 0.032$). The infection group had a higher frequency of the A allele of rs2149356 (57.0% vs. 36.2%, $P = 0.003$), as well as a higher frequency of the A/A genotype (37.2% vs. 13.8%, $P < 0.001$). There was no difference in the allele distribution or genotype of rs1927906 between the two groups.

Risk factors of bacterial infection: multivariate logistic regression analysis

Several clinical variables and genetic factors were considered to be potential risk factors of bacterial infection by univariate analysis. In the multivariate logistic regression model, bacterial infection was significantly associated with two factors: post-operation endotracheal intubation time ≥ 72 h ($P = 0.040$, OR=2.84, 95% CI 1.05-7.70) and rs2149356 (AA vs. AC/CC, $P = 0.003$, OR=4.24, 95% CI 1.65-10.93) (Table 4).

Rs2149356 and bacterial infection: Kaplan-Meier survival curves analysis

The genotype distribution of rs2149356 for the development of bacterial infection was evaluated using Kaplan-Meier estimates and the log-rank test. Infection occurred significantly earlier among patients carrying the genotype AA compared with those carrying the AC and CC

Table 4. Multivariate logistic regression analysis of risk factors associated with bacterial infections within 6 months after LT

Variables	OR (95% CI)	P-value
Endotracheal intubation time ≥ 72 h (1= endotracheal intubation time ≥ 72 h, 0= endotracheal intubation time < 72 h)	2.84 (1.05-7.70)	0.040
rs2149356 (1=AA, 0=AC/CC)	4.24 (1.65-10.93)	0.003

Variables with $P < 0.05$ were subsequently used in the multivariate analysis. LT, liver transplant; OR: Odds ratio; CI: Confidence interval.

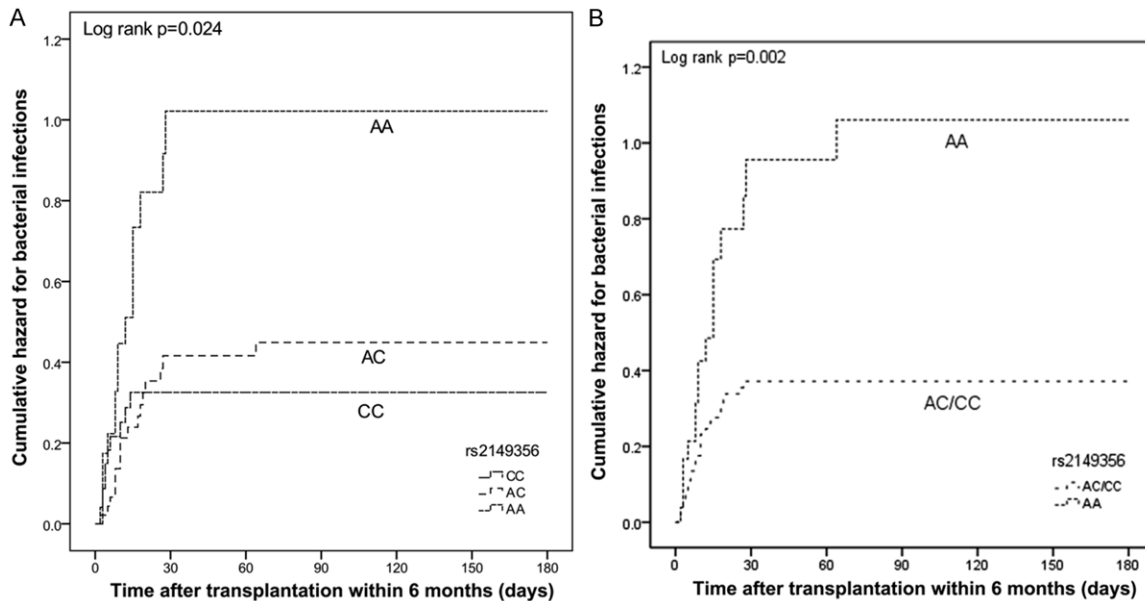


Figure 1. Kaplan-Meier curves of the infection incidence after liver transplantation within 6 months according to *TLR4* rs2149356 genotypes (A), log-rank: $P=0.024$. The Bonferroni method was used to correct for multiple comparisons. AA vs. CC, $P=0.011$ ($< 0.05/3=0.017$); AA vs. AC, $P=0.012$ (< 0.017); CC vs. AC, $P=0.059$ (> 0.017). (B) Polymorphism CC + AC and AA, log-rank: $P=0.002$.

genotypes, with the survival curves separating within a few days (**Figure 1A**). The frequency of bacterial infection was higher in the AA group than in the AC/CC group ($P=0.002$, **Figure 1B**).

Discussion

Infectious diseases are associated with morbidity and mortality after LT. The risk of bacterial infection after transplantation of a solid organ is the result of many factors [1, 2, 5-7]. Moreover, polymorphisms of recipient and/or donor organ may affect the innate immune system, which likely plays a crucial role in increasing the risk of infections [9-11].

In the present study, the association between recipient’s genotype polymorphisms of *TLR4*, a critical component of the innate immune system, and susceptibility to infection in patients after LT was assessed. Among the patients, 44 patients (38.9%) had bacterial infections within 6 months after LT. Of these, 19 and 25

patients were infected with Gram-positive and Gram-negative bacteria, respectively. Multivariate analysis revealed that the duration of endotracheal intubation and the rs2149356 SNP were independent risk factors for bacterial infection after LT, in agreement with previous reports [21, 23, 25]. Patients harboring the *TLR4* rs2149356 variant also showed a higher incidence of infection within 6 months after LT.

Multiple studies showed an association between *TLRs* gene polymorphisms and susceptibility to infectious diseases [9-11, 26]. Recently, studies have reported that *TLRs* SNPs can increase susceptibility to infection in severely injured trauma patients [27] and Gram-positive infection in sepsis [28]. In addition to binding to endogenous ligands released from damaged tissues and exogenous ligands such as LPS, *TLR4* also induces the proinflammatory response to Gram-negative bacteria [12]. There is evidence that *TLR4* SNPs increase the risk of Gram-negative bacterial infections in patients

[12, 13]. Indeed, studies have demonstrated that *TLR4* D299G and T399I SNPs confer increased risk to infection, as assessed by plasma LPS-binding protein, C-reactive protein, and white blood cell count [15]. The present study revealed that the *TLR4* rs1927907, rs1927914, and rs2149356 SNPs had an influence on bacterial infection after LT; furthermore, the rs2149356 SNP was shown to be an independent risk factor for infection after LT. Previous studies have reported that the *TLR4* rs2149356 SNP is associated with higher risk for sepsis in preterm infants, prostate cancer, normal tension glaucoma, and gouty arthritis [29]. In addition, the *TLR4* rs2149356 SNP plays an important role in susceptibility to some inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, and ulcerative colitis [13].

Nevertheless, few studies have investigated the association between the *TLR4* rs2149356 polymorphism and susceptibility to infection after transplantation, especially in LT. In this study, despite an association with the incidence of infection, there was no difference in long-term survival. In contrast, Lee et al. [30] showed that there was no significant association between the *TLR4* SNPs D299G and T399I, and the risk and outcomes of Gram-negative bacterial infections, while a previous study of 50 genetic variants showed no association between *TLR4* polymorphisms and susceptibility to bacterial and fungal infections after LT [31]. Nevertheless, previous studies also showed that *TLR4* polymorphisms are associated with susceptibility to LPS in animals and humans [32-34]. It should be mentioned that other *TLR4* polymorphisms have been widely implicated in infections and other diseases, but not in the context of LT; these include Asp299-Gly (rs4986790) and Thr399Ile (rs4986791), which are associated with viral and Gram-negative bacterial infections [35, 36].

The multivariate analysis showed that the duration of endotracheal intubation and the rs2149356 SNP were independent risk factors for bacterial infection after LT. Indeed, endotracheal intubation is a well-known risk factor for pneumonia, especially in patients with a compromised immune system [25, 37]. On the other hand, the role of the rs2149356 SNP in infections is less well known. A previous study showed that the rs2149356 SNP was associated with the development of invasive bacte-

rial infections in children [21]. Another study showed that this SNP played a role in hepatitis B recurrence after LT [38]. The rs2149356 SNP could also play a role in *Helicobacter pylori* infection and the risk of gastric cancer [39]. Nevertheless, these results are inconsistent and a number of factors (demographics, clinical, and genetic) could participate in the modulation of the risk of infection in different populations and different clinical settings. Additional studies are needed to solve this point.

This study suffers from some limitations. First, the sample size was small and the possibility of statistical errors cannot be excluded. In addition, we could not adequately compare the frequencies of various SNPs by bacterial infection type (Gram-positive vs. Gram-negative) due to the limited sample size. All patients were from a single center, and a selection bias cannot be ruled out. In addition, the immunosuppressant therapy was tailored to each patient's condition and was therefore variable among patients, leading to variable susceptibility to infections. Only *TLR4* polymorphisms were studied and additional ones should be studied such as NOD2 polymorphisms [40]. In addition, while studying single-gene variants, environmental factors might be responsible for the higher risk of dependent variables. Nevertheless, these results need to be confirmed in cohorts from other centers.

In summary, recipient *TLR4* rs1927907, rs1927914, rs11536889, and rs2149356 SNPs were associated with infections within 6 months after LT. Prolonged duration of endotracheal intubation and the *TLR4* rs2149356 SNP were independently associated with infections after LT. Patients with those factors should be strictly assessed in the early post-transplant period. Further multicenter studies with larger samples are required to identify gene polymorphisms of the innate immune system that influence the susceptibility to infection after LT.

Acknowledgements

The study was supported by the Science and Technology Department of Shanghai in China (No. 13JC1404600) and the National Natural Science Foundation of China (No. 81170447).

Disclosure of conflict of interest

None.

Address correspondence to: Fang Fang, Department of Nursing, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Shanghai 200080, China. E-mail: fangfang071@sina.com; Lin Zhong, Department of General Surgery, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, 100 Haining Road, Shanghai 200080, China. E-mail: zhonglin1@medmail.com.cn

References

- [1] Liou IW. Management of end-stage liver disease. *Med Clin North Am* 2014; 98: 119-152.
- [2] Itri JN, Heller MT and Tublin ME. Hepatic transplantation: postoperative complications. *Abdom Imaging* 2013; 38: 1300-1333.
- [3] Otero-Ravina F, Rodriguez-Martinez M, Selles CF, Gutierrez MG, Blanco MD, de Rituerto ST, Perez EV, Gonzalez-Juanatey JR and Sanchez-Guisande Jack D. Analysis of survival after liver transplantation in Galicia, Spain. *Transplant Proc* 2005; 37: 3913-3915.
- [4] Blair JE and Kusne S. Bacterial, mycobacterial, and protozoal infections after liver transplantation-part I. *Liver Transpl* 2005; 11: 1452-1459.
- [5] Dummer JS, Hardy A, Poorsattar A and Ho M. Early infections in kidney, heart, and liver transplant recipients on cyclosporine. *Transplantation* 1983; 36: 259-267.
- [6] Katsolis JG, Bosch W, Heckman MG, Diehl NN, Shalev JA, Pungpapong S, Gonwa TA and Hellinger WC. Evaluation of risk factors for cytomegalovirus infection and disease occurring within 1 year of liver transplantation in high-risk patients. *Transpl Infect Dis* 2013; 15: 171-180.
- [7] van Hoek B, de Rooij BJ and Verspaget HW. Risk factors for infection after liver transplantation. *Best Pract Res Clin Gastroenterol* 2012; 26: 61-72.
- [8] Zhong L, Men TY, Li H, Peng ZH, Gu Y, Ding X, Xing TH and Fan JW. Multidrug-resistant gram-negative bacterial infections after liver transplantation-spectrum and risk factors. *J Infect* 2012; 64: 299-310.
- [9] Razonable RR. Innate immune genetic profile to predict infection risk and outcome after liver transplant. *Hepatology* 2010; 52: 814-817.
- [10] Perkins JD. Predicting posttransplantation infection risk with gene polymorphisms. *Liver Transpl* 2006; 12: 488-489.
- [11] Sanclemente G, Moreno A, Navasa M, Lozano F and Cervera C. Genetic variants of innate immune receptors and infections after liver transplantation. *World J Gastroenterol* 2014; 20: 11116-11130.
- [12] Kawai T and Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nat Immunol* 2010; 11: 373-384.
- [13] Franchimont D, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Deviere J and Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; 53: 987-992.
- [14] Cervera C, Lozano F, Saval N, Gimferrer I, Ibanez A, Suarez B, Linares L, Cofan F, Ricart MJ, Esforzado N, Marcos MA, Pumarola T, Oppenheimer F, Campistol JM and Moreno A. The influence of innate immunity gene receptors polymorphisms in renal transplant infections. *Transplantation* 2007; 83: 1493-1500.
- [15] Ohto U, Yamakawa N, Akashi-Takamura S, Miyake K and Shimizu T. Structural analyses of human Toll-like receptor 4 polymorphisms D299G and T399I. *J Biol Chem* 2012; 287: 40611-40617.
- [16] Ducloux D, Deschamps M, Yannaraki M, Ferrand C, Bamouid J, Saas P, Kazory A, Chalopin JM and Tiberghien P. Relevance of toll-like receptor-4 polymorphisms in renal transplantation. *Kidney Int* 2005; 67: 2454-2461.
- [17] Singh K, Singh VK, Agrawal NK, Gupta SK and Singh K. Association of toll-like receptor 4 polymorphisms with diabetic foot ulcers and application of artificial neural network in DFU risk assessment in type 2 diabetes patients. *Biomed Res Int* 2013; 2013: 318686.
- [18] Wang Z, Wu S, Chen D, Guo F, Zhong L, Fan J and Peng Z. Influence of TLR4 rs1927907 locus polymorphisms on tacrolimus pharmacokinetics in the early stage after liver transplantation. *Eur J Clin Pharmacol* 2014; 70: 925-931.
- [19] Mansur A, von Gruben L, Popov AF, Steinau M, Bergmann I, Ross D, Ghadimi M, Beissbarth T, Bauer M and Hinz J. The regulatory toll-like receptor 4 genetic polymorphism rs11536889 is associated with renal, coagulation and hepatic organ failure in sepsis patients. *J Transl Med* 2014; 12: 177.
- [20] Zaki HY, Leung KH, Yiu WC, Gasmelseed N, Elwali NE and Yip SP. Common polymorphisms in TLR4 gene associated with susceptibility to pulmonary tuberculosis in the Sudanese. *Int J Tuberc Lung Dis* 2012; 16: 934-940.
- [21] Esposito S, Bosis S, Orenti A, Spina S, Montinaro V, Bianchini S, Zampiero A and Principi N. Genetic polymorphisms and the development of invasive bacterial infections in children. *Int J Immunopathol Pharmacol* 2016; 29: 99-104.
- [22] Kamath PS, Kim WR; Advanced Liver Disease Study Group. The model for end-stage liver disease (MELD). *Hepatology* 2007; 45: 797-805.

TLR4 SNPs, bacterial infection and transplantation

- [23] Zhong L, Li H, Li Z, Shi B, Wang P, Wang C, Fan J, Sun H, Wang P, Qin X, Peng Z. C7 genotype of the donor may predict early bacterial infection after liver transplantation. *Sci Rep* 2016; 6: 24121.
- [24] de Rooij BJ, van Hoek B, ten Hove WR, Roos A, Bouwman LH, Schaapherder AF, Porte RJ, Daha MR, van der Reijden JJ, Coenraad MJ, Ringers J, Baranski AG, Hepkema BG, Hommes DW and Verspaget HW. Lectin complement pathway gene profile of donor and recipient determine the risk of bacterial infections after orthotopic liver transplantation. *Hepatology* 2010; 52: 1100-1110.
- [25] Wade JJ, Rolando N, Hayllar K, Philpott-Howard J, Casewell MW and Williams R. Bacterial and fungal infections after liver transplantation: an analysis of 284 patients. *Hepatology* 1995; 21: 1328-1336.
- [26] Howell J, Gow P, Angus P and Visvanathan K. Role of toll-like receptors in liver transplantation. *Liver Transpl* 2014; 20: 270-280.
- [27] Bronkhorst MW, Boye ND, Lomax MA, Vossen RH, Bakker J, Patka P and Van Lieshout EM. Single-nucleotide polymorphisms in the Toll-like receptor pathway increase susceptibility to infections in severely injured trauma patients. *J Trauma Acute Care Surg* 2013; 74: 862-870.
- [28] Lee SO, Brown RA, Kang SH, Abdel-Massih RC and Razonable RR. Toll-like receptor 2 polymorphism and gram-positive bacterial infections after liver transplantation. *Liver Transpl* 2011; 17: 1081-1088.
- [29] Qing YF, Zhou JG, Zhang QB, Wang DS, Li M, Yang QB, Huang CP, Yin L, Pan SY, Xie WG, Zhang MY, Pu MJ and Zeng M. Association of TLR4 Gene rs2149356 polymorphism with primary gouty arthritis in a case-control study. *PLoS One* 2013; 8: e64845.
- [30] Lee SO, Brown RA, Kang SH, Abdel Massih RC and Razonable RR. Toll-like receptor 4 polymorphisms and the risk of gram-negative bacterial infections after liver transplantation. *Transplantation* 2011; 92: 690-696.
- [31] de Mare-Bredemeijer EL, Mancham S, Utomo WK, de Canck I, van Thielen M, de Meester E, Rossau R, van der Laan LJ, Hansen BE, Tilanus HW, Kazemier G, Janssen HL, Metselaar HJ and Kwekkeboom J. Genetic polymorphisms in innate immunity receptors do not predict the risk of bacterial and fungal infections and acute rejection after liver transplantation. *Transpl Infect Dis* 2013; 15: 120-133.
- [32] Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL and Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; 25: 187-191.
- [33] Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K and Akira S. Cutting edge: toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: evidence for TLR4 as the Lps gene product. *J Immunol* 1999; 162: 3749-3752.
- [34] Lorenz E, Mira JP, Frees KL and Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. *Arch Intern Med* 2002; 162: 1028-1032.
- [35] Al-Qahtani AA, Al-Anazi MR, Al-Zoghaibi F, Abdo AA, Sanai FM, Khan MQ, Albenmoussa A, Al-Ashgar HI and Al-Ahdal MN. The association of toll-like receptor 4 polymorphism with hepatitis C virus infection in Saudi Arabian patients. *Biomed Res Int* 2014; 2014: 357062.
- [36] Sampath V, Mulrooney NP, Garland JS, He J, Patel AL, Cohen JD, Simpson PM and Hines RN. Toll-like receptor genetic variants are associated with gram-negative infections in VLBW infants. *J Perinatol* 2013; 33: 772-777.
- [37] Melsen WG, Rovers MM, Groenwold RH, Bergmans DC, Camus C, Bauer TT, Hanisch EW, Klarin B, Koeman M, Krueger WA, Lacherade JC, Lorente L, Memish ZA, Morrow LE, Nardi G, van Nieuwenhoven CA, O'Keefe GE, Nakos G, Scannapieco FA, Seguin P, Staudinger T, Topeli A, Ferrer M and Bonten MJ. Attributable mortality of ventilator-associated pneumonia: a meta-analysis of individual patient data from randomised prevention studies. *Lancet Infect Dis* 2013; 13: 665-671.
- [38] Zhou L, Wei B, Xing C, Xie H, Yu X, Wu L and Zheng S. Polymorphism in 3'-untranslated region of toll-like receptor 4 gene is associated with protection from hepatitis B virus recurrence after liver transplantation. *Transpl Infect Dis* 2011; 13: 250-258.
- [39] Castano-Rodriguez N, Kaakoush NO, Pardo AL, Goh KL, Fock KM and Mitchell HM. Genetic polymorphisms in the Toll-like receptor signaling pathway in *Helicobacter pylori* infection and related gastric cancer. *Hum Immunol* 2014; 75: 808-815.
- [40] Janse M, de Rooij BJ, van Hoek B, van den Berg AP, Porte RJ, Blokzijl H, Coenraad MJ, Hepkema BG, Schaapherder AF, Ringers J, Weersma RK and Verspaget HW. Recipient's genetic R702W NOD2 variant is associated with an increased risk of bacterial infections after orthotopic liver transplantation. *PLoS One* 2013; 8: e72617.