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Features and clinical implications of hepatitis B virus genotypes and mutations in basal core promoter/precore region in 507 Chinese patients with acute and chronic hepatitis B

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ABSTRACT

Background: The association of hepatitis B virus (HBV) genotypes and basal core promoter (BCP) and precore (PC) mutations with the clinical characteristics is increasingly recognized.
Objective: To investigate virologic features and clinical implications of HBV genotypes, BCP and PC mutations between large-size patients with acute hepatitis B (AHB) and chronic hepatitis B (CHB).
Study design: One hundred and eighty-two AHB patients and 325 CHB patients were investigated. HBV genotypes and BCP/PC mutations were determined by direct sequencing. Mutations at 10 interested sites of the BCP/PC region were compared between the two groups of patients.
Results: AHB patients had a significantly higher ratio of genotype B to C than CHB patients (37.4–62.6% vs. 16.6–83.4%, $P < 0.001$). The prevalence of BCP/PC wild-type virus was 60.4% in AHB patients in contrast to 28.9% in CHB patients. Significantly lower prevalence of A1762T, G1764A, G1896A, and G1899A but higher prevalence of T1758C was found in AHB patients. Interestingly, T1758C and A1762T/G1764A appeared mutual restraint. Genotype B virus had lower BCP mutation frequency and similar PC mutation frequency compared to genotype C virus. AHB patients with BCP/PC mutant virus had higher viral load, whereas CHB patients with BCP/PC mutant virus had lower viral load and elevated alanine aminotransferase, in comparison with those with the wild-type virus.
Conclusion: Patients with genotype B virus, BCP/PC wild-type virus or T1758C mutant virus were more susceptible to develop AHB, whereas high prevalence of the BCP/PC mutations was associated with CHB development.

1. Background

Hepatitis B virus (HBV) chronically infected about 350 million people worldwide and 93 million people in China.[1,2] Acute HBV infection may lead to acute hepatitis B (AHB) with spontaneous clearance of virus within weeks to months, or infrequently lead to acute liver failure/fulminant hepatitis. By contrast, chronic HBV infection may lead to chronic hepatitis B (CHB) which is linked to persistence of viral replication and evolution to liver cirrhosis (LC) and hepatocellular carcinoma (HCC).[1] Chronic HBV infection develops in 90% of newborns, 29–40% of children and 5–10% of adults who were infected.[3] However, other factors associated with development of AHB or CHB remain largely unknown.

Resolution of HBV infection rests with interaction between the virus and host immune responses.[4] HBV as a highly variable virus is at least classified into eight genotypes based on nucleotide (nt) sequence divergence among strains of >8%. HBV genotype may vary in geographical distribution, viral characteristics, and relationship to clinical outcomes.[5] HBV with mutations in basal core promoter (BCP) and precore (PC) may enhance HBV replication *in vitro* and abrogates HBeAg translation, respectively; and HBeAg was considered to buffer immune attack of the infected hepatocytes.[6,7] Several studies have reported that the BCP/PC mutants may be associated with progression of fulminant hepatic failure,[8–12] while some other studies have debated this.[13–16] Although the prevalence of HBV genotypes and BCP/PC mutations in AHB and/or CHB patients have also been investigated,[17–22] these virologic features with their clin-

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ical implications between AHB and CHB patients have not been well documented in Chinese patients.

2. Objective

To investigate virologic features and clinical implications of HBV genotypes, BCP and PC mutations between large-size AHB and CHB patients.

3. Study design

3.1. Study population

This study was conducted at Beijing 302 Hospital among 507 patients who visited the hospital from January 2005 to December 2008. Among them, 182 patients were with AHB and 325 patients were with CHB. The diagnostic criteria were based on 2000 Xi'an Viral Hepatitis Management Scheme issued by the Chinese Society of Infectious Diseases and Parasitology, and the Chinese Society of Hepatology, of the Chinese Medical Association.[23] Briefly, a diagnostic criteria for AHB were as follows: without history of chronic HBV infection; acute onset of symptoms with positivity of biochemical and serological markers of hepatitis B at acute phase; and rapid drop of HBsAg titer, serum HBV DNA elimination and HBeAg seroconversion at convalescent phase. The diagnosis was confirmed by HBsAg clearance within 6 months after the initial onset. Sera from AHB patients were sampled on admission. CHB patients met the following criteria: a history of chronic hepatitis based on a histopathological diagnosis and/or compatible laboratory data and ultrasonographic findings, with mild to moderate liver inflammatory manifestations. For all patients, there was no evidence for HCC, concomitant of HCV, HDV, and HIV infection, metastatic or autoimmune liver disease. The study was approved by the Ethics Committee of Beijing 302 Hospital.

3.2. Serological markers and quantification of HBV DNA

Serum ALT, HBeAg/anti-HBe and other serological markers were routinely detected in the Central Clinical Laboratory of Beijing 302 Hospital. HBV DNA level was determined by a real-time PCR kit (Fosun Pharmaceutical Co., Ltd., Shanghai, China) with a lower limit of detection of 500 copies/mL (about 100 IU/mL).

3.3. Determination of the BCP/PC mutations

The sequences of HBV genomes were determined by direct sequencing method after nested PCR amplification. Outer primers were 5′-GACGTCCTTTGTYTACGTCC-3′ (sense, nt 1413–1432) and 5′-TCTGCGACGCGGCGATTGAG-3′ (antisense, nt 2403–2422). Inner primers were 5′-ACTTCGCTTCACCTCTGCAC-3′ (sense, nt 1583–1602) and 5′-ATCCACACTCCAAAAGAYACC-3′ (antisense, nt 2257–2277). The first-round PCR consisted of equilibrating at 94 °C for 3 min; 10 cycles of 94 °C for 35 s, 59 °C for 35 s (decreasing by 2 °C every other cycle), 72 °C for 70 s; and 30 cycles of 94 °C for 35 s, 56 °C for 35 s, 72 °C for 70 s. The second-round PCR consisted of 94 °C for 3 min; 35 cycles of 94 °C for 25 s, 56 °C for 25 s, and 72 °C for 50 s. Sequencing was mediated by a sequencing primer 5′-CATAAGAGGACTCTTGGAC-3′ (nt 1653–1671) and performed using an ABI 3730xl DNA Analyzers (Applied Biosystems, Foster City, CA).

Table 1
Demographic data, HBV genotype and BCP/PC mutation profiles of the studied 507 patients.

	AHB (n = 182)	CHB (n = 325)	P value
Gender (M/F)	151/31	279/46	.386
Age (years)	36.78 ± 12.18	38.34 ± 11.88	.164
Total bilirubin (μmol/L)	111.65 (6–672)	12.8 (4–77)	<.001
ALT (IU/L)	1479.5 (53–7932)	41 (7–665)	<.001
HBV DNA (logcps/mL)	4.13 ± 1.47	5.26 ± 1.65	<.001
HBeAg −/+ (negative %)	121/61 (66.5%)	134/191 (41.2%)	<.001
Anti-HBe −[a]/+ (negative %)	24/155 (13.4%)	232/93 (71.4%)	<.001
Genotype B	68 (37.4%)	54 (16.6%)	<.001
Genotype C	114 (62.6%)	271 (83.4%)	
Wild type	110 (60.4%)	94 (28.9%)	<.001
T1753C/A/G	20 (11.0%)	51 (15.7%)	.143
T1754G	5 (2.7%)	8 (2.5%)	.845
T1758C	18 (9.9%)	16 (4.9%)	.032
A1762T	40 (22.0%)	172 (52.9%)	<.001
G1764A	40 (22.0%)	177 (54.5%)	<.001
C1766T	3 (1.6%)	16 (4.9%)	.063
T1768A	1 (0.5%)	9 (2.8%)	.085
G1862T	2 (1.0%)	4 (1.2%)	.895
G1896A	18 (9.9%)	105 (32.3%)	<.001
G1899A	4 (2.2%)	22 (6.8%)	.025

AHB: acute hepatitis B; CHB: chronic hepatitis B.
[a] Records for three patients are not available.

3.4. HBV genotype analysis

HBV genotype assignment was based on phylogenetic analysis of the 1225 bp-long S/P-gene sequence (nt 54–3167). Similar methods have been used by other investigations.[15,24] The sense and antisense primers for first-round PCR were 5′-AGTCAGGAAGACAGCCTACTCC-3′ (nt 3146-3167) and 5′-AGGTGAAGCGAAGTGCACAC-3′ (nt 1577-1596), respectively. The sense and antisense primers for second-round PCR were 5′-TTCCTGCTGGTGGCTCCAGTTC-3′ (nt 54–75) and 5′-TTCCGCAGTATGGATCGGCAG-3′ (nt 1258–1278), respectively.

3.5. Statistical analysis

Values for results were expressed as means ± standard deviation or median. Differences in data between groups were examined by Chi-square test, Mann–Whitney U-test, Fisher's exact test or Student's t-test where appropriate. Statistical analysis was carried out in SPSS 16.0 software. A P value of <0.05 was considered statistically significant.

4. Results

4.1. Demographic data, genotypes and point mutations

The demographic data, genotypes and point mutations were shown in Table 1. Of all 507 patients, 122 patients were infected with genotype B HBV and 385 patients were with genotype C HBV. AHB patients had a significantly higher ratio of genotype B to C than

Table 2
Analysis of implications of T1758C variation.

	AHB	A1762/G1764	A1896	ALT (IU/L)	HBV DNA (logcps/mL)	HBeAg (−)	Anti-HBe (+)
C1758, n = 34	52.9%	11.8%	29.4%	474 (15–7932)	4.73 ± 13.17	44.1%	35.3%
T1758, n = 473	34.7%	42.7%	23.9%	81 (7–5315)	4.87 ± 1.67	50.7%	54.3%
P value	.032	<.001	.468	.152	.640	.456	.049

AHB: acute hepatitis B.

Table 3
Individual profiles of HBV BCP/PC mutations in different viral genotypes and clinical courses.

	Genotype B (n = 122)			Genotype C (n = 385)		
	AHB (n = 68)	CHB (n = 54)	P value	AHB (n = 114)	CHB (n = 271)	P value
Gender (M/F)	57/11	48/6	.422	94/20	231/40	.492
Age (years)	36.29 ± 11.92	33.58 ± 12.69	.230	37.08 ± 12.38	39.27 ± 11.51	.099
Total bilirubin (μmol/L)	100.5 (11–541)	12 (4–74)	<.001	114.7 (6–672)	12.9 (4–77)	<.001
ALT (IU/L)	1631 (53–5315)	41 (9–665)	<.001	1429.5 (113–7932)	40 (7–652)	<.001
HBV DNA (logcps/mL)	4.01 ± 1.58	5.14 ± 1.50	<.001	4.20 ± 1.41	5.28 ± 1.68	<.001
HBeAg −/+ (negative %)	49/19 (72.1%)	27/27 (50%)	<.001	72/42 (63.2%)	107/164 (39.5)	<.001
Anti-HBe −/+[a] (negative %)	8/59 (11.9%)	35/19 (64.8%)	<.001	16/96 (14.3%)	197/74 (72.7%)	<.001
Wild type	50	22	<.001	60	72	<.001
T1753C/A/G	7 (10.3%)	2 (3.7%)	.167	13 (11.4%)	49 (18.1%)	.104
T1754G	1 (1.5%)	3 (5.6%)	.228	4 (3.5%)	5 (1.8%)	.324
T1758C	1 (1.5%)	0	.371	17 (14.9%)	16 (5.9%)	.004
A1762T	14 (20.6%)	15 (27.8%)	.354	26 (22.8%)	157 (57.9%)	<.001
G1764A	14 (20.6%)	15 (27.8%)	.354	26 (22.8%)	162 (59.8%)	<.001
C1766T	1 (1.5%)	0	.557	2 (1.8%)	16 (5.9%)	.060
T1768A	1 (1.5%)	1 (1.9%)	.691	0	8 (2.95%)	.058
G1862T	0	1 (1.9%)	.443	2 (1.8%)	3 (1.1%)	.464
G1896A	7 (10.3%)	23 (42.6%)	<.001	11 (9.6%)	82 (30.3%)	<.001
G1899A	2 (3.0%)	4 (7.4%)	.238	2 (1.8%)	18 (6.6%)	.035
Substitutions[b]	0.71	1.19	<.001	0.88	1.90	<.001

AHB: acute hepatitis B; CHB: chronic hepatitis B.
[a] Records for three patients were not available.
[b] Average substitution number of each sample at the 10 analyzed sites.

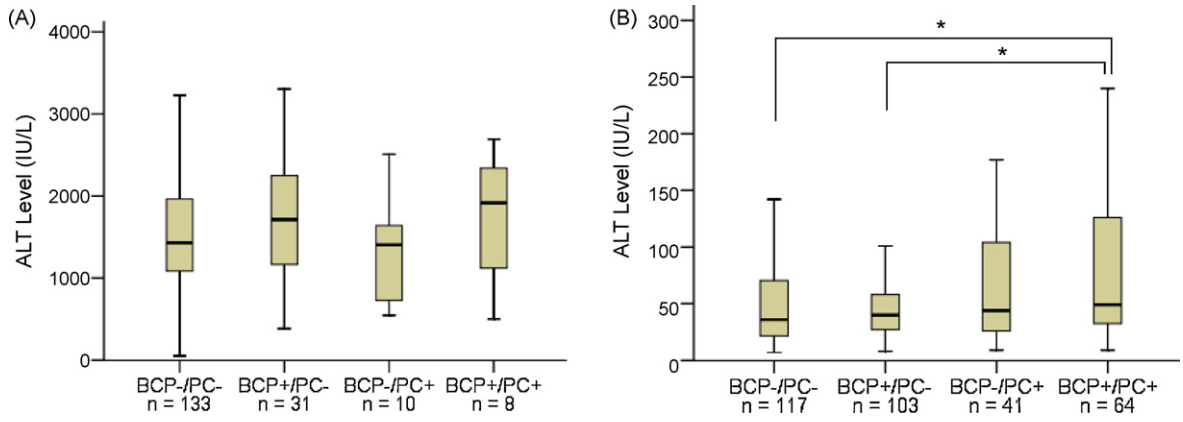

Fig. 1. HBV BCP/PC mutational patterns in relation to ALT level. Four mutational patterns (BCP−/PC−, BCP+/PC−, BCP−/PC+, and BCP+/PC+) were determined based on positivity (+) or negativity (−) of HBV BCP double mutations A1762T/G1764A and PC mutation G1896A. (A) Association of HBV BCP/PC mutational patterns with serum ALT level in patients with acute hepatitis B. (B) Association of HBV BCP/PC mutational patterns with serum ALT level in patients with chronic hepatitis B. Data are expressed as box plots, in which the horizontal lines illustrate the 25th, 50th, and 75th percentiles. *P < 0.05.

CHB patients (37.4–62.6% vs. 16.6–83.4%, P < 0.001). In an alternative word, 55.7% (68/122) patients with genotype B virus infection had AHB compared to 29.6% (114/385) patients with genotype C infection had AHB. In comparison with CHB patients, AHB patients had a significantly lower prevalence of mutants harboring A1762T, G1764A, G1896A and G1899A mutations individually but higher T1758C mutations, as well as higher prevalence of wild-type virus (Table 2).

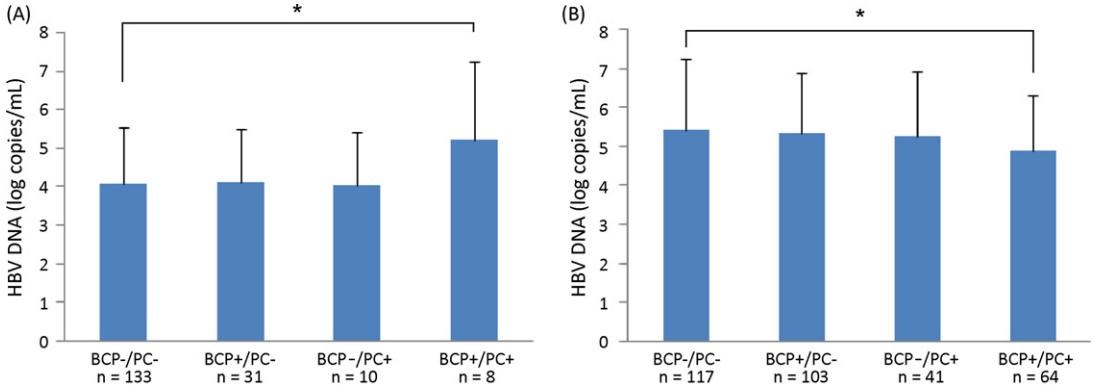

Fig. 2. HBV BCP/PC mutational patterns in relation to HBV DNA level. (A) Association of HBV BCP/PC mutational patterns with HBV DNA level in patients with acute hepatitis B. (B) Association of HBV BCP/PC mutational patterns with HBV DNA level in patients with chronic hepatitis B. Data are expressed as mean ± SD. *P < 0.05.

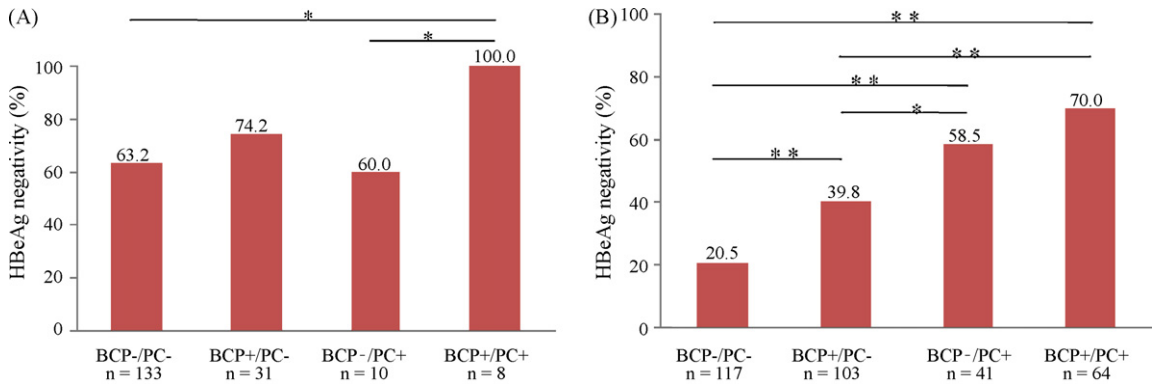

Fig. 3. HBV BCP/PC mutational patterns in relation to HBeAg negativity. (A) Association of HBV BCP/PC mutational patterns with HBeAg negative rate in patients with acute hepatitis B. (B) Association of HBV BCP/PC mutational patterns with HBeAg negative rate in patients with chronic hepatitis B. Data are expressed as mean ± SD. $^{*}P < 0.05$; $^{**}P < 0.01$.

In overall, significantly lower frequency of the mutations were found in genotype B virus than genotype C virus at the sites of 1753 (7.4% vs. 16.1%, $P = 0.016$), 1758 (0.8% vs. 8.6%, $P = 0.003$), 1762 (23.8% vs. 47.5%, $P < 0.001$), 1764 (23.8% vs. 48.8%, $P < 0.001$), and 1766 (0.8% vs. 4.7%, $P = 0.035$) in BCP region. No obvious difference in occurrence of PC mutations (G1862T, G1896A and G1899A) was observed between the two genotype viruses.

4.2. Mutation rate compared between acute and chronic hepatitis within genotype

Table 3 summarizes profiles of the BCP/PC mutations in patients infected with genotypes B or C virus separately. In genotype B virus infection, statistical difference in the mutation occurrence between AHB and CHB patients was only observed at G1896A. By contrast, in genotype C virus infection significant difference was found at T1758C, A1762T, G1764A, G1896A and G1899A between AHB patients and CHB patients. For both genotype B and C virus infections, AHB patients had significantly higher HBeAg negative rate and lower anti-HBe positive rate compared to CHB patients.

4.3. Mutation pattern in relation to ALT and HBV DNA levels, and HBeAg negativity

To simplify data analysis, we defined A1762T/G1764A as basic BCP mutation and G1896A as basic PC mutation, respectively. Accordingly, 4 basic mutational patterns were obtained as follows: BCP−/PC−, BCP+/PC−, BCP−/PC+ and BCP+/PC+. There was no significant difference in ALT levels among the AHB patients with each of the four patterns of mutant viruses (Fig. 1A). Among the CHB patients, higher ALT was observed in patients with PC mutation-positive virus than those with PC mutation-negative virus (Fig. 1B). AHB patients with BCP+/PC+ mutant virus had higher HBV DNA level (Fig. 2A). CHB patients with this pattern of virus, however, had lower viral load (Fig. 2B). AHB patients had a higher percentage of HBeAg negativity in general, and the HBeAg negative rate was further increased in those with BCP+/PC+ mutant virus (Fig. 3A). In CHB patients, accumulation of BCP/PC mutations was accompanied with escalation of HBeAg negative rate (Fig. 3B).

5. Discussion

HBV BCP and PC mutants have been reported to have various effects on the clinical course of patients with HBV-related liver diseases. In some reports, however, genotypes were not distinguished and relative small-size samples were investigated. Therefore bias may have been there which may partly account for discrepancy of conclusions from individual investigations. To our knowledge, there is still a paucity of data showing these virologic features between AHB and CHB patients in China.

Our results showed that patients infected with genotype B virus were more susceptible to develop AHB than those with genotype C virus. Unlike Japan where genotype A virus is commonly seen in AHB,[25,26] genotype A virus is not detected in our study. A recent study showed that genotypes had significant influence on HBV DNA level in chronic HBV carriers.[27] Our results in both AHB and CHB did not show obvious difference of HBV DNA level between genotypes B and C (Table 3), which was consistent with another investigation found between genotypes A and D in general.[20] BCP mutations T1758C, A1762T and G1764A, and PC mutations G1896A and G1899A were more frequently found in CHB patients than in AHB patients. Correspondingly, the wild-type virus was found more frequent in AHB patients. We noticed that genotype B virus compared to genotype C virus had lower occurrence of A1762T/G1764A (23.8% vs. 47.5%) in BCP region but similar mutation occurrence of G1896A (24.6% vs. 24.2%) in PC region. The results seemed not fully consistent with previous reports that G1896A was more frequently detectable in genotype B virus.[28,29] However, analysis of CHB patients alone indicated that G1896A frequency was higher in genotype B virus than genotype C virus (42.6% vs. 30.3%, $P < 0.01$, Table 3), suggesting that HBV PC mutation might have different effects on chronic and acute hepatitis. This may be partly due to smaller number of genotype B HBV-infected patients enrolled and partly due to particular genetic features of different viral genotypes. Interestingly, T1758C frequency was increased rather than decreased in AHB compared to CHB and was negatively associated with A1762T/G1764A occurrence (Table 2), suggesting a repulsion linkage might exist between them. The 1758 was the first nt of the second TA rich (nt 1758–1762, TTAAA) sequence of BCP region which would interact with the TATA binding proteins in transcription. Komatsu et al.[30] reported that virus clones from an infant with severe acute hepatitis B born to a HBeAg-positive mother all harbored T1758C but absented A1762T/G1764A. It is necessary to perform functional study to clarify whether T1758C mutation has influence on the viral replication.

The average substitution number in the BCP/PC region was significantly lower in AHB group compared to CHB group regardless of the virus genotypes, suggesting that high prevalence of BCP/PC mutations could be a distinguishing feature between AHB and CHB in general. As in most Asian countries, the persistent HBV carrier state had been established mainly through perinatal transmission and horizontal infection during the infancy in China. It can be reasoned that BCP/PC mutations gradually accumulate during long-term chronic HBV infection from that chronic hepatitis B evolves. Interestingly, we have found that BCP/PC mutation accumulation was positively associated with the severity of CHB (data not shown),

suggesting these mutations may play a role in pathogenesis of hepatitis B. How these mutations contribute to CHB development needs further study.

HBV harboring A1762T/G1764A and/or G1896A used to be termed as HBeAg negative mutants, and these mutations were defined as basic BCP/PC mutations in our study. AHB patients had a significantly lower prevalence of basic BCP and PC mutations compared to CHB patients. Elevated ALT level was found to be associated with the emergence of basic BCP/PC mutations in the virus in CHB patients rather than AHB patients (Fig. 1). Interestingly, HBV DNA level was increased in AHB patients but decreased in CHB patients with the emergence of basic BCP/PC mutations (Fig. 2). It might be speculated that BCP/PC mutant virus with enhanced viral replication and reduced/abrogated HBeAg expression were more likely to activate immune responses, leading to ALT elevation and viral load decline in chronic HBV infection. However, the alternation of ALT and HBV DNA levels may be influenced by the use of antiviral treatment and a blood test at a single time point may get biased evaluation of activity of liver disease for some CHB patients with fluctuating ALT and HBV DNA levels, although these influences were relatively proportionate in large-size samples of our study. HBeAg negative rate stepwisely increased along with the emergence of basic BCP mutation, basic PC mutation, and both in CHB patients, whereas AHB patients had an overall high HBeAg negative rate (Fig. 3). In light of this, diverse mechanisms for HBeAg clearance could be there, i.e., adaptive BCP/PC mutations are major contributor in CHB while immune responses play a major role in AHB.

In summary, patients with genotype B virus, BCP/PC wild-type virus or T1758C mutant virus were more susceptible to develop AHB, whereas high prevalence of the BCP/PC mutations was associated with CHB development. The study represents the first step toward understanding of virologic factors that may indicate and contribute to the resolution of HBV infection.

Conflicts of interest

The authors declare that they do not have an association that might pose a conflict of interest.

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