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Association of interleukin-28B polymorphisms with platelet count and liver function recovery after liver transplant

Yi Li, PhD[a], Tiantian Wei, MMed[b], Lin Yan, PhD[a], Zhiqiang Yang, PhD[c], Qian Huang, MMed[c], Yunying Shi, MD[b], Lanlan Wang[a,*]

Abstract

The present genome-wide association study investigated the relationship of interleukin 28B (IL-28B) genetic variants with HBV susceptibility and prognosis of HBV-infected patients. This study aims to examine the role of IL-28B polymorphisms on transplant etiologies and the liver function recovery in Chinese liver transplant recipients.

A total of 231 liver transplant recipients were enrolled in the study. The transplant etiologies included progressive HBV hepatitis, HBV-related liver cirrhosis (LC), HBV-related hepatocellular carcinoma (HCC), and non-HBV-related disease. All recipients were in stable condition before transplantation. Three single nucleotide polymorphisms (SNPs) of IL-28B (rs12979860, rs12980275, rs8099917) of recipients were analyzed by high-resolution melting (HRM) curve analysis. Liver function, blood cell count, and coagulation function were regularly tested before and for next 5 years after transplantation.

No significant association was found between IL-28B gene polymorphisms and transplant etiologies. Peripheral platelet count in the third and fourth days after transplantation were significantly higher in recipients carrying IL-28B rs12979860 T allele, or rs8099917 C allele ($P < .016666667$), while there were no significant differences between these variants and International Normalized Ratio (INR) levels. In addition, gamma-glutamyltransferase (GGT) levels in recipients with rs12980275 G allele were higher than those in the wide-type recipients before transplantation ($P < .016666667$, respectively); nevertheless, no influence of these variants on GGT recovery was observed after transplantation.

Genetic variations of IL-28B might impact on liver function recovery by influencing peripheral platelet counts and reducing liver inflammation, but have weak association with transplant etiologies.

Abbreviations: GGT = gamma-glutamyl transpeptidase, HCC = hepatocellular carcinoma, IFN-λ3 = interferon-lambda, LC = liver cirrhosis, LT = liver transplantation, SNPs = single nucleotide polymorphisms.

Keywords: coagulation function, gamma-glutamyltransferase, hepatitis B virus, IL-28B, liver transplantation, platelet

1. Introduction

Hepatitis B is one of the most common liver diseases worldwide, especially in China. It is often complicated by the development of cirrhosis and hepatocellular carcinoma (HCC). If HBV infection is not effectively treated, hepatocellular function may deteriorate progressively. For patients with end-stage liver diseases, liver transplantation (LT) is a highly effective treatment.[1–3] However, liver function recovery after LT varies between individuals and the recurrent HBV infection is one of the most important graft diseases that can occur after LT due to using the immunosuppressant therapy. The course of hepatitis B in a graft is usually more severe than that in nontransplant patients. In addition to viral, environmental, and behavioral factors, host genetic diversity is thought to contribute to the spectrum of the disease.[4]

Interleukin 28B (IL-28B), a member of type III interferons which termed interferon-lambda (IFN-λ3), plays an important role in antiviral immunity, especially in the IL-28B-mediated antiviral defense against hepatotropic viruses, such as HBV and HCV.[5,6] The gene for IL-28B is located on the long arm of chromosome 19 at position 19q13.13.[7,8] Recent studies have reported that IL-28B SNPs (rs1297860, rs12980275, and rs8099917) were associated with HCV spontaneous clearance as well as different outcomes of treatment with IFN/ribavirin therapy for chronic HCV infection.[9–16] In addition, studies indicate that IL-28B polymorphisms are also associated with HBsAg seroclearance, HBeAg seroconversion.[17–22] In our previous studies, we found that genotypes of IL-28B SNPs (rs8099917, rs12979860, and rs12980275) were associated with alanine aminotransferase (ALT) levels and aspartate aminotransferase (AST) levels in HBV-related LT recipients. Recipients with risk genes on IL-28B (the TT genotype of rs12979860, the GG

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[a] Department of Laboratory Medicine, [b] Kidney Research Institute, Division of Nephrology, West China Hospital, [c] West China Medical School, Sichuan University, Chengdu, China.

[*] Correspondence: Lanlan Wang, Department of Laboratory Medicine/Research Centre of Clinical Laboratory Medicine, West China Hospital, Sichuan University, No. 37, Guoxue Xiang, Wuhou District, Chengdu 610041, China (e-mail: wanglanlanhx@163.com).

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genotype of rs12980275, and the CC genotype of rs8099917) had a significantly higher AST concentration.[18,23] However, we have not found any association between IL-28B gene polymorphisms with the HBV recurrence in LT recipients.[20–22] The observations above suggest an important role of IL-28B SNPs against HBV infection. They also indicate that IL-28B SNPs might be associated with the recovery of HBV-related LT recipients.

As the IL-28B plays an important role in antiviral immunity and IL-28B SNPs can affect the outcomes of HCV or HBV infection, we analyzed whether IL-28B polymorphisms were associated with transplant etiologies and liver function recovery in liver transplant recipients.

2. Materials and methods

2.1. Patients

A total of 231 LT recipients, who underwent LT in West China Hospital from September 2000 to December 2011, were enrolled in this retrospective study. The HBV-related recipients were diagnosed by hepatitis B virus surface antigen (HBsAg) positive or high copies of serum HBV DNA ($>1 \times 10^4$ copies/mL) before transplantation. The differentiate of LC, HCC, and progressive hepatitis B was by histologic analysis of liver biopsy specimens during the liver transplant. All of these recipients included were in stable condition. Before transplantation, recipients were evaluated by Model for End-Stage Liver Disease (MELD) score and Child–Pugh score. Besides, those who had acute rejection, chronic rejection or platelet transfusion after LT were excluded from this study. After transplantation, all recipients received a calcineurin inhibitor (CNI)-based regimen (tacrolimus + mycophenolate mofetil + steroids). Steroids were discontinued in 3 to 6 months after transplantation. Liver function and coagulation function were monitored routinely in the next 5 years after LT. This study was approved by the ethics committee of West China Hospital and consistent with the guidelines of the Helsinki Declaration. All of the LT recipients signed an informed consent before inclusion in this study.

2.2. IL-28B gene polymorphisms

DNA was extracted from an EDTA anticoagulated peripheral whole blood using the whole blood DNA kit (Biotake Corporation, Beijing, China), diluted to 10 ng/mL by the AE buffer provided by the manufacturer and stored at −80°C until analyzed. The IL-28B gene polymorphisms in the promoter region (rs1297860, rs12980275, and rs8099917) were assessed. Some samples were previously genotyped by sequencing as controls for the 3 SNPs. The polymerase chain reaction (PCR) and melting curve analyses were performed under the same conditions in a 96-well plate on the Light Cycler480 (Roche Diagnostics, Penzberg, Bavaria, Germany). Primers for the 3 SNPs were as follows: rs12979860: 5′-ATTCCTGGACGTGGATGGGTAC-3′ (forward); 5′-AGCGC GGAGTGCAATTCA-3′ (reverse); rs8099917: 5′-TTGTCACTG TTCCTCCTTTTGTTT-3′ (forward); 5′-TGG GAGAATG-CAAATGAGAGATA-3′ (reverse); rs12980275: 5′-GCCAGTCT-CAAAAGAA CAAATGC-3′ (forward); 5′-CTACCCCGGCAA ATATTTAGACA-3′ (reverse). SNP genotyping was performed in a 10 μL reaction system contained 5 μL Roche Master Mix (Roche Applied Science, Mannheim, Germany) which comprises FastStart Taq DNA Polymerase and the High Resolution Melting Dye in a reaction buffer, 1.2 μL 25 mM $MgCl_2$, 0.1 μL 10 μmol/L forward primer and 0.1 μL 10 μmol/L reverse primer, 2.6 μL deionized water and finally 1 μL DNA sample. Real-time PCR was performed under the following conditions: a predenaturation step at 95°C for 15 minutes, continued with 50 cycles of 95°C for 10 seconds, 60°C for 15 seconds, and 72°C for 20 seconds. After the amplification phase, a melting curve analysis was performed at 95°C for 1 minute, 40°C for 1 minute, 65°C for 1 seconds, followed by slow heating at 0.01°C/second to 95°C. The results were analyzed by the Light Cycler 480 Gene Scanning software v1.2 (Roche Diagnostics). The genotype of subset was defined according to known genotypes of controls.

2.3. Laboratory tests measurement

HBV serological markers for HBsAg, HBeAg, anti-HBs, anti-HBc, and anti-HBe were conducted with ELISAs (Dade Behring, Marburg, Germany). All these recipients were divided into 4 groups by etiologies including liver cirrhosis (LC), HCC, progressive HBV hepatitis and non-HBV-related disease. Liver function, blood cell count, and coagulation function were regularly tested before LT, at the day of LT, in the 1st to 7th day, 14th day, 1st month, 3rd month, 6th month, 9th month, and the 1st to 5th year after LT. We tested alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (Alb), gamma glutamyl transpeptidase (GGT), total bilirubin (TB), direct bilirubin (DB), platelet count (PLT), prothrombin time (PT), international standardization ratio (INR), fibrinogen (FIB), activated partial thromboplastin time (APTT), and tacrolimus concentration.

2.4. Statistical analysis

Concordance of genotype distribution with Hardy–Weinberg equilibrium was assessed by Pearson Chi-square test. Clinical data were expressed as mean ± SD or median (interquartile range). Statistical analysis was performed by SPSS 19.0 (SPSS, Inc., Chicago, IL). Continuous variables with the normal distribution were analyzed using Student *t* test. Mann–Whitney *U* test or Wilcoson rank sum *W* test was used to compare the continuous variables that follow the skewed distribution. Pearson Chi-square test or Fisher exact test were used to analyze the categorical variables. A 2-sided P-value $< .05$ was considered statistically significant. We corrected the results with the Bonferroni method accounting for the number of SNPs ($P < .016666667$). When comparing the clinical indices, recipients were divided into 2 groups by genotypes: AA versus AB+BB (allele A vs allele B, A as the major allele, B as the minor allele). Because the minor alleles of the IL-28B SNPs are dominant and the number of recipients with BB genotype on rs12979860 was limited (<3). Haplotype analysis, which was performed to explore whether the selected SNPs were in strong linkage disequilibrium (LD) or they independently contribute to the transplant etiologies of liver transplant recipients, was evaluated by SHEsis online software (http://analysis.bio-x.cn/myAnalysis.php).

3. Results

3.1. Demographic characteristics

Total recipients (n = 231) were divided into different groups by their genotypes in IL-28B SNPs (rs12979860, rs12980275, and rs8099917). The detailed clinical characteristics of liver transplant recipients were depicted according to the different genotype groups in Table 1. There was no significant difference in age nor

Table 1

Clinical demographics of liver transplant recipients according to the different genotype groups.

	rs12979860			rs12980275			rs8099917		
Genotypes	**CC**	**CT+TT**	***P***	**AA**	**AG+GG**	***P***	**AA**	**AC+CC**	***P***
N, male/female	208 (169/39)	23 (19/4)	>.99	213 (173/40)	18 (15/3)	>.99	210 (170/40)	21 (18/3)	.77
Age, y*	44.80±9.74	45.00±12.04	.93	44.71±9.87	46.11±11.17	.57	44.98±9.94	43.24±10.28	.45
BMI, kg/m^2*	22.35±3.08	22.06±3.12	.67	22.30±3.07	22.66±3.24	.63	22.35±3.07	22.12±3.22	.75
MELD grade†	13 (9–20)	14 (10–18)	.58	13 (9–20)	14.5 (9.25–22.5)	.49	13 (9–20)	15 (10.5–21.75)	.25
Child–Pugh grade†	8 (6–10)	8 (5–9)	.47	8 (6–10)	8 (5–9)	.34	8 (6–10)	8.5 (6–10)	.63

BMI = body mass index, MELD = Model for End-Stage Liver Disease.

* Values were shown as mean ± SD.

† Values were shown as number or median (interquartile range).

Table 2

The distribution of IL-28B SNPs' alleles frequencies in patients with different transplant etiologies.

		HBV-related diseases							
SNPs	**Genotypes**	**HBV-related LC**	**HBV-related HCC**	**Progressive hepatitis B**	**Non-HBV diseases**	**OR (95%CI)***	***P****	χ^{2*}	***P**†*
rs12979860	CC (%)	86 (89.6)	58 (86.6)	18 (85.7)	45 (95.7)	1	.202	2.909	.41
	CT+TT (%)	10 (10.4)	9 (13.4)	3 (14.3)	2 (4.3)	3.06 (0.69–13.49)			
	C (%)	182 (94.8)	125 (93.3)	38 (90.5)	92 (97.9)	1	.186	3.829	.28
	T (%)	10 (5.2)	9 (6.7)	4 (9.5)	2 (2.1)	3.07 (0.71–13.25)			
rs12980725	AA (%)	87 (90.6)	60 (89.6)	18 (85.7)	46 (97.9)	1	.135	3.709	.30
	AG+GG (%)	9 (9.4)	7 (10.4)	3 (14.3)	1 (2.1)	5.30 (0.69–40.63)			
	A (%)	182 (94.8)	127 (94.8)	39 (92.9)	93 (98.9)	1	.124	3.616	.31
	G (%)	10 (5.2)	7 (5.2)	3 (7.1)	1 (1.1)	5.35 (0.71–40.35)			
rs8099917	AA (%)	86 (89.6)	60 (89.6)	19 (90.5)	43 (91.5)	1	.922	0.155	.99
	AC+CC (%)	10 (10.4)	7 (10.4)	2 (9.5)	4 (8.5)	1.24 (0.40–3.83)			
	A (%)	182 (94.8)	127 (94.8)	40 (95.2)	90 (95.7)	1	.924	0.147	.99
	C (%)	10 (5.2)	7 (5.2)	2 (4.8)	4 (4.3)	1.23 (0.41–3.69)			

* HBV-related vs non-HBV diseases.

† HBV-related LC vs HCC vs progressive HBV hepatitis vs non-HBV diseases. The *P*-value cutoff after multiple test correction is: $P < .00833333$.

CI = confidence interval, HCC = hepatocellular carcinoma, LC = liver cirrhosis, OR = odds ratio, SNPs = single nucleotide polymorphisms.

gender between each 2 groups with different genotypes in IL-28B SNPs ($P > .05$). The BMI and Child–Pugh grade before transplantation were also comparable between different groups ($P > .05$).

3.2. Association analysis of IL-28B polymorphisms with transplant etiologies

Three genotypes of IL-28B gene polymorphisms (rs1297860, rs12980275, and rs8099917) were distinguished by the melting profiles from the normalized melting curves. All the genotypes distribution of recipients were in Hardy–Weinberg equilibrium ($P > .05$). Distribution of IL-28B SNPs' genotypes and alleles in recipients are shown in Table 2. No significant association between IL-28B polymorphisms and different transplant etiologies was observed among LT recipients (rs12979860, $P = .406$ by genotype, $P = .280$ by allele; rs12980275, $P = .295$ by genotype, $P = .306$ by allele; rs8099917, $P = .985$ by genotype, $P = .986$ by allele) ($P > .000833333$ for multiple testing).

IL-28B haplotype was constructed from rs12979860 (minor allele T), rs12980275 (minor allele G), and rs8099917 (minor allele C). We set up the threshold frequency no. <0.03 to avoid some extremely rare haplotypes. LD value (D′) indicated the 3 SNPs were in high linkage disequilibrium (Fig. 1). Meanwhile, in liver transplant recipients, IL-28B haplotype block CAA and TGC showed no correlation with transplant etiologies between HBV-related recipients and non-HBV-related recipients (CAA: OR = 0.238, 95% CI = 0.244–1.285; TGC: OR = 4.206, 95% CI = 0.778–22.739) (Table 3).

To identify whether IL-28B polymorphisms were associated with HBV infection in the recipients, we divided the patients into HBV-related diseases group and non-HBV diseases group. No association was found in IL-28B polymorphism alleles between HBV-related diseases and non-HBV diseases (rs12979860, adjusted OR = 3.06, 95% CI = 0.69–13.49 by genotype, adjusted OR = 3.07, 95% CI = 0.71–13.25 by allele; rs12980275, adjusted OR = 5.30, 95% CI = 0.69–40.63 by genotype, adjusted OR =

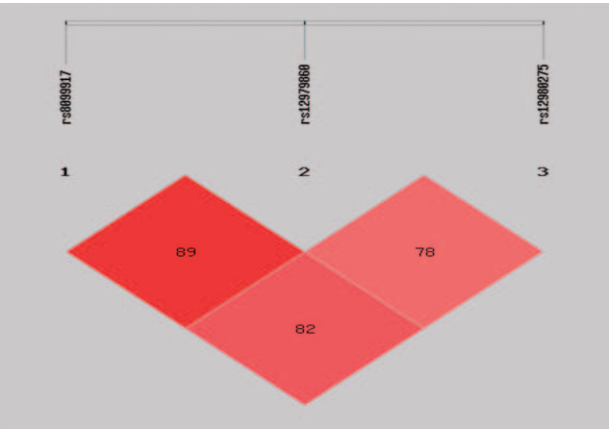

Figure 1. Linkage disequilibrium (LD) analysis of 3 polymorphisms in IL-28B gene (rs12979860, rs12980275, and rs8099917). The number in each square shows the LD value for each pair of SNPs.

Table 3

Haplotype analysis of the 3 IL-28B polymorphisms in liver transplant recipients.

	Case (freq.)	Control (freq.)	OR (95% CI)	*P*
CAA*	341.96 (0.929)	90.00 (0.957)	0.238 (0.044–1.285)	.195
TGC*	15.98 (0.043)	1.00 (0.011)	4.206 (0.778–22.739)	.195

CI = confidence interval, OR = odds ratio.

* Order of IL-28B haplotype block: rs12979860 (minor allele T), rs12980275 (minor allele G), and rs8099917 (minor allele C). Case: HBV-related recipients; Control: non-HBV-related recipients.

5.35, 95% CI = 0.71–40.35 by allele; rs8099917, adjusted OR = 1.24, 95% CI = 0.40–3.83 by genotype, adjusted OR = 1.23, 95% CI = 0.41–3.69 by allele) (Table 2).

3.3. Association of IL-28B gene with platelet counts and coagulation function

Platelet counts and coagulation function are directly associated with liver function, since liver products thrombopoietin and some clotting factors (I, II, VII, IX, X).[24] Therefore we tested the PLT counts and INR to evaluate the recovery of the patients' liver function after LT. Platelets are a component of blood whose function (along with the coagulation factors) is to stop bleeding by clumping and clotting blood vessel injuries, so the lower serum platelet counts may increase the risk of bleeding. PLT concentration was compared among HBV-related LT recipients with different genotypes. Patients who had different genotypes on rs12979860 were divided into CC and (CT+TT) group. For IL-28B gene rs12980275, the 2 genotypes groups were AA and (AG +GG). For rs8099917 the 2 groups were AA and (AC+CC). The numbers of the recipients with different genotypes in different time after LT are shown in Table 4. After Bonferroni correction ($P < .016666667$) we detected significant differences for rs12979860 on 3rd and 4th day and rs8099917 on the day of LT, 3rd and 4th day. HBV-related recipients with IL-28B genetic variants, rs12979860 T allele and rs8099917 C allele, had significantly higher serum platelet counts ($P < .016666667$, Table 4, Figs. 2–4). A week after LT, there seemed to be no difference between each 2 groups of recipients (Figs. 2–4). INR is derived from prothrombin time (PT) and widely used to evaluate the extrinsic pathway of coagulation. There was no difference in INR ratio among LT recipients with different IL-28B SNPs genotypes (Table 5). It seemed that HBV-related recipients with favorable IL-28B genetic genotypes on rs12979860 and rs8099917 SNPs (CC on rs12979860, AA on rs8099917) had lower serum platelet counts in the third and fourth days after LT but had similar coagulation function.

3.4. Association of IL-28B gene with liver function recovery

For all of the liver transplant recipients, routine liver function tests were done to evaluate the recovery of the patients after LT. We gathered the data before LT, on the day of LT, and 1st to 7th day, 14th day, 1st month, 3rd month, 6th month, 9th month, and the 1st to 5th year after LT. We evaluated the association between IL-28B SNPs and liver function tests.

The GGT concentration of HBV-related LT recipients with genotype of IL-28B gene of rs12980275 were AA: (AG+GG). The numbers of the recipients with different genotypes in different time are shown in Table 6. After Bonferroni correction ($P < .016666667$) we detected a significant association of rs12980275 with GGT concentration before transplant (Table 6). We observed that a lower GGT concentration was significantly associated with the AA genotype of rs12980275 in HBV-related recipients before LT (Table 6). It meant that HBV-related recipients with protective genotypes on IL-28B SNPs rs12980275 had lower serum GGT concentration and better liver function before LT. After LT, there seemed to be no differences between recipients with different genotypes. Other indices of liver function did not show significant difference before and after LT ($P > .016666667$).

4. Discussion

In this study, the relationship between IL-28B polymorphisms and transplant etiologies of patients who went through LT were analyzed. In addition, we analyzed the relationship between IL-28B polymorphisms and liver function recovery of HBV-related

Table 4

IL-28B genotypes and the platelet quantity of HBV-related recipients.

SNP	rs12979860					rs12980275					rs8099917				
Time	n	CC ($\times 10^9$/L)	n	CT+TT ($\times 10^9$/L)	*P*	n	AA ($\times 10^9$/L)	n	AG+GG ($\times 10^9$/L)	*P*	n	AA ($\times 10^9$/L)	n	AC+CC ($\times 10^9$/L)	*P*
Before LT	131	61 (34–107)	15	94 (36–142)	.24	134	51 (34–108)	12	81 (54–123)	.31	134	61 (34–107)	12	102 (57–140)	.06
The day of LT	121	57 (35–87)	13	109 (63–139)	.02	122	57 (34–87)	12	90 (62–131)	.02	122	57 (34–86)	12	114 (63–141)	**.01**
1st day after LT	138	51 (30–78)	16	80 (45–128)	.02	139	52 (32–78)	15	69 (38–122)	.15	139	51 (30–78)	15	80 (50–130)	.02
2nd day after LT	137	48 (27–73)	17	67 (51–85)	.04	139	48 (27–74)	15	66 (38–81)	.20	139	48 (27–74)	15	67 (42–89)	.07
3rd day after LT	131	49 (30–70)	16	74 (62–97)	**<.01**	132	50 (31–74)	15	68 (58–83)	.05	132	50 (30–72)	15	74 (58–101)	**.01**
4th day after LT	116	59 (35–84)	13	95 (79–150)	**<.01**	117	60 (37–85)	12	94 (67–158)	.02	117	60 (36–84)	12	94 (67–127)	**.01**
5th day after LT	113	65 (44–103)	13	99 (43–182)	.18	114	65 (44–103)	12	99 (40–197)	.16	115	65 (43–103)	11	90 (50–153)	.29
6th day after LT	97	88 (51–121)	12	106 (71–158)	.18	99	88 (52–122)	10	96 (66–185)	.38	97	88 (51–123)	12	96 (69–131)	.45
7th day after LT	130	101 (67–157)	14	119 (58–151)	.90	132	102 (67–158)	12	77 (49–129)	.21	130	102 (67–159)	14	99 (58–147)	.44
14th day after LT	139	190 (111–312)	17	163 (106–349)	>.99	141	192 (111–316)	15	137 (98–240)	.24	141	191 (111–316)	15	162 (98–260)	.42
1st month after LT	130	227 (133–328)	10	284 (131–451)	.33	131	229 (133–332)	9	282 (122–367)	.62	130	227 (133–332)	20	266 (131–371)	.55
3rd month after LT	92	141 (101–227)	4	117 (74–145)	.27	91	141 (99–228)	5	114 (85–137)	.18	91	140 (99–224)	5	120 (88–192)	.60
6th month after LT	83	128 (95–193)	6	175 (72–266)	.55	84	129 (95–193)	5	126 (50–236)	.96	82	129 (95–193)	7	128 (94–249)	.58
9th month after LT	85	130 (85–198)	4	112 (83–219)	.87	84	130 (87–199)	5	108 (75–185)	.52	84	129 (84–194)	5	115 (91–256)	.65
1st year after LT	118	136 (90–212)	15	121 (81–200)	.60	118	136 (90–214)	15	121 (81–161)	.42	120	136 (90–214)	13	121 (91–176)	.57
2nd year after LT	101	124 (94–193)	14	115 (101–195)	.91	101	124 (94–199)	14	115 (101–191)	.92	104	127 (95–196)	11	111 (101–187)	.41
3rd year after LT	86	124 (90–162)	12	124 (92–200)	.92	86	127 (90–164)	12	119 (95–170)	.78	88	127 (91–165)	10	110 (88–162)	.59
4th year after LT	69	130 (101–170)	10	105 (78–205)	.42	70	131 (102–174)	9	95 (77–152)	.15	69	131 (101–172)	10	103 (78–166)	.19
5th year after LT	53	128 (107–174)	5	143 (97–145)	.73	54	128 (107–173)	4	130 (88–146)	.57	53	128 (107–174)	5	129 (97–145)	.64

The unit of platelet quantity is (10^9/L). Continuous variables are expressed as median (interquartile range).

Bold values indicate statistically significant results. The *P*-value cutoff after multiple test correction is: $P < .016666667$.

LT = liver transplantation, n = number of patients, SNP = single nucleotide polymorphism.

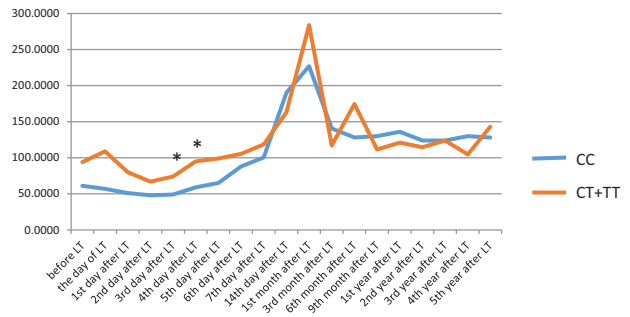

Figure 2. The association of IL-28B rs12979860 genotypes and the platelet quantity of HBV-related recipients. The unit of platelet quantity is (10^9/L). Continuous variables are expressed as median. LT = liver transplantation. *P-value cutoff after multiple test correction: $P < .016666667$.

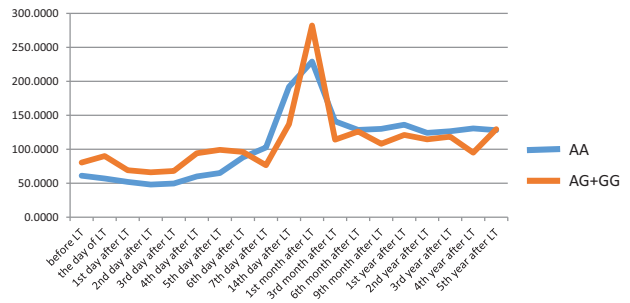

Figure 3. The association of IL-28B rs12980275 genotypes and the platelet quantity of HBV-related recipients. The unit of platelet quantity is (10^9/L). Continuous variables are expressed as median. LT = liver transplantation.

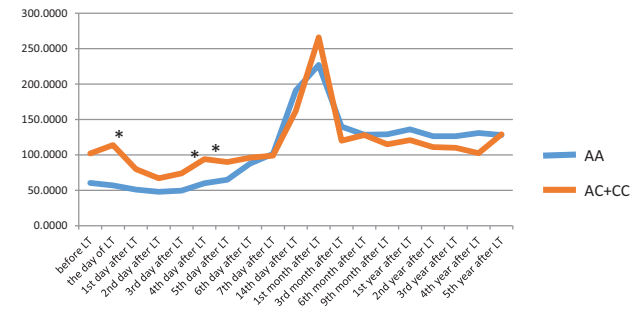

Figure 4. The association of IL-28B rs8099917 genotypes and the platelet quantity of HBV-related recipients. The unit of platelet quantity is (10^9/L). Continuous variables are expressed as median. LT = liver transplantation. *P-value cutoff after multiple test correction: $P < .016666667$.

recipients. It seemed that there was no significant difference in the frequencies of distribution among LT recipients with different transplant etiologies ($P > .00833333$). In the third and fourth days after LT, HBV-related recipients who had IL-28B protective genotypes on rs12979860 and rs8099917 SNPs (CC on rs12979860, AA on rs8099917) were significantly had lower platelet levels ($P < .016666667$) (Table 4, Figs. 2 and 4). However, the INR of recipients with different IL-28B polymorphisms had no difference (Table 4). Meanwhile, recipients who had protective genotypes on rs12980275 (AA on rs12980275) had lower GGT level before LT ($P < .016666667$) (Table 6).

It is well-known that blood platelets play an important role in hemostasis, with growing evidence showing that platelets could also affect tissue repair,[25] inflammation,[26] angiogenesis,[27] and ischemia/reperfusion injury.[28] In a previous study, it was found that platelets contribute to liver damage by promoting the intrahepatic accumulation of virus-specific CD8+ T cells and virus-nonspecific inflammatory cells.[29] Some studies indicated that antiplatelet therapy could prevent HBV-associated HCC without increasing bleeding risk.[30,31] In our study, recipients with protective genetic variants in IL-28B had lower serum platelet levels but the coagulation function measured by INR had no difference in the first 4 days after LT. This indicated that the lower platelet counts might be beneficial to the HBV-related recipients. Recent studies found that platelets played a critical role in liver injury and regeneration, which could also predict outcomes after LT.[32–34] Low perioperative platelet count ($<60 \times 10^9$/L) was an independent factor associated with severe complications and early graft and patient survival after LT.[33] These findings made low platelet count a risk factor for recipients. However, there was no definite conclusion on whether the low platelet counts was beneficial or risky to the HBV-related LT recipients.[34] More researches were needed to investigate this problem.

In addition, there was no direct evidence showing the relationship between IL-28B genetic variants and peripheral platelet counts in previous studies. Homoncik et al[35] found that single-dose interferon-α could decrease platelet counts in patients with chronic hepatitis C. Platelet counts were related with thrombopoietin produced by liver. We could guess that maybe the IL-28B polymorphisms can also influence the platelets counts by regulating the concentration of IFN-λ or thrombopoietin in patients with hepatitis B. Whether the IFN-λ could affect the

Table 5

IL-28B genotypes and International Normalized Ratio (INR) of HBV-related recipients.

SNP	rs12979860					rs12980275					rs8099917					
Time	n	CC	n	CT+TT	*P*	n	AA	n	AG+GG	*P*	n	AA	n	AC+CC	*P*	
Before LT	128	1.46 (1.13–1.92)	16	1.39 (1.29–1.55)	.90	131	1.43 (1.12–1.91)	13	1.43 (1.30–2.47)	.54	131	1.43 (1.12–1.91)	13	1.40 (1.30–2.37)	.53	
The day of LT	118	1.59 (1.35–1.92)	13	1.45 (1.28–2.77)	>.99	119	1.59 (1.33–1.91)	12	1.80 (1.34–2.96)	.63	120	1.59 (1.35–1.93)	11	1.86 (1.23–2.38)	.96	
1st day after LT	134	1.56 (1.37–1.82)	15	1.52 (1.38–2.05)	.92	135	1.57 (1.36–1.82)	14	1.49 (1.39–1.87)	.85	135	1.54 (1.37–1.81)	14	1.63 (1.37–2.14)	.70	
2nd day after LT	130	1.39 (1.20–1.59)	16	1.43 (1.24–1.83)	.41	132	1.39 (1.19–1.60)	14	1.43 (1.22–1.72)	.57	132	1.39 (1.19–1.59)	14	1.54 (1.33–1.96)	.12	
3rd day after LT	127	1.22 (1.12–1.40)	15	1.32 (1.19–1.63)	.13	128	1.23 (1.12–1.40)	7	1.32 (1.17–1.67)	.28	128	1.22 (1.12–1.40)	14	1.34 (1.22–1.67)	.08	
4th day after LT	105	1.21 (1.08–1.38)	9	1.24 (1.15–1.59)	.56	107	1.21 (1.08–1.38)	9	1.24 (1.14–1.66)	.66	104	1.21 (1.08–1.37)	10	1.31 (1.15–1.56)	.32	
5th day after LT	92	1.21 (1.06–1.35)	10	1.21 (1.06–1.43)	.87	93	1.21 (1.07–1.35)	8	1.23 (1.05–1.44)	>.99	93	1.21 (1.07–1.35)	9	1.36 (1.05–1.45)	.69	
6th day after LT	82	1.14 (1.03–1.30)	10	1.29 (1.06–1.45)	.47	84	1.14 (1.03–1.29)	11	1.38 (1.03–1.55)	.50	82	1.12 (1.03–1.28)	10	1.37 (1.11–1.45)	.12	
7th day after LT	110	1.10 (1.01–1.23)	12	1.17 (1.07–1.47)	.20	111	1.10 (1.01–1.24)	10	1.14 (1.06–1.28)	.59	109	1.10 (1.01–1.23)	13	1.20 (1.07–1.45)	.10	
14th day after LT	100	1.08 (0.99–1.17)	11	1.10 (1.02–1.23)	.70	101	1.08 (0.99–1.17)	5	1.10 (1.02–1.16)	.89	101	1.07 (0.99–1.17)	10	1.12 (1.02–1.40)	.46	
1st month after LT	76	1.09 (1.01–1.16)	7	1.12 (1.02–1.28)	.74	78	1.09 (1.01–1.15)	9	1.18 (1.07–1.84)	.38	76	1.08 (1.01–1.23)	7	1.18 (1.12–1.28)	.15	

Continuous variables are expressed as median (interquartile range). The *P*-value cutoff after multiple test correction is: $P < .016666667$.
LT = liver transplantation, n = number of patients, SNP = single nucleotide polymorphism.

Table 6

IL-28B genotypes and gamma glutamyl transpeptidase (GGT) concentration of HBV-related recipients.

SNP	rs12979860					rs12980275					rs8099917				
Time	n	CC ($\times10^9$/L)	n	CT+TT ($\times10^9$/L)	*P*	n	AA ($\times10^9$/L)	n	AG+GG ($\times10^9$/L)	*P*	n	AA ($\times10^9$/L)	n	AC+CC ($\times10^9$/L)	*P*
Before LT	127	55 (28–100)	15	88 (52–254)	.03	130	55 (28–100)	12	124 (72–238)	**<.01**	131	58 (29–101)	11	79 (25–188)	.25
The day of LT	114	43 (24–74)	14	45 (27–123)	.48	115	42 (24–74)	13	47 (26–126)	.46	117	45 (24–75)	11	37 (24–120)	.89
1st day after LT	135	46 (32–87)	16	51 (24–85)	.77	136	46 (32–84)	15	71 (23–95)	.74	137	46 (32–87)	14	38 (23–76)	.38
7th day after LT	133	165 (104–283)	15	177 (77–310)	.89	135	165 (104–290)	13	177 (66–305)	.67	133	168 (105–297)	15	168 (54–299)	.35
1st month after LT	133	178 (89–321)	12	174 (116–451)	.62	134	178 (97–281)	11	182 (104–356)	.65	133	178 (91–321)	12	174 (84–451)	.82
3rd month after LT	100	112 (44–206)	8	132 (38–335)	.75	99	111 (44–206)	9	142 (48–322)	.67	99	113 (44–206)	9	94 (46–322)	.90
6th month after LT	98	79 (33–205)	7	72 (24–297)	.96	98	79 (33–205)	7	63 (24–297)	.87	97	79 (32–205)	8	68 (28–247)	.77
9th month after LT	94	85 (38–201)	5	70 (18–303)	.42	93	85 (39–207)	6	51 (18–192)	.24	93	85 (37–207)	6	57 (18–192)	.29
1st year after LT	131	56 (25–173)	16	42 (23–190)	.95	131	56 (25–173)	16	41 (21–190)	.64	133	54 (25–171)	14	50 (25–197)	.75
2nd year after LT	114	48 (21–243)	14	65 (20–266)	.79	114	48 (22–243)	14	65 (17–266)	.99	116	45 (20–220)	12	108 (29–498)	.23
3rd year after LT	93	44 (19–159)	12	81 (19–152)	.69	93	49 (19–159)	12	61 (15–152)	.90	95	42 (17–156)	10	105 (32–197)	.17
4th year after LT	73	36 (17–123)	10	123 (48–183)	.04	74	37 (19–128)	9	61 (31–151)	.44	73	36 (17–131)	10	95 (48–141)	.10
5th year after LT	55	53 (18–168)	5	173 (91–247)	.12	56	54 (18–172)	4	171 (73–264)	.20	55	54 (19–173)	5	128 (34–247)	.66

The unit of platelet quantity is (10^9/L). Continuous variables are expressed as median (interquartile range).

Bold value indicates statistically significant results. The *P*-value cutoff after multiple test correction is: $P < .016666667$.

LT = liver transplantation, n = number of patients, SNP = single nucleotide polymorphism.

serum count of platelet remains to be tested by further molecular biology experiment. The relationship of IL-28B polymorphisms and peripheral platelet counts also needs further clarification.

GGT is an enzyme that transfers gamma-glutamyl functional groups. It is also predominantly used as a diagnostic marker for liver disease in medicine. Elevated serum GGT activity can be found in diseases of the liver and biliary system. This study showed that recipients who had IL-28B genotype on rs12980275 (AA on rs12980275) had lower GGT level before LT. This indicated that AA on rs12980275 of IL-28B polymorphisms were protective genotype while G on rs12980275, were risk alleles, which was consistent with our previous results.[18,22] Our previous studies showed that LT recipients with protective genes on IL-28B (the CC genotype on rs12979860, and the AA genotype on rs8099917) had a lower AST concentration and better liver function recovery after LT. Furthermore, we did not found the relationship between IL-28B polymorphisms and transplant etiologies of all patients who went through liver transplant. It might be related with the limitation of the total number of patients.

Previous studies have indicated that IL-28B rs12979860 C/T polymorphism T allele is more prevalent in bad progress. Carriage of this allele seems to enhance the risk for developing HCC in both HBV- and HCV-infected patients.[23,36,37] Liver transplant recipients with rs12980275 A/G polymorphism G allele and rs8099917 A/C polymorphism C allele have higher AST concentration and increasing risks of hepatic allograft dysfunction.[22] These researches indicate that recipients with favorable protective genotypes on IL-28B SNPs (CC on rs12979860, AA on rs12980275, and AA on rs8099917) may have mild outcome and recipients with risk genotypes (CT+TT on rs12979860, AG+GG on rs12980275, and AC+CC on rs8099917) would progress to worse outcome. Our study showed the relationship between IL-28B protective genetic variants (on rs12980275) and the liver function (GGT) before LT. We also found the association between IL-28B polymorphisms and peripheral platelet counts after LT of HBV-related recipients for the first time. This result could indicate the relationship between IL-28B SNPs, platelet counts and liver function recovery of recipients, which might provide a new therapy to regulate liver function of patients before and after LT. However, there were still some limitations in our study. Firstly, the difference of platelet counts between recipients with different IL-28B genetic variants only appeared in the first 4 days after LT. It is difficult to explain why this kind of difference concentrated in such a short time. Secondly, we did not test the relationship between IL-28B genetic variants, IFN-λ, thrombopoietin, and peripheral platelet by doing molecular biology experiment.

In conclusion, recipients with protective genotypes on IL-28B polymorphisms (the CC genotype of rs12979860 and the AA genotype of rs8099917) had lower peripheral platelet count but similar INR in the first 4 days after transplantation. Maybe lower peripheral platelet count also associated with liver function recovery by reducing liver inflammation and influencing thrombocytopoiesis. Besides, our study found that the genetic variation of IL-28B allele A of rs12980275 might be benefit to liver function because of the lower level of GGT before LT. However, we did not found significant association between IL-28B polymorphisms and liver transplant etiologies. This finding may provide a valuable gene therapy tool to regulate the liver function of recipients before and after LT.

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