Hepat Mon. January-December 2025; 25(1): e160428 https://doi.org/10.5812/hepatmon-160428

Published Online: 2025 July 23 **Research Article**

Effect of Promoter Region Polymorphisms in the IP-10 Gene on HBsAg Loss in Chronic Hepatitis B Patients

Melek Tutku Kaçar Şahin [1,*], Muhammed Burak Bereketoğlu [2], Hamide Dogan [3], Meryem Kose [4], Ferit Kuscu [1], Behice Kurtaran [5], Süheyla Kömür [1], Seza Ayse Inal [1], Damla Erturk [1], Yeşim Taşova [1], Yasemin Saygıdeğer [6,7], Aslıhan Candevir [1]

[1] Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Cukurova University, Adana, Turkey
[2] Department of Medical Genetics, Ege University Faculty of Medicine, İzmir, Turkey
[3] Pulmonology Research Laboratory, Biotechnology Research Center, Cukurova University, Adana, Turkey
[4] Department of Biotechnology, Institute of Health Sciences, Cukurova University, Adana, Turkey
[5] Department of Infectious Diseases and Clinical Microbiology, Acıbadem Hospital, Adana, Turkey
[6] Department of Pulmonary, Faculty of Medicine, Cukurova University, Adana, Turkey
[7] Department of Translational Medicine, Institute of Health Sciences, Cukurova University, Adana, Turkey

[*] ***Corresponding Author:*** Department of Infectious Diseases and Clinical Microbiology, Cukurova University Faculty of Medicine, Adana, Turkey. Email: melek_tutku_@hotmail.com

Received: 15 February, 2025; **Revised:** 23 June, 2025; **Accepted:** 7 July, 2025

Abstract

Background: Although vaccination programs have significantly reduced the incidence of hepatitis B, chronic infection with the hepatitis B virus (HBV) continues to be a major contributor to liver-related morbidity and mortality. Among available therapeutic outcomes, the clearance of hepatitis B surface antigen (HBsAg) is considered the most favorable endpoint.

Objectives: This study aims to evaluate genetic variations in the promoter region of the interferon gamma (IFN-γ)-inducible protein-10 (IP-10) gene — an important chemokine involved in inflammatory responses — which may play a role in the loss of HBsAg during chronic HBV infection.

Methods: This research was designed as a single-center case-control study. The study included 60 patients with documented HBsAg loss, retrospectively identified from a cohort of 1,950 chronic hepatitis B patients who were followed at the Infectious Diseases and Clinical Microbiology outpatient clinic of Cukurova University Hospital between 2005 and 2022. A control group of 60 patients who remained HBsAg positive was also included. Peripheral blood samples were collected from all participants, and deoxyribonucleic acid (DNA) was extracted to analyze IP-10 gene polymorphisms. The target gene region was amplified using polymerase chain reaction (PCR), followed by Sanger sequencing. Based on data from the Genome-Wide Association Studies (GWAS) database and published literature, three variants located within the IP-10 promoter, exon 1, intron 1, and exon 2 regions were selected for analysis. The resulting sequence data (in .ab1 format) were analyzed using the CLC Genomics Workbench 24 software.

Results: Between 2005 and 2022, the rate of HBsAg loss was calculated as 5.33%. The average age at which HBsAg loss occurred was 53.2±11.3 years, and 85% of the individuals experienced this loss after the age of 40. When comparing the case and control groups, no statistically significant differences were found in terms of gender, current age, age at diagnosis, Body Mass Index, alcohol use, or smoking habits ($P > 0.05$). Three polymorphisms — c.-135C>T (rs56061981), c.85C>T (rs11548618), and c.83T>G — were detected within the promoter and exon 2 regions of the IP-10 gene. Analysis of genotype and allele distributions revealed no significant differences between the two groups for any of these variants ($P > 0.05$).

Conclusions: Our findings suggest that the c.-135C>T, c.85C>T, and c.83T>G polymorphisms within the IP-10 gene region are not associated with HBsAg loss and therefore may not serve as reliable predictive markers.

Keywords: CXCL10, Cytokine, Polymorphisms, IP-10 Protein, Hepatitis B Surface Antigen

Copyright © 2025, Tutku Kaçar Şahin et al. This open-access article is available under the Creative Commons Attribution 4.0 (CC BY 4.0) International License (https://creativecommons.org/licenses/by/4.0/), which allows for unrestricted use, distribution, and reproduction in any medium, provided that the original work is properly cited.

How to Cite: Tutku Kaçar Şahin M, Burak Bereketoğlu M, Dogan H, Kose M, Kuscu F, et al. Effect of Promoter Region Polymorphisms in the IP-10 Gene on HBsAg Loss in Chronic Hepatitis B Patients. Hepat Mon. 2025; 25 (1): e160428. https://doi.org/10.5812/hepatmon-160428.

1. Background

Although hepatitis B virus (HBV) infection is preventable through vaccination, it continues to be a major cause of liver-related morbidity and mortality worldwide (1). According to a 2022 report by the World Health Organization (WHO), approximately 254 million people live with chronic HBV infection, which leads to around 1.1 million deaths annually (2). In Turkey, the seroprevalence of hepatitis B is reported to range from 2% to 8% (3). A crucial marker of a positive prognosis in chronic HBV infection is the loss of hepatitis B surface antigen (HBsAg) (4). However, HBsAg clearance is infrequent, primarily due to the persistence of covalently closed circular deoxyribonucleic acid (cccDNA) within hepatocytes. Spontaneous HBsAg loss is estimated to occur at an annual rate of approximately 0.5 - 1% (5). Recent studies have focused on identifying important clinical and viral factors that may help predict the spontaneous clearance of HBsAg. These factors encompass host-related variables such as gender, age, alanine aminotransferase (ALT) levels, presence of fatty liver or cirrhosis, as well as viral features including hepatitis B e antigen (HBeAg) status, HBV deoxyribonucleic acid (DNA) viral load, serum HBsAg levels, HBV genotype, and co-infection with hepatitis C virus (HCV) (6-11). Although these factors offer valuable information, accurately predicting HBsAg loss continues to be challenging. The diverse immune responses seen in chronic HBV patients are thought to be partly due to genetic variability among individuals (12).

Tremendous efforts have identified various host genetic variants influencing HBV infection susceptibility and outcomes, including polymorphisms in human leukocyte antigen (HLA) genes, cytokines, toll-like receptors (TLRs), microRNAs, the sodium taurocholate cotransporting polypeptide (NTCP, the HBV receptor), and vitamin D-related genes. A significant portion of genetic research has concentrated on cytokines and chemokines involved in modulating the immune response against HBV (13). Among these molecules, CXCL10 — also known as interferon gamma (IFN-γ)-inducible protein-10 (IP-10) — has gained attention for its involvement in HBV disease mechanisms. Research indicates that IP-10 facilitates the recruitment of leukocytes from circulation to the liver and stimulates natural killer (NK) cells to produce IFN-γ, contributing to the non-cytolytic clearance of intrahepatic cccDNA (14, 15). Additionally, the G-201A polymorphism located in the promoter region of the IP-10 gene has been associated with disease progression in patients with chronic HBV, likely through its effect on regulating IP-10 expression (16). The polymorphism known as G-201A in Human Genome 19 is specified as -135G/A in Human Genome 38 [(NM_001565.4) c.-135C>T]. Previous studies have examined the relationship between the c.-135C>T polymorphism and disease progression in hepatitis B and C (17-20); however, its direct association with HBsAg loss has not yet been explored.

2. Objectives

To our knowledge, this is the first study to directly investigate the relationship between IP-10 promoter polymorphisms and spontaneous HBsAg clearance, aiming to elucidate a potential genetic mechanism underlying functional cure in chronic HBV infection. Based on the existing data, our study hypothesizes that polymorphisms within the IP-10 promoter region could influence the immune response and HBsAg clearance by modulating IP-10 expression. In addition to this polymorphism, we also aimed to investigate other variants in the IP-10 promoter region potentially associated with HBsAg clearance through sequencing analysis.

3. Methods

3.1. Study Population and Design

This study was conducted as a single-center, observational case-control investigation. Patients included were followed at the Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Cukurova University. A retrospective screening was performed on 1,950 chronic HBV patients who attended the Infectious Diseases and Clinical Microbiology Outpatient Clinic between July 2005 and December 2022. A total of 104 patients with chronic HBV infection who experienced HBsAg loss during follow-up were identified. The inclusion criteria for the study were: Age 18 years or older, diagnosis of chronic HBV infection defined by HBsAg positivity lasting at least 6 months, follow-up at the Infectious Diseases and Clinical Microbiology Outpatient Clinic for a minimum of 1 year, and documentation of at least two consecutive HBsAg negative results at 6-month intervals. Exclusion criteria included acute HBV infection, coinfection with human immunodeficiency virus (HIV) or HCV, liver transplantation, cirrhosis, hepatocellular carcinoma, and patients with transient HBsAg loss. The control group consisted of chronic HBV patients who remained HBsAg positive, met the inclusion criteria, had a similar

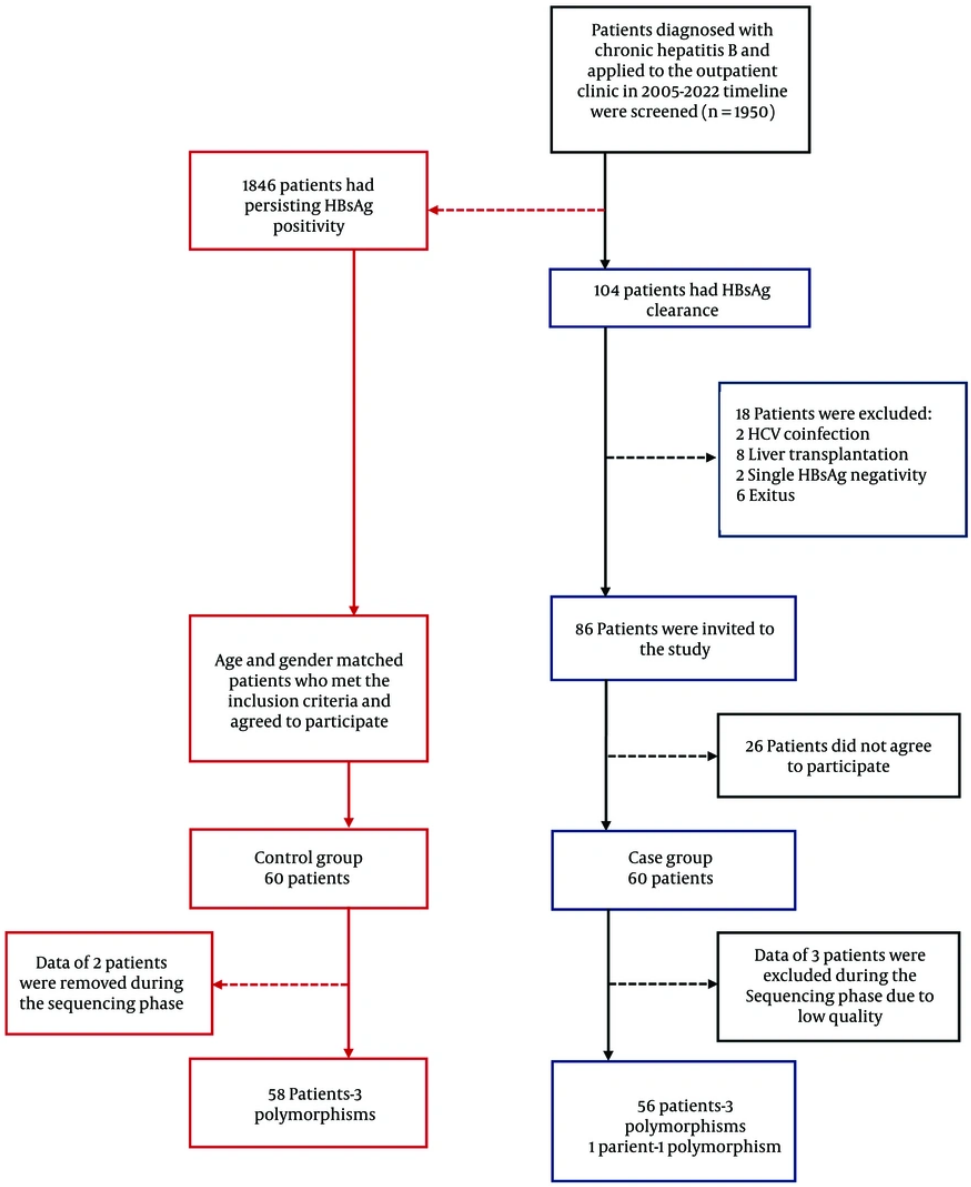

Figure 1. Flowchart of participant selection in this study (Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus).

age and gender distribution as the HBsAg loss group, and consented to participate in the study. These patients were selected from those who applied to the outpatient clinic since the start of the study. A total of 120 patients were included in the study, comprising 60 cases and 60 controls (Figure 1). Due to unsuitable Sanger sequencing results, polymorphism analysis could not be performed for a total of five patients — three from the case group and two from the control group. Peripheral blood samples were collected in vacuum tubes (BD Vacutainer®, England) containing 0.5 molar (M) ethylenediaminetetraacetic acid dipotassium (K2EDTA) (5.4 mg) and processed at the Chest Diseases Molecular Research Laboratory within the Biotechnology Center. The samples were stored at +4°C until DNA extraction. Genomic DNA was isolated, and primers targeting the

Table 1. Demographic Characteristics of the Case and Control Group

Variables	Case (N = 60)	Control (N = 60)	Total (N = 120)	P-Value
Male gender, n (%)	40 (66.7)	40 (66.7)	80 (66.7)	1.000 [a]
Alcohol use, n (%)	4 (6.7)	7 (11.7)	11 (9.2)	0.343 [a]
Cigarette use, n (%)	24 (40)	21 (35)	45 (37.5)	0.572 [a]
Age, mean ± SD	58.6 ± 10.5	57.4 ± 10.8	58.0 ± 10.6	0.522 [b]
Age at diagnosis, median (IQR)	38 (28.3 - 46)	33.5 (24 - 46.8)	35 (25 - 46)	0.105 [c]
HBsAg clearance age, mean ± SD/median (IQR)	53.2 ± 11.3/53 (47 - 60.8)	-	-	-
Body Mass Index, median (IQR)	27.9 (25.5 - 30.9)	27.2(24.8 - 28.4)	27.4 (25.1 - 29.9)	0.139 [c]
HBsAg clearance period, mean ± SD/median (IQR)	15.0 ± 7.3/15 (8 - 21)	-	-	-
Follow-up period, median (IQR)	19.5 (13 - 25)	23 (16 - 25)	22 (14 - 25)	0.199 [c]

Abbreviation: HBsAg, hepatitis B surface antigen.

[a] Chi-square test.

[b] Independent student *t*-test.

[c] Mann-Whitney U test.

Table 2. Comorbidity Distribution in Case and Control Groups [a]

Comorbidity	Case (N = 60)	Control (N = 60)	Total (N = 120)	P-Value [b]
Diabetes mellitus	7 (11.7)	12 (20)	19 (15.8)	0.211
Hypertension	19 (31.7)	17 (28.3)	36 (30)	0.690
Coronary artery disease	6 (10)	2 (3.3)	8 (6.7)	0.143
Malignancy	4 (6.7)	5 (8.3)	9 (7.5)	0.729
Other immunosuppressive diseases	3 (5)	4 (6.7)	7 (5.8)	0.697

[a] Values are expressed as No. (%).

[b] Chi-square.

IP-10 promoter region were designed for mutation analysis. While the primer design targeted the promoter region of the IP-10 gene, sequencing unexpectedly extended into exon 1, intron 1, and exon 2, allowing these regions to be analyzed as well. Based on a comprehensive search of the Genome-Wide Association Studies (GWAS) database and relevant literature, three variants were identified within these regions (c.-135C>T, c.85C>T, and c.83T>G). Sequence data were analyzed specifically with respect to these three variants.

3.2. Isolation of Genomic Deoxyribonucleic Acid from Blood Samples

Blood samples collected from 120 patients in EDTA tubes were centrifuged after the addition of Fixol, and the white layer containing leukocytes was separated. Genomic DNA was isolated using the GeneAll® ExgeneTM Blood SV Mini Kit according to the manufacturer's protocol. The purity and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer, and samples with absorbance ratios of 260/280 nm between 1.8 and 2.0, and 260/230 nm between 2.0 and 2.2 were included in the study.

3.3. Mutation Analyses

A 942-nucleotide segment covering the promoter, exon 1, exon 2, and intron 1 regions of the CXCL10 gene (NM_001565.4) was amplified using conventional polymerase chain reaction (PCR) with the forward primer (5′-3′) GAGGAGCAGAGGGAAATTCCG and reverse primer (5′-3′) CGTGGACAAAATTGGCTTGC. The PCR products were sequenced using Sanger sequencing on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). The sequencing data were analyzed with CLC Genomic Workbench 24 (Qiagen, Hilden, Germany) by aligning to the GRCh38/hg38 reference genome based on the CXCL10 gene (NM_001565.4). During analysis, primer binding sites, the first 20 and last 30 nucleotides, regions with a Phred quality score

Table 3. Baseline Alanine Aminotransferas and Hepatitis B Virus Deoxyribonucleic Acid Levels: Case vs. Control [a]

Parameters	Case (N = 60)	Control (N = 60)	Total (N = 120)	P-Value [b]
Baseline ALT (U/L)	27 (17.3 - 37.5)	26 (20 - 43.3)	26.5 (18 - 39.8)	0.560
Baseline HBV DNA (IU/mL)	223 (25 - 3, 120.5)	1580 (20.25 - 16, 240)	525 (22 - 5, 552.5)	0.134

Abbreviations: ALT, alanine aminotransferase; HBV DNA, hepatitis B virus deoxyribonucleic acid.

[a] Values are expressed as median (IQR).

[b] Mann Whitney U.

below 20, and sequences with background noise exceeding 20% were excluded.

3.4. Statistical Methods and Analyses

The required sample size was estimated using G*Power version 3.1.9.7. Based on this calculation, a minimum of 54 participants per group (total n = 108) was needed to achieve 80% statistical power with a significance level (α) of 0.05. The data were analyzed statistically using the Statistical Package for the Social Sciences (SPSS) version 25.0. Continuous variables were expressed as mean ± standard deviation or median with interquartile range (25th - 75th percentile) where appropriate, while categorical variables were presented as counts and percentages. The chi-square test was used to compare categorical variables. The Shapiro-Wilk test was applied to assess the normality of the data distribution. For normally distributed variables, the Independent Student's *t*-test was used, whereas the Mann-Whitney U test was applied for non-normally distributed variables in comparisons between two groups. Statistical significance was defined as $P < 0.05$ for all analyses.

4. Results

4.1. Characteristics of Patients

The study included a total of 120 patients, with 60 individuals in the case group (HBsAg loss) and 60 in the control group (HBsAg positivity). Among 1,950 chronic HBV patients evaluated between 2005 and 2022, the overall rate of HBsAg loss was 5.33%. There were no statistically significant differences between the case and control groups regarding gender, age, age at diagnosis, Body Mass Index, alcohol consumption, or smoking status ($P > 0.05$). The mean age at the time of HBsAg loss was 53.2±11.3 years, and 85% of patients experienced HBsAg loss after the age of 40. The average duration until HBsAg seronegativity was 15.0±7.3 years, with 65% (n = 39) of patients becoming seronegative after more than 10 years. Table 1 presents the distribution of demographic characteristics in the case and control groups.

The presence of comorbidities was comparable between the case and control groups ($P > 0.05$) (Table 2). Among the seven patients diagnosed with immunosuppressive conditions, one (14.3%) had ankylosing spondylitis, one (14.3%) had multiple sclerosis, one (14.3%) had myasthenia gravis, two (28.6%) had rheumatoid arthritis, and two (28.6%) had undergone renal transplantation.

There was no statistically significant difference in baseline ALT and HBV DNA levels between the case and control groups (Table 3).

Antiviral therapy was significantly more common in the control group ($P < 0.001$). The use of interferon (IFN) and nucleos(t)ide analogs (NAs) combined ($P = 0.042$), as well as NA monotherapy ($P < 0.001$), was also significantly higher among control group patients. Additionally, the duration of antiviral drug use was longer in the control group (Table 4).

4.2. Interferon Gamma-Inducible Protein-10 Polymorphisms

In addition to the c.-135C>T polymorphism identified in the promoter region, two additional polymorphisms — c.85C>T and c.83T>G — were detected in exon 2 of the IP-10 gene. Five patients were excluded from the study due to failure to meet the analytical criteria. Furthermore, in one patient from the case group, the region containing the c.-135C>T polymorphism was deemed suitable for analysis, whereas the regions for the c.85C>T and c.83T>G polymorphisms were not. As a result, only the c.-135C>T polymorphism was assessed in this individual. When the allele frequencies and genotypes of the c.-135C>T, c.85C>T, and c.83T>G polymorphisms were compared between the case and control groups, no statistically significant differences were observed ($P > 0.05$) (Table 5).

5. Discussion

As one of the main causes of cirrhosis, hepatocellular carcinoma, and liver-related mortality globally, chronic

HBV is still a significant infectious illness (21). It is evident that the host immune system plays a pivotal role in determining the development of persistence or clearance during the follow-up period of HBV infection (22). One of the most significant chemokines involved in the proinflammatory response is the IP-10 molecule. Several studies have demonstrated a link between serum IP-10 levels and HBsAg loss. For example, Jaroszewicz et al., Wong et al., and Yuan et al. have all reported that IP-10 levels — either elevated or reduced depending on the disease stage and sampling time — are significantly associated with the likelihood of HBsAg clearance (23-25). In our study, we examined the association between HBsAg loss and polymorphisms in the promoter, exon 1, intron 1, and exon 2 regions of the IP-10 gene in patients with chronic hepatitis B infection. While our study did not demonstrate a statistically significant association between the c.-135C>T, c.85C>T, and c.83T>G polymorphisms in the IP-10 gene and HBsAg loss, this finding should not be interpreted as conclusive evidence against a role for IP-10 in HBV immunity. Rather, our results suggest that these specific variants may have limited predictive value within the context of our cohort. The IP-10 is known to play a complex role in antiviral immune regulation, and its expression and function are likely influenced by a broader network of gene-environment interactions, epigenetic mechanisms, and immune signaling pathways. The lack of association observed in our study may reflect the multifactorial nature of HBsAg clearance, which cannot be explained by individual single nucleotide polymorphisms (SNPs) alone.

The rates of HBsAg clearance differ depending on the cohort studied and the inclusion criteria applied. For example, Buechter et al. followed 371 chronic HBV patients for approximately 12 years and reported an HBsAg loss rate of 7.8% (26). Similarly, Habersetzer et al. conducted a six-year follow-up study involving 315 chronic HBV patients in France, observing an HBsAg clearance rate of 9.2% (27). On the other hand, Chien et al. tracked 235 HBeAg-negative chronic HBV patients over seven years and found a lower HBsAg loss rate of 3.4% (28). In our own study, among 1,950 patients monitored from 2005 to 2022, 5.33% experienced HBsAg clearance. Consistent with previous research, these differences are likely influenced by multiple factors such as patient demographics, ethnic background, disease stage, duration of follow-up, and use of antiviral therapies. Studies have shown that HBsAg clearance is associated with older age and longer follow-up. Chu and Liaw demonstrated that clearance rates rise over time (6), and Buechter et al. reported a mean seroconversion period of 12 years (26). In line with these findings, our study observed a median HBsAg loss time of 15 years, with most cases occurring after 10 years. We also found that clearance was more frequent in individuals over 40. A large cohort study and a separate study from South Korea both reported higher rates of HBsAg seroclearance in patients above 40 compared to younger individuals (29, 30). In our cohort, the average age at the time of HBsAg loss was 53.2 years, and 85% of the cases occurred in patients older than 40. These results highlight age and follow-up duration as important factors in predicting HBsAg clearance.

Prior investigations suggest that certain SNPs in the IP-10 gene promoter are linked to the severity of infectious diseases like pulmonary tuberculosis and cerebral malaria (17). The c.-135C>T (also referred to as G-201A in earlier genome builds) variant has been shown in multiple studies to influence IP-10 expression levels, supported by findings from GWAS (31), protein QTL (32), and its association with HBV and HCV infections. While Talaat et al. reported no correlation between IP-10 polymorphisms and disease progression in chronic HCV patients, Thanapirom et al. highlighted the importance of the -135G/A polymorphism in achieving a sustained virologic response to pegylated IFN therapy (17, 18). The A allele of the G-201A polymorphism in the promoter region of the IP-10 gene has been associated with increased disease severity and progression. In a large-scale study conducted in China involving 2,400 individuals with chronic HBV infection, Deng et al. reported a higher frequency of disease progression among male carriers of the A allele (19). Similarly, Xu et al. found that this polymorphism was linked to liver disease development in HBV-infected patients, with the A allele being more prevalent in those with advanced disease (20). A separate study from Thailand demonstrated an association between the G-201A variant and both reduced HBsAg levels and improved virologic response to pegylated IFN therapy. Patients carrying the GG genotype exhibited lower HBsAg levels and higher response rates; however, no significant difference in HBsAg clearance rates was observed between GG and non-GG genotypes (16). Collectively, these studies suggest that the c.-135C>T polymorphism has previously been examined in the context of disease progression in HCV and HBV, as well as in relation to HBsAg level changes during IFN-based therapy. In contrast, our study directly investigated the association between this polymorphism and HBsAg clearance, finding no statistically significant correlation. Interestingly, we also identified a previously unreported CA genotype in one individual from the control group. Further genetic and functional analyses are needed to determine the clinical significance and potential

Table 4. Comparison of Treatment Use Between Case and Control Groups [a]

Parameters	Case (N = 60)	Control (N = 60)	Total (N = 120)	P-Value
Antiviral use	19 (31.7)	49 (81.7)	68 (56.7)	< 0.001 [b]
IFN and NA use	6 (10.0)	14 (23.3)	20 (16.7)	0.042 [b]
NA use only	13 (21.7)	34 (56.7)	47 (39.2)	< 0.001 [b]
IFN use only	-	1 (1.7)	1 (0.8)	0.315 [b]
IFN and NA use period	9.5 (6.25 - 12.0)	16.8 (13.3 - 24.1)	15.0 (10.5 - 21.0)	0.011 [c]
NA use only period	5.5 (3.75 - 8.0)	9.0 (6.0 - 15.0)	7.0 (5.0 - 13.5)	0.010 [c]

Abbreviations: IFN, interferon; NA, nucleos(t)ide analogs.

[a] Values are expressed as No. (%) or median (IQR).

[b] Chi-square test.

[c] Mann-Whitney U test.

biological implications of this novel variant. Although c.85C>T has not been linked to HBV, its potential role has been investigated in GWAS and protein QTL studies (31, 33). A study investigating the influence of genetic and lifestyle factors on circulating biomarkers related to inflammation and cancer identified the c.85C>T polymorphism in the IP-10 gene (34). In our research, neither the c.85C>T nor the c.83T>G polymorphism showed a significant association with HBsAg loss. The c.83T>G variant is a novel variant not reported in gnomAD v4.1, TOPMed, or the Turkish Variome, and we considered it potentially significant based on our observations. Therefore, we believe that our study is the first to evaluate the potential association of the c.-135C>T, c.85C>T, and c.83T>G polymorphisms with HBsAg loss in patients with chronic HBV infection. Given the absence of significant associations between the studied IP-10 polymorphisms and HBsAg loss, several methodological and biological factors should be considered. One key limitation is the relatively small sample size, which may have limited the statistical power to detect associations, particularly for low-frequency variants. The SNPs with low minor allele frequencies often require larger cohorts to reach adequate power, especially when investigating complex traits such as viral clearance. Additionally, population-specific genetic backgrounds may have influenced our results.

For instance, the allele frequency of the c.-135C>T polymorphism is reported as 0.402% in the Turkish population according to gnomAD v4.1, whereas it was 18.3% in our cohort. Similarly, the c.85C>T variant has a reported allele frequency of 10.737% in gnomAD, compared to 1.75% in our study population. These discrepancies likely reflect differences between the general population and disease-specific cohorts and may also point to underlying ethnic substructure within the Turkish population (35, 36). Moreover, previous studies that reported associations between IP-10 polymorphisms and HBV-related outcomes were primarily conducted in East Asian populations, where allele distributions and linkage disequilibrium patterns differ substantially from those in our cohort (19, 20). Therefore, population-specific factors must be taken into account when interpreting genetic association studies across diverse ethnic backgrounds. Beyond sample size and population variability, it is also important to consider the biological complexity of HBsAg clearance when interpreting these findings. The investigated polymorphisms may exert only a modest or indirect influence on IP-10 function or expression. HBsAg clearance is a multifactorial event involving a complex interplay between host genetics, immune response, viral factors, and treatment exposure. It is plausible that these individual variants alone are not sufficient to predict seroclearance without considering gene-gene and gene-environment interactions (37).

In addition to genetic and population-specific factors, treatment-related variables may have influenced our findings. Although we observed a significantly lower frequency and shorter duration of antiviral therapy among patients with HBsAg loss, treatment effects were not fully controlled for in the analysis. Given that antiviral therapy is a major modulator of immune-mediated viral clearance, such differences in treatment exposure may have confounded the genetic associations under investigation (38). Another important limitation of our study is the lack of functional analysis to support the observed genetic findings. Our investigation focused solely on genotyping, without evaluating the potential functional consequences of the identified polymorphisms — such

Table 5. Allele and Genotype Frequencies in Case and Control Groups [a]

Variant	Case (N = 57)	Control (N = 58)	Total (N = 115)	P-Value [b]
c.-135C>T genotype/allele				
CC	37 (61.7)	41 (68.3)	78 (65)	0.513
CT	18 (30)	12 (20)	30 (25)	0.513
TT	2 (3.3)	4 (6.7)	6 (5)	0.513
CA	-	1 (1.7)	1 (0.8)	-
C alleles	92 (80.7)	95 (81,9)	187 (81.3)	0.816
T alleles	22 (19.3)	20 (17.2)	42 (18.3)	0.952
A alleles	-	1 (0.9)	1 (0.4)	0.320
Variant	**Case (N = 56)**	**Control (N = 58)**	**Total (N = 114)**	**P-Value [b]**
c.85C>T genotype/allele				
CC	55 (91.7)	55 (91.7)	110 (91.7)	0.435
CT	1 (1.7)	3 (5)	4 (3.3)	0.435
C alleles	111 (99.1)	113 (97.4)	224 (98.2)	0.983
T alleles	1 (0.9)	3 (2.6)	4 (1.75)	0.322
c.83T>G genotype/allele				
TT	50 (83.3)	54 (90)	104 (86.7)	0.543
TG	6 (10)	4 (6.7)	10 (8.3)	0.543
T alleles	106 (94.6)	112 (96.55)	218 (95.6)	0.224
G alleles	6 (5.35)	4 (3.4)	10 (4.4)	0.500

[a] Values are expressed as No. (%).

[b] Chi-square.

as their effects on IP-10 mRNA or protein expression. However, functional validation through transcriptomic, proteomic, or promoter activity assays is essential to establish a mechanistic link between genotype and phenotype. In the absence of such data, the biological relevance of these variants remains speculative (20). Furthermore, the identification of a previously unreported genotype (CA at c.-135) and a novel SNP (c.83T>G) indicates additional genetic diversity within the IP-10 locus that has not been functionally characterized. These rare or population-specific variants may exert unique immunological effects that warrant further investigation in larger, well-characterized cohorts.

Overall, this study provides valuable negative data by demonstrating that the investigated polymorphisms are not reliable predictive markers for clinical outcomes in chronic hepatitis B. The lack of a clear association underscores the complex interplay of host genetic factors in immune-mediated viral clearance, which likely involves multiple genes and signaling pathways beyond IP-10. These findings further highlight the importance of investigating other genetic and immunological determinants — such as cytokine gene polymorphisms, HLA alleles, and components of the innate immune response — to better elucidate the mechanisms driving both spontaneous and treatment-induced HBsAg loss.

5.1. Conclusions

In conclusion, our findings indicate that the c.-135C>T, c.85C>T, and c.83T>G polymorphisms in the IP-10 gene are not significantly associated with HBsAg loss in patients with chronic hepatitis B. However, this result should not be interpreted as definitive evidence against the involvement of IP-10 in HBV-related immune responses. Given the complexity of HBsAg clearance, these specific SNPs may have limited predictive value in isolation. Moreover, the identification of a novel variant (c.83T>G) and an unreported genotype (CA at c.-135) suggests underlying genetic diversity within the IP-10 locus that warrants further exploration. To validate these findings and explore potential population-specific effects, future studies with larger and more ethnically diverse cohorts are needed. Furthermore, in-depth investigations should be conducted to assess the combined effects of IP-10 expression levels and promoter region polymorphisms on HBsAg clearance and treatment response. As a future direction, we also recommend integrating functional assays and epigenomic analyses, such as DNA methylation profiling

of the IP-10 promoter region, to better understand the regulatory mechanisms that may influence HBsAg loss. Additionally, employing omics approaches, such as transcriptomics and proteomics, to comprehensively investigate the effects of the IP-10 gene may contribute to a deeper understanding of the biological pathways influencing HBsAg loss.

Footnotes

Authors' Contribution: Study concept and design: M. T. K. S. and A. C.; Acquisition of data: M. T. K. S., B. K., A. S. I., and S. K.; Performance of the experiments: H. D., M. K., M. B. B., and Y. S.; Analysis and interpretation of data: Y. S. and M. B. B.; Drafting of the manuscript: M. T. K. S.; Critical revision of the manuscript for important intellectual content: A. C., Y. S., F. K., and D. E.; Statistical analysis: A. C.; Study supervision: A. C., Y. S., and Y. T.

Conflict of Interests Statement: The authors declare no conflict of interest.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication. The data are not publicly available due to institutional restrictions.

Ethical Approval: This study is approved under the ethical approval code of 2023-129/24 .

Funding/Support: This study was supported by Cukurova University Scientific Research Projects Fund as project number TTU-2023-15769 .

Informed Consent: Participants were informed about the purpose and content of the study before participating in the study, and their consent was obtained.

References

1. Huang CW, Yang CT, Su PY, Chen YY, Huang SP, Yen HH. Long-Term Hepatitis B Surface Antigen Profile and Seroclearance Following Antiviral Treatment: A Single-Center, Real-World Cohort Study. *Biomedicines*. 2023;**11**(11). [PubMed ID: 38001966]. [PubMed Central ID: PMC10669103]. https://doi.org/10.3390/biomedicines11112966.
2. World Health Organization. *Hepatitis B*. Geneva, Switzerland: World Health Organization; 2024. Available from: ttps://www.who.int/news-room/fact-sheets/detail/hepatitis-b.
3. Alp İ, ÖZtÜRk EngİN D, OĞUzoĞLu N, İNan A, Ceran N, DenİZlİ N, et al. Risk Factors and Seroprevalence of Hepatitis B, C, and D Virus in Hemodialysis Patients in Istanbul. *Mediterranean J Infect Microb Antimicrob*. 2014;**3**(1):1-6. https://doi.org/10.5578/mjima.6730.
4. Wong GLH, Gane E, Lok ASF. How to achieve functional cure of HBV: Stopping NUCs, adding interferon or new drug development? *J Hepatol*. 2022;**76**(6):1249-62. [PubMed ID: 35589248]. https://doi.org/10.1016/j.jhep.2021.11.024.
5. Zhu L, Zhai X, Wang Q, Jiang J, Peng H, Song C, et al. Incidence and determinants of spontaneous hepatitis B surface antigen seroclearance and seroconversion in hepatitis B e antigen-negative chronic infection patients: A population-based prospective cohort. *J Viral Hepat*. 2018;**25**(12):1588-98. [PubMed ID: 30112835]. https://doi.org/10.1111/jvh.12978.
6. Chu CM, Liaw YF. Hepatitis B surface antigen seroclearance during chronic HBV infection. *Antivir Ther*. 2010;**15**(2):133-43. [PubMed ID: 20386068]. https://doi.org/10.3851/IMP1497.
7. Ferreira SC, Chacha SG, Souza FF, Teixeira AC, Santana RC, Villanova MG, et al. Factors associated with spontaneous HBsAg clearance in chronic hepatitis B patients followed at a university hospital. *Ann Hepatol*. 2014;**13**(6):762-70. [PubMed ID: 25332262].
8. Chu CM, Lin DY, Liaw YF. Does increased body mass index with hepatic steatosis contribute to seroclearance of hepatitis B virus (HBV) surface antigen in chronic HBV infection? *Int J Obes (Lond)*. 2007;**31**(5):871-5. [PubMed ID: 17047638]. https://doi.org/10.1038/sj.ijo.0803479.
9. Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology*. 2002;**123**(6):1848-56. [PubMed ID: 12454842]. https://doi.org/10.1053/gast.2002.37041.
10. McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med*. 2001;**135**(9):759-68. [PubMed ID: 11694101]. https://doi.org/10.7326/0003-4819-135-9-200111060-00006.
11. Song A, Lin X, Chen X. Functional cure for chronic hepatitis B: accessibility, durability, and prognosis. *Virol J*. 2021;**18**(1):114. [PubMed ID: 34082765]. [PubMed Central ID: PMC8176700]. https://doi.org/10.1186/s12985-021-01589-x.
12. Seto WK, Wong DK, Kopaniszen M, Proitsi P, Sham PC, Hung IF, et al. HLA-DP and IL28B polymorphisms: influence of host genome on hepatitis B surface antigen seroclearance in chronic hepatitis B. *Clin Infect Dis*. 2013;**56**(12):1695-703. [PubMed ID: 23449268]. https://doi.org/10.1093/cid/cit121.
13. Xu J, Zhan Q, Fan Y, Yu Y, Zeng Z. Human genetic susceptibility to hepatitis B virus infection. *Infect Genet Evol*. 2021;**87**:104663. [PubMed ID: 33278635]. https://doi.org/10.1016/j.meegid.2020.104663.
14. Zhong S, Zhang T, Tang L, Li Y. Cytokines and Chemokines in HBV Infection. *Front Mol Biosci*. 2021;**8**:805625. [PubMed ID: 34926586]. [PubMed Central ID: PMC8674621]. https://doi.org/10.3389/fmolb.2021.805625.
15. Cornberg M, Wiegand SB. ImPortance of IP-10 in hepatitis B. *Antivir Ther*. 2016;**21**(2):93-6. [PubMed ID: 26598599]. https://doi.org/10.3851/IMP3014.
16. Limothai U, Chuaypen N, Khlaiphuengsin A, Posuwan N, Wasitthankasem R, Poovorawan Y, et al. Association of interferon-gamma inducible protein 10 polymorphism with treatment response to pegylated interferon in HBeAg-positive chronic hepatitis B. *Antivir Ther*. 2016;**21**(2):97-106. [PubMed ID: 26376789]. https://doi.org/10.3851/IMP2992.
17. Talaat RM, Elsharnoby S, Abdelkhalek MS, El-Shenawy SZ, Elmasry S. The Impact of Interferon-gamma (IFN-gamma) and IFN-gamma-Inducible Protein 10 (IP-10) Genes' Polymorphism on Risk of Hepatitis C Virus-Related Liver Cirrhosis. *Immunol Invest*. 2022;**51**(3):688-704. [PubMed ID: 33445993]. https://doi.org/10.1080/08820139.2020.1869251.
18. Thanapirom K, Suksawatamnuay S, Sukeepaisarnjaroen W, Tangkijvanich P, Treeprasertsuk S, Thaimai P, et al. Association between CXCL10 and DPP4 Gene Polymorphisms and a Complementary Role for Unfavorable IL28B Genotype in Prediction of Treatment Response in Thai Patients with Chronic Hepatitis C Virus Infection. *PLoS One*. 2015;**10**(9). e0137365. [PubMed ID:

26339796]. [PubMed Central ID: PMC4560372]. https://doi.org/10.1371/journal.pone.0137365.

19. Deng G, Zhou G, Zhang R, Zhai Y, Zhao W, Yan Z, et al. Regulatory polymorphisms in the promoter of CXCL10 gene and disease progression in male hepatitis B virus carriers. *Gastroenterology*. 2008;**134**(3):716-26. [PubMed ID: 18325387]. https://doi.org/10.1053/j.gastro.2007.12.044.
20. Xu Z, Liu Y, Liu L, Li X, Bai S, Rong Y, et al. Association of interferon-gamma induced protein 10 promoter polymorphisms with the disease progression of hepatitis B virus infection in Chinese Han population. *PLoS One*. 2013;**8**(9). e72799. [PubMed ID: 24023775]. [PubMed Central ID: PMC3762918]. https://doi.org/10.1371/journal.pone.0072799.
21. Toy M, Onder FO, Wormann T, Bozdayi AM, Schalm SW, Borsboom GJ, et al. Age- and region-specific hepatitis B prevalence in Turkey estimated using generalized linear mixed models: a systematic review. *BMC Infect Dis*. 2011;**11**:337. [PubMed ID: 22151620]. [PubMed Central ID: PMC3262158]. https://doi.org/10.1186/1471-2334-11-337.
22. Saraceni C, Birk J. A Review of Hepatitis B Virus and Hepatitis C Virus Immunopathogenesis. *J Clin Transl Hepatol*. 2021;**9**(3):409-18. [PubMed ID: 34221927]. [PubMed Central ID: PMC8237136]. https://doi.org/10.14218/JCTH.2020.00095.
23. Jaroszewicz J, Ho H, Markova A, Deterding K, Wursthorn K, Schulz S, et al. Hepatitis B surface antigen (HBsAg) decrease and serum interferon-inducible protein-10 levels as predictive markers for HBsAg loss during treatment with nucleoside/nucleotide analogues. *Antivir Ther*. 2011;**16**(6):915-24. [PubMed ID: 21900724]. https://doi.org/10.3851/IMP1866.
24. Wong GL, Chan HL, Chan HY, Tse CH, Chim AM, Lo AO, et al. Serum interferon-inducible protein 10 levels predict hepatitis B s antigen seroclearance in patients with chronic hepatitis B. *Aliment Pharmacol Ther*. 2016;**43**(1):145-53. [PubMed ID: 26526395]. https://doi.org/10.1111/apt.13447.
25. Yuan X, Fu T, Xiao L, He Z, Ji Z, Seery S, et al. Describing immune factors associated with Hepatitis B surface antigen loss: A nested case-control study of a Chinese sample from Wuwei City. *Front Immunol*. 2022;**13**:1025654. [PubMed ID: 36304473]. [PubMed Central ID: PMC9592898]. https://doi.org/10.3389/fimmu.2022.1025654.
26. Buechter M, Gunther AM, Manka P, Gerken G, Kahraman A. Factors Positively Correlated with Hepatitis B Surface Antigen Seroconversion in Chronic Hepatitis B. *J Pers Med*. 2024;**14**(4). [PubMed ID: 38673017]. [PubMed Central ID: PMC11051014]. https://doi.org/10.3390/jpm14040390.
27. Habersetzer F, Moenne-Loccoz R, Meyer N, Schvoerer E, Simo-Noumbissie P, Dritsas S, et al. Loss of hepatitis B surface antigen in a real-life clinical cohort of patients with chronic hepatitis B virus infection. *Liver Int*. 2015;**35**(1):130-9. [PubMed ID: 25145784]. https://doi.org/10.1111/liv.12661.
28. Chien TL, Wang JH, Kee KM, Chen CH, Hung CH, Lu SN. Factors Predicting HBsAg Seroclearance and Alanine Transaminase Elevation in HBeAg-Negative Hepatitis B Virus-Infected Patients with Persistently Normal Liver Function. *PLoS One*. 2016;**11**(12). e0166543. [PubMed ID: 27935953]. [PubMed Central ID: PMC5147825]. https://doi.org/10.1371/journal.pone.0166543.
29. Yeo YH, Tseng TC, Hosaka T, Cunningham C, Fung JYY, Ho HJ, et al. Incidence, Factors, and Patient-Level Data for Spontaneous HBsAg Seroclearance: A Cohort Study of 11,264 Patients. *Clin Transl Gastroenterol*. 2020;**11**(9). e00196. [PubMed ID: 33094953]. [PubMed Central ID: PMC7494149]. https://doi.org/10.14309/ctg.0000000000000196.
30. Park YM, Lee SG. Clinical features of HBsAg seroclearance in hepatitis B virus carriers in South Korea: A retrospective longitudinal study. *World J Gastroenterol*. 2016;**22**(44):9836-43. [PubMed ID: 27956808]. [PubMed Central ID: PMC5124989]. https://doi.org/10.3748/wjg.v22.i44.9836.
31. The NHGRI EBI GWAS Catalog. *CXCL10*. 2025, [cited 2025]. Available from: https://www.ebi.ac.uk/gwas/genes/CXCL10.
32. Wang Y, Zhou Q, Dong L, Xiong M, Jiang H, Guo M, et al. The effects of CXCL10 polymorphisms on COPD susceptibility. *Mol Genet Genomics*. 2018;**293**(3):649-55. [PubMed ID: 29285564]. https://doi.org/10.1007/s00438-017-1408-z.
33. Zhao JH, Stacey D, Eriksson N, Macdonald-Dunlop E, Hedman AK, Kalnapenkis A, et al. Author Correction: Genetics of circulating inflammatory proteins identifies drivers of immune-mediated disease risk and therapeutic targets. *Nat Immunol*. 2023;**24**(11):1960. [PubMed ID: 37679551]. [PubMed Central ID: PMC10602847]. https://doi.org/10.1038/s41590-023-01635-6.
34. Enroth S, Johansson A, Enroth SB, Gyllensten U. Strong effects of genetic and lifestyle factors on biomarker variation and use of personalized cutoffs. *Nat Commun*. 2014;**5**:4684. [PubMed ID: 25147954]. [PubMed Central ID: PMC4143927]. https://doi.org/10.1038/ncomms5684.
35. Hong EP, Park JW. Sample size and statistical power calculation in genetic association studies. *Genomics Inform*. 2012;**10**(2):117-22. [PubMed ID: 23105939]. [PubMed Central ID: PMC3480678]. https://doi.org/10.5808/GI.2012.10.2.117.
36. Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, et al. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*. 2024;**625**(7993):92-100. [PubMed ID: 38057664]. [PubMed Central ID: PMC11629659]. https://doi.org/10.1038/s41586-023-06045-0.
37. Nebbia G, Peppa D, Maini MK. Hepatitis B infection: current concepts and future challenges. *QJM*. 2012;**105**(2):109-13. [PubMed ID: 22252919]. [PubMed Central ID: PMC3259419]. https://doi.org/10.1093/qjmed/hcr270.
38. Rehermann B, Bertoletti A. Immunological aspects of antiviral therapy of chronic hepatitis B virus and hepatitis C virus infections. *Hepatology*. 2015;**61**(2):712-21. [PubMed ID: 25048716]. [PubMed Central ID: PMC4575407]. https://doi.org/10.1002/hep.27323.