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Characteristics of Core Promoter and Precore Stop Codon Mutants of Hepatitis B Virus in Vietnam

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In Asia, genotypes B and C are the most common genotypes of hepatitis B virus (HBV); and genotype C causes more severe liver disease. Core promoter/precore (CP/PC) mutants, known to be linked to these genotypes, could have an impact on the progression and severity of liver disease. Sera of 115 patients, including 39 acute and 76 chronic Vietnamese HBV infected patients, were tested for their liver profile, HBeAg, HBV genotypes, and HBV DNA level. Fragments of 282 nucleotides covering CP/PC were amplified, sequenced, and analysed. In the acute group, CP/PC mutants accounted for 38.4 and 25.6%, respectively. Genotype B was found to be predominant (74.3%, $P < 0.05$) and linked to the PC mutant (A1896) ($P < 0.05$). In the chronic group, CP/PC mutants accounted for 61.7 and 32.8%. CP mutants, especially the T1762/A1764 double mutant, were found to correlate with genotype C (81%, $P < 0.001$), liver cirrhosis, and hepatocellular carcinoma ($P < 0.05$). Therefore, genotype C in Vietnam, which carried high rate of C-1858 (70%), could play an important role in causing severe chronic liver disease. ***J. Med. Virol. 74:228–236, 2004.*** © 2004 Wiley-Liss, Inc.

KEY WORDS: HBV in Vietnam; HBV variant; corepromoter and precore mutant of HBV; HBV genotype

INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem [Lee, 1997]. The course of HBV infection and HBV-related liver injury depend on several host-viral factors [Lok, 2000]. Due to the lack of proof-reading capacity in the DNA polymerase, HBV carries high mutation rate, which is not limited to any open reading frame. Mutations in the core promoter (CP) region of HBV can be found in many hepatitis B e antigen (HBeAg)-negative patients [Chan et al., 2000; Lok et al., 2000]. The well-known mutation is the double nucleotide substitution: A to T at nucleotide 1762 and G to A at nucleotide 1764 (A1762T/G1764A). The CP mutants have been linked to the severity of liver diseases, especially hepatocellular carcinoma (HCC) [Kramvis and Kew, 1999]. Alternatively, the mutation in the precore (PC) region, substitution of G to A at nucleotide 1896 (A1896), creates a premature stop codon at codon 28 in the *PC* gene. This mutation prevents translation of the PC protein and completely abolishes the production of HBeAg. The role of A1896 to the HBV infection course is still controversial; although it has been thought to aggravate liver disease severity, especially fulminant hepatitis (FH) [Lok et al., 1994; Hunt et al., 2000]. In addition, different HBV genotypes have been associated with different mutant rates in the CP/PC regions of HBeAg-negative chronic hepatitis B [Funk et al., 2002]. Genotypes B and C are the most common genotypes in Asia, and the role of genotype C in the etiology of more severe liver disease has been demonstrated [Kao et al., 2002]. Recently, it has been found that genotype C, especially the C-1858 variant in Southeast Asia, is associated with a higher rate of CP mutants [Chan et al., 1999; Lindh et al., 1999].

A recent study in Vietnam showed that HBV was the most important causative agent correlated with liver disease [Tran et al., 2003]. To clarify the importance of HBV variants and HBV genotypes on the course of HBV infection, the correlation between CP/PC mutants, HBV genotypes and liver disease was analysed in Vietnamese patients.

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PATIENTS AND METHODS

Patients

A cross-sectional study was performed in 115 consecutive Vietnamese HBV infected patients within 1 year, between January 2000 and December 2000. There were 87 men and 28 women, aged from 16 to 83, with the mean age 45.62 ± 15.8 years. These patients were recruited from in-patients Gastroenterology wards at Cho Ray Hospital (Ho Chi Minh city, Vietnam) and Bach Mai Hospital (Hanoi, Vietnam). The diagnoses of HBV-related liver diseases were established based on clinical data, laboratory tests, and imaging studies (ultrasonography, computerised tomography (CT-scan), and/or Magnetic Resonance Imaging (MRI)). Among 115 patients, 39 were diagnosed as acute hepatitis B based on either the novo appearance of HBsAg or the presence of Immunoglobulin M antibody to hepatitis B core antigen (IgM anti-HBc). Patients with a prolonged prothrombin time over than 50% of control and/or hepatic encephalopathy during their acute hepatitis (AH) were diagnosed as FH. Seventy-six patients were diagnosed as chronic HBV infection; and the persistence of HBsAg of these patients were followed in more than 1 year. The chronic group included asymptomatic carriers (ASCs) with normal or mild elevated alanine transferase (ALT) (<2 times of upper normal limit); chronic hepatitis (CH) with mild symptoms and abnormal ALT; liver cirrhosis (LC), and HCC. Cirrhosis and HCC were defined on liver function test, alpha-fetoprotein level, imaging studies, and histology. None of the patients had co-infection with hepatitis C virus (HCV) and/or hepatitis D virus (HDV) and their serum samples were stored at $-70°C$ until used. Informed consent was obtained from all patients, and the study was approved by the local ethical committee.

Serologic Markers

All sera were screened for HBsAg, HBeAg, anti-HCV antibody, and anti-HDV antibody by enzyme linked immunosorbent assay (ELISA), using commercially available kits from Abbott (Abbott Laboratories, North Chicago, IL). Diagnosis of acute hepatitis was reconfirmed by IgM anti-HBc assay in all cases.

Extraction of DNA

Viral DNA was extracted from 100 μl of serum using the DNA/RNA extraction Kit (SepaGene RV-R, Sanko Junyaku Co., Ltd., Tokyo, Japan). The resulting pellet was eluted in 50 μl of RNase-free water and kept in $-20°C$ until use.

HBV Genotyping by PCR

Genotyping of HBV was identified by PCR using type-specific primers designed from pre-S1 through S genes of HBV [Naito et al., 2001]. Six genotypes (A to F) of HBV could be identified by specific bands of second PCR. To avoid false-positive results, instructions to prevent cross contaminations were strictly followed, and the results were considered valid only when they were consistently obtained in duplicate.

Amplification of the CP/PC Regions

Partial gene covering 282 nucleotides (nt) (from nt 1689 to 1970) of CP/PC region were amplified by nested PCR. Primer pair eP11: 5′-GCATGGAGACCACCGTGAAC-3′ (sense) and BG1R: 5′-ATAGGGGCATTTGGTGGTCT-3′ (antisense) was used for the first round PCR; and primer pairs PC1: 5′-CATAAGAGGACTCTTGGACT-3′ (sense), PC2: 5′-AAAGAATTCAGAAGGCAAAAAAGA-3′ (antisense) for the second round PCR. The PCR reaction was performed in 40 cycles (94°C 20 sec, 55°C 20 sec, and 72°C for 30 sec) followed by extension at 72°C for 7 min. PCR products were separated by 2% agarose gel electrophoresis and purified using the QIAquick gel extraction kit (Qiagen, Inc., Chatsworth, CA).

Nucleotide Sequencing and Phylogenetic Analysis

Purified PCR products were subjected to direct sequencing using the ABI PRISM™ Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA). The inner primer pair was used as sequencing primers. Sequences of amplified DNA were determined using automated DNA sequencer ABI 377 (Perkin Elmer, Norwalk, CT). Nucleotide sequences were multiple-aligned, analysed using Genetyx for Windows ver. 6.0 software (GENETYX, Tokyo, Japan), and corrected manually by visual inspection. Nucleotide consensus sequences of CP/PC regions of HBV genotypes B and C were taken from GenBank for multi-aligment and mutant analysis. Tree construction was analysed by neighbour-joining method with bootstrap resampling (1,000 times), using MEGA version 2.1 [Kumar et al., 2001].

Quantitation Assay of Viral Load

Quantitation of HBV DNA was performed by real-time PCR method [Chen et al., 2001]. The detection limit of this assay was 3.73×10^2 genome equivalents per ml. Sequences of primers and probes were HBc1: 5′-AGTGTGGATTCGCACTCCT-3′ (sense, nt 2269–2287), HBc1R: 5′-GAGTTCTTCTTCTAGGGGACCTG-3′ (antisense, nt 2387–2365) and HBcP1: 5′-CCAAATGCCCCTATCTTATCAACACTTCC-3′ (TaqMan probe, nt 2303–2331).

Statistical Analysis

Proportions of each factor were compared between the groups using Fisher exact 2-tail test, and the group means were compared using the Student's t-test. Differences were considered to be significant for $P < 0.05$. Mean of HBV DNA levels were compared after logarithmic transformation of the HBV DNA values from the real-time PCR assay.

RESULTS

Patients With Acute and Chronic HBV-Infected Diseases

Among 39 acute HBV-infected patients including acute hepatitis and fulminant hepatitis, there were 29 (74.3%) with genotype B and 10 (25.7%) with genotype C. Their mean age was 35.2 ± 12.8 years and 36 of them were men. In 76 chronic HBV-infected patients, there was an equal distribution of genotype B (39; 51%) and genotype C (37; 48.7%). The mean age of this group was 50.9 ± 14.4 years. The characteristics of the patients in each group and each diagnosis category were described in Table I. The acute group had a younger age ($P < 0.001$), a higher ALT ($P < 0.001$), and a higher HbeAg +ve rate ($P < 0.05$) than that of the chronic group. HBV DNA level among acute HBV infected patients was higher than that of chronic infection, however, there was no statistically significant difference ($P = 0.26$). Of note, the frequency of genotype B was found to be higher than that of genotype C in the acute group, in comparison with the chronic group (74.3% vs. 51.3%, $P < 0.05$).

CP/PC Mutant in Acute HBV-Infected Patients

In the CP region, the occurrence rate of the CP mutant was 15/39 (38.4%), in which the T1762/A1764 and deletion mutants accounted for 30.7 and 7.6%, respectively (Table II). Two out of three cases of fulminant hepatitis had a deletion in this region, spanning from 20 to 21 nucleotides (Fig. 1). The total rate of CP mutants in genotype B (9; 31%) was lower than that of genotype C (6; 60%) but the difference was not statistically significant ($P = 0.14$). In the PC region, 70% of genotype C isolates carried the C at nucleotide 1858 (C-1858) ($P < 0.001$), and there was no A1896 mutant among these isolates (Fig. 1B and Table II). However, genotype B isolates carried only T at nucleotide 1858 (T-1858), and the A1896 mutant was determined in 34.4% cases ($P < 0.05$) (Fig. 1A and Table II).

CP/PC Mutant in Chronic HBV-Infected Patients

In the CP region, the occurrence rate of the CP mutant was 51/76 (67.1%). Three kinds of mutants were detected in genotype B, i.e., T1762/A1764 (33.3%), T1762A alone (10.2%) and deletion mutant (7.6%) (Table III). The frequency of the T1762/A1764 double mutant was found to be higher in genotype C (81%) than in genotype B isolates (33.3%) ($P < 0.001$). In the PC region, the A1896 mutant was seen in 25/76 (32.8%). As reported in the acute group, C-1858 also possessed a strong link to genotype C (70.2%) ($P < 0.001$). The A1896 mutant, therefore, was less detectable in genotype C (5.4%) than in genotype B (58.9%) ($P < 0.001$). Furthermore, when only the T-1858 isolates were taken into account, the A1896 mutant rate was also lower in genotype C than in genotype B (2/11 (22.2%) and 23/39 (58.9%), respectively). In addition, there were correlations between cirrhosis and HCC with a high occurrence rate of T1762/A1764 in genotype C ($P < 0.01$); and A1896 in genotype B ($P < 0.05$) (Table III).

CP/PC Mutant, Virological Manifestations, and Liver Injury

In the acute group, the mean age, HBV DNA, and ALT level were not significantly different between the wild type and the CP/PC mutant type (Table IV). Conversely, in the chronic group, the CP mutant was detected more frequently in older age cases and associated with a lower HBV DNA level than that of the wild type. However, there was no statistically significant difference ($P = 0.6$ and 0.6, respectively). The same insignificant different finding was observed with the A1896 mutant, although it was detected in cases with a higher mean age, higher HBV DNA, and ALT level ($P = 0.7$; $P = 0.4$; and $P = 0.6$,

TABLE I. Characteristic of Patients of Acute and Chronic HBV Infection

Diagnosis	n	Sex (M/F)	Age (year)[a]	ALT (UI/L)	HBeAg (+ve/−ve)	HBV DNA (log_{10} copies/ml)	Genotype	
							B	C
Acute infection	39	36/3	35.2 (12.8)*	1,089 (892)**	9/30***	5.48 (1.33)****	29 (74.3)†	10 (25.7)
AH	36	26/10	35.2 (12.9)	1,137 (908)	8/28	5.50 (1.36)	28 (77.7)	8 (22.3)
FH	3	2/1	34.6 (15.1)	516 (373)	1/2	5.31 (1.38)	1 (33.3)	2 (66.7)
Chronic infection	76	59/17	50.9 (14.4)*	85 (120)**	5/71***	4.98 (1.21)****	39 (51.3)†	37 (48.7)
ASC	10	8/2	36.5 (18.1)	40 (5)	2/8	6.30 (0.74)	6 (60)	4 (40)
CH	4	3/1	47.2 (8.3)	69 (16)	0/4	4.45 (0.54)	0	4 (100)
LC	39	29/10	52.9 (12.8)	108 (163)	1/38	4.98 (1.36)	21 (53.8)	18 (46.2)
HCC	23	19/4	54.3 (12.7)	61 (28)	2/21	4.79 (0.93)	12 (52.1)	11 (47.9)

[a]Age, ALT, HBV DNA were denoted in mean with the standard deviation in parenthesis; sex, HBeAg were denoted in number of cases; and genotype was denoted in number of cases with percentage in parenthesis.
*$P < 0.001$.
**$P < 0.001$.
***$P < 0.05$.
****$P = 0.26$.
†$P < 0.05$.

TABLE II. Core Promoter and Precore Mutant in Acute HBV Infected Patients

	Core promoter (CP) region[a]			Precore region	
	T1762/A1764	T1762 alone	Deletion in CP	C-1858	A1896
Genotype B (n = 29)	8 (27.5)*	0	1 (3.4)	0***	10 (34.4)**
AH (n = 28)	7 (25.0)	0	1 (3.5)	0	9 (32.1)
FH (n = 1)	1 (100)	0	0	0	1 (100)
Genotype C (n = 10)	4 (40.0)*	0	2 (20.0)	7 (70.0)***	0**
AH (n = 8)	4 (50.0)	0	0	6 (75.0)	0
FH (n = 2)	0	0	2 (100)	1 (100)	0

[a]Number of cases with percentage in parenthesis.
*$P = 0.69$.
**$P < 0.05$.
***$P < 0.0001$.

respectively). HBeAg was still detected in patients with CP/PC mutants, however, the rate of HBeAg loss was found more frequent in CP mutant infected patients than those with A1896 mutant.

Phylogenetic Analyses

As shown in Figure 2, all of 115 analysed CP/PC sequences (282 nucleotides) were clustered in major branches of genotype B (68 isolates) and C (47 isolates). All genotype C isolates in this study was belonged to sub-branches that differed from Japanese isolates of genotype C (Accession D50520 and D50517). These genotype C isolates were closely related to branches including isolates from Vietnam and Thailand strains from database (AF223957 and AF068756, respectively). Genotype B isolates, however, were shown branching off from genotype C branch rather than from a more proximal node, with low bootstrap value (28%). The C-1858 strains which were only detected in genotype C isolates, assembled closely but did not form a unique phylogenetic entity.

DISCUSSION

It is known that the CP/PC plays a central role in HBV replication. CP directs the transcription of both pre-genomic RNA and precore mRNA [Kramvis and Kew, 1999]. PC and core genes are essential for the pre-genome encapsidation signal and for the core protein assembly [Tong et al., 1992]. The CP mutants have been found to correlate with the HBV genotypes, viral replication, and liver damage in East Asian HBV carriers [Lindh et al., 1999]. The PC stop codon mutant, A1896, has been considered an important factor for fulminant hepatitis and progressive liver disease [Lok et al., 1994; Hunt et al., 2000]. On the other hand, Vietnam has a high rate of endemic HBV infection, with an HBsAg carrier rate between 9–14% in urban areas [Tran et al., 1993; Nakata et al., 1994] and 12–20% in rural areas [Hipgrave et al., 2003]. More than 3.5 million Vietnamese are currently at risk of a premature death due to HBV infection [Ngoan Le et al., 2002; Hipgrave et al., 2003]. Therefore, virologic characterisation of this virus and the CP/PC mutant may be helpful for the understanding of HBV pathogenesis in this country.

In this study, genotype B was found to be the predominant genotype in the acute group (74.3%). As reported previously, genotypes B and C of HBV were equally distributed in Vietnam [Tran et al., 2003] and a similar result was also confirmed in chronic infected patients in the present study (51.3 and 48.7%, respectively). Interestingly, it was known that genotype B in Japan was linked to the acute form, specifically to fulminant but not acute hepatitis [Imamura et al., 2003]. Moreover, genotype B in Hong Kong patients was strongly associated with chronic hepatitis B exacerbations [Yuen et al., 2003a]. This finding suggested a correlation between genotype B with the acute forms of HBV infection. Recent studies have shown that genotype B might be more immunogenic, and patients infected with this genotype have earlier HBeAg seroconversion, in comparison to patients with genotype C [Chu et al., 2002; Yuen et al., 2003b]. Hence, an in-depth genomic sequence analysis of HBV in acute cases could be required to address this matter.

Among the investigated sequences of 39 acute and 76 chronic HBV-infected patients, there were different effects of genotypes on the CP/PC mutants. In the acute group, genotype B was found to correlate with the A1896 mutant. In the chronic group, genotype B was associated with the A1896 mutant, whereas genotype C was correlated with CP mutants. Interestingly, the T1762/A1764 double mutant in genotype C was found to be associated with cirrhosis and HCC. However, due to the small number of asymptomatic carriage and chronic hepatitis in this study, this result needs further confirmation. Nevertheless, similar findings were also reported by other Asian studies, suggesting that the high prevalence of the CP mutant in genotype C isolates could be one of the important factors causing a detrimental effect on the evolution of HBV infection [Takahashi et al., 1995; Lindh et al., 1999; Fang et al., 2002; Yotsuyanagi et al., 2002].

It has also been known that genotype C in Southeast Asian countries has a high prevalence of the C-1858 variant, which is base-paired to nucleotide 1896 and prevents the occurrence of the A1896 mutant [Lok et al.,

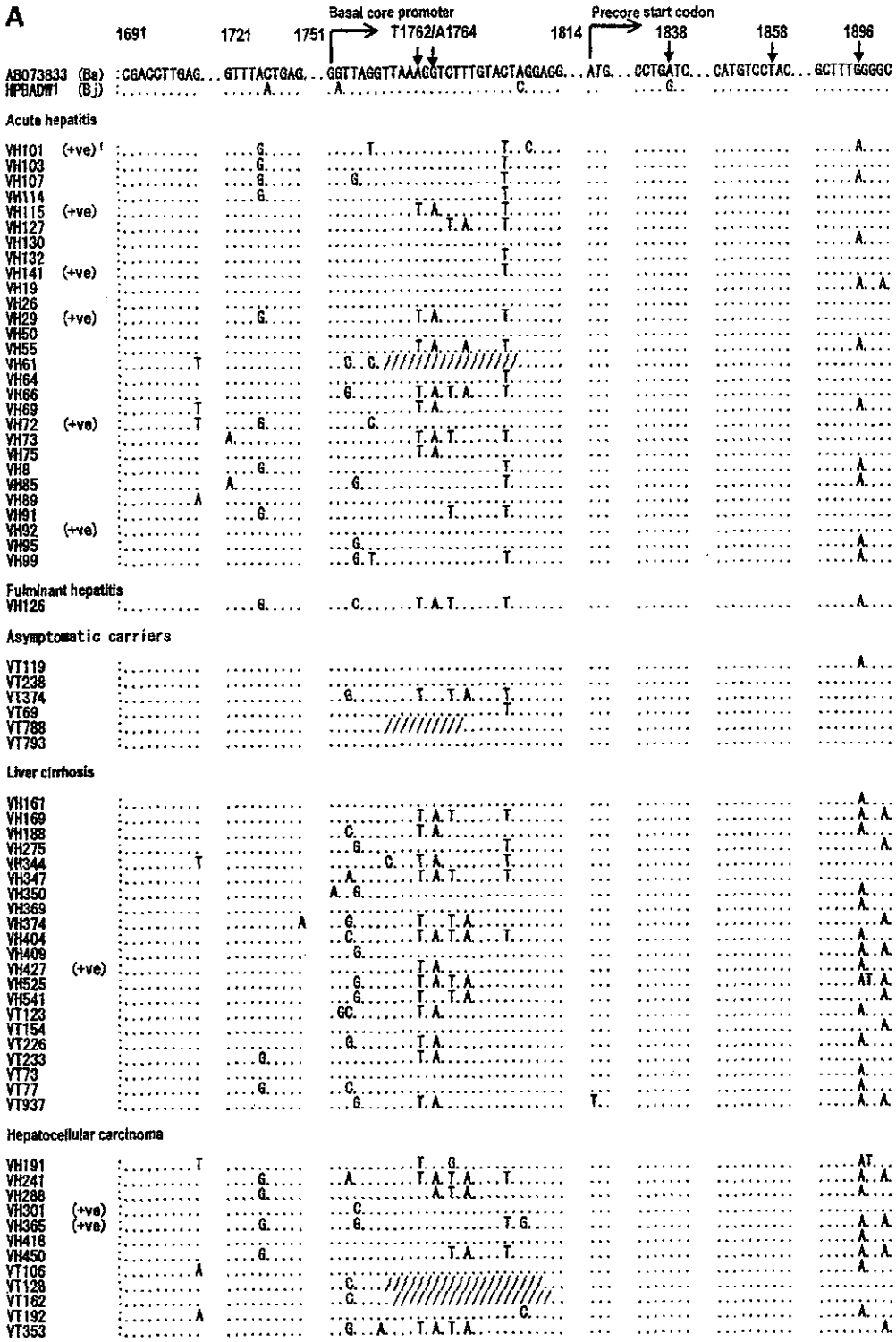

Fig. 1. Alignment of the CP/PC region (nt 1689–1970) from 68 Vietnamese genotype B (A) and 47 genotype C isolates (B). Dots represented nucleotides identical to the consensus sequences while deletions were represented as forward slashes. Isolates from each genotype were grouped based on clinical diagnosis. The top line(s) in each figure were wild type consensus sequences using nucleotide numbered by Okamoto et al. [1988]. The consensus of Ba and Bj subgroup of genotype B reported by Sugauchi et al. [2002] were shown (Accession No. AB073833 and D50521, respectively). The consensus of genotype C was the one reported in Japan (Accession No. D50517). Specific changes from G to A in nucleotide 1721 and 1727 were found in genotype C detected in Vietnam.

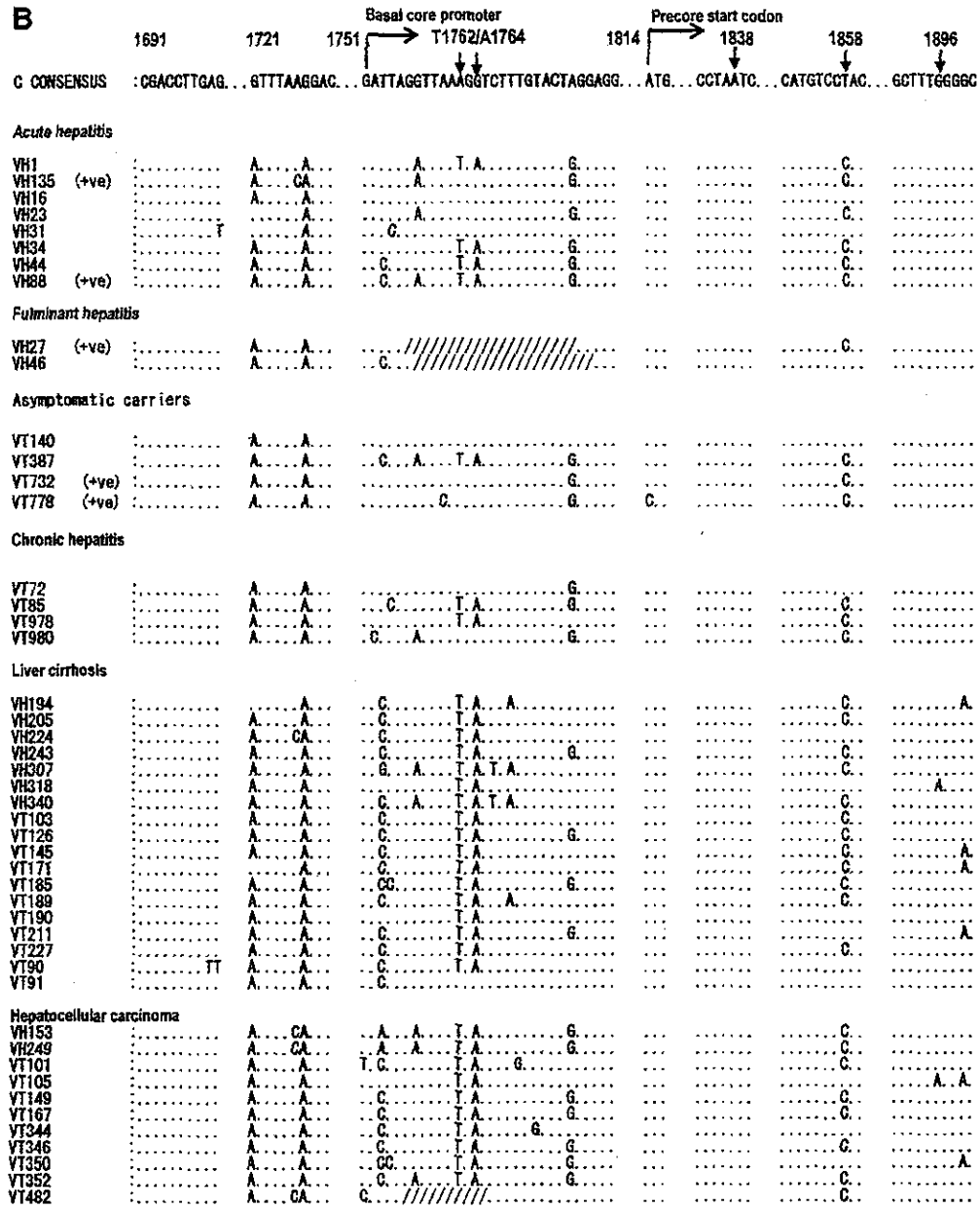

Fig. 1. (Continued)

1994]. C-1858 was also thought to correlate with the CP mutants [Chan et al., 1999]. In the present study, about 70% of genotype C isolates in Vietnamese patients possessed this variant. The result was remarkably different from a recent study in Japan [Kobayashi et al., 2004], in which no C-1858 variant was found in either genotype B or C. One of the possibly explanations is that genotypes B and C in Southeast Asia belong to subgroups that have a distinctive phylogenetic entity [Sugauchi et al., 2002; Huy et al., 2004]. In fact, the consensus nucleotide sequence at nt 1838 (genotype B, Ba specific) [Sugauchi et al., 2003] or nt 1721 and nt 1727 (genotype C) supports the notion that HBV genotypes B and C detected in Vietnam belong to subgroups of genotype B and C. In addition, phylogenetic tree analysis showed that C-1858 isolates have been grouped to each other, but cannot form a unique subgroup of C-1858 in genotype C (Fig. 2) as mentioned previously [Alestig et al., 2001]. In the present study, the analysed region is rather short (282 nucleotides) and partly covered the region of genotype B and C recombination in Southeast Asia (genotype Ba), which is spanned from nt 1740 to 2443 [Sugauchi et al., 2003]. In fact, all genotype B in this study were confirmed as subgroup Ba (data not shown). The resulting sub-branches reflect the recombination of genotype B and C in this region;

TABLE III. Core Promoter and Precore Mutant in Chronic HBV Infected Patients

	Core promoter (CP) region[a]			Precore region	
	T1762/A1764	T1762 alone	Deletion in CP	C-1858	A1896
Genotype B (n = 39)	13 (33.3)*	4 (10.2)	3 (7.6)	0***	23 (58.9)**
ASC (n = 6)	0	1 (16.6)	1 (16.6)	0	1 (16.6)
LC (n = 21)	11 (52.3)	2 (9.5)	0	0	14 (66.6)†
HCC (n = 12)	2 (16.6)	1 (8.3)	2 (16.6)	0	8 (66.6)†
Genotype C (n = 37)	30 (81.0)*	0	1 (27.0)	26 (70.2)***	2 (5.4)**
ASC (n = 4)	1 (25.0)	0	0	3 (75.0)	0
CH (n = 4)	2 (50.0)	0	0	3 (75.0)	0
LC (n = 18)	17 (94.4)****	0	0	12 (66.6)	1 (5.5)
HCC (n = 11)	10 (90.9)****	0	1 (9.0)	8 (72.7)	1 (9.0)

[a]Number of cases with percentage in parenthesis.
*$P < 0.001$.
**$P < 0.001$.
***$P < 0.0001$.
****$P < 0.01$.
†$P < 0.05$.

therefore it was associated with the low bootstrap values to differentiate the two genotypes; as well as the C-1858 variant.

Seven cases of deletion mutants, which spanned the TA-rich regions of the CP region, were found. These deletions have been known to result in a frame-shift and/or truncation of the X protein at the C terminal end [Kidd-Ljunggren et al., 1997]. Although two out of three cases of fulminant hepatitis had a deletion in the CP region, this mutant was also found in other diagnoses, and might have no significant role [Kramvis and Kew, 1999]. G1899A, a mutant that changes glycine at codon 29 to aspartic acid, has been linked to G1896A [Yuan et al., 1995]. However, in the present study, G1899A occurred independently with G1896A in 5/13 cases (38.4%) of genotype B and 5/6 cases (83.3%) of genotype C, respectively. Therefore, its role is not clearly identified in Vietnamese isolates.

In this study, the correlation between the HBV DNA level and CP/PC mutant was unclear in both the acute and chronic groups, although the HBV DNA level was found to be insignificantly lower in CP mutant isolates; and higher in the PC mutant isolates. In addition, the HBV DNA level was not significantly different between the acute and chronic states; and was in a lower range than in the previous studies, in which the level was usually around 10^8 copies/ml in patients with HBeAg +ve [Lindh et al., 1999]. One explanation relates to the time of collecting serum samples from these patients in the acute group. In the present study, up to 10 cases of acute group had A1896. These low viral titer samples might be approaching the period of seroconversion [Parekh et al., 2003] at the time of investigation.

In conclusion, mutants in the HBV CP/PC regions prevailed in chronic and acute hepatitis B patients in Vietnam. In chronic infection, CP mutants, especially

TABLE IV. Characteristic of CP/PC Mutant in Both Acute and Chronic Forms

	Core promoter		Precore	
	WT	MUT	WT	MUT
Acute forms				
Age[a]	34.7 (14.4)	36 (10.1)	33.6 (13.7)	39.0 (9.8)
HBeAg (+ve/−ve)	5/19**	4/11**	8/21	1/9
HBV DNA	5.27 (1.44)	5.78 (1.17)	5.34 (1.29)	5.81 (1.46)
ALT	1,171 (966)	959 (772)	1,120 (975)	1,011 (667)
Chronic Forms				
Age	46.8 (18.0)*	52.9 (11.9)*	50.5 (15.4)	51.7 (12.2)
HBeAg (+ve/−ve)	4/21***	1/50***	3/48	2/23
HBV DNA	5.50 (1.22)****	4.77 (1.15)****	4.69 (1.08)†	5.42 (1.29)†
ALT	91 (177)	81 (81)	69 (60)‡	116 (190)‡

[a]Age, ALT, HBV DNA were denoted in mean with the standard deviation in parenthesis; HBeAg was denoted in number of cases.
*$P = 0.465$.
**$P = 0.734$.
***$P < 0.05$.
****$P = 0.605$.
†$P = 0.490$.
‡$P = 0.606$.

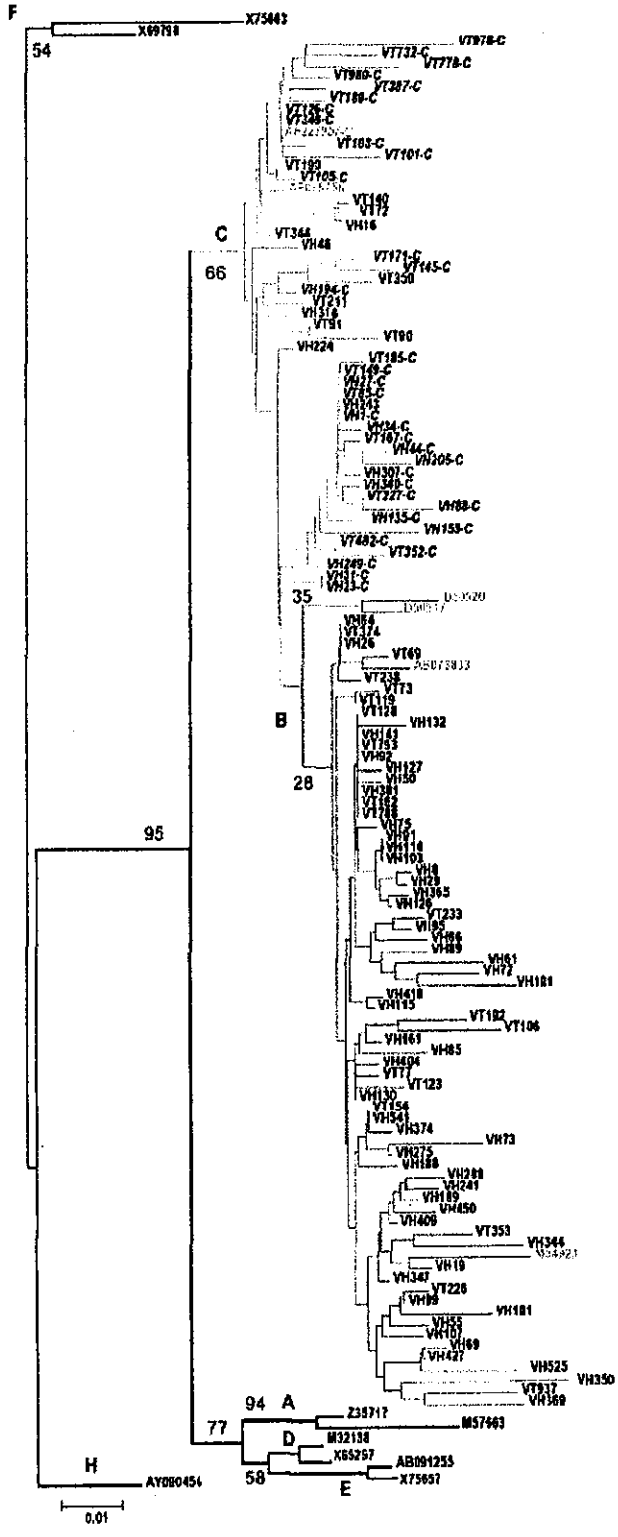

Fig. 2. Phylogenetic tree. This tree was constructed by the neighbour-joining method based on the partial nucleotide sequence of the CP/PC gene (282 nt, from nt 1691–1972) of 115 HBV Vietnamese isolates (VH and VT) and 15 reported isolates from genotype A–H in database. Bootstrap values were indicated in major branches and subbranches of genotype B and C. Genotype G was excluded from this analysis due to their common 36-nucleotide insertion in the core gene. Sequences of genotype C written in italic-C were those with C-1858.

the T1762/A1764 double mutant, were linked to genotype C of HBV, which had a high rate of C-1858 variants and could be associated with the more severe diseases. In acute infection, the influence of HBV genotypes on CP mutants was not clear, although genotype B, possessing a higher rate of the A1896 mutant, was linked to acute hepatitis manifestation in Vietnam.

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REFERENCES

Alestig E, Hannoun C, Horal P, Lindh M. 2001. Phylogenetic origin of hepatitis B virus strains with precore C-1858 variant. J Clin Microbiol 39:3200–3203.

Chan HL, Hussain M, Lok AS. 1999. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. Hepatology 29:976–984.

Chan HL, Leung NW, Hussain M, Wong ML, Lok AS. 2000. Hepatitis B e antigen-negative chronic hepatitis B in Hong Kong. Hepatology 31:763–768.

Chen RW, Piiparinen H, Seppanen M, Koskela P, Sarna S, Lappalainen M. 2001. Real-time PCR for detection and quantitation of hepatitis B virus DNA. J Med Virol 65:250–256.

Chu CJ, Hussain M, Lok AS. 2002. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology 122:1756–1762.

Fang ZL, Yang J, Ge X, Zhuang H, Gong J, Li R, Ling R, Harrison TJ. 2002. Core promoter mutations (A(1762)T and G(1764)A) and viral genotype in chronic hepatitis B and hepatocellular carcinoma in Guangxi, China. J Med Virol 68:33–40.

Funk ML, Rosenberg DM, Lok AS. 2002. World-wide epidemiology of HBeAg-negative chronic hepatitis B and associated precore and core promoter variants. J Viral Hepat 9:52–61.

Hipgrave DB, Van NT, Huong VM, Long HT, Dat DT, Trung TN, Jolley D, Maynard JE, Biggs B-A. 2003. Hepatitis B infection in rural Vietnam and the implications for a national program of infant immunization. Am J Trop Med Hyg 69:288–294.

Hunt C, McGill J, Allen M, Condreay L. 2000. Clinical relevance of hepatitis B viral mutations. Hepatology 31:1037–1044.

Huy TT-T, Ushijima H, Quang VX, Win KM, Luengrojanakul P, Kikuchi K, Sata T, Abe K. 2004. Genotype C of hepatitis B virus can be classified into at least two subgroups. J Gen Virol 85:283–292.

Imamura T, Yokosuka O, Kurihara T, Kanda T, Fukai K, Imazeki F, Saisho H. 2003. Distribution of hepatitis B viral genotypes and mutations in the core promoter and precore regions in acute forms of liver disease in patients from Chiba, Japan. Gut 52:1630–1637.

Kao JH, Chen PJ, Lai MY, Chen DS. 2002. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. J Clin Microbiol 40:1207–1209.

Kidd-Ljunggren K, Oberg M, Kidd AH. 1997. Hepatitis B virus X gene 1751 to 1764 mutations: Implications for HBeAg status and disease. J Gen Virol 78:1469–1478.

Kobayashi M, Arase Y, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, Suzuki F, Akuta N, Hosaka T, Someya T, Matsuda M, Sato J, Miyakawa Y, Kumada H. 2004. Wild-type precore and core promoter sequences in patients with acute self-limited or chronic hepatitis B. Scand J Gastroenterol 39:53–59.

Kramvis A, Kew MC. 1999. The core promoter of hepatitis B virus. J Viral Hepat 6:415–427.

Kumar S, Tamura K, Jakobsen IB, Nei M. 2001. MEGA2: Molecular evolutionary genetics analysis software. Bioinformatics 17:1244–1245.

Lee WM. 1997. Hepatitis B virus infection. N Engl J Med 337:1733–1745.

Lindh M, Hannoun C, Dhillon AP, Norkrans G, Horal P. 1999. Core promoter mutations and genotypes in relation to viral replication

and liver damage in East Asian hepatitis B virus carriers. J Infect Dis 179:775–782.

Lok AS. 2000. Hepatitis B infection: Pathogenesis and management. J Hepatol 32:89–97.

Lok AS, Akarca U, Greene S. 1994. Mutations in the pre-core region of hepatitis B virus serve to enhance the stability of the secondary structure of the pre-genome encapsidation signal. Proc Natl Acad Sci USA 91:4077–4081.

Lok AS, Hussain M, Cursano C, Margotti M, Gramenzi A, Grazi GL, Jovine E, Benardi M, Andreone P. 2000. Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. Hepatology 32:1145–1153.

Naito H, Hayashi S, Abe K. 2001. Rapid and specific genotyping system for hepatitis B virus corresponding to six major genotypes by PCR using type-specific primers. J Clin Microbiol 39:362–364.

Nakata S, Song P, Duc DD, Nguyen XQ, Murata K, Tsuda F, Okamoto H. 1994. Hepatitis C and B virus infections in populations at low or high risk in Ho Chi Minh and Hanoi, Vietnam. J Gastroenterol Hepatol 9:416–419.

Ngoanle T, Mizoue T, Yoshimura T. 2002. Estimates of cancer mortality in Hanoi and Ho Chi Minh City, Viet Nam in the 1990s. J Epidemiol 12:179–187.

Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. 1988. Typing hepatitis B virus by homology in nucleotide sequence: Comparison of surface antigen subtypes. J Gen Virol 69:2575–2583.

Parekh S, Zoulim F, Ahn SH, Tsai A, Li J, Kawai S, Khan N, Trepo C, Wands J, Tong S. 2003. Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants. J Virol 77:6601–6612.

Sugauchi F, Orito E, Ichida T, Kato H, Sakugawa H, Kakumu S, Ishida T, Chutaputti A, Lai CL, Ueda R, Miyakawa Y, Mizokami M. 2002. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J Virol 76:5985–5992.

Sugauchi F, Orito E, Ichida T, Kato H, Sakugawa H, Kakumu S, Ishida T, Chutaputti A, Lai CL, Gish RG, Ueda R, Miyakawa Y, Mizokami M. 2003. Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. Gastroenterology 124:925–932.

Takahashi K, Aoyama K, Ohno N, Iwata K, Akahane Y, Baba K, Yoshizawa H, Mishiro S. 1995. The precore/core promoter mutant (T1762A1764) of hepatitis B virus: Clinical significance and an easy method for detection. J Gen Virol 76:3159–3164.

Tong SP, Li JS, Vitvitski L, Trepo C. 1992. Replication capacities of natural and artificial precore stop codon mutants of hepatitis B virus: Relevance of pregenome encapsidation signal. Virology 191:237–245.

Tran VB, Buu M, Nguyenthi M, Morris GE. 1993. Hepatitis B in Ho Chi Minh City, Viet Nam. Trans R Soc Trop Med Hyg 87:262.

Tran HT, Ushijima H, Quang VX, Phuong N, Li TC, Hayashi S, Xuan Lien T, Sata T, Abe K. 2003. Prevalence of hepatitis virus types B through E and genotypic distribution of HBV and HCV in Ho Chi Minh City, Vietnam. Hepatol Res 26:275–280.

Yotsuyanagi H, Hino K, Tomita E, Toyoda J, Yasuda K, Iino S. 2002. Precore and core promoter mutations, hepatitis B virus DNA levels and progressive liver injury in chronic hepatitis B. J Hepatol 37:355–363.

Yuan TT, Faruqi A, Shih JW, Shih C. 1995. The mechanism of natural occurrence of two closely linked HBV precore predominant mutations. Virology 211:144–156.

Yuen MF, Sablon E, Wong DK, Yuan HJ, Wong BC, Chan AO, Lai CL. 2003a. Role of hepatitis B virus genotypes in chronic hepatitis B exacerbation. Clin Infect Dis 37:593–597.

Yuen MF, Sablon E, Yuan HJ, Wong DK, Hui CK, Wong BC, Chan AO, Lai CL. 2003b. Significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications, and hepatocellular carcinoma. Hepatology 37:562–567.