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HLA Variations and Association with Complicated Chronic Hepatitis B Virus Infection: A Prospective Cohort Study

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Abstract

Background: Human leukocyte antigen (HLA) variations are associated with variable hepatitis B virus (HBV) infection course, including viral clearance or progression to complicated disease (cirrhosis or hepatocellular carcinoma [HCC]).

Methods: We conducted a single-center prospective study including patients with either spontaneous clearance of hepatitis B surface antigen (HBsAg) (SC group) or progression to complicated HBV infection (ccHBV group). HLA alleles of patients with HBV clearance or complicated HBV infection, as well as that of healthy controls, were genotyped in low- and high-resolution analyses by single-specific primer polymerase chain reaction to identify genetic loci associated with variable disease course.

Results: Our analysis included 101 individuals in the SC group and 24 individuals in the ccHBV group. Low-resolution HLA analysis revealed that the prevalence of the HLA-A*01 (23.91% in the ccHBV group vs. 11.11% in the SC group, P = 0.03, OR = 2.50, 95% CI [1.0-5.98]) and HLA-B*57 (11.11% in the ccHBV group vs. 2.03% in the SC group, P = 0.01 < 0.05, OR = 5.82, 95% CI [1.2-30.68]) alleles were significantly higher in the ccHBV group compared to the SC group. Also, low-resolution analysis showed that the prevalence of the HLA-A*01 allele (23.91% in the ccHBV group vs. 9.7% in the control group, P = 0.004 < 0.05, OR = 2.93, 95% CI [1.34-5.9]) was significantly higher and that of the HLA-C*15 allele (8.1% in the control group vs. 0% in the ccHBV group, P = 0.04 < 0.05, OR = 2.85, 95% CI [0.5-16.16]) was significantly lower in the ccHBV compared to the control group Finally, high-resolution analysis showed that the prevalence of the HLA-DQB1*05:01 allele (13,64% in the ccHBV group vs. 13,16% in the control group, P = 0.02 < 0.05, OR = 2.82, 95% CI [1.17-6.33]) was significantly higher in the ccHBV compared to the control group.

Conclusion: In this analysis, HLA allele variations were associated with varying risk for progression to complicated HBV infection. Future larger studies are required to validate the results and ascertain whether HLA gene variations could have prognostic and therapeutic implications.

Keywords: HLA alleles; Complicated HBV infection; Single Nucleotide Polymorphisms; Cirrhosis; Hepatocellular Carcinoma

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Introduction

Chronic hepatitis B virus (HBV) infection affects approximately 296 million people worldwide [1], with prevalence varying significantly in different parts of the world, with central/east Asia, sub-Saharan Africa, and the Pacific region reporting a prevalence of about 5-8%, in contrast to the United States, where the respective number is close to 0.3% [2]. Chronic HBV infection can lead to hepatic decompensation, cirrhosis, and development of hepatocellular carcinoma (HCC) in up to 40% of untreated patients [3]. Regional differences with regards to chronic HBV incidence, as well as differential outcomes in chronic HBV-infected individuals, are attributed to an interplay of host genetic, viral, socioeconomic, and environmental factors [4].

Acute infection with HBV is characterized by activation of both CD8+ and CD4+ T cells that promote viral clearance and mediate liver inflammation through the production of cytokines, stimulation of humoral immunity, and direct cytotoxic effects [5]. Chronic HBV infection, on the other hand, is marked by impaired immune responses and inability of virus-specific T cells to effectively control viral replication [6]. Since T cell activation is dependent on Human Leukocyte Antigen (HLA) genes encoded by the Major Histocompatibility Complex (MHC) complex on chromosome 6, variations in HLA structure or function have been implicated in HBV clearance and persistence [7]. Indeed, previous studies have reported multiple HLA variations that influence the risk of chronic HBV development or progression to cirrhosis or HCC. These variations have been studied at the genome, allele and/or haplotype level, underlying the genetic substrate of chronic HBV infection and its complications [8-10].

It is expected that HLA variations are linked to chronic HBV susceptibility and progression to cirrhosis or HCC by modulating the pool of antigenic targets that can be presented to T cells, thus influencing the repertoire of both CD4+ and CD8+ T cells and their ability to control HBV replication [11]. Detection of specific HLA variations in patients with chronic HBV infection and HBV-related cirrhosis and HCC would enhance our understanding of disease pathogenesis and possibly yield more information about the quantification of risk and prognosis. In the present study, we evaluated the prevalence of specific HLA polymorphisms in a series of patients with chronic HBV infection and we compared the results to those of healthy individuals in order to assess whether HLA variations are associated with chronic HBV risk and its complications.

Materials and Methods

Study design

We conducted a single-center prospective study including patients ≥ 18 years of age with a diagnosis of chronic HBV infection who were examined at the outpatient clinic of Hippokration General Hospital of Thessaloniki between January 2018 and December 2022. The aim of the study was to calculate the prevalence of HLA polymorphisms in patients with chronic HBV infection with or without HBV-related complications (HCC or cirrhosis) and compare the prevalence of these polymorphisms with that of healthy individuals. DNA extraction for HLA analysis occurred at the time of study entry. This study was approved by the Institutional Review Board of Hippokration General Hospital (decision No. 29075/20-6-2024). All patients provided written informed consent in accordance with the Helsinki Declaration and were subsequently enrolled in the study.

Study population

Eligible patients were divided into two groups: a) The spontaneous clearance (SC) group included HBV-infected individuals who spontaneously cleared the hepatitis B surface antigen (HBsAg) and acquired natural immunity to the virus. Laboratory workup in the SC group was consistent with negative HBsAg testing, positive testing for the anti-hepatitis B surface antibody (anti-HBs) and anti-hepatitis B core antibody (anti-HBc), and normal liver function studies; b) The complicated chronic HBV (ccHBV) group included individuals with chronic HBV and a concomitant diagnosis of cirrhosis and/or HCC. Laboratory workup in the ccHBV group was consistent with positive HBsAg testing for at least 6 months prior to study entry, positive testing for anti-HBc and undetectable anti-hepatitis B core immunoglobulin M (anti-HBc IgM) antibody. The diagnosis of cirrhosis was based on liver biopsy results. Diagnosis of HCC was based on the diagnostic algorithms proposed by the American Association for the study of Liver Diseases (AASLD) [12] or the European Association for the Study of Liver – European Organization for Research and Treatment of Cancer (EASL-EORTC) [13]. Finally, a control group, consisting of healthy individuals from the Northern Greece Bone Marrow Donor Registry, was used to assess the differences in prevalence of HLA alleles between the ccHBV and control groups.

The control group consisted of healthy adult donors from the bone marrow bank of Northern Greece. Bone marrow donors were between 18 and 50 years old and they did not suffer from asthma, insulin-dependent diabetes, cancer, heart disease, multiple sclerosis, muscular dystrophy, schizophrenia, depression, infectious diseases, such as human immunodeficiency virus (HIV), hepatitis B and C, autoimmune disorders, vascular diseases, arterial or venous thrombosis and von Willebrand disease. In general, donors had a healthy medical record according to national guidelines for blood marrow donors. Individuals with a history of drug abuse, as well as those who were recipients of solid organ or hematopoietic cells in the past or were at risk for Creutzfeldt-Jacob disease, were excluded from the study. Healthy donors

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were recruited at the same time period when data specimen analysis for patients with HBV occurred, in order to analyze specimens from both HBV patients and controls using the same technique, as described below.

Exclusion criteria for this study included infection with other hepatotropic viruses, diagnosis of alcoholic hepatitis, steatotic liver disease, autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, non-HBV-related HCC, and presence of HCC-related metastatic disease.

HLA analysis

DNA extraction was performed from peripheral blood leucocytes using the iPrepTM PureLinkTM gDNA Blood kit (Invitrogen, USA), and the extracted DNA was spectrophotometrically quantified. HLA alleles of study participants were genotyped in low- and high-resolution analyses by single-specific primer polymerase chain reaction (SSP-PCR). Specifically, for the assessment of differences in HLA allele prevalence between the SC and ccHBV groups, two low-resolution HLA analyses were carried out, first one in a 1:1 patient ratio between the SC and ccHBV groups (for HLA-A, -B, -C, -DRB1, -DPB1 and -DQB1 alleles) and then another one in a 4:1 patient ratio between the SC and ccHBV group (for HLA-A, -B, -C, -DRB1, and -DQB1 alleles), respectively, in an effort to increase sample size and unmask differences in HLA allele prevalence between the groups that might have been missed in the 1:1 analysis. Also, low-resolution HLA analysis between the ccHBV and control groups was performed for estimating the prevalence of HLA-A, -B, -C, -DRB1 and -DQB1 alleles. High-resolution analysis between SC and ccHBV groups occurred for estimating the prevalence of HLA-DRB1, -DQB1, and -DPB1 alleles, whereas high-resolution analysis between the ccHBV and control groups occurred for estimating the prevalence of HLA-DRB1 and -DQB1 alleles.

Genotyping for two single nucleotide polymorphisms (SNPs) (rs9272105 and rs1110446) located in the HLA genome was further carried out between the SC and ccHBV group. Specifically, rs1110446 is located within the HLA class I region (and specifically mapped within the TRIM31 gene) and rs9272105 is located within the HLA class II region (and specifically mapped within the HLA-DQA1 region). Both SNPs have been associated with HBV-related HCC in different ethnic populations [14-18], and, as such, further analysis in these two SNPs was carried out to validate their association with HBV-related HCC in our population as well. Genotyping for SNP analysis was performed by using TaqMan SNP Genotyping assays (Assay IDs C_8942396_10 and C_30258340_10) with the QuantStudio 5 Real Time PCR System (Applied Biosystems TM). The reaction was performed in a 96-well format using 20 ng of genomic DNA in a total reaction volume of 25 ml. One of the allelic probes was labeled with FAM dye and the other with fluorescent VIC dye. PCR was run in the TaqMan MasterMix® (Applied Biosystems). The reaction plates were heated for 10 minutes at 95oC, followed by 40 cycles of 95oC for 15 seconds and 60oC for 1 minute. The fluorescence intensity of each well in TaqMan assay plate was read. Fluorescence data files from each plate were analyzed by QuantStudioTM Design and Analysis Software.

Statistical analysis

Statistical analysis was performed by using R programming language, version 4.2.2. [19]. The phenotypic frequencies of HLA-A, HLA-B, HLA-C, HLA-DRB1, HLA-DQB1 and HLA-DPB1 were compared among the groups of our study with the 2-sided Fisher's exact test. The Haldane-Anscombe correction was used when needed [20]. Results with p-value < 0.05 were considered statistically significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to further strengthen the results.

To analyze SNPs, the SNPStats online software [21] was used. Specifically, the SNPStats software was used to calculate allele and genotype frequencies, examine if the genotype frequencies are consistent with Hardy-Weinberg equilibrium (with p-value > 0.05 indicating equilibrium) and evaluate the association of the rs9272105 and rs1110446 polymorphisms with HBV in the different genetic models (co-dominant, dominant, recessive, over-dominant, and log-additive) to decide the best fit genetic model. The best model was selected based on the lowest Akaike Information Criterion (AIC). The AIC is a mathematical method for evaluating how well a model fits the data it was generated from [22]. Also, OR and 95% CI were calculated for each model.

Results

Patient characteristics

As shown in Table 1, we included 101 individuals in the SC group and 24 individuals in the ccHBV group. The mean age of the patients in the SC and ccHBV groups was 60.9 and 63.6 years, respectively. A total of 68 (67.3%) and 23 (95.8%) patients in the SC and ccHBV groups were male, respectively. In the ccHBV group, mean AST and ALT levels were 51.12 and 30.81 IU/L, respectively, while 12 (50%) had only cirrhosis and 12 (50%) patients had both cirrhosis and underlying HCC. In the SC group, AST and ALT levels were within the normal limits. HBV DNA was not detected in either group.

HLA analysis results

Low-resolution HLA analysis between patients in the SC and ccHBV groups

In Table 2, we present the low-resolution HLA analysis (in a 1:1 patient ratio) using Fisher's exact test assessing the prevalence of HLA alleles between the SC and ccHBV

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Table 1: Baseline characteristics of the patient groups included in the study

Variables	ccHBV group (n = 24)	SC group (n = 101)
Gender		
Male, number (%)	23 (95.8)	68 (67.3)
Female, number (%)	1 (4.2)	33 (32.7)
Age (years), mean ± SD	63.6 ± 7.1	60.9 ± 12.0
AST (IU/L), value ± SD	51.12 ± 12.0	WNL*
ALT (IU/L), value ± SD	30.81 ± 7.1	WNL*
HBV DNA undetectable, number (%)	24 (100)	101 (100)
Cirrhosis without HCC, number (%)	12 (50)	0
Cirrhosis with HCC, number (%)	12 (50)	0

*Patients in the SC group had AST and ALT levels withing the normal range, according to local laboratory test cutoff values.

Abbreviations: ALT: Alanine aminotransferase; **AST:** Aspartate aminotransferase; **ccHBV group:** Complicated chronic hepatitis B virus group; **DNA:** Deoxyribonucleic acid; **HBV:** Hepatitis B virus; **HCC:** Hepatocellular carcinoma; **IU:** International units; **L:** Liter; **SD:** Standard Deviation; **WNL:** Within Normal Limits.

Table 2: Low-resolution 1:1 HLA analysis between patients in the SC and ccHBV groups

Allele	Patients in the SC group (2N = 44)	Patients in the SC group, %	Patients in the ccHBV group (2N = 48)	Patients in the ccHBV group, %	P value	OR	95%CI
HLA-A							
A*01	**3**	**6.82**	**11**	**23.91**	**0.04**	**4.23**	**[1.01-25.48]**
A*02	15	34.09	9	19.57	0.15	0.47	[0.16-1.35]
A*03	4	9.09	6	13.04	0.74	1.49	[0.33-7.76]
A*11	5	11.36	3	6.52	0.48	0.55	[0.08-3.03]
A*24	6	13.64	8	17.39	0.77	1.33	[0.36-5.13]
A*26	1	2.27	2	4.35	1	1.94	[0.1-117.87]
A*29	1	2.27	2	4.35	1	1.94	[0.1-117.87]
A*32	2	4.55	1	2.17	0.61	0.47	[0.01-9.35]
A*33	1	2.27	2	4.35	1	1.94	[0.1-117.87]
HLA-B							
B*07	1	2.27	1	2.17	1	0.96	[0.01-76.69]
B*08	1	2.27	2	4.35	1	1.94	[0.1-117.87]
B*14	2	4.55	3	6.52	1	1.46	[0.16-18.29]
B*15	1	2.27	1	2.17	1	0.96	[0.01-76.69]
B*18	5	11.36	4	8.7	0.74	0.75	[0.14-3.74]
B*35	6	13.64	12	26.09	0.19	2.22	[0.68-8.03]
B*37	1	2.27	2	4.35	1	1.94	[0.1-117.87]
B*39	2	4.55	1	2.17	0.61	0.47	[0.01-9.35]
B*40	5	11.36	2	4.35	0.26	0.36	[0.03-2.34]
B*41	1	2.27	1	2.17	1	0.96	[0.01-76.79]
B*44	2	4.55	1	2.17	0.61	0.47	[0.01-9.35]
B*51	9	20.45	3	6.52	0.07	0.28	[0.04-1.21]
B*52	1	2.27	2	4.35	1	1.94	[0.1-117.87]
B*55	1	2.27	1	2.17	1	0.96	[0.01-76.69]
HLA-C							
C*01	2	4.55	1	2.17	0.61	0.47	[0.01-9.35]
C*02	4	9.09	2	4.35	0.43	0.46	[0.04-3.4]

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C*03	4	9.09	3	6.52	0.71	0.7	[0.1-4.42]
C*04	4	9.09	10	21.74	0.15	2.75	[0.71-13.08]
C*06	3	6.82	8	17.39	0.2	2.85	[0.62-17.87]
C*07	5	11.36	9	19.57	0.39	1.88	[0.51-7.85]
C*08	2	4.55	3	6.52	1	1.46	[0.16-18.29]
C*12	8	18.18	5	10.87	0.38	0.55	[0.13-2.12]
C*15	8	18.18	3	6.52	0.11	0.32	[0.05-1.45]
C*17	1	2.27	1	2.17	1	0.96	[0.01-76.69]
HLA-DRB1							
DRB1*01	5	11.36	5	10.42	1	0.91	[0.19-4.27]
DRB1*03	3	6.82	4	8.33	1	1.24	[0.2-8.98]
DRB1*04	5	11.36	4	8.33	0.73	0.71	[0.13-3.57]
DRB1*07	2	4.55	4	8.33	0.68	1.9	[0.26-22.0]
DRB1*10	1	2.27	2	4.17	1	1.86	[0.09-112.75]
DRB1*11	11	25	14	29.17	0.81	1.23	[0.45-3.48]
DRB1*12	1	2.27	2	4.17	1	1.86	[0.09-112.75]
DRB1*13	5	11.36	1	2.08	0.1	0.17	[0.0-1.6]
DRB1*14	3	6.82	2	4.17	0.67	0.6	[0.05-5.49]
DRB1*15	2	4.55	3	6.25	1	1.39	[0.15-17.47]
DRB1*16	5	11.36	7	14.58	0.76	1.33	[0.33-5.78]
HLA-DQB1							
DQB1*02	4	9.09	8	16.67	0.36	1.99	[0.48-9.75]
DQB1*03	19	43.18	20	41.67	1	0.94	[0.38-233]
DQB1*05	15	34.09	14	29.17	0.66	0.8	[0.3-2.11]
DQB1*06	6	13.64	6	12.5	1	0.91	[0.22-3.71]
HLA-DPB1							
DPB1*02	7	16.67	3	7.14	0.31	0.39	[0.06-1.87]
DPB1*03	3	7.14	1	2.38	0.62	0.32	[0.01-4.19]
DPB1*04	26	61.9	34	80.95	0.09	2.59	[0.88-8.13]
DPB1*13	2	4.76	1	2.38	1	0.49	[0.01-9.8]

Note: statistically significant associations are presented in bold.

Abbreviations: ccHBV: Complicated chronic hepatitis B virus; **CI:** Confidence interval; **HLA:** Human leucocyte antigen; **SC:** Spontaneous clearance.

groups. Results revealed that the prevalence of the HLA-A*01 allele (23.91% in the ccHBV group vs. 6.82% in the SC group, P = 0.04 < 0.05, OR = 4.23, 95% CI [1.01-25.48]) was significantly higher in the ccHBV group compared to the SC group. No other significant differences regarding the prevalence of HLA alleles were observed between the two groups.

In Table 3, we present the low-resolution HLA analysis (in a 4:1 patient ratio) using Fisher's exact test assessing the prevalence of HLA alleles between the SC and ccHBV groups. Results revealed that the prevalence of the HLA-A*01 (23.91% in the ccHBV group vs. 11.11% in the SC group, P = 0.03 < 0.05, OR = 2.50, 95% CI [1.0-5.98]) and HLA-B*57 (11.11% in the ccHBV group vs. 2.03% in the SC group, P = 0.01 < 0.05, OR = 5.82, 95% CI [1.2-30.68]) alleles were significantly higher in the ccHBV group compared to the SC group. No other significant differences regarding the prevalence of HLA alleles were observed between the two groups.

Low-resolution HLA analysis between patients in the ccHBV and control groups

In Table 4, we present the low-resolution HLA analysis using Fisher's exact test assessing the prevalence of HLA alleles between the ccHBV and control groups. Results revealed that the prevalence of the HLA-A*01 allele (23.91% in the ccHBV group vs. 9.7% in the control group, P = 0.004 < 0.05, OR = 2.93, 95% CI [1.34-5.9]) was significantly higher in the ccHBV group compared to control group. Also, the

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Table 3: Low-resolution 4:1 HLA analysis between patients in the SC and ccHBV groups

Allele	Patients in the SC group (2N = 44)	Patients in the SC group, %	Patients in the ccHBV group (2N = 48)	Patients in the ccHBV group, %	P value	OR	95%CI
HLA-A							
A*01	**22**	**11.11**	**11**	**23.91**	**0.03**	**2.5**	**[1 - 5.98]**
A*02	56	28.28	9	19.57	0.27	0.62	[0.25 - 1.41]
A*03	13	6.57	6	13.04	0.22	2.13	[0.62 - 6.45]
A*11	17	8.59	3	6.52	0.77	0.74	[0.13 - 2.74]
A*23	4	2.02	1	2.17	1	1.08	[0.02 - 11.23]
A*24	31	15.66	8	17.39	0.82	1.13	[0.42 - 2.78]
A*26	9	4.55	2	4.35	1	0.95	[0.1 - 4.85]
A*29	3	1.52	2	4.35	0.24	2.94	[0.24 - 26.46]
A*30	3	1.52	1	2.17	0.57	1.44	[0.03 - 18.44]
A*32	13	6.57	1	2.17	0.48	0.32	[0.01 - 2.22]
A*33	3	1.52	2	4.35	0.24	2.94	[0.24 - 26.46]
HLA-B							
B*07	4	2.03	1	2.22	1	1.07	[0.02 - 11.17]
B*08	7	3.55	2	4.44	0.68	1.23	[0.12 - 6.78]
B*14	7	3.55	3	6.67	0.41	1.89	[0.3 - 8.69]
B*15	4	2.03	1	2.22	1	1.07	[0.02 - 11.17]
B*18	24	12.18	4	8.89	0.62	0.69	[0.16 - 2.16]
B*35	31	15.74	12	26.67	0.13	1.88	[0.8 - 4.25]
B*37	2	1.02	2	4.44	0.16	4.39	[0.31 - 62.19]
B*39	5	2.54	1	2.22	1	0.85	[0.02 - 7.9]
B*40	14	7.11	2	4.44	0.74	0.6	[0.06 - 2.74]
B*41	4	2.03	1	2.22	1	1.07	[0.02 - 11.17]
B*44	15	7.61	2	4.44	0.32	0.27	[0.01 - 1.85]
B*49	3	1.52	1	2.22	0.57	1.43	[0.03 - 18.34]
B*51	30	15.23	3	6.67	0.15	0.39	[0.07 - 1.35]
B*52	10	5.08	2	4.44	1	0.85	[0.09 - 4.2]
B*55	4	2.03	1	2.22	1	1.07	[0.02 - 11.17]
B*57	**4**	**2.03**	**5**	**11.11**	**0.01**	**5.82**	**[1.2 - 30.68]**
B*58	1	0.51	1	2.22	0.34	4.32	[0.05 - 342.59]
HLA-C							
C*01	1	0.91	1	2.17	0.51	2.41	[0.03 - 191.55]
C*02	6	5.45	2	4.35	1	0.79	[0.08 - 4.64]
C*03	8	7.27	3	6.52	1	0.89	[0.15 - 3.94]
C*04	17	15.45	10	21.74	0.36	1.52	[0.56 - 3.90]
C*05	4	3.64	1	2.17	1	0.59	[0.01 - 6.19]
C*06	9	8.18	8	17.39	0.1	2.35	[0.73 - 7.43]
C*07	18	16.36	9	19.57	0.65	1.24	[0.45 - 3.23]
C*08	5	4.55	3	6.52	0.69	1.46	[0.22 - 7.89]
C*12	17	15.45	5	10.87	0.62	0.67	[0.18 - 2.06]
C*15	14	12.73	3	6.52	0.4	0.48	[0.08 - 1.85]
C*17	2	1.82	1	2.17	1	1.2	[0.02 - 23.57]
HLA-DRB1							
DRB1*01	23	11.73	5	10.42	1	0.88	[0.25 - 2.54]

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DRB1*03	17	8.67	4	8.33	1	0.96	[0.22 - 3.14]
DRB1*04	19	9.69	4	8.33	1	0.85	[0.2 - 2.73]
DRB1*07	9	4.59	4	8.33	0.29	1.88	[0.41 - 7.14]
DRB1*10	4	2.04	2	4.17	0.34	2.08	[0.18 - 15.02]
DRB1*11	55	28.06	14	29.17	0.86	1.06	[0.48 - 2.21]
DRB1*12	7	3.57	2	4.17	0.69	1.17	[0.12 - 6.44]
DRB1*13	22	11.22	1	2.08	0.06	0.17	[0 - 1.1]
DRB1*14	12	6.12	2	4.17	1	0.67	[0.07 - 3.16]
DRB1*15	13	6.63	3	6.25	1	0.94	[0.16 - 3.62]
DRB1*16	14	7.14	7	14.58	0.15	2.21	[0.71 - 6.31]
HLA-DQB1							
DQB1*02	7	11.67	8	16.67	0.58	1.51	[0.44 - 5.33]
DQB1*03	33	55	20	41.67	0.18	0.59	[0.25 - 1.35]
DQB1*05	16	26.67	14	29.17	0.83	1.13	[0.44 - 2.86]
DQB1*06	4	6.67	6	12.5	0.33	1.99	[0.44 - 10.2]

Note: statistically significant associations are presented in bold.

Abbreviations: ccHBV: complicated chronic hepatitis B virus; CI: confidence interval; HLA: human leucocyte antigen; SC: spontaneous clearance.

prevalence of the HLA-C*15 allele (8.1% in the control group vs. 0% in the ccHBV group, P = 0.04 < 0.05, OR = 2.85, 95% CI [0.5-16.16]) was significantly lower in the ccHBV group compared to control group. No other significant differences regarding the prevalence of HLA alleles were observed between the two groups.

High-resolution HLA analysis between patients in the SC and ccHBV groups

In Table 5, we present the high-resolution HLA analysis using Fisher's exact test assessing the prevalence of HLA alleles between the ccHBV and control groups. Results revealed no significant differences regarding the prevalence of HLA alleles between the two groups.

High-resolution HLA analysis results between patients in the ccHBV and control groups

In Table 6, we present the high-resolution HLA analysis using Fisher's exact test assessing the prevalence of HLA alleles between the ccHBV and control groups. Results revealed that the prevalence of the HLA-DQB1*05:01 allele (13,64% in the ccHBV group vs. 13,16% in the control group, P = 0.02 < 0.05, OR = 2.82, 95% CI [1.17-6.33]) was significantly higher in the ccHBV group compared to control group. No other significant differences regarding the prevalence of HLA alleles were observed between the two groups.

SNP association analysis results

SNP analysis for rs9272105 and rs1110446 occurred between 24 patients in the ccHBV group and 21 patients in the SC group.

SNP association analysis for rs9272105

In Supplementary Table 1 we present the allele and genotype distribution of rs9272105 between patients in the SC and ccHBV groups. Genotypic frequencies of rs9272105 were found to be in Hardy-Weinberg equilibrium (Supplementary Table 2). Association analysis of rs9272105 with cirrhosis or HCC development was tested under five different inheritance models. Using the AIC value to delineate the most appropriate inheritance model, a recessive inheritance pattern was chosen based on an AIC value of 63.6, which was lower compared with the AIC values of the remaining inheritance models. After correction with the Haldane-Anscombe method, patients with the GG genotype had higher odds of cirrhosis or HCC development compared with patients with the AG or AA genotypes, although differences in odds between the groups were not statistically significant (OR = 2.87, 95% CI [0.11 – 74.28]) (Supplementary Table 3).

SNP association analysis for rs1110446

In Supplementary Table 4 we present the allele and genotype distribution of rs1110446 between patients in the SC and ccHBV groups. Genotypic frequencies of rs1110446 were not found to be in Hardy-Weinberg equilibrium (Supplementary Table 5). Association analysis of rs1110446 with cirrhosis or HCC development revealed that there is a statistically significant higher odds of HBV-related complications in patients with the CT genotype compared to patients with the TT genotype (OR = 4.00, 95% CI [1.11 – 14.43]) (Supplementary Table 6).

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Table 4: Low-resolution HLA analysis between patients in the ccHBV and control groups

Allele	Healthy adults (2N = 29012)	Healthy adults, %	Patients in the ccHBV group (2N = 48)	Patients in the ccHBV group, %	P value	OR	95%CI
HLA-A							
A*01	**2814**	**9.7**	**11**	**23.91**	**0.004**	**2.93**	**[1.34 - 5.9]**
A*02	7921	27.3	9	19.57	0.32	0.65	[0.27 - 1.37]
A*03	2698	9.3	6	13.04	0.43	1.46	[0.51 - 3.48]
A*11	2002	6.9	3	6.52	1	0.83	[0.16 - 2.56]
A*23	928	3.2	1	2.17	1	0.67	[0.02 - 3.95]
A*24	4497	15.5	8	17.39	0.68	1.15	[0.46 - 2.5]
A*26	1625	5.6	2	4.35	1	0.77	[0.09 - 2.94]
A*32	1770	6.1	1	2.17	0.53	0.34	[0.01 - 2.01]
A*68	1161	4	0	0	1	0	[0 - 52.55]
HLA-B							
B*07	1230	4.2	1	2.17	1	0.51	[0.01 - 2.98]
B*08	1259	4.3	2	4.35	1	1.01	[0.12 - 3.89]
B*18	3396	11.6	4	8.7	0.82	0.73	[0.19 - 2]
B*35	5446	18.6	12	26.09	0.19	1.54	[0.73 - 3.06]
B*38	995	3.4	0	0	0.41	0	[0 - 2.38]
B*40	966	3.3	2	4.35	0.66	1.33	[0.16 - 5.12]
B*44	2196	7.5	2	4.35	0.58	0.55	[0.06 - 2.1]
B*51	4157	14.2	3	6.52	0.2	0.42	[0.08 - 1.32]
HLA-C							
HLA-C*01	38	4.2	1	2.17	0.72	0.43	[0.01 - 2.64]
HLA-C*02	58	6.3	2	4.35	1	0.67	[0.08-2.69]
HLA-C*03	46	5	3	6.52	0.5	1.32	[0.25 - 4.37]
HLA-C*04	156	17	10	21.74	0.42	1.35	[0.59 - 2.86]
HLA-C*05	20	2.2	1	2.17	1	1	[0.02 - 6.51]
HLA-C*06	88	9.6	8	17.39	0.12	1.98	[0.77 - 4.48]
HLA-C*07	164	17.9	9	19.57	0.7	1.12	[0.46 - 2.41]
HLA-C*08	20	2.2	3	6.52	0.09	3.12	[0.57 - 11.14]
HLA-C*12	164	17.9	5	10.87	0.32	0.56	[0.17 - 1.45]
HLA-C*14	34	3.7	3	6.52	0.42	1.81	[0.34 - 6.12]
HLA-C*15	74	8.1	0	0	0.04	2.85	[0.5 - 16.16]
HLA-C*17	10	1.1	1	2.17	0.42	2.01	[0.05 - 14.7]
HLA-DRB1							
DRB1*01	1283	6.7	5	10.42	0.25	1.62	[0.5 - 4.09]
DRB1*03	1110	5.8	4	8.33	0.36	1.48	[0.38 - 4.07]
DRB1*04	1838	9.6	4	8.33	1	0.86	[0.22 - 2.36]
DRB1*07	1513	7.9	4	8.33	0.78	1.06	[0.28 - 2.92]
DRB1*11	5169	27	14	29.17	0.75	1.11	[0.55 - 2.13]
DRB1*13	1895	9.9	1	2.08	0.09	0.19	[0 - 1.13]
DRB1*14	1149	6	2	4.17	1	0.68	[0.08 - 2.61]
DRB1*15	1455	7.6	3	6.25	1	0.81	[0.16 - 2.53]
DRB1*16	2259	11.8	7	14.58	0.5	1.28	[0.48 - 2.88]
HLA-DQB1							
DQB1*02	28	11.2	8	16.67	0.34	1.55	[0.57 - 3.83]
DQB1*05	54	22	14	29.17	0.27	1.46	[0.67 - 3.04]
DQB1*06	30	12.2	6	12.5	1	1.03	[0.33 - 2.73]

Note: statistically significant associations are presented in bold.
Abbreviations: ccHBV: Complicated chronic hepatitis B virus; **CI:** Confidence Interval; **HLA:** Human Leucocyte Antigen.

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Table 5: High-resolution HLA analysis between patients in the SC and ccHBV groups

Allele	Patients in the ccHBV group (2N = 48)	Patients in the SC group (2N = 46)	P value	95%CI	OR
HLA-DRB1					
DRB1*01:01	4	3	1	0.11-4.89	0.77
DRB1*01:02	1	1	1	0.013-84.16	1.05
DRB1*03:01	3	4	0.71	0.23-10.42	1.43
DRB1*04:03	2	1	1	0.01-10.27	0.52
DRB1*07:01	2	2	0.61	0.11-129.	2.13
DRB1*10:01	1	2	0.61	0.11-129.64	3.13
DRB1*11:01	5	7	0.54	0.38-6.80	1.55
DRB1*11:04	6	6	1	0.26-4.35	1.05
DRB1*12:01	1	2	0.61	0.11-129.64	2.13
DRB1*13:01	1	1	1	0.01-84.16	1.05
DRB1*14:54	2	1	1	0.01-10.27	0.52
DRB1*15:02	1	3	0.35	0.259-177.43	3.27
DRB1*16:01	4	7	0.35	0.46-10.04	1.98
HLA-DQB1					
DQB1*02:01	2	4	0.09	0.76-42.30	4.14
DQB1*02:02	2	3	0.16	0.62-36.93	3.54
DQB1*03:01	14	15	0.52	0.55-3.63	1.4
DQB1*03:02	2	1	0.44	0.37-26.74	2.42
DQB1*03:03	1	1	0.21	0.52-241.58	4.93
DQB1*05:01	6	6	0.42	0.49-6.14	1.66
DQB1*05:02	6	5	0.58	0.41-5.48	1.46
DQB1*05:03	3	2	0.49	0.38-12.78	1.94
DQB1*06:01	1	3	0.06	0.86-337.84	7.21
DQB1*06:03	2	3	0.16	0.62-36.93	3.54
HLA-DPB1					
DPB1*02:01	7	3	0.31	0.06-1.87	0.39
DPB1*03:01	3	1	0.62	0.01-4.19	0.32
DPB1*04:01	21	25	0.51	0.57-3.81	1.46
DPB1*04:02	5	9	0.38	0.54-8.41	2
DPB1*13:01	2	1	1	0.01-9.80	0.49

Abbreviations: ccHBV: Complicated chronic hepatitis B virus; **CI:** Confidence Interval; **HLA:** Human leucocyte antigen; **SC:** Spontaneous clearance.

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Table 6: High-resolution HLA analysis between patients in the ccHBV and control groups

Allele	Healthy adults (2N = 29012)	Healthy adults, %	Patients in the ccHBV group (2N = 48)	Patients in the ccHBV group, %	P value	OR	95%CI
HLA-DRB1							
DRB1*03:01	25	8.65	4	9.52	0.28	1.87	[0.45 - 5.83]
DRB1*07:01	27	9.34	2	4.76	1	0.82	[0.09 - 3.47]
DRB1*11:01	47	16.26	7	16.67	0.19	1.8	[0.64 - 4.42]
DRB1*11:04	84	29.07	6	14.29	0.68	0.77	[0.26 - 1.92]
DRB1*16:01	106	36.68	7	16.67	0.44	0.69	[0.25 - 1.63]
HLA-DQB1							
DQB1*03:01	158	51.97	15	34.09	0.43	1.29	[0.66 - 2.47]
DQB1*03:02	32	10.53	1	2.27	0.37	1.59	[0.46 - 4.42]
DQB1*05:01	**40**	**13.16**	**6**	**13.64**	**0.02**	**2.82**	**[1.17 - 6.33]**
DQB1*05:02	74	24.34	5	11.36	0.54	1.23	[0.5 - 2.73]

Note: statistically significant associations are presented in bold.
Abbreviations: ccHBV: Complicated chronic hepatitis B virus; **CI:** Confidence Interval; **HLA:** human leucocyte antigen.

Discussion

In this study, we compared the prevalence of HLA alleles between patients with natural immunity to HBV infection and complicated chronic HBV infection, and we further compared HLA allele distribution between healthy adult individuals and patients with complicated chronic HBV. Our results showed that the prevalence of HLA-A*01 and HLA-B*57 were significantly higher in patients with ccHBV compared with patients with natural immunity to HBV. Also, compared to the healthy adult individuals, patients with ccHBV had a significantly higher prevalence of HLA-A*01 and HLA-DQB1*05:01 and a significantly lower prevalence of HLA-C*15.

Compared to patients with natural immunity to HBV, patients in the ccHBV group had a significantly higher prevalence of HLA-A*01 and HLA-B*57 alleles. To the best of our knowledge, similar associations have not been reported in the literature, but other HLA-A and -B alleles have been described to occur with varying frequency in patients with persistence versus clearance of HBV. Thio et al. molecularly typed 194 Caucasian individuals with HBV persistence and 394 matched controls with HBV clearance, and showed that the frequency of HLA-A*03:01 was higher and that of HLA-B*08 and HLA-B*44 lower in patients clearance versus persistence of HBV, respectively [23]. Ramezani et al. assessed 33 patients with chronic HBV and 31 HBV carriers and found that the prevalence of HLA-A*33 was higher in the chronic HBV than in the carrier group. Depending on the sample size analyzed, ethnicity status, and molecular techniques used, other HLA class I associations have been found to modulate the natural course of HBV infection [7, 24-26]. Due to the highly polymorphic nature of HLA genes, future studies should consider the HBV genotype and clinical/laboratory characteristics of patients, and also perform analyses at the allele and haplotype levels.

Regarding HLA class II associations, our results showed that the prevalence of HLA-DQB1*05:01 was significantly higher in patients with chronic complicated HBV compared with healthy adult individuals. Other HLA-DQB1*05 allele polymorphisms, including HLA-DQB1*05:02 [27] and HLA-DQB1*05:03 [28], have been shown to confer susceptibility to chronic HBV infection. Polymorphisms in other HLA-DQB1 regions, including HLA-DQB1*02, HL-DQB1*03, and HLA-DQB1*06, are also associated with persistence of HBV virus [4]. However, literature evidence suggests that polymorphisms within the HLA-DQB1 region are also associated with lower risk of chronic HBV infection. Tălăngescu et al. [29] recruited 247 patients with chronic HBV infection and 304 healthy subjects and showed that polymorphisms within the HLA-DQB1*01, HLA-DQB1*06, HLA-DQB1*13, and HLA-DQB1*15 regions had a protective effect againt chronic HBV infection. Naderi et al. [30] included 90 patients with chronic HBV infection and 40 healthy subjects in their analysis and revealed that HLA-DQB1*06:04 had a protective role against viral persistence. HLA-DQB1 alleles have also been found to augment the response to HBV vaccines [31]. Collectively, it seems that distinct polymorphisms within the HLA-DQB1 region are associated with either higher or lower risk of chronic HBV infection, and, as such, it is critical to conduct high-resolution analyses to accurately define the genetic regions that positively or negatively influence viral persistence.

In our study, the presence of HLA-C*15 was significantly lower in the patients with chronic HBV infection compared with healthy adult subjects. Yengo et al. [32] analyzed a total of 136 patients with HBV or hepatitis C virus infection

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and 63 healthy subjects and found that the prevalence of HLA-C*15:05 was higher in patients with hepatitis compared with controls, but the results were not statistically significant. HLA-C associations have been described to alter the risk of chronic HBV infection in various ethnic cohorts, but their associations compared with other HLA class I molecules are more limited [7]. HLA-C molecules are classified as HLA-C1 and HLA-C2 based on amino acid substitutions at position 80 [33] and are recognized by distinct members of the killer-cell immunoglobulin-like receptor (KIR) family that interact with HLA class I ligands and are involved in viral disease control [34]. Auer et al. [35] recruited 511 chronic HBV patients and 140 healthy controls and revealed that KIR2DL2+HLA-C1 and KIR2DL3+HLA-C1 were associated with decreased chronic HBV risk, and KIR2DL3+HLA-C1 was associated with progression to HBV-related HCC. Although protective in our cohort, the effect of HLA-C*15 on chronic HBV progression should be interpreted with caution given the small sample size analyzed, and future studies with more patients involved should investigate the role of HLA-C molecules, both as independent alleles as well as part of disease-specific haplotypes that may modify chronic HBV risk.

Our study has several limitations. First, our sample size was small, and this might have prevented us from finding additional HLA variations that could be associated with varying chronic HBV risk. Next, our population consisted of individuals of Greek origin, and thus the results may not be generalizable to other ethnic populations. The group of complicated chronic HBV individuals consisted of patients with either HCC or cirrhosis; due to small sample size, we were unable to conduct subgroup analyses in patients with either complication. Additionally, high-resolution HLA analysis could only be carried out in 24 instead of the total 101 patients in the spontaneous clearance group due to reasons such as insufficient funding, inadequate genetic specimen stored for future analyses, and patients lost to follow-up. We acknowledge that such an approach could introduce selection bias, although at baseline, patients in the spontaneous clearance group presented with similar clinical and microbiological features. Finally, identification of the HBV genotype did not occur for each patient, and, as such, the risk of HBV persistence and development of HBV-related complications with specific HLA variations might be different according to HBV genotype analyzed.

In conclusion, our study found several HLA allele variations that are associated with either higher or lower risk of progression to complicated chronic HBV infection. Given the morbidity and mortality that accompanies HBV-related HCC and/or cirrhosis, it is crucial to identify factors that are associated with disease progression in an effort to develop prognostic indicators that could also have therapeutic implication. In the future, large scale, multi-ethnicity studies should verify the HLA alleles that have been described in the literature to modify the risk of chronic complicated HBV infection, but also assess whether distinct HLA polymorphisms are linked to differential treatment efficacy.

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Conflicts of Interest: None to disclose

Data availability statement:

Data sharing is available for this article and can be provided upon request.

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Supplementary Data

Supplement to: HLA Variations and Association with Complicated Chronic Hepatitis B Virus Infection: A Prospective Cohort Study

Table S1: Allele and genotype frequencies of rs9272105 between patients in the SC and ccHBV groups included in the analysis.

rs9272105 allele frequency (n=44)			
Allele	**Total population, number (%)**	**ccHBV group, number (%)**	**SC group, number (%)**
A	62 (70)	32 (70)	30 (71)
G	26 (30)	14 (30)	12 (29)
rs9272105 genotype frequency (n=45)			
A/A	19 (43)	10 (42)	9 (43)
A/G	24 (53)	12 (50)	12 (57)
G/G	1 (2)	1 (4)	0
NA	1 (2)	1 (4)	0

Abbreviations: ccHBV: complicated chronic hepatitis B virus; N/A: not available; SC: spontaneous clearance

Table S2: Testing for Hardy-Weinberg equilibrium for the rs9272105 polymorphism in the patients included in the analysis.

rs9272105 Hardy-Weinberg equilibrium testing (n=44)						
	A/A	**A/G**	**G/G**	**A**	**G**	**P-value**
Total population	19	24	1	62	26	0.069
ccHBV group	10	12	1	32	14	0.62
SC group	9	12	0	30	12	0.13

Abbreviations: ccHBV: complicated chronic hepatitis B virus; SC: spontaneous clearance

Table S3: Association of the rs9272105 polymorphism with ccHBV in the different genetic models.

Model	Genotype	CcHBV group, number (%)	SC group, number (%)	OR (95% CI)	P-value	AIC
Co-dominant	A/A	10 (43.5%)	9 (42.9%)	1	0.51	65.6
	G/A	12 (52.2%)	12 (57.1%)	1.11 (0.33-3.71)		
	G/G	1 (4.3%)	0 (0%)	0.00 (0.00-NA)		
Dominant	A/A	10 (43.5%)	9 (42.9%)	1	0.97	64.9
	G/A-G/G	13 (56.5%)	12 (57.1%)	1.03 (0.31-3.39)		
Recessive	A/A-G/A	22 (95.7%)	21 (100%)	1	0.25	63.6
	G/G	1 (4.3%)	0 (0%)	0.00 (0.00-NA)		
Over-dominant	A/A-G/G	11 (47.8%)	9 (42.9%)	1	0.74	64.8
	G/A	12 (52.2%)	12 (57.1%)	1.22 (0.37-4.02)		
Log-additive	---	---	---	0.88 (0.29-2.65)	0.82	64.9

Abbreviations: AIC: Akaike's information criterion; ccHBV: complicated chronic hepatitis B virus; NA: not available/applicable; OR: odds ratio; SC: spontaneous clearance.

Citation: Evangelia Myserli, Asimina Fylaktou, Konstantinos Ouranos, Maria Exindari, Polina Agorastou, Evangelia Sidira, Margarita Samali, Grigorios Myserlis, Ioannis Goulis, Georgia Gioula. HLA Variations and Association with Complicated Chronic Hepatitis B Virus Infection: A Prospective Cohort Study. Archives of Internal Medicine Research. 7 (2024): 175-188.

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Table S4: Allele and genotype frequencies of rs1110446 between patients in the SC and ccHBV groups included in the analysis.

rs1110446 allele frequency (n=42)			
Allele	**Total population, number (%)**	**ccHBV group, number (%)**	**SC group, number (%)**
T	63 (75)	35 (83)	28 (67)
C	21 (25)	7 (17)	14 (33)
rs1110446 genotype frequency (n=45)			
T/T	21 (50)	14 (58)	7 (33)
T/C	21 (50)	7 (29)	14 (67)
NA	0	3 (13)	0

Abbreviations: ccHBV: complicated chronic hepatitis B virus; N/A: not available; SC: spontaneous clearance

Table S5: Testing for Hardy-Weinberg equilibrium for the rs1110446 polymorphism in the patients included in the analysis.

rs1110446 Hardy-Weinberg equilibrium testing (n=42)						
	T/T	**T/C**	**C/C**	**T**	**C**	**P-value**
Total population	21	21	0	63	21	0.043
ccHBV group	14	7	0	35	7	1.00
SC group	7	14	0	28	14	0.05

Abbreviations: ccHBV: complicated chronic hepatitis B virus; SC: spontaneous clearance

Table S6: Association of the rs1110446 polymorphism with ccHBV.

Model	**Genotype**	**CcHBV group, number (%)**	**SC group, number (%)**	**OR (95% CI)**	**P-value**	**AIC**
N/A	T/T	14 (66.7)	7 (33.3)	1	0.03	57.5
	C/T	7 (33.3)	14 (66.7)	4.00 (1.11-14.43)		

Abbreviations: AIC: Akaike's information criterion; ccHBV: complicated chronic hepatitis B virus; NA: not available/applicable; OR: odds ratio; SC: spontaneous clearance.

Citation: Evangelia Myserli, Asimina Fylaktou, Konstantinos Ouranos, Maria Exindari, Polina Agorastou, Evangelia Sidira, Margarita Samali, Grigorios Myserlis, Ioannis Goulis, Georgia Gioula. HLA Variations and Association with Complicated Chronic Hepatitis B Virus Infection: A Prospective Cohort Study. Archives of Internal Medicine Research. 7 (2024): 175-188.