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Effect of HLA-DPA1 alleles on chronic hepatitis B prognosis and treatment response

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ABSTRACT

OBJECTIVE: Chronic hepatitis B (CHB) is a major health problem. The outcome of hepatitis B virus (HBV) infection is associated with variations in HLA-DPA1 alleles. The aim of this study was to investigate possible associations of HLA-DPA1 alleles with treatment response and with hepatitis B virus e antigen (HBeAg) seroconversion.

METHODS: Eight different HLA-DPA1 alleles from 246 CHB patients were genotyped by polymerase chain reaction with sequence-specific primers at high resolution to investigate the association of HLA-DPA1 alleles with treatment response, development of cirrhosis, HBeAg seroconversion, and disease reoccurrence upon HBeAg loss.

RESULTS: There was no significant association between HLA-DPA1 alleles and treatment response, development of cirrhosis, or HBeAg seroconversion. However, HLA-DPA1*04:01 allele was significantly more frequently found in patients who redeveloped disease upon HBeAg seroconversion (100% vs 36.8%: p=0.037; Fisher's exact test).

CONCLUSION: HLA-DPA1*04:01 allele may be a risk factor for reoccurrence of CHB after HBeAg seroconversion.

Keywords: Chronic hepatitis B; cirrhosis; HBeAg seroconversion; HLA-DPA1; treatment response.

Chronic hepatitis B (CHB) is a major global health issue, and despite national vaccination programs, between 350 million and 400 million people are infected with hepatitis B virus (HBV) worldwide [1]. Chronic HBV infection results in liver fibrosis, which can further develop into cirrhosis or hepatocellular carcinoma, both of which are major causes of liver-related death [2]. Number of annual deaths due to consequences of HBV infection is estimated to be nearly 600000 worldwide [3]. In Turkey, 5% of population is hepatitis B surface antigen positive, and our country currently has medium endemicity profile for hepatitis B [4].

Development of chronic HBV infection is a complex process that involves various viral, environmental and genetic components, such as HBV genomic variability, host age, and sex, as well as concurrent infection with hepatitis C virus (HCV), hepatitis D

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virus, and human immunodeficiency virus (HIV) [5, 6, 7, 8]. Segregation analysis and twin studies also suggest involvement of host genetic profile in development of chronicity in HBV infection [9, 10]. Human leukocyte antigen (HLA) class II loci have been proposed as major genetic host sites associated with HBV infection [11, 12, 13, 14]. HLA-restricted T lymphocytes and B lymphocytes in the humoral immune system are responsible for the generation of an accurate immunological response against the virus [5, 6]. When this cytolytic immune reaction is inaccurate and nonselective in hepatocytes, instead of virus eradication, necroinflammation and liver fibrosis occur [15]. Genome-wide association studies (GWAS) have also demonstrated an association between HLA class II gene region and HBV chronicity [16, 17]. A recent GWAS study reported specific association between HLA-DP locus and CHB in Japanese and Thai populations [18]. In the present study, 8 HLA-DPA1 polymorphisms in the Turkish population were screened in high resolution to investigate association between HLA-DPA1 polymorphisms and HBV treatment response, cirrhosis, hepatitis B e antigen (HBeAg) seroconversion, and recurrence of disease after HBeAg seroconversion.

MATERIALS AND METHODS

Study group

The present study, which was approved by the local ethics committee, included 246 CHB patients who were followed-up at the hepatology clinic of Goztepe Teaching and Research Hospital between August 2005 and August 2010. Patient case notes were carefully reviewed, and demographic data, laboratory results, endoscopy results, virological parameters, biopsy scores, and treatment status details were analyzed. Patients with delta virus co-infection, HCV, HIV, liver disease other than HBV, patients receiving immunosuppressive treatment, and patients under age of 18 were not selected for the study. Demographic data of the patients is provided in Table 1.

TABLE 1. Demographic data of patients

	n±SD	(Min.–Max.)
Gender		
Male	152 (61.8%)	
Female	94 (38.2%)	
Age		
Log DNA (IU/mL)	5.56±2.25	(1.15–10.89)
ALT (U/L)	92.03±100.72	(8–681)
AST (U/L)	61.51±59.32	(14–433)
HBeAg		
Positive	56 (22.8%)	
Negative	190 (77.2%)	
Cirrhosis		
Presence	53 (21.5%)	
Absence	193 (78.5%)	

SD: Standard deviation; Min.: Minimum; Max.: Maximum; DNA: Deoxyribonucleic acid; ALT: Alanine transaminase; AST: Aspartate aminotransferase; HBeAg: Hepatitis B e antigen.

HLA-DPA1 genotyping

In total, 5 mL of peripheral blood was collected from each patient and stored in ethylenediaminetetraacetic acid tubes at -80°C until extraction of deoxyribonucleic acid (DNA). Genomic DNA was extracted from 1 mL of blood using Invitrogen PureLink Genomic DNA purification kit (Thermo Fischer Scientific, Inc., Waltham, MA, USA) according to manufacturer's instructions. Eight different HLA DPA1 alleles were screened by polymerase chain reaction with sequence-specific primers (PCR-SSP) at high resolution [19]. Primer sequences and PCR product lengths are listed in Table 2. Internal positive control primers were included in the reaction system to exclude false negatives. Internal control was 439 bp fragment of human growth hormone gene 1 (Forward primer:5'GCCTTCCCAACC ATTCCCTTA3', Reverse primer: 5'TCACG-GATTTATGTTGTGTTTC3'). PCR was performed in 25 µL reaction mixture containing 0.5 units of DreamTaq Green DNA Polymerase (Thermo Fisher Scientific, Inc., Waltham, MA, USA), 1 mM magnesium chloride, 0.8 mM deoxynucleotide triphosphates, 0.2 mM primers, and 0.05 mM internal control primers. PCR amplification was achieved with initial denaturation at 94°C for 2 minutes followed by 30 amplification cycles. First 10 cycles consisted of denaturation at 94°C for 10

Table 2. List of primers used in the study

DPA1 allele	Primer	PCR product
*01:03/03:01	F: 5′GGGAGTTTATGTTTGAATTTGATGAA3′ R: 5′AGATAGGGCGTTACCGTTGG3′	209 bp
*01:03/01:04	F: 5′ATGCCGCGTTTGTACAGACG3′ R: 5′AGATAGGGCGTTACCGTTGGT3′	242 bp
*01:04	F: 5′TCTCTACTGTCTTTATGCAGCGG3′ R: 5′GATCCACATAGAACATCTCATCG3′	119 bp
*02:01:01/02:01:02	F: 5′GACCATGTGTCAACTTATGCCGC3′ R: 5′CTTTTTATCCAGATCCACATAGAACTG3′	108 bp
*02:02:01/02:02:02	F: 5′GACCATGTGTCAACTTATGCCA3′ R: 5′CTTGTCCAGATCCACATAGAACTG3′	103 bp
*03:01	F: 5′GACCATGTGTCAACTTATGCCAT3′ R: 5′AGATAGGGCGTTACCGTTGGT3′	258 bp
*04:01	F: 5′GCGTTTGTACAGACGCATAGAA3′ R: 5′GTGGTTGGAACGCTGGATAGC3′	207 bp
*02:01:01	F: 5′ATGCCGCGTTTGTACAGACC3′ R: 5′AGATGCCAGACGGTCTCCTTT3′	109 bp
*02:01:02	F: 5′TATGCCGCGTTTGTACACACG3′ R: 5′AGATGCCAGACGGTCTCCTTT3′	110 bp
*02:02:01	F: 5′GACCATGTGTCAACTTATGCCAT3′ R: 5′TGCCAGACGGTCTCCTTCTTA3′	122 bp
*02:02:01/03:01	F: 5′GACCATGTGTCAACTTATGCCA3′ R: 5′GCCAGACGGTCTCCTTCTTG3′	121 bp

PCR: polymerase chain reaction.

seconds followed by combined annealing-extension step at 65°C for 60 seconds. Remaining 20 cycles consisted of denaturation at 94°C for 10 seconds, annealing at 61°C for 50 seconds, and extension at 72°C for 30 seconds. After amplification, 10 µL of PCR products were loaded onto 2% agarose gel and stained with SYBR Green I Nucleic Acid Gel Stain (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Upon visualization under ultraviolet light, DPA1 allele types were determined according to presence or absence of a PCR product of corresponding length.

Statistical analysis

Statistical analyses were performed to evaluate differences between groups: patients with and without good treatment response to standard antiviral drugs, patients with and without good treatment response to nucleotide analogs, patients with and without good treatment response to interferon, patients with and without cirrhosis, patients with and without HBeAg seroconversion, and patients with and without disease progression after HBeAg seroconversion. For these analyses, 2x2 contingency tables and chi-square test were used. If the sample size was small, Fisher's exact test was applied.

For all analyses, SPSS Statistics 21 (IBM Corp., Armonk, NY, USA) was used; double-sided p values were calculated, and $p<0.05$ was considered statistically significant.

RESULTS

CHB patients were grouped and analyzed according to their treatment response to standard antiviral drugs, nucleotide analogs or interferon, presence of

TABLE 3. Number of patients in each study group

Study group		Number of patients
Treatment response to all antivirals	Yes	93
	No	99
Treatment response to nucleotide analogs	Yes	71
	No	73
Treatment response to interferon	Yes	69
	No	14
Cirrhosis	Present	53
	Absent	193
HBeAg seroconversion	Present	23
	Absent	34
Recurrence of disease after HBeAg seroconversion	Present	11
	Absent	12

HBeAg: Hepatitis B e antigen.

TABLE 4. Frequency of DPA1 alleles among chronic hepatitis B patients (n=246)

DPA1 alleles	Frequency (n=246)	
	n	%
DPA1*01:03	114	46.3
DPA1*01:04	46	18.7
DPA1*03:01	190	77.2
DPA1*04:01	55	22.4
DPA1*02:01:01	27	11.0
DPA1*02:01:02	31	12.6
DPA1*02:02:01	10	4.1
DPA1*02:02:02	9	3.7

cirrhosis, HBeAg seroconversion, and recurrence of disease after HBeAg seroconversion. Number of patients in each group can be seen in Table 3 and frequency of all DPA1 alleles among all patients (n=246) is listed in Table 4.

No significant association was observed between HLA DPA1 alleles and patients with or without cirrhosis (Table 5). Neither was there significant association between HLA DPA1 alleles and patients with or without HBeAg seroconversion. In addition, HLA DPA1 alleles were not found to be associated with treatment response to standard antiviral drugs (Table 6), nucleotide analogs, or interferon. However, when recurrence of disease after seroconversion was analyzed, HLA DPA1*04:01 allele was significantly more frequently found in patient CHB group with recurrence vs patients with inactive disease (100% vs 36.8%; p=0.037; Fisher's exact test) (Table 7).

DISCUSSION

CHB treatment is a challenging issue. In addition to viral factors, several host immune factors participate in drug resistance, HBeAg seroconversion, and reoccurrence of the disease. Since HLA-DPA1 and -DPB1 are less polymorphic than HLA-DR or -DQ, and HLA-DP cell surface expression levels are also likely to be lower, HLA-DP region was not thought to have as much clinical impact as other HLA alleles [20, 21]. However, recent GWAS studies have shown that HLA-DP region is associated with both protection against CHB and viral clearance [16, 17, 18].

HLA-DPA1 molecules, which are HLA class II molecules, are responsible for antigen presentation

Table 5. Frequency of HLA DPA1 alleles in patients with and without cirrhosis

DPA1 alleles	Cirrhotic patient group (n=53)		Non-cirrhotic patient group (n=193)		p
	n	%	n	%	
DPA1*01:03	29	54.7	85	44.0	0.167
DPA1*01:04	9	17.0	37	19.2	0.717
DPA1*03:01	39	73.6	151	78.2	0.474
DPA1*04:01	7	13.2	48	24.9	0.071
DPA1*02:01:01	6	11.3	21	10.9	0.928
DPA1*02:01:02	6	11.3	25	13.0	0.751
DPA1*02:02:01	3	5.7	7	3.6	0.507
DPA1*02:02:02	3	5.7	6	3.1	0.381

Table 6. Distribution of HLA DPA1 alleles according to treatment response

DPA1 alleles	Patients with treatment response (n=99)		Patients without treatment response (n=93)		p
	n	%	n	%	
DPA1*01:03	49	49.5	40	43.0	0.368
DPA1*01:04	19	19.2	18	194	0.977
DPA1*03:01	72	72.7	73	78.5	0.353
DPA1*04:01	16	16.2	22	23.7	0.193
DPA1*02:01:01	13	13.1	11	11.8	0.785
DPA1*02:01:02	12	12.1	10	10.8	0.766
DPA1*02:02:01	6	6.1	3	3.2	0.353
DPA1*02:02:02	5	5.1	3	3.2	0.527

Table 7. Frequency of HLA DPA1*04:01 allele in hepatitis B e antigen seroconverted group with and without disease recurrence

	Patients with disease recurrence after HBeAg seroconversion		Patients with inactive disease after HBeAg seroconversion	
	n	%	n	%
HLA DPA1*04:01 carrier	4	100	0	0
HLA DPA1*04:01 non- carrier	7	36.8	12	63.2

HBeAg: Hepatitis B e antigen. p=0.037 according to Fisher's exact test.

to CD4+ T helper cells. Since T cell helper response is critical for HBV clearance, there is a direct link between HBV clearance and increased CD4+ T cells response [22, 23]. Antigen-binding sites of HLA-DP molecules, which play a crucial role in the physical binding of peptides and subsequent recognition

by T-cells, are highly polymorphic [24, 25]. Therefore, diversity of HLA-DPA1 alleles due to variations in the HLA-DP coding regions is a key factor in antigen presentation and hence, viral clearance.

Recent studies demonstrated that HLA-DPA1 SNPs rs3077 and rs9277378, which are located in the 3'UTR and 2Kb upstream intronic site, respectively, are associated with spontaneously resolved HBV infection [26, 27] and chronicity of HBV [28, 29]. In this study, no significant association between HLA-DPA1 alleles and HBeAg seroconversion was found. However, we observed higher frequency of HLA-DPA1*04:01 allele in patients with active disease vs inactive disease after HBeAg seroconversion (100% vs 36.8%; p=0.037; Fisher's exact test), thus showing an association between HLA-DPA1 and recurrence of disease after HBeAg loss. Lack of association between HLA-DPA1 alleles and HBeAg seroconversion has also been stated in the literature [30]. However, HLA-DPA1 alleles were shown to be associated with HBeAg loss, anti-HBe seroconversion, and HBV DNA level suppression in HBeAg seropositive CHB patients upon interferon alpha or pegylated interferon treatment [31]. In conclusion, presence of HLA-DPA1*04:01 allele may be a risk factor for recurrence of the disease upon HBeAg seroconversion, and thus may be a potential biomarker to predict patients in need of prolonged antiviral therapy. Nonetheless, a larger cohort is required to validate this finding.

Conflict of Interest: None declared.

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