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ORIGINAL ARTICLE

Haplotype-dependent HLA-DRB1-DQB1 susceptibility to occult HBV infection in Xi'an Han population

Tianju Wang | **Chunmei Shen** | **Jun Qi** | **Liping Chen** | **Sheng Liu** | **Hengxin Li**

Shaanxi Province Blood Center, Xi'an, People's Republic of China

Correspondence
Hengxin Li, Shaanxi Province Blood Center, 407# Zhuque Ave, Xi'an, Shaanxi Province 710061, People's Republic of China.
Email: hengxin127@163.com

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Abstract

Background: Occult hepatitis B virus (HBV) infection (OBI) is primarily characterized by the persistence of HBV-DNA in the liver tissues and/or in the serum without detectable HBsAg. Human leukocyte antigen (HLA) polymorphisms have been found to be strongly associated with HBV in different ethnic backgrounds. The association of HLA-DRB1-DQB1 haplotypes with OBI has not been previously reported in China. The aim of this study was to identify the potential association of HLA-DRB1-DQB1 haplotypes that may be involved in OBI genetic susceptibility.

Methods: A case–control study was conducted between 107 OBI subjects and 280 healthy controls from the blood donors in the Shaanxi Province Blood Center. The HLA-DRB1, DQB1 loci were genotyped using polymerase chain reaction-sequence based typing (PCR-SBT). Based on the genotype data of the two loci, haplotype estimation was performed.

Results: HLA-DRB1*07:01-DQB1*02:02 ($pc = 0.344 \times 10^{-3}$, OR = 3.489, 95%CI = 2.000–6.088) and HLA-DRB1*09:01-DQB1*03:03 ($pc = 0.02$, OR = 2.370, 95%CI = 1.450–3.873) serve as the possible risk and susceptibility haplotypes for OBI in Xi'an Han after Bonferroni correction.

Conclusions: This study demonstrated that HLA II haplotypes were significantly associated with OBI in the Xi'an Han population. To the best of our knowledge, this is the first study to associate HLA-DRB1-DQB1 haplotypes with OBI, which can provide valuable insights into the relationship between the various genetic factors and immune responses in the Xi'an population. The findings can also form the basis for future studies about the role of HLA in OBI.

KEYWORDS

haplotype frequency, human leukocyte antigen, occult HBV infection, Xi'an Han population

1 | INTRODUCTION

Occult HBV infection (OBI) is defined as the presence of replication-competent HBV DNA (i.e., epitomal HBV covalently closed circular DNA [cccDNA]) in the liver and/or HBV DNA in the blood of persons testing negative for hepatitis B surface antigen (HBsAg) based on the currently available assays (Raimondo et al., 2010, 2019). OBI can lead to serious clinical conditions such as increased risk of reactivation, possible transmission of infection, contribution to cirrhosis, and hepatocellular carcinoma (Malagnino et al., 2018; Raimondo et al., 2010, 2013). OBI prevalence has been reported to vary significantly between the different geographical areas and population according to HBV endemicity. The prevalence of OBI among the blood donor was reported as 1:7517 in China (Zheng et al., 2011), 1:3832 in Thailand (Phikulsod et al., 2009), 1:108 in Mozambique (Mabunda et al., 2020), and 1:204 in Brazil (Nishiya et al., 2021).

The causes of OBI have not been completely elucidated. Genetic factors act as important determinants of HBV risk (Ahn et al., 2000; Amarapurpar et al., 2003; Doganay et al., 2014; Han et al., 2005). Most OBI cases have relatively low levels of HBV cccDNA in the liver. Overall replication activity and viral protein expression are markedly suppressed by the host immunological and epigenetic mechanisms (Raimondo et al., 2010). T cells and humoral antibody responses also appear to play an important role in host control of OBI (Loomba & Liang, 2017).

The human leukocyte antigen (HLA) is located at the chromosome 6p21.31 (Shiina et al., 2009). HLA complex is specialized in the presentation of short peptides to T cells and plays a vital role in the body's immune defense (Holoshitz, 2013). An association between HBV infection and HLA was first reported in 1981 (Sampliner et al., 1981) and HLA is one of the most intensively studied genetic factors in HBV. Several previous studies have shown that HLA haplotype may be associated with increased susceptibility to HBV infection in the different populations. It has also been observed that the different haplotypes can correlate to susceptibility in distinct ethnic groups. For instance, HLA-DRB1*03:01 and DQA1*05:01, DQB1*03:01 are protective for HBV infection in Chinese population (Jiang et al., 2003). HLA-DRB1*07, DRB1*13, and DQB1*03 are associated with high risk for chronic HBV infection in the Turkish population (Karan et al., 2002). DQA1*05:01, DQB1*03:01 (Thio et al., 1999) and DRB1*09 (Ahn et al., 2000) were associated with HBV persistence.

We have previously analyzed the potential association between OBI and HLA alleles polymorphism. The aim of this study was to investigate the HLA-DRB1 and HLA-DQB1 gene profiles from 107 OBI carriers and 280 ethnically matched healthy control subjects to explore the associations between OBI and HLA haplotypes in Xi'an Han population. This is the first study to evaluate the possible role of HLA-DRB1-DQB1 haplotypes in OBI carriers from Xi'an Han population.

2 | MATERIALS AND METHODS

Ethical compliance, blood collection, and DNA preparation was performed as described previously (Wang et al., 2017). The details related to the blood collection and testing are listed in Figure 1. All DNA samples were stored at −80°C.

2.1 | Genotyping of HLA-DRB1 and HLA-DQB1

All the samples were genotyped for HLA-DRB1 and HLA-DQB1 loci based on PCR-SBT methods at the high-resolution levels according to the method described in the previous study (Wang et al., 2017). SeCore™ Sequencing Kits (Invitrogen, Carlsbad, CA, USA) and the Applied Biosystems Inc. ABI 3730XL platform (Applied Biosystems, Foster City, CA, USA) were applied for all HLA II genotyping. The genotypes of HLA-DRB1 and HLA-DQB1 were assigned using HLA SBT uTYPE software version 7.2 (Invitrogen, Carlsbad, CA, USA). The ambiguous samples within the relevant exon were resolved by group-specific sequencing primers (GSSP), sequence-specific primers (SSP), or according to the standards of the China Marrow Donor Program (CMDP) (He et al., 2012, 2018). However, partial G group alleles were not discriminated in the present study, including DRB1*12:01G (DRB1*12:01/12:06/12:10) and DRB1*14:01G (DRB1*14:01/54).

2.2 | Statistical analyses

In this study, the analyses of HLA-DRB1-DQB1 haplotypes were performed at the high-resolution levels. The haplotype frequencies were calculated based on EM algorithm using Arlequin software version 3.11 (http://cmpg.unibe.ch/software/arlequin3) (Excoffier et al., 2007). The chi-squared tests and Fisher's exact tests were conducted to compare the distribution of HLA-DRB1-DQB1 haplotypes between OBI and control groups. The statistical analyses were performed using SPSS software version 19.0 (IBM Corp, Armonk, NY, USA), and the odds ratio (OR) and 95% confidence interval (95% CI) were also calculated. Besides, the p values were corrected (pc) for the multiple testing using Bonferroni's method to avoid the errors caused by multiple comparisons between the two

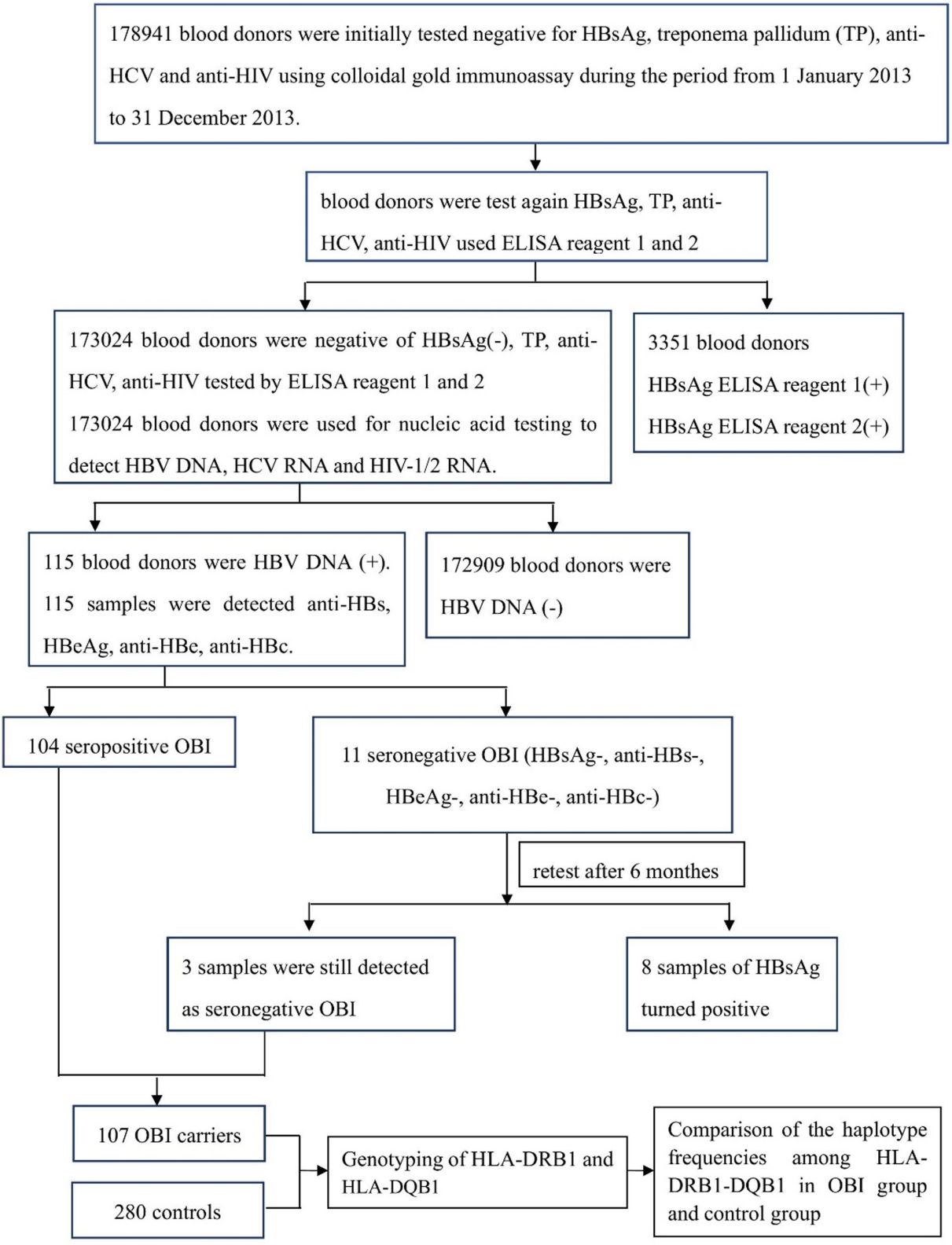

FIGURE 1 Schematic collection of the sample under study. 178,941 blood donors were initially tested negative for HBsAg, anti-TP, anti-HCV, and anti-HIV using colloidal gold immunoassay during the period from January 2013 to December 2013. A total of 107 OBI samples were collected (negative for anti-HCV and anti-HIV and HBsAg, positive for HBV DNA), of which 104 were seropositive OBI and three were seronegative OBI. A total of 280 healthy volunteers matched with OBI individuals in age and gender (negative for anti-HCV and anti-HIV, HBsAg and HBV DNA) were randomly selected from the same geographical regions.

samples. In addition, the *pc* was corrected by multiplying the numbers of comparisons observed. A level of $pc < 0.05$ was considered as statistically significant.

To examine whether association between HLA-DRB1 and HLA-DQB1 alleles were independent of other alleles in the linkage disequilibrium, multivariate logistic regression analyses were carried out with the different combinations of HLA alleles as independent variables, and the presence or absence of OBI was regarded as the dependent variable.

3 | RESULTS

3.1 | Demographic and biochemical information of subjects investigated in the study

The general information of the studied donors has been summarized in Table 1. A total of 107 OBI carriers were enrolled, 75 men and 32 women, with an age range from 18 to 55 years (mean 36.7 ± 11.1 years). A total of 280 healthy control subjects were involved from the voluntary blood donors whose blood samples were negative for anti-HCV and anti-HIV, HBsAg and HBV DNA, 174 men and 106 women, with an age range from 19 to 55 years (mean 34.8 ± 11.2 years). None of the participants had a familial relationship with each other.

TABLE 1 Demographic and biochemical information of subjects investigated

Parameter	OBI carriers	Control subjects
Number of subjects	107	280
Age mean (years) at the time of study (mean ± *SD*)	36.7 ± 11.1	34.8 ± 11.2
Gender		
Male	75	174
Female	32	106
Hemoglobin (g/L)	≥115	≥115
ALT ± SD (IU/L)	<40	<40
ELISA tests		
HBsAg	–	–
Anti-HIV	–	–
Anti-HCV-1/2 and P24	–	–
Anti-Treponema Pallidum	–	–
Nucleic Amplification Technique		
HBV DNA	+	–
HIV-1/2 RNA	–	–
HCV RNA	–	–

3.2 | Comparison of the haplotype frequencies among HLA-DRB1-DQB1 in OBI group and control group at high resolution

The results of HLA-DRB1-DQB1 haplotypes at high resolution in OBI group and control group are presented in Table 2 and Figure 2. A total of 19 different HLA-DRB1-DQB1 haplotypes were detected in OBI group at high resolution. Twenty-four haplotypes were detected in the control group. Among the DRB1-DQB1 haplotypes, only four showed a significantly different distribution between the OBI group and the control group, which included DRB1*07:01-DQB1*02:02 (14.02% vs. 4.39%, $p = 0.1376 \times 10^{-4}$), DRB1*09:01-DQB1*03:03 (15.41% vs. 7.17%, $p = 0.0008$), DRB1*14:05-DQB1*05:03 (3.27% vs. 0.89%, $p = 0.0240$), and DRB1*15:04-DQB1*06:02 (1.40% vs. 0.00%, $p = 0.0209$). After Bonferroni correction, the haplotypes DRB1*07:01-DQB1*02:02 ($pc = 0.344 \times 10^{-3}$, OR = 3.489, 95%CI = 2.000–6.088), and DRB1*09:01-DQB1*03:03 ($pc = 0.02$, OR = 2.370, 95%CI = 1.450–3.873) were found to exhibit a higher frequency in the OBI group than in the controls. The haplotype DRB1*09:01-DQB1*03:03 was found to be associated with OBI carriers with a statistical significance, but neither DRB1*09:01 (16.36% vs. 12.86%) nor DQB1*03:03 (20.09% vs. 14.46%) alone was associated with OBI (Wang et al., 2017). However, a haplotype consisting of these two alleles was observed to be associated with OBI.

3.3 | Multivariate logistic regression analyses

HLA-DRB1*07:01 was observed in strong (linkage disequilibrium, LD) with HLA-DQB1*02:02. To identify causative HLA alleles and haplotypes, and to distinguish them from HLA alleles that were associated with OBI only due to LD, we performed a multivariate logistic regression analysis to investigate whether the HLA*07:01 association was independent of HLA-DQB1*02:02. Individual HLA-DRB1*07:01 and HLA-DQB1*02:02 were included as the separate independent variables in the model. After inclusion of all these variables, it was observed that the primary association of OBI was with HLA-DRB1*07:01, and that this was independent of the HLA-DQB1*02:02. Table 3 shows that HLA-DRB1*07:01 ($p = 0.030$, OR = 1.722, 95%CI = 1.053–2.819) was directly associated with OBI independent of HLA-DQB1*02:02 ($p = 0.179$, OR = 1.455, 95%CI = 0.842–2.513).

HLA-DRB1*09:01 was found to be in strong LD with HLA-DQB1*03:03. We investigated whether HLA-DRB1*09:01 might also form part of an extended haplotype with HLA*03:03, thereby explaining the observed

TABLE 2 Comparison of the haplotype frequencies among HLA-DRB1-DQB1 in OBI group and control group at high resolution

	OBI	Control				
HLA-haplotypes	**HF(%)**	**HF(%)**	***p***	***pc***	**OR**	**95%CI**
DRB1*01:01-DQB1*05:01	0.00	1.24	0.1993	–	–	–
DRB1*03:01-DQB1*02:01	3.74	2.83	0.4957	–	–	–
DRB1*04:01-DQB1*03:01	0.00	1.07	0.1950	–	–	–
DRB1*04:05-DQB1*04:01	2.80	2.49	0.8026	–	–	–
DRB1*04:06-DQB1*03:01	0.00	1.26	0.1993	–	–	–
DRB1*04:06-DQB1*03:02	1.40	1.23	1.0000	–	–	–
DRB1*07:01-DQB1*02:02	**14.02**	**4.39**	**0.1376×10^{-4}**	**0.344×10^{-3}**	**3.489**	**2.000–6.088**
DRB1*07:01-DQB1*03:01	0.48	1.75	0.3061	–	–	–
DRB1*07:01-DQB1*03:03	3.26	1.42	0.1399	–	–	–
DRB1*08:03-DQB1*06:01	1.40	2.46	0.4247	–	–	–
DRB1*09:01-DQB1*03:01	0.94	1.31	1.0000	–	–	–
DRB1*09:01-DQB1*03:03	**15.41**	**7.17**	**0.0008**	**0.02**	**2.370**	**1.450–3.873**
DRB1*11:01-DQB1*03:01	6.53	3.36	0.0713	–	–	–
DRB1*11:01-DQB1*03:03	0.00	1.18	0.1993	–	–	–
DRB1*11:04-DQB1*03:01	0.47	1.42	0.4570	–	–	–
DRB1*12:01G-DQB1*03:01	6.07	4.26	0.3457	–	–	–
DRB1*12:02-DQB1*03:01	8.87	5.42	0.0973	–	–	–
DRB1*14:05-DQB1*05:03	**3.27**	**0.89**	**0.0240**	–	–	–
DRB1*15:01-DQB1*03:01	0.00	1.10	0.1950	–	–	–
DRB1*15:01-DQB1*03:03	0.00	1.19	0.1993	–	–	–
DRB1*15:01-DQB1*06:01	1.40	1.36	1.0000	–	–	–
DRB1*15:01-DQB1*06:02	3.27	4.73	0.5506	–	–	–
DRB1*15:02-DQB1*06:01	2.33	1.91	0.7791	–	–	–
DRB1*15:04-DQB1*06:02	**1.40**	**0.00**	**0.0209**	–	–	–
DRB1*16:02-DQB1*05:02	1.40	0.71	0.4025	–	–	–

Note: Only haplotypes with significant differences as determined by chi-square test or Fisher exact test are listed; haplotypes with significant differences after Bonferroni correction are shown in bold.

Abbreviations: HF, haplotype frequency; OR, odds ratio; *pc*, corrected *p*; 95%CI, 95% confidence interval.

association with OBI. Individual HLA-DRB1*09:01 and HLA-DQB1*03:03 were also included as the separate independent variables in the same model. Table 4 shows that HLA-DRB1*09:01 ($p = 0.417$, OR = 1.207, 95%CI = 0.766–1.902) and HLA-DQB1*03:03 ($p = 0.102$, OR = 1.424, 95%CI = 0.933–2.173) were associated with OBI dependently.

4 | DISCUSSION

OBI is defined by the presence of HBV-DNA in the liver tissues and/or in the serum of HBsAg-negative individuals (Allain, 2004). OBI can exhibit long-lasting specific T-cell immune response against different HBV epitopes. The activity of HBV-specific CD4+ T cell has been reported to be markedly reduced in patients with persistent infection as compared with the subjects who have self-limiting infection (Ferrari et al., 1990; Thursz et al., 1995). *HLA* genes are highly polymorphic and play a major role in the regulation of immune response against exogenous infections. This property makes HLA a very important candidate biomarker to an infectious disease. HLA-DRB1 and DQB1 molecules have been reported to be critical for the development of CD4+ T cell. In addition, specific HLA-DRB1-DQB1 haplotype polymorphisms have been associated with various diseases including pemphigus vulgaris (Zivanovic et al., 2016), primary biliary cirrhosis (Umemura et al., 2012), Vitiligo (Bouayad et al., 2013), keloids (Lu et al., 2011), paroxysmal nocturnal hemoglobinuria (Nowak et al., 2010), and celiac disease (Eller et al., 2006). Moreover, several previous studies have suggested that extended HLA haplotypes provide an even greater risk compared with alleles. Thus, we mainly focused on the susceptibility of haplotypes for HLA-DRB1-DQB1 in this study.

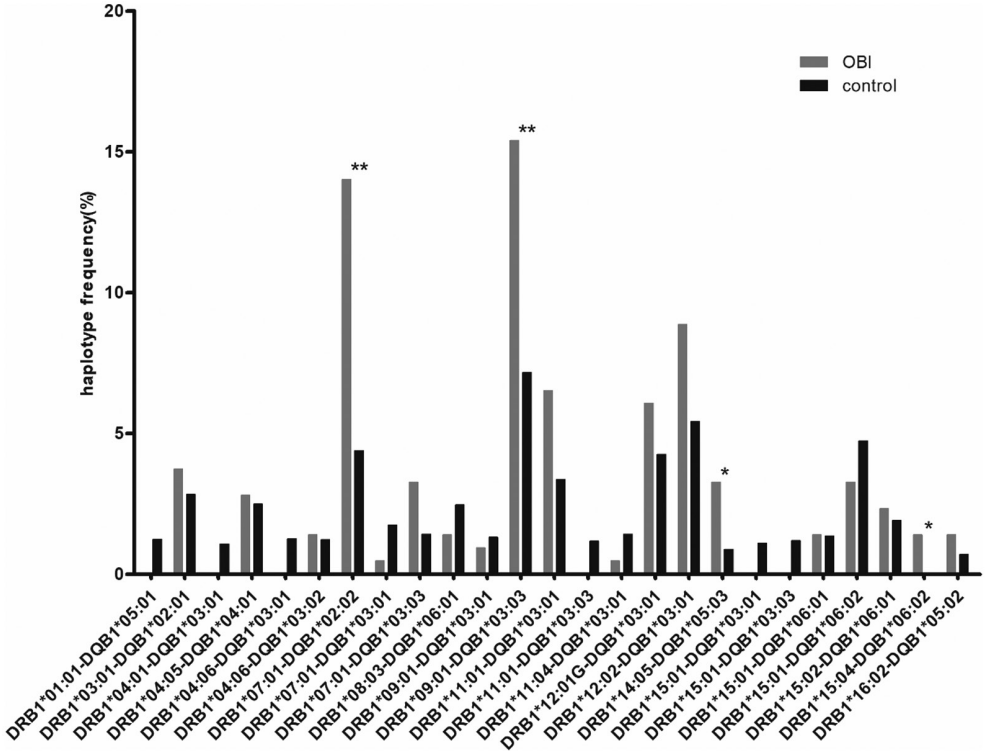

FIGURE 2 Comparisons of the frequencies of HLA II haplotypes at high resolution between OBI carriers from Xi'an Han and controls. Frequencies of HLA-DRB1-DQB1 between OBI group (gray column) and control group (blank column) were compared. *p* values for multiple comparisons (*pc*) were corrected by Bonferroni correction. *$p < 0.05$, **$pc < 0.05$.

TABLE 3 Multivariate logistic regression analysis to demonstrate the association of HLA-DRB1*07:01 with OBI, independent of HLA-DQB1*02:02

	Regression coefficient	*SE*	OR	95%CI	*p*
DRB1*07:01	0.544	0.251	1.722	1.053–2.819	0.030
DQB1*02:02	0.375	0.279	1.455	0.842–2.513	0.179
Constant	−1.083	0.090			

Note: Multivariate logistic regression analysis with presence or absence of OBI as the dependent variable. All independent variables were adjusted for each other in the model. *p* represents the significance of each variable compared with individuals negative for that variable.

Abbreviations: CI, confidence interval; OBI, occult HBV infection; OR, odds ratio.

TABLE 4 Multivariate logistic regression analysis to demonstrate the association of HLA-DRB1*09:01 with OBI, dependent of HLA-DQB1*03:03

	Regression coefficient	SE	OR	95%CI	*p*
DRB1*09:01	0.188	0.232	1.207	0.766–1.902	0.417
DQB1*03:03	0.353	0.216	1.424	0.933–2.173	0.102
Constant	−1.050	0.093			

Note: Multivariate logistic regression analysis with presence or absence of OBI as the dependent variable. All independent variables were adjusted for each other in the model. *p* represents the significance of each variable compared with individuals negative for that variable.

Abbreviations: CI, confidence interval; OBI, occult HBV infection; OR, odds ratio.

HLA-DRB1*13 has been associated with HBV viral clearance in Africans and Europeans (Höhler et al., 1997; Thursz et al., 1995). On the contrary, HLA-DRB1*13 can function as a susceptibility gene for chronic HBV infection in Turkish populations (Karan et al., 2002). HLA-DRB1*07 and DRB1*15 were found to be protective against HBV infection in Chinese population but were associated with chronic infection in Turkish and Indian populations (Amarapurpar et al., 2003; Han et al., 2005). HLA-DRB1*11 and DRB1*12 alleles were associated with HBV clearance

in Chinese population (Jiang et al., 2003; Meng et al., 2003). HLA-DRB1*09 was associated with HBV persistence and disease severity in Chinese (Meng et al., 2003) and Korean populations (Ahn et al., 2000). Extensive allele diversity has been also observed in HLA associations with HBV infections in different ethnic populations. However, racial diversity, methodology, and complex immune-regulatory mechanisms and variations in the study design make it difficult to find consistent associations among the different studies. Overall, HLA-DRB1*07 and DRB1*09 were found to be associated with HBV infection and disease chronicity in most of the previous reports.

HLA-DRB1*07 acts as a risk factor for HBV infection (Almarri & Batchelor, 1994; Thio et al., 2003), and the results have indicated that patients with HLA-DRB1*07 allele are unable to mount a specific immune reaction to HBV and are thus predisposed to chronic infection (Doganay et al., 2014). The haplotypes containing HLA-DRB1*07:01, such as B*44-DRB1*07:01 and B*44-C*16:01-DRB1*07:01 have been significantly associated with HBV persistence. We confirmed an association between HLA-DRB1*07:01, DQB1*02:02, and OBI that has been previously reported in a case–control comparison study. Moreover, DRB1*07:01-DQB1*02:02 haplotype was found to be correlated with OBI. In summary, this study extended the findings of the previous work and indicated a remarkable consistency of the HLA association. Since DRB1*07 exists as DRB1*07:01 at high resolution among Chinese population (Chen et al., 2019), the potential association between DRB1*07 and HBV chronicity was consistent with previous finding. Thus, identification of the same HLA allele from the various population strongly suggested that there was at least one common element persisting infection of HBV in humans.

In this study, the associations between HLA-DRB1*07:01-DQB1*02:02 and OBI could be attributed to the effect of individual allele that displayed susceptibility toward OBI. Although HLA-DRB1*07:01 (OR = 2.012) appeared to contribute more significantly toward OBI than HLA-DQB1*02:02 (OR = 1.919) alone does (Wang et al., 2017), the data strongly indicated that a combination of these two alleles (DRB1*07:01-DQB1*02:02, OR = 3.489) exhibited an additive effect on OBI.

Interestingly, as a single locus allele, neither DRB1*09:01 nor DQB1*03:03 was found to be associated with OBI, whereas, the HLA-DRB1*09:01-DQB1*03:03 haplotype emerged as a risk haplotype, displaying a 2.37-fold increased risk for the development of OBI. The results suggested that LD between DRB1*09:01 and DQB1*03:03 might be important for regulating the progression of OBI. The LD of DRB1*09:01-DQB1*03:03 was also observed in Asian population in the previous reports (In et al., 2015; Trachtenberg et al., 2007). The mechanism underlying the genetic association remains to be elucidated. For HLA, the study of haplotypes might be more important than individual alleles, because immune factors work together in body's immune defense against viruses.

In order to avoid a type I error in the analysis (a false-positive), Bonferroni correction was applied to the *p* value. In statistics, the Bonferroni correction is a method used to counteract the problem of multiple comparisons. It is considered as the simplest and most conservative method to control type I error. However, the routine use of Bonferroni correction has been criticized as deleterious to arrive at the statistical judgment, resting the wrong hypothesis, and reducing the chance of a type I error but at the expense of introducing a type II error (Armstrong, 2014). Hence, in order to reduce the number of the comparisons with inadequate power, the haplotypes with low frequencies (<1%, <2%, <3%, or <5%) have not been involved in some previous reports (Cao et al., 2014; Hwang et al., 2007; Sippert et al., 2015; Thio et al., 2003). For HLA-DRB1-DQB1 haplotypes, the number of haplotypes used for Bonferroni correction was 25, since some reports did not follow a uniform standard. Although Bonferroni correction for the multiple comparisons has been applied in this study, this statistical method likely can lead to type II error (Cardon & Bell, 2001). Therefore, we have listed the various haplotypes in which significance was lost after application of Bonferroni correction, as potential susceptible haplotypes in the study.

This study has several limitations. First, we focused on the HLA-DRB1-DQB1 haplotypes, not on alleles and epitopes analysis, and HBV antigens were not investigated in this reported. In the next step, we investigated these research areas and analyzed structural elements present in HLA. Second, the number of OBI was relatively small, however, this is one of the largest OBI cohorts with HLA-DRB1-DQB1 haplotypes at high-resolution level in northern Chinese Han. Hence, further studies with a larger sample size are necessary in future. Finally, the HBV-infected patients have not been studied in this study and our group will also analyze them in future studies.

To the best of our knowledge, this is the first study that has investigated the contribution of HLA-DRB1-DQB1 haplotypes to the susceptibility to OBI. In this case–control study in Xi'an Han population, OBI carriers served as the controls were fine-matched. Our results demonstrated that OBI carriers differed significantly in HLA-DRB1-DQB1 haplotypes compared with the normal controls. The frequency of haplotype HLA-DRB1*07:01-DQB1*02:02, and HLA-DRB1*09:01-DQB1*03:03 were markedly increased in the OBI carriers. Our results further indicated that HLA-DRB1-DQB1 haplotype was involved in genetic susceptibility to OBI, and this information may help to develop novel approaches for both prevention and treatment of OBI.

AUTHOR CONTRIBUTIONS

TJW and CMS designed the experiments; LPC, SL, and TJW performed the experiments and analyzed the data; TJW and JQ analyzed the data and wrote the manuscript, HXL and CMS gave the idea behind the manuscript compilation; all authors read and approved the manuscript prior to submission.

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ETHICS STATEMENT

The experimental procedures were conducted in line with the principles of the World Health Organization and the Declaration of Helsinki. The study was approved by the Medical Ethics Committee of Shaanxi Blood Center. According to the policies of the China Health Ministry, the purpose of the research was explained to all participants. Written informed consents were obtained from all the volunteers involved before participated in our study.

FUNDING INFORMATION

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Tianju Wang https://orcid.org/0000-0001-7900-4757
Jun Qi https://orcid.org/0000-0002-8795-3140
Hengxin Li https://orcid.org/0000-0002-5547-0080

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