Variation of HLA-DPB1 Gene in Hepatitis B Infection

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Research

Keywords: HLA-DPB1 gene, Expression, HBV susceptible, HBV spontaneous clearance

Posted Date: August 19th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-52937/v1>

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Abstract

Background: Hepatitis B virus (HBV) affects approximately 68 million people in China. 10-15% of adult infected with HBV will develop to chronic HBV, liver cirrhosis (LC), liver failure and hepatocellular carcinoma (HCC). The human leukocyte antigen HLA-DPB1 gene polymorphism and expression were identified to be associated with HBV susceptible and spontaneous clearance. We evaluated the role of HLA-DPB1 gene polymorphism in HBV infection.

Methods: In this study, HLA-DPB1, rs9277535 polymorphisms were investigated in 259 HBV infection patients (CHB) and 442 healthy controls (HC) by using a sequence based typing. The mRNA of HLA-DPB1 were measured by real-time polymerase chain reaction (RT-PCR).

Results: The results shown that DPB1 gene, rs9277535 were all associated with HBV infection in the Sichuan Han population. Rs9277535A, DPB1*04:02 play a protected role in HBV infection, Rs9277535G, DPB1*05:01 prone to susceptible to HBV infection. rs9277535GG have significantly higher HLA-DPB1 mRNA expression in HBV group compared that in HC group. DPB1*05:01 and DPB1*21:01 have a significantly lower HLA-DPB1 mRNA expression in HBV infection group compared than HC group. The meta-analysis revealed that HLA-DPB1*02:01, DPB1*02:02, DPB1*04:01 and DPB1*04:02 protected against HBV infection, while DPB1*05:01, DPB1*09:01, and DPB1*13:01 were risk factors for HBV infection susceptibility. DPB1*02:01 and DPB1*04:01 were prone to HBV spontaneous clearance, while DPB1*05:01 and DPB1*13:01 were associated with chronic HBV infection.

Conclusions:

DPB1 alleles and rs9277535 have a major effect on the risk of HBV infection, HBV infection associated with lower HLA-DPB1 expression. DPB1 alleles have important role in HBV susceptible and spontaneous clearance.

Background

As a relatively high prevalence HBV in China, the seropositive of HBsAg was estimated about 5.49%^[1] in 2015, that means approximately 93 million people infected by HBV, and 10–20% of adult HBV infections will progress to chronic HBV infection, alone or in combination with liver cirrhosis (LC) and/or hepatocellular carcinoma (HCC)^[2], lead to a heavy public health burden of HBV related liver disease in China^[3].

Multiple factors attributed the risk of chronic HBV infection, such as age, gender, BMI, ethnicity, viral mutation and genotypes, host genetic variations and related host immune responses and so on. A genome-wide association study^[4] (GWAS) in Japanese and other Asian populations found a significant association of chronic hepatitis B with several polymorphisms of the HLA loci including HLA-DPB1 and associated SNP rs9277535A/G. Subsequently, they revealed risk alleles DPB1*0501 and DPB1*0301, and protective alleles DPB1*0402 and DPB1*0401, respectively. Many researches^[5–11] verified these results in

different ethnic genetic populations, they revealed that HLA-DPB1 and rs9277535A/G not only related with susceptible HBV infection but also affect the results of HBV infection, to be spontaneous clearances or to be chronic HBV infections.

Recent years, researchers^[12] found that risk alleles of HLA-DP decrease liver mRNA expression of HLA-DP in HBV patients, suggesting that expression of HLA-DP genes is important in control of HBV in non-Hispanic European. Thomas and his colleagues found^[13] that the rs9277534A/G variant distinguishes the most protective HLA-DPB1 allele (DPB1*04:01) from the most susceptible (DPB1*01:01) after HBV infection, they also confers rs9277534GG have significantly higher levels of HLA-DPB1 surface protein and transcript level expression in healthy donors compared than rs9277534AA genotype, rs9277534A/G can be an HLA-DPB1 expression marker^[14], Decreased expression of DPB1 mRNA are associated with HBV reactivation in patients treated with immunomodulatory agents^[15], and increased HCV-related liver disease and correlated with HCV related disease progression^[16]. These results suggesting that differences HLA-DPB1 alleles and DPB1 expression may influence the risk of persistent HBV infection.

The latest research found^[17] that subset of HLA-DP molecules, such as HLA-DP401, which interact with NKp44 trigger functional NK cell responses. This interaction between DP alleles and NKp44 implicates HLA class II as a component of innate immune response, much like HLA-C and KIR molecular. It may provide a potential mechanism^[18] for the relationship between HLA-DP alleles and disease outcomes, including HBV infection. It's speculates that during acute HBV infection, NKp44 interacts with HLA-DP401 allele expressed on the surface of infected hepatocytes, IFN- γ was secreted both by Th1 and NK cells which contributing to lysis of HBV infected cells. In HBV infected individuals carrying HLA-DP301, NKp44 is unable to bind to HLA-DP301 molecules, resulting in inefficient lysis of infected hepatocytes by NK cells and lead to higher risk of chronic HBV infection.

The distribution of HLA-DPB1 molecules in Chinese populations were similar to those in Asian populations, HLA-DPB1 expression in HBV infection in Asian population haven't reported yet. In this research, we typed HLA-DPB1 and associated rs9277535A/G in a population-based case control study in Chinese Han population, including 259 HBV cases and 441 controls from Sichuan province, also identified the different HLA-DPB1 expression in different alleles of these two groups.

Methods

Samples

The samples involved in this study were from physical examination center of Deyang people hospital, Sichuan province, China. A total of 499 participants, including 259 chronic HBV carriers (CHB) and 441 healthy controls (HC).

The HBV subjects were determined based on the serological results of Hepatitis B surface antigen (HBsAg), antibody against Hepatitis B surface antibody (HBsAb), Hepatitis Be antigen (HBeAg), antibody against Hepatitis Be (HBeAb) and total antibody against HBV core antigen (HBcAb) tests. Volunteers and

patients with seropositive for HBsAg combine with seropositive for HBeAg and HBcAb or HBeAb and HBcAb and positive of HBsAg at least lasting 6 months were considered to be CHB carriers, the HC who were seronegative for both HBsAg and HBeAg. All blood samples were negative for Hepatitis C virus (HCV) and Human immunodeficiency virus (HIV).

Subjects who were seropositive for HBsAg, had normal serumalanine aminotransferase (ALT) levels (lower than 35 U/L), had no obvious clinical symptoms were considered to be asymptomatic carriers and had not use any antiretroviral drugs. Each subject gave his/her written informed consent before enrolment.

rs9277535 and HLA-DPB1 genotyping

The peripheral blood of all samples and DNA extraction were performed as consistent with previous reported [19, 20]. Genotyping was performed using sequence based typing (PCR-SBT). Amplification primers were following: rs9277535: Forward was 5'- TAACTGTGTGTGTTGCTGC, reward was 5'- CTGCGTGGTGAGAAAACAGG, for amplify HLA-DPB1 exon 2, forward primer was 5'- CTGCGTGGTGAGAAAACAGG, 5'- CCTGACAAGCTCCAGATGGG, reward primer was 5'- TTCCTTTATGGTGTGCTCCT. For each PCR mix (total volume, 10uL) contained 1ul DNA and 5uM primers and 5uL GoTaq Green Master Mix (Promega, Madison, USA). The DPB1 ambiguities were resolved by group-specific sequencing primers (GSSPs) sequencing, each primer was referenced by EBI-IMG database. primers were shown in Table 1. Thermal cycling conditions were as followed: 96°C for 3 min, 30 cycles at 95°C for 20s, 62°C for 15s, 72°C for 1min, and 72°C for 5 min. The PCR products were analyzed by an ABI 3730 DNA Sequencer (Applied Biosystem, Foster City, CA).

Table 1
Group-specific sequencing primers for DPB1 high resolution typing

Primers	Directions	Sequences	Positions
DPB1-Z38R1	Reward	CGA CGT CCC AGT GCC <u>GGA</u>	341
DPB1-Z38R2	Reward	GAG CCG CGA CGT CCC AGT <u>G</u>	346
DPB1-Z39F1	Forward	CCC CGC AGA GAA TTA <u>CC</u>	109
DPB1-Z39F2	Forward	GC AGA GAA TTA CCT <u>TTI</u>	113
DPB1-Z62R	Reward	AGC ATC AAC ACA GAC <u>GTG</u>	315
DPB1-Z64R	Reward	ACA AGG TCA TGA GGC <u>GTG</u>	251
DPB1-Z65R	Reward	CCG ACA AGG TCA TGA <u>GGG</u>	294

Group-specific sequencing primers used to resolve alternative genotypes, the primers based on ambiguous genotypes obtained

HLA-DPB1 mRNA level measurement

RNA were prepared from cell suspensions of each sample freshly isolated peripheral blood mononuclear cell (PBMC) using a TRIzol method (Invitrogen), the RNA extraction and cDNA preparation were also consistent with previous reported [19, 20].

The expression of HLA-DPB1 were quantified by SYBR green quantitative PCR (qPCR) using the threshold cycle (CT) method in CFX96 Touch PCR machine (Bio-Rad) with primers Forward: 5'-GTGCATTGCAGAAGGTCAGA-3' Reverse: 5'-CTGGTGATAGGCCATCAGGT-3'. Each PCR were triplicate including 12.5ul of FastStart Universal SYBR Green Master (Roche), 200nM primers, and 2.5ul of cDNA in a total volume of 25ul, qPCR protocol was using the recommended by Roche specification. The specificity of the DPB1 primers were confirmed by melt curve analysis using the dissociation step with single-peaked. All reactions were standardized to the reference gene GAPDH expression.

Linkage of HLA-DPB1 and SNPs

Genotype frequency was tested for Hardy–Weinberg equilibrium using Arlequin v 3.5.1.2. D' was calculated as described by Paximzadis et al [21]. The maximum-likelihood (ML) method was used to estimate the LD, an Expectation-Maximization (EM) algorithm for multi-locus genotypic data were used by the observed data with an unknown gametic phase. ELB algorithm was used to estimate the gametic phase of the genotype data generated at all the polymorphic positions within the gene region when the recessive alleles are present. The statistical significance of the linkage disequilibrium between each of the allele pairs was evaluated by the approximate X^2 reported by previously [19]. All statistical significance was defined as $P < 0.05$.

Meta-analysis study

In order to improve the influence of the HLA-DPB1 on the susceptible and spontaneous clearance of HBV infection, we performed a meta-analysis of them. Relevant studies were searched and identified by a computerized literature search of electronic databases, including Pubmed, EBSCO, Elsevier, and Web of Science, with English only as the language restriction.

The following index terms were used: 'Hepatitis B' and 'HLA-DP'. The inclusion criteria were: (a) genotype frequencies of HLA-DPB1 can be obtained in healthy controls, HBV carriers, chronic HBV infections and HBV spontaneous clearance; (b) the study included specific criteria for enrolling the samples; (c) the study had a case–control design; and d) the numbers of cases and controls, and the alleles frequencies were clear stated. Studies that did not meet these criteria were excluded.

Statistics analysis

The Hardy–Weinberg equilibrium (HWE) of the genotype distributions and the linkage disequilibrium (LD) of the SNPs were examined using Arliquin 3.5 software, the differences for categorical variables and continuous variables were compared using the χ^2 test and Student's t test, respectively. We used a logistic regression model to calculate the age- and gender-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) between the HLA-DPB1 variants and the risk of HBV infection. The meta-analysis was performed by Review Manager 5.2 software, the odd ratios (OR) with 95% confidence intervals (CI) were used to assess the strength of HLA-DPB1 polymorphisms in HBV susceptibility. Values of P lower than 0.05 were considered statistically significant.

Results

Relationship between HLA-DPB1 and rs9277535A/G in HBV infection

The carriage of the HLA-DPB1 rs9277535A (0.52, 95%CI: 0.41–0.65) indicated a stronger protective factors with HBV infection, while rs9277535G (1.94, 95%CI: 1.54–2.46) was susceptible to HBV infection (Table 2).

Table 2
Distribution of rs9277535 alleles in HBV and HC groups

	N	AA	AG	GG	A	G
HBV group	259	19 (7.3%)	107 (41.3%)	133 (51.4%)	0.28	0.72
HC	441	77 (17.5%)	213 (48.3%)	153 (34.7%)	0.42	0.58
HBV versus HC		AA versus GG + AG	GG + AG versus AA	A versus G	G versus A	
P value		0.001 ^a	0.001 ^a	0.001 ^a	0.001 ^a	
Risk ratio		0.37	1.99	0.52	1.94	
95% CI		(0.22–0.63)	(1.45–2.72)	(0.41–0.65)	(1.54–2.46)	
^a P < 0.01 (after Bonferroni correction), HBV: hepatitis B virus, HC: healthy control,						
CI: confidence interval.						

HLA-DPB1 shows highly polymorphic, the most prevalence allele in our populations were HLA-DPB1*05:01, which (1.41, 95%CI: 1.14–1.76) was significantly associated with HBV susceptibility in Sichuan Han population. Both HLA-DPB1*04:02 (0.31, 95%CI: 0.16–0.59) were significantly associated with HBV protection in our study population (Table 3).

Table 3
Distribution of the HLA-DPB1 alleles in different groups.

DPB1*	HC	F(%)	HBV carriers	F(%)	P (HBV VS.HC)	Risk ratio 95% CI
	N = 441		N = 259			
01:01	8	0.9	2	0.4	0.22	0.42(0.09-2.00)
02:01	141	16.0	73	14.0	0.19	0.86(0.64–1.17)
02:02	62	7.0	30	5.8	0.22	0.81(0.52–1.28)
03:01	36	4.1	20	3.9	0.48	0.94(0.54–1.65)
04:01	67	7.6	31	6.0	0.15	0.77(0.50–1.20)
04:02	40	4.5	13	2.5	0.001 ^a	0.31(0.16–0.59)
04:03	1	0.1	—	—	—	—
05:01	362	41.0	257	50	0.001 ^a	1.41(1.14–1.76)
09:01	5	0.6	5	1.0	0.29	1.71(0.49–5.93)
13:01	65	7.4	31	6.0	0.19	0.80(0.51–1.25)
14:01	24	2.7	12	2.3	0.39	0.85(0.42–1.71)
15:01	1	0.1	1	0.2	—	—
16:01	1	0.1	1	0.2	—	—
17:01	20	2.3	14	2.7	0.37	1.20 (0.60–2.39)
19:01	3	0.3	4	0.8	0.23	2.28(0.51–10.23)
21:01	28	3.2	21	4.1	0.24	1.29(0.72–2.29)
26:01	1	0.1	—	—	—	—
28:01	6	0.7	—	—	—	—
31:01	2	0.2	—	—	—	—
35:01	—	—	1	0.2	—	—
36:01	2	0.2	1	0.2	—	—
38:01	3	0.3	—	—	—	—

^a $P < 0.01$ (after Bonferroni correction), HBV: hepatitis B virus, HC: healthy control,
CI: confidence interval.

DPB1*	HC	F(%)	HBV carriers	F(%)	<i>P</i> (HBV VS.HC)	Risk ratio 95% CI
41:01	1	0.1	—	—	—	—
45:01	1	0.1	—	—	—	—
51:01	—	—	1	0.2	—	—
57:01	1	0.1	—	—	—	—
59:01	1	0.1	—	—	—	—
^a <i>P</i> < 0.01 (after Bonferroni correction), HBV: hepatitis B virus, HC: healthy control, CI: confidence interval.						

Table 4
Linkage disequilibrium between the HLA-DPB1 alleles and rs9277535 in different groups.

HLA-DPB1	HC				HBV carriers			
	Rs9277535	F(%)	D'	<i>P</i>	Rs9277535	F(%)	D'	<i>P</i>
*02:01	A	16.0	0.95	< 0.001	A	11.4	0.73	< 0.001
*02:02	A	7.0	0.	< 0.001	A	4.4	0.68	< 0.001
*03:01	G	4.1	1	< 0.001	G	3.7	0.82	0.02
*04:01	A	7.6	0.95	< 0.001	A	5.0	0.78	< 0.001
*04:02	A	4.4	0.99	< 0.001	A	1.9	0.68	< 0.001
*05:01	G	40.4	0.95	< 0.001	G	48.5	0.92	< 0.001
*13:01	G	7.3	0.99	< 0.001	G	5.2	0.87	< 0.001
*14:01	G	24	1	< 0.001	G	2.1	0.78	NS
*17:01	A	2.3	1	< 0.001	A	1.9	0.6	< 0.001
*21:01	G	3.1	0.99	< 0.001	G	4.1	1	< 0.001
HBV: hepatitis B virus, HC: healthy control.								

Table 5
Characteristics of the studies regarding hepatitis B virus infection susceptibility.

First author	year	Ethnicity	Total		Genotype method	Refs
			Case	Control		
Donaldson PT	2001	Hong Kong	121	123	PCR-SSO	[5]
Kamatani Y	2009	Japanese	607	934	PCR-SBT	[4]
Nishida N	2014	Japanese	488	464	PCR-SSO	[11]
Nishida N	2014	Korean	251	140	PCR-SSO	[11]
Nishida N	2014	Hong Kong	280	156	PCR-SSO	[11]
Nishida N	2014	Thai	369	122	PCR-SSO	[11]
Nishida N	2015	Japanese	1357	1225	PCR-SSO	[6]
Nishida N	2016	Japanese	2278	805	Array assay	[9]
Zhu M	2016	Chinese Han	951	937	Array assay	[22]

Table 6
Overall results regarding HLA-DPB1 polymorphisms in hepatitis B virus-susceptible patients.

HLA-DPB1*	N	Heterogeneity			Overall relationship	
		I ² ,%	P	Model	OR (95% CI)	P value
02:01	9	14	0.32	F	0.73 (0.68–0.78)	< 0.00001
02:02	9	0	0.79	F	0.81 (0.71–0.93)	0.002
03:01	9	23	0.24	F	1.05 (0.92–1.19)	0.49
04:01	9	58	0.01	R	0.50 (0.40–0.63)	< 0.00001
04:02	8	61	0.01	R	0.49 (0.39–0.61)	< 0.00001
05:01	9	22	0.24	F	1.39 (1.32–1.47)	< 0.00001
09:01	9	58	0.01	R	1.55 (1.30–1.86)	< 0.00001
13:01	9	40	0.40	F	1.26 (1.08–1.47)	0.003
14:01	9	0	0.67	F	0.97 (0.79–1.20)	0.80
17:01	5	0	0.80	F	1.15 (0.88–1.51)	0.31

Table 7
Meta-analysis of HLA-DPB1 polymorphisms and hepatitis B virus clearance.

First author	year	Ethnicity	Total		Genotype method	Refs
			Case	Control		
Cho SW	2008	Koreans	80	384	PCR-SBT	[7]
Thomas R	2012	Americans	421	241	PCR-SBT	[13]
Nishida N	2014	Japanese	570	488	PCR-SSO	[11]
Nishida N	2014	Korean	106	251	PCR-SSO	[11]
Nishida N	2014	Hong Kong	84	280	PCR-SSO	[11]
Nishida N	2014	Thai	109	369	PCR-SSO	[11]
Katrinli S	2017	Turkey	85	94	PCR-RFLP	[8]

Table 8
Meta-analysis of HLA-DQB1 alleles associated with hepatitis B virus spontaneous clearance

HLA-DPB1*	N	Heterogeneity		Overall relationship		
		$I^2, \%$	P	Model	OR (95% CI)	Pvalue
02:01	6	10	0.35	F	1.30 (1.15–1.46)	< 0.0001
02:02	5	6	0.37	F	1.22 (0.94–1.59)	0.14
03:01	6	36	0.23	F	1.17 (0.91–1.52)	0.23
04:01	7	62	0.01	R	1.56 (1.16–2.11)	0.004
04:02	6	78	0.0004	R	1.77 (0.98–3.02)	0.06
05:01	5	75	0.003	R	0.78 (0.66–0.92)	0.004
09:01	5	59	0.04	F	1.01 (0.58–1.77)	0.97
13:01	6	35	0.16	F	0.65 (0.51–0.84)	0.0008
14:01	6	0	0.87	F	0.68(0.43–1.07)	0.09
17:01	5	0	0.68	F	1.28 (0.79–2.08)	0.32

After meta-analysis, we found that the DPB1*02:01, DPB1*02:02, DPB1*04:01 and DPB1*04:02 were paly protect role to HBV infection, however, DPB1*05:01, DPB1*09:01 and DPB1*13:01 associated with HBV susceptible. DPB1*02:01, DPB1*04:01 and DPB1*04:02 were associated with HBV spontaneous clearance in Asian population, while DPB1*05:01 and DPB1*13:01 were associated with chronic HBV infection,

The expression of DPB1 mRNA levels in HBV infection

The HLA-DPB1 mRNA expression was significantly lower in the rs9277535AA genotype compared than the rs9277534GG genotype. The HLA-DPB1*05:01 and DPB1*21:01 in HBV group showed significantly lower HLA-DPB1 mRNA expression levels compared to the healthy control groups. As shown in table

The linkage disequilibrium between rs9277535A/G and HLA-DPB1 alleles

The LD test of HLA-DPB1 and rs9277535A/G indicated that the association between DPB1 alleles with different rs9277535A/G that make up the haplotypes. The HLA-DPB1*04:01 and 04:02 had strong linkage with rs9277535A, which play protective role in HBV infection, the HLA-DPB1*05:01 had strong linkage with rs9277535G, which susceptible to HBV infection.

Discussion

China is a relatively high endemic area of HBV infection, there are many HBV carriers, chronic hepatitis patients, cirrhosis and hepatocellular carcinoma patients caused by HBV infection, which cause heavy social burden^[22]. After the introduction of the HBV vaccine into the immunization program in China in 1992, the government administered free HBV vaccine to newborns to reduce the spread of HBV. At the same time, China has adopted a variety of methods to prevent and control HBV, strengthen the standardized management of HBV screening and medical treatment for blood donors, and effectively control iatrogenic HBV infection. All the above measures have significantly reduced the infection rate of new HBV infections, and the total HBV prevalence rate in China has dropped to 5.49% by 2015^[1]. Therefore, it is of great significance to study the infection mechanism of hepatitis B for the prevention and treatment of hepatitis B.

HLA-II genes play a key role in viral antigen presentation to mediate cellular and humoral immune responses. Since Kamatani and his colleagues^[4] did GWAS in large HBV infection cohort shown that HLA-DPB1 and related single nucleotide polymorphism rs9277535A/G are a strongly risk factors for persistent infection with hepatitis B virus. Thereafter, many researches focused on and successfully repeated the association between HLA-DPB1 and HBV infection and infection outcomes. they found^[11] that HLA-DPB1 *09:01 increased HBV susceptibility, DPB1*02:01 was not susceptible to HBV, and DPB1*02:01 was also associated with reduced risk of HBV progression to chronic HBV. Studies^[13] in American populations also found that the allele with the greatest risk of infection for HBV was DPB1*01:01, and the allele with the greatest protection against HBV infection was DPB1*04:01. Subsequently, the genotypes of DPB1 * 05:01, 09:01, and DPB1 * 02:01, 04:01, and 04:02 were again found to be susceptible to HBV in Japanese^[9] and Chinese^[10] populations. The studies also found that some DPB1 alleles and rs9277535G related to weak HBV vaccines response^[23], low sensitive of HBV drug

therapy^[15], incidence of occult HBV infection^[24], and also prone to hepatocellular carcinoma development^[25]. In our study, we found the strongly association between rs9277535 and HLA-DPB1 alleles and HBV infection susceptibility. We found the rs9277535A, HLA-DPB1*04:02 were protective factors for chronic HBV infection in this study population, the results were accordingly to previously. rs9277535G and HLA-DPB1*05:01 were significantly associated with HBV susceptibility in Sichuan Han population. Evidence reported here in suggests that HLA-DP have key role in HBV infection progression.

The influence of the HLA-DPB1 region on HBV recovery is due to levels of HLA-DPB1 expression and less likely to differences in the peptides presented by different HLA-DPB1 alleles. The HLA-DPB1 expression were significantly different rs9277535AA and rs9277535GG, the protect genotype 9277535AA has significantly lower HLA-DPB1 mRNA expression level compared than the risk genotype rs9277535GG in healthy controls, Thomas^[13] and his colleagues also demonstrated that rs9277534GG genotype, which confers susceptibility to HBV persistence with significantly higher levels of HLA-DP surface protein and transcript level expression in healthy donors, rs9277534GG have lower levels of HLA-DPB1 mRNA in HBV group, O'Brien^[12] had reported that rs9277535AA with higher HLA-DPB1 mRNA expression in liver associated with lower HBV odds ratios with chronic HBV, our study also get a similar result with HLA-DPB1 expression in PBMC. The HLA-DPB1*05:01 and DPB1*21:01 in HBV group showed significantly lower HLA-DPB1 mRNA expression levels compared to the healthy control groups. Previous research^[7] observed that decreased expression levels of DPA1 and DPB1 mRNA were correlated with the HBV activation after 2 years of treatment with HBV drugs, the high expression of DPA1 mRNA was associated with lower HBV viral load^[21]. These results suggesting that the persistence HBV infection may influenced by differences HLA-DPB1 expression, lower HLA-DPB1 expression may lower the function of antigen peptide present, at last, decrease the immune response to HBV viral, resulting HBV persistence infection risks.

Studies also found^[10] that amino acids at position 84–87 of the second exon antigen presentation sequence of DPB1 had a complete linkage with the DPB1 alleles, and there were two major amino acids at position 84–87 with GGPA and DENA, through bioinformatics analysis found that the four amino acids is located at the groove contacting peptide residues pocket-1, can be caused different antigen presenting functions, at last, caused different immunity function to HBV infection. Class II HLA molecules expression on the surface of antigen-presenting cells, to combine with antigenic peptide to CD4 + T helper cells. The T cell paly a crucial role in HBV response in host immune response.

Usually, HLA class II molecules present exogenous antigen peptide, However, HLA-DP molecules with beta-chains encoding DPGly84^[26] does not bind invariant chain (Ii) via the class II-associated invariant chain peptide (CLIP) region to constitutively present endogenous peptides. And processed by the proteasome and transported to the ER by the transporter associated with antigen processing (TAP). Therefore, DP84Gly can uniquely uses both class I and II antigen processing pathways to present peptides derived from intracellular and extracellular sources, DP84Asp has not such endogenous antigen

presentation function. Therefore^[27], this polymorphism has different functions in autoimmune, antiviral and tumor mechanisms through the different function of antigen presentation .

The DP84Gly genotype not only plays a unique antiviral function in adaptive immunity, but also as a ligand of NKp44^[17] to activate NKT cells to play an antiviral role in natural immune function. Compared with healthy controls, NK cells in CHB patients show inhibitory phenotypes, in which the expression of activated receptors NKp44 and NKp46 are down-regulated^[28]. Whether the DP84Gly genotype can promote the spontaneous clearance of HBV by binding NKp44 to activated NK after HBV infection has not been studied.

HLA-DPB1 expression associated with HBV infection was not verified in Chinese population, to the best of our knowledge, this is the first research to investigate the HLA-DPB1 mRNA expression in different HLA-DPB1 alleles and SNPs alleles, and the association between mRNA expression and HBV infection. Further research should be clearly to verified the role of NK cells and CD4 + T cells in DP84Gly including DPB1*04:01, DPB1*04:02 DPB1*02:01 and DPB1*02:02 in HBV protection and clearance.

Conclusion

HLA-DPB1 gene have a major effect on the risk of HBV infection, Rs9277535A, HLA-DPB1*02:01, DPB1*02:02, DPB1*04:01 and DPB1*04:02 protected against HBV infection, while Rs9277535G, HLA-DPB1*05:01, DPB1*09:01, and DPB1*13:01 prone to susceptible to HBV infection. HLA-DPB1*02:01 and DPB1*04:01 were prone to HBV spontaneous clearance, while HLA-DPB1*05:01 and DPB1*13:01 were associated with chronic HBV infection. Rs9277535GG have significantly higher HLA-DPB1 mRNA expression in HBV group compared that in HC group. Alleles with susceptible to HBV infection seems to decrease HLA-DPB1 mRNA expression.

Abbreviations

HBV: hepatitis B virus

HLA: human leukocyte antigens

APCs: antigen-presenting cells

CHB: chronic HBV carriers

HC: healthy controls

HBsAg: hepatitis B surface antigen

HBsAb: Hepatitis B surface antibody

HBeAg: Hepatitis Be antigen

HBeAb: Hepatitis Be antibody

HBcAb: Hepatitis core antibody

HCV: Hepatitis C virus

HIV: Human immunodeficiency virus

HWE: Hardy–Weinberg equilibrium

Declarations

Acknowledgments

The authors thank the participants for generously providing the venous blood samples.

Authors' contributions

Ou GJ, Xu HX, and Ji X performed the experiments. Liu X collected and evaluated the samples. Ou GJ and Liu XJ wrote the original draft of the manuscript. Wang J and Liu XJ Wang J and Liu XJ designed the experiments and performed the data analysis, discussed the results, and substantially revised the manuscript.

Competing interests

The authors declare that they have no competing interests.

Data availability

All the data and materials supporting the conclusions were included in the main paper.

Consent for publication

Not applicable.

Funding

This work was supported by the CAMS Innovation Fund for Medical Sciences[CIFMS] under contract 2016-I2M-3-024, Funding of Sichuan Science and Technology Department under contract 2017RZ0047 and Ministry of Science and Technology of China, Grant/Award number: 2014EG150133. The CIFMS

program provided us blood samples and relevant data, funding of Sichuan Science and Technology Department project provide us the cytokine detect methods and methods of HLA-DPB1 typing, program of Ministry of Science and Technology of China provide us HBV relevant detect methods.

Ethics approval and consent to participate

This study was approved by the ethic committees of the Institution of Blood Transfusion—CAMS&PUMC, and was conducted according to the principles of the Declaration of Helsinki. All participants provided written informed consent before enrolment, and the study's protocol was approved by the ethic committees of the Institution of Blood Transfusion, CAMS&PUMC.

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Figures

Figure 1

HLA-DPB1 mRNA expression in rs9277535 alleles

Figure 2

HLA-DPB1 mRNA expression levels of rs9277535 alleles in the hepatitis B virus-infected (HBV) and healthy control (HC) groups.

Figure 3

HLA-DPB1 mRNA expression levels in the hepatitis B virus-infected (HBV) and healthy control (HC) groups.

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