The Lower Pre-Transplant Hepatitis C Antibody in Il-28B RS8099917 Tt Genotype with Higher miRNA-122 Expression May Correlate to the Acute Cellular Rejection After Ldlt

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Article

The Lower Pre-Transplant Hepatitis C Antibody in IL-28B RS8099917 TT Genotype with Higher miRNA-122 Expression May Correlate to the Acute Cellular Rejection after LDLT

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Abstract: Background miRNA-122 has been reported to be a useful tool for monitoring ACR after LT. IL-28B SNP rs8099917 genotype is known to contribute to the control of HCV infection. We sought to investigate the relationship between IL-28B SNP rs8099917 genotype and miRNA-122 expression and immune mechanism of ACR after LT using hepatitis C antibody calibration. **Patients and Methods** A total of 45 patients with chronic hepatitis C received LDLT. IL-28B SNP rs8099917 genotyping was used to divide TT and GT groups. The relative expression levels of miRNA-122 were calculated by quantitative PCR as $2^{[(Ct\ of\ miR\text{-}122)\ (Ct\ of\ U6)]}$ normalized to a reference gene and compared to a control sample, and the anti-HCV titers before and after LT were tracked to observe the relationship with ACR. **Results** In the genotype of rs8099917, TT was significantly more associated with higher serum miRNA-122 levels than GT ($p = 0.029$). The ACR was significantly higher in genotype TT than in GT ($p = 0.002$). TT was significantly associated with low levels of pre-LT anti-HCV compared with GT ($p = 0.022$). Multivariate analysis with 95% confidence intervals showed a significant association between rs8099917 genotype TT, lower pre-LT and higher post-LT anti-HCV and ACR. **Conclusion** Based on our current data, IL-28B SNP rs8099917 genotype TT may express higher miRNA-122 and correlate with lower Pre-LT and higher Post-LT anti-HCV titers, and may correlate to the pathogenesis of ACR after LT.

Keywords: acute cellular rejection; hepatitis C virus; IL-28B; liver transplantation; microRNA-122; single nucleotide polymorphism

1. Introduction

Acute cell rejection (ACR) remains an important problem after liver transplantation (LT). It represents a genetic pathological response of the immune system to the potential effects of immunosuppressants, particularly hepatitis C virus (HCV)-associated LT [1]. Liver grafts may face an immune response from the host and a residual viral biological attack. IL-28B single nucleotide polymorphism (SNP) rs8099917 is known to help fight and spontaneously clear HCV infection by the genetic characteristics [2,3]. In addition, the host's own small non-coding RNAs, including miRNA-122, miRNA-301, miRNA-133a, and miRNA-21, may be expressed in the liver with varying degrees of genetic upregulation and downregulation through different pathogenic conditions [4]. In particular, miRNA-122 may play an important role in ACR after LT [5]. Here, we would like to explore the relationship between the IL-28B SNP rs8099917 genotyping associated with miRNA-122 expression and the pathological mechanism of anti-HCV levels on ACR after LT.

2. Results

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Although IL-28B SNP rs8099917 can be defined as three genotypes of TT, GT or GG, only two genotypes of TT and GT were detected in this study. Therefore, the two types were divided into TT group and GT group for further discussion.

2.1. The Relationship between miRNA-122 and Expression IL-28B rs8099917

In the genotype of rs8099917, TT was significantly more associated with higher serum miRNA-122 levels than GT (p = 0.029) (Table 1).

Table 1. The relationship between IL-28B SNP rs8099917 and miRNA-122 ($2^{-\Delta\Delta CT}$) in patients with HCV relative living donor liver transplantation.

IL-28B SNP rs8099917	miRNA-122	P value
Genotype TT, n = 29	0.0029±0.0034	0.029
Genotype GT, n = 16	0.0010±0.0029	

HCV: hepatitis C virus; IL: interleukin; SNP: single nucleotide polymorphism; $2^{-\Delta\Delta CT}$: a method used in qPCR to calculate the relative expression levels of a target gene. The $2^{\Delta\Delta CT}$ method involves comparing the cycle threshold (CT) values of the target gene to a reference gene and then normalizing to a control sample. Student's t-test (tails = 1, type = 2) <0.05 indicates a significant difference.

2.2. The Relationship between Acute Cellular Rejection and IL-28B rs8099917

The ACR was significantly higher in genotype TT than in GT (p = 0.002) (Table 2).

Table 2. The relationship between acute cellular rejection and IL-28B SNP rs8099917 in patients with HCV relative living donor liver transplantation.

IL-28B SNP rs8099917	Acute cellular rejection	P value
Genotype TT, n = 29	9	0.002
Genotype GT, n = 16	10	

HCV: hepatitis C virus; IL: interleukin; SNP: single nucleotide polymorphism. A chi-square test p-value of <0.05 indicates significant differences.

2.3. The Relationship between anti-HCV Titer and IL-28B rs8099917

TT was significantly associated with low levels of pre-LT anti-HCV compared with GT (p = 0.022) (Table 3). In Tables 4 and 5, the intercept was 27.847 and IL-28B TT was 24.846 (p = 0.001, 95% CI: 10.375 to 39.317, Observed Power = 0.923). The IL-28B TT genotype is associated with an increase of 24.846 units in pre-LT anti-HCV titer compared to the reference group (IL-28B GT). This relationship was statistically significant (p = 0.001), indicating a strong positive association.

Table 3. The relationship between IL-28B SNP 8099917 and the anti-HCV titer on pre-/post-liver transplantation.

Category	IL-18B SNP rs8099917		P value
	TT	GT	
Pre-LT anti-HCV titer	54.24±30.63	38.13±21.08	0.022
Post-LT ant-HCV titer	73.93±44.00	64.33±47.64	0.255

HCV: hepatitis C virus; IL: interleukin; LT: liver transplantation; SNP: single nucleotide polymorphism; Student's t-test (tails = 1, type = 2) <0.05 indicates a significant difference.

Multivariate analysis with 95% confidence intervals showed the correlation between rs8099917 genotype TT, lower pre-LT anti-HCV and ACR (Table 4; Table 5).

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Table 4. Multivariate Tests for IL-28B SNP rs8099917 and co-factors.

Effect		Value	F	Hypothesis df	Error df	Sig.	Observed Power[a]
Intercept	Pillai's Trace	.934	141.163[b]	4.000	40.000	.000	1.000
	Wilks' Lambda	.066	141.163[b]	4.000	40.000	.000	1.000
	Hotelling's Trace	14.116	141.163[b]	4.000	40.000	.000	1.000
	Roy's Largest Root	14.116	141.163[b]	4.000	40.000	.000	1.000
IL-28B	Pillai's Trace	.282	3.922[b]	4.000	40.000	.009	.865
	Wilks' Lambda	.718	3.922[b]	4.000	40.000	.009	.865
	Hotelling's Trace	.392	3.922[b]	4.000	40.000	.009	.865
	Roy's Largest Root	.392	3.922[b]	4.000	40.000	.009	.865

a: Computed using alpha = 0.05; b: Exact statistic; c: Design = Intercept+IL28B; **b**: Design: Intercept+IL-28B.

Table 5. Parameter Estimates for IL-28B SNP rs8099917 and co-factors.

Dependent Variable	Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Observed Power[a]
						Lower Bound	Upper Bound	
Pre-LT anti-HCV	Intercept	27.847	5.760	4.834	.000	16.231	39.464	.997
	[IL-28B TT]	24.846	7.176	3.463	.001	10.375	39.317	.923
	[IL-28B GT]	0[b]	.	.	.	.	.	.
Post-LT anti-HCV	Intercept	47.797	11.027	4.335	.000	25.560	70.035	.989
	[IL-28B TT]	30.947	13.736	2.253	.029	3.246	58.649	.596
	[IL-28B GT]	0[b]	.	.	.	.	.	.
miRNA122	Intercept	.002	.001	2.032	.048	1.19E-005	.003	.510
	[IL-28B TT]	6.77E-005	.001	.068	.946	-.002	.002	.051
	[IL-28B GT]	0[b]	.	.	.	.	.	.
ACR	Intercept	1.375	.120	11.430	.000	1.132	1.618	1.000
	[IL-28B TT]	.315	.150	2.100	.042	.012	.617	.537
	[IL-28B GT]	0[b]	.	.	.	.	.	.

a: Computed using alpha =.05; b: This parameter is set to zero because it is redundant.

1. Pre-LT anti-HCV Titer: Significant positive association with IL-28B TT.
2. miRNA-122: No significant association with IL-28B TT.
3. ACR: Significant positive association with IL-28B TT.

2.3.1. Pre-LT anti-HCV Titer

- Intercept: 27.847
- IL-28B TT: 24.846 (p = 0.001, 95% CI: 10.375 to 39.317, Observed Power = 0.923)

The IL-28B TT genotype is associated with an increase of 24.846 units in pre-LT anti-HCV titer compared to the reference group (IL-28B GT). This relationship is statistically significant (p = 0.001), indicating a strong positive association.

2.3.2. miRNA-122

- Intercept: 0.002
- IL-28B TT: 0.0000677 (p = 0.946, 95% CI: -0.002 to 0.002, Observed Power = 0.051)

For miRNA-122, the IL-28B TT genotype shows a very small increase of 0.0000677 units compared to the reference group. However, this relationship is not statistically significant (p = 0.946), suggesting no meaningful association.

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2.3.3. ACR

- Intercept: 1.375
- IL-28B TT: 0.315 (p = 0.042, 95% CI: 0.012 to 0.617, Observed Power = 0.537)

The IL-28B TT genotype is associated with an increase of 0.315 units in ACR compared to the reference group. This relationship is statistically significant (p = 0.042), indicating a positive association.

For the intercept between IL-28B TT and ACR, it means that the intercept value for ACR is 1.375, which is the baseline value when IL-28B GT is the reference group. For the IL-28B TT, the parameter estimate (B) for IL-28B TT is 0.315, with a standard error of 0.150. The t-value is 2.100, and the p-value (Sig.) is 0.042, which is less than 0.05, indicating that this result is statistically significant. The 95% confidence interval for this parameter ranges from 0.012 to 0.617 (Table 5). This means that individuals with the IL-28B TT genotype have an ACR that is, on average, 0.315 units higher than those with the IL-28B GT genotype. The positive B value suggests a positive association between the IL-28B TT genotype and ACR. The observed power for this parameter is 0.537, indicating a moderate level of statistical power. In summary, the IL-28B TT genotype was significantly associated with a higher ACR compared to the IL-28B GT genotype.

3. Discussions

The immune mechanism of HCV infection is very complex, involving the interaction of multiple hosts and viral agents [6], and this study aims to explore the performance of IL-28B and microRNA that have a positive effect on HCV in order to develop the dilemma of no effective vaccine. When a patient is infected with HCV, the immune system produces antibodies to against HCV, but these antibodies are not protective antibodies and cannot eliminate the virus from the human being body. Non-protective antibodies do not provide immune protection against the immune effect of anti-HCV, which may be due to the high mutation rate of HCV, which makes it difficult for antibodies to effectively neutralize the virus [7,8]. It can only be used as an indicator for diagnosing HCV infection as well as anti-HCV antibodies can be used for clinical diagnosis to determine whether you have been infected or are now infected with HCV. Based on our current results, anti-HCV titers may have a signal associated with ACR after LDLT. In particular, lower pre-LT anti-HCV titers and higher post-LT anti-HCV titers may enhance the immune response to liver grafts. The immune expression mechanism of HCV infection activates an innate immune response, including the production of interferons, which can inhibit viral replication [9,10]. In addition, host cytotoxic T lymphocytes and natural killer cells play an important role in the process of viral clearance, but hepatitis C virus has a variety of strategies to evade the host immune response, including avoiding the role of neutralizing antibodies and spreading through cells [11,12]. Although anti-HCV antibodies are not protective antibodies, they are the immune response of the host immune system to HCV infection. As a result, HCV antibody titers become well worth exploring [13].

It was well known that IL-28B SNP rs8099917 genotype should be benefit for HCV clearance even spontaneous or PEG interferon administration [14,15]. In our current study, it is suggested that rs8099917 genotype TT may be significantly associated with ACR after LT compared to genotype GT. The IL-28B genotype, specifically the TT variant of the rs8099917 SNP, has been studied in the context of liver transplantation. Research indicates that this genotype can influence the immune response and outcomes post-transplantation. The TT genotype is associated with a different immune response compared to other genotypes. This can affect the likelihood and severity of ACR after LT [14,16]. Therefore, patients with the TT genotype may have a higher risk for ACR, necessitating closer monitoring and potentially different immunosuppressive strategies.

In our recent report, different clinicopathological situations express different miRNAs and different up and down regulation effects in the field of liver transplantation [4], especially miRNA-122 has a close correlation with ACR in LT patients. Expression of liver miRNAs was significantly higher compared to serum miRNAs. When organ tissues are not being used, serum miRNAs should be one of the simple methods that can help with different diagnoses of acute jaundice after LT. miRNA-122 plays a significant role in liver function and has been studied in the context of LT,

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particularly regarding ACR. miRNA-122 levels can be altered in patients experiencing ACR after LT. Recent reported showed lower levels of miRNA-122 have been associated with increased risk rejection. Hence, monitoring miRNA-122 levels could potentially serve as a biomarker to predict or diagnose ACR, allowing for earlier intervention and management [4].

There were some limitations to our study due to funding constraints, the relatively small sample size of this study, and the single centered of the study. In particular, a simple Student's t-test interpretation was statistically significant in the analysis of IL-28B TT and miRNA-122, but there was no significant correlation in the multivariate analysis. Nonetheless, we judged the risk of bias to be very small, as standardization of pre-LT clinical and laboratory assessments, monitoring of immunosuppressive regimens and graft outcomes after LT, the use of multivariate analyses to examine the strength of statistical data to confirm their reliable associations, and increased study funding with a view to collecting more cases may be more convincing.

In conclusion, our current data suggest that the IL-28B SNP rs8099917 genotype TT may express higher mRNA-122 and be associated with lower anti-HCV titers in pre-LT, resulting in an association with post-LT ACR. Thus, when liver graft rejection occurs after LT, anti-HCV titers are increased compared to pre-LT, and individuals with specific IL-28B SNPs 8099917 TT genotypes have stronger miRNA-122 expression in LT.

4. Materials and Methods

4.1. Patients

A total of 45 anti-HCV-positive patients receiving LDLT were included in this study, including 29 males and 16 females, with a mean age of 58.16±7.02 years, of which 42.2% had hepatocellular carcinoma. The complete clinical patient profile was shown in Table 6. According to IL-28B SNP rs8099917 genotyping, they were divided into two groups, TT 29 cases and GT 16 cases. All of our patients received interferon plus ribavirin or direct-acting antiviral agents including Harvoni (i.e., 400 mg of sofosbuvir plus 90 mg of ledipasvir), or a combination of 400 mg of sofosbuvir/60 mg of daclatasvir/800 mg of ribavirin for HCV infection whenever possible. All recipients received the same immunosuppression protocol, being administered with mycophenolate mofetil at 250 mg Q12H PO and tacrolimus at 1 mg QD PO, and the doses were adjusted according to mycophenolic acid plasma levels > 3.0 μg/mL and tacrolimus blood concentrations of 5–10 ng/mL. Our exclusion criteria included pediatric transplantation, deceased donor liver transplantation, HBsAg-positive patients, primary biliary cholangitis, biliary atresia, Wilson's disease, polycystic disease, and re-transplantation.

Table 6. Clinical profiles of 45 recipients with positive serum anti-HCV antibody underwent living donor liver transplantation.

Category	No. (%)	
Cases	45 (100)	
Gender, M/F	29/16	
Age (mean ± SD)	58.16 ± 7.02	
Combined with HCC	19 (42.2)	
HCV genotype (1a/1b/2/ undetected)	2/16/11/16	
IL-28B, rs8099917 (GT/TT)	16/29	
	Pre-LT	**Post-LT**
AST	64.14 ± 47.60	50.00 ± 72.20
ALT	49.76 ± 61.18	41.45 ± 33.60
Bil. T	2.05 ± 1.33	1.80 ± 5.85
HCV RNA	17 (37.8)	14 (31.1)
Anti-HCV antibody titer (mean ± SD)	43.86 ± 25.77	67.74 ± 46.11

Alb: albumin; Bil. T: total bilirubin; INR: international normal range; LT: liver transplantation.

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4.2. Anti-HCV Antibody

Serum samples of anti-HCV were collected on the day before LT and at the same time as liver biopsy was clinically necessary after LT. Anti-HCV was identified using the COBAS TaqMan HCV Assay version 2.0 (Roche Diagnostics Corporation, Indianapolis, IN, United States) [17].

4.3. IL-28B Single Nucleotide Polymorphism

IL-28B SNP rs8099917 genotypes were studied using a 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with Custom TaqMan SNP Genotyping Assays (Applied Biosystems) for allele discrimination. The real-time PCR reactions were performed in 96-well microplates, using the ABI 7500 Fast Real-Time Polymerase Chain Reaction System (Applied Biosystems International, Framingham, MA) in accordance with the manufacturer's instructions. The IL-28B SNP rs8099917 was defined as TT, GT or GG genotype as recommended by the manufacturer. All genotypes of IL-28B SNP rs8099917 were assayed in duplicate in order to assess inter-assay precision [14,15].

4.4. MicroRNA-122

MiRNA-122 levels was extracted by using the miRNeasy Mini Kit (Qiagen217004) according to the manufacturer's protocol. Reverse transcription (RT) was performed with 1 μg RNA using the First-Strand cDNA Synthesis Kit (Promega, Madison, WI, USA) or miScript RT Kit (Qiagen, Hilden, Germany) for the transcription of miRNA according to the manufacturer's instructions. Using the ABI TaqMan Fast Universal PCR master mix or TaqMan Universal PCR master mix for miRNA (Applied Biosystems, Foster City, CA, USA), we performed RT-PCR on an ABI 7500 Fast Real-Time PCR System with the SDS 1.4 program. The cycle threshold (Ct value), which is inversely correlated with miRNA level, was defined as the number of cycles required for the fluorescent signal to cross the threshold in quantitative PCR. Comparative RT-PCR data, including non-template controls, were obtained in triplicate. The fold increase in cytokine mRNA expression was calculated using the comparative $2^{-\Delta\Delta Ct}$ method, where Ct represents the threshold cycle for each transcript. The expression of the miRNA was defined based on Ct, and relative expression levels were calculated as $2^{[(Ct\ of\ miR\text{-}122)\ (Ct\ of\ U6)]}$ after normalization with reference to the expression of small nuclear RNA U6 [18]

4.5. Liver Biopsy Interpretation

All of the suspected clinical organ rejection after LT was performed by the first author of this study, and the histopathological diagnostic criteria for all pathologic graft rejection were defined based on the 1995 Banff classification, and the severity grades were characterized by the rejection activity index [19].

4.6. Ethics

All procedures involving human participants were performed in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study protocol was approved and authorized by the Ethics Committee of our hospital (approval number: 202300159B0). Informed consent was obtained from all patients included in the study. We followed the Strengthening the Reporting of Observational Studies in Epidemiology statement guidelines for reporting observational studies. No allograft donors or recipients were from a vulnerable population.

4.7. Statistics

Statistical analyses were performed using the SPSS (version 22.0; SPSS Inc., Chicago, IL, USA). Descriptive values were expressed as mean ± standard deviation and percentages. Categorical variables were compared using the chi-squared or Fisher's exact test, and continuous variables were compared using Student's *t*-test. All tests were two-tailed, and a *p*-value of <0.05 was considered

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statistically significant. Multivariate analysis was used to correlate the relationship between the co-factors.

Author Contributions: K-WC and Y-CL were responsible for the study concept and design; W-FL, K-TH and L-WH performed data analysis and interpretation; and K-WC and C-CW drafted the manuscript and provided critical revisions.

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Institutional Review Board Statement: The Institutional Review Board of Kaohsiung Chang Gung Memorial Hospital approved this study (Reference number: 202300159B0).

Informed Consent Statement: Informed consent was obtained from all patients included in the study.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

ALF	acute liver failure
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
HCV	hepatitis C virus
HCC	hepatocellular carcinoma
INR	international normalized ratio
LT	liver transplantation
LDLT	living-donor liver transplantation
miRNA	micro RNA
SNP	single nucleotide polymorphism

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