

Clinical Significance of the Genotype and Core Promoter/Pre-Core Mutations in Hepatitis B Virus Carriers

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Key Words

Hepatitis B virus · Genotype · Core promoter · Pre-core

Abstract

It has been shown that clinical and virological characteristics vary among hepatitis B virus (HBV) genotypes. In this study, we measured the virus level, disease severity, and presence or absence of core promoter (CP)/pre-core (PC) mutations in 241 HBV carriers, and investigated the clinical significance of measuring the HBV genotype. In genotype C HBV carriers, the proportion of hepatitis B e antigen (HBeAg)-positive patients was significantly higher than that in genotype B HBV carriers (0 vs. 34.4%, $p < 0.05$), and the virus level was higher (4.9 vs. 4.05 LGE/ml). In the genotype B HBV carriers, the incidence of PC mutation was significantly higher (69 vs. 34%, $p < 0.05$). In the genotype C HBV carriers, the incidence of CP mutation was significantly higher (13 vs. 78%, $p < 0.05$). We compared patients with the wild (W)/mutant (M) pattern in the CP/PC regions to those with the M/W pattern in the CP/PC regions among the genotype C HBV carriers. Both the proportion of HBeAg-positive patients (65.8 vs. 15.4%, $p < 0.05$) and the alanine aminotransferase (ALT) level (48 vs. 21.5 IU, $p < 0.05$) were higher in the patients

with the M/W pattern in the CP/PC regions, and the disease severity was deteriorated. In conclusion, genotype B HBV may more frequently induce HBe seroconversion via PC mutation compared to genotype C HBV. Among the genotype C HBV carriers, hepatitis activity and the deterioration of the disease severity were significantly inhibited in the group in which PC mutation initially occurred, in comparison to the group in which CP mutation initially occurred.

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Introduction

The natural courses in hepatitis B virus (HBV) carriers vary; HBe seroconversion at a young age decreases the HBV-DNA level, inhibiting hepatitis in some patients [1, 2], whereas the deterioration of liver fibrosis leads to liver cirrhosis (LC) or hepatocellular carcinoma (HCC) in other patients [3]. Furthermore, the mode of infection varies; vertical infection from a mother to an infant is observed in Asia, and infection during adulthood rarely makes the patient a carrier. However, in Europe and the USA, infection during adulthood makes the patient a carrier, resulting in chronic hepatitis in many patients [4, 5].

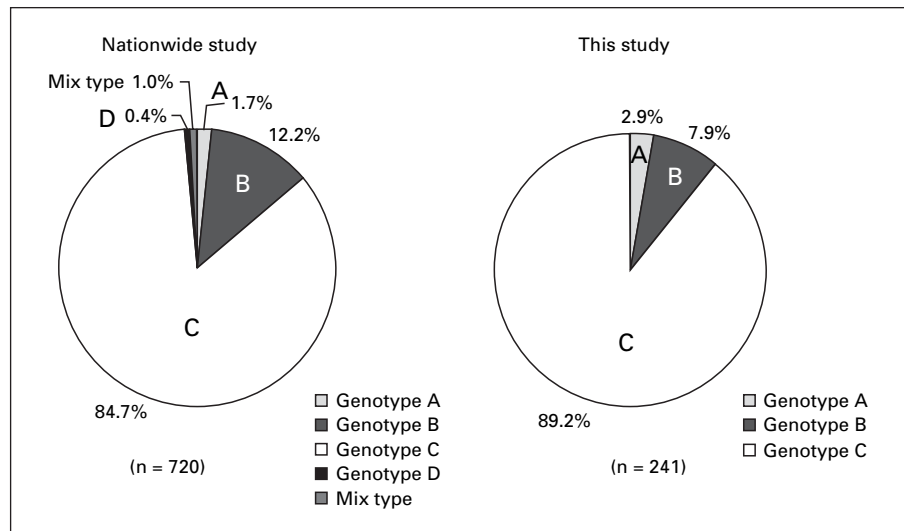


Fig. 1. Distribution of HBV genotypes: in this study (right), genotype A was observed in 7 subjects (2.9%), genotype B in 19 (7.9%), and genotype C in 215 (89.2%). The incidence of genotype B was slightly lower in our study than in the previous study by Orito et al. (left) on the distribution of HBV genotype in 720 subjects.

What contributes to the variety of these clinical courses has remained to be clarified.

Previously, HBV was classified into four subtypes by serological differences in the antigenic determinant. However, currently, HBV is classified into genotypes A to G by phylogenetic differences in the base sequence [6–13]. Clinical and virological characteristics vary among the genotypes [5, 14–19]. Furthermore, HBV is a DNA virus; however, this virus is replicated via reverse transcription in infected hepatocytes [20], and mutation frequently occurs, as reported for RNA viruses [21–29]. Core promoter (CP)/pre-core (PC) mutations reduce the production of hepatitis B e antigen (HBeAg), and are clinically important. In this cooperative study involving four hospitals, we investigated the clinical significance of measuring the genotype and CP/PC mutations in 241 HBV carriers in whom the genotype could be measured.

Subjects and Methods

The subjects were 241 HBV carriers (155 males, 86 females, age 51 years). Serum samples were collected from these patients for analysis. There were 47 asymptomatic carriers (ASCs). The disorders consisted of chronic hepatitis (CH) in 113 patients, LC in 32 patients, and HCC in 49 patients. The following four hospitals participated in this study: Department of Gastroenterology, Osaka Red Cross Hospital, Department of Gastroenterology, Japan Red Cross Wakayama Medical Center, Department of Gastroenterology, Kishiwada City Hospital, and Department of Gastroenterology, Kinki University Medical School Hospital.

Serologic Markers. HBsAg, HBeAg, anti-HBe, and anti-hepatitis C virus (HCV) were determined by enzyme immunoassay (Sysmex, Kobe, Japan). Serum HBV DNA was quantified by poly-

merase chain reaction (PCR) assay (Amplicor HBV Monitor; Roche Diagnostics, Tokyo, Japan). The linear range of quantification in this assay was 2.6–7.6 log copies/ml.

Genotyping of HBV. The HBV genotype was determined using patients' sera by PCR of the S-gene region and the restriction fragment length polymorphism pattern was analyzed, as described by Mizokami et al.

Detection of Pre-Core and Core Promoter Mutation. The G to A mutation at nt 1,896 in the PC region (A1896 mutation), and the A to T mutation at nt 1,762 and G to A mutation at nt 1,764 in the CP region (T1762 and A1764 mutations) were determined by the direct sequence method after PCR amplification, basically as previously described.

Analysis

We compared the background factors in our genotype B/C HBV carriers using the Mann-Whitney U test. The incidences of CP/PC mutations in these patients were compared using Fisher's exact test. In examining the background factors in the genotype C HBV carriers with respect to CP/PC mutations, variance analysis (Tukey method) was performed, considering multiplicity. $p < 0.05$ was regarded as significant.

Results

Figure 1 shows the distribution of HBV genotypes; 7 patients (2.9%) had genotype A HBV, 19 patients (7.9%) had genotype B HBV, and 215 patients (89.2%) had genotype C HBV. Previously, Orito et al. [18] published the distribution of HBV genotypes in 720 patients in Japan in a nationwide study. In our study, the percentage of genotype B HBV carriers was slightly lower than that in their study. Subsequently, we investigated differences in the clinical background in genotype B/C HBV carriers, who

Fig. 2. CP mutation in the genotype B/C HBV carriers: the incidence of genotype B was higher than genotype C (88 vs. 18%, $p < 0.05$) for the wild-type sequence and that of genotype C was higher than genotype B (13 vs. 78%, $p < 0.05$) for the mutant type. The mixed type showed no difference.

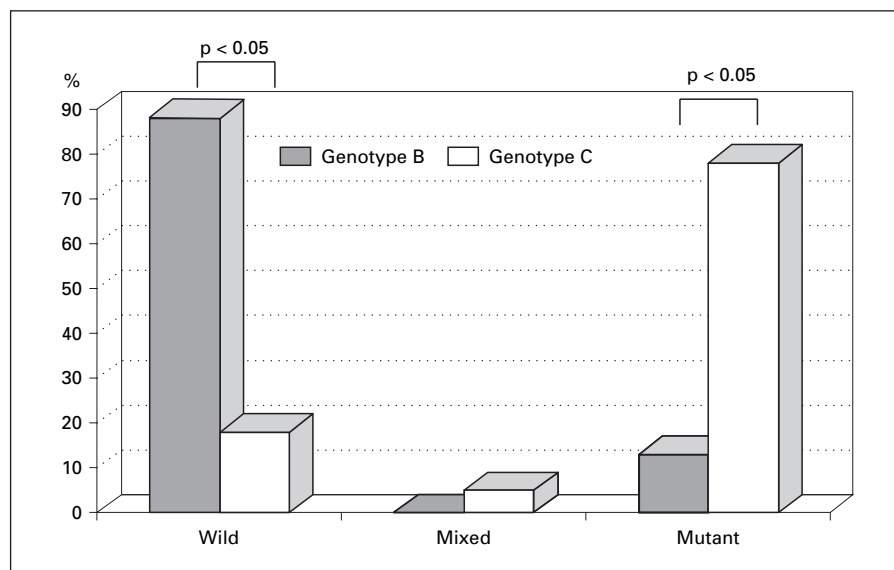


Table 1. Comparison of background factors in the genotype B/C HBV carriers

	B (n = 19)	C (n = 215)	p
Age, years	59.0 (26–76)	50 (18–86)	<0.05
Gender, M:F	12:7	138:77	n.s.
Condition			
ASC·CH·LC·HCC	4·10·0·5	43·97·31·44	n.s.
Total bilirubin, mg/dl	0.8 (0.3–8.1)	0.8 (0.3–10.6)	n.s.
ALT, IU	44.0 (15–407)	38 (9–1,222)	n.s.
HBeAg-positive, %	0	34.4	<0.05
HBV-DNA (PCR), log copies/ml	4.05 (2.5–7.7)	4.9 (2.5–7.7)	n.s.

ASC = Asymptomatic carriers; CH = chronic hepatitis; LC = liver cirrhosis; ALT = alanine aminotransferase; HBeAg = hepatitis B e antigen; PCR = polymerase chain reaction.

Table 2. Incidences of CP/PC mutations in the genotype B/C HBV carriers

CP	PreC	
	wild	mutant
Wild (genotypes B/C)	3/12	11/18
Mutant (genotypes B/C)	1/42	1/97

are most frequent in Japan (table 1). There were no significant differences in gender, total bilirubin (T-Bil), or alanine aminotransferase (ALT). However, in the genotype B HBV carriers, the age was significantly more advanced, and the proportion of HBeAg-positive patients was significantly lower. In addition, the HBV-DNA level was lower in these carriers. There was no significant difference in the disease severity between the two groups. Subsequently, we investigated CP/PC mutations, which may be associated with the genotype-related differences. Figure 2 shows the incidence of CP mutation in the genotype B/C HBV carriers. The wild type was more frequent in the genotype B HBV carriers, and the mutant type was more frequent in the genotype C HBV carriers, with significant differences. There was no significant difference in the percentage of the mixed type. Figure 3 shows the incidence of PC mutation. There was no significant difference in the percentage of the wild type between the two groups. The mixed type was more frequent in the genotype C HBV carriers, and the mutant type was more frequent in the genotype B HBV carriers, with significant differences.

Table 2 shows the incidences of CP/PC mutations with respect to the wild (W) and mutant (M) patterns in the genotype B/C HBV carriers. Of the genotype B HBV carriers, 11 showed the W/M pattern, 1 showed the M/W pattern, and 1 showed the M/M pattern. Of the genotype C HBV carriers, 18 showed the W/M pattern, 42 showed the M/W pattern, and 97 showed the M/M pattern.

Table 3 shows the background factors in the genotype C HBV carriers with respect to CP/PC mutations. We

Fig. 3. PC mutation in the genotype B/C HBV carriers: the wild-type sequence showed no difference between the two groups. The incidence of genotype C was higher than genotype B (6 vs. 33%, $p < 0.05$) for the mixed type and that of genotype B (69 vs. 34%, $p < 0.05$) for the mutant type.

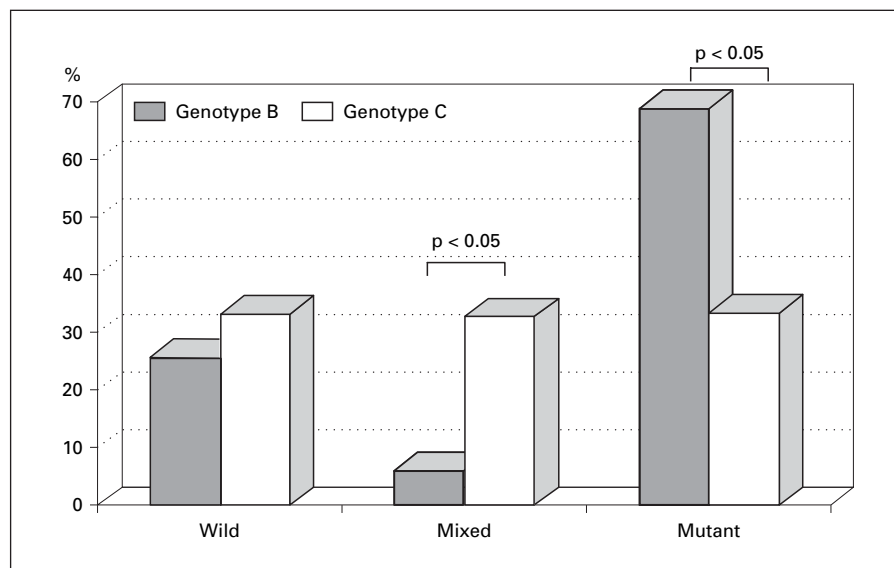


Table 3. Background factors in the genotype C HBV carriers with respect to CP/PC mutations

	W/W (n = 12)	W/M (n = 18)	M/W (n = 42)	M/M (n = 97)
Age, years	40 (22–86)	52 (18–77)	40 (19–69)	50.5 (20–78)
Males:females	8:4	9:9	26:16	67:30
T-Bil, mg/dl	0.95 (0.3–10.6)	0.8 (0.3–1.4)	0.75 (0.3–2.1)	0.8 (0.3–4.0)
ALT, IU	111 (12–1,590)	21.5 (9–114)	48 (11–428)	42 (11–599)
HBeAg-positive, %	50	15.4	65.8*	27
HBV-DNA (PCR), log copies/ml	6.6 (3.4–7.7)	3.6 (2.8–7.6)	6.7 (2.5–7.7)	4.6 (2.5–7.7)

* $p < 0.05$.

CP = Core promoter; PC = pre-core; T-Bil = total bilirubin; ALT = alanine aminotransferase; IU = international units; HBeAg = hepatitis B e antigen; PCR = polymerase chain reaction.

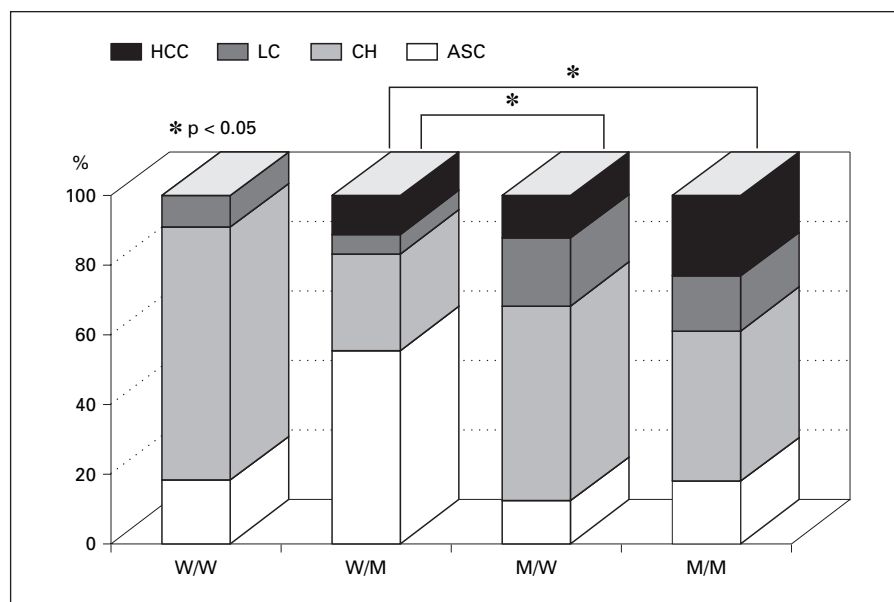
compared the type W/M carriers with the type M/W carriers, and the proportion of HBeAg-positive patients was significantly higher in the type M/W carriers. The ALT and HBV-DNA levels were higher in these carriers. We compared the type M/W carriers with the type M/M carriers, and the proportion of HBeAg-positive patients was significantly higher in the type M/W carriers. The HBV-DNA level was higher in these carriers; however, there was no significant difference in the ALT level. When comparing the type W/M carriers with the type M/M carriers, there were no significant differences in the proportion of HBeAg-positive patients or the HBV-DNA level. Fig-

ure 4 shows the association between these mutations and disease severity. In the type M/W and M/M carriers, the disease severity was more advanced than in the type W/M carriers. In tables 2 and 3 and figure 4, the mixed type was included in the mutant type for both CP and PC.

Discussion

Previously, Orito et al. [18] reported the distribution of HBV genotypes in 720 patients in Japan. In this study, the proportion of genotype B HBV carriers was slightly

Fig. 4. Association between CP/PC mutations and disease severity in the genotype C HBV carriers: the pathologic condition was significantly more progressed for M/W than for W/M and for M/M than for W/M.



lower than that in their study. This was possibly because this study investigated only the Kansai District; genotype B HBV is more frequent in the Tohoku and Okinawa Districts.

In Japan, genotypes B and C comprise approximately 97% of HBV carriers, and clinical and virological differences between these genotypes have been reported. The two genotypes are frequent in Asia, and the proportion of HBeAg-positive patients, ALT level, HBV-DNA level, and liver tissue inflammation score are lower in genotype B HBV carriers. In these carriers, the proportion of ASCs is high, and the incidences of LC and HCC are low [4, 16, 17, 30]. In Taiwan, the incidence of HCC at <math>< 51</math> years of age, which is rare in Japan, is high in genotype B HBV carriers. In particular, according to a study [14], approximately 90% of HCC patients aged <math>< 36</math> years had genotype B HBV. Briefly, the clinical course depends on the genotype. In this study, there were no significant differences in the ALT level or disease severity; however, the proportion of HBeAg-positive patients was significantly lower in the genotype B HBV carriers, and the HBV-DNA level was lower. Subsequently, we investigated CP/PC mutations, which may be associated with differences among the genotypes. CP mutation was significantly more frequent in the genotype C HBV carriers (fig. 2), and PC mutation was significantly more frequent in the genotype B HBV carriers (fig. 3). Therefore, a lower percentage of genotype B HBV carriers may be positive for HBeAg in comparison to genotype C HBV carriers, and

genotype B HBV carriers may show a lower virus level. According to Orito et al. [16], in the PC region, the wild type is significantly more frequent in HBeAg-positive patients regardless of the HBV genotype, and the mutant type is significantly more frequent in hepatitis B e antibody (HBeAb)-positive patients. Briefly, PC is associated with the proportion of HBeAg-positive patients, and PC mutation may improve hepatitis via seroconversion into HBeAb. In the CP region, the wild type is significantly more frequent in genotype B HBV carriers regardless of the HBeAg/HBeAb states, and the mutant type is significantly more frequent in genotype C HBV carriers. Briefly, CP is associated with the genotype C, and multivariate analysis has shown that CP is associated with the genotype C and the deterioration of the disease severity. Similarly, Kao et al. [31] investigated 250 HBV carriers, and reported that CP mutation was in parallel to the deterioration of the disease severity, and that CP mutation was detected in 3% of the ASCs and in 64% of the patients with HCC. In addition, multivariate analysis suggested that CP mutation increases the risk of HCC. Briefly, CP mutation may frequently occur in genotype C HBV carriers, leading to the deterioration of disease severity [14, 16–18, 32, 33].

Yatsunami et al. have reviewed the spontaneous course of hepatitis B with respect to gene mutation in the CP/PC regions. According to them, wild strains are observed in the PC and CP regions in HBeAg-positive ASCs with a normal ALT level, and when liver dysfunction per-

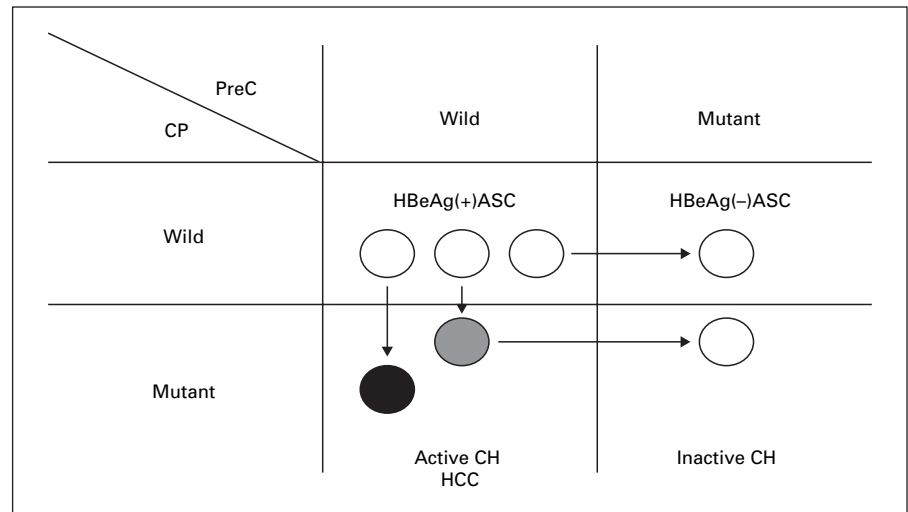


Fig. 5. Association between CP/PC mutations and clinical findings.

sists for 1 to >2 years after the onset of hepatitis, gene mutation occurs in the CP region. Thereafter, predominant mutant strains in the PC region cause seroconversion from HBeAg into HBeAb, decreasing the virus level and improving hepatitis. In most patients with active CH, wild strains are observed in the PC region, and mutant strains in the CP region. In mild hepatitis, patients who do not consult a physician without symptoms and achieve the healing via HBeAg seroconversion, gene mutation may occur only in the PC region without gene mutation in the CP region, making them HBeAg-negative ASCs with a normal ALT level (fig. 5).

We investigated the incidences of CP/PC mutations with respect to the HBV genotype. In most genotype B HBV carriers, PC mutation occurred without CP mutation. However, in many genotype C HBV carriers, CP mutation occurred prior to PC mutation. Briefly, the spontaneous course of hepatitis B depends on the HBV genotype. Generally speaking, among patients with HBV-related CH, disease activity is higher in genotype C HBV carriers; genotype C HBV is clinically important. Therefore, we subsequently examined the clinical background of genotype C HBV carriers with respect to CP/PC mutations. We compared patients with the W/M pattern to those with the M/W pattern, and the proportion of HBeAg-positive patients was significantly higher in the M/W pattern patients, and the virus and ALT levels were higher, suggesting higher hepatitis activity and an advanced stage. In other words, in patients with CP mutation prior to PC mutation, hepatitis activity persists, deteriorating the disease severity. In the M/M pattern patients, the proportion of HBeAg-positive patients was

significantly lower than in the M/W pattern patients, as demonstrated in the W/M pattern patients, and the HBV-DNA level was lower. However, the disease severity was more advanced than in the W/M pattern patients; there is a marked difference in the severity between patients with PC mutation alone before improvement of hepatitis and those with PC mutation following CP mutation, even when hepatitis subsides, which influences the prognosis. Therefore, in the treatment of hepatitis B, it may be important to shorten the period of CP mutation and accelerate PC mutation. In the phase in which the virus level and hepatitis activity are high, as they are in M/W pattern patients, viral proliferation should be aggressively inhibited by administration of lamivudine, or PC mutation should be induced by IFN administration to obtain the M/M pattern earlier. In this study, the ALT level was high in the W/W pattern group, and most patients had CH. This was possibly because the age was relatively advanced (median 40 years), and because many patients consulted the hospitals after the onset of hepatitis; at a younger age prior to the onset of hepatitis, there may be many ASCs with a normal ALT level, as previously reported.

It has been shown that the clinical features of hepatitis B markedly differ among the HBV genotypes. Furthermore, the incidences of gene mutations in the CP/PC regions differed among the genotypes, suggesting the association between these gene mutations and clinical findings. The measurement of the HBV genotype and CP/PC mutations may be clinically important for determining therapeutic strategies and predicting the prognosis. In the future, a larger number of patients should be investigated.

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