

# Basal core promoter mutation is associated with progression to cirrhosis rather than hepatocellular carcinoma in chronic hepatitis B virus infection

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**BACKGROUND:** As most cases of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) have concurrent cirrhosis, viral factors identified to be associated with HCC might be related to cirrhosis rather than HCC.

**METHODS:** Hepatitis B virus DNA levels, genotypes and precore/basal core promoter (BCP) mutants were compared between cirrhotic HCC and non-cirrhotic HCC patients. Age- and sex-matched case-control studies were performed to identify the risk factors.

**RESULTS:** Hepatitis B virus DNA levels showed no significant difference between non-cirrhotic HCC patients ( $n = 20$ ) and cirrhotic HCC patients ( $n = 140$ ) or 1:3 age- and sex-matched cirrhotic HCC patients ( $n = 60$ ), but genotype C and BCP mutant were significantly more prevalent in the latter than in the former. In multiple logistic regression, BCP mutant but not genotype C correlated significantly with the presence of cirrhosis in HCC patients. Compared with inactive carriers ( $n = 60$ ), non-cirrhotic HCC patients ( $n = 20$ ) had significantly higher HBV DNA levels but no difference in HBV genotypes and precore/BCP mutants. Furthermore, HBV DNA levels, the distribution of HBV genotypes and the prevalence of precore/BCP mutants all failed to show any significant difference between cirrhotic HCC patients ( $n = 60$ ) and cirrhotic patients without HCC ( $n = 60$ ).

**CONCLUSION:** Basal core promoter mutant is associated with progression to cirrhosis rather than HCC in chronic HBV infection.

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumours worldwide. Infection with hepatitis B virus (HBV) has, by far, the strongest association with HCC of any aetiological agents (Liaw and Chu, 2009). Viral factors such as HBV genotypes, viral mutants and viral load may have an utmost important role in the pathogenesis of HBV-related HCC (Chan, 2011). The most prevalent HBV genotypes in Asia are genotypes B and C (Kao, 2002; Chu and Liaw, 2005). Several cross-sectional or longitudinal studies reported a higher risk of HCC among genotype C infected patients (Kao, *et al*, 2000; Fujie, *et al*, 2001; Yu *et al*, 2005). Virologically, genotype C HBV has a higher frequency of basal core promoter (BCP) mutation than genotype B HBV (Lindh *et al*, 1999; Orito *et al*, 2001). The frequency of BCP mutation increases with progression of liver disease (Takahashi *et al*, 1995; Fang *et al*, 2002; Kao *et al*, 2003; Chen *et al*, 2005; Lin *et al*, 2005). In addition, a recent prospective longitudinal study from Taiwan revealed that the risk for cirrhosis and HCC increased with increasing HBV DNA levels in a dose-response relationship and that elevated serum HBV DNA level  $\geq 10^4$  copies  $\text{ml}^{-1}$  was a strong predictor of cirrhosis and HCC (Chen *et al*, 2006; Iloeje *et al*, 2006).

The independent and interactive effects of individual viral factor on the development of HCC have been studied by multivariate analysis but the results are still inconclusive. In several case-control studies, HBV DNA levels  $\geq 10^4$  copies  $\text{ml}^{-1}$  (Yuen *et al*, 2008) or  $\geq 10^5$  copies  $\text{ml}^{-1}$  (Liu *et al*, 2006) and BCP T1762/A1764 mutant (Liu *et al*, 2006; Tong *et al*, 2007; Yuen *et al*, 2008), but not genotype C (Liu *et al*, 2006; Yuen *et al*, 2008) were independent risk factors for HCC development; precore A1896 mutant was independently associated with HCC development in some studies (Liu *et al*, 2006; Tong *et al*, 2007) but not in others (Yuen *et al*, 2008). The results of prospective longitudinal studies also were controversial. In one study from southern China, BCP T1762/A1764 mutant was a significant aetiological factor for HCC (Fang *et al*, 2008), in another study from Hong Kong, genotype C, but not BCP T1762/A1764 mutant, increased the risk of HCC (Chan *et al*, 2004), and in the other study from Taiwan, HBV DNA level, genotype C and BCP T1762/A1764 mutant all were independent predictors for HCC, while precore A1896 mutant was significantly associated with low risk of HCC (Yang *et al*, 2008). The reasons for such remarkable discrepancies remain unclear. In a recent meta-analysis of 43 studies enrolling 11 582 patients with chronic HBV infection, BCP T1762/A1764 mutant is the most common mutation that is statistically significantly associated with the development of HCC; the presence of BCP T1762/A1764 mutant is associated with an odds ratio of 3.79 for HCC development (Liu *et al*, 2009).

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The majority of patients with HCC have concurrent liver cirrhosis. Unfortunately, most previous studies did not particularly categorise the HCC patients into cirrhotic and non-cirrhotic. Viral factors identified to be associated with HCC might be related to cirrhosis rather than HCC. In this study, we first compared the clinical and virological characteristics including HBV DNA levels, HBV genotypes, precore A 1896 and BCP T1762/A1764 mutants between cirrhotic and non-cirrhotic patients with HBV-related HCC. We then performed case-control studies by comparing the virological characteristics between non-cirrhotic patients with HCC and age- and sex-matched inactive carriers to identify factors significantly associated with HCC development in non-cirrhotic patients, and between cirrhotic patients with HCC and age- and sex-matched cirrhotic patients but without HCC to identify factors significantly associated with HCC development in cirrhotic patients. Meanwhile, we also performed a case-control study including cirrhotic patients without HCC and age- and sex-matched inactive carriers to identify viral factors responsible for progression to cirrhosis.

## MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Chang Gung Memorial Hospital at Taipei, and was carried out in accordance with the Helsinki Declaration of 1975.

### Patients and controls

Between 2007 and 2008, 160 consecutive patients with newly diagnosed HBV-related HCC were recruited from our unit. All these patients were positive for hepatitis B surface antigen (HBsAg) but negative for antibodies against hepatitis C virus (anti-HCV) and antibody against hepatitis D virus (anti-HDV). Patients who had other concomitant diseases including alcoholic liver disease, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis and Wilson's disease were excluded. The diagnosis of HCC was based on histopathology (fine needle aspiration, core liver biopsy or surgical resection) or typical radiological features (hypervascularity on the arterial phase and washout during the venous phase in dynamic computed tomography or magnetic resonance imaging) (Bruix and Sherman, 2005). The radiological diagnosis of HCC for 1–2 cm tumour needed at least two dynamic imaging techniques showing typical features of HCC and only a single dynamic study showing the typical features of HCC was necessary to confirm the diagnosis of HCC for a tumour > 2 cm. If the radiological appearances were not typical, the diagnosis of HCC was confirmed by histopathological examination. The diagnosis of cirrhosis was confirmed histologically or was based on clinical parameters and liver ultrasonographic findings (Lin *et al*, 1993).

Two groups of controls with no evidence of HCC based on clinical assessment and liver ultrasonographic findings were included during the same study period: (1) age- and sex-matched, hepatitis B e antigen (HBeAg)-negative and antibody against HBeAg (anti-HBe)-positive inactive carriers with persistently normal ALT levels ( $\leq 36 \text{ U l}^{-1}$ ) for more than 10 years, as previously reported (Chu *et al*, 2010), and (2) age- and sex-matched HBsAg-positive patients with cirrhosis. All these two controls also had no other concomitant diseases, including alcoholic liver disease, autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis or Wilson's disease.

### Methods

Hepatitis B surface antigen, HBeAg, anti-HBe and anti-HDV were assayed using radioimmunoassay or enzyme immunoassay kits (Abbott Diagnostics, North Chicago, IL, USA). Anti-HCV

was using a third-generation enzyme immunoassay (Abbott Diagnostics). Serum HBV DNA levels were assayed using the COBAS AmpliCor HBV Monitor Test (Roche Diagnostics, Branchburg, NJ, USA; lower limit of detection  $300 \text{ copies ml}^{-1}$ ). The conversion in  $\text{IU ml}^{-1}$  (1 IU is equivalent to 5.26 HBV DNA copies) was made according to the manufacturer's instructions. Hepatitis B virus genotypes were determined by using the PCR-restriction fragment length polymorphism of the surface gene of HBV, as previously described (Chu and Liaw, 2005), or by using a commercial genotype-specific probes assay (SMITEST HBV Genotyping Kit, Medical and Biological Laboratories Co., LTD, Nagoya, Japan) (Sugauchi *et al*, 2003). Precore A1896 mutant was detected by amplification-created restriction site method, as described before (Chu *et al*, 1996). BCP genes were amplified by PCR, and nucleotide sequences of the amplified products were directly determined by using an automatic sequencer.

### Statistical analyses

Data were presented as mean  $\pm$  s.d., median (interquartile range) or number (%). To compare characteristics between groups, either the  $\chi^2$ -test or Fisher's exact test was used to analyse categorical variables and the Student's *t*-test or Mann-Whitney *U* nonparametric test was used to analyse continuous variables. Univariate and multivariate logistic regression analyses were performed to identify the factors that correlated with the development of cirrhosis or HCC. Variables with *P* values < 0.1 in the univariate models were tested in a multivariate setting. Significant associations identified in multivariate analysis were presented as odds ratio (95% confidence interval). Statistical procedures were performed using the SPSS statistical software (version 13.0; SPSS, Chicago, IL, USA). *P* values < 0.05 were considered significant.

## RESULTS

Of the 160 HCC patients, 140 had cirrhosis, based on pathological examination in 50 (18 by percutaneous liver biopsy before the detection of HCC and 32 during hepatic resection or liver transplantation), and on liver ultrasonographic findings in 90. Among 50 patients with pathology-verified cirrhosis, oesophageal or gastric varices was detected in 68.9% (22/32), splenomegaly by ultrasonography in 62% (31/50) and thrombocytopenia in 64% (32/50). Totally, 38 patients (76%) had at least one sign of portal hypertension. Among 90 patients with ultrasonographic diagnosis of cirrhosis, oesophageal or gastric varices was detected in 70.8% (51/72), splenomegaly by ultrasonography in 64.4% (58/90) and thrombocytopenia in 72.2% (65/90). Totally, 71 patients (78.9%) had at least one sign of portal hypertension. The remaining 20 patients had non-cirrhotic liver, based on pathological examination during hepatic resection in 9 and on liver ultrasonographic findings in 11. All these 20 patients had normal spleen size by ultrasonography and normal platelet count and none of 12 patients who received esophagogastroduodenoscopy examination had oesophageal or gastric varices.

### Comparison of clinical, demographic and virological characteristics between cirrhotic patients with HCC and non-cirrhotic patients with HCC

The comparison of clinical, demographic and virological characteristics between cirrhotic patients with HCC and non-cirrhotic patients with HCC are summarised in Table 1.

Non-cirrhotic patients were significantly younger than cirrhotic patients. The frequency of HBeAg and levels of HBV DNA showed no significant difference between them. However, cirrhotic patients had significantly higher frequency of genotype C and BCP T1762/A1764 mutant than non-cirrhotic patients. The same

**Table 1** Clinical and virological characteristics of HBV-related HCC: comparison between cirrhotic and non-cirrhotic patients

Characteristics	All	Cirrhotic HCC		Non-cirrhotic HCC
		Series A <sup>a</sup>	Series B <sup>b</sup>	
Number of patients	160	140	121	20
Male gender	125 (78.1)	111 (79.3)	94 (77.7)	14 (70)
Age (years)	58.9 ± 11.5	60.0 ± 10.8*	60.2 ± 10.9**	51.0 ± 13.4***
$\alpha$ -fetoprotein (ng ml <sup>-1</sup> )	21 (8–105)	22 (18–90)	24 (8–90)	9 (3–420)
> 20 ng ml <sup>-1</sup>	81 (50.6)	73 (52.1)	66 (54.5)	8 (40)
HBeAg positivity	24 (15)	20 (14.3)	7 (14.0)	4 (20)
HBV DNA (IU ml <sup>-1</sup> )	17 419 (390–17 419)	19 545 (408–174 192)	24 421 (753–188 421)	15 172 (100–115 088)
< 200	35 (21.9)	30 (21.4)	25 (20.7)	5 (25)
200–1999	16 (10)	15 (10.7)	11 (9.1)	1 (5)
2000–19 999	31 (19.4)	26 (18.6)	22 (18.2)	5 (25)
20 000–199 999	41 (25.6)	37 (26.4)	34 (28.1)	4 (20)
200 000–1 999 999	30 (18.8)	27 (19.3)	24 (19.8)	3 (15)
≥ 2 000 000	7 (4.4)	5 (3.6)	5 (4.1)	2 (10)
Genotype				
B	79 (63.2)	66 (60) <sup>†</sup>	56 (58.3) <sup>‡</sup>	13 (86.7) <sup>†‡</sup>
C	46 (36.8)	44 (40)	40 (41.7)	2 (13.3)
Precore 1896				
Wild-type (G1896)	28 (22.4)	24 (21.8)	23 (24.0)	4 (26.7)
Mutant (A1896)	97 (77.6)	86 (78.2)	73 (76.0)	11 (73.3)
Basal core promoter 1762/1764				
W wild-type (A1762/G1764)	37 (29.6)	27 (24.5) <sup>§</sup>	22 (22.9) <sup>¶</sup>	10 (66.7) <sup>§¶</sup>
M mutant (T1762/A1764)	88 (70.4)	83 (75.5)	74 (77.1)	5 (33.3)

Abbreviations: HBV = hepatitis B virus; HCC = hepatocellular carcinoma; HBeAg = hepatitis B e antigen. Data are presented as n (%), mean ± s.d. or median (interquartile range). \* $P = 0.008$ , \*\* $P = 0.008$ , <sup>†</sup> $P = 0.044$ , <sup>‡</sup> $P = 0.035$  <sup>§</sup> $P = 0.0008$ , <sup>¶</sup> $P = 0.0005$ . <sup>a</sup>Series A: including 50 patients with pathological diagnosis of cirrhosis and 90 with ultrasonographic diagnosis of cirrhosis. <sup>b</sup>Series B: including 50 patients with pathological diagnosis of cirrhosis and 71 with ultrasonographic diagnosis of cirrhosis who had at least one sign of portal hypertension.

findings were present if patients in the cirrhotic group without histological confirmation and any sign of portal hypertension were excluded. The association of older age, genotype C and BCP T1762/A1764 mutant with cirrhosis in univariate analysis were then analysed by using multiple logistic regression analysis. As shown in Table 2, older age and BCP T1762/A1764 mutant, but not genotype C ( $P = 0.35$ ), correlated significantly with the presence of cirrhosis in HCC patients.

As the non-cirrhotic patients were significantly younger than cirrhotic patients by a mean of 9 years (Table 1), further analysis was performed by comparing the virological characteristics between 20 non-cirrhotic patients with HCC and 1:3 age- and sex-matched cirrhotic patients with HCC. As shown in Table 3, the virological differences between them were essentially the same as those seen in Table 1.

### Comparison of virological characteristics between non-cirrhotic patients with HCC and age- and sex-matched inactive carriers

To identify viral factors associated with the development of HCC in non-cirrhotic patients, a case-control study was performed by including 20 non-cirrhotic patients with HCC and 1:3 age- and sex-matched inactive carriers. As shown in Table 3, non-cirrhotic patients with HCC had significantly higher frequency of HBeAg and higher levels of HBV DNA than inactive carriers. However, the distribution of HBV genotypes and the frequency of precore A1896 and BCP T1762/A1764 mutants all showed no significant difference between them. In multiple logistic regression, HBV DNA level  $\geq 20\,000$  IU ml<sup>-1</sup>, but not HBeAg ( $P = 0.97$ ), was

**Table 2** Association of older age and basal core promoter mutant with cirrhosis in patients with HCC: multiple logistic regression analysis

Variables	Odds ratio (95% CI)	P-value
Age (per year increase)	1.07 (1.01–1.12)	0.015
Basal core promoter 1762/1764		
Wild-type (A1762/G1764)	1	
Mutant (T1762/A1764)	4.403 (1.17–16.59)	0.029

Abbreviations: CI = confidence interval; HBV = hepatitis B virus; HCC = hepatocellular carcinoma.

significantly associated with HCC in non-cirrhotic patients (Table 4).

### Comparison of virological characteristics between cirrhotic patients with HCC and age- and sex-matched cirrhotic patients without HCC

To identify viral factors correlated with the development of HCC in cirrhotic patients, a case-control study was performed by including 60 cirrhotic patients with HCC and 60 age- and sex-matched cirrhotic patients without HCC. As shown in Table 3, virological characteristics including HBV DNA levels, the distribution of HBV genotypes and the prevalence of precore A1896 and BCP T1762/A1764 mutants all showed no significant difference between cirrhotic patients with and without HCC.

**Table 3** Comparison of virological characteristics among age- and sex-matched non-cirrhotic HCC, cirrhotic HCC, inactive carriers and cirrhotics with HCC

Characteristics	Non-cirrhotic HCC (n = 20)	Cirrhotic HCC (n = 60)	Inactive carriers (n = 60)	Cirrhosis (n = 60)
Age (years)	51.2 ± 13.3	51.3 ± 9.1	50.7 ± 11.6	51.2 ± 12.4
Male gender	14 (70)	48 (80)	42 (70)	49 (81.7)
HBeAg positivity	4 (20)*	8 (13.3)	0 (0)*	4 (6.7)
HBV DNA (IU ml <sup>-1</sup> )	15 172 (100–115 088)**	43 755 (1517–219 296)	399 (60–2761)**†	10 257 (283–120 512)†
<200	5 (25)	10 (16.7)	23 (38.3)	14 (23.3)
200–1999	1 (5)	5 (8.3)	20 (33.3)	6 (10)
2000–19 999	5 (25)	12 (20)	13 (21.7)	15 (25)
20 000–199 999	4 (20)	17 (28.3)	4 (6.7)	14 (13.3)
200 000–1 999 999	3 (15)	13 (21.7)	0 (0)	8 (8.4)
≥2 000 000	2 (10)	3 (5)	0 (0)	3 (5)
Genotype				
B	13 (86.7%)‡	28 (56)‡	32 (86.5)§	24 (52.2)§
C	2 (13.3%)	22 (44)	5 (13.5)	22 (47.8)
Precore 1896				
Wild-type (G1896)	4 (26.7)	11 (22)	7 (18.9)	11 (23.9)
Mutant (A1896)	11 (73.3)	39 (78)	30 (81.1)	35 (76.1)
Basal core promoter 1762/1764				
Wild-type (A1762/G1764)	10 (66.7)¶	14 (28)¶	29 (78.4)¶¶	14 (30.4)¶¶
Mutant (T1762/A1764)	5 (33.3)	36 (72)	8 (21.6)	32 (69.6)

Abbreviations: HBV = hepatitis B virus; HCC = hepatocellular carcinoma; HBeAg = hepatitis B e antigen. Data are presented as n (%), mean ± s.d. or median (interquartile range). \*P = 0.003, \*\*P = 0.004, †P = 0.004, ‡P = 0.03, §P = 0.0009, ¶P = 0.0065, ¶¶P < 0.0001.

**Table 4** Association of HBV DNA levels with HCC in non-cirrhotic patients: multiple logistic regression analysis

HBV DNA (IU ml <sup>-1</sup> )	Odds ratio (95% CI)	P
<2000	1	
2000–19 999	2.21 (0.54–9.03)	0.27
≥20 000	10.75 (2.34–49.84)	0.0023

Abbreviations: CI = confidence interval; HBV = hepatitis B virus; HCC = hepatocellular carcinoma.

### Comparison of virological features between cirrhotic patients and age- and sex-matched inactive carriers

In the meanwhile, to identify viral factors involved in the pathogenesis of HBV-related cirrhosis, a case-control study was performed by including 60 cirrhotic patients without HCC and 60 sex- and age-matched inactive carriers. As shown in Table 3, the cirrhotic patients had significantly higher levels of HBV DNA, higher frequency of genotype C and BCP T1762/A1764 mutant, compared with inactive carriers. Multiple logistic regression analysis identified HBV DNA levels ≥20 000 IU ml<sup>-1</sup> and BCP T1762/A1764 mutant but not genotype C (P = 0.43) were independent predictors for progression to cirrhosis (Table 5).

### DISCUSSION

Among 160 HBV-related HCC in this series, 20 were non-cirrhotic and the other 140 were cirrhotic. Only 9 and 50 patients, respectively, were verified to have non-cirrhotic and cirrhotic liver by pathological examination. A high-resolution real-time ultrasound was used to determine the presence or absence of cirrhosis in the remaining patients. This technique is somewhat limited with sensitivity and specificity in the 80% range (Lin *et al*, 1993). However, clinical signs of portal hypertension, such as oesophageal or gastric varices, splenomegaly or thrombocytopenia, were not seen in any patients with ultrasonographic diagnosis of

**Table 5** Association of HBV DNA levels and basal core promoter mutant with cirrhosis: multiple logistic regression analysis

Variables	Odds ratio (95% CI)	P
HBV DNA (IU ml <sup>-1</sup> )		
<2000	1	
2000–19 999	1.77 (0.45–6.90)	0.41
≥20 000	7.26 (1.49–35.42)	0.014
Basal core promoter 1762/1764		
Wild-type (A1762/G1764)	1	
Mutant (T1762/A1764)	3.76 (1.07–13.21)	0.039

Abbreviations: CI = confidence interval; HBV = hepatitis B virus.

non-cirrhotic liver but were evident in ~70% of patients with ultrasonographic diagnosis of cirrhosis. These figures were similar to those observed in the pathology-verified patients.

In this investigation, we have shown that, among patients with HBV-related HCC, there was no significant difference in gender distribution, but the non-cirrhotic patients were significantly younger than the cirrhotic patients by a mean of about 10 years (Table 1). The frequency of HBeAg and levels of HBV DNA did not show significant difference between cirrhotic patients and non-cirrhotic patients. However, the non-cirrhotic patients had significantly higher frequency of genotype B and lower frequency of BCP T1762/A1764 mutant than the cirrhotic patients (Table 1). The findings of younger age and higher frequency of genotype B in non-cirrhotic patients than cirrhotic patients in this series were consistent with the early observations reported by others from Taiwan (Kao *et al*, 2000). In a cross-sectional study, Kao *et al* (Kao *et al*, 2000) first reported that genotype B was associated with HCC in patients younger than 50 years (the majority of them were non-cirrhotic) and genotype C was associated with HCC in patients older than 50 years (the majority of them were cirrhotic). The lower frequency of BCP T1762/A1764 mutant in non-cirrhotic patients in our series might be attributed to the lower frequency of genotype C in these patients, as genotype C HBV has higher



frequency of BCP mutant than genotype B HBV (Lindh *et al*, 1999; Orito *et al*, 2001). As the number of non-cirrhotic patients with HCC in this series was relatively small, further studies of a larger series of patients are needed to confirm these observations.

By using multiple logistic regression analysis, the present investigation showed that older age and BCP T1762/A1764 mutant but not genotype C independently correlated with the presence of cirrhosis in HCC patients (Table 2). These findings highly suggested that BCP T1762/A1764 mutant may have an important role in the pathogenesis of HBV-related cirrhosis.

Several previous case series also reported a higher frequency of BCP T1762/A1764 mutant in patients with cirrhosis than in inactive carriers or patients with chronic hepatitis (Takahashi *et al*, 1995; Fang *et al*, 2002; Kao *et al*, 2003; Chen *et al*, 2005; Lin *et al*, 2005). The present investigation is the first case-control study to identify viral factors significantly associated with progression to cirrhosis in chronic HBV infection. In this analysis, the inactive carriers had persistently normal ALT levels for at least 10 years and were presumed at minimal potential for progression of liver disease (Tai *et al*, 2009), and thus could be served as suitable controls. Moreover, the inactive carriers were age- and sex-matched for patients with cirrhosis. Both age and sex are important host factors in the pathogenesis of HBV-related cirrhosis (Liaw *et al*, 1988; Fattovich *et al*, 1991). Our results identified two factors significantly predictive for progression to cirrhosis (Table 5). Patients with HBV DNA levels  $\geq 20\,000$  IU ml<sup>-1</sup> were approximately seven times more likely to have cirrhosis than those with levels  $< 2000$  IU ml<sup>-1</sup>. Another factor predictive for progression to cirrhosis was the presence of BCP T1762/A1764 mutant, which was associated with about 4-fold increased risk of cirrhosis. Genotype C correlated with progression to cirrhosis in univariate analysis (Table 3), but it became non-significant in the multivariate analysis (Table 5). As genotype C HBV tends to have a high prevalence of BCP T1762/A1764 mutant (Lindh *et al*, 1999; Orito *et al*, 2001), the effect of BCP T1762/A1764 mutant on the development of cirrhosis might be strong enough to mask the effect of genotype C. Further prospective studies are needed to clarify the role of each viral factor in the progression of liver diseases. The frequency of precore A1896 mutant was high in patients with cirrhosis (76%), but this figure was similar to that observed in inactive carriers (81%), as shown in Table 3. These findings are in keeping with our previous observations that precore A1896 mutant is highly prevalent in patients with chronic HBV infection and does not seem to have an important role in the progression of liver disease in our area (Chu *et al*, 1996).

This investigation then analysed viral factors significantly associated with the development of HCC in non-cirrhotic patients by comparison of virological characteristics between non-cirrhotic patients with HCC and age- and sex-matched inactive carriers. As shown in Table 3, the frequency of HBeAg and levels of HBV DNA were significantly higher in non-cirrhotic patients with HCC than in inactive carriers, while HBV genotypes, precore A1896 and BCP T1762/A1764 mutants showed no significant difference between them. Multiple logistic regression analysis revealed that HBV DNA level was the only factor significantly correlated with HCC development in non-cirrhotic patients (Table 4). The results of this investigation argued against the role of genotype C, precore A1896 or BCP T1762/A1764 mutant in the pathogenesis of HCC in non-cirrhotic patients.

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Perhaps the more significant finding of the present study is that virological characteristics including HBV DNA levels and the distribution of HBV genotypes and the prevalence of precore A1896 and BCP T1762/A1764 mutants all failed to show any significant difference between cirrhotic patients with HCC and age- and sex-matched cirrhotic patients without HCC (Table 3). These findings highly suggested that all these viral factors are not directly involved in the pathogenesis of HCC in patients with HBV-related cirrhosis. In the previous meta-analysis, the pooled frequencies of BCP T1762/A1764 mutant in the control groups were 46.2%, significantly lower than that of 70.6% in HCC patients (Liu *et al*, 2009). However, it should be pointed out that the control groups consisted of asymptomatic carriers, chronic hepatitis patients and cirrhotic patients, with the pooled frequencies of BCP T1762/A1764 mutant being 28.3%, 49.9% and 70.6%, respectively (Liu *et al*, 2009). The frequency of BCP T1762/A1764 mutant in HCC patients and cirrhotic controls indeed was similar. The age and gender distribution of HCC patients and controls enrolled in that meta-analysis was unknown. In this investigation, the frequencies of BCP T1762/A1764 mutant among age- and sex-matched inactive carriers, non-cirrhotic HCC, cirrhotic patients and cirrhotic HCC were 21.6%, 33.3%, 69.6% and 72%, respectively. These results highly suggested that BCP T1762/A1764 mutant is associated with progression to cirrhosis but not directly related to HCC development. Further prospective studies are needed to ascertain whether the risk of HCC development might be different between cirrhotic patients with and without BCP T1762/A1764 mutant. Interestingly, in a study using laser capture microdissection of hepatocytes from patients with HBV-related HCC, there is no difference in the mutation profile at BCP region between tumour and non-tumour cells (Iavarone *et al*, 2003). It seems likely that cirrhosis *per se* is a pre-malignant lesion, but whether other viral mutations (Liu *et al*, 2009) or host and environmental factors (Fattovich *et al*, 2004) might predispose the cirrhotic patients to development of HCC warrants further investigation.

In conclusion, the present study revealed that (1) BCP T1762/A1764 mutant is an independent risk factor for progression to cirrhosis; (2) the prevalence of BCP T1762/A1764 mutant in non-cirrhotic patients with HCC is low, as in inactive carriers; and (3) the prevalence of BCP T1762/A1764 mutant is high in both cirrhotic patients with HCC and cirrhotic patients without HCC. Taken together, these data highly suggest that BCP T1762/A1764 mutant is associated with progression to cirrhosis rather than HCC in chronic HBV infection.

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## Conflict of interest

Liaw YF has been involved in clinical trials or served as a global advisory board member of Roche, Bristol-Myers Squibb, GlaxoSmithKline, Novartis and Gilead Sciences. The other authors declare no conflict of interest.

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