

SD. Statistical analyses were performed using χ^2 test and Fisher's exact test for categorical variables. Mann-Whitney *U* test or 1-way analysis of variance were used for continuous variables, as appropriate. Mantel-Haenszel χ^2 test was used to analyze the trend of frequencies of viral mutations. Multivariate analyses with logistic regression were used to determine the independent factors associated with HCC and T1653. Differences were considered to be significant for *P* values <.05. The statistical analysis software used was Stata software, version 8.0 (StataCorp).

RESULTS

Table 1 compares ALT level, platelet count, and HBV DNA level, as well as mutations in the box α (enhancer II), core promoter, and preC region, among 40 carriers of inactive virus, 40 patients with chronic hepatitis, and 40 patients with HCC who were infected with HBV genotype C in an age-matched case-control study. ALT and HBV DNA levels were significantly lower among carriers of inactive virus than among patients with chronic hepatitis or patients with HCC (*P* < .0001 and *P* = .001, respectively). Platelet count was lower among patients with HCC than among carriers of inactive virus or patients with chronic hepatitis (*P* < .0001).

The frequency of the T1653 mutation in the box α was significantly higher among patients with HCC (70%) than

among carriers of inactive virus (25%) or patients with chronic hepatitis (35%; *P* < .0001) (table 1). Of interest, the T1653 mutation had an opposite correlation with the M1753 mutation. The prevalence of T1762/A1764 was high in all clinical status groups, with no statistically significant difference between groups (table 1). The trend of the frequency of T1653, increasing from carriers of inactive virus to patients with chronic hepatitis to patients with HCC, was analyzed by Mantel-Haenszel χ^2 test (OR, 2.48; 95% CI, 1.59–3.85; *P* = .0001) (figure 1). The trend of the frequency of T1762/A1764 was not statistically significant (*P* = .1502) (figure 1).

The attributable risk of multiple factors, including sex, HBV DNA level, ALT level, platelet count, and the presence of the T1653, M1753, T1762/A1764, and A1896 mutations for HCC in the HBV carriers was determined by multiple logistic regression analysis (table 2). There was a statistically significant association between development of HCC and ALT level >37 U/L (OR, 12.56; 95% CI, 0.55–6.21; *P* < .0001) and platelet count <18 × 10⁴ platelets/mm³ (OR, 11.5; 95% CI, 3.47–38.21; *P* < .0001). The T1653 mutation was still significantly associated with the development of HCC (OR, 5.05; 95% CI, 1.56–16.35; *P* = .007).

The attributable risk of multiple factors, including HBV DNA level, ALT level, platelet count, α -fetoprotein level, protein in-

Table 1. Demographic, clinical, and virologic characteristics of patients infected with hepatitis B virus (HBV) genotype C who were matched for age and hepatitis B e antigen (HBeAg) status.

Variable	Clinical status			<i>P</i>
	Carriage of inactive virus (n = 40)	Chronic hepatitis (n = 40)	Hepatocellular carcinoma (n = 40)	
Male sex	31 (77.5)	37 (92.5)	36 (90)	.171
Age, years	50.9 ± 11.1	50.2 ± 8.9	50.7 ± 9.4	Matched
HBeAg positive	0 (0)	0 (0)	0 (0)	Matched
Anti-HBeAg positive	40 (100)	40 (100)	40 (100)	Matched
HBV genotype C	40 (100)	40 (100)	40 (100)	Matched
Alanine transaminase level, U/L ^a	20.8 ± 7.6	102 ± 108.7	83.2 ± 84.8	.0001
Platelet count, ×10 ⁴ platelets/mm ^{3b}	20.7 ± 3.1	17.4 ± 4.1	12.8 ± 5.7	.0001
HBV DNA level, LGE/mL ^c	4.3 ± 0.8	5.9 ± 1.5	5.4 ± 1.5	<.0001
Mutation in the box α : T1653 ^d	10 (25)	14 (35)	28 (70)	<.0001
Mutation in the core promoter				
M1753	10 (25)	6 (15)	9 (22.5)	.609
T1762/A1764	31 (77.5)	31 (77.5)	36 (90)	.289
Mutation in the precore region: A1896	25 (62.5)	26 (65)	25 (62.5)	1.0

NOTE. Data are no. (%) of patients or mean value ± SD. Anti-HBeAg, antibody to HBeAg; LGE, log genome equivalents.

^a *P* < .0001 for carriers of inactive virus vs. patients with chronic hepatitis; *P* = .002 for carriers of inactive virus vs. patients with hepatocellular carcinoma.

^b *P* < .0001 for patients with hepatocellular carcinoma vs. carriers of inactive virus or patients with chronic hepatitis; *P* = .002 for carriers of inactive virus vs. patients with chronic hepatitis.

^c *P* < .0001 for carriers of inactive virus vs. patients with chronic hepatitis; *P* = .001 for carriers of inactive virus vs. patients with hepatocellular carcinoma.

^d *P* < .0001 for carriers of inactive virus vs. patients with chronic hepatitis; *P* = .001 for carriers of inactive virus vs. patients with hepatocellular carcinoma.

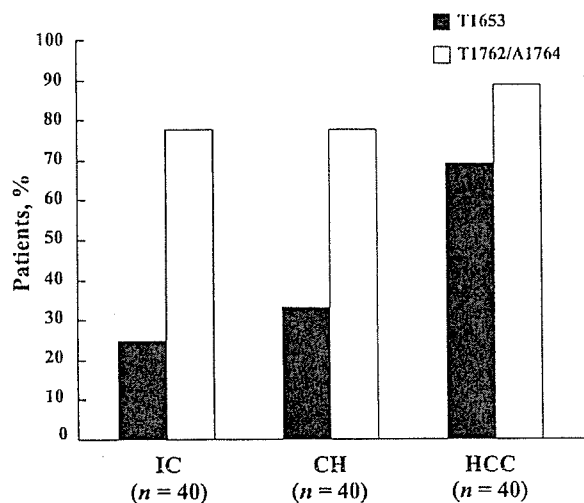


Figure 1. Prevalence of T1653 box α and T1762/A1764 basic core promoter mutations among patients with chronic hepatitis B virus infection, stratified by clinical status. The trend of the frequency of the T1653 mutation was analyzed by Mantel-Haenszel χ^2 test. The OR estimate is an approximation of the OR for carriers of inactive virus (IC), patients with chronic hepatitis (CH), and patients with hepatocellular carcinoma (HCC) having a strain with the mutation (OR, 2.48; 95% CI, 1.59–3.85; $P = .0001$). The trend of the frequency of the T1762/A1764 mutation was not statistically significant according to the Mantel-Haenszel χ^2 test ($P = .1502$).

duced by the absence of vitamin K (antagonist II) level, for T1653 in patients with HCC with HBV genotype C infection was determined by multiple logistic regression analysis (table 3). An α -fetoprotein level >300 ng/mL was the only independent predictive value for the presence of the T1653 mutation in patients with HCC with HBV genotype C infection (OR, 12.67; 95% CI, 1.19–134.17; $P = .035$).

Table 4 compares sex, age, and mutations in the box α , core promoter, and preC region among patients infected with HBV genotypes Aa (10 patients), Ae (13), Ba (20), Bj (20), C (15), and D (13) with the same variables among patients with HCC. Mean age was significantly higher among patients with HBV genotype Bj infection, compared with patients with HBV genotype Ba, genotype C, and genotype D infection ($P < .05$). The prevalence of T1653 among patients with HBV genotype C infection (66.7%) was significantly higher than it was among patients infected with other genotypes (15%–25%; $P < .05$), excluding patients infected with HBV genotype Aa. The prevalence of T1762/A1764 among patients with HBV genotype Ba infection (85%) and HBV genotype C infection (86.7%) was also significantly higher than it was among patients infected with other genotypes (20%–50%; $P < .05$). The prevalence of A1896 among patients with HBV genotype Aa infection and HBV genotype Ae infection was significantly lower than it was among patients infected with other genotypes ($P < .05$).

DISCUSSION

Many previous studies have reported that the clinical course of chronic HBV infection may be modified by several specific viral mutations [5, 20, 21], although the significance of such specific mutations in patients with chronic hepatitis B remains controversial. Because most studies have not controlled for different variables, such as age, HBV genotype, and HBe status, it is unknown whether the mutations were associated with disease progression, greater age of the patient, the specific HBV genotype or subtype, or HBe status. In this study, to exclude any biases, we performed an age-matched case-control study involving only anti-HBe-positive patients infected with HBV genotype C.

In the present case-control study, the prevalence of T1653 was found to be significantly higher among patients with HCC, compared with carriers of inactive virus and patients with chronic hepatitis with HBV genotype C infection; however, the prevalence of T1762/A1764 was high in all clinical status groups. During the anti-HBe-positive phase of infection, T1653 was more reliable than T1762/A1764 as a predicting factor for

Table 2. Multivariate analysis of variables with independent predictive value for development of hepatocellular carcinoma among a group of 120 patients with hepatitis B virus infection.

Variable	OR (95% CI)	<i>P</i>
Sex		
Female	1	
Male	5.06 (0.85–30.15)	.075
HBV DNA level		
<4.8 LGE/mL	1	
≥ 4.8 LGE/mL	0.34 (0.09–1.21)	.096
Alanine transaminase level		
<37 U/L	1	
≥ 37 U/L	12.56 (3.05–51.77)	.0001 ^a
Platelet count		
$\geq 18 \times 10^4$ platelets/mm ³	1	
$<18 \times 10^4$ platelets/mm ³	11.51 (3.47–38.21)	.0001 ^a
T1653 mutation		
No	1	
Yes	5.05 (1.56–16.35)	.007 ^a
M1753 mutation		
No	1	
Yes	1.23 (0.31–5.04)	.770
T1762/A1764 mutation		
No	1	
Yes	2.67 (0.57–12.54)	.214
A1896 mutation		
No	1	
Yes	0.96 (0.29–3.11)	.943

NOTE. Each OR was adjusted for age and other variables in the analysis. LGE, log genome equivalents.

^a Statistically significant.

Table 3. Multivariate analysis of variables with independent predictive value for the presence of the T1653 mutation among 40 patients with hepatocellular carcinoma.

Variable	OR (95% CI)	P
HBV DNA level		
<4.9 LGE/mL	1	
≥4.9 LGE/mL	0.89 (0.16–4.79)	.899
ALT level		
<53 U/L	1	
≥53 U/L	1.72 (0.29–9.96)	.541
Platelet count		
≥12 × 10 ⁴ platelets/mm ³	1	
<12 × 10 ⁴ platelets/mm ³	1.39 (0.28–7.02)	.683
α-Fetoprotein level		
<300 ng/mL	1	
≥300 ng/mL	12.67 (1.19–134.17)	.035 ^a
PIVKA-2 level		
<50 mAU/mL	1	
≥50 mAU/mL	0.25 (0.05–1.43)	.120

NOTE. Each OR was adjusted for age and other variables in the table. PIVKA-2, protein induced by the absence of vitamin K (antagonist II).

^a Statistically significant.

the development of HCC. In fact, in the multivariate analysis, the presence of T1762/A1764 was not an independent predictor of HCC, but ALT level >37 U/L, platelet count <18 × 10⁴ platelets/mm³, and the presence of T1653 were independent predictors of HCC. The T1653 mutation had also been reported by Takahashi et al. [17]; they reported that this specific mutation was prevalent among Japanese patients with HCC, although their study was not a case-control study. These results do not deny that T1762/A1764 is associated with hepatocarcinogenesis, because poor prognosis associated with HBV ge-

notype C infection, compared to that associated with HBV genotype B (Ba and Bj) infection, correlated with a high prevalence of T1762/A1764 [2, 9, 16], indicating that the BCP double mutation is associated with a high potential for hepatocarcinogenesis. The appearance of the T1653 mutation after the occurrence of the T1762/A1764 mutation (the T1762/A1764 mutation usually occurs earlier than the T1653 mutation) could indicate that the virulence of HBV is increasing, which could result in the development of HCC. In the multivariate analysis, however, HBV DNA level was no longer a predicting factor for HCC. One of the reasons for this is that the HBV DNA data used in this study were obtained at the time of diagnosis of HCC. A recent prospective study from Taiwan has indicated that high HBV DNA levels at baseline and infection with HBC genotype C were independent predictors for HCC, but the mean viral load at the time of diagnosis of HCC was significantly lower than at baseline [27]. Although our data could not indicate an association between HBV DNA level and hepatocarcinogenesis, if we could measure the HBV DNA level before diagnosis of HCC, it might found to be a predicting factor for HCC. Furthermore, an examination of the characteristics of patients with HCC who had the T1653 mutation showed that an elevated α-fetoprotein level (≥300 ng/mL) was the only predictor for the development of HCC in patients with the T1653 mutation. It has been reported that α-fetoprotein level is useful not only for diagnosis but also as a prognostic indicator for patients with HCC [22, 23], suggesting that the T1653 mutation might be associated with poor prognosis for patients with HCC.

The prevalence of several mutations among patients with HCC differed from that among patients with different HBV genotypes (Aa, Ae, Ba, Bj, C, and D) (table 4). The prevalence

Table 4. Demographic and virological characteristics of patients with hepatocellular carcinoma who were positive for antibody to hepatitis B e antigen (anti-HBe), by hepatitis B virus (HBV) genotype.

Variable	HBV genotype						P
	Aa (n = 10)	Ae (n = 13)	Ba (n = 20)	Bj (n = 20)	C (n = 15)	D (n = 13)	
Male	10 (100)	12 (92.3)	18 (90)	15 (75)	15 (100)	13 (100)	.10
Age, years ^a	54.4 ± 7.7	55.3 ± 4.4	54.4 ± 14.8	64.9 ± 9.6	47.9 ± 7.6	53.5 ± 8.3	.0002
HBeAg positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Matched
Anti-HBe positive	10 (100)	13 (100)	20 (100)	20 (100)	15 (100)	13 (100)	Matched
Mutation in the box α: T1653 ^b	4 (40)	2 (15.4)	5 (25)	5 (25)	10 (66.7)	3 (23.1)	.039
Mutations in the core promoter region							
M1753	3 (30)	3 (23.1)	5 (25)	4 (20)	2 (13.3)	1 (7.7)	.759
T1762/A1764 ^c	5 (50)	6 (46.2)	17 (85)	4 (20)	13 (86.7)	5 (38.5)	<.0001
Mutation in the precore region: A1896 ^d	0 (0)	0/13 (0)	9/20 (45)	15/20 (75)	9/15 (60)	8/13 (61.5)	<.0001

NOTE. Data are no. (%) of patients or mean value ± SD. HBeAg, hepatitis B e antigen.

^a P < .05 for Bj vs. Ba or D; P < .0001 for Bj vs. C.

^b P < .05 for C vs. Ba or Bj or D; P < .01 for Ae vs. C.

^c P < .05 for Ae vs. Ba or C; P < .01 for D vs. Ba or C; P < .0001 for Bj vs. Ba or C.

^d P < .05 for Ba vs. Aa or Ae; P < .005 for Aa vs. C or D and for Ae vs. Ba or C or D; P < .0001 for Bj vs. Aa or Ae.

of T1653 was the highest among patients with HBV genotype C infection, followed by those with HBV genotype Aa infection, although the number of patient with HBV genotype Aa infection was too small for any conclusions to be drawn. The prevalence of T1762/A1764 was higher among patients with HBV genotype Ba and HBV genotype C infection than among patients infected with other genotypes. HBV genotype Ba has a sequence that closely resembles that of HBV genotype C in the core promoter region, because it is recombinant HBV between HBV genotype Bj and HBV genotype C from nucleotides 1740 to 2485. Although A1896 was not found in HBV genotype Aa and HBV genotype Ae, as has been reported elsewhere [15], HBV genotype Aa had some specific mutations upstream of the preC initiation codon and encapsidation signal site. Therefore, several HBV genotype-specific mutations would be associated with different mechanisms on seroconversion or HBV replication for each genotype or subtype.

Buckwold et al. [24] reported that T1762/A1764 can no longer bind liver-enriched transcription factors and that the transcription of precore RNA and the expression of HBeAg were reduced. Thereafter, Li et al. [25] reported that this mutation not only removed the nuclear receptor-binding site but also created a hepatic nuclear factor 1 transcription factor-binding site. As for a factor correlated with BCP, the core upstream regulatory sequence, which has a strong stimulation effect on the BCP, was reported. In an earlier article by Yu et al. [28], the box α elements (nucleotides 1646–1668) individually stimulated promoter activity >100-fold. The T1653 mutation converts the box α binding site for CCAAT/enhancer-binding protein and related factors into the perfect palindromic sequence 1648-TCTTATATAAGA, which might enhance binding affinity and core promoter/enhancer II activity. Therefore, it is possible that the mutation in the box α influenced the HBe production and viral replication through the BCP activity. In addition, the T1653 mutation corresponds to an amino acid change from histidine to tyrosine at aa 94 of the X protein, so this alteration of X protein might be hepatocarcinogenesis. Gunther et al. [26] analyzed T1653, T1762, and A1764 mutations in the context of an in vitro study involving wild-type HBV (genotype D, AF043594), and they reported that the preC mRNA and HBeAg secretion was reduced, but the amount of progeny virus DNA in the cells and in the culture medium increased only marginally (if at all), as determined by Southern blot analysis. However, because the genotype was different from that in our study (genotype D vs. genotype C) and the mutant type included not only T1653, T1762, and A1764 mutations but also other mutations in the core promoter, it is possible that some other mutation influenced the results in the earlier study.

In conclusion, the addition of the T1653 mutation in the box α to the BCP mutation increases the risk of HCC in patients

with HBV genotype C infection, suggesting that HBV with both the T1653 mutation and the BCP double mutation in patients with chronic hepatitis B should be eradicated by antiviral therapy. Functional analyses of HBV strains with the T1653 mutation are needed in vitro and in vivo.

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A case–control study of response to lamivudine therapy for 2 years in Japanese and Chinese patients chronically infected with hepatitis B virus of genotypes Bj, Ba and C

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Abstract

Background/aims: In eastern Asian countries, hepatitis B virus (HBV) genotype Ba (HBV/Ba), HBV/Bj and HBV/C are prevalent. The aim was to investigate the response or resistance to lamivudine therapy among patients with different HBV genotypes.

Methods: Of 67 Japanese and Chinese patients with chronic hepatitis B, 18 patients with HBV/Bj, 15 with HBV/Ba and 34 with HBV/C were selected for a case–control study matched according to gender and age. All the patients were treated with lamivudine for 2 years and evaluated the response or emergence of the YMDD mutation at year 2 during the treatment. HBV genotypes were detected by the restriction fragment length polymorphism. The YMDD mutation was detected by the direct sequencing after amplification by PCR.

Results: At year 2 during therapy, 44.8% of the patients showed normalization of ALT and undetectable HBV DNA (favorable response), 35.8% developed the YMDD mutation. There was no significant difference of response to the therapy among the three genotype groups. The emergence of the YMDD mutation was associated with HBV/C. By the multiple logistic regression analysis, however, the significant factor of a favorable response was a higher pretreatment ALT level and negative HBeAg status and the significant factor of the emergence of the YMDD mutation was HBV/C.

Conclusions: Higher pretreatment ALT level, HBeAg status or HBV genotype may affect the response or resistance to lamivudine therapy. © 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: HBV genotype Ba, Bj, C; Lamivudine; Response to therapy; Resistant mutation

1. Introduction

Hepatitis B virus (HBV) is one of the major causative agents of acute or chronic liver diseases in Asian countries [1]. All strains of HBV isolated from various countries can be classified into eight HBV genotypes, HBV genotype A

Abbreviations: HBV/B, hepatitis B virus genotype B; HBV/Ba, hepatitis B virus subtype Ba; HBV/Bj, hepatitis B virus subtype Bj; HBV/C, hepatitis B virus genotype C

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(HBV/A) to HBV/H, according to their phylogenetic relationship [2–4]. As reported previously, patients with different HBV genotypes show genotype-specific manifestations in their clinical and virologic features [5–8]. In addition, geography-specific distributions of HBV genotypes have been demonstrated among areas and countries in world wide [5,7,9]. In south-east Asian countries, such as Japan, Taiwan or China, HBV/B and HBV/C are most prevalent [6,7,9]. Patients with HBV/B tend to have anti-HBe at a younger age and less chance to develop hepatocellular carcinoma compared to those with HBV/C [6–8,10].

Recently, we have demonstrated that HBV/B strains should be divided into two subtypes, HBV/Ba and HBV/Bj, according to their genetic relationship, and that HBV/Ba is found ubiquitously in Asian countries, while HBV/Bj is found only in Japan [11,12]. We also demonstrated that a higher proportion of patients with HBV/Ba have a HBeAg-positive status and hepatocellular carcinoma at a younger age than those with HBV/Bj [12,13].

Lamivudine is one of the widely available anti-virus drugs for patients with chronic HBV infection, which strongly suppress the reverse transcriptase of HBV. However, some patients develop unsatisfactory anti-viral effects during therapy because of the emergence of lamivudine-resistant strains during therapy [14,15]. It is still controversial whether the efficacy of lamivudine therapy may be associated with the difference of HBV genotypes. Therefore, the aim of this study was to investigate the response to lamivudine therapy for 2 years among patients with different genotypes of HBV/Ba, HBV/Bj or HBV/C.

2. Patients and methods

2.1. Patient selection

One hundred and twenty-two patients with chronic HBV infection were treated with lamivudine, 100 mg daily, for 2 years in various hospitals in Japan and Hong Kong. All patients were consecutively enrolled in this study, and informed consent was obtained. The patients with normal ALT levels (40 IU/L or less) before commencement of the treatment were excluded. All patients were positive for HBsAg for more than 6 months and negative for both anti-HCV and anti-HIV. All patients were tested by ultrasonography or CT scan to rule out decompensated cirrhosis or hepatocellular carcinoma. For the comparison of the response to lamivudine therapy among different genotypes, 18 patients with HBV/Bj, 15 patients with HBV/Ba and 34 patients with HBV/C were selected and matched according to gender and age for a case–control study. Liver biopsy was performed in the 45 patients who agreed.

For the HBeAg-positive patients, a complete response to lamivudine therapy was defined as the normalization of ALT, undetectable serum HBV DNA by the real-time PCR method, and seroconversion from HBeAg to anti-HBe at year

2 during therapy. For all the patients, including both the HBeAg-positive and -negative groups, a favorable response to lamivudine therapy was defined as the normalization of ALT and undetectable serum HBV DNA at year 2 during therapy.

Lamivudine resistance was defined as the emergence of the YMDD motif mutation (YIDD and/or YVDD) at year 2. Breakthrough hepatitis was designated when emergence of the YMDD mutation and re-elevation of ALT level and HBV DNA level were found.

2.2. Virologic assays

HBsAg, HBeAg and anti-HBe were tested by chemiluminescence enzyme immunoassay (CLEIA). The serum HBV DNA level in all the samples was quantified by the real-time PCR method (HBV RTD-Direct test, SRL Inc., Tokyo) in Nagoya City University. The detection limit of this assay was 100 copies/mL. The HBV genotype was determined by restriction fragment length polymorphism as described previously [16]. When the test results were inconclusive, the sequences of the S region were determined directly, then the genotype was decided by phylogenetic analysis [16,17]. The subtypes of HBV/Ba and HBV/Bj were also determined by restriction fragment length polymorphism [11]. The YMDD motif mutations (M204I/V) during lamivudine therapy were detected by the direct sequencing method after amplification by PCR. The Pre-C mutation at nucleotide position 1896 and the basal core promoter double mutations at nucleotide positions 1762 and 1764 were detected using the direct sequencing method [18].

2.3. Statistical analysis

The data were statistically analyzed by Student's *t*-test, the non-parametric Mann–Whitney test and the Chi-square test where appropriate. The multivariate analysis was accomplished by multiple logistic regression analysis. A *p*-value less than 0.05 was regarded as statistically significant.

3. Results

3.1. Baseline differences among the three HBV genotype groups

In the various baseline clinical and virologic factors, there were significant differences among the HBV/Bj, HBV/Ba and HBV/C groups, while age and gender were matched (Table 1). In the HBV/C group, a higher proportion of the patients at the F3 or F4 stage were found compared to the HBV/Bj or HBV/Ba group. The mean ALT level before treatment in the HBV/Ba group was significantly lower than the HBV/Bj or HBV/C group. Only 3% of the HBV/Bj group showed positive HBeAg, compared to 80.0% of the HBV/Ba group and 79.4% of the HBV/C group. The mean serum HBV

Table 1
Baseline clinical and virologic characteristics of the patients studied

Characteristics	HBV genotype			p-Value
	HBV/Bj (n = 18)	HBV/Ba (n = 15)	HBV/C (n = 34)	
Gender (M:F)	18:0	15:0	34:0	Matched
Age (year, mean \pm S.D.)	43.4 \pm 11.4	40.5 \pm 15.2	43.2 \pm 8.9	Matched
Ethnic (Japanese:Chinese)	18:0	5:10	24:10	<0.01
Stage (F1:F2:F3:F4:ND)	10:0:2:0:6	4:1:1:0:9	6:6:7:8:7	<0.01
ALT (IU/L, mean \pm S.D.)	293.3 \pm 353.0	152.5 \pm 130.8	309.9 \pm 288.3	<0.05
Positive for HBeAg	3 (16.7%)	12 (80.0%)	27 (79.4%)	<0.01
HBV DNA level (log copies/mL, mean \pm S.D.)	5.96 \pm 1.69	8.17 \pm 0.81	7.40 \pm 1.09	<0.01
Pre-C (nt. 1896) mutation (wild:mutant)	4:14	10:5	26:8	<0.01
Core promoter (nt. 1762/1764) mutations (wild:mutant)	16:2	13:2	5:27 (ND:2)	<0.01

DNA level of the HBV/Bj group was significantly lower than the HBV/Ba or HBV/C group. A higher proportion of the HBV/Bj group had pre-C mutation compared to the HBV/Ba or HBV/C group. In contrast, a higher proportion of the HBV/C group had the core promoter mutations compared to the HBV/Bj or HBV/Ba group.

3.2. Response to lamivudine therapy

Of all the 67 patients, 44.8% showed a favorable response to lamivudine therapy (Fig. 1a). 61.1% of the HBV/Bj group,

40.0% of the HBV/Ba group and 38.2% of the HBV/C group showed a favorable response to the therapy, respectively. However, there was no significant difference in the favorable response rate among the different HBV genotype groups.

When the patients were stratified into the HBeAg-positive or -negative group, 33.3% showed complete response in the patients with positive HBeAg, compared to 60.0% in the negative HBeAg group ($p < 0.05$; Fig. 1b). There was no significant difference in the favorable response rate among the three HBV genotype groups, even if the

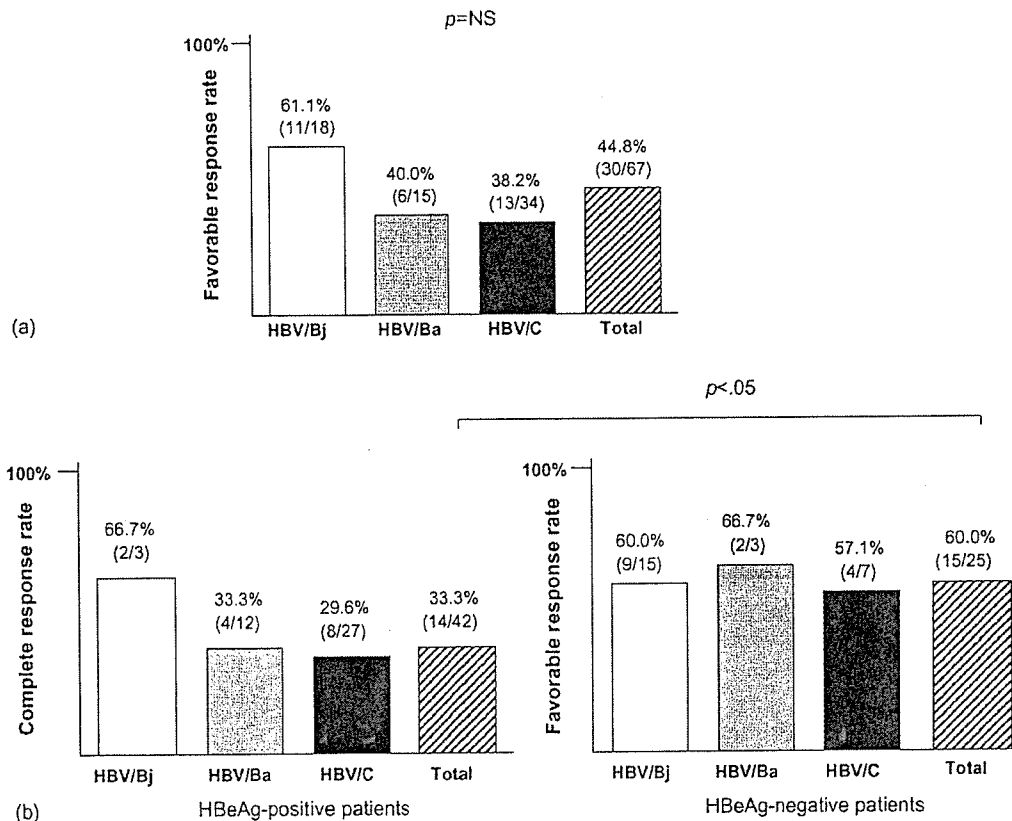


Fig. 1. The rate of favorable responses to lamivudine therapy in both HBeAg-positive and -negative patients among three different genotype groups (a) and the rate of complete response in the HBeAg-positive patients and the rate of favorable response in the HBeAg-negative patients (b).

Table 2
Complete response rate in HBeAg-positive patients with HBV/Ba and HBV/C

Characteristics	HBV genotype		p-Value
	HBV/Ba (n=12)	HBV/C (n=27)	
Age (years)			
<40	4/8 (50.0)	2/13 (15.4)	0.22
≥40	0/4 (0)	6/14 (42.9)	0.31
ALT (IU/L)			
<200	1/9 (11.1)*	1/9 (11.1)	0.45
≥200	3/3 (100)*	7/18 (38.9)	0.18
HBV DNA level (log copies/mL)			
<7.5	1/1 (100)	2/10 (20.0)	0.59
≥7.5	3/11 (27.3)	6/17 (35.3)	0.97
Pre-C (nt. 1896) mutation			
Wild	2/9 (22.2)	6/25 (24.9)*	0.72
Mutant	2/3 (66.7)	2/2 (100)*	0.81
Core promoter (nt. 1762/1764) mutations			
Wild	4/12 (33.3)	1/2 (50.0)	0.73
Mutant	0/0 (0)	7/23 (30.4)	–

Values in parenthesis are given in percentage.

* $p < 0.05$.

patients were stratified into HBeAg-positive or -negative groups.

In the patients with positive HBeAg, pretreatment factors associated with complete response were analyzed in 12 patients in the HBV/Ba group and 27 in the HBV/C group (Table 2). Between the two genotype groups, there were no significant differences of the factors predicting complete response to the therapy in age, ALT level, HBV DNA level, pre-C mutation and core promoter mutations. However, in the HBV/Ba group, the patients who had 200 IU/L or higher ALT levels showed complete response more frequently than those with less than 200 IU/L. In the HBV/C group, a higher proportion of the patients with pre-C mutation tended to have a complete response compared to those with wild pre-C.

Of the patients with negative HBeAg, 15 in the HBV/Bj group and 7 in the HBV/C group, there were no significant differences in the predicting factors for a favorable response to the therapy between the HBV/Bj and HBV/C groups (Table 3).

To investigate the significance of predicting factors associated with complete response to lamivudine therapy in HBeAg-positive patients, stepwise logistic regression analysis was performed (Table 4). A factor of the pretreatment ALT level of 200 IU/L or higher and pre-C mutation were significant factors (odds ratio: 12.056, $p = 0.03$ and 25.553, $p = 0.03$, respectively).

In all the patients with both positive and negative HBeAg, the significant factors predicting a favorable response to the therapy were a pretreatment ALT level of 200 IU/L or higher (odds ratio: 3.715, $p = 0.02$) and a negative HBeAg status (odds ratio: 3.472, $p = 0.03$; Table 5). The other factors were not significant.

Table 3
Favorable response rate in HBeAg-negative patients with HBV/Bj and HBV/C

Characteristics	HBV genotype		p-Value
	HBV/Bj (n=15)	HBV/C (n=7)	
Age (years)			
<40	1/2 (50.0)	1/1 (100)	0.67
≥40	8/13 (61.5)	3/6 (50.0)	0.98
ALT (IU/L)			
<200	4/8 (50.0)	1/3 (33.3)	0.85
≥200	5/7 (71.4)	3/4 (75.0)	0.56
HBV DNA level (log copies/mL)			
<7.5	7/13 (53.8)	1/4 (25.0)	0.66
≥7.5	2/2 (100)	3/3 (100)	–
Pre-C (nt. 1896) mutation			
Wild	2/9 (22.2)	1/1 (100)	0.65
Mutant	7/13 (53.8)	3/6 (50.0)	0.74
Core promoter (nt. 1762/1764) mutations			
Wild	7/13 (53.8)	2/3 (66.7)	0.81
Mutant	2/2 (100)	2/4 (50.0)	0.76

Values in parenthesis are given in percentage.

Table 4
Significant factors associated with complete response to lamivudine therapy in HBeAg-positive patients by the stepwise logistic regression analysis

Factors	Odds ratio	95% CI	p-Value
ALT level (IU/L)			
<200	1		
≥200	12.056	1.322–109.907	0.03
Pre-C (nt. 1896) mutation			
Wild	1		
Mutant	25.553	1.391–469.526	0.03

3.3. Emergence of YMDD mutation

Of all the patients, 35.8% emerged the YMDD mutation at year 2 during therapy (Fig. 2a). In the HBV/C group, 50.0% had the YMDD mutation compared to 27.8% in the HBV/Bj and 13.3% in the HBV/Ba group ($p < 0.05$, respectively). When the patients were stratified into positive or negative HBeAg status, 51.9% of the HBV/C group with positive HBeAg emerged the YMDD mutation compared to 8.3% of the HBV/Ba group with positive HBeAg, even though HBeAg positivity and HBV DNA levels at baseline

Table 5
Significant factors associated with favorable response to lamivudine therapy in both HBeAg-positive and -negative patients by the logistic regression analysis

Factors	Odds ratio	95% CI	p-Value
ALT level (IU/L)			
<200	1		
≥200	3.715	1.269–10.875	0.02
HBeAg status			
Positive	1		
Negative	3.472	1.156–10.417	0.03

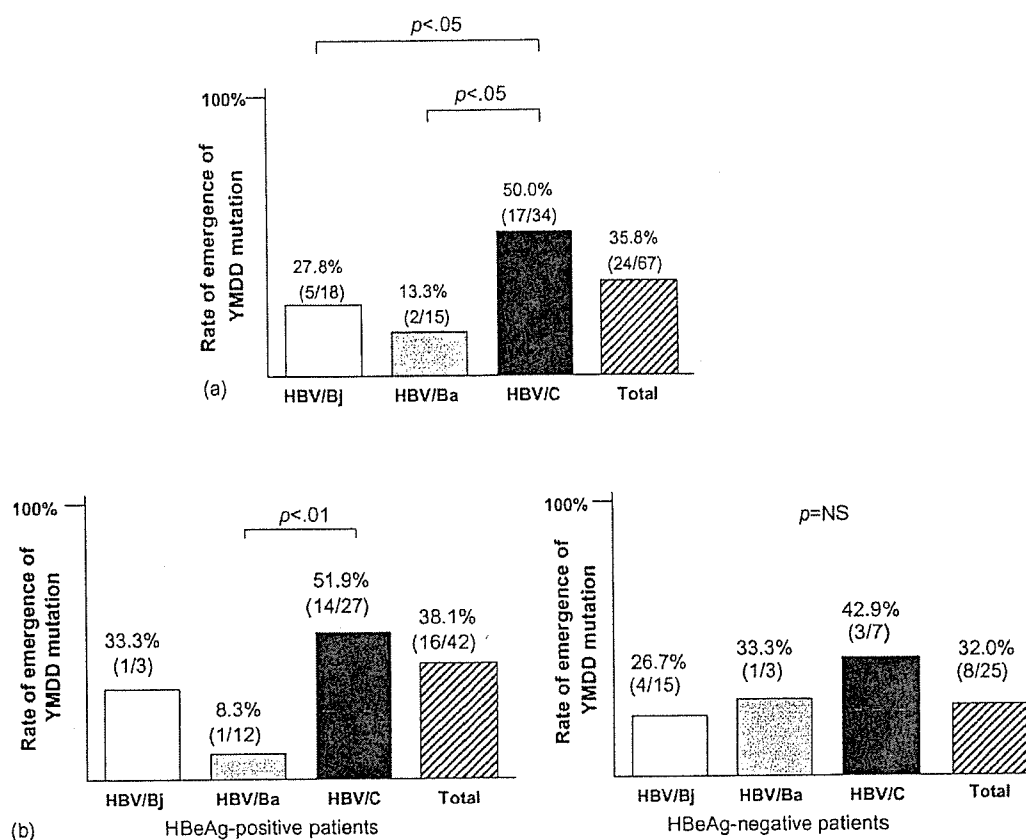


Fig. 2. The emergence rate of the YMDD mutation among three different genotype groups (a) and the emergence rate of the YMDD mutation stratified into HBeAg-positive and -negative patients (b).

were almost same between the HBV/Ba and HBV/C groups ($p < 0.01$; Fig. 2b). There was a trend for a higher proportion of the HBV/C group to have the YMDD mutation in the patients with negative HBeAg. However, the statistical differences were obscure because the numbers of the patients were small when they were stratified into HBeAg-positive or -negative groups.

The pretreatment factors predicting the emergence of the YMDD mutation were analyzed among three HBV genotype groups (Table 6). In the patients aged less than 40 years, 57.1% in the HBV/C group emerged the YMDD mutation compared to 12.5% in the HBV/Ba group ($p < 0.05$). In the patients with wild pre-C, the YMDD mutation emerged more frequently in the HBV/C group than in the HBV/Ba group ($p < 0.05$). In the patients with 7.5 or more log copies/mL of the HBV DNA level, the YMDD mutation emerged more frequently in the HBV/C group than in the HBV/Ba group ($p < 0.05$). Stratified in the positive HBeAg group, 58.9% in the HBV/C group with 7.5 or more log copies/mL of the HBV DNA level emerged the YMDD mutation compared to 40.0% in the HBV/C group with less than 7.5 log copies/mL of the HBV DNA level. However, in the HBV/Ba group with positive HBeAg, there was no difference of the frequency of the YMDD mutation between the higher or the lower HBV DNA groups.

The significant factor predicting emergence of the YMDD mutation by the stepwise logistic regression analysis was HBV/C, compared to HBV/Bj or HBV/Ba (odds ratio: 3.714, $p = 0.02$; Table 7). The other factors such as HBeAg status, HBV DNA levels and ALT levels at baseline were not significant.

3.4. Breakthrough hepatitis

Of all the patients, 11.9% had breakthrough hepatitis at year 2 during therapy (Fig. 3). There was no significant difference in the frequency of breakthrough hepatitis among the three HBV genotype groups.

4. Discussion

It is well-known that the clinical and virologic manifestations of patients with chronic HBV infection depend upon their HBV genotypes [5–8]. However, it is still controversial whether the response or resistance to lamivudine therapy is associated with HBV genotypes. To analyze the relationship between the response or resistance to lamivudine therapy and pretreatment factors, for the first time we conducted an age and gender matched case-control study among Japanese and

Table 6
Emergence of the YMDD mutation in patients among three HBV genotype groups

Characteristics	HBV genotype			p
	HBV/Bj (n=18)	HBV/Ba (n=15)	HBV/C (n=34)	
Age (year)				
<40	2/5 (40.0%)	1/8 (12.5%) *	8/14 (57.1%)	<.05
≥40	3/13 (23.1%)	1/7 (14.3%)	9/20 (45.0%)	.22
ALT				
<200	3/11 (27.3%)	2/11 (18.2%) *	8/12 (66.7%)	<.05
≥200	2/7 (28.6%)	0/4 (0%)	9/22 (40.9%)	.26
HBV DNA level (log copies/mL)				
<7.5	4/15 (26.7%)	1/2 (50.0%)	7/14 (50.0%)	.41
≥7.5	1/3 (33.3%)	1/13 (7.7%) *	10/20 (50.0%)	<.01
Pre-C (nt.1896) mutation				
Wild	0/4 (0%)	1/10 (10.0%) *	14/26 (53.8%)	<.05
Mutant	5/14 (35.7%)	1/5 (20.0%)	3/8 (37.5%)	.77
Core promoter (nt.1762/1764) mutations				
Wild	5/16 (31.3%)	1/13 (7.7%)	1/5 (20.0%)	.30
Mutant	0/2 (0%)	1/2 (50.0%)	15/27 (55.6%)	.31

* $p < 0.05$

Table 7
Significant factors associated with emergence of the YMDD mutation by the stepwise logistic regression analysis

Factors	Odds ratio	95% CI	p-Value
HBV genotype			
Bj or Ba	1		
C	3.714	1.272–10.847	0.02

Chinese patients with HBV genotypes Bj, Ba and C. In this study, it was indicated that the significant factors predicting a favorable response to lamivudine therapy do not include the HBV genotype, but a higher pretreatment ALT level or negative HBeAg status and that the significant factor predicting emergence of the YMDD mutation is the HBV genotype (HBV/C).

The natural course of chronic HBV infection is usually affected by age and gender [19]. The natural seroconversion from positive HBeAg to anti-HBe is sometimes observed

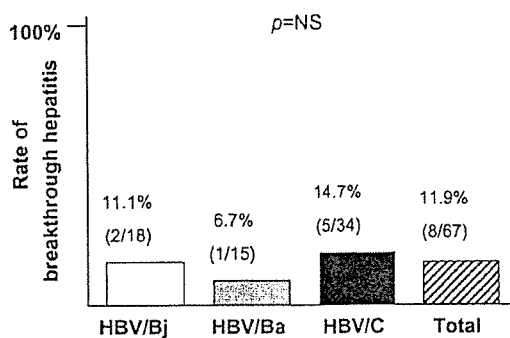


Fig. 3. The rate of breakthrough hepatitis among the three different genotype groups.

with the normalization of ALT in younger adults [8,10]. In addition, females often experience seroconversion after delivery [20]. So, age and sex are major factors influencing the natural course of chronic HBV infection. Thus, this report is a first case–control study to investigate the relationship between HBV genotype and response to lamivudine therapy.

In all the patients, there was no significant difference of the rate of favorable response to the therapy among the three HBV genotype groups, even if the patients were stratified into an HBeAg-positive or -negative status. Although this study was an age and gender matched case–control study, the frequency of positive HBeAg was very low in the HBV/Bj group compared with the HBV/Ba or HBV/C groups because a higher proportion of patients with HBV/Bj have negative HBeAg status compared to those with HBV/Ba or HBV/C, as previously reported [12]. So, to investigate the relationship of the HBV genotype with the response to the therapy, we also compared the positive HBeAg patients between the HBV/Ba and HBV/C group. There was no significant difference in the response to the therapy between two groups. In addition, in the patients with negative HBeAg, there was also no significant difference of response to the therapy between the HBV/Bj and HBV/C group. The multiple logistic regression analysis revealed that a higher pretreatment ALT level and pre-C mutation were significant factors predicting complete response in the patients with positive HBeAg, and that higher ALT level and negative HBeAg status were significant factors predicting favorable response in all the patients.

Recently, some papers have reported the relationship between the HBV genotype and the response to lamivudine therapy, while the other paper denied this relationship [21–27]. Therefore, it is still controversial whether the

HBV genotype is associated with the response to lamivudine therapy for more than 1 year. Thus, we conducted a case–control study which revealed no association of HBV genotype with response to therapy. However, there remained to be a significant bias in the baseline features, such as a positive rate of HBeAg. It is ideal that age, gender and HBeAg status are matched. However, there are very few adult patients with HBV/Bj who are positive HBeAg and still have indication for lamivudine therapy, since those patients with HBV/Bj tend to experience seroconversion at a younger age. So, each paper might conduct various results in different backgrounds.

A higher pretreatment ALT level was one of the important predictors for a complete response to lamivudine therapy [28,29]. In this study, an ALT level greater than 200 IU/L and the pre-C mutation were significant factors in the patients with positive HBeAg. It was considered that the pre-C mutation is associated with the induction of a negative HBeAg status [30]. In all the patients, a higher ALT level and a negative HBeAg status were predicting factors for a favorable response, as previously reported [31,32].

It was reported previously that higher HBV DNA level or positive HBeAg status were significant predicting factors for the emergence of the YMDD mutation among the consecutive patients with HBV genotypes A–C in the cross-section studies [22,23]. In general, a higher proportion of Japanese patients with HBV/C tend to have a higher HBV DNA level and a positive HBeAg status which may lead a trend of emergence of the YMDD mutation [22,29,32]. In this study, there was also a trend of higher frequency of emergence of the YMDD mutation in the HBV/C group with positive HBeAg and a higher HBV DNA level. However, the significant predicting factor of emergence of the YMDD mutation was HBV genotype (HBV/C) by the multivariate analysis in the present study. It is still unclear why the viral factors, such as genotype, HBV DNA level or HBeAg status, may affect the chance of the emergence of the YMDD mutation. Further *in vitro* or *in vivo* studies are warranted.

In this study, the number of patients (only eight) who developed breakthrough hepatitis was too small to investigate the factors affecting breakthrough hepatitis. If we were to observe this cohort of patients for a longer term, we may investigate more information, such as the L180M mutation or other mutations of the HBV genome [33].

The factors associated with a favorable response to lamivudine therapy or the emergence of the YMDD mutation may vary according to the baseline features of the patients studied. In this case–control study, a higher ALT level and negative HBeAg status were significant factors predicting a favorable response to lamivudine therapy, and HBV genotype C was a significant factor predicting the emergence of the YMDD mutation.

However, the patient numbers of this case–control study were not enough to confirm these results. Thus, further studies are warranted with a large number of the patients in each HBV genotype group.

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Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan

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Abstract

Genotypes of hepatitis B virus (HBV) were determined in 485 patients with acute hepatitis B from all over Japan. They were A in 92 (19%), Ba in 26 (5%), Bj in 32 (7%), C in 330 (68%) and D in 5 (1%). Sexual contacts were the main route of transmission in them. Overall,

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HBV persisted in only 5 of the 464 (1%) followed patients. Genotypes C accounted for more than 68% in northern as well as southern areas, contrasting with genotype A accounting for 34% in and around the Metropolitan areas. During 24 years from 1982 to 2005, genotype A increased from 5% to 33%, while genotype B gradually decreased from 26% to 8%. Fulminant hepatitis was significantly more frequent in infection with genotype B_j (41%) than those with the other genotypes ($p < 0.01$). The core-promoter double mutation (T1762/A1764) and precore stop-codon mutation (A1896) were more frequent in patients with fulminant than acute self-limited hepatitis (57% versus 15% and 58% versus 10%, respectively, $p < 0.01$ for both). In conclusion, genotype A distributes unevenly over Japan, prevails in younger patients through sexual transmission and has increased with years. Furthermore, fulminant outcome was more frequent in patients with genotype B_j than those with the other genotypes.

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Keywords: Chronic hepatitis; Fulminant hepatitis; Hepatitis B e antigen; Hepatitis B surface antigen; Sexual transmission

1. Introduction

Mass vaccination with plasma-derived or recombinant vaccines has been effective in Asian countries hyperendemic with hepatitis B virus (HBV), as well as in the United States and France. In Japan, perinatal transmission of HBV from mothers with hepatitis B surface antigen (HBsAg) and hepatitis B e antigen (HBeAg) in serum used to be the principal route to establish the persistent carrier state [1]. Hence, passive and active immunoprophylaxis of babies born to carrier mothers with HBeAg by combined hepatitis B immunoglobulin and vaccine was mandated since 1986, and extended to carrier mothers without HBeAg in 1995. As the results, HBsAg has become rare in the Japanese born after 1986, and is detected in merely 0.3% of the first-time blood donors younger than 19 years at present [2].

There is an increasing trend, however, for acute HBV infection to occur preferentially in young men through promiscuous sexual contacts [3–7]. Foreign sexual workers from neighboring Asian countries are suspected as significant sources of de novo HBV infection in them [4,8]. Thus, patients with acute hepatitis visiting hospitals are increasing all over Japan. Since the majority of acute HBV infections ran subclinical courses, hospitalized cases of acute hepatitis B would represent the tip of an iceberg.

Eight genotypes have been detected by the sequence divergence >8% in the entire HBV genome composed of approximately 3200 nucleotides (nt), and designated by capital alphabet letters from A to H in the order of documentation [9–12]. They have distinct geographical distribution and are associated with severity of liver disease as well as response to antiviral therapies [13–15]. Furthermore, subgenotypes have been reported for HBV/A, B and C, and named Aa (Asian/African type) and Ae (European type) [16], B_j (Japanese type) and Ba (Asian type) [17], as well as Ce (east Asian type) and Cs (southeast Asian type) [18]. There have been increasing lines of evidence that Aa and Ae, as well as Ba and B_j, influence the replication of HBV and bear clinical relevance [19–21].

Over 24 years from 1982 to 2005, a multicenter study was conducted throughout Japan on 547 patients with acute hepatitis B. Genotypes were determined on viral isolates recovered from them, and analyzed for distributions changing with time and in place. The results highlighted foreign HBV genotypes represented by HBV/A which have increased

through sexual contacts, and HBV/B_j prevalent in patients with fulminant hepatitis.

2. Patients and methods

2.1. Patients with acute hepatitis B

During 1982 through 2005, 547 patients with acute hepatitis B were registered in 25 hospitals throughout Japan, of them, 147 and 336 cases are overlapping with previous report from Yotsuyanagi et al. [7] and Ozasa et al. [22], respectively, and 64 cases were newly registered in this study. These hospitals were grouped into the following eight areas: Hokkaido, Tohoku, Kanto, Koshin, Tokai, Kinki, Chugoku/Shikoku and Kyushu/Okinawa. The diagnosis of acute hepatitis B was contingent on a sudden onset of clinical symptoms of hepatitis and detection of high-titered antibody to hepatitis B core antigen (anti-HBc) of IgM class in serum. The great majority of them were followed for clinical outcomes until the disappearance of HBsAg through 24 weeks or longer after the presentation. HBV genotypes were determined in sera stored at -40°C , and their geographical distributions and chronological changes were analyzed. Further, they were correlated with sources of infection and clinical outcomes of acute hepatitis. The study protocol conformed to the 1975 declaration of Helsinki, and was approved by Ethics Committees of institutions. Every patient or his/her next of kin gave an informed consent on the purpose of this study.

2.2. Serological markers of HBV infection

HBsAg was determined by hemagglutination (MyCell, Institute of Immunology Co. Ltd., Tokyo, Japan) or enzyme-linked immunosorbent assay (ELISA) (AxSYM, Abbott Japan, Tokyo, Japan), and HBeAg by ELISA (ELISA, F-HBe, Kokusai Diagnostic, Kobe, Japan). Anti-HBc of IgM class was determined by ELISA (HBc-antiM RIA, Dainabot, Tokyo, Japan).

2.3. Genotypes and subgenotypes of HBV

The six major HBV genotypes (A–F) were determined serologically by ELISA using commercial kits (HBV GENO-

TYPE EIA, Institute of Immunology). The method depends on the combination of epitopes on preS2-region products detected by monoclonal antibodies that is specific for each of them [23,24]. Genotypes were confirmed by restriction fragment length polymorphism (RFLP) when required [25].

HBV/Bj (Japanese type) without the recombination with genotype C over the precore region and the core gene and Ba (Asian type) with the recombination were determined by its absence or presence on HBV DNA sequences, as well as RFLP involving on specific nucleotide substitutions, by the method described previously [26].

2.4. Point mutations in the precore region and basic core-promoter (BCP)

Mutations in the precore region for A1896 and BCP for T1762/A1764 were detected by enzyme-linked minisequence assay (Smitest HBV Pre-CELMA, Roche Diagnostic, Tokyo, Japan) according to the manufacturer's instructions, or by sequencing the precore region and BCP using the method described previously [27]. The results were recorded as "the wild-type" or "the mutant-type" expressed dominantly by HBV isolates.

2.5. Statistical analysis

Categorical variables were compared between groups by the χ^2 -test or Fisher's exact test, and non-categorical variables by the Mann-Whitney's *U*-test. A *p*-value less than 0.05 was considered significant.

3. Results

3.1. Clinical profiles of patients with acute hepatitis B

During 1982 through 2005, 547 patients with acute hepatitis B were registered in 25 hospitals from all over Japan. Genotypes of HBV were unclassifiable in 40 (7%) and sufficient clinical data not available in 22 (4%) of them. Exclusive of these 62 patients, 485 (89%) were left for the evaluation of geographic distribution of HBV genotypes, as well as their changes with time, transmission routes and relevance with clinical outcomes.

The 485 patients with acute hepatitis B had the mean \pm S.D. age of 35.7 ± 13.7 years, and included 338 (70%) men. Their peak alanine aminotransferase (ALT) averaged 2576 ± 1673 IU/L and peak total bilirubin 9.5 ± 9.5 mg/dL. They all possessed anti-HBc of IgM class in high titers, and HBeAg was detected in sera from 338 (70%) of them at the presentation. Fulminant hepatitis with coma of grade >II and prothrombin time <40% developed within 8 weeks after the onset in 45 (9%) of them.

Fulminant hepatitis led to death in 18 (5%) patients, and 3 (1%) received liver transplantation. Exclusive of these 21

patients, HBV persisted in only 5 of the 464 (1%) studied patients with acute hepatitis. They represented 3 of the 92 (3%) infected with HBV/A, 1 of the 58 (2%) with B and 1 of the 330 (0.3%) with C. None of the five patients with chronic outcome had received antiviral or steroid treatment during their acute phase of illness.

3.2. Geographic distribution of HBV genotypes in patients with acute hepatitis B

Overall, HBV/A was detected in 92 (19%), Ba in 26 (5%), Bj in 32 (7%), C in 330 (68%) and D in 5 (1%). Distribution of HBV genotypes over Japan is illustrated in Fig. 1. They dispersed unevenly in place. HBV/C accounted for more than 68% in both northern and southern areas, contrasting with HBV/A accounting for 34% in and around the Metropolitan area. Among HBV/B infections, the proportion of HBV/Ba was higher in Koshin, Kinki and Kyushu/Okinawa (14/17, 1/1 and 2/3), while that of HBV/Bj was higher in Hokkaido, Tohoku, Kanto and Tokai (4/4, 6/7, 13/21 and 5/5).

3.3. Demographic and clinical differences of patients infected with various genotypes

Table 1 compares demographic and clinical characteristics of patients with different HBV genotypes. Patients with HBV/D were excluded from the analysis due to their small numbers. The mean age was lower in patients with HBV/A (31.8 ± 10.9 years) than HBV/Ba (40.7 ± 10.9 , $p < 0.01$), HBV/Bj (41.2 ± 17.0 , $p = 0.01$) and HBV/C (35.8 ± 13.9 , $p < 0.03$); it was higher in patients with HBV/Ba than HBV/C (40.7 ± 10.9 versus 35.8 ± 13.9 , $p = 0.02$). The proportion of patients aged <30 years was significantly greater in HBV/A (58%) than HBV/Ba (12%, $p < 0.01$), HBV/Bj (38%, $p < 0.04$) or HBV/C infection (42%, $p < 0.01$). Men predominated ($p < 0.01$) in infections with HBV/A and HBV/Ba (92% and 88%, respectively) than those with HBV/Bj and HBV/C (56% and 64%, respectively). The peak ALT level was higher in HBV/Bj (3371 ± 2342 IU/L) than HBV/A (2051 ± 1009 , $p = 0.04$) or HBV/C (2650 ± 1747 , $p < 0.03$) infection. HBeAg was detected in 84% of patients with HBV/A at the frequency much higher than that in those with HBV/Ba (54%, $p < 0.01$), HBV/Bj (59%, $p < 0.01$) or HBV/C (60%, $p < 0.01$).

The routes of transmission were sexual contacts in 216 (45%) patients, followed by medical accidents in 14 (8%), blood transfusion in 4 (1%) and drug in 1 (0.2%); transmission routes were not identified in the remaining 245 (51%) patients. Sexual transmission was the most frequent cause of infection in 57% of HBV/A, 73% of HBV/Ba, 34% of HBV/Bj and 40% of HBV/C infections.

Fulminant hepatitis was significantly more frequent in patients infected with HBV/Bj (41%) than the other genotypes ($p < 0.01$); it occurred in 2 of the 5 (40%) patients with HBV/D, also. In reflection of severe clinical courses, the peak ALT level tended to be high in patients with HBV/Bj.

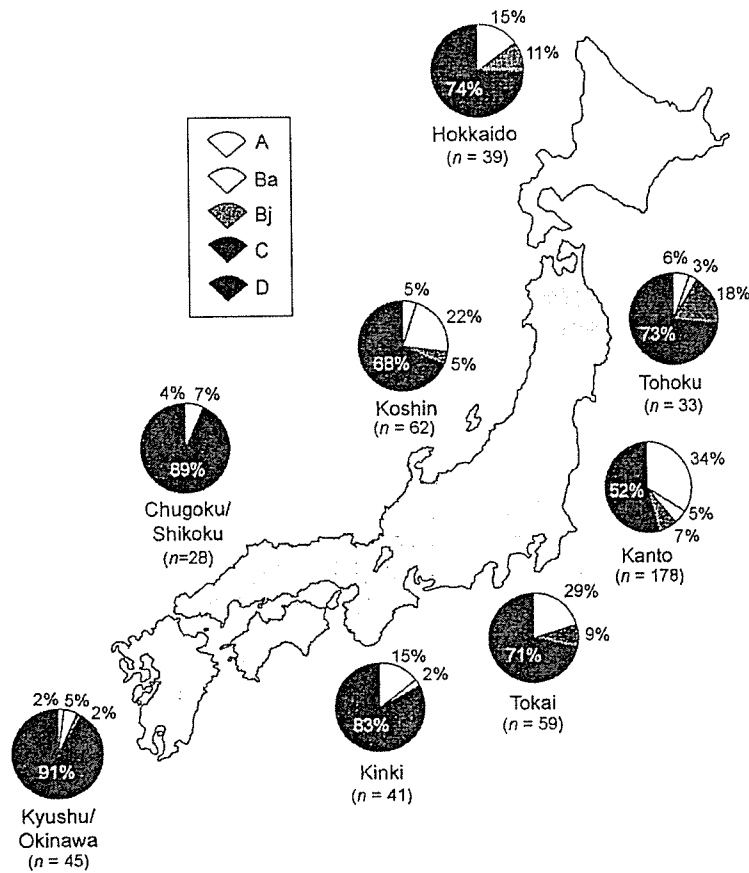


Fig. 1. Geographic distribution of HBV genotypes among 485 patients with acute hepatitis in Japan during 1982–2005.

The core-promoter double mutation (A1762T/G1764A) was more frequent in patients with fulminant than acute self-limited hepatitis infected either with HBV/Bj (70% versus 0%, $p < 0.01$) or HBV/C (50% versus 18%, $p < 0.01$). Also, precore stop-codon mutation (G1896A) was more often in patients with fulminant than acute self-limited hepatitis who were infected with HBV/Ba (100% versus 5%, $p = 0.01$), HBV/Bj (60% versus 0%, $p < 0.01$) or HBV/C (50% versus 14%, $p < 0.01$).

3.4. Changes in the distribution of HBV genotype with the age and time

Fig. 2 depicts the distribution of HBV genotypes stratified by the age. Prevalence of HBV/A decreased with the age, and was higher in the patients aged <30 years (26%) than in the 40s (14%, $p = 0.03$) or aged ≥ 50 years (8%, $p < 0.01$). Prevalence of HBV/B increased with the age, in converse, and was lower in the patients aged <30 years (7%) than in the 40s (18%, $p = 0.01$) or aged ≥ 50 years (17%, $p = 0.02$). No significant differences were observed in the distribution of HBV/Ba or HBV/Bj among all age groups.

Fig. 3 illustrates changes in HBV genotypes through the four time spans covering 24 years. HBV/A accounted

for 5% (2/42) in 1982–1990, 5% (4/78) in 1991–1995 and 14% (18/133) in 1996–2000, and thereafter increased to 33% (58/176) in 2001–2005. There was significant difference between 1982–1995 and 1996–2005 ($p < 0.01$). HBV/B accounted for 26% (11/42) in 1982–1990, 17% (13/78) in

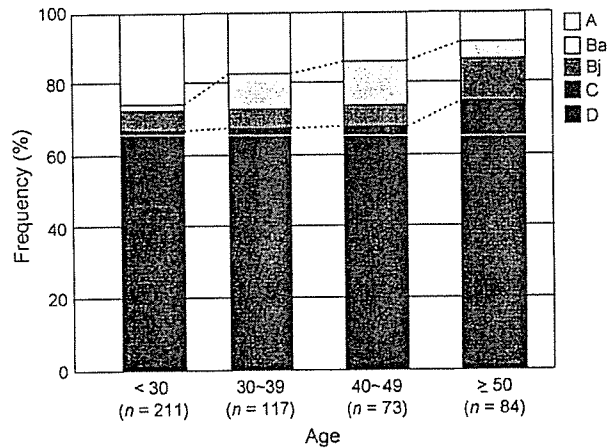


Fig. 2. Age-specific prevalence of HBV genotypes among 485 patients with acute hepatitis.

Table 1
Demographic and clinical differences among patients infected with HBV of distinct genotypes

	Genotypes			
	A (n=92)	Ba (n=26)	Bj (n=32)	C (n=330)
Mean age (years)	31.8 ± 10.9 ^a	40.7 ± 10.9 ^b	41.2 ± 17.0	35.8 ± 13.9
Age <30 years	54 (58%) ^c	3 (12%) ^d	12 (38%)	140 (42%)
Male	85 (92%) ^e	23 (88%) ^f	18 (56%)	210 (64%)
Peak ALT (IU/L)	2051 ± 1009 ^g	2536 ± 1104	3371 ± 2342 ^h	2650 ± 1747
Peak total				
Bilirubin (mg/dL)	10.3 ± 10.4	8.9 ± 5.8	10.5 ± 8.1	9.3 ± 9.7
HBeAg positive	78 (84%) ⁱ	14 (54%)	19 (59%)	199 (60%)
Transmission routes				
Sexual	52 (57%)	19 (73%)	11 (34%)	134 (40%)
Medical accident	0	0	3 (9%)	11 (3%)
Drug/tattoo	0	0	0	1 (1%)
Blood transfusion	0	0	1 (4%)	3 (1%)
Unknown	40 (43%)	7 (23%)	17 (53%)	181 (55%)
Metropolitan areas	60 (65%) ^j	8 (31%)	13 (41%)	93 (28%)
Fulminant hepatitis	1 (1%)	2 (8%)	13 (41%) ^j	29 (9%)
Mutations in HBV DNA				
BCP (1762T/1764A)				
Acute self-limited	2/67 (3%)	4/22 (18%)	0/13 (0%) ^k	41/223 (18%) ^k
Fulminant	0/1 (0%)	0/2 (0%)	7/10 (70%)	10/20 (50%)
Precore (1896A)				
Acute self-limited	1/67 (1%)	1/22 (5%) ^k	0/13 (0%) ^l	31/223 (14%) ^k
Fulminant	1/1 (100%)	2/2 (100%)	6/10 (60%)	10/20 (50%)

$p < 0.01$, acute vs. fulminant.

^a $p < 0.01$, A vs. Ba. $p = 0.01$, A vs. Bj. $p = 0.03$, A vs. C.

^b $p = 0.02$, Ba vs. C.

^c $p < 0.01$, A vs. Ba. $p < 0.04$, A vs. Bj. $p < 0.01$, A vs. C.

^d $p < 0.01$, Ba vs. C. $p < 0.04$, A vs. Bj. $p < 0.01$, A vs. C.

^e $p < 0.001$, A vs. Bj. $p < 0.01$, A vs. C.

^f $p < 0.01$, Ba vs. Bj. $p < 0.01$, Ba vs. C.

^g $p = 0.04$, A vs. Ba. $p < 0.01$, A vs. B1. $p < 0.01$, A vs. C.

^h $p = 0.03$, Bj vs. C.

ⁱ $p < 0.01$, A vs. Ba, Bj or C.

^j $p < 0.01$, Bj vs. A, Ba or C.

^k $p < 0.01$, acute vs. fulminant.

^l $p = 0.01$, acute vs. fulminant.

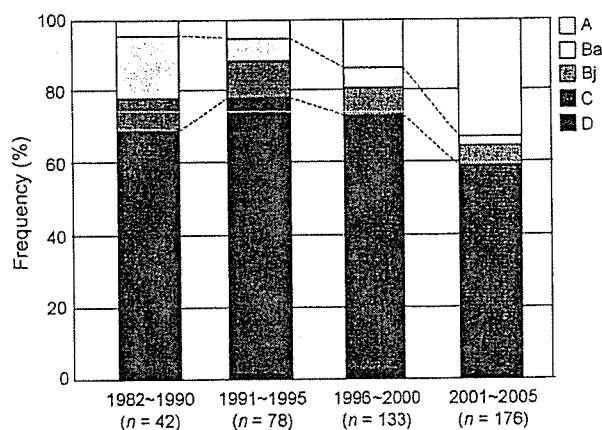


Fig. 3. Chronological changes in the distribution of HBV genotypes among 429 patients with acute hepatitis.

1991–1995 and 14% (18/133) in 1996–2000, and thereafter decreased to 8% (14/176) in 2001–2005; there was significant difference between 1982–1990 and 2001–2005 ($p < 0.01$). However, no significant differences were observed in the distribution of HBV/Ba or HBV/Bj among the four time spans.

4. Discussion

As in most Asian countries, the persistent HBV carrier state had been established mainly through perinatal transmission and horizontal infection during the infancy in Japan, until 1986 when combined active and passive immunoprophylaxis was started in the national program. There still are approximately million HBV carriers estimated by the prevalence of HBsAg in the first-time blood donors [28]; they had been infected with HBV before the prophylaxis started. The age-specific prevalence is high in the elderly, and some of them develop chronic liver disease culminating in cirrhosis and hepatocellular carcinoma. By far the majority of genotypes in

patients with persistent HBV infection are HBV/B (approximately 30%) and HBV/C (about 70%) [4,29]. In patients with acute hepatitis in hospitals in the Metropolitan Tokyo, however, HBV/A was detected in 23% with HBV/Ae prevailing [5], in contrast to HBV/B in 14% and HBV/C in 44% [4]. It is not certain if a high prevalence of HBV/A in acute HBV infection in Tokyo is extrapolated to the other areas in Japan.

The present study was performed on 547 patients with acute hepatitis B visiting 25 hospitals in Japan during 24 years from 1982 to 2005. They include the 147 (27%) patients reported by Yotsunangi et al. [7] and the 336 (61%) by Ozasa et al. [22]; the remaining 64 (12%) patients were recruited anew. Yotsunangi et al. [7] dealt with a peculiar distribution of HBV/A clustering in and around Metropolitan areas and clinical manifestation dependent on genotypes, while Ozasa et al. [22] focused on the influence of HBV genotypes on clinical outcomes, in terms of fulminant hepatitis and viral persistence, as well as the occurrence of precore stop-codon mutation. The assortment of these cohorts of patients have given us the power to precisely picture the epidemiology of genotypes in acute HBV infection all over Japan and shift thereof during the past quarter of century. In addition, it confirmed the results of previous studies on the precore mutations, and extended them to the core-promoter double mutation that can make differences in fulminant or self-limited outcome of acute HBV infection under the influence of HBV genotypes. In the present multicenter study on patients with acute hepatitis B, HBV/C was the most frequent and HBV/A was the second most common in central areas in Japan harboring big cities (Fig. 1). Along with HBV/A, the other foreign genotypes (Ba and D) accounted for 123 of the 485 (25%) acute HBV infections. Furthermore, foreign origins of acute HBV infections would increase further, if overseas origins of HBV/C (Ce and Cs) are examined in the future.

Clinical relevance of HBV genotypes, in terms of severity of liver disease and response to antiviral treatment, has been evaluated mostly in patients with chronic liver disease [27,30,31]. Due to uneven distributions of HBV genotypes over the world, however, comparison is largely restricted to only two genotypes prevailing in each country. All in all, it would be reasonable to state that patients with HBV/A or HBV/B fare better than those with HBV/D or HBV/C [21,27,30–32]. Influence of HBV genotypes on acute hepatitis B is also reported [33]. In the present study, in which patients with acute hepatitis infected with HBV/A, HBV/Ba, HBV/Bj or HBV/C were compared, there were significant differences in age, gender, peak ALT levels, HBeAg positivity and outcome (Table 1). These results indicate that HBV genotypes influence the clinical profile in not only chronic, but also acute hepatitis B.

Fulminant hepatitis developed rather frequently in 45 of the 485 (9%) patients, although it would be overrepresented in them who visited hospitals due to overt liver disease. Remarkably, chances for patients infected with HBV/Bj to develop fulminant hepatitis were significantly higher than those with

HBV/A, HBV/Ba or HBV/C (41% versus 1%, 8% or 9%, $p < 0.01$). These results were consistent with those in the previous study [22]. In patients with acute hepatitis B from Chiba in Japan [34], HBV/B was more frequent in those with fulminant than self-limited hepatitis (63% versus 31%, $p = 0.027$); however, some cases of acute exacerbation of persistent HBV infection were included in their study.

The precore stop-codon mutation (G1896A) was detected significantly more frequently in patients with fulminant than acute self-limited hepatitis, in confirmation of our previous report [22]. Furthermore, the BCP double mutation (A1762T/G1764A) was detected more often in fulminant than acute self-limited hepatitis. In infection with HBV/Bj, in particular, the frequency of BCP mutation was much higher in the patients with fulminant (70%) than that reported in those with chronic hepatitis (16%) [27]. Precore and BCP mutations are very frequent in patients with fulminant hepatitis in Asia [35–37] and the Middle East [38], but rare in Western countries [39–41]. These differences may be explained by distinct geographical distributions of HBV genotypes.

An extremely wide range (0–12%) has been reported in the rate of persistence after acute HBV infection in the adulthood [42–49]. Why such a big difference arises needs to be clarified, because the HBV genotype is implicated in a high chronicity rate in European countries where HBV/A is predominant. In accordance with this view, HBV/A was more frequent than HBV/D in 32 patients with chronic active hepatitis (80% versus 11%), while the reverse was the case in acute hepatitis (10% versus 80%) in Switzerland [33]. In Japan, also, infection with HBV/A tends to prolong longer than that with HBV/B or HBV/C in the adulthood (23% [3/13] versus 13% [1/8] or 12% [3/25]) [4]. In the present series on 464 patients with acute hepatitis B in Japan, for whom genotyping was feasible, HBV infection persisted in only 5 (1%) representing 3 of the 92 (3%) with HBV/A, 1 of the 58 (2%) with HBV/B and 1 of the 330 (0.3%) with HBV/C. Hence the infection with HBV/A would persist longer than those with the other genotypes.

The present study highlighted the recent rapid increase in cases of acute hepatitis B in Japan, which gained about five-fold from 4.7 per year before 1990 to 33.1 after 2000 in participant hospitals. Primary HBV infection in the adulthood can occur by homo/heterosexual contacts, intravenous drug use, medical accidents and blood transfusion [50–54]. Acute HBV infection in the 485 patients studied was transmitted by sexual contacts in 216 (45%), medical accidents in 14 (3% (none since 2001)) and blood transfusion in 4 (1% (none since 2001)). The route is unknown for the remaining 245 (51%) patients; some of them might have been infected by undeclared sexual contacts and/or intravenous drug use, although recreational drugs are still uncommon in Japan. It comes as a surprise that the overall proportion of foreign genotypes (A, Ba and D) in patients with acute hepatitis B was high at 25%. Of these foreign genotypes, Asian genotypes such as HBV/Ba have decreased gradually, while HBV/A has increased in converse (Fig. 3). Furthermore,

some of “domestic” HBV/C infections that accounted for the majority (68%) may well have been imported by sexual workers from foreign countries where this genotype is prevalent. Combined, a substantial part of acute HBV infection in Japan does seem to have been imported.

In conclusion, the distribution of HBV genotypes in patients with acute hepatitis B is different geographically, and has changed with time in Japan. Recently, acute HBV infection is increasing among the Japanese adults, although it rarely becomes chronic. A significant part of it is transmitted sexually with foreign HBV genotypes. Furthermore, a fulminant outcome is frequent after infection with HBV/Bj having precore and/or BCP mutations. These facts should be made open to the public for making them aware of the risk and taking measures to prevent it.

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