Prevalence of HBV Precore/Core Promoter Variants in the United States

Chi-Jen Chu,[1] Emmet B. Keeffe,[2] Steven-Huy Han,[3] Robert P. Perrillo,[4] Albert D. Min,[5] Consuelo Soldevila-Pico,[6] William Carey,[7] Robert S. Brown, Jr.,[8] Velimir A. Luketic,[9] Norah Terrault,[10] Anna S. F. Lok,[1] and The U.S. HBV Epidemiology Study Group

Variants in the precore ($G_{1896}A$) and core promoter ($A_{1762}T$, $G_{1764}A$) regions of hepatitis B virus (HBV) may be related to serum HBV DNA levels and severity of liver disease. The aims of this nationwide study were to determine the prevalence of HBV precore/core promoter variants in the United States and the association between these variants and patient demographics, HBV genotypes, serum HBV DNA level, and severity of liver disease. A total of 694 consecutive chronic HBV-infected patients seen in 17 U.S. liver centers during a 1-year period were enrolled. Demographic, clinical, and laboratory data were collected. Sera were tested for HBV genotypes as well as precore and core promoter variants by line-probe assays. Quantitative HBV DNA levels were determined using Cobas Amplicor HBV Monitor kits. Precore and core promoter variants were found in 27% and 44% of patients with chronic HBV infection in the United States. Precore and core promoter variants were more common in hepatitis B e antigen (HBeAg)-negative than in HBeAg-positive patients (precore, 38% vs. 9%; core promoter, 51% vs. 36%; respectively, $P < .001$). The prevalence of these variants was related to ethnicity, place of birth, and HBV genotypes. Patients with core promoter variants were more likely to have hepatic decompensation. Precore and/or core promoter variants were associated with higher serum HBV DNA levels in HBeAg-negative but not in HBeAg-positive patients. In conclusion, HBV precore and core promoter variants are not rare in the United States. Physicians should be aware of the existence of HBV precore and core promoter variants and the clinical condition of "HBeAg-negative chronic hepatitis." (HEPATOLOGY 2003;38:619-628.)

Hepatitis B virus (HBV) replicates asymmetrically via reverse transcription of an RNA intermediate,[1] making it prone to mutations. The most common naturally occurring HBV variants include the precore stop codon mutation ($G_{1896}A$), which abolishes hepatitis B e antigen (HBeAg) production,[2-4] and the dual mutation in the core promoter region ($A_{1762}T$, $G_{1764}A$), which down-regulates HBeAg production.[5,6] These variants, particularly the precore variant, are found in the vast majority of patients with HBeAg-negative chronic hepatitis, in which HBV replication and hepatic inflammation persist despite the absence of HBeAg. Precore and core promoter variants have been reported in up to 50% to 80% of patients with HBeAg-negative chronic hepatitis B in Europe and Asia.[7] Selection of precore variant is dependent on HBV genotype; it is most common in patients with HBV genotype D and rare in patients with HBV genotype A.[8-11]

The relationship between precore/core promoter variants, serum HBV DNA levels, and severity of liver disease is unclear. Some[5,12,13] but not all[14] *in vitro* studies suggest that core promoter mutations increase HBV replication. One study using branched DNA assay found that serum HBV DNA and alanine aminotransferase (ALT) levels were comparable among patients with and without precore/core promoter variants, regardless of HBeAg status.[15] Another study also using branched DNA assay reported that the presence of precore/core promoter vari-

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; ALT, alanine aminotransferase; WT, wild type.

From the [1]Division of Gastroenterology, University of Michigan, Ann Arbor, MI; [2]Stanford University, Stanford, CA; [3]University of California, Los Angeles, Los Angeles, CA; [4]Ochsner Clinic; [5]Mount Sinai School of Medicine, New York, NY; [6]University of Florida, Gainesville, FL; [7]Cleveland Clinic, Cleveland, OH; [8]Columbia-Presbyterian Medical Center, New York, NY; [9]Virginia Commonwealth University, Richmond, VA; and [10]University of California, San Francisco, San Francisco, CA.

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Address reprint requests to: Anna S. F. Lok, M.D., Division of Gastroenterology, University of Michigan Medical Center, 3912 Taubman Center, Box 0362, Ann Arbor, MI 48109-0362. E-mail: aslok@umich.edu; fax: 734-936-7392.

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ants was associated with lower serum HBV DNA levels, but this study did not separately analyze HBeAg-positive and HBeAg-negative patients.[11] A third study based on polymerase chain reaction assay found that among patients with severe HBeAg-negative hepatitis, those with precore variant had higher HBV DNA levels compared with patients with wild-type (WT) precore sequence.[16] Precore/core promoter variants have been reported to be associated with fulminant hepatitis,[17-20] more active chronic liver disease,[4,8,9,21] and hepatocellular carcinoma.[22-24] However, other studies found that these variants can also be found in hepatitis B surface antigen carriers who had inactive liver disease.[25,26] Thus, the pathogenic roles of precore/core promoter variants and their relationship to quantitative serum HBV DNA levels need to be further examined.

To date, there are little data on the prevalence of precore/core promoter variants in the United States. Previous small-scale studies suggest that precore variant as well as HBeAg-negative chronic hepatitis B are rare in the United States.[27,28] The aims of this nationwide study were to determine the prevalence of HBV precore/core promoter variants in the United States and the association between these variants and patient demographics, HBV genotypes, serum quantitative HBV DNA level, and severity of liver disease. The prevalence of HBV genotypes in different regions of the United States and the relation between HBV genotypes, mode of transmission, ethnicity, and place of birth are separately reported.[29]

Patients and Methods

Patients. This was a cross-sectional study of adults with chronic HBV infection in the United States. Consecutive chronic HBV-infected patients seen in 17 U.S. liver centers during a 1-year period were enrolled. The 17 participating centers were distributed across the United States. Patients currently receiving antiviral treatment and those with recurrent hepatitis B after liver transplantation were excluded. Patients on antiviral therapy were excluded because of the effects of treatment on serum HBV DNA levels and the possibility that antiviral therapy might have an impact on precore/core promoter variants. The protocol was approved by the institutional review boards of each of the participating centers. Eligible patients were asked to sign a written consent form before participating in this study.

Demographic, clinical, and laboratory data were collected during clinic visits. The demographic data included sex, age, place of birth, and ethnicity. The clinical information included presumed source of infection, mode of presentation (asymptomatic, symptoms of chronic liver disease [such as fatigue, right upper abdominal discomfort], or decompensated cirrhosis [variceal hemorrhage, ascites or hepatic encephalopathy]), liver histology, and history of hepatitis B treatment. Laboratory results on hepatitis B markers (hepatitis B surface antigen, HBeAg, and hepatitis B e antibody), liver chemistry, platelet count, and prothrombin time were also recorded. A 15-mL blood sample was collected during clinic visit, centrifuged, aliquoted, and stored frozen at −20°C before shipment to the central laboratory at the University of Michigan.

HBV Genotyping. HBV genotyping was determined by a line-probe assay (Inno-Lipa HBV genotyping assay; Innogenetics Inc., Ghent, Belgium).[11,30,31] All samples with unclassified genotypes and 8% of samples that could be genotyped were randomly selected and sent for bidirectional automated sequencing at the DNA sequencing core facility (University of Michigan Medical Center) using the standard protocol for the Applied Biosystems DNA sequencer 377 (Perkin Elmer Corp., Foster City, CA). Sequencing results were compared with published sequences to ascertain HBV genotypes.[32,33]

Precore Stop Codon Variant ($G_{1896}A$) and Core Promoter Variant ($A_{1762}T$, $G_{1764}A$). Serum samples were tested for precore stop codon ($G_{1896}A$) and core promoter ($A_{1762}T$, $G_{1764}A$) variants using Inno-Lipa precore/core promoter kits (Innogenetics Inc.).[11,30,31] Except for the primers, the procedure was similar to that for HBV genotyping. All samples with indeterminate results and 10% of randomly selected samples with precore and/or core promoter variant were sent for direct sequencing as previously described.

Quantification of Serum HBV DNA Levels. Serum HBV DNA level was quantified by a polymerase chain reaction assay with a lower limit of detection of 2×10^2 copies/mL and a linearity range of 2×10^2 to 2×10^5 copies/mL (Cobas Amplicor HBV Monitor; Roche Diagnostic Systems Inc., Pleasanton, CA).[34-37] All samples with positive HBeAg were prediluted up to 10^6 fold, and a 10^5-fold dilution was used for initial testing. For samples with negative HBeAg, undiluted serum was used for initial testing, retesting after dilution was performed when results of the initial testing were greater than 1×10^5 copies/mL.

Statistical Analyses. Results were expressed as mean ± SE. Data were analyzed using SPSS version 10.0 software package (SPSS Inc., Chicago, IL). Statistical analyses were performed using χ^2 and Fisher's exact test for categorical variables. Independent t test or one-way ANOVA with Tukey test were used for continuous variables as appropriate. Multivariate analyses with logistic regression were used to determine the independent factors

Table 1. Comparison Between Patients With or Without Detectable Serum HBV DNA by Polymerase Chain Reaction Assay

	HBV DNA Positive	HBV DNA Negative	*P*
No.	530	164	
Age (y)	43 ± 1	44 ± 1	NS
Male (%)	68	69	NS
Born in the United States (%)	35	36	NS
Ethnicity (W/A/B/O %)	32/56/9/3	28/53/13/6	NS
HBeAg positive (%)	37	2	<.001
Abnormal ALT (%)	58	35	<.001
ALT (IU/L)	105 ± 9	46 ± 4	<.001
AST (IU/L)	81 ± 7	43 ± 3	<.001
Albumin (g/dL)	3.9 ± 0.3	4.0 ± 0.4	NS
Total bilirubin (mg/dL)	1.2 ± 0.1	1.0 ± 0.1	NS
Platelet count (1,000 K/mm^3)	187 ± 4	192 ± 7	NS
Prothrombin time (s)	12.5 ± 0.1	12.6 ± 0.2	NS

Abbreviations: NS, not significant; W, white; A, Asian; B, black; O, other; AST, aspartate aminotransferase.

that correlate with serum quantitative HBV DNA level and severity of liver disease. Results were considered statistically significant at $P < .05$.

Results

A total of 694 patients were enrolled. Serum HBV DNA was detected in 530 patients (76%): 196 of 199 (98%) HBeAg-positive and 334 of 495 (68%) HBeAg-negative patients. The mean age of these 530 patients was 43 ± 1 years (range, 13-80 years), and 68% were men; 32% were non-Hispanic white, 9% black, 56% Asian, and 3% of other races. Table 1 summarizes the comparison of patients with or without detectable serum HBV DNA. Patients with detectable serum HBV DNA had significantly higher prevalence of HBeAg and abnormal ALT levels.

A total of 453 patients were excluded. The demographics of these patients were comparable to that of the patients enrolled. The mean age was 45 ± 1 years, and 66% were men; 34% were non-Hispanic white, 5% black, 56% Asian, and 5% of other races. The reasons for exclusion included currently receiving antiviral therapy (69%), refusal to participate (12%), prior liver transplantation (4%), and poor venous access or blood sample not drawn (15%). Of the patients who were excluded because they were receiving antiviral treatment, approximately 75% were HBeAg positive when treatment was initiated.

The prevalence of HBV genotypes in the study population was as follows: A, 34.7%; B, 22.0%; C, 30.8%; D, 10.4%; E, 0.4%; F, 0.6%; G, 1.1%. All except 4 samples (99%) could be genotyped by Inno-Lipa assay. Sequencing results of 53 randomly selected samples that could be genotyped were completely concordant with the Inno-Lipa assay.

Indeterminate results were obtained in 1.5% (n = 8) samples in the precore and 8.7% (n = 46) in the core promoter region using the Inno-Lipa assay. The main reason for indeterminate results in the precore region was due to a point mutation ($G_{1897}A$) in the region of the probe. Deletions (n = 19), single rather than dual mutations ($A_{1762}T$ or $G_{1764}A$) (n = 17), and other point mutations in the region of the probe (n = 10) were the main reasons for indeterminate results in the core promoter region. Of the samples that could be typed, the concordance between the Inno-Lipa assay and sequencing was 99%. Line-probe assay was more sensitive in detecting mixed sequences than direct sequencing.

Prevalence of Precore/Core Promoter Variants in Relation to HBeAg Status. Precore ($G_{1896}A$) variant was found in 27% and core promoter ($A_{1762}T$, $G_{1764}A$) variant in 44% of the study population. Compared with HBeAg-positive patients, HBeAg-negative patients were more likely to have precore (38% vs. 9%, $P < .001$) or core promoter variants (51% vs. 36%, $P < .001$). Mutations in both precore and core promoter regions were found in 4% of HBeAg-positive and 19% of HBeAg-negative patients, respectively ($P < .001$). WT sequences in both precore and core promoter regions were found in 57% of HBeAg-positive and 27% of HBeAg-negative patients, respectively ($P < .001$).

Prevalence of Precore/Core Promoter Variants in Relation to HBV Genotypes. A strong correlation was found between HBV genotypes and precore as well as core promoter variants ($P < .001$) (Fig. 1). Patients with genotype A were most likely to have WT sequence in both precore and core promoter regions, whereas patients with genotype D were most likely to have mutations in both regions. Precore variant (alone or in combination with core promoter variant) was most common among patients with genotypes D (55%) and B (44%), followed by genotype C (22%), and very rare in patients with genotype A (3%). Core promoter variant (alone or in combination with precore variant) was more common among patients with genotype C (60%), followed by genotype A (41%) and D (40%), and less common in patients with genotype B (26%).

Despite differences in prevalence of HBV genotypes in different regions of the United States, the prevalence of precore as well as core promoter variants was similar across the country. WT sequences in both precore and core promoter regions ranged from 36% in the West to 37% in the East and Midwest to 43% in the South.

Prevalence of Precore/Core Promoter Variants in Relation to Ethnicity and Place of Birth. The prevalence of precore variants was significantly related to the ethnicity of the subjects ($P < .01$) (Fig. 2). Precore variants (alone or in combination with core promoter variant)

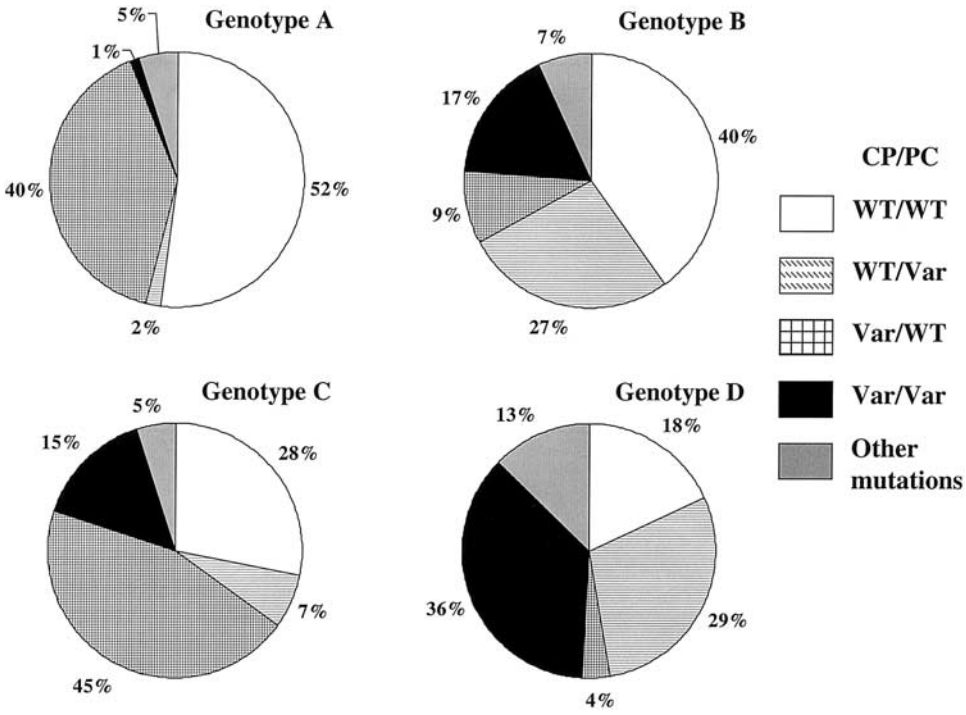

Fig. 1. Prevalence of precore (PC)/core promoter (CP) variants in relation to HBV genotypes. Var, variant.

were found in 32% of Asian patients, 19% of white patients, and only 6% of black patients. The prevalence of core promoter variants (alone or in combination with precore variants) was also lower among black patients (31%), but the difference across the ethnic groups was less marked, being present in 45% of Asian and white patients ($P = .96$). Mutations in both precore and core promoter regions were most common among Asian patients (17%) and very rare in black patients (2%).

The prevalence of precore variants ($P < .001$) was also related to place of birth. Precore variant was found in only 10% of patients born in the United States, 33% of those born in East Asia, and 56% of those born in Europe. Patients born in the United States were more likely to

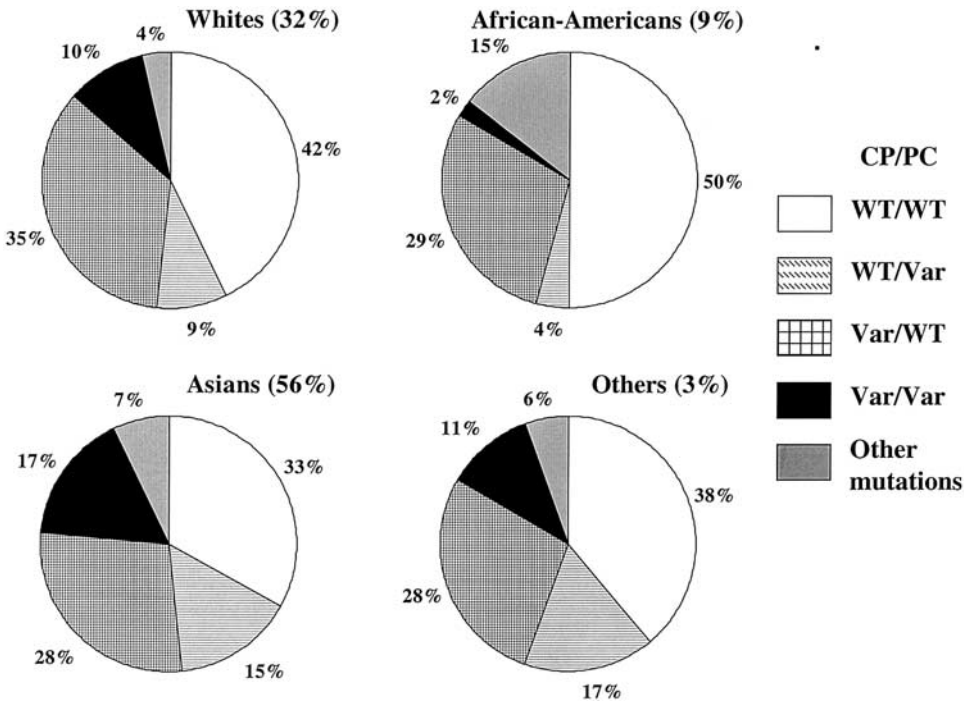

Fig. 2. Prevalence of precore (PC)/core promoter (CP) variants in relation to ethnicity. Var, variant.

Table 2. Comparisons Between Different Patterns of Precore/Core Promoter Variants

	CP:WT PC:WT	CP:WT PC:Var	CP:Var PC:WT	CP:Var PC:Var	Other Mutations	*P*
No. of patients	201	65	160	69	35	
% of population	38	12	30	13	7	
Male	139 (69)	49 (75)	104 (65)	46 (67)	23 (66)	NS
Age (yr)	42 ± 1	41 ± 1	43 ± 1	46 ± 2	44 ± 2	NS
Born in the United States	92 (46)	9 (14)	64 (40)	9 (13)	11 (31)	<.001
HBeAg positive	111 (55)	8 (12)	62 (39)	9 (13)	6 (17)	<.001
Abnormal ALT level	122 (61)	35 (55)	93 (58)	33 (48)	24 (69)	NS
Decompensated cirrhosis	14 (7)	4 (6)	24 (15)	9 (13)	6 (17)	.08
Liver biopsy within past 2 y	69 (34)	19 (29)	73 (46)	18 (26)	16 (46)	NS
Biopsy-proven cirrhosis (cirrhosis/biopsies)	23 (34)	5 (28)	27 (38)	8 (44)	5 (31)	NS
Prior antiviral treatment	40 (20)	13 (20)	40 (25)	11 (16)	6 (17)	NS
Response to previous antiviral treatment	9 (23)	1 (9)	12 (35)	1 (9)	2 (40)	NS
ALT (IU/L)	123 ± 20	133 ± 35	80 ± 9	77 ± 11	110 ± 24	NS
AST (IU/L)	93 ± 14	83 ± 20	73 ± 8	60 ± 6	78 ± 12	NS
Albumin (g/dL)	3.9 ± 0.1	4.0 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	3.8 ± 0.1	NS
Total bilirubin (mg/dL)	1.1 ± 0.1	1.4 ± 0.3	1.2 ± 0.2	1.5 ± 0.3	0.9 ± 0.1	NS
Platelet count (K/mm^3)	203 ± 6	202 ± 9	171 ± 6	167 ± 10	178 ± 13	<.05
Prothrombin time (s)	12.3 ± 0.2	12.1 ± 0.2	12.8 ± 0.3	13.1 ± 0.4	11.9 ± 0.3	NS

NOTE. Results of continuous variables are expressed as mean ± SEM, and results of categorical variables are expressed as number (%). Response to prior antiviral treatment could only be obtained in 100 of 110 patients.

Abbreviations: CP, core promoter; PC, precore; Var, variant; NS, not significant; AST, aspartate aminotransferase.

have WT sequence in both precore and core promoter regions than those born outside the United States (51% vs. 32%; $P < .001$).

Precore/Core Promoter Variants and Liver Disease. Patients with WT and variant precore/core promoter sequences were comparable in age and sex (Table 2). Patients with core promoter variant alone or in association with precore variant were more likely to have decompensated cirrhosis ($P < .05$) (Table 2) and to have lower platelet count (Fig. 3) compared with those with precore variant only or WT sequence in both precore/core promoter regions. Based on a blood test at a single time point, there was no difference in liver chemistries among the patients with different patterns of precore/core promoter sequences (Table 2 and Fig. 4).

To determine if precore/core promoter variants were related to liver disease, we used ALT level as a surrogate marker of hepatic inflammation and platelet count (<150,000 mm^3) as a surrogate marker of liver fibrosis. Potential factors that may be related to ALT level and platelet count, including sex, age (older than 40 years or 40 years or younger), ethnicity, HBV genotype, core promoter variant, precore variant, liver chemistries, previous antiviral therapy, and $\log_{10}$ HBV DNA level, were analyzed. Multivariate analyses with logistic regression identified male sex as the only predictor of abnormal serum ALT level among HBeAg-positive patients and male sex and high HBV DNA level ($>10^5$ copies/mL) as predictors among HBeAg-negative patients (Table 3). In HBeAg-positive patients, older age and presence of core promoter variants were independently associated with lower platelet counts; in HBeAg-negative patients, older age, aspartate aminotransferase level, and clinical evidence of decompensated cirrhosis were predictors of lower platelet counts (Table 4).

Precore/Core Promoter Variants and Quantitative Serum HBV DNA Levels. Serum HBV DNA levels were significantly higher among the HBeAg-positive patients. Among the patients with detectable serum HBV DNA, the mean level for HBeAg-positive patients was 3 $\log_{10}$ higher (mean ± SEM, 8.0 ± 0.1; median, 8.5; range, 2.9-10.3 $\log_{10}$ copies/mL) than for HBeAg-negative patients (mean ± SEM, 4.7 ± 0.1; median, 4.4; range, 2.4-10.0 $\log_{10}$ copies/mL) ($P < .001$). This difference was more marked when patients with undetectable serum HBV DNA were included and an arbitrary value of 1 $\log_{10}$ copies/mL was assigned to those patients; the mean HBV DNA level for HBeAg-positive patients was 4 $\log_{10}$ higher (mean ± SEM, 7.9 ± 0.1; median, 8.5; range, 1.0-10.3 $\log_{10}$ copies/mL) than the HBeAg-negative patients (mean ± SEM, 3.6 ± 0.1; median, 3.6; range, 1.0-10.0 $\log_{10}$ copies/mL) ($P < .001$).

HBeAg-positive patients with core promoter variant had significantly lower serum HBV DNA levels compared with those with WT sequence in the core promoter region (Fig. 5A). The reverse was true among the HBeAg-negative patients. HBeAg-negative patients with either core promoter or precore variants had significantly higher

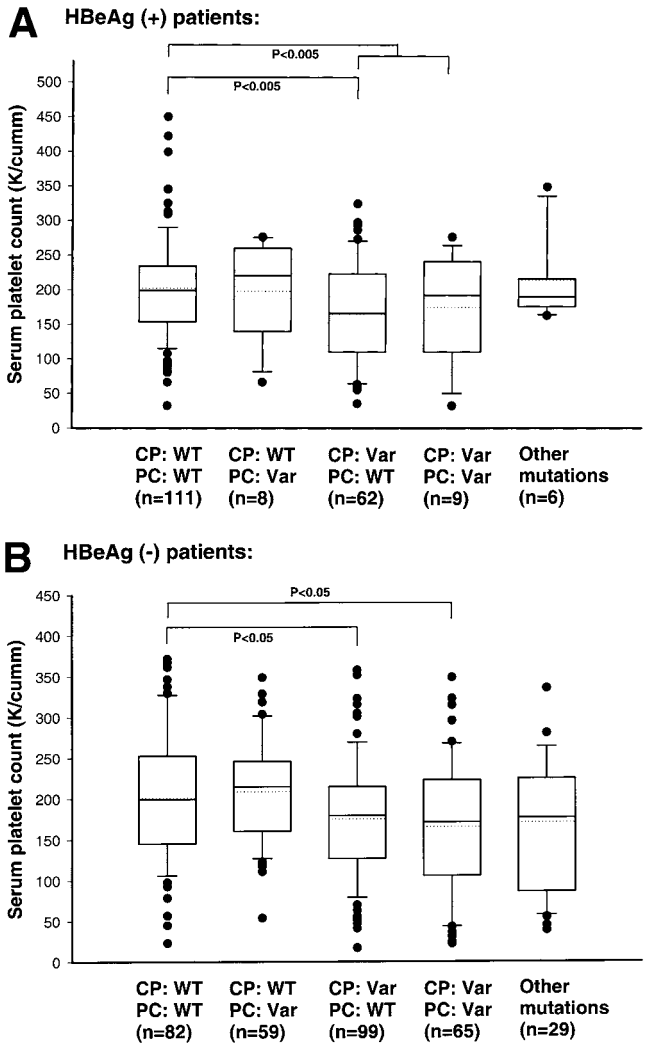

Fig. 3. Relationship between different precore (PC)/core promoter (CP) patterns and platelet count in (A) HBeAg-positive and (B) HBeAg-negative patients. The box plots show the 10th, 25th, 50th, 75th, and 90th percentiles and outliers (**circles**). Mean HBV DNA level is indicated as a **dotted line**. Var, variant.

HBV DNA levels compared with those with WT sequence in both precore/core promoter regions (Fig. 5B).

Potential factors that may be related to serum HBV DNA levels, including sex, age, ethnicity, HBV genotype, precore/core promoter variant, previous antiviral therapy, liver chemistries, and hepatic decompensation, were analyzed. Multivariate analysis with logistic regression identified compensated liver disease, WT sequence in the core promoter region, and age younger than 40 years as predictors of higher serum HBV DNA level among HBeAg-positive patients, whereas abnormal ALT levels and the presence of core promoter or precore variant were predictors of higher serum HBV DNA level among HBeAg-negative patients (Table 5). HBV genotype was not related to serum HBV DNA level among HBeAg-positive as well as HBeAg-negative patients.

Discussion

In this study, precore and core promoter variants were found in 27% and 44%, respectively, of patients with chronic HBV infection in the United States. Several factors may account for the higher prevalence of precore and core promoter variants in our study compared with earlier studies.[27,28] First, the influx of immigrants from countries with a high prevalence of precore and core promoter variants in recent years may have altered the epidemiology of HBV infection in this country. This was supported by the fact that precore variant was found in only 10% of the patients born in the United States but in 36% of those born outside this country. Second, our study included a larger number of patients from different regions of the

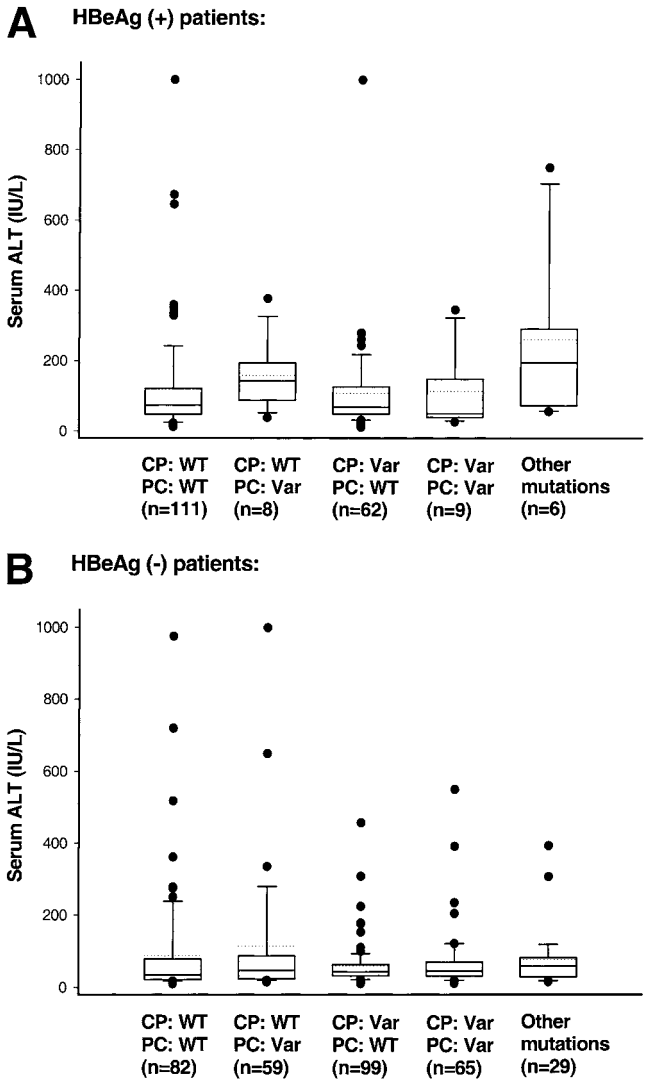

Fig. 4. Relationship between different precore (PC)/core promoter (CP) patterns and serum ALT value in (A) HBeAg-positive and (B) HBeAg-negative patients. The box plots show the 10th, 25th, 50th, 75th, and 90th percentiles and outliers (**circles**). Mean HBV DNA level is indicated as a **dotted line**. Var, variant.

Table 3. Independent Factors Associated With Abnormal Serum ALT (>45 IU/L)

Factors	Odds Ratio	95% CI	*P*
HBeAg-positive patients			
Male sex	4.28	2.00-9.17	<.001
HBeAg-negative patients			
Serum HBV DNA $>10^5$ copies/mL	6.16	3.59-10.56	<.001
Male sex	2.09	1.19-3.66	.01

United States. Third, it is possible that differences in pathogenicity and responsiveness to treatment may have resulted in global changes in the prevalence of precore/core promoter variants over time. Finally, the exclusion of patients currently receiving antiviral treatment, most of whom were HBeAg positive, may have resulted in a bias toward HBeAg-negative patients, who are more likely to harbor these variants.

We acknowledge that enrollment of patients from tertiary care liver centers and exclusion of patients currently receiving antiviral therapy may have introduced selection bias and our results may not be generalized to all patients with chronic HBV infection in the United States. The most common reason for exclusion was current antiviral therapy, and approximately 75% of patients currently receiving antiviral therapy were HBeAg positive. The disproportionate exclusion of HBeAg-positive patients might have resulted in an overestimation of precore and, to a lesser extent, core promoter variants. It is also possible that patients with more active liver disease may have been disproportionately excluded. However, this is the largest cross-sectional study of the prevalence and clinical significance of precore/core promoter variants in the United States. Our study represents the first step toward understanding the molecular epidemiology of HBV infection in this country.

In accordance with previous reports, our study showed that precore variants were predominantly detected in HBeAg-negative patients, whereas core promoter variants were found in both HBeAg-negative and HBeAg-positive patients.[15,38] A more marked increase in the prevalence of precore versus core promoter variants in HBeAg-negative patients is attributed to the fact that precore ($G_{1896}A$) variants abolish whereas core promoter variants ($A_{1762}T$, $G_{1764}A$) only down-regulate HBeAg production.

Table 4. Independent Factors Associated With Lower Platelet Count (<150,000/mm³)

Factors	Odds Ratio	95% CI	*P*
HBeAg-positive patients			
Age >40 years	4.95	2.41-10.16	<.001
With CP variant	2.89	1.43-5.86	.003
HBeAg-negative patients			
Decompensated cirrhosis	11.16	4.33-28.78	<.001
Age >40 years	3.40	1.75-6.58	.001
Abnormal AST level	2.32	1.31-4.09	.024

Abbreviation: CP, core promoter; AST, aspartate aminotransferase.

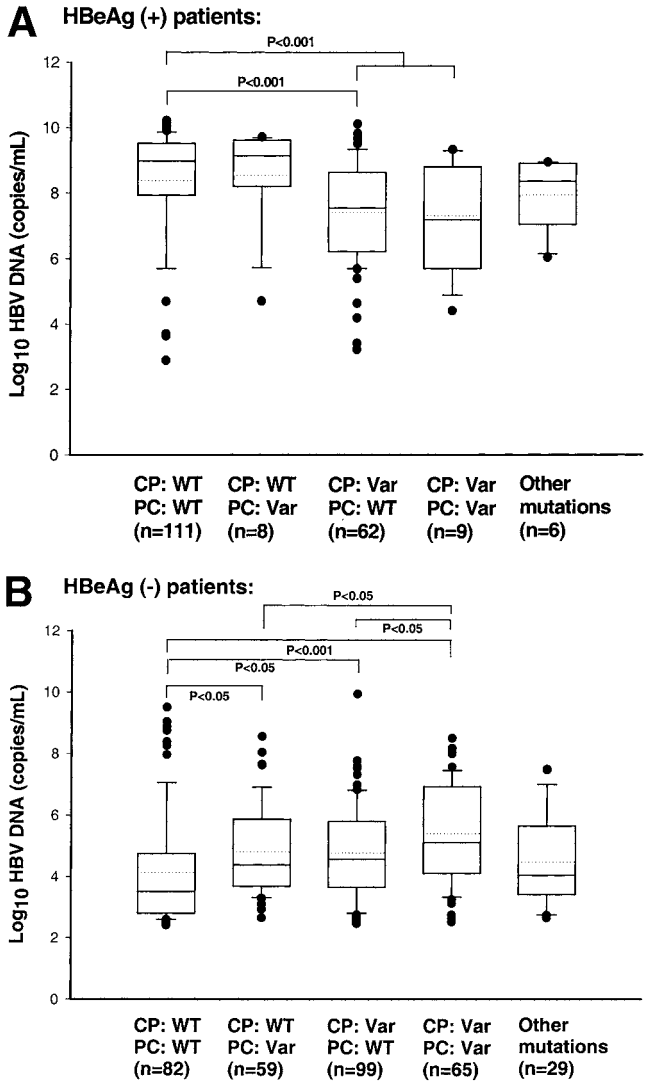

Fig. 5. Relationship between different precore (PC)/core promoter (CP) patterns and serum HBV DNA level (log_{10} copies/mL) in (A) HBeAg-positive and (B) HBeAg-negative patients. The box plots show the 10th, 25th, 50th, 75th, and 90th percentiles and outliers (**circles**). Mean HBV DNA level is indicated as a **dotted line**. Var, variant.

We confirmed the strong relationship between precore variant and HBV genotypes.[8-11] We found that core promoter variant was more evenly distributed among the 4 major HBV genotypes. Nevertheless, as in our previous study,[29] core promoter variants tended to be more prevalent among HBV genotypes (A and C) that are less often associated with precore variants. The association between HBV genotypes and precore ($G_{1896}A$) variant is related to base pairing in the stem-loop structure of the pregenome encapsidation sequence,[10,11,39,40] but the basis for the as-

Table 5. Independent Factors Associated With Higher Serum HBV DNA Level in HBeAg-Positive ($>10^8$ copies/mL) and HBeAg-Negative Patients ($>10^5$ copies/mL)

Factors	Odds Ratio	95% CI	P
HBeAg-positive patients			
Without CP variant	3.55	1.67-7.53	.001
Compensated liver disease	4.74	1.36-16.55	.015
Age ≤40 years	2.49	1.14-5.42	.022
HBeAg-negative patients			
Abnormal ALT level	5.49	2.80-10.73	<.001
With CP variant	3.42	1.90-6.16	<.001
With PC variant	1.87	1.05-3.32	.032

NOTE. Different cutoffs in serum HBV DNA levels were used for analysis because HBeAg-positive patients had serum HBV DNA levels that were 3-4 $\log_{10}$ higher than HBeAg-negative patients.

Abbreviations: CP, core promoter; PC, precore.

sociation between HBV genotypes and core promoter variants has not been deciphered.

The clinical significance of precore and core promoter variants is not yet fully understood. We analyzed our data of a large number of unselected patients to determine if precore and core promoter variants are associated with more active or advanced liver disease. Based on blood tests at a single time point, we found no difference in liver chemistries between patients with WT versus variant precore/core promoter sequences. We acknowledge that patients with chronic hepatitis B may have fluctuating serum aminotransferase levels, and liver chemistries may not accurately reflect hepatic necroinflammation.[4,41,42] Thus, a blood test at a single time point cannot definitively exclude a relationship between precore/core promoter variants and activity of liver disease. Our study did show a correlation between core promoter variant and advanced liver disease (cirrhosis and hepatic decompensation). We found that patients with core promoter variant alone or in association with precore variant were more likely to have decompensated cirrhosis (defined as current or previous history of variceal hemorrhage, ascites, or hepatic encephalopathy) compared with those with precore variant only or WT sequence in both precore/core promoter regions. In addition, other than age, core promoter variant was independently associated with low platelet count, a reliable predictor of liver fibrosis, in HBeAg-positive patients.

In this study, we found no correlation between ALT and serum HBV DNA levels among HBeAg-positive patients. This may be related to the high percentage of Asian patients, many of whom may be in the immune-tolerant phase.[43,44] However, high serum HBV DNA level was the most important predictor of abnormal ALT values among HBeAg-negative patients. Lindh et al.[16] also reported an association between serum HBV DNA level and histologic inflammation and fibrosis scores and ALT values in HBeAg-negative but not HBeAg-positive patients. These findings suggest that the mechanisms of liver damage may be different in HBeAg-positive and in HBeAg-negative chronic hepatitis, with host immune response more important in HBeAg-positive patients and direct viral effects in HBeAg-negative patients.

Our study showed that HBeAg-positive patients with selection of core promoter variants had lower serum HBV DNA levels, whereas HBeAg-negative patients with core promoter or precore variants had higher serum HBV DNA levels compared with those with WT sequence in both precore and core promoter regions. The discrepant effects of precore/core promoter mutation on serum HBV DNA levels in HBeAg-positive versus HBeAg-negative patients may be related to the modulating effect of the host immune response. During the HBeAg-positive phase, selection of core promoter variant occurs during the process of immune clearance, accounting for the lower serum HBV DNA levels. After HBeAg seroconversion, there is an overall 3 to 4 $\log_{10}$ reduction in serum HBV DNA levels.[31] HBeAg-negative patients with WT sequence in both the precore and core promoter regions have lower serum HBV DNA levels because virus replication is adequately suppressed by the host immune system, whereas HBeAg-negative patients with core promoter or precore variants have higher serum HBV DNA levels because HBV variants with deficient HBeAg production can evade host immune response and are therefore able to maintain higher levels of HBV replication. We acknowledge that our observation was based on serum HBV DNA levels at a single time point and that patients with chronic hepatitis B, particularly HBeAg-negative patients, tend to have fluctuating serum HBV DNA levels. Nevertheless, compared with other reports, our study included the largest number of patients and used a sensitive polymerase chain reaction assay to quantify serum HBV DNA levels.

In summary, our nationwide study showed that precore and core promoter variants could be found in approximately one third of patients with chronic HBV infection in the United States. The prevalence of these variants was related to ethnicity, place of birth, and HBV genotypes. Nevertheless, precore as well as core promoter variants were detected at similar rates across the country. Our study also showed that patients with core promoter variants were more likely to have cirrhosis and hepatic decompensation. In addition, HBeAg-negative patients with core promoter and/or precore variants had higher serum HBV DNA levels than those with WT sequence. Thus, physicians practicing in the United States should be aware of the existence of HBV precore and core promoter variants and the clinical condition "HBeAg-negative

chronic hepatitis" because the diagnosis, natural course, and treatment of this condition differs from classic "HBeAg-positive chronic hepatitis." At the moment, the clinical entity "HBeAg-negative chronic hepatitis" can be diagnosed by the absence of HBeAg, presence of hepatitis B e antibody, serum HBV DNA level greater than 5 $\log_{10}$ copies/mL, and biochemical/histologic evidence of active liver disease. Further studies are needed to determine if additional testing for HBV genotype as well as precore and core promoter variants will help in prognostication and in guiding treatment decisions.

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