J Appl Biomed
DOI: 10.32725/jab.2025.017
journal homepage: http://jab.zsf.jcu.cz

Journal of Applied Biomedicine

Original research article

Relationship between interleukin-37 genetic polymorphisms and HBV-related liver disease in a Chinese Han cohort

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Abstract
Hepatitis B virus (HBV)-related liver disease is an inflammatory-associated disease, with diverse clinical phenotypes ranging from asymptomatic HBV carriers to hepatocellular carcinoma. Interleukin-37 (IL-37), a cytokine that effectively inhibits innate and adaptive immunity, has powerful anti-inflammatory and anti-tumor effects. Several single nucleotide polymorphisms (SNPs) in the *IL37* gene are genetic predictive risk factors for HBV infection and HBV-mediated liver disease progression. However, different ethnic groups may have different allele frequencies and linkage disequilibrium structures. The effect of SNPs in *IL37* on HBV infection and its relationship with different clinical outcomes have not been clarified among the Han people in southern China. Based on *in silico* functional prediction and previously reported in the literature to be potentially associated with diseases, we screened seven potentially functional SNPs (rs3811046, rs3811047, rs2723176, rs2723186, rs4611652, rs4392270, and rs4241122) located in the *IL37* genomic region and 3-kb upstream and downstream of the gene body. 1,582 subjects were included in the study, including 747 patients with HBV-related liver disease, 405 patients who cleared HBV, and 430 healthy controls. The seven SNPs were genotyped using the SNaPshot SNP assay, and co-dominant, dominant, and recessive models were used to explore the association of each SNP with HBV infection and clinical outcomes after HBV infection. The rs4241122 demonstrated a significant association with both HBV infection and its clinical outcomes, with the GG genotype identified as an independent protective factor for spontaneous clearance of HBV. The rs2723186 and rs4392270 were also significantly associated with HBV clearance under specific genetic models. Furthermore, rs3811046 and rs3811047 were correlated with the progression of liver abnormalities following HBV infection. Our data suggests that SNPs at the *IL37* locus are associated with susceptibility to HBV infection and clinical outcomes after HBV infection.

Keywords: Chinese; Hepatitis B virus; Interleukin-37; Single-nucleotide polymorphisms; SnaPshot

Highlights:
- SNPs at the *IL37* locus were associated with HBV infection.
- SNPs at the *IL37* locus were associated with HBV clearance.
- SNPs at the *IL37* locus were also correlated with the progression of liver abnormalities after HBV infection.

Introduction

Hepatitis B virus (HBV) is a hepatotropic DNA virus that can be transmitted from mother to child and in blood and body fluids. Although most patients with HBV infection clear the virus within six months, approximately 5–10% of patients become chronically infected (Cheng et al., 2023). HBV-related liver disease refers to a variety of liver diseases caused by chronic and persistent HBV infection, including chronic hepatitis B, cirrhosis, and hepatocellular carcinoma (HCC) (Cheng et al., 2023). China has a large burden of HBV infection (Liu et al., 2023), and the proportion of HCC caused by HBV-persistent infection is as high as 86% (Wang et al., 2017), which seriously endangers the life and health of Chinese people.

However, the etiology of HBV infection and clearance is complex, and the exact mechanism is not clear. The clinical phenotype of individuals infected with HBV is diverse: from self-limited infection to acute hepatitis, asymptomatic viral carrier (chronic inactive hepatitis), chronic active hepatitis, and even cirrhosis and HCC. At present, most studies suggest that HBV itself and environmental factors do not fully explain

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http://doi.org/10.32725/jab.2025.017

Submitted: 2025-01-24 • Accepted: 2025-10-24 • Prepublished online: 2025-10-30
J Appl Biomed 23/4: 184–196 • EISSN 1214-0287 • ISSN 1214-021X

the clinical phenotypic differences in HBV-related liver diseases. The interaction between HBV, the host immune response and the molecular genetic mechanism of inflammatory cytokines may be important factors affecting different clinical outcomes after HBV infection (Al-Anazi et al., 2019; Cheng et al., 2018). Cellular innate immunity is the host's first line of defense against HBV infection. After infection, HBV can use an escape mechanism to evade the surveillance of the immune system (Yang et al., 2022). The host's immune response to virus infection leads to liver cell damage, lost ability to clear the HBV, and the patient becoming a long-term virus carrier (Asín-Prieto et al., 2019; Li et al., 2019a). Additionally, chronic HBV-persistent infection can inhibit adaptive immune cells, causing dysfunction of the adaptive immune response (Li et al., 2019b).

Interleukin 37 (IL-37) is a member of the IL-1 family and a cytokine that effectively inhibits innate and adaptive immunity (Allam et al., 2020). IL-37 plays an important protective role in the development of infectious, allergic, metabolic, and autoimmune diseases and tumors through its powerful anti-inflammatory and anti-tumor effects (Gu et al., 2023; Jiang et al., 2023; Su and Tao, 2021). Several studies in China have reportedly shown significant associations between genetic variants in the *IL37* gene and several diseases (Liu et al., 2017; Tan et al., 2016; Yan et al., 2015; Yin et al., 2017). The *IL37* serum level increased significantly in the patients with chronic hepatitis B (Liu et al., 2023), and decreased significantly after antiviral treatment (Meng et al., 2020). Al-Anazi et al. (2019) reported that SNPs in *IL37* were closely related to HBV infection and HBV clearance in the Saudi Arabian population. However, SNPs in *IL37* did not correlate with chronic HBV infection in the Han population in central China (Cheng et al., 2023). The rs4241122 was not a valuable biomarker for predicting liver disease progression in HBV-infected patients in the Iranian population (Molaei et al., 2023).

Since these studies involved different populations and diseases, differences in their results could be attributed to differences in ethnicity or pathological disease mechanisms. In this study, based on the 1000 Genomes Project Database (GPD) and functional prediction using bioinformatics databases and reported in the literature, we screened potentially functional SNPs at the *IL37* locus. Then, we investigated whether these SNPs are associated with susceptibility to HBV infection and the risk of different clinical outcomes after HBV infection in the Han population in southern China.

Materials and methods

SNP selection

SNPs in the *IL37* gene were downloaded from the 1000 GPD (http://www.internationalgenome.org). All genetic variants with a minor allele frequency (MAF) >0.05 and <1 and located within the *IL37* genomic region plus 3 kb upstream and downstream of the gene (chromosome 2:113,670,548–113,676,459, GRCh37), 21 SNPs, were obtained. The 21 SNPs were further evaluated with the Ensembl, GTEx, Combined Annotation Dependent Depletion, Regulome DB, genome-wide annotation of variants, PolyPhen-2, and Varcards databases. The SNPs were prioritized using the annotation databases described above. The SNPs that were predicted to be functional in at least one of the annotation tools were selected as candidate SNPs (rs3811046, rs4611652, and rs4241122). Additionally, a literature search was conducted on these 21 SNPs to screen for reported SNPs that may be associated with diseases. Four SNPs reported to be possibly associated with diseases were selected as supplementary candidate SNPs (rs4392270, rs3811047, rs2723176, and rs2723186). However, as this study is preliminary and exploratory in nature, practical constraints such as genotyping costs and research timeline necessitated a phased analytical strategy: priority was given to genotyping and analyzing SNPs with reliable functional predictions or existing literature support. By integrating these two approaches, a total of seven SNPs were ultimately included in this study for subsequent experiments and evaluation.

Patients

1,582 subjects meeting the inclusion criteria were selected from the Huadu District People's Hospital of Guangzhou, including 430 controls, 405 patients who cleared HBV, 338 patients with inactive HBV infection, 269 patients with active HBV infection, 102 patients with HBV-related cirrhosis, and 38 patients diagnosed with HBV-related HCC. Among these, patients with inactive HBV infection, active HBV infection, HBV-related cirrhosis, and HBV-related HCC were collectively referred to as HBV-related liver disease. Controls were characterized by the absence of any known serological markers for HBV. Patients who cleared HBV without a hepatitis B virus treatment history were defined by being positive for HBsAb, HBcAb, and HBeAb, but negative for HBsAg, HBeAg, and HBV DNA. Patients with inactive HBV infection were defined by being positive for HBsAg, HBcAb, and HBeAb but negative for HBsAb, HBeAg, and HBV DNA. Patients with active HBV infection were defined by being positive for HBsAg, HBeAg and/or HBeAb, and HBV DNA. Viral markers of both patients with HBV-related cirrhosis and HCC were defined by being positive for HBsAg, or negative for HBsAg but with a clear history of hepatitis B infection. In addition to the laboratory and clinical diagnosis of cirrhosis and HCC, cirrhosis was confirmed by CT, ultrasound and/or MRI imaging, and HCC was confirmed by pathological diagnosis. All subjects were Han from southern China, and peripheral blood DNA was extracted for genotyping. Informed consent was obtained from all participating individuals, and the study was approved by the Medical Ethics Committee of Huadu District People's Hospital of Guangzhou (2022070).

SNaPshot SNP typing

DNA was extracted from peripheral blood leukocytes using a standard phenol/chloroform method. DNA quantity and quality were evaluated using a nucleic acid quantifier (NanoDrop 2000 spectrophotometer, ThermoFisher) and 2% agarose gel electrophoresis. The seven selected SNPs were genotyped using SNaPshot SNP typing. Polymerase chain reaction (PCR) was performed with Applied Biosystems (2720). The forward primers, reverse primers, and SNaPshot PCR primers of each SNP are shown in Table 1, and the specific operations were as previously reported (Fang et al., 2021).

Statistical analysis

In this study, we conducted case-control tests on the seven selected SNPs in six subgroups: negative controls vs. cases with HBV-related liver disease to explore genetic factors associated with HBV-related liver disease; patients with HBV clearance vs. those with HBV-related liver disease to explore the genetic factors related to HBV clearance; patients with inactive hepatitis vs. those with HBV severe liver disease (including active hepatitis, cirrhosis, and HCC) to explore genetic factors related to severe HBV-related liver disease; patients with hepatitis and cirrhosis vs. those with HBV-HCC to explore genetic factors

Table 1. Sequences of forward primers, reverse primers, and SNaPshot PCR primers of 7 SNPs

SNPs	Sequences
Forward primers and reverse primers	
rs3811046-rs3811047-F	CCAAACCCGTGATCCAGTTC
rs3811046-rs3811047-R	AAAGACTTCAGCCCCATCCA
rs2723176-F	TCAAACGTCAGCAGCATCAG
rs2723176-R	AGTGGTGTTCTCGCTCATGA
rs2723186-F	CTGTGTTTTCTGCCTCCACC
rs2723186-R	ATGTGTGGGTTCTGAGAGCA
rs4611652-F	ACGCCTTCCTCGCTAATTTG
rs4611652-R	TTGGCTGGATTGTGACTTGC
rs4241122-rs4392270-F	TAGCAGAAAGGAGATGGGGC
rs4241122-rs4392270-R	CTGTGTTAGTGCAGGGGTTG
SNaPshot PCR primers	
rs3811046	TTTTTTTTTTTTTTTTTTTTTTGACTGCAGACCCGGCTG
rs2723186	TTTTTTTTTTTTTTTTTTTTTGGAAGAAGAGGAGGCTTAAACC
rs2723176	TTTTTTTTTTTTTTTTTTTTTTTTCTCTAGGGGCTGCTTTAACAAG
rs4611652	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCAGTCTGAGTGCAATACTTGCAG
rs4241122	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGACTTCAGCTTCCAAATACTCATCC
rs3811047	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTACTTGTGTGAACAAAATTCATGG
rs4392270	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCCTGATGAGATATAGTGAGTCACTATGAC

Note: F – forward primer, R – reverse primer

related to HBV-HCC susceptibility. The genotype frequencies of the seven SNPs in negative controls were tested for accordance with Hardy–Weinberg equilibrium. χ^2 tests were used to compare the SNP alleles and genotype frequencies. Codominant, dominant, and recessive models were applied to analyze the correlation between the genotypes of the seven selected SNPs and the development of disease by unconditional logistic regression analysis. The odds ratio (OR) and 95% confidence intervals (95% CI) were calculated. A p-value < 0.05 was considered statistically significant. SPSS 20.0 statistical software was used for data processing and analysis, and HaploView4.2 software was used for haplotype construction and disease association analyses.

Results

Characteristics of the study subjects

We divided 1,582 subjects into 6 subgroups: inactive infection, active infection, cirrhosis, HCC, viral clearance, and negative controls. The inactive, active, cirrhosis, and HCC groups were collectively referred to as HBV-related liver diseases. As shown in Table 2, the average age (years) of the six subgroups was the highest in the HCC group at 60.00 ± 14.16; the lowest was in the active group at 38.32 ± 10.18; followed by the negative control group at 40.29 ± 15.82; the inactive group at 44.31 ± 10.35; 51.15 ± 15.53 in the clearance group; and 56.78 ± 12.48 in the cirrhosis group. The age difference among the six groups was statistically significant (p < 0.001). Among the six subgroups, the incidence of HCC among males was the highest at 81.58%, followed by the cirrhosis group at 74.51%, the active group at 67.46%, the inactive group at 64.31%, negative controls at 52.33%, and the clearance group at 48.89%. The sex differences of the six subgroups were statistically significant (p < 0.001). It should be noted that this study primarily focuses on genetic-level analysis. During the retrospective data collection process, it was found that clinical biochemical indicators were missing or incomplete for some cases, which prevented a comprehensive analysis of systematic clinical data across all samples. In addition, since lifestyle information (such as smoking and alcohol consumption) is not routinely systematically documented in standard clinical practice, such data were also not included in this study.

Table 2. Characteristics of the study subjects

Variable	Inactive (n = 338)	Active (n = 269)	Cirrhosis (n = 102)	HCC (n = 38)	Control (n = 430)	Clearance (n = 405)	p-value
Age (years)	44.31 ± 10.35	38.32 ± 10.18	56.78 ± 12.48	60.00 ± 14.16	40.29 ± 15.82	51.15 ± 15.53	<0.001
Sex/Male count (%)	228 (67.46%)	173 (64.31%)	76 (74.51%)	31 (81.58%)	225 (52.33%)	198 (48.89%)	<0.001

Note: Age (years) – expressed as Mean ± SD, p-value – Kruskal–Wallis test for age and chi-squared test for sex

Hardy–Weinberg equilibrium tests

After genotyping the seven SNPs included in this study, Hardy–Weinberg equilibrium was tested for the genotype frequencies in the negative controls, as shown in Table 3. All seven sites were in line with Hardy–Weinberg equilibrium ($p > 0.05$), indicating that the study population was representative.

Table 3. Hardy–Weinberg equilibrium test for the 7 SNPs in negative control individuals

SNP ID	Pos (GRCh37)	Chinese_MAF	Genotype	Number	HWE χ^2	HWE p
rs3811046	113671378	T: 0.800	TT	319	3.982	0.137
			GT	99		
			GG	12		
rs3811047	113671410	G: 0.800	GG	318	3.489	0.175
			GA	100		
			AA	12		
rs2723176	113672509	C: 0.871	CC	338	4.720	0.094
			CA	84		
			AA	8		
rs2723186	113675080	G: 0.871	GG	338	4.720	0.094
			GA	84		
			AA	8		
rs4611652	113676659	C: 0.871	CC	338	4.720	0.094
			CT	84		
			TT	8		
rs4392270	113678629	G: 0.871	GG	338	4.936	0.085
			GA	84		
			AA	8		
rs4241122	113678856	A: 0.805	AA	318	5.010	0.082
			GA	100		
			GG	12		

Note: HWE – Hardy–Weinberg equilibrium; MAF – Minor allele frequency, note – when MAF ≦ 0.8 indicates the major allele frequency

Genotype and allele frequency distributions of IL37 *SNPs associated with HBV-related liver disease*

Table 4 summarizes the genotype and allele frequency distributions of the seven selected SNPs in the HBV-related liver disease groups and controls. Our results showed that rs4241122 was associated with HBV-related liver disease. In the codominant model, individuals homozygous for rs4241122-GG were less likely to develop liver disease than those homozygous for the reference genotypes ($p = 0.049$, OR = 0.402, 95% CI = 0.163–0.995). In the recessive model, GA+AA of rs4241122 increased the disease risk compared with GG ($p = 0.028$, OR = 2.652, 95% CI = 1.075–6.540). No significant difference in the genotype and allele distributions of rs3811046, rs3811047, rs2723176, rs2723186, rs4611652, and rs4392270 SNPs was observed in patients infected with HBV compared to controls (Table 4).

Table 4. Comparison of genotype/allele distributions between HBV-related liver disease and controls

SNPs	Genotype/Allele distribution	Controls (n = 430)	HBV patients (n = 747)	Models	OR (95% CI)	χ^2	p
rs3811046	T	737 (85.70%)	1264 (84.61%)		0.917 (0.724–1.163)	0.511	0.475
	G	123 (14.30%)	230 (15.39%)				
	TT	319 (71.19%)	527 (70.55%)		Reference		
	GT	99 (23.02%)	210 (28.11%)	codominant	1.284 (0.974–1.693)	3.141	0.076
	GG	12 (2.79%)	10 (1.34%)		0.504 (0.215–1.181)	2.486	0.115
	GG+GT/TT	111/319	220/527	dominant	1.200 (0.918–1.567)	1.786	0.181
	GG/GT+TT	12/418	10/737	recessive	2.116 (0.906–4.939)	3.317	0.077

Table 4. ***(continued)***

SNPs	Genotype/Allele distribution	Controls (n = 430)	HBV patients (n = 747)	Models	OR (95% CI)	χ^2	p
rs3811047	G	736 (85.58%)	1267 (84.81%)		1.063 (0.839–1.348)	0.259	0.611
	A	124 (14.42%)	227 (15.19%)				
	GG	318 (73.95%)	530 (70.95%)		Reference		
	GA	100 (23.26%)	207 (27.71%)	codominant	1.242 (0.942–1.637)	2.365	0.124
	AA	12 (2.79%)	10 (1.34%)		0.500 (0.214–1.171)	2.551	0.110
	AA+GA/GG	112/318	217/530	dominant	1.163 (0.890–1.518)	1.222	0.269
	AA/GA+GG	12/418	10/737	recessive	2.116 (0.906–4.939)	3.137	0.077
rs2723176	C	760 (88.37%)	1307 (87.48%)		0.920 (0.710–1.191)	0.403	0.526
	A	100 (11.63%)	187 (12.52%)				
	CC	338 (78.60%)	564 (75.50%)		Reference		
	CA	84 (19.53%)	179 (23.96%)	codominant	1.277 (0.953–1.710)	2.691	0.101
	AA	8 (1.86%)	4 (0.54%)		0.300 (0.090–1.003)	3.825	0.050
	AA+CA/CC	92/338	183/564	dominant	1.192 (0.897–1.584)	1.467	0.226
	AA/CA+CC	8/422	4/743	recessive	3.521 (1.054–11.763)	3.525	0.060
rs2723186	G	760 (88.37%)	1307 (87.48%)		1.087 (0.839–1.408)	0.403	0.526
	A	100 (11.63%)	187 (12.52%)				
	GG	338 (78.60%)	564 (75.50%)		Reference		
	GA	84 (19.53%)	179 (23.96%)	codominant	1.277 (0.953–1.710)	2.691	0.101
	AA	8 (1.86%)	4 (0.54%)		0.300 (0.090–1.003)	3.825	0.050
	AA+GA/GG	92/338	183/564	dominant	1.192 (0.897–1.584)	1.467	0.226
	AA/GA+GG	8/422	4/743	recessive	3.521 (1.054–11.763)	3.525	0.060
rs4611652	C	760 (88.37%)	1307 (87.48%)		1.087 (0.839–1.408)	0.403	0.526
	T	100 (11.63%)	187 (12.52%)				
	CC	338 (78.60%)	564 (75.50%)		Reference		
	CT	84 (19.53%)	179 (23.96%)	codominant	1.277 (0.953–1.710)	2.691	0.101
	TT	8 (1.86%)	4 (0.54%)		0.300 (0.090–1.003)	3.825	0.050
	TT+CT/CC	92/338	183/564	dominant	1.192 (0.897–1.584)	1.467	0.226
	TT/CT+CC	8/422	4/743	recessive	3.521 (1.054–11.763)	3.525	0.060
rs4392270	G	760 (88.37%)	1305 (87.35%)		1.101 (0.850–1.425)	0.530	0.467
	A	100 (11.63%)	189 (12.65%)				
	GG	338 (78.60%)	562 (75.23%)		Reference		
	GA	84 (19.53%)	181 (24.23%)	codominant	1.296 (0.968–1.735)	3.031	0.082
	AA	8 (1.86%)	4 (0.54%)		0.301 (0.090–1.006)	3.802	0.051
	AA+GA/GG	92/338	185/562	dominant	1.209 (0.910–1.607)	1.723	0.189
	AA/GA+GG	8/422	4/743	recessive	3.521 (1.054–11.763)	3.525	0.060
rs4241122	A	736 (85.58%)	1266 (84.74%)		0.935 (0.738–1.185)	0.305	0.581
	G	124 (14.42%)	228 (15.26%)				
	AA	318 (73.95%)	527 (70.55%)		Reference		
	GA	100 (23.26%)	212 (28.38%)	codominant	1.279 (0.971–1.685)	3.069	0.080
	GG	12 (2.79%)	8 (1.07%)		**0.402 (0.163–0.995)**	**3.886**	**0.049**
	GG+GA/AA	112/318	220/527	dominant	1.185 (0.908–1.548)	1.562	0.211
	GG/GA+AA	12/418	8/739	recessive	**2.652 (1.075–6.540)**	**4.832**	**0.028**

Note: OR – odds ratio; CI – confidence interval. Bold indicates significance

Genotype and allele frequency distributions of IL37 *SNPs associated with HBV clearance*

Table 5 summarizes the genotype and allele frequency distributions of the seven *IL37* SNPs in the HBV-related liver disease group and clearance group. Our results showed that rs2723186, rs4392270, and rs421122 were associated with HBV clearance compared to patients with HBV-related liver disease. We observed that non-reference heterozygous genotype carriers of rs2723186 and rs4392270 were risk factors, and individuals with these genotypes were less likely to clear HBV than homozygotes carrying the reference allele. In a codominant model, the HBV clearance ability of individuals carrying homozygous reference genotypes of rs2723186 and rs4392270 was significantly increased (both $p < 0.05$) compared with those carrying heterozygous non-reference genotypes. In addition, in the recessive model, the HBV-related liver disease group was compared with the clearance group, and rs421122 was significantly correlated with clearance (GA+AA vs. GG, $p = 0.036$, OR = 2.579, 95% CI = 1.029–6.464). No significant difference was found between the HBV-related liver disease group and the clearance group in the remaining SNPs (Table 5).

Table 5. Comparison of genotype/allele distributions between HBV-related liver disease and the clearance group

SNPs	Genotype/Allele distribution	Controls (n = 405)	HBV patients (n = 747)	Models	OR (95% CI)	χ^2	p
rs3811046	T	687 (84.81%)	1264 (84.61%)		0.984 (0.776–1.248)	0.018	0.894
	G	123 (15.19%)	230 (15.39%)				
	TT	294 (72.59%)	527 (70.55%)		Reference		
	GT	99 (24.44%)	210 (28.11%)	codominant	1.183 (0.896–1.563)	1.406	0.236
	GG	12 (2.96%)	10 (1.34%)		0.465 (0.198–1.089)	3.110	0.078
	GG+GT/TT	111/294	220/527	dominant	1.106 (0.845–1.447)	0.536	0.464
	GG/GT+TT	12/393	10/737	recessive	2.250 (0.964–5.255)	3.699	0.054
rs3811047	G	690 (85.19%)	1267 (84.81%)		1.030 (0.810–1.309)	0.059	0.808
	A	120 (14.81%)	227 (15.19%)				
	GG	297 (73.33%)	530 (70.95%)		Reference		
	GA	96 (23.70%)	207 (27.71%)	codominant	1.208 (0.913–1.600)	1.747	0.186
	AA	12 (2.96%)	10 (1.34%)		0.467 (0.199–1.094)	3.075	0.080
	AA+GA/GG	108/297	217/530	dominant	1.126 (0.859–1.476)	0.736	0.391
	AA/GA+GG	12/393	10/737	recessive	2.250 (0.964–5.255)	3.699	0.054
rs2723176	C	720 (88.89%)	1307 (87.48%)		0.874 (0.669–1.141)	0.981	0.322
	A	90 (11.11%)	187 (12.52%)				
	CC	322 (79.51%)	564 (75.50%)		Reference		
	CA	76 (18.77%)	179 (23.96%)	codominant	1.345 (0.995–1.817)	3.713	0.054
	AA	7 (1.73%)	4 (0.54%)		0.326 (0.095–1.123)	3.155	0.076
	AA+CA/CC	83/322	183/564	dominant	1.259 (0.939–1.688)	2.371	0.124
	AA/CA+CC	7/398	4/743	recessive	3.267 (0.951–11.227)	2.791	0.095
rs2723186	G	721 (89.01%)	1307 (87.48%)		1.159 (0.886–1.516)	1.165	0.281
	A	89 (10.99%)	187 (12.52%)				
	GG	323 (79.75%)	564 (75.50%)		Reference		
	GA	75 (18.52%)	179 (23.96%)	codominant	**1.367 (1.010–1.849)**	**4.105**	**0.043**
	AA	7 (1.73%)	4 (0.54%)		0.327 (0.095–1.126)	3.137	0.077
	AA+GA/GG	82/323	183/564	dominant	1.278 (0.952–1.715)	2.680	0.102
	AA/GA+GG	7/398	4/743	recessive	3.267 (0.951–11.227)	2.791	0.095
rs4611652	C	719 (88.77%)	1307 (87.48%)		1.130 (0.866–1.476)	0.814	0.367
	T	91 (11.23%)	187 (12.52%)				
	CC	321 (79.26%)	564 (75.50%)		Reference		
	CT	77 (19.01%)	179(23.96%)	codominant	1.323 (0.980–1.786)	3.341	0.068
	TT	7 (1.73%)	4 (0.54%)		0.325 (0.094–1.119)	3.172	0.075
	TT+CT/CC	84/321	183/564	dominant	1.240 (0.926–1.661)	2.082	0.149
	TT/CT+CC	7/398	4/743	recessive	3.267 (0.951–11.227)	2.791	0.095

Table 5. ***(continued)***

SNPs	Genotype/Allele distribution	Controls (n = 405)	HBV patients (n = 747)	Models	OR (95% CI)	χ^2	p
rs4392270	G	720 (88.89%)	1305 (87.35%)		1.159 (0.887–1.513)	1.170	0.279
	A	90 (11.11%)	189 (12.65%)				
	GG	322 (79.51%)	562 (75.23%)		Reference		
	GA	76 (18.77%)	181 (24.23%)	codominant	**1.365 (1.010–1.844)**	**4.099**	**0.043**
	AA	7 (1.73%)	4 (0.54%)		0.327 (0.095–1.127)	3.134	0.077
	AA+GA/GG	83/322	185/562	dominant	1.277 (0.953–1.712)	2.685	0.101
	AA/GA+GG	7/398	4/743	recessive	3.267 (0.951–11.227)	2.791	0.095
rs4241122	A	691 (85.31%)	1266 (84.74%)		0.956 (0.752–1.216)	0.133	0.715
	G	119 (14.69%)	228 (15.26%)				
	AA	297 (73.33%)	527 (70.55%)		Reference		
	GA	97 (23.95%)	212 (28.38%)	codominant	1.232 (0.932–1.628)	2.141	0.143
	GG	11 (2.72%)	8 (1.07%)		0.410 (0.163–1.030)	3.597	0.058
	GG+GA/AA	108/297	220/527	dominant	1.148 (0.876–1.505)	1.000	0.317
	GG/GA+AA	11/394	8/739	recessive	**2.579 (1.029–6.464)**	**4.382**	**0.036**

Further analysis by considering the age or sex

Variable	B	S.E.	Wald	OR (95% CI)	p
codominant model					
sex	0.772	0.130	35.155	**2.165 (1.677–2.795)**	**0.000**
age	0.033	0.005	50.471	**1.033 (1.024–1.042)**	**0.000**
rs4392270		8.509		0.014	
rs4392270(GA)	–0.356	0.160	4.954	**0.701 (0.512–0.958)**	**0.026**
rs4392270(AA)	1.155	0.652	3.143	3.175 (0.885–11.393)	0.076
dominant model					
sex	0.777	0.130	35.899	**2.175 (1.687–2.805)**	**0.000**
age	0.032	0.005	49.137	**1.033 (1.023–1.042)**	**0.000**
recessive model					
sex	0.778	0.130	35.808	**2.177 (1.687–2.809)**	**0.000**
age	0.032	0.005	50.042	**1.033 (1.024–1.042)**	**0.000**
rs4241122(GG)	–1.085	0.485	5.003	**0.338 (0.131–0.874)**	**0.025**

Note: OR – odds ratio; CI – confidence interval, B – partial regression coefficient; S.E. – standard error of partial regression coefficient. Bold indicates significance

The results of further analysis considering age and sex showed that male gender (OR ≈ 2.17, $p < 0.001$) and increasing age (OR = 1.033, $p < 0.001$) were independent risk factors for HBV infection clearance. In the codominant model, the GA genotype at the rs4392270 exhibited a protective effect (OR = 0.701, 95% CI: 0.512–0.958, p = 0.026), while the AA genotype, although not statistically significant, still showed a trend toward increased risk (OR = 3.175, p = 0.076). In the recessive model, the GG genotype at the rs4241122 demonstrated a significant protective effect (OR = 0.338, 95% CI: 0.131–0.874, p = 0.025).

Genotype and allele frequency distributions of IL37 *SNPs associated with HBV severe liver disease*

Table 6 summarizes the genotype and allele distributions of the seven SNPs in the inactive group vs. patients with active HBV, cirrhosis, and HCC. Our results showed that rs3811046, rs3811047, and rs4241122 were associated with HBV progression to more severe liver abnormalities compared to the inactive group. Under the co-dominant model, the GT genotype of rs3811046 was compared with TT genotype carriers (p = 0.009, OR = 0.652, 95% CI = 0.473–0.899), the GA genotype of rs3811047 were compared with the GG genotype (p = 0.012, OR = 0.661, 95% CI = 0.478–0.912), and carriers of the GA genotype of rs4241122 were compared with the AA genotype (p = 0.013, OR = 0.665, 95% CI = 0.483–0.916). All showed a reduced risk of HBV progression to more severe liver abnormalities. In the dominant model, rs3811046 was associated with the HBV severe liver disease group (GG+GT vs. TT, p = 0.030, OR = 0.705, 95% CI = 0.514–0.967), and rs3811047 was associated with the HBV severe liver disease group (AA+GA vs. GG, p = 0.038, OR = 0.715, 95% CI = 0.521–0.982). Rs4241122 was statistically significant in the dominant model, and individuals carrying the GG+GA genotype were less likely to progress to severe liver abnormalities than those carrying the AA genotype (p = 0.030, OR = 0.705, 95% CI = 0.514–0.967) (Table 6).

Table 6. Comparison of genotype/allele distributions between the inactive group and patients with active HBV, cirrhosis and HCC

SNPs	Genotype/Allele distribution	Initiative (n = 338)	Active, cirrhosis and HCC (n = 409)	Models	OR (95% CI)	χ^2	p
rs3811046	T	562 (83.14%)	702 (85.82%)		1.228 (0.927–1.626)	2.046	0.153
	G	114 (16.86%)	116 (14.18%)				
	TT	225 (66.57%)	302 (73.84%)		Reference		
	GT	112 (33.14%)	98 (23.96%)	codominant	**0.652 (0.473–0.899)**	**6.808**	**0.009**
	GG	1 (0.30%)	9 (2.20%)		6.705 (0.843–53.308)	3.236	0.072
	GG+GT/TT	113/225	107/302	dominant	**0.705 (0.514–0.967)**	**4.708**	**0.030**
	GG/GT+TT	1/337	9/400	recessive	0.132 (0.017–1.046)	3.743	0.053
rs3811047	G	564 (83.43%)	703 (85.94%)		0.824 (0.621–1.093)	1.809	0.179
	A	112 (16.57%)	115 (16.57%)				
	GG	227 (67.16%)	303 (74.08%)		Reference		
	GA	110 (32.54%)	97 (23.72%)	codominant	**0.661 (0.478–0.912)**	**6.340**	**0.012**
	AA	1 (0.30%)	9 (2.20%)		6.743 (0.848–53.601)	3.255	0.071
	AA+GA/GG	111/227	106/303	dominant	**0.715 (0.521–0.982)**	**4.304**	**0.038**
	AA/GA+GG	1/337	9/400	recessive	0.132 (0.017–1.046)	3.743	0.053
rs2723176	C	582 (86.09%)	725 (88.63%)		1.259 (0.927–1.711)	2.174	0.140
	A	94 (13.91%)	93 (11.37%)				
	CC	245 (72.49%)	319 (78.00%)		Reference		
	CA	92 (27.22%)	87 (21.27%)	codominant	0.726 (0.518–1.017)	3.458	0.063
	AA	1 (0.30%)	3 (0.73%)		2.304 (0.238–22.286)	0.520	0.471
	AA+CA/CC	93/245	90/319	dominant	0.743 (0.532–1.038)	3.038	0.081
	AA/CA+CC	1/337	3/406	recessive	0.402 (0.042–3.878)	0.097	0.755
rs2723186	G	582 (86.09%)	725 (88.63%)		0.794 (0.584–1.079)	2.174	0.140
	A	94 (13.91%)	93 (11.37%)				
	GG	245 (72.49%)	319 (78.00%)		Reference		
	GA	92 (27.22%)	87 (21.27%)	codominant	0.726 (0.518–1.017)	3.458	0.063
	AA	1 (0.30%)	3 (0.73%)		2.304 (0.238–22.286)	0.520	0.471
	AA+GA/GG	93/245	90/319	dominant	0.743 (0.532–1.038)	3.038	0.081
	AA/GA+GG	1/337	3/406	recessive	0.402 (0.042–3.878)	0.097	0.755
rs4611652	C	582 (86.09%)	725 (88.63%)		0.794 (0.584–1.079)	2.174	0.140
	T	94 (13.91%)	93 (11.37%)				
	CC	245 (72.49%)	319 (78.00%)		Reference		
	CT	92 (27.22%)	87 (21.27%)	codominant	0.726 (0.518–1.017)	3.458	0.063
	TT	1 (0.30%)	3 (0.73%)		2.304 (0.238–22.286)	0.520	0.471
	TT+CT/CC	93/245	90/319	dominant	0.743 (0.532–1.038)	3.038	0.081
	TT/CT+CC	1/337	3/406	recessive	0.402 (0.042–3.878)	0.097	0.755
rs4392270	G	581 (85.95%)	724 (88.51%)		0.794 (0.585–1.078)	2.198	0.138
	A	95 (14.05%)	94 (11.49%)				
	GG	244 (72.19%)	318 (77.75%)		Reference		
	GA	93 (27.51%)	88 (21.52%)	codominant	0.726 (0.519–1.016)	3.491	0.062
	AA	1 (0.30%)	3 (0.73%)		2.302 (0.238–22.266)	0.519	0.471
	AA+GA/GG	94/244	91/318	dominant	0.743 (0.532–1.036)	3.072	0.080
	AA/GA+GG	1/337	3/406	recessive	0.402 (0.042–3.878)	0.097	0.755

Table 6. *(continued)*

SNPs	Genotype/Allele distribution	Initiative (n = 338)	Active, cirrhosis and HCC (n = 409)	Models	OR (95% CI)	χ^2	p
rs4241122	A	562 (83.14%)	704 (86.06%)		1.253 (0.945–1.661)	2.453	0.117
	G	114 (16.86%)	114 (13.94%)				
	AA	225 (66.57%)	302 (73.84%)		Reference		
	GA	112 (33.14%)	100 (24.45%)	codominant	**0.665 (0.483–0.916)**	**6.228**	**0.013**
	GG	1 (0.30%)	7 (1.71%)		5.215 (0.637–42.690)	2.371	0.124
	GG+GA/AA	113/225	107/302	dominant	**0.705 (0.514–0.967)**	**4.708**	**0.030**
	GG/GA+AA	1/337	7/402	recessive	0.170 (0.021–1.392)	2.292	0.130

Note: OR – odds ratio; CI – confidence interval. Bold indicates significance

Genotype and allele frequency distributions of IL37 *SNPs associated with HBV-HCC*

The genotype and allele distributions of the *IL37* SNPs in the HCC group and patients with inactive, active HBV, and cirrhosis are summarized in Table 7. No statistical differences were found in the distribution of genotype frequencies of these SNPs between the two groups ($p > 0.05$ for all).

Table 7. Comparison of genotype/allele distributions between the HCC group and patients with inactive, active and cirrhosis

SNPs	Genotype/Allele distribution	Initiative, active and cirrhosis (n = 709)	HCC (n = 38)	Models	OR (95% CI)	χ^2	p
rs3811046	T	1201 (84.70%)	63 (82.89%)		0.876 (0.474–1.618)	0.180	0.672
	G	217 (15.30%)	13 (17.11%)				
	TT	501 (70.66%)	26 (68.42%)		Reference		
	GT	199 (28.07%)	11 (28.95%)	codominant	1.065 (0.516–2.197)	0.029	0.864
	GG	9 (1.27%)	1 (2.63%)		2.141 (0.261–17.541)	0.503	0.478
	GG+GT/ TT	208/501	12/26	dominant	1.112 (0.550–2.245)	0.087	0.768
	GG/GT+TT	9/700	1/37	recessive	0.476 (0.059–3.855)	NA	0.409
rs3811047	G	1203 (84.84%)	64 (84.21%)		0.953 (0.506–1.796)	0.022	0.882
	A	215 (15.16%)	12 (15.79%)				
	GG	503 (70.94%)	27 (71.05%)		Reference		
	GA	197 (27.79%)	10 (26.32%)	codominant	0.946 (0.449–1.990)	0.022	0.883
	AA	9 (1.27%)	1 (2.63%)		2.070 (0.253–16.937)	0.460	0.498
	AA+GA/GG	206/503	11/27	dominant	0.995 (0.484–2.043)	0.000	0.989
	AA/GA+GG	9/700	1/37	recessive	0.476 (0.059–3.855)	NA	0.409
rs2723176	C	1242 (87.59%)	65 (85.53%)		0.837 (0.434–1.617)	0.280	0.597
	A	176 (12.41%)	11 (14.47%)				
	CC	536 (75.60%)	28 (73.69%)		Reference		
	CA	170 (23.98%)	9 (23.68%)	codominant	1.013 (0.469–2.190)	0.001	0.973
	AA	3 (0.42%)	1 (2.63%)		6.381 (0.643–63.317)	2.505	0.113
	AA+CA/CC	173/536	10/28	dominant	1.107 (0.527–2.324)	0.072	0.789
	AA/CA+CC	3/706	1/37	recessive	0.157 (0.016–1.548)	NA	0.189
rs2723186	G	1242 (87.59%)	65 (85.53%)		0.837 (0.434–1.617)	0.280	0.597
	A	176 (12.41%)	11 (14.47%)				
	GG	536 (75.60%)	28 (73.69%)		Reference		
	GA	170 (23.98%)	9 (23.68%)	codominant	1.013 (0.469–2.190)	0.001	0.973
	AA	3 (0.42%)	1 (2.63%)		6.381 (0.643–63.317)	2.505	0.113
	AA+GA/GG	173/536	10/28	dominant	1.107 (0.527–2.324)	0.072	0.789
	AA/GA+GG	3/706	1/37	recessive	0.157 (0.016–1.548)	NA	0.189

Table 7. *(continued)*

SNPs	Genotype/Allele distribution	Initiative, active and cirrhosis (n = 709)	HCC (n = 38)	Models	OR (95% CI)	χ^2	p
	C	1242 (87.59%)	65 (85.53%)		0.837 (0.434–1.617)	0.280	0.597
	T	176 (12.41%)	11 (14.47%)				
	CC	536 (75.60%)	28 (73.69%)		Reference		
rs4611652	CT	170 (23.98%)	9 (23.68%)	codominant	1.013 (0.469–2.190)	0.001	0.973
	TT	3 (0.42%)	1 (2.63%)		6.381 (0.643–63.317)	2.505	0.113
	TT+CT/CC	173/536	10/28	dominant	1.107 (0.527–2.324)	0.072	0.789
	TT/CT+CC	3/706	1/37	recessive	0.157 (0.016–1.548)	NA	0.189
	G	1241 (87.52%)	64 (84.21%)		0.761 (0.403–1.437)	0.714	0.398
	A	177 (12.48%)	12 (15.79%)				
	GG	535 (75.46%)	27 (71.05%)		Reference		
rs4392270	GA	171 (24.12%)	10 (26.32%)	codominant	1.159 (0.550–2.443)	0.150	0.699
	AA	3 (0.42%)	1 (2.63%)		6.605 (0.665–65.613)	2.597	0.107
	AA+GA/ GG	174/535	11/27	dominant	1.253 (0.609–2.578)	0.376	0.540
	AA/GA+GG	3/706	1/37	recessive	0.157 (0.016–1.548)	NA	0.189
	A	1203 (84.84%)	63 (82.89%)		0.866 (0.469–1.601)	0.211	0.646
	G	215 (15.16%)	13 (17.11%)				
	AA	501 (70.66%)	26 (68.42%)		Reference		
rs4241122	GA	201 (28.35%)	11 (28.95%)	codominant	1.055 (0.511–2.175)	0.021	0.886
	GG	7 (0.99%)	1 (2.63%)		2.753 (0.326–23.212)	0.867	0.352
	GG+GA/AA	208/501	12/26	dominant	1.112 (0.550–2.245)	0.087	0.768
	GG/GA+AA	7/702	1/37	recessive	0.369 (0.044–3.077)	NA	0.343

Note: OR – odds ratio; CI – confidence interval; NA – Not Applicable

Haplotype analysis

The haplotype distributions of the *IL37* polymorphisms in the HBV-related liver disease group vs. negative controls, the HBV-related liver disease group vs. the clear group, and the inactive hepatitis vs. the active hepatitis, cirrhosis, and HCC combined group were determined. We found that rs3811046, rs3811047, rs2723176, rs2723186, rs4611652, rs4392270, and rs4241122 formed three haplotypes (T-G-C-G-C-G-A; G-A-A-A-T-A-G; and G-A-C-G-C-G-G). However, these haplotypes were not associated with the risk of HBV infection-associated liver disease, HBV clearance, or HBV severe liver disease (Table 8).

Table 8. Haplotype frequencies of IL-37

Haplotypes							Freq.	Case, Control, Frequencies	χ^2	p
rs3811046	rs3811047	rs2723176	rs2723186	rs4611652	rs4392270	rs4241122				
Controls vs patients infected with HBV										
T	G	C	G	C	G	A	0.852	0.850, 0.857	0.202	0.6530
G	A	A	A	T	A	G	0.122	0.124, 0.117	0.321	0.5712
G	A	C	G	C	G	G	0.026	0.026, 0.027	0.026	0.8728
Clearance vs patients infected with HBV										
T	G	C	G	C	G	A	0.847	0.846, 0.851	0.105	0.7454
G	A	A	A	T	A	G	0.119	0.124, 0.111	0.900	0.3427
G	A	C	G	C	G	G	0.029	0.026, 0.034	1.025	0.3112
The inactive group vs patients with active HBV, cirrhosis and HCC										
T	G	C	G	C	G	A	0.846	0.858, 0.831	2.059	0.1513
G	A	A	A	T	A	G	0.124	0.113, 0.138	2.162	0.1415
G	A	C	G	C	G	G	0.026	0.025, 0.028	0.196	0.6579

Discussion

HBV-related liver disease is an important disease that endangers the health of the Chinese population and people worldwide. Its clinical phenotype is complex and diverse; however, the mechanisms underlying this clinical diversity have not been clarified. Studies have found that individual differences in HBV-persistent infection and different clinical outcomes after infection may be related to polymorphisms of host immune response-related genes (Al-Anazi et al., 2019). Because polymorphisms can affect gene expression, different genotypes may produce different proteins or change the transcriptional efficiency, thereby affecting the immune response to HBV and the disease phenotype (Ben Selma et al., 2021; Dondeti et al., 2022). IL-37 is a cytokine that effectively inhibits innate and adaptive immunity, and its role in disease occurrence has received increasing attention. Studies on the relationship between *IL37* polymorphisms and disease occurrence have attracted much attention (Al-Anazi et al., 2019; Liu et al., 2017; Molaei et al., 2023; Tan et al., 2016; Yan et al., 2015; Yin et al., 2017). Given that different ancestries may have different allele frequencies and linkage disequilibrium structures (Munkongdee et al., 2021), the same SNP may be associated with different clinical classifications or pathological mechanisms of disease in different populations (Cheng et al., 2023).

As shown in Table 2, the HBV-HCC group had the highest proportion of males and the oldest average age, followed by the cirrhosis group. The distribution of sex and age among the six subgroups was statistically significant ($p < 0.001$). This indicates that being male and of older age are important risk factors for the development of HBV chronic infection into cirrhosis, HCC, and other serious liver diseases in China. This may be attributed to the stimulating effect of androgens (Li et al., 2019c) and the long duration of harboring HBV, because HBV infection mainly occurs in infants and young children in China (Indolfi et al., 2019). The risk of chronic and persistent HBV infection in infants and young children is as high as 90% (Indolfi et al., 2019), and the longer a patient carries HBV, the higher the risk of developing severe HBV-related liver disease.

Two *IL37* SNPS included in this study, rs3811046 (Gly-31Val) and rs3811047 (Thr42Ala), are missense mutations in exon 2, resulting in different amino acid changes. The results of this study showed that rs3811046 and rs3811047 were in complete linkage disequilibrium (LD). Rs3811046 and rs3811047 both correlated with HBV-severe liver disease. Among these, under the codominant model, individuals carrying heterozygous genotypes were less likely to progress to severe liver disease than those carrying the reference homozygous genotypes. Populations carrying the non-reference homozygous and risk heterozygous genotypes in dominant models were less likely to progress to severe liver disease. This seems to indicate that in the current study population, once infected with HBV, individuals carrying rs3811046 and rs3811047 heterozygous genotypes were prone to becoming long-term HBV carriers with benign liver disease. The T-G haplotype, consisting of the protective alleles rs3811046-T and rs3811047-G, is associated with a markedly reduced risk of progression to severe liver disease following HBV infection (all ORs <1). Consequently, individuals carrying the IL37 Val31-Ala42 protein variant exhibit a significantly lower likelihood of developing advanced liver pathology after HBV infection. This protective effect is likely mediated through enhanced immunosuppressive function of the Val31-Ala42 IL-37 variant, which more potently suppresses the production of pro-inflammatory cytokines such as IL-1β during chronic HBV infection. This attenuated inflammatory response consequently reduces hepatocyte injury and fibrotic progression, thereby delaying the development of cirrhosis and HCC. Although rs3811046 or rs3811047 are associated with Graves' disease (Yan et al., 2015), Behcet's disease (Tan et al., 2016), tuberculosis (Liu et al., 2017), and coronary artery disease (Yin et al., 2017) in China, no other studies have confirmed that these two SNPs are associated with HBV chronic liver disease (Al-Anazi et al., 2019; Cheng et al., 2023). In one study, Al-Anazi et al. (2019) in a Saudi Arabian population and Cheng et al. (2023) in a Han population in central China conducted research on the association of rs3811046 and rs3811047 with HBV-related liver disease. No significant relationship was found between these two SNPs and HBV infection and infection outcome. These inconsistencies may be influenced by sample size, geographic region, ethnicity, and disease, so relevant population trials and functional tests are needed to confirm these findings.

A study in a Saudi Arabian population (Al-Anazi et al., 2019) found that rs2723176, rs2723186, and rs4392270 were associated with self-healing after HBV infection but not with liver disease with HBV infection. The results of this study showed that rs2723176, rs2723186, and rs4392270 were in complete LD. The GA genotypes of rs2723186, and rs4392270 were significantly associated with difficulty in clearing HBV after infection in the codominant model (OR ≈ 1.37, $p < 0.05$). That is, individuals carrying (GA) genotypes of rs2723186, and rs4392270 had approximately a 37% higher risk of developing chronic liver disease after HBV infection compared to those with the wild-type (GG) genotype. This suggests that the GA genotype may impair the host's ability to clear the virus, thereby hindering the self-healing process following HBV infection. The rs4392270 is annotated in the Ensembl database as a transcription factor binding site variant. This locus is situated in a non-coding regulatory region of the gene, and the nucleotide change (G>A) may alter the binding affinity of the local DNA sequence to specific transcription factors, thereby directly modulating the expression of downstream target genes. This mechanism likely influences the HBV clearance process, suggesting that rs4392270 may represent a potential causal variant. Although rs2723186 is classified as an intron variant, it also demonstrates a significant association with disease risk in genetic analyses, which can be attributed to its strong LD with the putative functional variant rs4392270. However, while univariate analysis indicated a significant association between the GA genotype and increased disease risk, multivariate analysis adjusting for age and gender revealed a statistically significant protective effect. Although the variant was initially hypothesized to confer risk based on its location within a regulatory region, the multivariable model suggests a potential protective role. One plausible biological mechanism is that the G>A substitution enhances the binding affinity of a transcriptional repressor, leading to downregulation of key genes involved in inflammatory responses or viral persistence pathways. This altered gene expression may promote a more balanced immune regulation, ultimately facilitating viral clearance. In addition, there were no significant differences in the distribution of the rs2723176, rs2723186, and rs4392270 genotypes between HBV-associated liver disease patients and healthy controls, which were consistent with those of al-Anazi et al. (2019).

To our knowledge, there are no similar studies on the relationship between rs4611652 and HBV infection outcomes. Our results revealed that rs4611652 was observed to be not

associated with HBV-related liver disease and self-healing after HBV infection in our study.

This study demonstrated significant differences in rs4241122 between HBV-related liver disease and healthy controls under the codominant and recessive models. The GG genotype of rs4241122 was significantly associated with decreased risk of HBV-related liver disease, suggesting this is a protective factor for HBV-associated liver disease. Compared with the GG genotype, the GA+AA genotype of rs4241122 was significantly associated with an increased risk of HBV-related liver disease. In the recessive model (GG vs. GA+AA), the GG genotype of rs4241122 demonstrated a significant protective effect against HBV viral clearance. Univariate analysis indicated that, compared to individuals with non-GG genotypes (GA/AA), carriers of the GG genotype had a significantly reduced risk of developing HBV-related liver disease (OR = 0.338, 95% CI: 0.131–0.874, $p = 0.025$). To further validate the independence of this association, we incorporated age and sex into a multivariate adjustment. The results remained significant (adjusted OR = 0.338, 95% CI: 0.131–0.874, $p = 0.025$), indicating that the protective effect is independent of these confounding factors. Although the univariate analysis produced an OR of 2.579 when using GG as the reference – showing an inverse relationship with the multivariate result – both analyses consistently support the conclusion that the GG genotype acts as a protective factor. Moreover, the significance of the model improved after adjustment (p decreased from 0.036 to 0.025), further strengthening evidence for an independent association between the rs4241122 and viral clearance. In addition, compared with the GG+GA genotype, the AA genotype of rs4241122 was more frequently distributed in the severe liver disease group than in the inactive group, and individuals carrying the GA genotype were more likely to develop severe liver abnormalities. We found that rs4241122-GA was associated with severe liver abnormalities, which was inconsistent with a study on the Iranian population and may be caused by population differences (Molaei et al., 2023).

This study identifies the GG genotype of rs4241122 as an independent protective factor associated with spontaneous clearance of HBV. Notably, according to the Ensembl database (https://useast.ensembl.org/index.html), rs4241122 is annotated as residing within a transcription factor binding site and has been linked to elevated levels of IL-1 in gingival crevicular fluid. We propose that this regulatory influence may extend to systemic modulation of IL-1β expression. As a pivotal pro-inflammatory cytokine, IL-1β exerts a dual role in antiviral immunity, often described as a "double-edged sword". We hypothesize that the A allele (risk allele) of rs4241122 may strengthen transcription factor binding affinity, thereby promoting excessive IL-1β production. In the setting of HBV infection, such heightened and sustained inflammation could not only aggravate hepatic injury but also drive immune exhaustion, ultimately impairing viral clearance. In contrast, the G allele (protective allele) appears to facilitate a more balanced IL-1β response – sufficient to activate antiviral immunity effectively while minimizing collateral inflammatory tissue damage, thus favoring viral elimination. Although the documented phenotypic association originates from local IL-1β measurements in the oral cavity, the immunomodulatory effect of this SNP is likely systemic. This provides a plausible explanation for its ability to shape infection outcomes in a distal organ such as the liver. Our findings offer novel insights into host genetic susceptibility to HBV infection, underscoring the importance of genetic variants that fine-tune innate immune inflammatory responses as decisive factors in infection resolution. Future studies should prioritize functional validation of this SNP's role in IL-1β regulation and elucidate its mechanistic contributions within the inflammatory network of chronic hepatitis.

It is important to note that the data obtained from this study should be interpreted carefully and cannot be directly extrapolated to other populations. There are several limitations: (1) We did not collect comprehensive clinical data from participants. The retrospective design limited the availability and completeness of clinical data (e.g., liver enzymes, bilirubin, AFP, globulin), preventing adjustment for these confounders in multivariate or stratified analyses. Lifestyle data (smoking, alcohol) were also unavailable, restricting interaction analyses. While these constraints focus the conclusions on genetic associations and do not invalidate the core findings, unmeasured confounding cannot be excluded. These limitations are common in retrospective genetic studies. Future prospective cohorts with standardized collection of clinical, biochemical, and lifestyle data are needed to better evaluate gene-environment interactions in HBV progression; (2) The sample size of this study was not large, especially the sample sizes of the cirrhosis and HCC groups were small. Furthermore, this retrospective analysis focused solely on *IL37* and did not incorporate other established biomarkers – such as HBcrAg, HBV RNA, FIB-4, Mac-2, or sCD14 – for a more comprehensive diagnostic or predictive assessment; (3) The mechanisms by which statistically significant SNPs function are not fully understood, so functional verification is needed.

Conclusion

Taken together, rs4241122 was found to be associated with HBV infection and its clinical outcomes, with the GG genotype identified as an independent protective factor for spontaneous clearance of HBV. The rs2723186 and rsrs4392270 were also significantly associated with HBV clearance under specific genetic models. Furthermore, rs3811046 and rs3811047 were correlated with the progression of liver abnormalities following HBV infection. The heterozygous genotypes of these SNPs are strong disease-progression risk-related variants. These findings suggest that some *IL37* polymorphisms may be associated with susceptibility to HBV infection in the Chinese population and may be valuable biomarkers for predicting liver disease progression in patients with HBV infection.

Acknowledgements
The authors would like to thank all participants for consenting to this study. The authors also appreciate the anonymous reviewers; their comments and suggestions contributed greatly to improving the quality of the manuscript.

Funding
This study was partially supported by the Guangzhou Health Technology Project (No. 20231A011116), the Huadu District Basic and Applied Basic Research Joint Funding Project (District-Hospital Collaboration), Guangzhou (No. 24HDQYLH12) and Guangzhou Medical Key Subject Construction Project [2025–2027].

Ethical aspects and conflict of interest
The authors have no potential conflict of interest to declare with respect to the research, authorship, and/or publication of this article.

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