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Association of polymorphisms of programmed cell death–1 gene with chronic hepatitis B virus infection

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ABSTRACT

Programmed cell death–1 (PD-1) plays a critical role in regulating T-cell function during hepatitis B virus (HBV) infection. The present study investigated the relationships between the polymorphisms of the *PD-1* gene and the susceptibility to chronic HBV infection. Single nucleotide polymorphisms (SNPs) in *PD-1* gene at positions −606G/A (PD-1.1) and +8669 G/A (PD-1.6) were analyzed by bidirectional PCR amplification of specific alleles (Bi-PASA) in 198 chronic HBV patients and 280 controls. Although the genotype and allele frequencies of PD-1.1 were not different between chronic HBV patients and controls, the genotype and allele frequencies of PD-1.6 were significantly different. PD-1.6 GG genotype and the combination of genotypes with G allele were less frequent in HBV patients than in controls ($p = 0.007$ and $p = 0.031$, respectively). The allele G was also less frequent in patients than in controls ($p = 0.006$). Haplotype PD-1.1G/PD-1.6G was less frequent in patients than in controls ($p = 0.001$). Cirrhosis patients had a lower frequency of PD-1.6 G allele compared with controls ($p = 0.007$). Our findings, firstly reporting the association between *PD-1* polymorphism and HBV infection, suggest that *PD-1* gene may be one of the genes predisposing to chronic HBV infection and disease progression.

1. Introduction

Hepatitis B virus (HBV) infection remains a major public health problem, with approximately 2 billion persons infected, 350 million chronic virus carriers, and 60,000 deaths each year worldwide [1,2]. HBV infection can cause clinical conditions varying from spontaneous recovery in an acute hepatitis, to chronic infection predisposing persons at high risk for death from hepatic cirrhosis and hepatocellular carcinoma (HCC) [1,2]. The diversity of the HBV disease spectrum and clinical course was attributed, in a very large extent, to the host immunologic [3–5] and genetic factors, including single nucleotide polymorphisms (SNPs) of a variety of genes, such as major histocompatibility complex (MHC) [6,7], cytokines [8–10], and cytotoxic T-lymphocyte antigen 4 (CTLA-4) genes [11–13].

The programmed cell death–1 (*PDCD1*, also known as *PD-1*) gene is one of the costimulatory molecule genes located in chromosome 2q37.3, and encodes a cell surface receptor PD-1 [14]. PD-1 is an inhibitory immunoreceptor expressed on activated T-cells, B-cells, and myeloid cells [15]. It belongs to the immunoglobulin superfamily B7-CD28 and is mainly involved in T-cell activation [16,17]. When engaged by its ligands PDL-1 (B7-H1) or PDL-2 (B7-DC), PD-1 inhibits T-cell receptor–mediated proliferation and cytokine production in activated T lymphocytes [18] and thereby plays a crucial role in the downregulation of immune responses. As for infections, the PD-1:PD-L pathway is involved in the T-cell dysfunction and lack of viral control in established chronic infection [19,20].

PD-1 is also expressed on $CD8^+$ T cells and $CD4^+$ $CD25^+$ T cells. Studies have shown that HBV-specific $CD8^+$ T cells express PD-1 and intrahepatic HBV-specific $CD8^+$ cells express higher levels of PD-1 than their peripheral counterparts [21]. PD-1 upregulation on HBV-specific $CD8^+$ T cells is engaged in the dysfunction of T cells and high viremia in chronic HBV patients. PD-1:PDL-1 interactions contribute to the functional suppression of virus-specific $CD8^+$ T lymphocytes in the liver and the antiviral T-cell responses, such as $CD8^+$ T-cell proliferation and interferon-γ and interleukin-2 production by intrahepatic lymphocytes, which could be improved by the blockade of the inhibitory PD-1/PD-L1 pathway [21–24]. Investigations in HBV transgenic mice have suggested that antigen recognition in the liver and PD-1 upregulation are associated with the oscillations of noncytolytic and cytolytic effector functions and expansion–contraction kinetics of the cytotoxic T lymphocytes,

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maximizing viral clearance and minimizing tissue injury during HBV infection [25]. However, poor coordination of these events could lead to viral persistence and chronic liver disease [25].

Studies have also demonstrated that chronic HBV patients present with an increased percentage of $CD4^+$ $CD25^+$ regulatory T cell (Treg) in peripheral blood, and that Tregs play an active role in modulating effectors of immune response to HBV infection and in influencing the disease prognosis in hepatitis B patients [26,27]. The HBV-specific Tregs have an immunosuppressive effect on HBV-specific T helper cells, contributing to an inadequate immune response against the virus and leading to chronic infection [28]. Longitudinal investigation of the relationship between PD-1 expression, viral load, and HBeAg in chronic hepatitis B patients undergoing oral antiviral treatment (lamivudine or telbivudine) revealed a strong correlation between HBV viremia and hyperexpression of PD-1 on all T cells. Treatment-induced suppression of HBV replication results in a significant reduction of PD-1 transcription and PD-1 expression on the T-cell surface, thus reducing its negative impact on T-cell activation and function [29]. Observation also showed that expression of PD-1 is upregulated in $CD4^+$ $CD25^+$ FoxP3+ Treg of nonresponders after HBsAg vaccine immunization [30]. All of these studies strongly indicate the important role of the regulatory function by PD-1 in the host immune response against HBV infection.

Considering the important role of PD-1 in T-cell function of HBV-specific immune response, and the possible effects of expression or functional alteration of PD-1 resulting from gene polymorphisms on the immune response, the present study aimed to examine the role of two common SNPs of *PD-1* gene in patients with chronic HBV infection.

2. Subjects and methods

2.1. Patients and control subjects

A total of 198 unrelated patients with chronic HBV infection (143 male and 55 female; 18–78 years of age, mean age 36.54 ± 12.76 years) from Shaanxi Province, China, were studied. All patients were randomly selected. The clinical diagnoses in the patients were mainly based on the diagnostic criteria of the Proposal of Prevention and Treatment of Viral Hepatitis, Xi'an, 2000, issued by the Chinese Society of Infectious Diseases and Parasitology and the Chinese Society of Hepatology of the Chinese Medical Association [31]. Of the 198 patients, 31 were diagnosed as asymptomatic carriers, 92 with chronic hepatitis, 55 with liver cirrhosis, and 20 with HCC. Patients aged less than 18 years, pregnant women, patients who had other hepatic viral infection (hepatitis A virus, hepatitis C virus, hepatitis D virus, or hepatitis E virus), autoimmune hepatitis and drug-induced hepatitis or alcoholic hepatitis, patients with severe complications of the cardiovascular, renal, or respiratory system, patients with mental disease, and patients with concurrent infection of human immunodeficiency virus-1 were all excluded. The demographic and laboratory characteristics of the HBV patients are shown in Table 1.

A total of 280 healthy subjects (181 male and 99 female; 18–76 years of age, mean age 35.45 ± 14.41 years) from the same area as the HBV patients were recruited as healthy controls. All the subjects, including patients and controls, are unrelated Chinese Han individuals. The distribution of age and gender did not differ between HBV patients and healthy controls. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee and informed consent was obtained from each patient and control subject included in the study.

2.2. Genotyping of PD-1 polymorphisms by bidirectional polymerase chain reaction amplification of specific alleles

Blood samples were collected from the patients and healthy controls in the morning after an overnight fast. Genomic DNA was extracted from 200 μl of ethylenediaminetetraacetic acid (EDTA)–treated peripheral blood using TIANamp Genomic DNA Kit (Tiangen Biotech, Beijing, China) according to manufacturer's instructions. Genotyping of PD-1.1 and PD-1.6 was performed by bidirectional polymerase chain reaction (PCR) amplification of specific alleles (Bi-PASA) using two outer (P and Q) and two inner allele-specific (A and B) primers [32]. The primers were designed according to the PD-1 sequence (EF064716) using Oligo6.0 software (Molecular Biology Insights, Cascade, CO).

For PD-1.1 SNP, -606G/A (previously called PD-1.1 at -531G/A from the transcriptional start site), the primers are P: 5′-TCTAGCCTCGCTTCGGTTAT-3′, Q: 5′-CTCACTGCTGTGGCCTCTTT-3′, A: 5′-GGGGCGGGGCGCGAAGGGGGATGGGCCA-3′ (variant-specific) and B: 5′-GGCCGGGGGGTGCCTGGCCTCTGCCTTCCC-3′ (wild-type specific).

For PD-1.6 SNP, +8669 G/A (previously called PD-1.6 at +8738 G/A in the 3′ untranslated region), the primers are P: 5′′-GAAGTT-TCAGGGAAGGTCAG-3′, Q: 5′-CAGTGTGTGGATGTGAGGAG-3′, A: 5′-GGGCGGGCGGACCTAGGGCCCCCCATA-3′ (variant-specific) and B: 5′-GGGCGGGCGGAGCTCCCAGGGTGGGCAC-3′ (wild-type specific). The primers were synthesized and purified by Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China).

A 6.0 μl (0.3 μg) quantity of genomic DNA was amplified in each 25 μl Bi-PASA reaction containing 12.5 μl of 2×Master Mix (2×Taq PCR Mix, Xi'an Runde Biotechnology, Xi'an, China), 1.0 μl (10 μmol/l) of each of P primer and Q primer, 0.5 μl (10 μmol/l) of each of A primer and B primer, and 3.5 μl of double-distilled H_2O. The reaction mixture was first heated at 94°C for 3 minutes, and amplification was done for 30 cycles in PTC-200 TM Peltier thermal cycler (MJ Research, Waltham, MA) by denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds and extension at 72°C for 1 minute in each cycle and a final extension at 72°C for 10 minutes. The products were analyzed by electrophoresis on 2% agarose gels stained with ethidium bromide and revealed under UV light using UV-transilluminator (UVP Model White /2 UV; UVP, Inc, Upland, CA). The Bi-PASA of PD-1.1 produced two fragments of 730 and 473

Table 1
Demographic and laboratory characteristics of hepatitis B virus patients

	Asymptomatic carrier (n = 31)	Chronic hepatitis (n = 92)	Liver cirrhosis (n = 55)	Hepatocellular carcinoma (n = 20)
Mean age (y)	30.81 ± 10.92	33.60 ± 12.12	40.60 ± 11.96	47.75 ± 10.73
Gender (M/F)	16/15	67/25	44/11	16/4
Mean ALT (IU/l)	25.52 ± 7.22	291.10 ± 364.78	97.79 ± 117.45	99.95 ± 86.15
Mean AST (IU/l)	25.63 ± 7.41	239.31 ± 348.25	161.17 ± 166.05	168.15 ± 228.24
Mean total bilirubin (μmol/l)	11.99 ± 3.75	60.98 ± 93.65	97.13 ± 141.54	75.25 ± 153.88
Mean direct bilirubin (μmol/l)	3.61 ± 1.23	27.74 ± 47.61	43.04 ± 70.60	23.45 ± 41.79
Mean albumin (g/L)	40.43 ± 2.93	39.34 ± 5.74	31.08 ± 6.98	33.07 ± 4.56

ALT, alanine aminotransferase; AST, aspatate aminotransferase; F, female; M, male.

Table 2
Genotype and allele frequencies of PD-1.1 and PD-1.6 polymorphisms in hepatitis B virus (HBV) patients and controls

Polymorphism	HBV patients (n = 198)	Controls (n = 280)	p	OR	95% CI
PD-1.1					
Genotype					
GG	50 (25.3)	72 (25.7)	1	1	
GA	106 (53.5)	138 (49.3)	0.688	1.095	0.702–1.708
AA	42 (21.2)	70 (25.0)	0.493	0.831	0.489–1.412
GA+AA	148 (74.7)	208 (74.3)	0.979	1.006	0.660–1.533
Allele					
G	206 (52.0)	282 (50.4)		1	
A	190 (48.0)	278 (49.6)	0.612	0.918	0.709–1.190
PD-1.6					
Genotype					
AA	116 (58.6)	135 (48.2)	1	1	
GA	76 (38.4)	121 (43.2)	0.135	0.748	0.511–1.095
GG	6 (3.0)	24 (8.6)	0.007	0.276	0.108–0.705
GA+GG	82 (41.4)	145 (51.8)	0.031	0.667	0.461–0.965
Allele					
A	308 (77.8)	391 (69.8)		1	
G	88 (22.2)	169 (30.2)	0.006	0.662	0.491–0.893

Data are presented as n (%).

bp for GG genotype, two fragments of 730 and 294 bp for AA genotype and three fragments of 730, 473 and 294 bp for GA genotype. The Bi-PASA of PD-1.6 produced two fragments of 490 and 353 bp for GG genotype, two fragments of 490 and 171 bp for AA genotype and three fragments of 490, 353 and 171 bp for GA genotype.

2.3. Statistical analysis

Statistical analysis was performed by SPSS software version 16.0 (SPSS, Inc., Chicago, IL). The frequency of genotypes and alleles was determined by direct gene counting method. Hardy–Weinberg equilibrium was tested for each polymorphism included in this study with χ^2 test. Comparisons were made by χ^2 test. For the association between genotype, allele and haplotype of the polymorphisms and the disease, univariate logistic regression analysis was used to calculate the χ^2 value, p value, odds ratios (OR), and 95% confidence interval (CI) after adjusting for age and gender. A p value less than 0.05 was regarded to be statistically significant. The linkage disequilibrium and haplotype construction were performed from the observed genotypes using SHEsis method [33].

3. Results

3.1. Distributions of the genotypes and alleles of PD-1.1 and PD-1.6 polymorphism between patients and controls

The frequencies of PD-1.1 genotypes had no significant differences between HBV patients and controls (Table 2). The frequencies of PD-1.6 genotypes were significantly different between patients and controls. Using genotype AA as reference, genotype GG was less frequent in patients than in controls (p = 0.007, OR = 0.276, 95% CI = 0.108–0.705). When the genotypes with G allele were combined, the patients also had lower frequencies of the genotypes with G allele compared with controls (p = 0.031, OR = 0.667, 95% CI = 0.461–0.965, Table 2).

Allele frequency analysis showed that the frequency of PD-1.1 alleles was not significantly different between HBV patients and controls (Table 2). However, the allele G of PD-1.6 was less frequent in patients than in controls (p = 0.006, OR = 0.662, 95% CI = 0.491–0.893, Table 2).

3.2. Haplotype analysis of PD-1.1 and PD-1.6 between patients and controls

The haplotypes of PD-1.1 and PD-1.6 were constructed and compared between patients and controls. Using haplotype PD-1.1G/PD-1.6A as reference, haplotype PD-1.1G/PD-1.6G was less frequent in HBV patients than in controls (p = 0.001, OR = 0.530, 95% CI = 0.363–0.773, Table 3).

3.3. Combined analysis of PD-1.1 and PD-1.6 genotypes between patients and controls

Compared with the combination of PD-1.1 GG genotype and PD-1.6 AA genotype, the combination of PD-1.1 GG genotype and PD-1.6 GA or GG was less frequent in patients (p = 0.016, OR = 0.381, 95% CI = 0.174–0.834, Table 4), and the combination of PD-1.1 GA or AA and PD-1.6 GA or GG was also less frequent in HBV patients than in controls (p = 0.031, OR = 0.452, 95% CI = 0.220–0.930, Table 4).

Compared with the OR 0.667 of PD-1.6GA + GG genotypes in patients with chronic HBV infection (Table 2), the combination with PD-1.1 GG genotype decreased the risk of OR to 0.381, and this effect was more obvious than the combination with PD-1.1 genotype GA or AA, which had an OR of 0.452 (Table 4).

3.4. Distributions of the genotypes and alleles of PD-1.1 and PD-1.6 polymorphism in HBV patients with different clinical diagnoses

After adjusting for age and gender, the distribution of genotypes and alleles of both PD-1.1 and PD-1.6 polymorphisms were compared among HBV patients with different clinical diagnoses and controls. The frequency distributions of PD-1.6 genotype and allele were different (p = 0.043 and p = 0.026, respectively, Table 5). Using A allele as reference, patients with liver cirrhosis had a lower frequency of PD-1.6 G allele compared with controls (p = 0.007, OR = 0.483, 95% CI, 0.285–0.818, Table 5).

4. Discussion

In the present study we genotyped two polymorphisms at −606G/A (previously called PD-1.1 at −531G/A from the transcriptional start site) and +8669 G/A (previously called PD-1.6 at +8738G/A in the 3′ untranslated region) on the *PD-1* gene, which have been shown to be common among the Chinese population [34,35], in patients with chronic HBV infection. The results indicated that the PD-1.1 SNP of *PD-1* gene had no association with chronic HBV infection. However, the PD-1.6 SNP was significantly different between chronic HBV patients and controls. The patients had lower frequencies of genotype GG and genotypes with the G allele, and lower frequencies of G allele at the PD-1.6 SNP locus. The haplotype composed of PD-1.1 G allele and PD-1.6 G allele was also less frequent in HBV patients. The combination of PD-1.6 GA or GG genotype with PD-1.1 GG genotype was also less frequent in

Table 3
PD-1.1 and PD-1.6 haplotype frequency in hepatitis B virus patients and controls

PD-1.1	PD-1.6	Patients (n = 198)	Controls (n = 280)	p	OR	95% CI
G	A	143 (36.1)	154 (27.5)	1		1
G	G	63 (15.9)	128 (22.8)	0.001	0.530	0.363–0.773
A	A	165 (41.7)	237 (42.3)	0.062	0.750	0.554–1.014
A	G	25 (6.3)	41 (7.4)	0.132	0.657	0.380–1.135

Data are presented as n (%).

Table 4
Combined comparison of PD-1.1 and PD-1.6 genotypes in hepatitis B virus patients and controls

PD-1.1	PD-1.6	Patients (n = 198)	Controls (n = 280)	p	OR	95% CI
GG	AA	22 (11.1)	17 (6.1)	1	1	
GG	GA or GG	28 (14.1)	55 (19.6)	0.016	0.381	0.174–0.834
GA or AA	AA	94 (47.5)	118 (42.2)	0.131	0.586	0.292–1.172
GA or AA	GA or GG	54 (27.3)	90 (32.1)	0.031	0.452	0.220–0.930

Data are presented as n (%).

chronic HBV patients. These findings suggested that the *PD-1* gene may play a role in chronic HBV infection in the Chinese population.

We found that the frequencies of PD-1.6 genotype and allele were different in patients with different clinical diagnoses. Cirrhosis patients had a lower frequency of PD-1.6 G allele. This is consistent with the findings in all the studied subjects with chronic HBV infection. It was suggested that the PD-1.6 polymorphism may also have predisposing role in the disease progress.

Alteration of expression and function in PD-1 conferred by *PD-1* polymorphism may affect the T-cell activation and antiviral response via PD-1: PD-L pathway in chronic infection [19,20]. One recent report demonstrated a significant association of PD-1.1 SNP and the expression levels of PD-1 with subacute sclerosing panencephalitis (SSPE) in Japanese and Filipinos population [36]. We showed a significant association of PD-1.6 SNP rather than PD-1.1 SNP with chronic HBV infection in Chinese population. In view of the high similarity of *PD-1* SNPs in Asian populations [34,35,37], this may suggest the role of disease-specific mechanisms in the function of PD-1 as proposed in Graves' disease [38].

Previous studies showed that some SNPs of the *PD-1* gene may alter the expression and/or function of PD-1. For instance, the 7078G/A SNP (7146G/A from the transcriptional initiation start site and previously called PD-1.3), which is not polymorphic in Chinese populations studied [34,35], in intron 4 of the *PD-1* gene may affect the binding affinity of a transcriptional factor, RUNX, and the *PD-1* mRNA levels in the peripheral mononucleocytes [39]. Even though the biologic effect of PD-1.6 SNP remains unknown, the increased PD-1 expression on HBV-specific $CD8^+$ cells and $CD4^+$ $CD25^+$ Treg cells in HBV patients have been demonstrated to be related to the dysfunction of antiviral T-cell response and persistence of the viral infection [21–24]. It is therefore possible that the PD-1.6 SNP, either by itself or by other functional SNPs in a haplotype under linkage disequilibrium, may affect the expression or function of PD-1 and thereby modify the immune response against the virus infection. The PD-1.6 SNPs with G allele, which showed lower frequencies in chronic HBV infection, may be involved in the downregulation of PD-1 expression in the T cells associated with the immune responses, including T-cell activation and the production of antiviral cytokines, such as interferon-γ and tumor necrosis factor, via so far unknown mechanism in chronic HBV infection, conferring a protective effect. Recently, it has been demonstrated that the promoter activity in the construct with −606A allele was significantly lower than that with −606G allele [36]. In our study, the combination of PD-1.6 genotype containing G allele (GA or GG) with the PD-1.1 GG genotype decreased the OR from 0.667 to 0.381 and the PD-1 haplotype comprising PD-1.6 G and PD-1.1 G alleles decreased the OR from 0.662 in PD-1.6 G allele to 0.530. This may be a reflection of the minor contribution of PD-1.1 G allele to the protective role of PD-1.6 G allele in chronic HBV infection through its effect on the promoter activity. It is speculated that the PD-1.6 GG genotype and G allele of *PD-1* gene, alone or in a haplotype with PD-1.1 G allele, may have inhibitory effect on the PD-1 function and thus reduce its negative impact on T-cell activation and function, exhibiting a protective role in chronic HBV infection.

SNPs in the gene of cytotoxic T-lymphocyte antigen 4 (CTLA-4), another immunoinhibitory receptor, have been shown to be implicated in the susceptibility of HBV infection [11–13], proposing the possibility of the interactions between PD-1 and CTLA-4 SNPs in determining the disease diversity and clinical course of HBV infection. Therefore, further interaction study between the polymorphisms of the two genes is needed to elucidate the role of coordination of the PD-1 and CTLA-4 polymorphisms in HBV infection.

Our study has several limitations, which may preclude the interpretation of our results, including the relatively small number of patients, the diversity of the HBV disease, the numbers of SNP sites examined, the lack of functional elucidation of the PD-1.6 SNP, and the ethnic differences of SNPs [40]. The explanation for our findings may also be procluded by the multiple factors involved in the disease *per se*, and therefore further investigation is warranted to clarify the implications.

Table 5
Genotype and allele frequencies of PD-1.1 and PD-1.6 polymorphisms in hepatitis B virus patients with different clinical diagnoses

Polymorphism	Controls (n = 280)	Asymptomatic carrier (n = 31)	Chronic hepatitis (n = 92)	Liver cirrhosis (n = 55)	Hepatocellular carcinoma (n = 20)	P
PD-1.1						
Genotype						
GG	72 (25.7)	6 (19.4)	28 (30.4)	12 (21.8)	4 (20.0)	1
GA	138 (49.3)	19 (61.3)	46 (50.0)	30 (54.5)	11 (55.0)	0.650
AA	70 (25.0)	6 (19.4)	18 (19.6)	13 (23.6)	5 (25.0)	0.749
Allele						
G	282 (50.4)	31 (50.0)	102 (55.4)	54 (49.1)	19 (47.5)	0.750
A	278 (49.6)	31 (50.0)	82 (44.6)	56 (50.9)	21 (52.5)	
PD-1.6						
Genotype						
AA	135 (48.2)	20 (64.5)	48 (52.2)	36 (65.5)	12 (60.0)	1
GA	121 (43.2)	10 (32.3)	39 (42.4)	19 (34.5)	8 (40.0)	0.326
GG	24 (8.6)	1 (3.2)	5 (5.4)	0 (0)	0 (0)	0.043
Allele						
A	391 (69.8)	50 (80.6)	135 (73.4)	91 (82.7)	32 (80.0)	0.026
G	169 (30.2)	12 (19.4)	49 (26.6)	19 (17.3)*	8 (20.0)	

Data arepresented as n (%).
*Compared with controls, χ^2 = 7.343, p = 0.007, odds ratio = 0.483%, 95% confidence interval = 0.285–0.818, using A allele as reference.

Inclusion of individuals who cleared HBV infection could help to clarify whether there is an association with the predisposition to chronicity. However, neonatal infection and horizontal transmission during early childhood are the most common routes of HBV transmission in China, and the outcome of HBV infection is closely related to the age of infection acquisition [41]. The recruitment of individuals who cleared HBV infection and are also comparable in the age at which the infection was acquired with the individuals with chronic HBV infection is practically difficult in China. Therefore, as this is the first report of the association between PD-1 polymorphism and chronic HBV infection, further comprehensive investigation in large sample population of different ethnic origin and different outcomes of infection with comparable ages of infection acquisition is required to confirm and extend our findings.

In conclusion, the findings of the present study showed that polymorphism in the 3′ untranslated region of the *PD-1* gene (PD-1.6) may be implicated in the susceptibility to chronic HBV infection and disease progression, adding new evidence of SNPs in the *PD-1* gene to the genetically predisposed multiple factors associated with HBV infection. Further elucidation of the biologic functions of PD-1 SNPs and their coordination with other regulatory molecules such as CTLA-4 may lead not only to an in-depth understanding of the genetic component in the susceptibility of chronic HBV infection and disease progression but also to novel therapeutic designs by precise manipulation of these inhibitory receptors and individualized considerations for chronic HBV infection.

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