Different Hepatitis B Virus Genotypes Are Associated With Different Mutations in the Core Promoter and Precore Regions During Hepatitis B e Antigen Seroconversion

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Mutations in the core promoter and precore regions are frequently found in hepatitis B e antigen (HBeAg)-negative patients, but precore stop codon mutation is restricted to hepatitis B virus (HBV) genotypes that have T at nucleotide 1858. The aims of this study were to determine the role of core promoter and/or precore mutations in HBeAg seroconversion and their impact on the subsequent course of liver disease, and to determine if core promoter mutations are more frequently selected in patients with HBV genotypes that preclude the development of precore stop codon mutation. Serial sera from 45 patients with chronic HBV infection were polymerase chain reaction (PCR)-amplified, and the HBV core promoter and precore regions were sequenced. Ninety-two percent of patients had core promoter or precore mutations after HBeAg seroconversion: 42% had core promoter changes only, 38% had precore stop codon mutations only, and 12% had changes in both regions. Seventy-three percent of the patients had persistently normal aminotransferases, and only 8% had multiple flares in aminotransferases after HBeAg seroconversion. Core promoter changes were significantly more common in patients infected with HBV who have C at nucleotide 1858 (91% vs. 27%; $P < .01$), while precore stop codon changes were exclusively found in patients infected with HBV who have T at nucleotide 1858 (87% vs. 0; $P < .01$). The vast majority of our patients had core promoter and/or precore mutations after HBeAg seroconversion. Nevertheless, most patients had sustained remission of liver disease. Our data suggest that core promoter changes are preferentially selected in patients infected with HBV genotypes that preclude the development of precore stop codon mutation. (HEPATOLOGY 1999;29:976-984.)

Mutations in the precore region of the hepatitis B virus (HBV) genome have been reported in many hepatitis B e antigen (HBeAg)-negative patients with chronic HBV infection.[1-7] The predominant mutation involves a G-to-A change at nucleotide 1896 (A1896), which creates a premature stop codon at codon 28 (Fig. 1). This mutation prevents the translation of the precore protein and completely abolishes the production of HBeAg. Longitudinal studies found that A1896 emerges or is selected around the time of HBeAg seroconversion.[2,5-7] These findings suggest that A1896 plays an important role in HBeAg clearance. However, not all patients develop A1896 after HBeAg seroconversion; some patients retain wild-type precore sequence, while others have undetectable HBV DNA in serum (by polymerase chain reaction [PCR] assay). It is now recognized that the occurrence of A1896 is restricted to HBV genotypes with T at nucleotide 1858.[4,8-10] A change from G to A at nucleotide 1896 increases the stability of the stem-loop structure of the pregenome encapsidation sequence (ϵ) when the opposite nucleotide at 1858 is a T (T1858), but this change disrupts a pre-existing C-G pair when the nucleotide at 1858 is a C (C1858).[4] The restriction of A1896 to specific HBV genotypes accounts for its high prevalence in Asia and the Mediterranean basin, where the predominant HBV genotypes (B, C, and D) frequently have T1858, and its low prevalence in North America and Europe, where the predominant HBV genotype (A) almost always has C1858.[9,10] In our previous studies of Chinese patients from Hong Kong, we found that only 60% of patients were infected with HBV genotypes that have T1858.[4] Nevertheless, patients infected with HBV genotypes that have C1858, which precludes the development of A1896, appeared to clear HBeAg at rates similar to those infected with HBV genotypes that have T1858.[11]

Recent studies reported that mutations in the core promoter region can be found in many HBeAg-negative patients; the most common mutations involve a two-nucleotide substitution: A-T at nucleotide 1762 and G-A at nucleotide 1764 (TA) (Fig. 2).[12-15] In some patients, the TA changes were not associated with A1896, suggesting that mutations in the core promoter region alone may play a role in HBeAg clearance. This hypothesis is supported by *in vitro* observations that the TA changes decrease transcription of precore mRNA and secretion of HBeAg.[16-20] However, TA changes have also been

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen; A1896, G-A change at nucleotide 1896 in the precore region; T1858/C1858, T at nucleotide 1858/C at nucleotide 1858 in the precore region; PCR, polymerase chain reaction; TA, A-T change at nucleotide 1762 and G-A change at nucleotide 1764 in the core promoter region; anti-HBe, hepatitis B e antibody; ALT, alanine transaminase.

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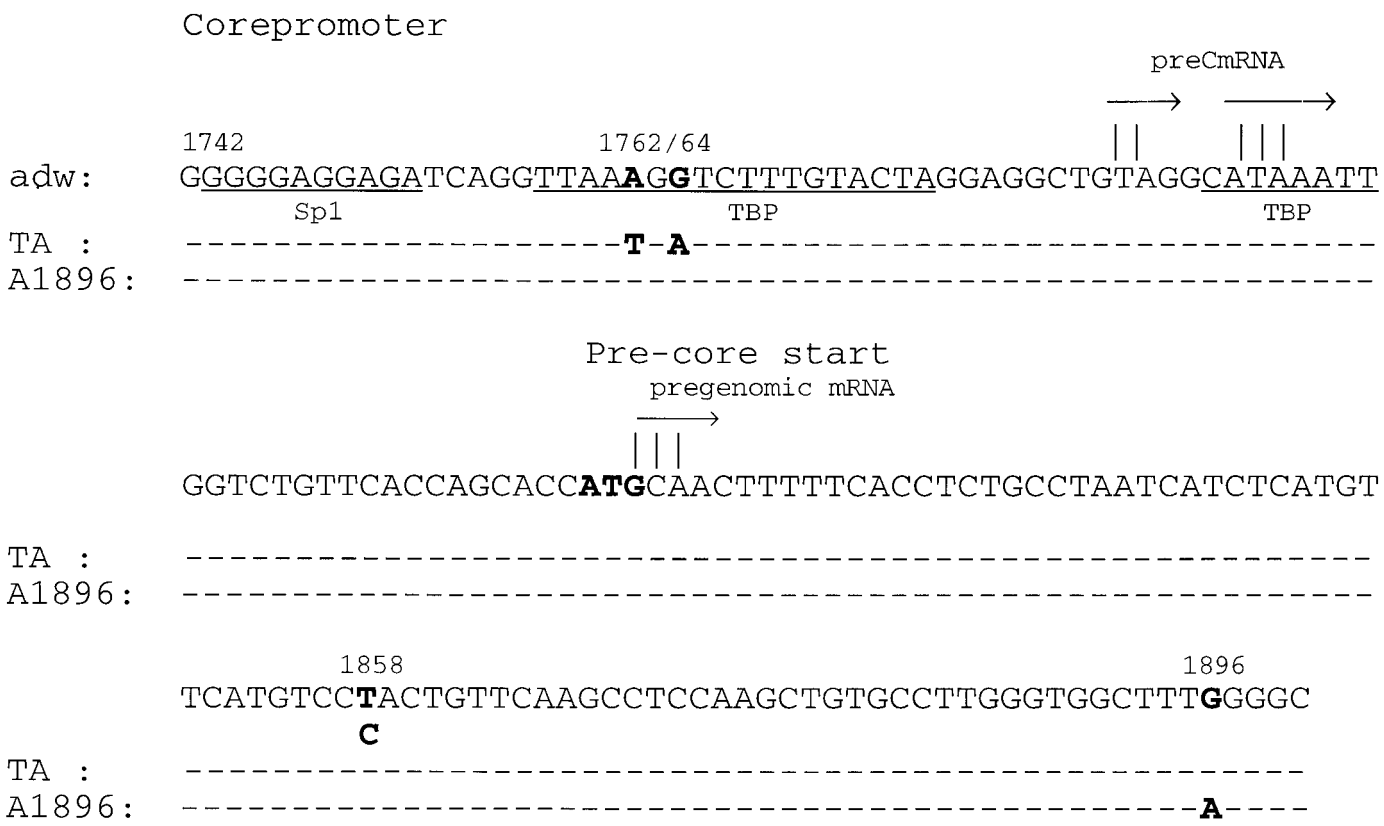

FIG. 1. The core promoter and precore sequences and the location of the TA change and A1896 (stop codon) mutation. The transcription initiation sites of the precore messenger RNA and the pregenomic RNA are indicated by *arrows*. Transcription factor binding sites (Sp1 and TBP) are *underlined*.

found in some HBeAg-positive patients, especially those with chronic hepatitis.[12,13] Thus, the role of core promoter mutations in HBeAg clearance is unclear.

Most reports of core promoter mutations came from Japan. There are very little data on the prevalence of core promoter mutations in other parts of the world. It is not clear if the prevalence of core promoter mutations differ in different geographical regions and if the occurrence of these mutations is restricted to specific HBV genotypes.

Initial studies reported that A1896 was predominantly found in patients with fulminant hepatitis or chronic active hepatitis, suggesting that the precore stop codon mutation may be more pathogenic.[2,3,21,22] Subsequent studies showed that A1896 can also be found in asymptomatic carriers.[23,24] Nevertheless, there remains lingering concerns that HBeAg seroconversion associated with the development of A1896 is less likely to result in remission of liver disease. Similarly, early studies reported that core promoter mutations were mainly found in patients with fulminant hepatitis or chronic active hepatitis,[12,13,15,25] but later studies showed that TA changes were also found in asymptomatic carriers.[12,14] Thus, it is unclear if TA changes influence the severity or course of liver disease after HBeAg seroconversion.

In this study, we analyzed serial HBV precore and core promoter sequences in Chinese patients with chronic HBV infection before and after HBeAg seroconversion to determine whether: 1) core promoter and/or precore mutations were detected in all patients after HBeAg seroconversion; 2) core promoter mutations were more frequently selected in patients with HBV genotypes that have C1858, which precludes the development of A1896; and 3) the development of core promoter and/or precore mutations was associated with active liver disease after HBeAg seroconversion.

PATIENTS AND METHODS

Patients. Forty-five Chinese patients with chronic HBV infection who were seen in the Hepatitis Clinic, Queen Mary Hospital, Hong Kong, were studied. These patients were selected based on the availability of two or more serum samples over a follow-up period of at least 2 years. They included 27 (60%) males and 18 (40%) females, aged 10 to 40 years (mean ± SD: 25 ± 7 years). All patients were negative for hepatitis C and hepatitis D antibodies. All patients were HBeAg-positive at presentation. Group I included 26 patients with sustained HBeAg to HBe antibody (anti-HBe) seroconversion. Group II included 19 controls who were persistently HBeAg-positive during the follow-up period. These patients were further divided into groups A (C1858) and B (T1858) based on the sequence at nucleotide position 1858 in the precore region. The HBV S gene sequence was determined in previous studies in 13 patients. Five patients (patients 13, 15, 18, 21, and 40) were infected with HBV genotype B, all of whom had T1858. Eight patients (patients 8, 10, 12, 38, 34, 35, 36, and 44) were infected with HBV genotype C; all but 1 (patient 44) had C1858. Twenty-three (51%) patients, 14 in group I and 9 in group II, received interferon alfa treatment for 3 to 6 months in two previously reported interferon trials,[26,27] while the remaining 22 patients (49%) never received interferon or other antiviral treatment.

Materials. An aliquot of serum was collected at each clinic visit (every 1 to 6 months) and stored at −70°C for HBV DNA assay. The earliest and latest available serum samples of each patient were analyzed. The interval between these samples ranged from 31 to 93 months (mean ± SD: 63 ± 20 months). In group I patients, one serum sample must be available before and the other one after HBeAg seroconversion. Additional interval samples were studied in

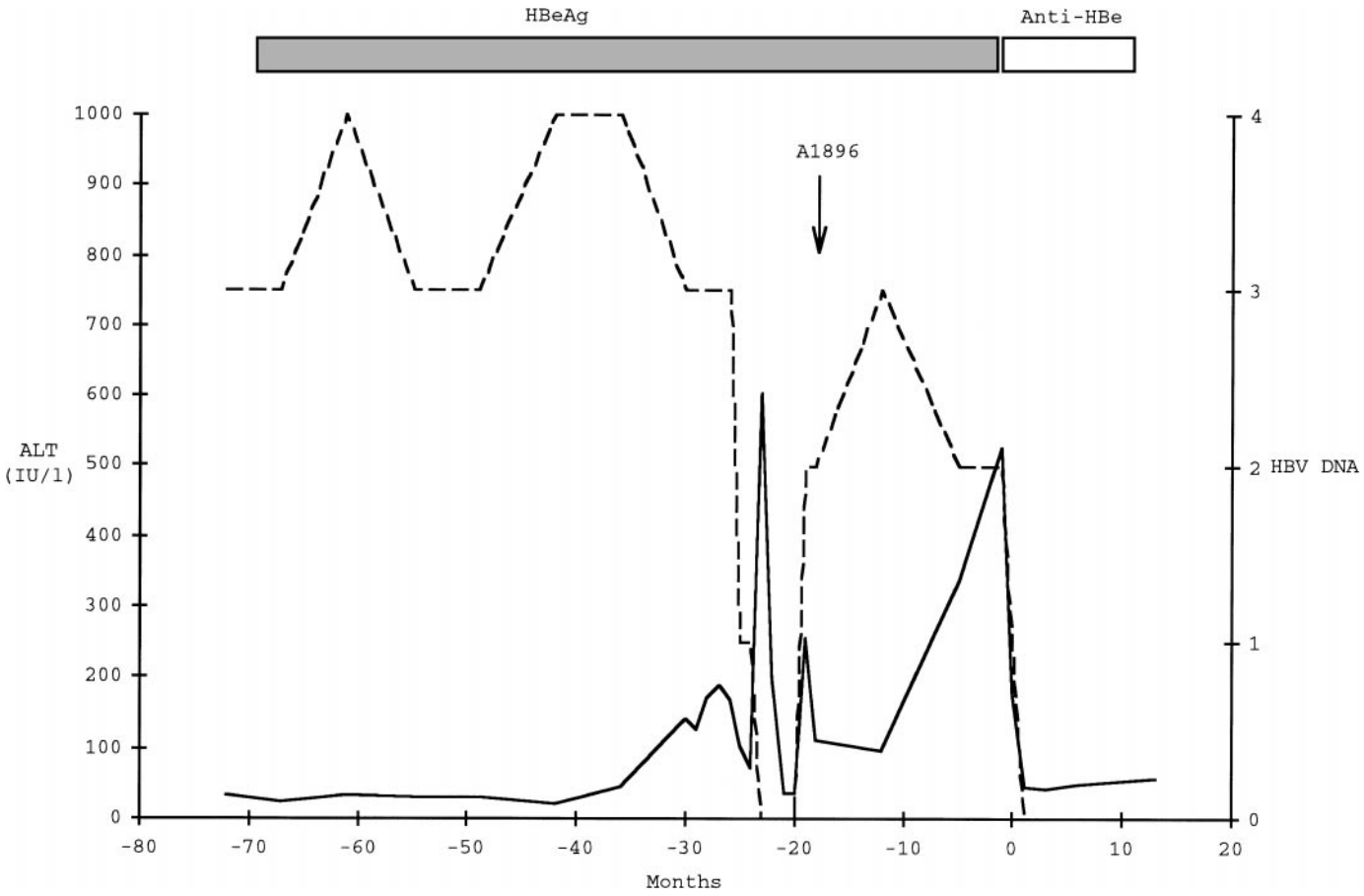

FIG. 2. Patient 14, who had T1858 in the precore region and normal ALT levels during the first 3 years of follow-up. HBeAg seroconversion was preceded by multiple flares in ALT and emergence of precore stop codon mutation (A1896). ALT levels normalized just before HBeAg seroconversion and remained normal during the course of follow-up. *Broken line,* HBV DNA; *straight line,* ALT.

patients who had changes in the core promoter and precore regions to determine the timing of emergence of these mutants. The time of emergence of these mutations was defined as the time when the initial sample was collected if the mutation was detected in that sample, and the midpoint between the last sample when the mutation was absent and the first sample when the mutation was detected if the mutation developed during follow-up. The initial samples of all the 23 patients who received interferon were collected before initiation of therapy.

Hepatitis Serology and HBV DNA Assay. Hepatitis B, C, and D virus serological markers were tested using commercially available enzyme-linked immunosorbent assay kits from Abbott Laboratories (North Chicago, IL). HBV DNA was determined by a semiquantitative dot-blot hybridization assay.[27]

PCR Amplification and Direct Sequencing. DNA was extracted by incubating 100 μL serum with 50 μL buffer containing (final concentration) 0.5 mg/mL proteinase K, 10 mmol/L Tris-HCl (pH 8.0), 20 mmol/L ethylenediaminetetraacetic acid, and 0.5% sodium dodecyl sulfate at 50°C for 2 hours. The samples were extracted by Qiaquick spin columns (Qiagen Inc., Chatsworth, CA) according to the manufacturer's instructions. The extracted DNA was eluted with 30 μL H_2O.

First-round PCR was performed on 5 μL of DNA extract in a 50-μL reaction mix containing (final concentration) 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.25 μmol/L of each of the four deoxynucleotide triphosphates, 2.5 U Taq polymerase (Perkin Elmer, Foster City, CA), and 0.6 mmol/L of each of the external primers. The reaction was performed in a Personal Cycler (Biometra Inc., Tampa, FL) for 36 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 3 minutes, with a 7-minute extension step at 72°C at the end. For the second-round PCR, 2 μL of first-round PCR product was added to 48 μL of reaction mix with the same composition as the first-round reaction, except that internal primers were used. Five microliters of the second-round PCR products was analyzed by electrophoresis in a 1% agarose gel stained with ethidium bromide, and visualized with an ultraviolet transilluminator.

External primers were P1 (5′-TCGCATGGAGACCACCGTGA-3′, positions 1604-1623) and P2 (5′-ATAGCTTGCCTGAGTGC-3′, positions 2076-2060); internal primers were P3 (5′-CATAAGAG-GACTCTTGGACT-3′, positions 1653-1672) and P4 (5′-GGAA-AGAAGTCAGAAGGC-3′, positions 1974-1957). All necessary precautions to prevent cross-contamination were observed, and negative controls were included in each assay.

Amplified PCR products were purified by Qiaquick spin columns (Qiagen Inc.) according to the manufacturer's instructions. Purified HBV DNA was directly sequenced by the DNA sequencing core facility at the University of Michigan Medical Center using the standard protocol for the Applied Biosystems DNA sequencer 373A (Perkin Elmer Corp.) using primers P4 and P3.

Statistical Analyses. The two-tailed Student *t* test was used to compare continuous variables, and the Fisher's two-tailed exact test was used to compare categorical data.

RESULTS

At presentation, patients who had sustained HBeAg seroconversion (group I) were significantly older than those who were persistently HBeAg-positive (group II), mean ages for groups I and II patients being 29 ± 5 years versus 21 ± 7

years, respectively ($P < .01$) (Table 1). There was no difference in sex distribution, percent of patients who received interferon treatment, or duration of follow-up between the two groups of patients.

Core Promoter and Precore Mutations in Relation to HBeAg Seroconversion (Group I). Among the patients who had sustained HBeAg seroconversion (group I), 11 (42%) had core promoter, but only 2 (8%) had precore stop codon mutations at presentation (Table 2). During follow-up, 3 (12%) patients developed core promoter, while 11 (42%) developed precore stop codon changes. Overall, 92% of the patients had core promoter or precore mutations after HBeAg seroconversion: 42% had core promoter changes only, 38% had precore stop codon mutations only, and 12% had changes in both regions. Core promoter changes were significantly more common in patients infected with HBV who have C1858 (91% vs. 27%; $P < .01$), while precore stop codon changes were exclusively found in patients infected with HBV who have T1858 (87% vs. 0; $P < .01$).

Mutations in the core promoter region appeared earlier and were detected in almost all (93%) patients during the HBeAg-positive phase, while precore stop codon mutations tended to appear around the time of HBeAg seroconversion. The mean interval between the first detection of core promoter mutations and HBeAg seroconversion was significantly longer: 18 ± 22 months (range, −58 to +24 months) than the mean interval between the first detection of precore stop codon mutation and HBeAg seroconversion: 2 ± 16 months (range, −18 to +35 months) ($P < .05$) (Table 3). Of the 3 patients who had both core promoter and precore mutations, TA changes appeared 7 and 25 months before A1896 in 2 patients (patients 12 and 25) and simultaneously with A1896 in the third patient (patient 24).

Core Promoter and Precore Mutations in Patients Infected With HBV Who Have C1858 (Groups IA and IIA). Among the patients infected with HBV who have C1858, a significantly higher percentage of patients who had sustained HBeAg seroconversion (group IA) had core promoter changes compared with those who were persistently HBeAg-positive (group IIA) in both the initial (82% vs. 9%) as well as the last available samples (91% vs. 36%; [$P = .02$]) (Table 2).

Nine of 11 (82%) group IA patients had core promoter changes in the initial serum samples collected 4 to 59 months before HBeAg seroconversion (Table 3). Another patient (patient 6) developed core promotor changes during follow-up, 24 months after HBeAg seroconversion. Only 1 (9%) patient (patient 2) had no detectable core promoter change up to 46 months after HBeAg seroconversion. In contrast, in group IIA patients, only 1 of 11 (9%) had core promoter changes in the initial serum sample (Table 4). Three patients (patients 29, 30, 32) developed core promotor changes after 9, 17, and 74 months of follow-up. Seven patients had no core promoter changes after a mean follow-up period of 63 ± 18 months (range, 33-84 months).

All patients who had core promoter changes retained those changes throughout the course of follow-up. Of the 14 patients who had core promoter changes, T1762 and A1764 (TA) were simultaneously detected in 10 patients (Tables 3 and 4). In the remaining 4 patients, 3 (patients 7, 9, 10) had A1764 change only (AA) in the initial samples, 1 of whom (patient 10) developed additional an T1762 change during follow-up, while the other 2 still had an isolated A1764 change 9 and 75 months after HBeAg seroconversion. The fourth patient (patient 29) evolved from wild-type core promoter sequence (AG) in the initial sample to isolated A1764 change (AA) after 74 months, with an additional T1762 change (TA) 4 months later. None of our patients had an isolated T1762 change.

None of the patients infected with HBV who have C1858 had a precore stop codon mutation (A1896) (Table 2). However, 1 patient (patient 4) had a mutation of the precore start codon.

Core Promoter and Precore Mutations in Patients Infected With HBV Who Have T1858 (Groups IB and IIB). Among the patients infected with HBV who have T1858, patients who had sustained HBeAg seroconversion (group IB) had a significantly higher prevalence of precore stop codon mutation than those who were persistently HBeAg-positive (group IIB) in the last available samples: 87% vs. 25% ($P < .01$), but this difference was not evident in the initial samples: 13% vs. 0% (NS) (Table 2). There was no difference in the prevalence of core promoter changes in both the initial and the last available samples between these two groups (Table 2).

Two (13%) of 15 patients (patients 20, 23) who had sustained HBeAg seroconversion (group IB) had precore stop codon mutation in the initial samples collected 8 and 10 months before HBeAg seroconversion (Table 3). Eleven additional patients developed precore stop codon mutation during follow-up; in 6 patients, A1896 was detected 6 to 18 months before HBeAg seroconversion, while in the other 5 patients, A1896 was first detected 1 to 35 months after HBeAg seroconversion. Among the 13 patients with precore stop codon mutations, 3 also had core promoter changes (patients 12, 24, and 25). In the remaining 2 patients who did not have precore stop codon mutation, 1 (patient 21) developed a 21-bp deletion (nucleotides 1748-1768) in the core promoter region, while the other patient (patient 16) had no detectable change in both the core promoter and precore regions. None of the patients who remained HBeAg-positive (group IIB) had precore stop codon mutation in the initial samples; only 2 (patients 40, 45) developed A1896 during follow-up (Table 4). None of the group IIB patients had core promoter changes in any of the samples studied.

Effect of Interferon Treatment on the Development of Core Promoter and Precore Mutations. Fourteen group I and 9 group II patients received interferon therapy during the study period. New mutations appeared in similar proportions of

TABLE 1. Characteristics of the Patients at Presentation

Group	No. of Patients	Sex (M/F)	Age* (yr)	HBeAg Seroconversion	No. Treated With Interferon (%)	Duration of Follow-up (mo)*
I	26	14/12	29 ± 5 (13-37)	yes	14 (54)	63 ± 19 (31-92)
II	19	13/6	21 ± 7 (10-40)	no	9 (47)	62 ± 20 (32-93)

*Mean ± SD (range).

TABLE 2. Core Promoter (TA Changes) and Precore Stop Codon (A1896) Mutations in Patients With Different HBV Genotypes

				Initial Samples		Changes During FU		Last Samples	
				No. of Patients With		No. of Patients With		No. of Patients With	
Patient Group	No. of Patients	Sequence at nt 1858	HBeAg Seroconversion	TA Changes (%)	A1896	TA Changes	A1896	TA Changes	A1896 (%)
I	26	C/T	yes	11 (42)	2 (8)	3*	11	14 (54)	13 (50)
IA	11	C	yes	9 (82)	0	1	0	10 (91)	0
IB	15	T	yes	2 (13)	2 (13)	2*	11	4 (27)*†	13 (87)
II	19	C/T	no	1 (5)	0	3	2	4 (21)	2 (11)
IIA	11	C	no	1 (9)	0	3	0	4 (36)	0
IIB	8	T	no	0	0	0	2	0	2 (25)

*One patient had a 21-bp deletion in the core promoter region.
†Three patients had coexistent A1896.

patients who did or did not receive interferon treatment (Table 5).

Correlation of Core Promoter and Precore Mutations With Remission of Liver Disease After HBeAg Seroconversion. Of the 26 patients who had sustained HBeAg seroconversion, 18 had elevated alanine transaminase (ALT) levels at presentation; an additional 7 patients developed intermittent or persistently elevated ALT levels 2 to 50 months before HBeAg seroconversion. Only 1 patient (patient 2) had persistently normal ALT levels throughout the study period.

Most (92%) patients had core promoter and/or precore stop codon mutations after HBeAg seroconversion. Nevertheless, 19 (73%) had persistently normal ALT levels during a follow-up period of 36 ± 18 months (range, 13-75 months) after HBeAg seroconversion (Fig. 2); only 7 (27%) had active liver disease after HBeAg seroconversion (Table 6). Three patients continued to have elevated ALT levels for a few months after HBeAg seroconversion, followed by persistently normal ALT levels (Fig. 3). Two patients had a transient ALT flare 18 and 32 months after HBeAg seroconversion. Two patients had multiple flares in ALT levels despite sustained HBeAg seroconversion (Fig. 4). There was no correlation

TABLE 3. Serial Core Promoter and Precore HBV Sequences of Patients Who Had Sustained HBeAg Seroconversion (Group I)

		Initial Samples			Follow-up Samples					
Patient No.	Sex/ Age	CP nt 1762/1764	Precore nt 1896	Time (mo)	CP nt 1762/1764	Precore nt 1896	Time (mo)	Timing of A1896 (mo)	Timing of TA Change (mo)	IFN R_x
Group IA: C1858										
1	F/31	TA	G	−10	TA	G	+46	NA	−10	Y
2	M/31	AG	G	−18	AG	G	+46	NA	Nil	Y
3	F/26	TA	G	−58	TA	G*	+26	NA	−58	Y
4	M/32	TA	G†	−9	TA	G†	+26	NA	−9	Y
5	M/51	TA	G	−37	TA	G	+15	NA	−37	Y
6	M/13	AG	G	−19	TA	G	+61	NA	+24	N
7	F/24	AA	G	−4	AA	G	+9	NA	−4	N
8	F/35	TA	G	−50	TA	G	+2	NA	−50	N
9	F/28	AA	G	−3	AA	G	+75	NA	−3	N
10	F/31	AA	G	−11	TA	G‡	+9	NA	−11	N
11	F/31	TA	G	−1	TA	G	+26	NA	−1	N
Group IB: T1858										
12	M/29	TA	G	−19	TA	A	+34	−12	−19	Y
13	M/32	AG	G	−5	AG	A	+50	+35	Nil	Y
14	M/26	AG	G	−62	AG	A	0	−16	Nil	Y
15	F/27	AG	G	−22	AG	A	+5	−6	Nil	Y
16	F/26	AG	G	−11	AG	G	+17	Nil	Nil	Y
17	M/27	AG	G	−23	AG	A	+2	−17	Nil	Y
18	M/29	AG	G	−7	AG	A	+34	+17	Nil	Y
19	F/32	AG	G	−48	AG	A	+29	+15	Nil	Y
20	F/26	AG	A	−10	AG	A	+16	−10	Nil	Y
21	M/13	AG	G	−44	Del	G	+1	Nil	Nil	N
22	M/30	AG	G	0	AG	A	+51	+1	Nil	N
23	M/36	AG	A	−8	AG	A	+16	−8	Nil	N
24	M/24	AG	G	−23	TA	A	+43	−13	−13	N
25	F/37	AA§	G	−43	AA§	A	+15	−18	−43	N
26	M/35	AG	G	−4	AG	A	+68	+9	Nil	N

NOTE. Time zero = time of HBeAg seroconversion.
Abbreviations: CP, core promoter; IFN R_x, interferon treatment (Y = yes, N = no).
*G-A1898, †precore start codon mutation ATG-AGG; ‡G-A1899; §C-T1766 and T-A1768.

TABLE 4. Serial Core Promoter and Precore HBV Sequences of Patients Who Were Persistently HBeAg-Positive (Group II)

Patient No.	Sex/ Age	Initial Samples: CP nt 1762/1764	Initial Samples: Precore nt 1896	Follow-up Samples: CP nt 1762/1764	Follow-up Samples: Precore nt 1896	Time (mo)	Timing of TA Change (mo)	IFN R_x
Group IIA: C1858								
27	M/28	TA	G	TA	G	+81	0	Y
28	F/13	AG	G	AG	G	+73	Nil	Y
29	M/14	AG	G	TA	G	+93	+74	Y
30	M/22	AG	G	TA	G	+52	+17	Y
31	F/18	AG	G	AG	G	+69	Nil	Y
32	F/29	AG	G	TA	G	+38	+9	Y
33	F/18	AG	G	AG	G	+46	Nil	N
34	M/10	AG	G	AG	G	+84	Nil	N
35	M/26	AG	G	AG	G	+46	Nil	N
36	M/17	AG	G	AG	G	+75	Nil	N
37	M/15	AG	G	AG	G	+33	Nil	N
Group IIB: T1858							Timing of A1896 (mo)	
38	F/19	AG	G	AG	G	+72	Nil	Y
39	M/18	AG	G	AG	G	+84	Nil	Y
40	M/40	AG	G	AG	A	+86	+32	Y
41	M/19	AG	G	AG	G	+76	Nil	N
42	M/26	AG	G	AG	G	+32	Nil	N
43	F/23	AG	G	AG	G	+33	Nil	N
44	M/28	AG	G	AG	G	+71	Nil	N
45	M/14	AG	G	AG	A	+41	+38	N

NOTE. Time zero = time of first sample.
Abbreviations: CP, core promoter; IFN, interferon treatment (Y = yes, N = no).

between the presence of core promoter or precore mutations and ALT pattern after HBeAg seroconversion (Table 6). All 7 patients with elevated ALT levels after HBeAg seroconverson were negative for hepatitis C and hepatitis D antibodies and had detectable serum HBV DNA by hybridization assay.

DISCUSSION

In this study, we found that 92% of Chinese patients in Hong Kong had mutations in the core promoter and/or precore regions after sustained HBeAg seroconversion: 42% had core promoter changes only, 38% had precore changes only, and 12% had changes in both regions. Similar to other reports, the most common core promoter mutations in our patients involved an A-T change at nucleotide 1762 and a G-A change at nucleotide 1764, while the most common precore mutation was a G-A change at nucleotide 1896. We found that the core promoter changes appeared significantly earlier than the precore changes. In many patients, changes at both nucleotide positions 1762 and 1764 were already present at presentation. However, in some patients, progression from wild-type (AG) to mutant (TA) sequence was observed during the course of infection. In a few patients, an intermediate step involving an isolated change at nucleotide 1764 (AA) was noted. None of our patients was found to have an isolated change at nucleotide 1762 (TG). Despite the early appearance of the TA changes, several lines of evidence support a role (albeit small) for the TA changes in the clearance of HBeAg. *In vitro* studies showed that the TA changes can prevent binding of a liver-enriched factor to the core promoter region, resulting in decreased transcription of precore RNA and thus secretion of HBeAg.[16] We found a significantly higher prevalence of core promoter changes among the patients who subsequently cleared HBeAg compared with those who remained HBeAg-positive: 91% versus

TABLE 5. Effect of Interferon Treatment on the Development of Core Promoter and/or Precore Stop Codon Mutations

Patient Group	Interferon Treatment	No. of Patients	No. of Patients Who Developed New Mutations in: Core Promoter	No. of Patients Who Developed New Mutations in: Precore Region
IA	yes	5	0	0
	no	6	1	0
IB	yes	9	0	7
	no	6	2	4
IIA	yes	6	3	0
	no	5	0	0
IIB	yes	3	0	1
	no	5	0	1

TABLE 6. Serum Aminotransferase Levels After HBeAg Seroconversion in Relation to Core Promoter and Precore Mutations

Type of Mutation	No. of Patients	No. of Patients With: Persistent/ Intermittent Elevation in ALT	No. of Patients With: Sustained Normalization in ALT
TA change only	10	2	8
TA + A1896	3	1	2
A1896 only	10	3	7
21-bp deletion in core promoter region	1	1	0
No change	2	0	2
Total	26	7 (27%)*	19 (73%)

*Three patients had elevated ALT levels during the first 6 months after HBeAg seroconversion.

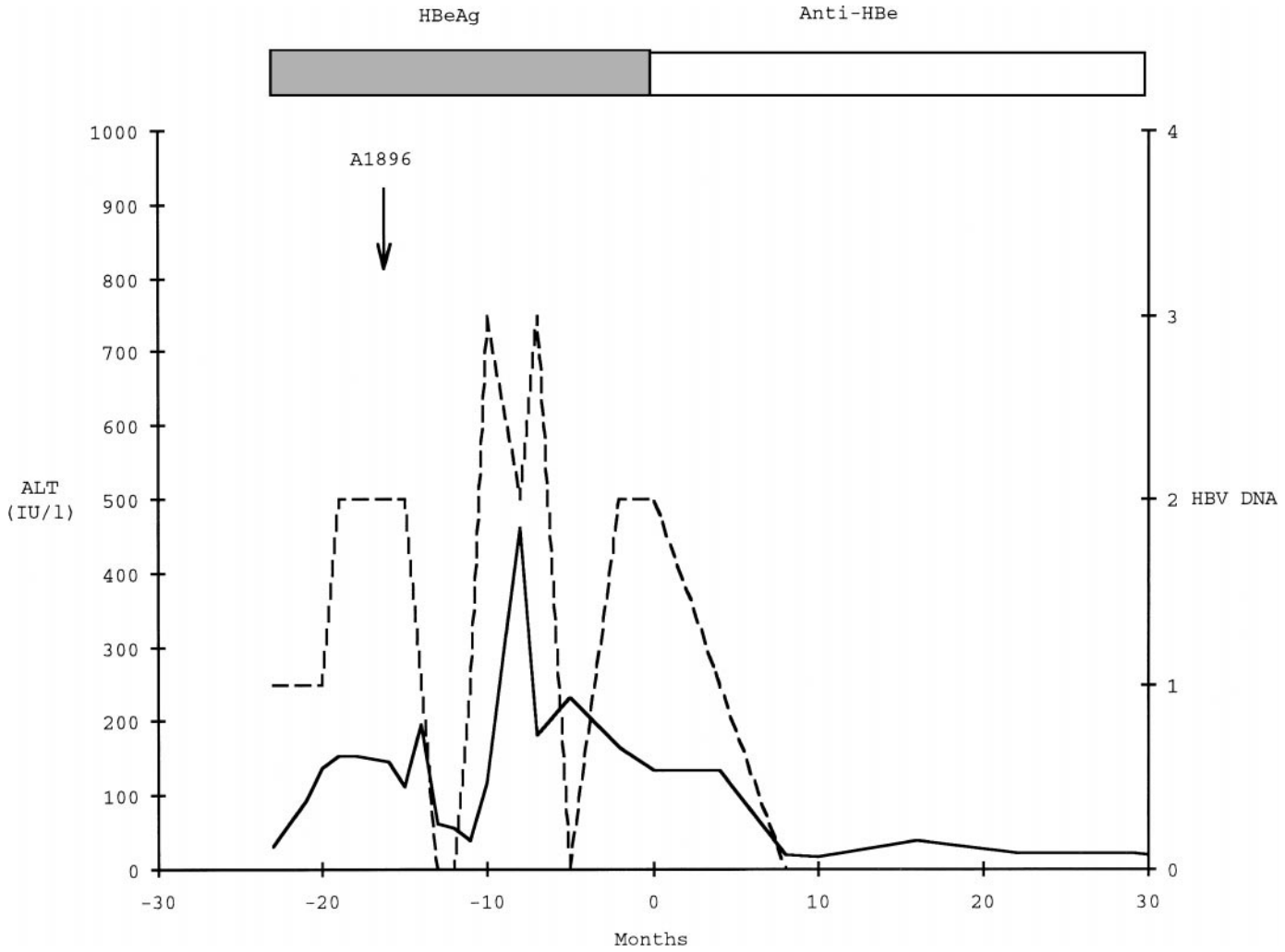

FIG. 3. Patient 15, who had T1858 in the precore region and normal ALT levels at presentation. Shortly after presentation, this patient developed elevated ALT levels and precore stop codon mutation (A1896). Serum HBV DNA remained detectable by hybridization assay, and ALT levels continued to be elevated for 4 months after HBeAg seroconversion, followed by sustained disappearance of serum HBV DNA and normalization of ALT. *Broken line*, HBV DNA; *straight line*, ALT.

36%. The early emergence of the TA changes compared with A1896 may be related to the inefficiency of the core promoter changes in preventing HBeAg production. The TA changes decrease, but do not completely abolish, HBeAg secretion.[16-20] Thus, additional steps such as immune clearance or development of other mutations such as A1896 may be needed for HBeAg clearance. An isolated A1764 change has also been reported in a small percentage of patients by other investigators.[13] *In vitro* studies showed that an A1764 change has no effect on precore RNA transcription, while an isolated T1762 change has a similar suppressive effect on precore RNA transcription as the combined TA changes.[28] It is therefore not surprising that an A1764 change appeared earlier in the course of infection.

The overall prevalence of core promoter and/or precore mutations in our patients was very similar to that reported by Okamoto et al.,[12] but the pattern of changes was markedly different. In Okamoto's study, 98% of 57 anti-HBe–positive patients had core promoter and/or precore changes, 9% had core promoter changes only, 33% had precore changes only, and 56% had changes in both regions. Several reasons may account for the differences in pattern of core promoter and precore mutations in the two studies. Firstly, all our patients were observed to seroconvert from HBeAg to anti-HBe, while Okamoto's patients were anti-HBe–positive at presentation. A longer interval between HBeAg seroconversion and testing may account for a higher prevalence of A1896 in Okamoto's patients. A1896 was first detected more than 1 year after HBeAg seroconversion in 3 (23%) of our patients. It is also possible that some of Okamoto's patients were infected with the precore stop codon mutant *ab initio*. Secondly, 42% of our patients were infected with HBV who have C1858. Although data on the prevalence of HBV with C1858 were not provided in Okamoto's article, other reports suggest that HBV genotypes with C1858 are uncommon in Japan. A higher percentage of patients infected with HBV genotypes who have C1858 may account for a lower frequency of A1896 and a higher frequency of TA changes in our patients (see below).

In accordance with our previous observations,[4] we confirmed that the precore stop codon mutation (A1896) was exclusively found in patients infected with HBV who have T1858. We also found that the core promoter mutations were significantly more common in patients infected with HBV who have C1858, which precludes the development of

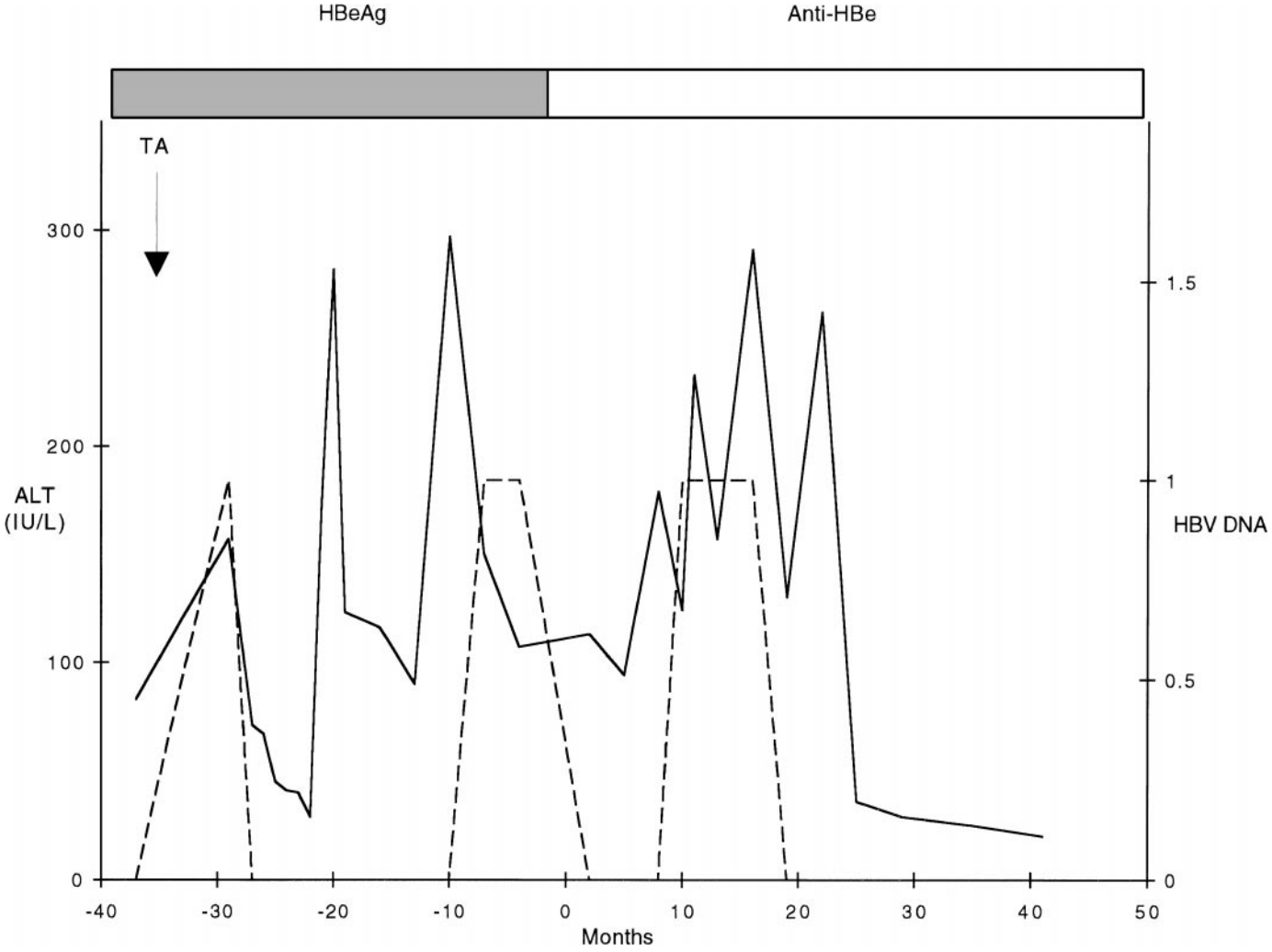

FIG. 4. Patient 5, who had recurrent flares in ALT and TA changes in the core promoter region before HBeAg serconversion. He had multiple flares in ALT, accompanied by fluctuations in serum HBV DNA levels after HBeAg seroconversion. *Broken line*, HBV DNA; *straight line*, ALT.

A1896. To our knowledge, this is the first report that suggests a preferential selection of core promoter mutations in patients infected with HBV genotypes who have C1858. Although there have been very little data on the prevalence of core promoter mutations in countries in which HBV genotypes with C1858 are common, a study in the United States reported that 4 (50%) of 8 anti-HBe–positive chronic hepatitis B patients had TA changes.[29] Further studies are needed to confirm if core promoter mutations are preferentially selected in HBV genotypes that cannot develop precore stop codon mutations and the exact mechanisms by which these mutations are selected. TA changes in the core promoter region have been reported to be associated with enhanced replication in *in vitro* studies,[16,17,19] but these findings have been refuted by other investigators.[18] TA changes have also been postulated to alter the secondary structure of the 3′-end of the pregenomic RNA, thus facilitating DNA minus-strand translocation to DR1,[30] but these effects have not been proven.

Almost all our patients had core promoter and/or precore stop codon mutations after HBeAg seroconversion. Nevertheless, most had sustained remission of liver disease. Our experience suggests that HBeAg seroconversion is usually associated with sustained remission of liver disease, even when accompanied by the development of core promoter and/or precore mutations. We acknowledge that our study population is small and our data may not be applicable to patients in other geographical regions. However, our data corroborate previous reports that core promoter and precore mutations can be detected in anti-HBe–positive carriers with normal ALT levels. Our findings suggest that core promoter and/or precore mutations are not necessarily pathogenic and that additional factors such as mutations in other regions of the HBV genome, host immune response, or *ab initio* infection with the mutants may be important in determining the course of liver disease in anti-HBe–positive patients.

In summary, we found that the vast majority of our patients had core promoter and/or precore stop codon mutations after HBeAg seroconversion. Our data suggest that the core promoter changes are preferentially selected in patients infected with HBV genotypes who have C1858 that preclude the development of precore stop codon mutation. The basis for this selection is not clear, but the core promoter changes may provide an alternative mechanism for HBeAg clearance in these patients. Despite the frequent occurrence of core

promoter and/or precore mutations, most patients had sustained remission of liver disease after HBeAg seroconversion.

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