

Association of human leukocyte antigen DQB1 and DRB1 alleles with chronic hepatitis B

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Supported by Internal research funds of Istanbul Technical University, No. 36403

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Received: December 12, 2013 Revised: February 17, 2014

Accepted: April 1, 2014

Published online: July 7, 2014

Abstract

AIM: To investigate the effect of human leukocyte antigen (HLA) DRB1 and DQB1 alleles on the inactive and advanced stages of chronic hepatitis B.

METHODS: Patient records at a single institution's hepatology clinic were reviewed. Demographic data, laboratory results, endoscopy results, virological parameters, biopsy scores and treatment statuses were recorded. In total, 355 patients were eligible for the

study, of whom 226 (63.7%) were male. Overall, 82 (23.1%) were hepatitis B early antigen (HBeAg) positive, 87 (24.5%) had cirrhosis, and 66 (18.6%) had inactive disease. The presence of DQB1 and DRB1 alleles was determined by polymerase chain reaction with sequence-specific primers. The distribution of the genotyped alleles among patients with cirrhosis and patients with chronic active hepatitis was analyzed.

RESULTS: The most frequent HLA DQB1 allele was *DQB1*03:01* (48.2%), and the most frequent HLA DRB1 allele was *DRB1*13/14* (51.8%). *DQB1*05:01* was more frequent in patients with active disease than in inactive patients (27% vs 9.1%; $P = 0.002$, $P_c = 0.026$). *DRB1*07* was rare in patients with cirrhosis compared with non-cirrhotics (3.4% vs 16%; $P = 0.002$, $P_c = 0.022$). Older age ($P < 0.001$) and male gender ($P = 0.008$) were the other factors that affected the presence of cirrhosis. In a multivariate logistic regression analysis, *DRB1*07* remained a significant negative predictor of cirrhosis ($P = 0.015$). A bioinformatics analysis revealed that a polymorphic amino acid sequence in *DRB1*07* may alter interaction with the T-cell recognition site.

CONCLUSION: This study demonstrates that HLA alleles may influence cirrhosis development and disease activity in Turkish chronic hepatitis B patients.

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Key words: Chronic active hepatitis; Cirrhosis; Hepatitis B; Human leukocyte antigen DQ; Human leukocyte antigen DR

Core tip: Chronic hepatitis B is a major health problem worldwide. Recent genome-wide association studies revealed a significant association between the human leukocyte antigen (HLA) class II region and chronic hepatitis B. In the present study, we genotyped HLA

DQB1 and DRB1 alleles in 355 chronic hepatitis B patients and investigated the effects of the HLA alleles on disease activity and cirrhosis development. We found that *DQB1*05:01* was a risk factor for chronic active hepatitis and that *DRB1*07* was a protective factor against cirrhosis. A bioinformatics analysis revealed that *DRB1*07* might be associated with a hypimmune response.

Doganay L, Fejzullahu A, Katrinli S, Yilmaz Enc F, Ozturk O, Colak Y, Ulasoglu C, Tuncer I, Dinler Doganay G. Association of human leukocyte antigen DQB1 and DRB1 alleles with chronic hepatitis B. *World J Gastroenterol* 2014; 20(25): 8179-8186 Available from: URL: <http://www.wjgnet.com/1007-9327/full/v20/i25/8179.htm> DOI: <http://dx.doi.org/10.3748/wjg.v20.i25.8179>

INTRODUCTION

Hepatitis B virus (HBV) is observed all around the world, and nearly 350 million people have chronic HBV infections^[1]. The estimated number of annual deaths due to the consequences of HBV infection is nearly 600000^[2]. The virus itself is non-cytopathic, and liver damage during chronic infection is due to the host immune reaction against the virus. Several host immune mechanisms have been proposed to be involved in viral persistence, beyond viral factors^[3]. Human leukocyte antigen (HLA)-restricted T lymphocytes, B lymphocytes in the humoral immunity system, dendritic cells, natural killer cells and numerous cytokines are required to generate an accurate immunologic response against the virus^[4,5]. An inaccurate, nonselective cytolytic immune reaction to hepatocytes is believed to cause necroinflammation and further liver fibrosis, rather than eradicating the virus^[3].

In the majority of chronic hepatitis B patients, an inactive state, with low DNA levels (HBV DNA levels less than 2000 IU/mL) and normal liver enzyme levels, is observed. Nonetheless, nearly 30% of these inactive carriers (hepatitis B early antigen (HBeAg) negative, normal serum liver enzyme level and low serum HBV DNA level) will develop active disease, and nearly 10% will develop cirrhosis^[6,7]. To date, there is no defined test to predict which patient will remain in the inactive state without treatment and which patient will progress to chronic active hepatitis, which may culminate in cirrhosis unless specific antiviral treatment is introduced. It should be noted that when cirrhosis develops, the expected five-year survival decreases to 50% in untreated hepatitis B patients^[8]. Additionally, for cirrhotics, the annual risk of developing hepatocellular carcinoma is 5 times higher than for non-cirrhotics^[9]. Furthermore, in general, cirrhosis is the most common non-neoplastic cause of death related to the digestive system in the United States^[10].

Studies investigating the effect of HLA polymorphism on the disease state of different populations with HBV

infection have been performed. Studies in the Chinese population showed that *HLA DRB1*03:01*, *DQB1*03:01*, *DQB1*03:03*, *DQB1*05:03*, *DRB1*06*, *DRB1*08* and *DRB1*16* are associated with viral persistence^[11-13]. In Taiwanese patients, *DRB1*12:02* was shown to be related to viral persistence, whereas the *DRB1*04:01* and *DRB1*07:01* alleles were related to viral clearance^[14]. In a study from the United States, the *DQB1*03:01* allele was related to viral persistence in Afro-Americans^[15]. A study from Turkey revealed that HLA DR7, DR13 and DQ3 alleles were related to susceptibility to chronic infection^[16].

Recent genome-wide association studies revealed an association between the HLA class II gene region (DR, DQ and DP) and HBV chronicity^[17-19]. Moreover, a recent study revealed the effect of DQB1 alleles on the treatment response to nucleoside/nucleotide analogs in chronic HBV infection^[20]. In this study, we aimed to investigate the association between HLA DQB1 and DRB1 alleles and the outcome of chronic HBV disease (inactive state, active disease or cirrhosis).

MATERIALS AND METHODS

Patients

Chronic hepatitis B patients who were followed up at a hepatology clinic at a single institution between August 2005 and August 2010 were included in this study. The local ethics committee approved the study. The patients' case notes were reviewed, and demographic data, laboratory results, endoscopy results, virological parameters, biopsy scores and treatment statuses were noted. There were 628 patients. Patients with delta virus co-infection, hepatitis C virus (HCV) or human immunodeficiency virus infection or liver disease rather than hepatitis B; patients receiving immunosuppressive treatment; and patients under age 18 were excluded. Patients with cirrhosis, patients with inactive disease and patients with active disease were identified according to the following definitions.

Cirrhosis: Cirrhosis was confirmed by biopsy unless portal hypertension (portal ascites or esophageal varices and thrombocytopenia) was present.

Chronic inactive disease: HBeAg-negative patients with persistently normal serum alanine transferase (ALT) levels (lower than 30 IU/mL) and persistently low HBV DNA levels (lower than 2000 IU/mL) for at least three years of follow-up were defined as inactive. A sonographic examination that revealed normal liver function, without any sign of portal hypertension, was mandatory. If the liver was biopsied, the fibrosis score had to be 0, and the hepatic activity index (HAI) had to be less than 4.

Chronic active disease (the patients being treated): The patients who fulfilled the American Association for the Study of Liver Diseases (AASLD) 2009 criteria and were receiving treatment were included^[21]. Any patient who received treatment without fulfilling the AASLD

Table 1 List of primers used in the study

DQB1 allele	Primers	PCR product	DRB1 allele	Primers	PCR product
DQB1*0501	5' 5' CGGAGCGCGTGC GGGG 3' 3' 5' GCTGTCCAGTACTCGGCAA 3'	128	DRB1*01	5' TTGTGGCAGCTTAAGTTTGAAT 3' 5' GCTGTCCAGTACTCGGCAT 3'	168
DQB1*0502	5' 5' TGCGGGGTGTGACCAGAC 3' 3' 5' TGTTCAGTACTCGGCGCT 3'	117	DRB1*15/16	5' TCCTGTGGCAGCCTAAGA G 3' 5' CGCTGCACTGTGAAGCTCTC 3'	310
DQB1*0503	5' 5' TGCGGGGTGTGACCAGAC 3' 3' 5' GCGGCGTACC GCCCGA 3'	87	DRB1*03	5' GTTCTTGGAGTACTCTAGGTC 3' 5' TGCAGTAGTTGTCCACCCG 3'	222
DQB1*0601	5' 5' GCCATGTGCTACTTCACCAAT 3' 3' 5' CACCGTGTCCAACCTCCGCT 3'	198	DRB1*04	5' GTTCTTGGAGCAGGTTAAACA 3' 5' CGCTGCACTGTGAAGCTCTC 3'	262
DQB1*0602	5' 5' CGTGCCTCTTGTGACCAGAT 3' 3' 5' GCTGTCCAGTACTCGGCAT 3'	121	DRB1*11	5' CACGTTTCTTGGAGTACTCTAC 3' 5' CTGGCTGTCCAGTACTCTC 3'	179
DQB1*0603	5' 5' GGAGCGCGTGCCTTGTGA 3' 3' 5' GCTGTCCAGTACTCGGCAT 3'	127	DRB1*12	5' AGTACTCTACGGGTGAGTGT 3' 5' CTGTCCAGGACTCGGCGA 3'	198
DQB1*0604	5' 5' CGTGTACCAGITTAAGGGCA 3' 3' 5' GCAGGATCCCGCGTACC 3'	254	DRB1*13/14	5' GTTCTTGGAGTACTCTAGGTC 3' 5' CGTAGTGTGTCTGCA(GA)TAGG 3'	234
DQB1*0201	5' 5' GTGCGTATTGTGAGCAGAAG 3' 3' 5' GCAAGTTCGTGCGGAGCT 3'	205	DRB1*07	5' CCTGTGGCAGGG AAGTATA 3' 5' CCCGTAGTTGTGTCTGCACAC 3'	232
DQB1*0201/0302	5' 5' GACGGAGCGGTGCGTCT 3' 3' 5' CTGTTCAGTACTCGGCGG 3'	129	DRB1*08	5' AGTACTCTACGGGTGAGTGT 3' 5' CCCGTATTGTGTCTGCAG 3'	227
DQB1*0301	5' 5' GACGGAGCGGTGCGTCT 3' 3' 5' AGTACTCGGCGTACGGCG 3'	122	DRB1*09	5' GTTCTTGAAGCAGGATAAGTTT 3' 5' CCCGTAGTTGTGTCTGCACAC 3'	236
DQB1*0302/0303	5' 5' GACGGAGCGGTGCGTCT 3' 3' 5' AGTACTCGGCGTACGGCG 3'	122	DRB1*10	5' CGGTGCTGGAAGACGGCG 3' 5' CTGCACGTGAAGCT CTCAC 3'	204
DQB1*0303	5' 5' GACGGAGCGGTGCGTCT 3' 3' 5' CTGTTCAGTACTCGGCGT 3'	129	DRB1-Exon2	5' TTCGTGTCACACAGCAGTTC 3' 5' GCGCTGCACTGTGAAGCTCTC 3'	295
DQB1*0401	5' 5' CACCAACGGACCGAGCT 3' 3' 5' GGTAGTTGTGTCTGCATACG 3'	200	Internal control (HGH)	5' CAGTGCCTTCCCAACCATTCCTTA 3' 5' ATCCACTACGGATTCTGTGTGTTTC 3'	439
DQB1*0402	5' 5' CACCAACGGACCGAGCG 3' 3' 5' GGTAGTTGTGTCTGCATACG 3'	200			

criteria was excluded from the study.

In total, 378 patients met the given criteria, among whom 23 did not give consent or did not show up for their appointment (for blood sample collection for HLA analysis). The remaining 355 patients were included and formed the study population. All patients were Caucasian; were born in Turkey; and, when asked, identified themselves to be of Turkish descent. Among the study patients, 63.7% ($n = 226$) were male, 23.1% ($n = 82$) were HBeAg positive, 24.5% ($n = 87$) had cirrhosis, and 18.6% ($n = 66$) had inactive disease. In all, 81.4% of the patients had active disease and had received at least one course of treatment. The mean age, mean log DNA level and mean ALT level at diagnosis were 37.2 ± 15.1 years, 5.4 ± 2.2 IU/mL and 91.8 ± 35 IU/mL, respectively. The mean follow-up time was 85.5 ± 11 mo. The liver histology scores of 194 patients were available. The mean HAI was 6.8 ± 3.2 , and the mean fibrosis score was 2.1 ± 1.8 .

HLA analysis

In total, 5 mL of blood was taken from patients and stored in EDTA tubes at -80°C until the day of DNA extraction. Genomic DNA was extracted from 1 mL of peripheral blood using an Invitrogen PureLink Genomic DNA purification kit (Grand Island, NY). The alleles of HLA DQB1 and DRB1 were detected by a polymerase chain reaction with sequence-specific primers (PCR-SSP)^[22]. DQB1 alleles were defined at high resolution, and DRB1 alleles were defined at low resolution. Primer

sequences and PCR product sizes are listed in Table 1. Internal positive-control primers were included in the reaction system to eradicate false negatives. The internal control was a fragment of human growth hormone gene 1 (chr 17), consisting of 439 bp. PCR was performed in a 25 μL reaction mixture containing 100 ng genomic DNA, 1 U Crimson Taq DNA Polymerase (New England Biolabs, Ipswich, MA), optimized Crimson Taq polymerase buffer (New England Biolabs, Ipswich, MA) at one-fifth of the total volume, 1 $\mu\text{mol/L}$ MgCl_2 (Fermentas, Glen Burnie, MD), 0.2 $\mu\text{mol/L}$ dNTPs (Fermentas, Glen Burnie, MD), 0.2 $\mu\text{mol/L}$ primers and 0.04 $\mu\text{mol/L}$ internal-control primers (C5 and C3). The following cycling conditions were employed: 95°C for 5 min; 30 cycles at 95°C for 30 s, 66°C for 30 s and 68°C for 1 min; and a single final extension at 68°C for 7 min. The PCR conditions were optimized to obtain a higher yield and greater fidelity. Following amplification, 10 μL of products stained with SYBR Green were loaded onto a 2% agarose gel in the presence of an O'Range GeneRuler Ultra Low Range marker (Fermentas, Glen Burnie, MD) and then identified under ultraviolet light after electrophoresis. The allelic type was determined according to the presence or absence of PCR products of the desired length.

Statistical analyses

Statistical analyses were performed to evaluate the difference between cirrhotic and non-cirrhotic patients and between patients with active and inactive disease. During

Table 2 DQB1 alleles among patients with active disease or inactive disease *n* (%)

DQB1 allele	Patients with active disease <i>n</i> = 289	Patients with inactive disease <i>n</i> = 66	χ^2	<i>P</i> value	OR	95%CI
1	*05:01	78 (27)	9.52	0.002 ^a	3.7	1.5-8.9
2	*05:02	27 (9.3)	0.1	0.75	0.9	0.4-2.1
3	*05:03	53 (18.3)	0.29	0.59	0.83	0.4-1.6
4	*06:01	40 (13.8)	0.81	0.37	0.7	0.4-1.5
5	*06:02	0	NA	NA	NA	NA
6	*06:03	0	NA	NA	NA	NA
7	*06:04	1 (0.3)	b	0.34	0.2	0.01-3.7
8	*02:01	2 (0.7)	b	0.46	0.5	0.04-5.1
9	*03:02	94 (32.5)	0.12	0.72	1.1	0.6-2
10	*03:01	135 (46.7)	1.32	0.25	0.7	0.4-1.3
11	*03:03	27 (9.3)	0.2	0.65	1.3	0.4-3.4
12	*04:02	7 (2.4)	b	0.36	NA	NA
13	*04:01	0	NA	NA	NA	NA

^a*P* < 0.01 (after Bonferroni correction). b: Fisher’s exact test was applied; NA: Not available.

Table 3 DRB1 alleles among patients with cirrhosis or without cirrhosis *n* (%)

DRB1 allele	Patients with cirrhosis <i>n</i> = 87	Patients without cirrhosis <i>n</i> = 268	χ^2	<i>P</i> value	OR	95%CI
1	*01	2 (2.3)	b	1	0.9	0.2-4.3
2	*15/16	17 (19.5)	0.07	0.79	0.9	0.5-1.7
3	*03	9 (10.3)	0.03	0.8	1.1	0.5-2.4
4	*04	2 (2.3)	b	0.6	2.1	0.3-12.7
5	*11	3(3.4)	b	0.5	1.5	0.4-6.4
6	*12	5 (5.7)	0.9	0.34	0.6	0.2-1.7
7	*13/14	45 (51.7)	0.01	0.98	1	0.6-1.6
8	*07	3 (3.4)	9.2	0.002 ^a	0.2	0.06-0.6
9	*08	4 (4.6)	b	0.2	2.5	0.7-9.7
10	*09	3 (3.4)	b	0.37	2.3	0.5-10.7
11	*10	4 (4.6)	b	0.77	1.1	0.4-3.6

^a*P* < 0.05 (after Bonferroni correction). b: Fisher’s exact test was applied; NA: Not available.

the HLA analysis, 2 × 2 tables and the chi square test were used. When the sample sizes were small or when the expected values in the cells of the chi square table were less than 5, Fisher’s exact test was used. Parametric variables were analyzed using Student’s *t* test. To analyze the effect of multiple variables on the presence of cirrhosis, multivariate logistic regression was applied.

All *P* values were double sided, and if the *P* value was below 0.05, it was considered as statistically significant unless there were multiple comparisons. Bonferroni correction was applied for multiple comparisons, and corrected *P* values are given. SPSS 20 (Chicago, IL) was used for the statistical analysis. Genotypic analysis was performed using Genepop Population Genetics Software Package Version 4.2^[23].

Sequence alignment analysis

The sequence alignment of HLA class II proteins was performed using ClustalW^[24].

RESULTS

HLA and active disease

The most frequent HLA DQB1 allele was *DQB1*03:01* (48.2%), and the most frequent HLA DRB1 allele was

*DRB1*13/14* (51.8%). In total, 66 patients had inactive hepatitis B, and 289 patients had chronic active hepatitis. The *DQB1*05:01* allele was significantly more frequent in patients with active disease compared with inactive patients (27% *vs* 9.1%; *P* = 0.002, *P_c* = 0.026). The other DQB1 alleles (Table 2) and DRB1 alleles did not have a significant association with inactive disease. In the multivariate logistic regression analysis, the *DQB1*05:01* allele had a significant association with chronic active hepatitis (Beta = 1.29, Wald = 7.5, Beta (exp) = 3.63, *P* = 0.006). This effect was independent of age, HBeAg status and gender.

HLA and cirrhosis

In this study, 87 patients had cirrhosis. Patients without cirrhosis carried *HLA DRB1*07* more frequently than did the cirrhotic patients (16% *vs* 3.4%; *P* = 0.002, *P_c* = 0.022). The other DRB1 alleles (Table 3) and all of the DQB1 alleles did not exhibit a statistically significant association with the presence of cirrhosis.

Gender influenced the presence of cirrhosis (28.3% in males *vs* 17.8% in females; *P* = 0.03), and cirrhotic patients were older (47.4 *vs* 33.9; *P* < 0.001). HBeAg-negative patients were more likely to have cirrhosis compared with HBeAg-positive patients (27.1% *vs* 15.9%; *P* = 0.04).

Table 4 Factors affecting the presence of cirrhosis (multivariate logistic regression analysis)

	Beta	SE	Wald	Exp (Beta)	P value	95%CI
HBeAg (positive)	-0.08	0.38	0.05	0.92	0.83	0.44-1.93
DRB1*07	-1.59	0.65	5.9	0.2	0.015	0.06-0.73
Age at diagnosis	0.07	0.01	39.9	1.07	< 0.001	1.05-1.09
Gender (male)	0.78	0.30	6.7	2.18	0.01	1.2-3.9

HBeAg: Hepatitis B early antigen.

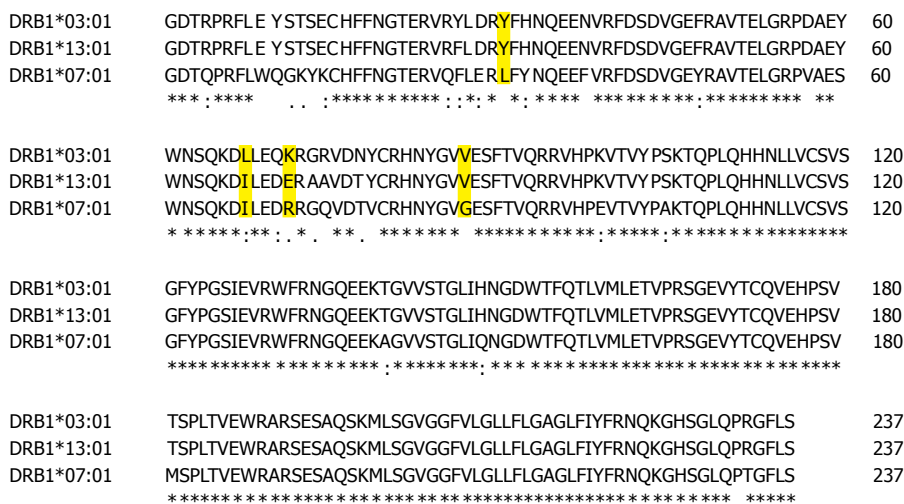


Figure 1 Sequence similarity analysis of the β subdomain of three different major histocompatibility complex class II proteins. An asterisks underneath a capital letter denotes a single, fully conserved residue. The semicolons and dots represent amino acids with conservation of strong groups or weak groups, respectively. For a mismatch between amino acids, a space is left underneath. The residues determined to be important for T-cell receptor recognition are shown with yellow shading.

Age, HBeAg status, gender and *DRB1*07* were analyzed in the multivariate logistic regression analysis. *DRB1*07* status remained independently related to the presence of cirrhosis ($P = 0.015$), in addition to age ($P < 0.001$) and gender ($P = 0.01$) (Table 4).

Genotypic analysis

The most frequent DQB1 genotypes were *DQB1*03:01* and *DQB1*03:02* ($n = 35, 9.9\%$), *DQB1*03:01* and *DQB1*05:01* ($n = 30, 8.5\%$) and *DQB1*06:01* and *DQB1*03:01* ($n = 20, 5.6\%$). The distribution of these DQB1 genotypes did not reveal a significant difference between cirrhotic patients and non-cirrhotics or between inactive patients and patients with chronic active hepatitis (data not shown).

The most frequent DRB1 genotypes were *DRB1*13/14* and *DRB1*15/16* ($n = 28, 7.9\%$) and *DRB1*03* and *DRB1*13/14* ($n = 14, 3.9\%$). These genotypes did not significantly correlate with the presence of cirrhosis or active disease (data not shown).

In silico analysis of DRB1*07

When the identified polymorphic sites were mapped to the amino acid sequence of HLA class II molecules, four substitutions were observed at the T-cell interaction site. These substitutions were located at $\beta 30, \beta 67, \beta 71$ and $\beta 86$, as obtained by ClustalW analysis (Figure 1).

DISCUSSION

In this study, we compared the allele distribution among chronic active hepatitis patients with the distribution among patients in the chronic inactive stage. We defined inactivity according to histology, persistently normal ALT levels and persistently low viremia. Chronic active hepatitis patients more frequently carried the *HLA DQB1*05:01* allele than did inactive patients. This allele has been found to be related to a better response to the HBV vaccine^[25] and to be a protective factor against chronic HBV infection^[17]. Previous data have associated *DQB1*05:01* with an appropriate immunologic response, but in the present study, *DQB1*05:01* was related to active disease, thus revealing an inappropriate immune response. This finding could be a reflection of genetic divergence between different populations or a consequence of the genotype of the virus; only the D genotype is present in Turkey^[26].

Another finding of this study was the more frequent presence of *HLA DRB1*07* in the non-cirrhotic group compared with the cirrhotics. Liver histology scores were better in patients with the *DRB1*07* allele, and the mean fibrosis score and HAI score of these patients were lower ($P = 0.001$ and $P = 0.008$, respectively). Chronic HBV infection is an immunologically driven disease, and HBV is a non-cytopathic virus. Host genetic factors and viral

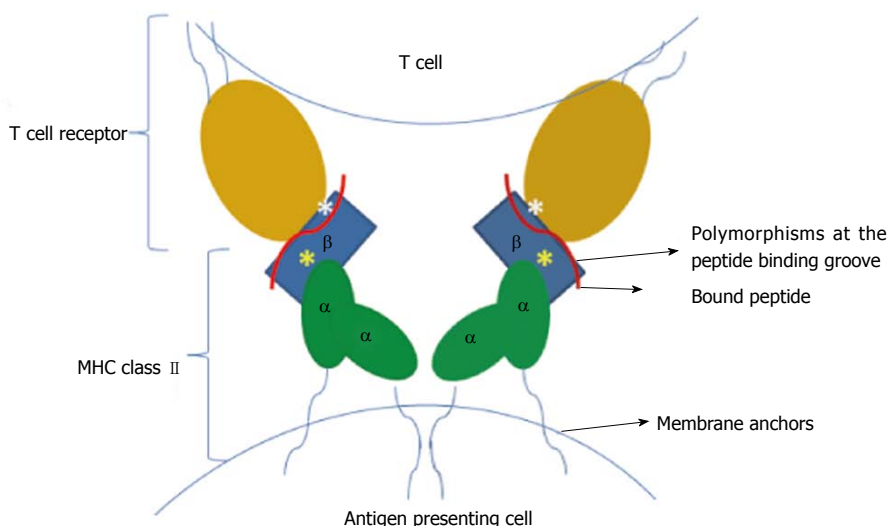


Figure 2 Possible model illustrating the single-nucleotide polymorphic regions of major histocompatibility complex class II that affect T-cell receptor recognition. The yellow asterisks show mutational sites that are buried in the peptide-binding pocket, whereas the white asterisks indicate mutations in the solvent-exposed T-cell recognition regions. This model was built according to the available crystal structures, which were published previously^[41].

and environmental factors have been proposed to determine disease progression or regression^[3]. Immune action against the virus involves HLA-restricted T lymphocytes, antibody-secreting B lymphocytes, natural killer cells and numerous cytokines. The immune recognition of viruses requires the presentation of viral antigens to CD4-positive and CD8-positive lymphocytes by HLA class II and class I molecules, after which viral clearance or persistence occurs^[27,28]. When an adequate vigorous polyclonal, multispecific response to HBV antigens is not produced, CD4-positive and CD8-positive T cells that are capable of terminating HBV infection do not accumulate in the liver^[4,5]. If HBV-specific CD8-positive and CD4-positive T cell responses are absent, the immune response then becomes nonselective and results in chronic necroinflammation and fibrosis. This long-lasting cytolytic and inappropriate immune response against infected hepatocytes is responsible for liver damage. Previous reports have suggested an association between cirrhosis and HLA DRB1; for instance, two studies from China have demonstrated an association between cirrhosis and *HLA DRB1*12*^[29,30]. Another study, from Brazil, revealed the protective effect of *HLA DRB1*11* against severe liver damage in HCV patients^[31]. Other studies and a recent meta-analysis implicated *DRB1*07* as a risk factor for persistent HBV infection^[16,32,33]. Studies from Turkey^[34], the United Kingdom^[35], Belgium^[25], Spain^[36], Germany^[37] and China^[38,39] revealed that the presence of *DRB1*07* (or DR7) causes unresponsiveness to the HBV vaccine. Furthermore, in a Turkish study exploring HLA alleles in renal transplant candidates, panel-reactive antibodies were less frequently detected in the patient group with the *DRB1*07* allele, despite the fact that this group had a higher predisposition to developing these antibodies^[40]. This finding indicates a hypoimmune profile associated with *DRB1*07*. In the current study, the *DRB1*07* allele was less frequent in cirrhotic patients

compared with non-cirrhotics. Together with previous reports, the results of the present study may indicate that patients with the *DRB1*07* allele are not able to mount a specific immune reaction to HBV and are predisposed to chronic infection. This hypoimmune response also yields less hepatocyte damage and less cirrhosis. The puzzling immune response observed in patients carrying the *DRB1*07* allele may be attributable to this allele's interaction with the T-cell recognition site. The available crystal structures of HLAs reveal that these proteins carry allele-specific motifs^[41]. The antigenic peptide specificity of HLA class II proteins comes from the determined polymorphic pockets in the peptide-binding cleft. T-cell receptor recognition is accomplished by both the peptide and HLAs^[41]. Certain HLA DRB1 types show polymorphisms specifically at the T-cell recognition site, without significantly altering peptide binding^[42,43]. Interestingly, our bioinformatics analysis revealed that *DRB1*07* contains four substitutions at the T-cell interaction site, which are located at $\beta 30$, $\beta 67$, $\beta 71$ and $\beta 86$, according to ClustalW analysis (Figure 1). Two of these residues, $\beta 67$ and $\beta 71$, have been determined to be important for peptide binding and T-cell receptor recognition in mutagenesis studies^[42,43]. These residues are solvent exposed and are predicted to be critical for direct T-cell receptor binding, so any alteration at these sites would change T-cell receptor recognition. Additionally, the buried residues $\beta 30$ and $\beta 86$ have been shown to be important for T-cell recognition in mutagenesis studies^[41,42]. Although these residues cannot have a direct effect on T-cell recognition, they might have an indirect conformational influence that can be communicated to the T-cell recognition site (Figure 2). Due to changes in the T-cell recognition residues in the *HLA DRB1*07* variant, the immune response can be weakened, resulting in chronic infection without cirrhosis.

This study has certain limitations. A larger sample size

could be used to identify further associations between HLA subgroups and disease outcomes. This is a cohort study, so it is not possible to conclude that there is a direct causative relationship between our findings and the disease state. Molecular studies are necessary to investigate HBV-specific antigenic peptides and the specific structure of the rearranged T-cell receptor. However, the well-defined patient groups, the long duration of the follow-up period and the medium- to high-resolution genotyping of HLA alleles are the strengths of this study.

In conclusion, the natural course of HBV disease is not easily predictable; viral factors, environmental factors and host factors may affect the disease course. This study suggests that the *HLA DQB1*05:01* allele is associated with chronic persistent disease and that the *DRB1*07* allele is a protective factor against cirrhosis.

ACKNOWLEDGMENTS

We are grateful to Asli Kumbasar for critically editing the manuscript.

COMMENTS

Background

In certain patients, chronic hepatitis B infection may culminate in cirrhosis, whereas other patients will remain in the inactive state. There is no single test for predicting patient outcomes.

Research frontiers

Recent genome-wide association studies revealed a relationship between human leukocyte antigen (HLA) class II and hepatitis B chronicity.

Innovations and breakthroughs

The authors genotyped HLA DQB1 and DRB1 alleles in their patient cohort. They revealed that the *HLA DQB1*05:01* allele is related to chronic persistent disease and that the *DRB1*07* allele is a protective factor against cirrhosis.

Applications

Host genetic factors are associated with hepatitis B outcomes. To understand and identify the exact mechanism, future molecular research is needed.

Terminology

The HLA system is the name given to the genes that encode the major histocompatibility complex (MHC) proteins. MHC proteins/antigens are essential for the immune system. MHC class II presents antigens to T-helper cells, which then stimulate B lymphocytes to commence specific antibody production.

Peer review

The authors present the results of a retrospective cohort study in patients with chronic hepatitis B, in which they evaluated the association between HLA DQB1 and DRB1 alleles and the stage of the disease. They identified an association between *DQB1*05:01* and active hepatitis B disease. Furthermore, *DRB1*07* was associated with the absence of cirrhosis. The topic of the study is very interesting.

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P- Reviewers: Gigi E, Gevers TJG

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ISSN 1007-9327

