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Association between donor and recipient Interleukin-18 gene polymorphisms and the risk of infection after liver transplantation

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Abstract

Purpose: The purpose of this study was to retrospectively evaluate the association between Interleukin-18 (IL-18) gene polymorphisms of the donor and recipient in liver transplant patients with bacterial infections.

Methods: Five single nucleotide polymorphisms (SNPs) (rs7106524, rs5744247, rs1946518, rs549908 and rs187238) of the IL-18 gene from the donors were genotyped and their association with post-operative bacterial infections was evaluated in liver transplant patients (N=113). A second independent group of liver transplant patients from a different organ transplant centre was also recruited for validation purposes (N=44).

Results: IL-18 mRNA mean expression levels and protein levels were significantly lower in liver transplant patients with bacterial infections. For the donor SNP rs1946518, more recipients carried the A allele in the bacterial-infected group than the uninfected group (61.4% vs 39.7%; $P \leq 0.002$). The mean IL-18 mRNA expression and protein levels were significantly lower in the transplanted livers of recipients carrying the rs1946518 AA genotype compared with those from recipients with CC genotype (3.64, 3.33 vs. 2.75, $P \leq 0.048$). The A allele of rs1946518 also resulted in lower luciferase activity than the C allele in a reporter assay. The area under ROC curve indicated that the rs1946518 SNP genotype in the donor liver predicted an increased risk of bacterial infection after liver transplantation (AUROC > 0.82).

Conclusions: These findings indicate that the IL-18 rs1946518 SNP in the donor liver is a risk factor for developing bacterial infection after liver transplantation.

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Advances in organ procurement, improved surgical techniques, earlier detection and treatment of postoperative complications and new immunosuppressive drugs have made liver transplantation an effective and cost-beneficial option for the treatment of end-stage liver disease. The United Network for Organ Sharing (UNOS) has stated that most transplant centers report a one-year survival rate exceeding 80% [1]. Bacterial infection is one of the most frequently occurring post-transplant complications, being responsible for approximately 70% of complications, and is one of the leading causes of morbidity and mortality in liver transplant recipients [2-5]; therefore, identifying patients at a high risk of developing a bacterial infection would be beneficial for preventing disease and for improving the long-term prognosis after liver transplantation.

Several demographic and clinical risk factors have been implicated in the development of bacterial infection following liver transplant [6-10]; however, some patients who do not have these risk factors are still predisposed to serious infections, suggesting a genetic influence. It is possible that genetic backgrounds of either and/or both the liver donor and the recipient may influence the risk of developing pathogenic infections [11-13].

The Th1 and Th2 immune responses normally play key roles in the host defense mechanism to eliminate intruding bacteria [14]. These immune response mechanisms are severely compromised as a result of surgical stress, making patients susceptible to bacterial infections [14]. Interleukin-18 (IL-18) is a member of the IL-cytokine family and up regulates both Th1 and Th2 [14,15]. IL-18 also activates cytotoxic T lymphocytes and natural killer cells [13,16] and participates in both innate and acquired immunity. The IL-18 gene has been shown to map to chromosome 11q22.2-22.3 [17]. Single nucleotide polymorphisms (SNPs) in the IL-18 gene have been identified and these SNPs have been shown to affect IL-18 protein levels and influence viral and bacterial infections [18-24].

Although IL-18 clearly plays a role in the host immune response, whether different polymorphisms in the gene affect the incidence of bacterial infection in patients following liver transplantation is not yet known. Prior work performed by our group found that the genotype of the C7 complement factor was associated with bacterial infection in liver transplant recipients [25]: liver recipients with the C7 rs6876739 CC genotype were more prone to infection than were recipients with the rs6876739 TT genotype and the rs6876739 CC genotype was associated with lower levels of C7 mRNA and protein. C7 and the other terminal complement proteins

interact to form the MAC, which directly lyses foreign pathogens; hence, a reduction in C7 may impact MAC function. We also found that MAC also triggers NLRP3 inflammasome activation and IL-1 β release from Th1 cells. The inflammasome activates intracellular caspase-1 autocatalytically, which cleaves the inactive precursors of IL-1 β and IL-18 into bioactive cytokines. Due to the interconnection between the complement system and IL-18 activity, we hypothesized that SNPs that alter IL-18 protein levels may also impact the risk of bacterial infection in liver transplant recipients. The purpose of this study was to investigate whether polymorphisms of IL-18 in the liver of the donor influence the development of bacterial infection after liver transplantation in a Han Chinese population.

Methods

Patients

This was a retrospective study in patients undergoing primary liver transplantation between July 2007 and January 2011 at Shanghai Jiaotong University Affiliated First Peoples Hospital. The hospital is a 1586-bed tertiary-care institute in China and specializes in organ transplantation. Informed consent was obtained from all recipients. Each organ donation and transplant was approved by the hospital's Institutional Review Board and the study was performed under the guidelines of the Ethics Committee of the hospital, the current regulations of the Chinese government and the Declaration of Helsinki. Orthotopic liver transplants were performed using cadaveric livers and all had end-to-end biliary anastomosis constructed without T-tube drainage. No donor livers were harvested from executed prisoners. The original data from other transplant centres were sent to the authors, who performed all the analyses.

Study patients

Eligible patients (≥ 18 years of age) who underwent primary liver transplantation, had compatible blood and tissue type with donors and were not receiving combined liver/kidney transplant were recruited for the study. If a patient received two liver-transplant surgeries, only the data from the first surgery were included in the analysis. All liver-transplant recipients were evaluated using UNOS MELD scoring system to determine the severity of the liver disease [26]. The experimental group was patients with bacterial infection following liver transplantation and the comparator population was transplant patients from the same hospital who had no infections. To further test the possible role of IL-18 SNPs in

bacterial infection, a second patient population of liver transplant recipients, recruited from another transplant center (Qianfo Mountain Hospital affiliated with Shandong University) and selected using the same inclusion/exclusion criteria as above, was evaluated. All patients received the same treatment designed to prevent infection.

Data collection

Data were collected from hospital and operative records of each liver transplantation recipient, including perioperative demographics, clinical characteristics, operative information (such as time, blood loss, etc.) and clinical events within six months post-transplantation (i.e., duration of initial intubation, length of intensive care unit stay, another operation, dialysis and liver rejection).

Definition of infection

The definitions for bacterial infection were published in our previous studies [10,26]. Infection was confirmed by a single culture after observing clinical signs of infection (e.g., chills, fever, hypotension and the presence of infection determined by computer tomography [CT] or chest X-ray) or isolation of a microorganism from two consecutive cultures associated with signs of infection. Specimens were taken from corresponding infected sites for identification of bacterial species. Multiple samples from the same patient were taken at different time points.

IL-18 genetic and molecular analysis

Recipient and donor genotypes were determined separately. Genomic DNA was extracted and the presence and frequency of the IL-18 gene SNPs, rs7106524, rs5744247, rs1946518, rs549908 and rs187238 were evaluated.

To assess IL-18 RNA levels, IL-18 cDNA was generated from total RNA using RT-PCR, and the levels quantitated using Sybgreen PCR mix (Qiagen, Venlo, Netherlands) and ABI ViiA7 Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Actin mRNA level was used as an internal control.

The effect of the rs1946518 SNP on promoter activity of IL-18 was evaluated using LO2 (a human normal hepatocyte cells) transiently co-transfected with pGL3-IL-18 promoter (rs1946518A) and pRL-TK, or pGL3-IL-18 promoter (rs1946518C) (both constructs contained a 752-bp fragment of the promoter region of the human IL-18 gene either with or without the SNP) and pRL-TK by lipofectamine (Life Technologies, Carlsbad CA, USA) according to the

manufacturer's instructions. Twenty-four hours after transfection, the cells were lysed and assayed for firefly luciferase activity (Dual-Luciferase Reporter Assay System, Promega, Madison WI, USA) according to the manufacturer's instructions. Transfection and luciferase assays were performed in triplicate.

IL-18 serum levels

Blood was collected from recipients before and at one, three and seven days after liver transplantation. Serum was prepared by centrifugation and stored at -20°C. Serum IL-18 levels were measured by ELISA kit (BMS 267/2, Bender MedSystems, Vienna, Austria) in accordance with the manufacture's instruction.

IL-18 immunohistochemistry

For immunohistochemistry, a rabbit polyclonal anti-human IL-18 antibody (1:800 dilution; Abcam, Cambridge, UK) was used to detect IL-18 protein expression in tissue sections.

Statistical analysis

Continuous variables were presented as median and interquartile ranges (IQRs), since the Kolmogorov-Smirnov tests showed these variables were not normally distributed. The Mann-Whitney U tests were performed for the comparisons between the bacteria-infected and non-infected groups. Categorical variables were presented as counts and percentages, with Chi-square tests or Fisher's exact tests for group comparisons. Logistic regression was utilized to investigate the risk factors associated with bacterial infection. Variables reaching statistical significance in univariate analyses were included in multivariable analyses, with stepwise model selection.

The area under ROC (AUROC) curve was performed to evaluate the predictive ability among different models. The Kaplan-Meier curves with log-rank test were used to evaluate the survival rates among donor and recipient IL-18 gene genotypes. Statistical analyses were considered significant when two-sided *P*-value < 0.05. Statistical analyses were performed using SAS 9.2 statistics software (SAS Institute, Cary, NC USA). The Kaplan-Meier curves were performed by SPSS 17.0 statistics software (IBM, Chicago, IL USA).

Supplemental data

Data on bacterial infections and antibiotic treatments, and experimental details of IL-18 genetic, molecular analyses and immunohistochemistry are available upon request.

Results

Demographics and clinical characteristics

Of the included patients (N=113), 69 had no bacterial infection and 44 had infection following surgery (Table 1). All infections occurred within six months of transplantation. Of the 44 infected patients, 21 had pulmonary infection, nine had sepsis, nine had cholangitis and five had other infections. The types of bacteria responsible for each infection varied across the patients. Most patients (in both groups) were male and the median age was 48 years. There was no difference in the MELD score ($P=0.410$), encephalopathy grade ($P=0.960$) and Child-Pugh scores ($P=0.599$) between groups (Table 1). A greater percentage of subjects in the infected group had used broad-spectrum antibiotics (38.7%) prior to surgery compared with the non-infected group (11.6%) ($P=0.001$). Fewer than 10% of patients had diabetes and operative and post-operative variables were similar between the groups. Reasons for liver transplant are summarized in Table 1.

IL-18 genotype distributions between patients with and without bacterial infection

Donor and recipient genotype frequencies were all in Hardy-Weinberg equilibrium. The allele frequency of IL-18 SNPs rs1946518, rs7106524, rs549908, rs5744247 and rs187238 were 0.48, 0.47, 0.10, 0.40 and 0.11, respectively. The frequency of alleles for the recipients for the SNPs rs1946518 rs7106524, rs549908, rs5744247, rs187238 and were 0.49, 0.50, 0.14, 0.35 and 0.14. All analyzed IL-18 SNPs were in Hardy-Weinberg equilibrium within each patient group, except for IL-18 rs7106524 and rs1946518 ($D' = 0.990$, $r^2 = 0.895$).

For donor livers, the genotype and alleles differed significantly between the bacteria-infected and non-infected recipient patient groups for SNPs rs1946518 and rs7106524 ($P \leq 0.036$) (Table 2). No significant differences were found between groups for the other three donor SNPs ($P \geq 0.094$). For rs1946518, a greater percentage of patients in the infected group had received livers with the AA genotype (43.2%) and A allele (61.4%) compared with the non-infected group (14.7% for the AA genotype and 39.7% for the A allele) ($P \leq 0.003$). For rs7106524, a greater portion of patients in the infected

group compared with the non-infected group had received livers that carried the GG genotype (45.2% vs. 22.4%, respectively; $P=0.036$) and the G allele (64.3% vs. 46.3%; $P=0.009$). In recipients, there were no significant differences across the five SNPs between the infected and non-infected groups ($P \geq 0.183$). There was also no association in donor livers or recipients between IL-18 SNPs genotypes and the different types of infections ($P > 0.05$).

Multivariate analysis found that donor livers with rs1946518 SNP, but not rs710652, were associated with bacterial infection; hence, we focused our analysis on rs1946518.

To further investigate the possible role of the donor liver rs1946518 genotype on the risk of infection following liver transplantation, 44 liver-transplant patients from a different organ transplant center were recruited. Similar to our original group of patients, the percentages of this second group of patients, who received livers with donor rs1946518 AA genotype and the A allele, were higher in the bacteria-infected group compared with the non-infected group (AA genotype: 53.3% vs. 17.2%; $P=0.037$, A allele: 66.7% vs. 34.5%; $P=0.004$).

IL-18 gene and protein expression

IL-18 gene expression and protein levels from recipients, with and without an infection and who carried donor livers with different rs1946518 genotypes, were assessed. Immunohistochemistry staining found that IL-18 protein expression in infected patients was lower than that in non-infected patients (Figure 1A). Consistent with the lower protein expression, we found that the mean IL-18 mRNA expression levels was significantly lower in patients with infections compared with those without infections (3.04 vs. 3.5; $P=0.015$) (Table 3; Figure 1B). The mean of IL-18 mRNA expression was also significantly higher in patients who received livers from donors carrying the rs1946518 AC and CC genotypes than those who received livers with the AA genotype (3.64, 3.33 vs. 2.75; $P \leq 0.001$).

The mean IL-18 serum levels before liver transplantation were low (28.4 pg/mL) in all liver recipients. Following liver transplantation, IL-18 protein serum levels increased in liver recipients and was higher in recipients who received livers from donors carrying the rs1946518 CC genotype compared with donor livers carrying the rs1946518 AA genotype ($P < 0.05$) (Figure 1C).

The rs1946518 SNP is located in the promoter region of the IL-18 gene and the different alleles may influence promoter activity. To test this idea, rs1946518 C and A alleles

FIGURE 1. (A) Immunohistochemistry staining of IL-18 staining in donor liver tissue based on (left) the patient being infected and (right) not infected. The images showed that IL-18 protein expression in infected patients is lower than that in non-infected patients. (B) The relative expression of IL-18 mRNA in the donor liver of infected and non-infected patients. IL-18 mRNA levels were lower in infected group compared with the non-infected group. The independent t-test was performed to compare the differences between infected and non-infected patients. (C) The relationship between serum IL-18 level and infection following transplant. The IL-18 serum level of the recipients with donor rs1946518 CC was higher compared with those with donor rs1946518 AA genotype. (D). Comparison of promoter activity between AA and CC alleles of the IL-18 promoter rs1946518. The luciferase activity of the rs1946518 CC allele was significantly higher than that of the rs1946518 AA allele.

in front of the luciferase gene were cloned and luciferase activity assayed in LO2 cells. The C allele resulted in greater luciferase gene transcription resulting in higher levels of luciferase activity compared with the A allele ($P=0.026$) (Figure 1 D).

Risk factors associated with infection

Univariate logistic regression analysis indicated that gender, the use of broad-spectrum antibiotics prior to liver transplantation, prolonged endotracheal intubation of ≥ 72

hours, additional post-transplant operations, biliary complications, stay in the ICU after liver transplantation, the rs1946518 and rs7106524 genotype of the donor liver were all statistically significant risk factors for developing bacterial infection (all $P \leq 0.034$) (Table 4).

Multivariate analysis employed two different models, both of which used stepwise selection of variables found significant in the univariate analysis: Model 1 included clinical factors and Model 2 included both clinical and genetic factors. Model 1 found that gender, use of broad-spectrum antibiotics prior to transplant surgery and length of stay in the ICU following

TABLE 1. Demographic and clinical characteristics of subjects

	Total (N=113)	Non-infection (N=69)	Infection (N=44)	<i>P</i> value
Pre-transplant variables				
Age (years)	48 (42, 55)	48 (42, 54)	49 (41.5, 55)	0.748
Gender				0.018*
Male	94 (83.19%)	62 (89.86%)	32 (72.73%)	
MELD score	10 (8, 14)	10 (8, 13)	11 (8, 14)	0.410
Encephalopathy grades II-IV	5 (4.42%)	3 (4.35%)	2 (4.55%)	0.960
Child-Pugh Score	7 (5, 9)	7 (5, 8)	7 (5, 9)	0.599
Pre-LT broad-spectrum antibiotics	25 (22.12%)	8 (11.59%)	17 (38.64%)	0.001*
Diabetes mellitus	8 (7.08%)	6 (8.7%)	2 (4.55%)	0.480
Reasons leading to LT				
Hepatocellular carcinoma	60 (53.1%)	40 (57.97%)	20 (45.45%)	0.386
Cirrhosis related to hepatitis B viruses	33 (29.2%)	21 (30.43%)	12 (27.27%)	
Hepatitis C viruses and alcohol-related cirrhosis	3 (2.65%)	1 (1.45%)	2 (4.55%)	
Autoimmune cirrhosis	4 (3.54%)	2 (2.9%)	2 (4.55%)	
Non-functional graft	6 (5.31%)	2 (2.9%)	4 (9.09%)	
Other etiologies	7 (6.19%)	3 (4.35%)	4 (9.09%)	
Operative variables				
Packed red cell transfusion (U)	8 (2, 14)	6 (0, 12)	9 (5, 14)	0.243
Blood loss during LT (mL)	2550 (1200, 5250)	2550 (1300, 5000)	2750 (1200, 6250)	0.672
Operation time (hrs)	6 (6, 8)	6 (5.5, 7)	6.5 (6, 8)	0.096
Anhepatic time (min)	60 (55, 60)	60 (50, 60)	60 (59, 60)	0.450
Post-transplant variables				
Post-LT transfusion (U)	0 (0, 4)	0 (0, 3)	0 (0, 7.5)	0.122
Post-LT antibiotics (days)	17 (13, 23)	16 (12, 21)	18 (14, 26)	0.077
ICU stay after LT (hrs)	384 (264, 552)	312 (216, 480)	504 (336, 648)	<0.001*
Post-transplant renal dysfunction	7 (6.19%)	3 (4.35%)	4 (9.09%)	0.428
Prolonged endotracheal intubation (≥72 hrs)	22 (19.47%)	9 (13.04%)	13 (29.55%)	0.031*
Endotracheal reintubation	5 (4.42%)	1 (1.45%)	4 (9.09%)	0.075
Post-transplant re-operative episodes	10 (8.85%)	3 (4.35%)	7 (15.91%)	0.045*
Acute rejection with high-dose corticosteroids or ATG therapy	20 (17.7%)	11 (15.94%)	9 (20.45%)	0.540
Biliary complications	15 (13.27%)	5 (7.25%)	10 (22.73%)	0.018*
Post-LT without application of prednisone	32 (33.33%)	16 (26.23%)	16 (45.71%)	0.051

Continuous variables were presented as median and interquartile ranges (IQRs). Categorical variables were presented as counts and percentages.

* $P < 0.05$ significant differences between infection and non-infection groups.

Abbreviations: LT, liver transplant

FIGURE 2. Receiver operating characteristic curve of models for the prediction of bacterial infection. Model 1 included only clinical parameters; Model 2 included both clinical parameters and genetic risk factors. The predictive ability by AUROC was significantly better in Model 2 than Model 1 ($P=0.025$).

surgery were significantly associated with infection. Model 2 found that gender, use of broad-spectrum antibiotics before surgery, length of ICU stay after surgery and rs1946518 genotype of the donor liver were significant risk factors for bacterial infection (all $P \leq 0.034$) (Table 3). For both Models 1 and 2, the use of broad spectrum antibiotics prior to liver transplant had the greatest odds ratio for developing bacterial infection (OR: 3.2 [95% confidence interval [95% CI]: 1.09-9.36] and 4 [95% CI: 1.22-13.12]) compared with the other associated factors (Table 4).

There was no association of rs1946518 SNP with either gender or the use of broad spectrum antibiotics prior to liver transplant (all $P < 0.05$). The frequency of different donor alleles of rs1946518, but not recipient alleles, differed significantly with respect to ICU stay ($P=0.04$ vs. 0.093, respectively).

FIGURE 3. Survival curve among recipient with rs1946518 genotypes in liver transplant patients. Kaplan-Meier curves with log-rank test were used to evaluate the survival rates among recipient rs1946518 gene genotypes. The recipient rs1946518 genotype CC had lower survival rate compared with the AA and AC genotypes.

Predictive abilities of models on bacterial infection

The predictive abilities of the two models on bacterial infection were evaluated by areas under ROC curve (AUROC) analysis. According to the AUROC, both models had relative good predictive abilities (AUROC > 0.70) and good fits, suggesting both models were appropriate for predicting bacterial infection. Significant differences were found between the two models in predictive abilities (AUC of Model 1: 0.73; AUC of Model 2: 0.82; $P=0.025$), demonstrating the predictive ability was significantly better when genetic risk factors were included (Figure 2).

Survival analysis

There were no significant differences among donor and recipient genotypes in survival rates, with the exception of recipients carrying rs1946518 genotype CC who had lower survival rates compared with those with AA and AC genotypes ($P=0.036$) (Figure 3).

TABLE 2. IL-18 genotype distributions between infection and non-infection

Donor		Genotype distribution, n (%)		P value
		No infection (N=69)	Infection (N=44)	
rs1946518	Genotype*			0.003
	AA	10 (14.7%)	19 (43.2%)	
	AC	34 (50%)	16 (36.4%)	
	CC	24 (35.3%)	9 (20.5%)	
	Alleles			0.002
	A	54 (39.7%)	54 (61.4%)	
	C	82 (60.3%)	34 (38.6%)	
rs7106524	Genotype*			0.036
	AA	20 (29.9%)	7 (16.7%)	
	AG	32 (47.8%)	16 (38.1%)	
	GG	15 (22.4%)	19 (45.2%)	
	Alleles			0.009
	A	72 (53.7%)	30 (35.7%)	
	G	62 (46.3%)	54 (64.3%)	

*Genotype was undetermined in one donor rs1946518 and four donor rs7106524.

TABLE 3. IL-18 mRNA expression in the patients with and without infection and in the different genotypes

	IL-18 mRNA expression	P value
Infection		0.015
No	3.50±0.90	
Yes	3.04±1.08	
Donor 1946518 type		<0.001*
AA	2.75±0.93	
AC	3.64±0.93*	
CC	3.33±0.94*	

* $P < 0.05$ significant differences compared with donor 1946518 type AA.

TABLE 4. Risk factors associated with infection assessed by multivariate analysis

	Odds ratio (95%CI)	<i>P</i> value
Model 1*		
Gender (ref=female)	0.23 (0.07-0.77)	0.017
Pre-LT broad-spectrum antibiotics (ref=no)	3.2 (1.09-9.36)	0.034
ICU stay after LT (hours)	1.004 (1-1.01)	0.002
Model 2†		
Gender (ref=female)	0.15 (0.04-0.54)	0.004
Pre-LT broad-spectrum antibiotics (ref=no)	4 (1.22-13.12)	0.022
ICU stay after LT (hours)	1.003 (1-1.01)	0.013
Donor rs1946518 genotype (ref=AA)		
AC	0.13 (0.04-0.42)	0.001
CC	0.19 (0.05-0.68)	0.011

*Model 1 included only clinical factors.

†Model 2 included both clinical and genetic factors.

Abbreviations: LT, liver transplant

Discussion

Bacterial infection in post-liver transplant surgery is a major complication resulting in increased morbidity and mortality. IL-18 binds to the interleukin-18 receptor and, together with IL-12, induces cell-mediated immunity following infection with microbial organisms or by compounds such as lipopolysaccharides. In this study, we evaluated the association between five IL-18 SNPs of donor livers and recipient patients with bacterial infection, risk factors for bacterial infection and survival in a population of Han Chinese who received liver transplants. Specifically, we retrospectively reviewed a group of 113 liver-transplant patients in which 38.9% of the patients had bacterial infections within the first six months post-transplantation. Among the patients with infections, 43.2% were infected by gram-positive bacilli and 56.8% were infected by gram-negative bacilli.

Of the five IL-18 SNPs evaluated, rs1946518 and rs7106524 were not in Hardy Weinberg equilibrium in the infected and non-infected patient groups. A greater percentage of patients in the infected group carried the donor rs1946518 AA (or A allele) or rs7106524 GG (or G allele) genotypes compared with the non-infected group. There were no significant differences in the five SNPs in the recipients' genotypes between the infected and non-infected groups.

The relationship of the IL-18 rs1946518 genotype of the donor liver and the risk of infection is supported by the observation that the IL-18 mRNA and protein levels were lower in patients who received livers with the rs1946518 AA genotype compared with patients who received livers with the CC genotype. In addition, multivariable analysis indicated that the rs1946518 SNP was a risk factor for developing bacterial infection and the AA genotype was associated with greater risk of bacterial infection compared with the AC and CC genotypes. The AUROC analysis for the model that contained the donor liver rs1946518 genotype was 0.82, suggesting that rs1946518 has very good predictability for increased risk of bacterial infection after liver transplantation. Other risk factors for infection included being female, the use of broad spectrum antibiotics before transplant surgery and the length of stay in the ICU after surgery. In support of these findings, among the second independent population of patients from a different transplant center, we found the same risk factors. Comparison of the use of only clinical risk factors (Model 1) with employing both clinical and genetic risk factors (Model 2) found greater predictive accuracy of about 10% when both clinical and genetic risk factors were included (AUC 0.73 for Model 1 and AUC 0.82 for Model 2; $P=0.025$). An improvement of 10% is important in the clinic. Our findings

support the use of both clinical and genetic risk factors (i.e., Model 2) for predicting the chance of infection, due to the higher accuracy than with the use of clinical risk factors alone.

There were no significant differences among donor and recipient genotypes in survival rates, with the exception of recipients carrying rs1946518 CC genotype who had lower survival rates compared with the AA and AC genotypes. The fact that the risk of bacterial infection and survival are associated with different rs1946518 genotypes may indicate that these two outcomes are controlled by different cellular processes and the different genotypes affect these processes differently. It is possible that soon following post-transplantation, the liver expression of the donor's liver IL-18 gene is greater compared with the recipient and, consequently, has a greater effect on infection rate. The absence of an effect of rs1946518 CC on survival rates may result from survival being a longer time point and it is possible that the levels of the donor and recipient IL-18 expression become more comparable over time. In our study, all infections occurred within six months post-transplantation. In addition, reduction in long-term survival was mainly due to tumor recurrence. These findings are consistent with other studies that found liver cancer recurrence is the most important factor affecting the prognosis of liver transplantation [27].

Although, the current study found IL-18 gene polymorphism of the donor liver did not affect survival following transplantation, prior studies have also found that infection may impact survival, as 50% to 90% of the deaths after liver transplantation were associated with infection [28,29]. It should be noted that the survival data were only analyzed by univariate analysis and that multivariate analysis, as well as further in-depth studies, are needed to confirm these results.

Our findings indicate that the genotype of rs1946518 in the donor liver may influence the development of infection following transplant surgery, suggesting for a period of time following transplantation, the IL-18 expression in the recipients is coming from the donor liver. Previous studies found rs1946518 was associated with hepatitis B viral infection [21], hepatitis C viral infection [30] and tuberculosis [23]. There is evidence that other IL-18 promoter polymorphisms (IL-18-105A/C and IL-18-137 G/C) affected IL-18 levels in monocytes [31]. Promoter polymorphisms may reduce IL-18 gene expression and, consequently, compromise the host response. Consistent with this, we found that rs1946518 AA genotype was associated with a greater risk of bacterial infection and that A allele reduced luciferase expression in our reporter gene assay.

We also found that rs7106524 in the donor liver was associated with infection; however, rs7106524 was not found to be a risk factor by multivariate analysis. Both rs7106524 and rs1946518 had strong linkage disequilibria suggesting there may be some selection on the presence of these SNPs. The rs7106524 is located in an intron and may influence splicing or translation, which may reduce the levels of IL-18 protein. Further studies are required to test this idea. The rs187238 (-137 C/G) SNP is also located in the IL-18 promoter regions; however, we found no association of this SNP with post-liver transplant infection. In addition, we did not find an association between rs549908 (+105 A/C) or rs5744247 (-380 G/C) alleles and bacterial infections, suggesting these SNPs may not significantly influence IL-18 levels or activity.

How the presence of these genotypes in the donated liver influence bacterial infection is not clear. It is possible that ongoing inflammatory response to injury in donor livers [32] and the specific genotype of the donor IL-18 may influence the rate of bacterial infection. It is unlikely that IL-18 levels due to the polymorphism influence risk of infection; otherwise the same recipient genotype would also have influenced the bacterial infection rate. Additional studies are necessary to further explore these findings.

The association of the use of broad spectrum antibiotics before transplant surgery and length of stay in the ICU after surgery were found to be risk factors for infection. The increased risk due to ICU length of stay may reflect longer mechanical ventilation and endotracheal intubation times, which are known to increase the risk of bacteremia by almost 17-fold [7] and to increase the risk of pneumonia by almost 8-fold after liver transplantation [33]. The increased risk in patients treated with broad-spectrum antibiotics may reflect the development of drug-resistant pathogens.

Hepatocellular carcinoma and hepatitis B-related cirrhosis were the main reasons for liver transplants in this study. This may reflect the high rate of hepatitis B virus infections in mainland China which often results in hepatocellular carcinoma. With the exception of gender (female), there were not pre-surgery clinical or demographic factors that appeared to influence the risk of developing post-liver transplantation bacterial infection. The incidence of infection (about 40%) was consistent with previous studies [10,34]. The most common infection post-liver transplantation was pulmonary infection.

How IL-18 expression influences the host immune system is not well understood. Critically ill patients following major surgery, trauma injury or burn injury are highly susceptible to bacterial infections/sepsis leading to extremely poor outcomes as a result of infectious complications. Their host defense

systems against infections, such as Th1-mediated cellular immunity, Th2-mediated humoral immunity, and neutrophil-mediated immunity, are severely impaired. IL-18 has the potential to up-regulate the Th1 and Th2 immune responses. It is known that IL-18, in synergy with IL-12, augments the Th1 response to bacterial infections. IL-18 also augments the Th2 response to allergic disorders in the absence of IL-12 [14]. IL-18 may influence other processes that may impact immunosuppression in liver transplant patients, hepatitis B recurrence and allograft function [35-37].

There are several limitations of this study that need to be acknowledged. The size of the population was small, which may have limited the ability to detect minor effects of the different SNPs. When single-gene variants are studied, environmental factors are usually responsible for the higher risk of the dependent variable. It should be noted that the survival data was only analyzed by univariate analysis and that multivariate analysis, as well as further in-depth studies, are needed to confirm survival analysis.

Conclusion

Our findings indicate that donor IL-18 rs1946518 SNP (A allele) was associated with an increased risk of bacterial infection after liver transplantation. These findings suggest that patients carrying this SNP may benefit from preoperative routine use of antibiotics. The study also found that the high AUROC curve suggested that rs1946518 SNP has a potential clinical value for the prediction of bacterial infection. The findings support the use of the combination of clinical risk factors and donor IL-18 gene polymorphism to predict the risk of infection following liver transplantation and to identify patients, prior to transplant, who may benefit from active therapies to prevent and control infection.

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