

Correlation of HLA-DP/DQ polymorphisms with transplant etiologies and prognosis in liver transplant recipients

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Abstract

Previous study has identified that the genetic variants in the human leukocyte antigen (HLA)-DP/DQ region were strongly associated with hepatitis B virus (HBV) infection. But their roles in liver function recovery after hepatic transplantation were still obscure. This study aimed to investigate whether HLA-DP/DQ polymorphisms were associated with post-transplant etiologies and prognosis in Chinese liver transplant recipients.

A total of 144 liver transplant recipients were enrolled, which were divided into 2 groups according to the transplant etiology: HBV-related disease and non-HBV-related disease. HBV-related disease includes 3 subgroups: liver cirrhosis, hepatocellular carcinoma, and progressive HBV hepatitis. Three single-nucleotide polymorphisms HLA-DP (rs3077 and rs9277535) and HLA-DQ (rs7453920) were studied in all recipients by high-resolution melting curve analysis. Liver function indices (albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyltranspeptidase, direct bilirubin, total bilirubin) and coagulation indices (prothrombin time, platelet, international normalized ratio, fibrinogen) were routinely tested. After transplant, 10 recipients who were positive for HBsAg or with elevation in HBV virus load were regarded as HBV recurrence.

No significant association of HLA-DP/DQ polymorphisms with HBV recurrence or transplant etiology was observed ($P < .05$). Recipients with HLA-DQ (rs7453920) AG and AA genotype had lower direct bilirubin levels than GG genotype individuals, especially on the 14th day after surgery (17.80 vs. 5.35, $P = .038$). Patients with A alleles displayed earlier liver function recovery than patients with G alleles (7 vs. 6 months). No significant correlation was shown in HLA-DP rs3077 and rs9277535 with HBV infection or liver function recovery ($P < .05$).

Our study concluded that HLA-DP (rs3077 and rs9277535) and HLA-DQ (rs7453920) were not significantly associated with HBV recurrence or HBV susceptibility, but HLA-DQ rs7453920 was related to prognosis of liver transplant recipients. HLA-DQ rs7453920 A might be used as an indicator of earlier recovery and better prognosis after transplantation.

Abbreviations: Alb = albumin, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, DBil = direct bilirubin, FIB = fibrinogen, GGT = gamma-glutamyltranspeptidase, HCC = hepatocellular carcinoma, INR = international normalized ratio, LC = liver cirrhosis, NBD = none-HBV disease, PHH = progressive HBV hepatitis, PLT = platelets, PT = prothrombin time, TB = total bilirubin.

Keywords: direct bilirubin, HLA polymorphisms, liver function, Liver transplantation, transplant etiology

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1. Introduction

At present, approximately 240 million people worldwide have been infected chronically with hepatitis B virus (HBV).^[1] In China, the number is nearly 10 million.^[2] After HBV infection, people may manifest as asymptomatic carriers, or have occult hepatitis, acute hepatitis, chronic active hepatitis, and hepatic cirrhosis, or even develop into hepatic carcinoma.^[3] In China, the main reason for liver transplantation is HBV-related liver diseases, including hepatitis, cirrhosis, liver cancer, among others.^[4]

The outcome of liver transplantation owing to hepatitis B-related end-stage liver disease is remarkably affected by recurrent infection and subsequent allograft failure. There are diverse factors determining the outcome, such as host factor, virus factor, and the immunosuppression drugs.^[5] However, not all of the liver transplant recipients suffer from HBV recurrence under the same conditions of immunosuppressant administration and viral infection. Patients' immunity is a critical factor that affects HBV recurrence following liver transplantation.

Several recent studies suggested that human leukocyte antigen (HLA) class DP, DQ (HLA-DP/DQ) controlling the host immune response could play an important role in determining HBV infection outcome. HLA-DP/DQ has been demonstrated to assist in antigen recognition, immune response, and virus eradication. Recently, one genome-wide association study (GWAS)^[4] conducted in Japanese and Thai population proposed that 11 single-nucleotide polymorphisms (SNPs) were associated with persistent HBV infection, especially HLA-DP rs3077, HLA-DP rs9277535. Meantime, at the validation stage, several cohorts of different ethnicities also confirmed that rs3077 and rs9277535 strongly correlated with HBV clearance. Another GWAS^[6] conducted in Japanese population suggested that not only HLA-DP polymorphisms, but also HLA-DQ-related SNPs were pertinent to chronic HBV infection, with rs7453920 presenting the strongest associations. Subsequently, large quantity of studies validated this conclusion in Asian population, including Chinese Han, Korean, and Japanese among others.^[7,8] The results showed that HLA-DP/DQ correlated not only with persistent HBV infection, but also with disease progression.

However, the association of HLA gene polymorphism with pathogenesis as well as prognosis after liver transplantation have not been investigated. Therefore, this study aims to investigate the associations of 3 HLA-DP SNPs rs3077, rs9277535, and HLA-DQ rs7453920 with transplant etiologies and prognosis after liver transplantation in the Chinese population.

2. Materials and methods

2.1. Patients

We enrolled 144 liver transplantation recipients in West China Hospital, including 73 liver cirrhosis (LC) subjects, 37 hepatocellular carcinoma (HCC) patients, 9 progressive HBV hepatitis (PHH) patients, and 25 non-HBV disease population subjects. All the recipients underwent operations between 2006 and 2012. And they all have received a tacrolimus-based immunosuppressive regimen for >6 months without suspension after liver transplantation. None of the recipients were positive for HCV or HDV. Those who have had acute or chronic graft rejection were excluded from the study. All recipients were negative for hepatitis C virus (HCV) or anti-hepatitis D virus (HDV).

All donors were negative for HBV. The diagnostic criteria for hepatitis B before transplantation were positive for HBsAg in blood test during 6 months before transplant or positive for HBsAg in liver biopsy during liver transplant. The total recipients (n=144) were divided into end-stage liver disease secondary to hepatitis B (n=119) and end-stage liver disease secondary to other diseases (n=25) by the criteria for hepatitis B before transplantation. HBV-related disease was divided into LC (n=73) and HCC (n=37) by liver biopsy during liver transplant. The PHH (n=9) was defined by positive for HBsAg and without LC or HCC in biopsy. After transplantation, low-dose intramuscular hepatitis B immunoglobulin in combination with lamivudine (100 mg/day) has been used for the prevention of post-transplant HBV recurrence in recipients. The recipients who were positive for HBsAg or elevation in HBV DNA after transplantation were regarded as HBV recurrence. There were 10 recipients positive for HBsAg after liver transplantation. Among the 10 patients, there were only 3 patients who had elevation in HBV DNA. All the liver transplantation patients volunteered for the study and had signed informed consent. This study was approved by the Ethics Committee of West China Hospital.

Dynamic analyses of liver function and coagulation indices were conducted before surgery and 7 days, 14 days, 1 month, 6 months, and 1 year after surgery. The indices are albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), platelets (ALT), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT), total bilirubin (TB), prothrombin time (PT), international normalized ratio (INR), fibrinogen (FIB), tacrolimus (FK), direct bilirubin (DBil), respectively. The basic characteristics of the recipients were shown in Table 1.

Table 1

Basic characteristics of liver transplant recipients.

| Characteristics | LC | HCC | PHH | NBD | P |
|-------------------|------------------|------------------|------------------|------------------|-------|
| Age, y | 40 (37–45) | 49 (40–56) | 40 (31–45) | 51 (38–58) | .003 |
| Weight, kg | 61.0 (55.0–69.0) | 65.0 (60.0–71.0) | 57.0 (50.5–75.0) | 54.0 (50.0–62.5) | .002 |
| N (male; female) | 73 (65/8) | 37 (33/4) | 9 (8/1) | 25 (15/10) | .013 |
| MELD scores | 18 (13–24) | 11 (8–13) | 28 (21–40) | 17 (12–22) | <.001 |
| Child-Pugh scores | 9 (7–11) | 6 (5–8) | 10 (9–12) | 8 (7–10) | <.001 |
| HBsAg+ | 73 | 37 | 9 | 0 | <.001 |
| HBsAb+ | 2 | 0 | 0 | 13 | <.001 |
| HBeAg+ | 19 | 7 | 0 | 0 | .001 |
| HBeAb+ | 35 | 23 | 7 | 6 | .006 |
| HBcAb+ | 65 | 36 | 8 | 8 | <.001 |

Values are shown as median (interquartile range) or number of cases. HBcAb=antibody to the hepatitis B core antigen, HBeAb=antibody to the hepatitis Be surface antigen, HBeAg=hepatitis Be antigen, HBsAb=antibody to the hepatitis B surface antigen, HBsAg=hepatitis B surface antigen, HCC=hepatocellular carcinoma, LC=liver cirrhosis, NBD=none-HBV disease, PHH=progressive HBV hepatitis.

2.2. Genomic DNA extraction

Genomic DNA was extracted from peripheral venous blood using the blood DNA kit (Biotake corporation). DNA was isolated from the whole blood using the AE buffer provided by the producer. The concentration of DNA was diluted to 10 ng/ μ L for working solutions. And the isolated DNA was stored at -20°C .

2.3. HLA gene polymorphism typing

Three SNPs (rs3077, rs743920, rs9277535) were analyzed using the polymerase chain reaction (PCR) and melting curve analyses. The condition was in a 96-well plate on the Light Cycler480 (Roche Diagnostics, Penzberg, Bavaria, Germany). And the primers were designed into a small fragment surrounding the polymorphisms to avoid the presence of other sequence variations. The primers for the PCR amplification are as follows: rs3077: TCAGCTTTTCTTCTCACTTCATGTG (forward); GAGCTTGAAGGGTCAGCAAjTTC (reverse); rs9277535: AATGGTGAGCAGACTGCAAA TCT (forward); TGGTAATGATAAAACATGCTCTCAGTAA (reverse); rs7453920: TTTAGGGAGGTAAGAGGGAAAGC (forward); CGAGAACGCCCTGATCTAAGA (reverse).

The PCR conditions were performed as follows: the first step was to denature at 95°C for 10 seconds, then amplify for 50 cycles at 95°C for 10 seconds, anneal at 60°C for 15 seconds, and extend at 72°C for 25 seconds. After amplification, the high-resolution melting analyses were performed by rising the temperature from 65°C to 95°C at $0.01^{\circ}\text{C}/\text{s}$. Finally, cool it down to 40°C .

2.4. Statistical analysis

We used SPSS, version 20.0 (SPSS Inc, Chicago, IL) for statistical analysis. The Kruskal-Wallis test was used to compare several groups. χ^2 analysis was used to test the distribution of genotypes. Data are expressed as median (range) or just number of cases. Statistic significance was defined by $P < .05$.

3. Results

3.1. Clinical characteristics of included subjects

The LC population consisted of 73 subjects (65 males and 8 females), and their mean age was 40 (range 37–45) years. The HCC population was comprised of 37 individuals (33 males and 4 females) and their mean age was 49 (range 40–56) years. The HPP patients composed of 9 people (8 males and 1 female) and their mean age was 40 (31–45) years. And the non-HBV disease population was made up of 25 subjects (15 males and 10 females) and their mean age was 51 (38–58) years. Significant differences

were observed among the 4 groups in all clinical characteristics ($P < .05$), which might be because of the epidemiology basis. All the details were shown in Table 1. We analyzed the sexes and other baseline information among different gene groups. No difference was found in the distribution of clinical characters among different gene groups, except the model for end-stage liver disease (MELD) score in rs9277535, which means that the baseline clinical characteristics are matched among different gene groups (Table 2).

3.2. Association analysis of HLA-DP/DQ polymorphisms With HBV recurrence

All 3 polymorphisms of the 4 groups met the conditions of Hardy-Weinberg equilibrium ($P > .05$). No strong linkage disequilibrium was observed among the 3 SNPs (rs7453920, rs3077, and rs9277535). $D' < 0.6$ and $r^2 < 0.3$ in the block (supplement Figure 1, <http://links.lww.com/MD/B755>).

We made a statistic analysis of the 3 SNPs (rs3077, rs9277535, and rs7453920) according to the recurrence of HBV. There was no significant difference in the allele frequency of HLA-DP gene (rs3077 and rs9277535) and HLA-DQ gene (rs7453920) between HBV recurrence group and non-HBV recurrence group.

We found no significant difference in the allele frequency of rs3077 (A:G, 30%:70% vs. 27.7%:72.3%, $P = .22$) between HBV recurrence group and non-HBV recurrence group.

No significant difference existed in the allele frequency of rs9277535 (A:G, 5%:95% vs. 9.1%:90.9%, $P = .38$) between HBV recurrence group and non-HBV recurrence group.

There was no significant difference in the allele frequency of rs7453920 (A:G, 30%:70% vs. 22.5%:77.5%, $P = .28$) between HBV recurrence group and non-HBV recurrence group (Table 3).

3.3. Association analysis of HLA-DP/DQ polymorphisms with transplant etiologies

We divided the recipients into HBV-related disease group and non-HBV disease group to identify whether HLA-DP/DQ polymorphisms were associated with HBV infection in the recipients. However, only negative results were perceived (rs3077: odds ratio [OR]=0.89, 95% confidence interval [CI]=0.37–2.14, $P = .793$ about genotype, OR=0.94, 95% CI=0.46–1.91, $P = .857$ about allele; rs7453920: OR=1.88, 95% CI=0.61–5.79, $P = .269$ about genotype, OR=1.34, 95% CI=0.51–3.49, $P = .55$ about allele; rs9277535: OR=0.95, 95% CI=0.40–2.30, $P = .913$ about genotype, OR=0.92, 95% CI=0.44–1.91, $P = .816$ about allele) (Table 4).

Table 2

Clinical demographics of the liver transplant recipients.

| | rs3077 | | | rs7453920 | | | rs9277535 | | |
|--------------------------------|-----------------|-----------------|-----|-----------------|-----------------|-----|-----------------|-----------------|------|
| | GG | AG+AA | P | GG | AG+AA | P | GG | AG+AA | P |
| N (male/female) [†] | 83 (69/14) | 61 (52/9) | .73 | 125 (105/20) | 19 (16/3) | .00 | 85 (73/12) | 59 (48/11) | .47 |
| Weight, kg [‡] | 62.6 \pm 10.4 | 61.4 \pm 11.2 | .59 | 62.6 \pm 10.5 | 59.1 \pm 11.7 | .58 | 61.6 \pm 10.1 | 62.8 \pm 11.5 | .19 |
| Age, y [§] | 43 (38–53) | 43 (36–53) | .56 | 44 (38–53) | 38 (34–40) | .65 | 42 (37–53) | 43 (38–53) | .59 |
| MELD scores | 14 (12–20) | 18 (11–24) | .12 | 15 (12–22) | 18 (11–27) | .49 | 15 (12–21) | 17 (11–24) | .01* |
| Child-Pugh scores [§] | 8 (6–10) | 9 (7–11) | .11 | 8 (6–10) | 9 (7–11) | .34 | 8 (6–10) | 8 (7–10) | .07 |

[†] Values were shown as number.

[‡] Values were shown as mean \pm SD.

[§] Values were shown as median (interquartile range).

* Values are regarded as statistically significant.

Table 3

Correlation of HLA-DP and HLA-DQ polymorphisms with HBV recurrence in liver transplant recipients.

| | rs3077 | | | rs7453920 | | | rs9277535 | | |
|---------------------|-----------|------------|-----|-----------|------------|-----|-----------|------------|-----|
| | A (%) | G (%) | P | A (%) | G (%) | P | A (%) | G (%) | P |
| HBV recurrence | 6 (30.0) | 14 (70.0) | .22 | 1 (5.0) | 19 (95.0) | .38 | 6 (30.0) | 14 (70.0) | .28 |
| None-HBV recurrence | 60 (27.7) | 158 (72.3) | | 20 (9.1) | 198 (90.9) | | 49 (22.5) | 169 (77.5) | |

HBV=hepatitis B virus, HLA=human leukocyte antigen.

Table 4

The distribution of HLA-DP/DQ SNPs' alleles frequencies in patients with HBV-related diseases and none-HBV disease.

| | HBV-related diseases | | | | NBD | OR (95% CI) | P |
|------------------|----------------------|-----------|-----------|-----------|-----|------------------|------|
| | LC | HCC | PHH | | | | |
| rs3077 | | | | | | | |
| GG (%) | 39 (53.4) | 26 (70.3) | 3 (30.0) | 15 (60.0) | | 1 | |
| AG+AA (%) | 34 (46.6) | 11 (29.7) | 6 (60.0) | 10 (40.0) | | 0.89 (0.37–2.14) | .793 |
| G (%) | 105 (71.9) | 63 (85.1) | 10 (55.6) | 38 (76.0) | | 1 | |
| A (%) | 41 (28.1) | 11 (14.9) | 8 (44.4) | 12 (24.0) | | 0.94 (0.46–1.91) | .857 |
| rs7453920 | | | | | | | |
| GG (%) | 64 (87.7) | 35 (94.6) | 6 (60.0) | 20 (80.0) | | 1 | |
| AG+AA (%) | 9 (12.3) | 2 (5.4) | 3 (30.0) | 5 (20.0) | | 1.88 (0.61–5.79) | .269 |
| G (%) | 129 (88.4) | 72 (97.3) | 15 (83.3) | 44 (88.0) | | 1 | |
| A (%) | 17 (11.6) | 2 (2.7) | 3 (16.7) | 6 (12.0) | | 1.34 (0.51–3.49) | .550 |
| rs9277535 | | | | | | | |
| GG (%) | 43 (58.9) | 24 (64.9) | 3 (33.3) | 15 (60.0) | | 1 | |
| AG+AA (%) | 30 (41.1) | 13 (35.1) | 6 (66.7) | 10 (40.0) | | 0.95 (0.40–2.30) | .913 |
| G (%) | 113 (77.4) | 59 (79.7) | 10 (55.6) | 39 (78.0) | | 1 | |
| A (%) | 33 (22.6) | 15 (20.3) | 8 (44.4) | 11 (22.0) | | 0.92 (0.44–1.91) | .816 |

CI=confidence interval, HCC=hepatocellular carcinoma, HLA=human leukocyte antigen, LC=liver cirrhosis, NBD=non-HBV disease, OR=odds ratio, PHH=progressive HBV hepatitis, SNP=single-nucleotide polymorphism.

We then divided the HBV-related disease patients into 3 subgroups according to etiologies (LC, HCC, PHH), and analyze the correlation between them and non-HBV disease group. We found that there was a significant association of the rs3077 and rs7453920 variant with HCC patients compared with LC patients. The minor allele A may be correlated with a reduced risk of HCC. However, after adjusting age and sex to control potential confounders, we observed that the association of the rs3077 and rs7453920 variant with HCC patients was missing. This indicated that the age, weight, and sex were the confounding factors. We found no significant association of HLA-DP/DQ polymorphism with other groups (Table 5).

3.4. Association analysis of HLA-DP/DQ polymorphisms with prognosis in liver transplant recipients

All of the liver transplant recipients did routine blood liver function tests and coagulation function tests to evaluate the recovery after LT. The data were collected before liver transplantation, the day of LT, 7th day, 14th day, 1st month, 6th month, and the 1st year after liver transplantation. We evaluated the association between HLA-DP/DQ SNPs and liver function tests (such as Alb, AST, ALP, GGT, DBIL, among others) and coagulation function tests (TB, PT, PLT, INR, among others).

When identifying the associations of these genetic variations with the indices of liver function, we found that HLA-DQ

Table 5

The distribution of HLA-DP/DQ SNPs' alleles frequencies in patients with different etiologies.

| | rs3077 | | | | rs7453920 | | | | rs9277535 | | |
|-----|-----------|------------|-------|------|-----------|------------|-------|------|-----------|------------|-------|
| | A (%) | G (%) | P | P' | A (%) | G (%) | P | P' | A (%) | G (%) | P |
| LC | 41 (28.1) | 105 (71.9) | .029* | .239 | 17 (11.6) | 129 (88.4) | .048* | .326 | 33 (22.6) | 113 (77.4) | .692* |
| HCC | 11 (14.9) | 63 (85.1) | .575† | .589 | 2 (2.7) | 72 (97.3) | .946† | .798 | 15 (20.3) | 59 (79.7) | .930† |
| PHH | 8 (44.4) | 10 (76.0) | .199‡ | .233 | 3 (16.7) | 15 (83.3) | .090‡ | .256 | 8 (44.4) | 10 (55.6) | .816‡ |
| NBD | 12 (24.0) | 38 (76.0) | .426§ | .596 | 6 (12.0) | 44 (88.0) | .924§ | .987 | 11 (22.0) | 39 (78.0) | .069§ |

HCC=hepatocellular carcinoma, HLA=human leukocyte antigen, LC=liver cirrhosis, NBD=non-HBV disease, PHH=progressive HBV hepatitis, SNP=single-nucleotide polymorphism. P': Logistic regression controlling for age, sex, and weight.

* P was LC vs. HCC.

† P was LC vs. NBD.

‡ P was HCC vs. NBD.

§ P was PHH vs. NBD.

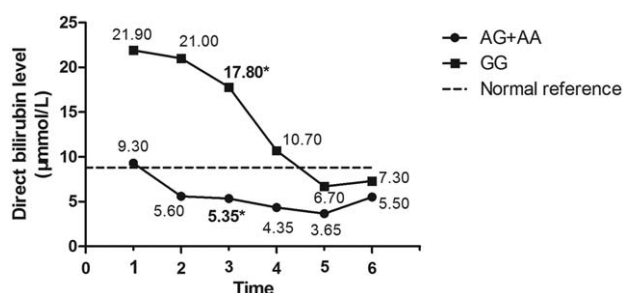


Figure 1. The association of HLA-DQ rs7453920 and the recipients' direct bilirubin levels at different time points. *Values are statistically different ($P < .05$). 1 represents before surgery; 2, 7 days after surgery; 3, 14 days after surgery; 4, 1 month after surgery; 5, 6 months after surgery; 6, 1 year after surgery.

rs7453920 significantly correlated with prognosis in liver transplant recipients. rs7453920 AG and AA genotype subjects always had significantly lower DBil levels compared with GG genotype individuals, especially on the 14th day after surgery (17.80 vs. 5.35, $P = .038$). And rs7453920 allele A had protective function in liver function. It costs less time for the DBil levels in AG and AA genotyp recipients to be back to normal compared with GG genotype individuals (7 days vs. 6 months, shown in Fig. 1). Besides, similar outcomes were also observed in HLA-DP rs9277535, but its significance only showed on the 7th day after surgery (14.50 vs. 45.00, $P = 0.028$). Before surgery, rs9277535 AG and AA genotype patients had even higher DBil levels (Fig. 2). However, such associations were not shown in HLA-DP rs3077 polymorphism.

Additionally, when analyzing the correlations of these genetic factors with the indices of coagulation function, we found that both on the 7th and 14th day after liver transplantation, recipients who had A alleles of rs3077 in HLA-DP gene had significantly longer PT (14.10 vs. 13.50, $P = .042$; 13.90 vs. 12.90, $P = .029$; Table 5). But 6 months after surgery, the results had a transient reversal. AG and AA genotype recipients had shorter PT than GG genotype individuals. INR, an international normalized ratio used to measure the coagulation function, presented the same result as PT did. As for the HLA-DP rs9277535 and HLA-DQ rs7453920, no significance was observed (supplement Table 1–6, <http://links.lww.com/MD/B755>).

4. Discussion

In this study, we investigated the associations of HLA-DP/DQ polymorphisms with HBV recurrence, HBV susceptibility, and

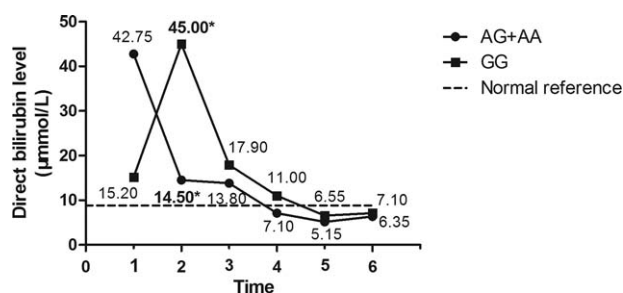


Figure 2. The association of HLA-DP rs9277535 and the recipients' direct bilirubin levels in different time points. *Values are statistically different ($P < 0.05$). 1 represents before surgery; 2, 7 days after surgery; 3, 14 days after surgery; 4, 1 month after surgery; 5, 6 months after surgery; 6, 1 year after surgery.

transplant prognosis of Han population in Southwestern China. And we demonstrated that all of the 3 SNPs (HLA-DP rs3077, rs9277535, HLA-DQ rs7453920) were not significantly correlated with HBV recurrence. Furthermore, HLA-DQ rs7453920 was significantly associated with HBV susceptibility as well as liver function recovery after transplantation. As for HLA-DP rs3077 and rs9277535, they might have no impact on HBV susceptibility or transplant prognosis.

We first analyzed the associations of the 3 SNPs with the HBV recurrence, but only got negative results. Neither of HLA-DP rs3077, rs9277535, or HLA-DQ rs7453920 was found to have significant correlation with HBV recurrence. Based on our experimental data, we could only conclude that HLA-DP/DQ polymorphisms were not pertinent to HBV recurrence.

As listed before, several studies evaluated the correlation of HLA-DQ rs7453920 with HBV susceptibility,^[6,7,9–14] but quite conflicting results were obtained. To clarify this obscure association, we reappraised this experiment and found that HLA-DQ rs7453920 was not related to HBV susceptibility ($P > .05$). Other studies have shown that HLA-DP rs3077 and rs9277535 seemed to be significantly correlated with HBV susceptibility as well as viral clearance.^[15–22] But according to our current data analysis, no significant correlation was shown. This might be because of our limited sample size.

HLA-DP and HLA-DQ molecules, both belonging to the HLA II genes, can produce antigens on the surface of antigen-presenting cells. When infected with HBV, the host launches adaptive immune responses that CD4+ T lymphocytes recognize antigenic peptides and then activate CD8+ cytotoxic T lymphocytes,^[23,24] which resulted in the clearance of the HBV. One recent study^[25] implied that patients with G alleles of HLA-DP rs3077 and rs9277535 were more inclined to have lower mRNA expressions. However, there is no related evidence to identify the association of HLA-DQ polymorphisms with mRNA expression.

DBil level is an important indicator of liver function as both of its production and metabolism were related to hepatocyte activity. A few studies^[26,27] have confirmed that DBil concentrations in blood had prognostic value on liver function recovery after liver transplantation. In our study, we found that HLA-DQ rs7453920 was significantly correlated with prognosis of liver transplant recipients, and rs7453920 allele A had a protective function in prognosis. Patients carrying A alleles of rs7453920 had better liver functions and recovered more quickly than those with G alleles. Besides, similar outcomes were also observed in HLA-DP rs9277535. However, such associations were not shown in HLA-DP rs3077 polymorphism.

PT is an indicator of the hemostatic functions of coagulation factors synthesized in the liver. The increase of PT in blood after liver transplantation reveals that the recovery of hepatic protein-synthetic ability is not so good.^[28] Our present data demonstrated that patients with HLA-DP rs3077 A alleles had significantly longer PT on the 7th and 14th day after liver transplantation, which were linked to bad protein-synthetic ability of liver transplant recipients and was discrepant with previous findings.^[20,29,30] However, the results on 6 months after surgery showed a transient reversal. Recipients with AG and AA genotype had shorter PT than GG genotype individuals. Further studies are needed to identify whether there are relationships between the SNP and the PT levels.

Apart from what were mentioned above, there were some limitations in our study. First, the small sample size restricted us from detecting the correlation of the 2 SNPs (rs3077 and

rs9277535) with transplant etiologies as well as prognosis. Additionally, owing to the limited sample size, we failed to further study the association of transplant etiologies with DBIL concentrations in the rs7453920 group. Thirdly, we lacked the virus genotype information of the recipients. Zhang et al.^[18] has pointed out that virus genotypes had impact on the HLA-DP polymorphisms, thus influencing the outcomes of HBV infection. Finally, we only selected 3 SNPs to represent the HLA-DP/DQ polymorphisms, which may omit some important information from other SNPs.

5. Conclusions

Notwithstanding these limitations, our study is the first study dissecting the association of genetic variants with prognosis of liver transplant recipients. We proved that HLA-DQ (rs7453920) was associated with prognosis of liver transplant recipients. The A allele of rs7453920 served as a protective factor in liver function recovery. As for the HLA-DP (rs3077 and rs9277535), experimental data did not show any correlation with the HBV infection and prognosis.

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