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Original Article

Donor SGT1 gene polymorphisms influence the incidence of bacterial infection after liver transplantation

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Abstract: Background and aim: The present study aimed to evaluate the association of donor suppressor of G-two allele of SKP1 (SGT1) gene polymorphisms with a bacterial infection after liver transplantation in a Han Chinese population. Bacterial infection represents the most important clinical problem after liver transplantation (LT). SGT1 has a positive impact on the innate defense against pathogens. Methods: A total of 127 Chinese patients undergoing primary LT in the hospital from May 2008 to November 2011 were included in this study to examine the possible association of *SGT1* gene polymorphisms with a bacterial infection. SGT1 expression was assessed by immunohistochemistry and multiplex reverse transcription polymerase chain reaction. Results: Donor rs9526974 polymorphisms (CG+GG vs CC genotype) were found to be significantly associated with bacterial infection. The mRNA and protein levels were lower in patients with the rs9526974 CG+GG genotype compared with patients with the CC genotype. The multivariatelogistic analysis revealed that donor rs9526974 genetic variation [95% confidence interval (CI) 1.674-9.678; $P = 0.002$)], intensive care unit stay after LT. Conclusions: The present findings suggested that the donor SGT1 rs9526974 CG+GG genotype was associated with an increasing risk for post-transplant bacterial infection, which might contribute to a novel strategy for preventing infection and improving postoperative outcomes.

Keywords: Bacterial infection, liver transplantation, SGT1 polymorphisms

Introduction

Owing to advanced operation techniques and new immunosuppressive drugs, infections instead of acute graft rejection have been a paramount cause of mortality and morbidity after liver transplantation (LT), which occurs with a high rate of up to 80% [1]. Furthermore, bacterial infections account for approximately 70% infectious complications post-transplantation [2]. A previous study revealed that high-risk clinical parameters including critical illness, prolonged operation time, overall level of immunosuppression, postoperative care, and technical complexity of LT surgery, as well as occurrence of invasive diagnostic procedures were associated with bacterial infections [3, 4]. However, it is still inevitable that some patients who have none of these high-risk infection clinical parameters acquire infection after LT, indicating that inherent susceptibility factors contribute to infection. Genetic polymorphisms in the innate immune system, from both donor and recipient, have been identified as important risk factors for infection after LT. For instance, de Rooij has demonstrated that donor and recipient lectin complement pathway gene polymorphisms determine the risk of bacterial infections after orthotopic LT [5, 6].

The suppressor of G-two allele of SKP1 (SGT1) is highly conserved among all eukaryotes [7]. SGT1 binds to different cochaperones and is involved in several specific cellular functions including ubiquitination, cyclic adenosine monophosphate pathway, centrosome maturation, kinetochore assembly, and immune response. SGT1 has been reported to be overexpressed and regulate the Akt, also known as

protein kinase B (PKB), signaling pathway by promoting the degradation of beta-TrCP-dependent PH domain leucine-rich repeat protein phosphatase 1 (PHLPP1) in gastric cancer [8, 9]. Meanwhile, with or without required for Mla12 resistance (RAR1), SGT1 protein and heat shock protein (HSP)90 consist of a chaperone that controls nucleotide-binding domain and leucine-rich repeat receptor stability and activity in response to pathogen attack [10, 11]. Silencing of SGT1 results in the abrogation of inflammasome activity [12].

Although SGT1 clearly plays a role in host immune response, it is not known whether different polymorphisms in the SGT1 gene affect the incidence of bacterial infection in patients following LT. This study was designed to evaluate the association of donor SGT1 genotype polymorphisms with the risk of bacterial infection after LT in a Han Chinese population.

Materials and methods

Patients

This study retrospectively analyzed 127 patients undergoing orthotopic LT in the transplant center for end-stage liver diseases from May 2008 to November 2011. Patients aged more than 18 years who were blood and tissue matched with donors and did not have combined liver/kidney transplantation were included in the study. The study population was Han Chinese. The data from the first surgery of the patients who underwent two LT surgeries were included for the analysis.

Ethics statement

Each organ donation or transplantation was approved by the Institutional Review Board, Shanghai Jiaotong University Affiliated First People's Hospital, Shanghai, strictly under the guidelines of the ethics committee of the hospital and the Declaration of Helsinki [13]. All the LT recipients were evaluated using the Model for End-stage Liver Disease (MELD) scoring system implemented by the United Network for Organ Sharing [14].

Data collection

Hospital medical records and follow-up data of each LT recipient were the main resources, including preoperative demographic and clinical characteristics (i.e., age, gender, Child-Pugh and MELD), operative variables (i.e., operation time and blood loss), and clinical events within 6 months post-transplant [i.e., duration of initial intubation, intensive care unit (ICU) stay, reoperation, dialysis, and rejection]. For analyzing risk factors, control subjects were recruited from the same institution. All LT recipients were divided into two groups: cases with bacterial infection and controls without bacterial infection.

Infection definition

Bacterial infection definitions were as described previously [3, 15]. The diagnosis of bacterial infection was based on fever (>38°C), elevation of C-reactive protein, and specific clinical symptoms. The infection could be categorized as sepsis, pneumonia, wound infections, peritonitis, urinary tract infections, or cholangitis, confirmed by a single culture after observing clinical signs of infection (e.g., chills, fever, or hypotension, or by imaging such as computed tomography or chest x-ray) or isolation of a bacterial agent in two consecutive cultures associated with signs of infection. Specimens were taken from corresponding infected sites for identifying bacterial species. Multiple samples from the same patient were taken at different time points.

Pneumonia is defined as fever, cough, dyspnea, reduced arterial oxygen, typical pulmonary infiltrate on chest x-ray, and a positive culture from sputum or bronchoalveolar lavage. Urinary tract infection is defined as dysuria, leukocyturia, and a positive urine culture with $>10^5$ colony-forming units/mL. Wound infections are defined as detection of pus in the wound and a positive bacterial culture. Sepsis is defined as fever, low arterial blood pressure, systemic inflammatory response, and a positive bacterial blood culture. Cholangitis is defined as fever, elevation of cholestatic enzymes, and dilated bile ducts detected by ultrasound.

DNA extraction

Genomic DNA of patients was extracted from the EDTA-anticoagulated whole blood of recipients using the QIAamp DNA Blood Mini Kit (Qiagen, CA, USA) and from the fresh-frozen

Table 1. Summary of patients' demographic and clinical characteristics

	Total	No infection	Infection	*P*
N	127	77 (60.6%)	50 (39.4%)	
Age		48.18±8.72	47.70±10.56	0.774
Sex				
Male		64 (63.4%)	37 (36.6%)	0.262
Female		13 (50.0%)	13 (50.0%)	
Hepatocellular carcinoma	72	48 (66.7%)	24 (33.3%)	0.111
HBV-related hepatocirrhosis	109	67 (61.5)	42 (38.5)	0.634
Hepatitis B viruses	105	66 (62.9%)	39 (37.1%)	0.262
MELD score >30	6	3 (50.0%)	3 (0.530)	0.530
Hepatic encephalopathy	11	3 (27.3%)	8 (72.7%)	0.016
Child-Pugh				
A (5-6)	53	34 (64.2%)	19 (35.8%)	0.461
B (7-9)	49	31 (63.3)	18 (36.7%)	
C (>9)	24	12 (50.0%)	12 (50.0%)	
Pre-LT broad-spectrum antibiotics	18	11 (61.1%)	7 (38.9%)	0.964
Diabetes mellitus	16	12 (75.0%)	4 (25.0%)	0.264
Operative variables				
Packed red cell transfusion (U)		11.64±11.85	10.39±8.27	0.519
Operation time (hrs)		7.74±2.36	7.84±2.172	0.810
Blood loss during LT (mL)		3925.66±5373.43	4321.4±6325.55	0.707
Anhepatic time (min)		58.83±10.97	58.38±9.12	0.810
Post-transplant variables				
Post-transplant renal dysfunction	8	5 (62.5%)	3 (37.5%)	0.911
Prolonged endotracheal intubation (≥72 hrs)	25	9 (36.0%)	16 (64.0%)	0.005
Endotracheal reintubation or tracheotomy	3	1 (33.3%)	2 (66.7%)	0.320
Acute rejection with corticosteroids or ATG therapy	16	8 (50.0%)	8 (50.0%)	0.352
Post-LT antibiotics (days)		20.05±11.45	24±11.73	0.132
ICU stay after LT (hrs)		293.57±174.19	454.08±315.68	0.002
Diets restore(hrs)		77.43±54.24	106.59±100.68	0.093
Biliary complications	13	4 (30.8%)	9 (69.2%)	0.020
Post-transplant reoperative episodes	9	2 (22.7%)	7 (77.8%)	0.028
Post-LT with application of prednisone	85	47 (55.3%)	38 (44.7%)	0.080

Continuous data was presented by median with inter-quartile range (IQR); Categorical data was presented by count and percentage. *$P<0.05$ significant differences between infection and non-infection groups.

liver tissue of donors using the Maxwell 16 Tissue DNA Purification Kit (Promega, WI, USA) in accordance with the manufacturer's instructions. Single-nucleotide polymorphisms (SNPs) were detected using Applied Biosystems SNaPShot and TaqMan technology.

SGT1 immunohistochemical staining

A rabbit polyclonal anti-human SGT1 antibody (Abcam; 1:250 dilution) was used to detect SGT1. Sections were treated with a protease at 37°C for 20 min for antigen retrieval. Staining intensity was graded as follows: 0, no staining; 1+, mild staining; 2+, moderate staining; and 3+, intense staining. The staining area was scored using the following scale: 0, no staining of cells; 1+, <10% of tissue stained positive; 2+, 10%-50% stained positive; and 3+, >50% stained positive. The sum of the final staining score index was designated as follows: 0-2, negative expression; 3-4, weak expression; and 5-6, strong expression [16].

SGT1 cloning by reverse transcription polymerase chain reaction

The primer sequences for SGT1 mRNA were as follows: sense, 5'-CTG ACT AAG GCTTTG GAA CAG AA-3'; antisense, 5'-CTG TAA AAG TTTCTA

Table 2. Primary diseases leading to liver transplantation

	Total	No infection	Infection
Hepatocellular carcinoma related to cirrhosis	66	42	24
Hepatocellular carcinoma irrelated to cirrhosis	8	7	1
Cirrhosis related to hepatitis B viruses	36	23	13
Hepatitis B viruses	4	2	2
Autoimmune liver disease	4	3	1
Cirrhosis related to hepatitis C viruses	1	0	1
Alcoholic cirrhosis	4	2	2
Primary cholangitis	2	1	1
Budd Chiari syndrome	1	1	0
Liver diffuse hemangioma	1	0	1

Table 3. Donor SGT1 genotype association with bacterial infection

SNP Donor Rs9526974		Genotype distribution		*P* value
Genotype		No Infection	Infection	
Dominant model	CC	51 (69.9%)	22 (30.1%)	0.013
	GC+GG	26 (48.1%)	28 (51.9%)	
Recessive model	GC+CC	74 (62.2%)	45 (37.8%)	0.262
	GG	3 (37.5%)	5 (62.5%)	
Superdominant model	CC+GG	54 (66.7%)	27 (33.3%)	0.065
	GC	23 (50%)	23 (50%)	
Codominant model	CC	51 (63.9%)	22 (30.1%)	0.037
	GC	23 (50.0%)	23 (50.0%)	
	GG	3 (37.5%)	5 (62.5%)	

GGG CAG CA-3'. Real-time monitoring of polymerase chain reaction (PCR) was performed using the SYBR Green I dye (Roche, Basel, Switzerland). The PCR cycling started at 95°C for 30 s followed by 40 cycles of 95°C for 5 s, 60°C for 30 s, with a final step at 72°C for 20 min to allow for a complete extension of all PCR fragments.

Statistical analysis

Quantitative variables were compared using the Student's *t* test or Wilcoxon test. Differences in composition and distribution of categorical variables were assessed by Pearson's X^2 test or Fisher's exact test. Risk factors for bacterial infection were evaluated by a logistic regression analysis. A *P* value less than 0.05 represented statistical significance for all statistical comparisons. Statistical analyses were performed using SPSS19.0 software (SPSS, IL, USA).

Results

Demographic and clinical data

Patients' characteristics are shown in **Table 1**. A total of 127 LT patients included 26 females and 101 males with a median age of 47.85 years (range 20-67 years). Primary diseases in these cases are shown in **Table 2**: hepatocellular carcinoma related to cirrhosis (51.97%, *n* = 66), hepatocellular carcinoma unrelated to cirrhosis (6.30%, *n* = 8), and cirrhosis related to hepatitis B viruses (28.35%, *n* = 36). Fifty patients suffered from bacterial infections within 6 months after LT.

Table 1 shows that hepatic encephalopathy (27.3% vs 62.7%; *P* = 0.016), length of stay in the ICU postoperatively (293.57±174.19 vs 454.08±315.68; *P* = 0.002), percentage of patients with ≥ 72 h of endotracheal intubation (36.00% vs 64.00%; *P* = 0.005), percentage of patients with biliary complications (30.8% vs 69.2%; *P* = 0.020), and post-transplant reoperative episodes (22.7% vs 72.3%; *P* = 0.028) were significantly different between the two groups.

All the variables such as age, gender, MELD score, packed red cell transfusion, blood loss, operation time, and an hepatic time were not significantly different between the bacterial infection group and noninfection group.

The donor SGT1 rs9526974 CG+GG genotype was associated with an increasing risk for post-transplant bacterial infection

SGT1 rs9526974 alleles and genotype distributions in bacterial infection cases and controls are shown in **Tables 3** and **4**. A significant association between G allele and development of infection was found in allele comparison (G vs C) (OR = 1.931; 95% CI 1.067-3.484; *P* = 0.028). Similarly, the altered distribution of genotypes and alleles was observed in rs-

Table 4. Alleles distributions of donor rs9526974 between control and infection group

rs9526974(Allele)	No infection	Infection	*P* value	*OR* (95% CI)
C	126 (64.3%)	70 (35.7%)	0.028	1.931 (1.067-3.484)
G	28 (48.3%)	30 (51.7%)		

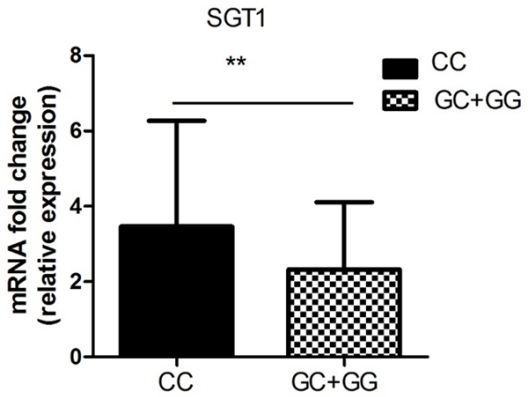

Figure 1. The relative expression of Sgt1 in donor liver based on rs9526974 genotype by RT-PCR. (**P<0.01 *P<0.05).

9526974 (C>G) between the two groups. The incidences of bacterial infection were remarkably lower in patients with the donor rs9526974 CC genotype than in the noninfection group. In a dominant model (GC+GG vs CC), a decreased risk of infection after LT was observed in individuals with CC genotype compared with those with the GC+GG genotype (adjusted OR = 2.497; 95% CI 1.201-5.187; P = 0.013).

Detection of SGT1 mRNA expression in the donor liver tissue by reverse transcription PCR

To further prove the association of rs9526974 genotype with SGT1 expression, reverse transcription (RT)-PCR was performed on these donor tissue samples, as shown in **Figure 1**. The CC donor liver tissue had higher mRNA expression of SGT1 compared with the GG+CG donor liver tissue (P = 0.0071), revealing a significant difference between the two groups.

SGT1 protein was overexpressed in donor liver tissue with rs9526974 CC genotype

Immunohistochemistry was performed on the donor sample to determine the level of SGT1 protein in the donor liver tissue (**Figure 2**). The immunoreactivity of SGT1 was lower in the rs9526974 GC+GG genotype compared with the CC genotype (P = 0.001) (**Table 5**).

Risk factors for bacterial infection: multivariate logistic regression analysis

Donor SGT1 rs9526974 polymorphisms, ICU stay after LT, prolonged endotracheal intubation, post-transplant reoperative episodes, biliary complications, and hepatic encephalopathy were considered as potential risk factors in the logistic regression analysis. The multivariate logistic regression analysis showed that donor SGT1 rs9526974 polymorphisms, ICU stay after LT, and hepatic encephalopathy were independent risk factors after LT (**Table 6**).

Discussion

The present study analyzed the association between the clinical characters and the incidence of infection after LT. The data showed that clinical features such as length of stay in the ICU postoperatively, percentage of patients with ≥ 72 h of endotracheal intubation, percentage of patients with biliary complications, and post-transplant reoperative episodes were potential predictors of infection for patients with LT, which was in accordance with foreign research [17]. When considering these clinical features with the genetic mutation, the genotype of SGT1 was also found to be associated with infection after LT.

SNP rs9526974, as a novel locus, lies in the intron variation region of SGT1. It was initially discovered as an essential component in yeast and now has been found to be highly conserved among all eukaryotes [6, 18]. SGT1 contains five distinct domains: tetratricopeptide repeat domain, two variable regions (VR1 and VR2), CS motif (present in CHP and SGT1 proteins), and SGS motif. SGT1 binds specifically to the molecular chaperone-HSP90 via its CS domain [19]. Additionally, SGT1 can interact with HSP70 through the SGS domain [20]. Findings indicated that SGT1 was a regulator of SCF and other cullin-based ubiquitin ligases [21-23]. In plants, SGT1 positively regulates disease resistance conferred by resistance (R)

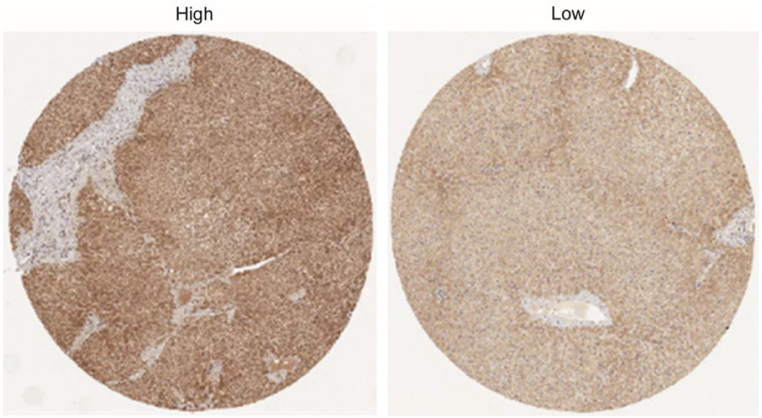

Figure 2. Immunohistochemistry staining of Sgt1 staining in donor liver tissue based on rs9526974 genotype. Representative IHC images of Sgt1 in donor tissue with CC and GC+GG genotype (magnification × 200)

Table 5. IHC scores of staining SGT1 in donor liver tissue according to rs9526974 genotype

Tissue	SGT1 expression		*P* value
	Low	High	
CC	19 (29.2%)	46 (70.8%)	0.001
GC+GG	37 (59.7%)	25 (40.3%)	

proteins [24]. R proteins have homologous structural features with the mammalian nucleotide oligomerization domain (NOD)-like receptors (NLR). NLR family, including Nod1 and Nod2, plays a crucial role in antipathogens as a pattern recognition receptor of intracellular bacteria [25]. The SGT1 plays a vital role as a sensor in NLR immune activity [10, 26]. It has other functions such as cell apoptosis and an impact on T-cell differentiation. Treatment with the siRNAs specifically targeted human SGT1 mRNA in MCF-7 Nod1 and Nod2 cells. Interestingly, it was found that SGT1 protein expression was almost completely abolished in MCF-7 Nod1 cells, but it was not impaired by Nod2 signaling [27], supporting the contention that SGT1 is essential for a functional Nod1 signaling pathway but does not appear to be required for the same responses induced by Nod2 pathway activation.

SGT1 was reported to be a promising antipathogen target against diseases. However, whether SGT1 gene polymorphisms are related to infection after LT in the Chinese population is still unknown. The present study retrospectively reviewed 127 LT patients of which 50 (39.4%) patients had a bacterial infection, and 77 patients (60.4%) had no infection after liver transplantation within the first 6 months. Donor SGT1 rs9526974 polymorphisms were found to be associated with bacterial infection. The patients who received the donor liver with the rs9526974 CG+GG genotype were more likely to acquire bacterial infection compared with those with the donor rs9526974 CC genotype (51.9% vs 30.1%, $P = 0.013$). RT-PCR was performed in this study to prove that the mRNA expression was lower in the rs9526974 GC+GG genotype compared with the CC genotype. The SGT1 protein level was less in the GC+GG donor liver tissue than in the CC donor liver tissue ($P = 0.001$) in accordance with the mRNA level. The multivariate logistic regression analysis revealed that the donor rs9526974 genotype was an independent risk factor for bacterial infection after LT (95% CI 1.674-9.678, $P = 0.002$). Patients with the donor rs9526974 CC genotype are less likely to have infection compared with those with the non-CC genotype. Rs9526974 C →G leads to a reduction in SGT1 expression, which is essential for NOD1 activation and degrades its disease resistance ability.

The present study had two limitations. First, SNP rs9526974 was the only SNP existing on SGT1 examined, and the potential combined influence of SNPs at other loci was neglected in this study. Hence, the data might not fully reveal the genotype distributions of the whole population. Second, follow-up functional studies should be extremely crucial to elucidating the direct mechanism underlying this association.

Altogether, our findings indicated that the genotype of rs9526974 in the donor liver might influence the development of infection following ICU stay after LT, and hepatic encephalopathy. The present findings revealed that SGT1 gene polymorphism was an important independent risk factor for early bacterial infection and mortality in LT patients. Donor rs95-

Table 6. Multivariate logistic regression analysis of risk factors associated with bacterial infection after liver transplantation

	Odds ratio (95% CI)	*P* value
SNP	4.025 (1.674-9.678)	0.002
ICU stay after LT	1.002 (1.000-1.004)	0.027
Hepatic encephalopathy	5.837 (1.216-28.030)	0.028

26974 CG+GG genotype was associated with an increasing relative risk of post-transplant bacterial infection, which was known to increase the risk of bacterial infection by almost 4-fold.

The results suggested that the donor SGT1 rs9526974 genotype might be a potential new marker of bacterial infection to predict recipient bacterial infection before transplantation, and might be beneficial for preventing disease and improving the long-term prognosis after liver transplantation identified as important risk factor of developing bacterial infection. Further mechanistic studies of this genetic variant are needed to clarify the present findings.

Disclosure of conflict of interest

None.

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