

Human Leukocyte Antigen Class II Association with Spontaneous Recovery from Hepatitis B Virus Infection in Koreans: Analysis at the Haplotype Level

It has been speculated that human leukocyte antigen (HLA) alleles are associated with the outcome of hepatitis B virus (HBV) infection although the data obtained from various populations have shown some inconsistencies. A total of 464 HBV-infected Korean individuals (80 spontaneously recovered [SR] and 384 chronically infected [CI]) were selected to investigate the association of HLA class II alleles with the viral clearance and persistence. Our results showed that: 1) multiple HLA class II alleles and haplotypes were associated with viral clearance (DRB1*1302, DRB1*1502, DQB1*0302, DQB1*0609, and related-haplotypes) and persistence (DRB1*0701, DQB1*0301, and related-haplotypes); 2) DRB1*1302 and DQB1*0609 were more strongly associated with viral clearance. And the association of DQB1*0609 ($p=0.0084$; OR, 7.24) with viral clearance was much stronger than previously recognized, DRB1*1302 ($p=0.0038$; OR, 4.34); and 3) linkage to a specific DPB1 allele in a haplotype strengthened the association with viral clearance, although DPB1 itself was not associated with the outcome. These results indicate the existence of multiple factors controlling viral clearance in the HLA class II gene region. Further extended investigation on the genetic factors related to the outcome of HBV infection will provide valuable insights into the understanding of the mechanisms involved.

Key Words : Hepatitis B Virus; Clearance; Persistence; DQB1*0609; DRB1*1302

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INTRODUCTION

Infection with human hepatitis B virus (HBV) can lead to high morbidity and mortality due to the development of end-stage liver diseases. It is estimated that more than 350 million people worldwide are infected with HBV (1). Among infected individuals, about 5-10% of adults and more than 90% of children become persistently infected by the virus and develop liver diseases. The clinical outcome of the infection is variable and may result in spontaneous recovery, inactive HBV surface antigen (HBsAg) carrier state, chronic hepatitis, liver cirrhosis, or hepatocellular carcinoma (2, 3). The precise mechanisms leading to these various outcomes are not clearly defined yet but it has been perceived that host immune responsiveness is the critical factor.

It has been demonstrated that vigorous CD4⁺ and CD8⁺ T lymphocyte responses to various HBV antigens were associated with HBV self-elimination, while insufficient CD4⁺ T cell help and defective CD8⁺ T cell repertoire at the early stages of infection were connected to viral persistence (4, 5).

Due to the specific antigen presenting function of human leukocyte antigens (HLA) in immune responsiveness, the contribution of HLA to the outcome of HBV infection has been studied extensively in different populations. However, data have shown some inconsistencies with regard to HLA effects on HBV clearance or persistence in different ethnic and racial groups. Extensive HLA polymorphism contributes to the selection of antigenic peptides for presentation to T lymphocytes resulting in different immune responses to infection amongst individuals. Furthermore, HLA molecules differing only at 1-2 amino acids showed different peptide binding preference (6). This implies that HLA analysis for disease association studies requires high resolution typing to better understand the mechanisms involved.

Korea is an endemic area for HBV infection and about 5% of the population has been shown to be chronically infected (7). Two studies regarding HLA association in Koreans have been reported and consistently demonstrated that DR13 was associated with self-elimination of HBV (8, 9). However, these studies to clarify the HLA association in the popula-

tion were limited in several respects. They examined only HLA-A, -B, and -DR at low resolution and used patients with organ transplants as their study subjects. Therefore, the purpose of this study was to further investigate the HLA association with the outcome of HBV infection using high resolution typing of HLA class II loci (HLA-DRB1, -DQB1, and -DPB1). The impact of HLA diversity was evaluated on viral clearance and persistence.

MATERIALS AND METHODS

Study subjects

A total of 464 Koreans who had been exposed to HBV as detected by the presence of antibody to HBV core antigen (anti-HBc) IgG, were enrolled from the outpatient clinics of the Gastroenterology Department of Ajou University Hospital (Suwon, Korea) between March and December 2002. Study subjects were divided into two groups based on the status of HBV serologic markers exhibited for more than 6 months. The spontaneously recovered (SR) group included 80 individuals (60 males and 20 females, aged 19-70 yr, mean \pm SD, 47.9 ± 10.3) who were HBsAg negative, anti-HBc positive and antibody to HBV surface antigen (anti-HBs) positive, and had no evidence of liver function abnormalities and of previous chronic liver disease based on their medical history. The chronically infected (CI) group included 384 individuals (283 males and 101 females, aged 16-77 yr, mean \pm SD, 41.0 ± 10.3) who were HBsAg and anti-HBc positive, and anti-HBs negative. They were regularly followed with blood tests for serum alanine aminotransferase (ALT)/aspartate aminotransferase (AST), hepatitis B e antigen (HBeAg)/antibody to hepatitis B e antigen (anti-HBe) and alpha fetoprotein (AFP), and with radiographic tests of the liver (computed tomography or ultrasonography) at an interval of every 6 months for more than a year. HBV-DNA was detected by Digene Hybrid Capture II Assay (Digene Diagnostics, Beltsville, MD, U.S.A.); the detection limit of the test was 0.5 pg/mL. None of the patients had hepatocellular carcinoma. Patients who were positive for anti-HBs and negative for anti-HBc IgG, and patients with other types of chronic liver disease such as alcoholic liver disease, chronic hepatitis C, steatohepatitis, and Wilson's disease, were excluded from this study. Informed consents were obtained from each subject, and the Institutional Review Board of Human Research of Ajou University Hospital approved the study protocol.

HLA allelic typing

Genomic DNA was extracted from peripheral blood mononuclear cells of the study subjects using the method of Miller and colleagues (10). Allele-level genotypes of the HLA-DRB1, -DQB1, and -DPB1 genes from each study sample

were obtained by direct DNA sequence analysis based on the procedures described previously (11-13). Briefly, the polymorphic exon 2 of the gene was amplified using group-specific (HLA-DRB1) or locus-specific (HLA-DQB1, and -DPB1) primer sets. Automated cycle sequencing using dye terminator chemistry (v3.1, Applied Biosystems, Foster City, CA, U.S.A.) was performed on the amplified gene product and the amplicon was analyzed on an ABI3100 Genetic Analyzer (Applied Biosystems). Data analysis was performed with Sequence Navigator and MatchTool software (PE-Applied Biosystems Inc., U.S.A.). Homozygosity was assumed when no other HLA-DRB1, -DQB1, and -DPB1 allele was detected upon direct DNA sequence analysis.

Statistical analysis

Statistical analyses were done using SAS (version 8.01, SAS institute, Cary, NC). Haplotype reconstruction was implemented by PHASE (14). Hardy-Weinberg equilibrium for all loci and linkage disequilibrium for all pairs of loci were tested using Arlequin (version 2.0, Genetics and Biometry Laboratory, University of Geneva, Switzerland). Phenotype frequencies of HLA-DRB1, -DQB1, and -DPB1 alleles as well as haplotype frequencies of DRB1-DQB1, DQB1-DPB1, and DRB1-DQB1-DPB1 in two study groups were compared. Alleles (<3%) and haplotypes (<2%) with low frequencies were not analyzed due to inadequate statistical power. Some general characteristics were compared between the two study groups using t-test and chi-square test. Odds ratios (ORs) with 95% confidence intervals (95% CI) and *p* values for alleles and haplotypes associated with the outcome of HBV infection were calculated by logistic regression analysis adjusting for age and sex. When one element in the 2×2 table was zero, the *p* value was calculated by a chi-square test in haplotype analysis. Statistical significance was assumed at $p < 0.05$. When the *p* value was less than 0.05, a Bonferroni correction was applied by multiplying the *p* value by the number of comparisons made.

RESULTS

Characteristics of study groups

Among HBV-exposed subjects including SR group (*n*=80) and CI group (*n*=384), the SR group (47.9 ± 10.3 yr old) was somewhat older than the CI group (41.0 ± 10.3 yr old) ($p < 0.0001$). However, regarding gender, there was no significant difference between the SR group (% male=75.0) and the CI group (% male=73.7) ($p > 0.1$).

Alleles associated with HBV clearance or persistence

Using direct DNA sequencing analysis, class II allele geno-

types at the four-digit level were obtained from the study subjects. A total of 31 HLA-DRB1, 16 HLA-DQB1, and 23 HLA-DPB1 alleles were identified. Distribution of the alleles for all loci were consistent with Hardy-Weinberg equi-

librium in the SR and CI groups, respectively ($p > 0.05$).

Phenotype frequencies of HLA class II alleles in the two study groups are listed in Table 1. The frequencies of DRB1*1302 (17.50% vs. 4.95%, $p = 0.0002$, OR, 4.34) and DQB1*

Table 1. Comparison of HLA class II phenotype frequencies between HBV spontaneously recovered (SR) and chronically infected (CI) groups

Allele	SR n=80 (PF, %)	CI n=384 (PF, %)	p value	p_c value	OR (95% CI)
DRB1*					
0101	7 (8.75)	38 (9.90)	0.9797	-	-
0301	2 (2.50)	20 (5.21)	0.4144	-	-
0403	5 (6.25)	14 (3.65)	0.5676	-	-
0405	10 (12.50)	57 (14.84)	0.5411	-	-
0406	10 (12.50)	27 (7.03)	0.1351	-	-
0410	3 (3.75)	8 (2.08)	0.1776	-	-
0701	7 (8.75)	69 (17.97)	0.0458	NS	0.43 (0.19-0.98)
0802	6 (7.50)	16 (4.17)	0.2157	-	-
0803	10 (12.50)	75 (19.53)	0.2037	-	-
0901	18 (22.50)	83 (21.61)	0.9582	-	-
1101	8 (10.00)	47 (12.24)	0.7600	-	-
1201	6 (7.50)	35 (9.11)	0.7519	-	-
1202	5 (6.25)	39 (10.16)	0.1581	-	-
1301	3 (3.75)	7 (1.82)	0.2292	-	-
1302	14 (17.50)	19 (4.95)	0.0002	0.0038	4.34 (2.00-9.40)
1401	5 (6.25)	27 (7.03)	0.9343	-	-
1405	6 (7.50)	26 (6.77)	0.9780	-	-
1501	12 (15.00)	59 (15.36)	0.6098	-	-
1502	12 (15.00)	32 (8.33)	0.0376	NS	2.21 (1.05-4.67)
DQB1*					
0201	2 (2.50)	20 (5.21)	0.4144	-	-
0202	7 (8.75)	65 (16.93)	0.0761	-	-
0301	17 (21.25)	126 (32.81)	0.0470	NS	0.55 (0.30-0.99)
0302	20 (25.00)	49 (12.76)	0.0172	NS	2.12 (1.14-3.93)
0303	21 (26.25)	96 (25.00)	0.9281	-	-
0401	11 (13.75)	56 (14.58)	0.7121	-	-
0402	6 (7.50)	22 (5.73)	0.3534	-	-
0501	9 (11.25)	50 (13.02)	0.8812	-	-
0502	1 (1.25)	27 (7.03)	0.0852	-	-
0503	8 (10.00)	41 (10.68)	0.8454	-	-
0601	20 (25.00)	99 (25.78)	0.8720	-	-
0602	12 (15.00)	54 (14.06)	0.8134	-	-
0604	6 (7.50)	14 (3.65)	0.1274	-	-
0609	8 (10.00)	6 (1.56)	0.0006	0.0084	7.24 (2.35-22.27)
DPB1*					
0201	32 (40.00)	144 (37.50)	0.8808	-	-
0202	7 (8.75)	26 (6.77)	0.8295	-	-
0301	6 (7.50)	27 (7.03)	0.8432	-	-
0401	9 (11.25)	36 (9.38)	0.5104	-	-
0402	11 (13.75)	40 (10.42)	0.4062	-	-
0501	51 (63.75)	264 (68.75)	0.4223	-	-
0901	10 (12.50)	26 (6.77)	0.0690	-	-
1301	5 (6.25)	50 (13.02)	0.0710	-	-
1401	2 (2.50)	17 (4.43)	0.4373	-	-
1701	4 (5.00)	17 (4.43)	0.9014	-	-

PF, phenotype frequency of HLA allele is presented in percentage; p_c value, p value corrected by multiplying the number of alleles tested (19 for DRB1, 14 for DQB1); OR, odds ratio (SR group vs. CI group); CI, confidence interval; NS, statistically not significant after correction.

Alleles with a phenotype frequency >3% are listed.

Only significant p_c values (<0.05) are presented.

0609 (10.00% vs. 1.56%, $p=0.0006$, OR, 7.24) were significantly higher in the SR group compared to the CI group. Their statistical significance was maintained after correction of the p values ($p_c=0.0038$ and $p_c=0.0084$, respectively). The frequencies of DRB1*1502 and DQB1*0302 were higher in the SR group, while the frequencies of DRB1*0701 and DQB1*0301 were higher in the CI group. However, p values were not statistically significant after the correction (Table 1).

Comparison of the impact of DRB1*1302 and DQB1*0609 on HBV clearance

As described above, two alleles (DRB1*1302 and DQB1*0609) were significantly associated with HBV clearance. Interestingly, all of the individuals carrying DQB1*0609 were DRB1*1302-positive in this study. This is due to the strong linkage disequilibrium between these two alleles in Koreans as previously described (15). Thus, the statistics for these two alleles might be distorted and an additional analysis was necessary to estimate the impact of the two alleles on HBV clearance. Fortunately, DRB1*1302 also showed strong linkage disequilibrium with DQB1*0604 in the study population (15). About 42% of DRB1*1302+ Korean individu-

als carry DQB1*0609 and the remainder (58%) carry DQB1*0604. Thus, the significance of those two clearance alleles were indirectly evaluated by comparing haplotype frequencies of DRB1*1302-DQB1*0609 and DRB1*1302-DQB1*0604 in the two study groups. As shown in Table 2, the frequency of DRB1*1302-DQB1*0609 was much higher in the SR group than in the CI group with a statistical significance (5.63% vs. 0.78%, $p=0.0002$, OR, 7.57), and its significance remained after correcting the p value ($p_c=0.0040$). Although it was still elevated in the SR group (3.75% vs. 1.82%), the frequency of the second haplotype, DRB1*1302-DQB1*0604, was not statistically different between the two groups. In conclusion, DQB1*0609 was the primary allele associated with HBV clearance. The observed association of clearance with DRB1*1302 might be due to the DRB1 allele itself and/or to the linked DQB1*0609.

Haplotypes associated with HBV clearance or persistence

Two- and three-locus HLA class II haplotypes associated with viral clearance and persistence are listed in Table 3. All of the haplotypes, except two (DQB1*0303-DPB1*0201, DRB1*0901-DQB1*0303-DPB1*0201), carried at least

Table 2. Comparison of DRB1*1302-related haplotype frequencies between the SR and CI groups

DRB1*-DQB1*	SR n=160 (HF, %)	CI n=768 (HF, %)	p value	p_c value	OR (95% CI)
1302-0604	3.75	1.82	0.1267	-	-
1302-0609	5.63	0.78	0.0002	0.0040	7.57 (2.66-21.58)

SR, spontaneously recovered; CI, chronically infected; HF, haplotype frequency is presented in percentage; p_c value, p value corrected by multiplying the number of haplotypes tested (20 for DRB1-DQB1); OR, odds ratio (SR group vs. CI group); CI, confidence interval. Only significant p_c values (<0.05) are presented.

Table 3. Two- and three-locus haplotypes associated with viral clearance and persistence

Haplotype	SR n=160 (HF, %)	CI n=768 (HF, %)	p value	p_c value	OR (95% CI)
Viral clearance					
DRB1*1302-DQB1*0609	5.63	0.78	0.0002	0.0040	7.57 (2.66-21.58)
DQB1*0609-DPB1*0201	3.75	0.39	0.0013	0.0260	9.93 (2.46-40.15)
DRB1*1302-DQB1*0609-DPB1*0201	3.75	0.26	0.0010	0.0190	14.92 (2.98-74.63)
DRB1*1502-DQB1*0601	8.12	3.91	0.0239	NS	2.18 (1.11-4.27)
DQB1*0601-DPB1*0901	6.83	2.78	0.0144	NS	2.55 (1.21-5.40)
DRB1*1502-DQB1*0601-DPB1*0901	6.88	2.84	0.0149	NS	2.53 (1.20-5.33)
DQB1*0302-DPB1*0501	7.26	2.56	0.0043	NS	2.97 (1.41-6.26)
DQB1*0303-DPB1*0201	7.44	2.98	0.0091	NS	2.61 (1.27-5.38)
DRB1*0901-DQB1*0303-DPB1*0201	6.60	2.53	0.0103	NS	2.74 (1.27-5.91)
Viral persistence*					
DQB1*0301-DPB1*0201	0	4.01	0.0100	NS	NA
DQB1*0202-DPB1*1301	0	3.81	0.0121	NS	NA
DRB1*0701-DQB1*0202-DPB1*1301	0	3.82	0.0121	NS	NA

SR, spontaneously recovered; CI, chronically infected; HF, frequency of allele or haplotype is presented in percentage; p_c value, p value corrected by multiplying the number of haplotypes tested (20 for DRB1-DQB1; 20 for DQB1-DPB1; 19 for DRB1-DQB1-DPB1); OR, odds ratio (SR group vs. CI group); CI, confidence interval; NS, statistically not significant after correction ($p_c>0.05$); NA, not applicable.

*, p value was calculated by chi-square test.

one of the outcome-related alleles (Table 1). For example, three DRB1*1302- and DQB1*0609-related haplotypes (DRB1*1302-DQB1*0609 ($p_c=0.0040$), DQB1*0609-DPB1*0201 ($p_c=0.0260$), DRB1*1302-DQB1*0609-DPB1*0201 ($p_c=0.0190$)) were strongly associated with viral clearance as DRB1*1302 and DQB1*0609 themselves were. The haplotypes maintained statistical significances after correction of the p values. Other haplotypes associated with viral clearance were DRB1*1502- and DQB1*0302-related haplotypes. Haplotypes associated with viral persistence were DQB1*0301- and DRB1*0701-related haplotypes. However, p values for those haplotypes were not statistically significant after the correction (Table 3).

Although none of the DPB1 allele appeared to be associated with the outcome of HBV infection (Table 1), there was an indirect association of DPB1 with viral clearance observed at the haplotype level (Table 3). On a haplotype (DQB1*0303-DPB1*0201, $p=0.0091$, OR, 2.61) associated with viral clearance with a statistical significance, neither DQB1*0303 (26.25% vs. 25.00%) nor DPB1*0201 (40.00% vs. 37.50%) alone was associated with viral clearance as shown in Table 1. However, a haplotype consisting of these two alleles appeared to be associated with viral clearance. Furthermore, the OR value of its 3-locus haplotype, DRB1*0901-DQB1*0303-DPB1*0201 (OR, 2.74), was similar to that of DQB1*0303-DPB1*0201 (OR, 2.61). We could observe a similar phenomenon in another example. DQB1*0609 (OR, 7.24) was strongly associated with viral clearance as described above. However, linking this allele to statistically insignificant DPB1*0201 again in a haplotype DQB1*0609-DPB1*0201 increased the OR value (OR, 9.93). In a three-locus haplotype, DRB1*1302-DQB1*0609-DPB1*0201, the OR value was even higher (OR, 14.92).

DISCUSSION

From the study of the HLA association with the outcome of HBV infection, we found that two HLA class II alleles are strongly associated with viral clearance. The first allele was DRB1*1302 and this result was concordant with two previous Korean studies that demonstrated that DR13 was associated with self-elimination of HBV (8, 9). In addition, DRB1*1302 has been consistently reported as a factor associated with protection against persistent HBV infection in two other racial groups, Africans (16) and Caucasians (17, 18). Identification of the same HLA allele and/or serologic type from various populations strongly suggests that there is at least one common element restricting HBV in humans. A beneficial effect of DR13 on HBV infection has also been confirmed by functional assay. Diepolder *et al.* demonstrated that DR13-positive individuals showed a more vigorous CD4+ T cell response to HBV core antigen (HBcAg) than DR13-negative patients (19). DR13-restricted CD4+ T cell

epitopes of HBcAg were later discovered (20).

The second allele associated with viral clearance was DQB1*0609, which is in strong linkage disequilibrium with DRB1*1302 in the study population. The impact of the DQB1*0609 allele on HBV clearance was indirectly analyzed by comparing frequencies of the two DRB1*1302-positive haplotypes, DRB1*1302-DQB1*0609 and DRB1*1302-DQB1*0604, between the two study groups. The result indicates that DQB1*0609 has a much stronger association with HBV clearance than DRB1*1302 and we believe that DQB1*0609 is the primary class II allele associated with viral clearance. This hypothesis is supported by several previous studies. First, there has been a report that the phenotype frequency of DQw1 was significantly lower in Dutch patients with chronic active HBV infection (21) (DQB1*0609 is one of the alleles encoding the DQw1 molecule). In addition, a study in Gambians indicated that the frequency of DQB1*06 linked to DRB1*1302 was significantly higher in children (11.9% vs. 6.5%, $p=0.06$) and adults (9.2% vs. 0%, $p=0.05$) who cleared the virus than in individuals who carried the virus persistently (16). Later, DQB1*0609 was found to be relatively frequent in Gambians (DQB1*0609=4.4%; DQB1*0604=0.7%) (22). Lastly, it has been demonstrated that DR13-positive individuals exhibited a more vigorous CD4+ T cell response to HBcAg than DR13-negative individuals during acute HBV infection (19). However, less than 10% of the HBc-specific CD4+ T cell clones derived from DR13-positive patients were DR13-restricted. This result left open the possibility that the majority of the CD4+ T cell clones (>90%) might be restricted to other HLA class II molecules including DQw1 encoded by a DQB1*06 allele in DR13-positive individuals.

Therefore, for the first time, we have shown that DQB1*0609 is the primary allele associated with HBV clearance and its association is much stronger than previously found for DRB1*1302. In most of the previous association studies on HBV clearance, DQB1 high resolution typing was not performed. Only three studies so far analyzed DQB1 at allele resolution; however, the frequencies of DQB1*0609 might have been too low in the study populations to catch its statistical significance (18, 23, 24). That could be the reason why DQB1*0609 has not been identified thus far with HBV clearance. Our hypothesis needs to be confirmed by further studies in populations expressing a high frequency of this allele.

Several additional alleles were found to be associated with the outcome of HBV infection in this study, although the result did not reach statistical significance. The increased frequency of DRB1*1502 in the SR group was consistent with previous studies in Qatar (25) and China (26). Likewise, the higher frequencies of DRB1*0701 (9, 25) and DQB1*0301 (23, 24) in the CI group were consistent with previous reports. One of the previous studies analyzed a Korean population and found significant association of DR7 with

HBV persistence ($p < 0.001$) (9). Another study in the same population found a high frequency of DR9 ($p < 0.001$), instead of DR7, in the CI group (8). These discrepancies might stem from differences in the study subjects. In our study, the study samples were recruited from outpatient clinics based on the HBV serologic markers. Previous studies analyzed the compiled HLA typing data of individuals undergoing organ transplantation and their donors where HLA matching between patient and donor is beneficial. Thus, the HLA frequencies in those studies could have been biased. DQB1*0302, found in this study associated with HBV clearance, has not been identified previously.

During the analysis of HLA class II multi-locus haplotypes, we found several related haplotypes carrying outcome-related alleles. Interestingly, association with viral clearance is significantly intensified on DQB1-DPB1 haplotypes consisting of a DQB1 allele (either a statistically insignificant allele [e.g., DQB1*0303] or a clearance-associated allele [e.g., DQB1*0609]) and the statistically insignificant DPB1*0201, compared to OR values of the DQB1 allele alone. These results indicate that a DPB1 allele in combination with a DQB1 allele has either a cooperative (or synergistic) protective effect since DPB1*0201 (40.0% vs. 37.5%) alone has no effect. Alternatively, there might be another gene influencing the outcome of HBV infection located near the DPB1 gene. Several polymorphic genes, such as LMP, DM, and DO, whose gene products assist in antigen binding to the HLA molecules in a cell, are known to be located between DQB1 and DPB1 genes (27). Further extended investigation of these polymorphic genes might elucidate the cooperative (or synergistic) modification of immune responsiveness to HBV infection. Whether it is a cooperative (or synergistic) interaction or a closely linked gene, factor other than DR and DQ alleles might be involved in viral clearance.

The DRB1*1302-DQB1*0609-DPB1*0201 haplotype shows the strongest protective effect against HBV ($p = 0.0190$, OR, 14.92). Based on the results obtained from this study, it is most likely that the strong protective effect of the haplotype against HBV persistence might derive from three different factors: DRB1*1302, DQB1*0609, and another unknown factor linked to DPB1 as just described. Further investigations should be made to identify this.

In summary, multiple HLA class II alleles and related haplotypes are associated with viral clearance and persistence in Koreans. In specific, DRB1*1302 and DQB1*0609 are strongly associated with viral clearance. However, unexpectedly, the association of DQB1*0609 is much stronger than that of DRB1*1302. In addition, the association with a statistically insignificant DPB1 allele in a haplotype strengthens the association with viral clearance, hinting that another unknown factor might be involved in viral clearance. These results may reflect the existence of multiple factors controlling viral clearance encoded in the HLA class II gene region. Further extended investigation on the genetic factors relat-

ed to the outcome of HBV infection will provide valuable insights into the understanding of the mechanisms involved.

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