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Original Article

HBV Subgenotype C2 Infection, A1762T/G1764A Mutations May Contribute To Hepatocellular Carcinoma with Cirrhosis in Southeast China

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Abstract

Background: To glean insights into the relationship among hepatitis B virus (HBV) genotype/subgenotypes, A1762T/G1764A mutations and advanced liver disease such as liver cirrhosis (LC) and hepatocellular carcinoma (HCC) in Southeast China.

Methods: A case-control study was performed, consisting of chronic hepatitis B (CHB) patients (n=160), LC patients (n=150), and HCC patients (n=156). Fluorescence quantitative polymerase chain reaction (FQ-PCR) was used to detect A1762T/G1764A mutations. HBV genotypes/subgenotypes were determined by multiplex PCR. All patients' clinical data was systematically collected from the hospital records.

Results: Our study revealed HBV genotypes C (63.95%) and B (33.69%) were predominant in chronically infected patients, subgenotype B2, C2 and C1 were the major subgenotypes. Both subgenotype C2 infection and A1762T/G1764A mutations were associated with LC and HCC with cirrhosis, subgenotype C2 (OR=2.033, 95%CI=1.246-3.323, *P*=0.003 for LC *vs* CHB; OR=3.247, 95%CI=1.742-6.096, *P*=0.001 for HCC with cirrhosis *vs* CHB; respectively), and A1762T/G1764A mutations (OR=1.914, 95%CI=1.188-3.085, *P*=0.005 for LC *vs* CHB; OR=2.996, 95%CI=1.683-5.353, *P*=0.002 for HCC with cirrhosis *vs* CHB; respectively), but no differences in the frequencies of both variants between LC and HCC with cirrhosis groups were found.

Conclusions: HBV subgenotype C2 infection and A1762T/G1764A mutations are both risk factors of LC and HCC with cirrhosis development in the patients with CHB in Southeast China, but all no helpful for predicting HCC development in LC patients.

Keywords: Hepatitis B Virus, Basal core promoter, Liver cirrhosis, Hepatocellular carcinoma

Introduction

Hepatitis B virus (HBV) infection can cause acute and chronic hepatitis, liver cirrhosis (LC), and heaptocellular carcinoma (HCC). The prevalence of HBV infection varies markedly throughout the world. Hepatitis B is highly endemic in developing regions such as Southeast Asia, sub-Saharan Africa and the Amazon Basin. There are about 120 million people with chronic HBV infection in China, and the incidence of HCC in the natural population in Shanghai is about 20/100,000 every year who commonly carry with HBV surface antigen. The prevalence of HBV infection has become a serious public health problem (1).

Mutations in the HBV genome may occur as a result of natural selection pressure, host immune response, anti-virus drug administration and lack of a correction function of reverse transcriptase during virus replication. HBV mutations were often observed in the pre-C and basal core promoter (BCP) regions, such as T1753C, A1762T, G1764A,

G1862T, G1896A and G1899A nucleotide acid substitution. Among these, A1762T was almost accompanied with G1764A nucleotide acid substitution. It has received much interest since it seemed to show related to clinical outcomes of HBV infection. Hou (2) and Ledesma (3) described that A1762T/G1764A mutations were associated with chronic hepatitis B (CHB) with negative HBV e antigen (HBeAg). Yotsuyanagi (4) and Liu (5) found that the mutations could increase the risks of LC and HCC. Controversially, some studies suggested that the mutations were no relationship with HBeAg status in CHB patients and did not show any difference in the distribution frequencies of the mutations between CHB and HCC groups (6, 7).

In Asia, HBV genotypes mostly prevalent are genotypes B and C. Subgenotype B2 (Ba) is found throughout Asia, including mainland China, whereas the prevalence of subgenotype B1 (Bj) is restricted to Japan. Subgenotype C1 (Cs) is prevalent in Southern Asia, whereas C2 (Ce) is predominantly of genotype C in the Far East. Studies suggested that genotype C infection could induce A1762T/G1764A mutations easier and it was related to LC and HCC, but no relationship with advanced liver disease (LC and HCC) was also reported (8, 9). The details in the prevalence of HBV subgenotypes in Southeast China are still unclear. Meanwhile, the data is limited on the relationship among HBV genotypes/subgenotypes, A1762T/G1764A mutations and Clinicopathological characteristics in HCC patients.

In this study, we randomly collected the sera of 160 CHB patients, 150 LC patients, and 156 HCC patients to investigate the risk factors that affect the progression of chronic HBV infection, including HBV genotypes/subgenotypes, A1762T/G1764A mutations, and some serologic markers.

Materials and Methods

Patients

All of 466 patients with chronic HBV infection were recruited randomly in this study, who visited Hanzhou First People's Hospital from June 2007 to December 2010. All patients were infected with HBV for 8 months to 25 years, and the average infection time was 16.1 ± 7.5 years. All participants were divided into three age- and gender-matched groups, consisted of 160 CHB patients (140 males and 20 females, age range 35 to 65 years, mean age 49.5 ± 11.8 years), 150 LC patients (136 males and 14 females, age range 33 to 67 years, mean age 49.3 ± 10.1 years), and 156 HCC patients (140 males and 16 females, age range 38 to 69 years, mean age 50.1 ± 9.3 years). CHB patients met the diagnostic criteria in serological and histopathological examination, and without impressions of LC and HCC detected by liver ultrasound. LC patients were diagnosed by liver ultrasound, computed tomography (CT), and magnetic resonance imaging (MRI), and accompanying with portal hypertension and hypersplenism. HCC patients were determined by liver ultrasound, CT, MRI and serum α fetoprotein (AFP) level, which met the diagnostic criteria for HCC, and confirmed by histopathological examination finally. 2 milliliters of sera were collected from each patient and stored at -70℃ until use. In addition, all participants never accepted anti-virus administration and the patients presenting other liver diseases were excluded from this study, such as autoimmune hepatitis, alcoholic hepatitis, Wilson disease and other types of hepatitis virus infection.

Serological test

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) levels of all patients were detected with Hitachi 7600 automatic biochemical analyzer and supporting reagents (Hitachi Co., Ltd., Japan). The results of blood platelet count were from the hospital patient records. Serum HBV markers were detected by ELISA kits (Kehua Bio-engineering Co., Ltd., Shanghai, China). Serum AFP level was measured with chemiluminescence method, using Bayer ACS-180 instrument and supporting reagents (Bayer, Co., Ltd., Germany).

PCR and sequencing

Serum HBV DNA level was quantified by ABIPRISM®7000 instrument (ABI Co., Ltd.,

USA), the reagent kits provided by DaAn Gene Diagnostic Co., Ltd of SUN YAT-SEN University (Guangzhou, China) with a lower limit of detection of 500 copies/mL. HBV DNA was extracted using the boiling method. The extraction protocol in brief as below, about 50 μ l serum was added to equal volume of lysis buffer (NaCl 0.1M, EDTA 0.01M, Tris-HCl 0.1M, SDS 1%), boiling for 10 min, and centrifuged in 12000 rpm for 5 min, then supernatants were used for PCR amplification directly. HBV A1762T/G1764A mutations were determined by real-time fluorescence quantitative polymerase chain reaction (FQ-PCR), according to previous report (10) with some modifies. The primer pair sequences for FQ-PCR are 5'-CCG ACC TTG AGG CAT ACT TCA -3' for the forward primer and 5'- CCA ATT TAT GCC TAC AGC CTC CTA -3' for the reverse primer. The wildtype- and mutant-specific probe sequences are VIC-AGG TTA AAG GTC TTT GTA C and 6FAM-AGG TTA ATG ATC TTT GTA C, respectively (Oligonucleotides synthesized by Dalian TaKaRa Biotech Co., Ltd., China). The FQ-PCR was done in total volume of 50 μL consisting of TaKaRa Ex Taq (5 U / μL) 0.6μL, 10 × Ex Taq Buffer (Mg2+ Plus) 5 μL, dNTP Mixture (2.5 mM for each) 5 μL, primer (50μM) 0.3 μL for each, probe (50μM) 0.1μL for each, template DNA 5μL and distilled water 33.6μL. PCR conditions were as follows: 50°C for 2 minutes, then 95°C for 3 minutes, followed by 40 cycles of 94°C for 15 seconds and 60°C for 1 minute. The sera containing the mutations was confirmed by direct sequencing acted as positive control, distilled water acted as negative control in this study.

HBV genotypes and subgenotypes were typed by multiplex PCR with type-specific primers (11). The samples with low levels of HBV DNA were determined by nested multiplex PCR (12).

Statistical analysis

Data analysis was performed using SPSS11.0 software (SPSS Inc., Chicago, IL, USA). $\chi 2$ test or Fisher exact test was used to distinguish the differences of frequency distribution among the groups. Measurement data was presented as mean ± standard deviation. The differences between two groups with normal distribution or nor-normal distribution were detected by *t* test or Mann-Whitney U test, respectively. ANOVA test was performed to determine the differences among the three groups. Association was expressed as odds ratio (OR) as risk estimates with 95% confidence intervals (95% CI). All statistical tests were two-sided, and a probability level of $P< 0.05$ was considered to be statistically significant.

Ethical approval

The current study was approved by the Medical Ethics Committee of Hangzhou First People's Hospital and all participants completed an informed consent process.

Results

Serological markers, HBV DNA, HBV genotypes/subgenotypes and A1762T/G1764A mutations in 466 patients

The results of serological tests, HBV genotypes/subgenotypes and A1762T/G1764A mutations in subjects with CHB (n=160), LC (n=150) and HCC (n=156) were listed in Table 1. We found there were obviously significant differences in the levels of serum ALT, AST, TBIL, AFP and blood Platelet count among the three groups ($P<$ 0.05 for all). CHB patients showed higher ALT, lower AST and AFP levels than either LC or HCC patients. LC patients showed higher TBIL level and lower Platelet count than the other two groups. HBV DNA level was higher in both LC and HCC patients than CHB patients, but no difference was found between LC and HCC patients. The frequency of serum HBeAg positive patients among the three groups showed no difference.

We found 157 subjects infected with HBV genotype B, 298 with genotype C, and 11 with genotype B/C mixture in 466 patients with chronic HBV infection. HBV genotypes C (63.95%) and B (33.69%) were predominant. Genotype B was all classified into subgenotype B2 (100%). C2 (98.32%) and C1 (1.68%) were the major subgenotypes of genotype C. The results showed both subgenotype C2 (68.67% *vs.* 51.88%, P=0.003 for LC *vs.* CHB; 68.59% *vs.* 51.88%,

P=0.002 for HCC *vs.* CHB; respectively) and A1762T/G1764A mutations (57.33% *vs.* 41.25%, *P*=0.005 for LC *vs.* CHB; 58.97% *vs.* 41.25%, *P*=0.002 for HCC *vs.* CHB; respectively) were more often detected in LC and HCC patients than in CHB patients, but there were no differences in both variants between LC and HCC patients (Table 1).

Table 1: Serum markers, HBV genotypes/ subgenotypes, A1762T/G1764A mutations in 466 patients with chronic HBV infection

Clinical parameters	CHB (*n*=160)	LC (*n*=150)	HCC (*n*=156)	P^a	P^b	P^c
ALT (U/L)	133.62±96.37	98.76±66.08	75.38±51.69	0.010	0.003	0.016
AST (U/L)	48.32±36.35	75.18±60.27	125.20±66.51	0.001	<0.0001	0.002
TBIL (μmol/L)	48.39±23.42	79.83±46.40	29.12±16.70	0.001	0.004	<0.0001
Platelet count (10^9/L)	12.73±6.19	6.62±2.58	10.93±5.47	<0.0001	0.007	<0.0001
HBeAg (+)	94 (58.75)	76 (50.67)	76 (48.72)	0.153	0.074	0.733
AFP (ng/mL)	12.1±4.8	47.9±16.2	942.3±426.4	0.004	<0.0001	0.002
HBV-DNA (log_{10}copies/mL)	5.42±1.42	5.86±1.37	5.93±1.02	0.006	0.003	0.612
Genotypes / subgenotypes						
B	70 (43.75)	42 (28.0)	45 (28.85)	0.006	0.005	0.898
B1	0(0.0)	0(0.0)	0(0.0)			
B2	70 (43.75)	42 (28.0)	45 (28.85)	0.006	0.005	0.898
C	85 (53.13)	105 (70.0)	108 (69.23)	0.002	0.003	0.884
C1	2 (1.25)	2 (1.33)	1 (0.64)			
C2	83 (51.88)	103 (68.67)	107 (68.59)	0.003	0.002	0.988
Genotype mixture						
B/C	5 (3.13)	3 (2.0)	3 (1.92)			
A1762T/G1764A mutations	66 (41.25)	86 (57.33)	92 (58.97)	0.005	0.002	0.771

Data was presented as mean ± standard deviation or number (%).

[a], LC *vs.* CHB ; [b], HCC *vs.* CHB ; [c], HCC *vs.* LC.

P values for HBV subgenotype C1 and genotype mixture were not shown because of small sample size.

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; ALT, alanine aminotransferase; AST , aspartate aminotransferase; TBIL , total bilirubin; HBeAg, HBV e antigen; AFP, α fetoprotein

A1762T/G1764A mutations, HBV subgenotypes and serum HBeAg status

We focused on the relationship of serum HBeAg status, HBV genotypes/subgenotypes and A1762T/G1764A mutations. The patients with positive serum HBeAg had a significantly lower prevalence of the mutations than that with negative HBeAg (47.15% *vs.* 58.18%, *P*=0.017), but those infected with subgenotype C2 had higher mutations prevalence than that infected with subgenotype B2 (65.87% *vs.* 26.11%, *P*<0.0001) (Table 2).

HBV subgenotypes, A1762T/G1764A mutations and clinicopathological characteristics in HCC patients

To investigate whether HBV subgenotypes and A1762T/G1764A mutations were responsible to clinicopathological characteristics in HCC patients, we gathered four important clinicopathological parameters from 156 HCC patients which could represent for clinical behaviors of tumor. The relationship among subgenotypes, the mutations and these parameters was analyzed. We found the frequencies of subgenotype C2 and the mutations were no significant differences between two HCC subgroups that were stratified according to tumor stage, vascular invasion or lymph node metastasis and tumor size respectively, but the prevalence of these two variants in HCC with cirrhosis were

more frequently than in those without cirrhosis (65.42% *vs.* 34.58%, *P*=0.016 for subgenotype C2; 66.30% *vs.* 33.70%, *P*=0.009 for the mutations; respectively) (Table 3).

Table 2: Frequencies of A1762T/G1764A mutations in the patients with different serum HBeAg status and HBV subgenotypes infection

Groups	n	A1762T/G1764A mutations		*P*
		(+) (n=244)	(-) (n=222)	
HBeAg (+)	246	116(47.15)	130 (52.85)	0.017
HBeAg (-)	220	128 (58.18)	92 (41.82)	
Subgenotype B2	157	41 (26.11)	116 (73.89)	<0.0001
Subgenotype C2	293	193 (65.87)	100(34.13)	

Data was presented as number (%)
The data of HBV subgenotype C1 and genotype mixture was not shown because of small sample size

Table 3: The relationship among HBV subgenotypes, A1762T/G1764A mutations and clinicopathological characteristics in HCC patients

Clinicopathological parameters	HCC patients (*n*=156)					
	A1762T/G1764A mutations		*P*	HBV subgenotypes		*P*
	(+) (*n*=92)	(-) (*n*=64)		B2 (*n*=45)	C2 (*n*=107)	
Tumor stage						
I + II	40 (43.48)	30 (46.88)	0.786	22 (48.89)	38 (35.51)	0.124
III + IV	52 (56.52)	34 (53.12)		23 (51.11)	69 (64.49)	
Cirrhosis						
With	61 (66.30)	29 (45.31)	0.009	20 (44.44)	70 (65.42)	0.016
without	31 (33.70)	35 (54.69)		25 (55.56)	37 (34.58)	
Vascular invasion or lymph node metastasis						
With	64 (69.57)	42 (65.63)	0.910	30 (66.67)	74 (69.16)	0.763
Without	28 (30.43)	22 (34.37)		15 (33.33)	33 (30.84)	
Tumor size (cm)						
≥5	62 (67.39)	48 (75.0)	0.416	33 (73.33)	68 (63.55)	0.244
＜5	30 (32.61)	16 (25.0)		12 (26.67)	39 (36.45)	

Tumor stage was classified according to the TNM criteria of International Union Against Cancer
HCC, hepatocellular carcinoma.
The data of HBV subgenotype C1 and Genotype mixture was not shown because of small sample size

Association analysis of HBV subgenotype C2, A1762T/G1764A mutations with progression of CHB

To further understand the risks in progression of chronic HBV infection for HBV subgenotype C2 infection and A1762T/G1764A mutations, the association analysis was used in this study. The results showed both subgenotype C2 infection and the mutations were associated with LC and HCC with cirrhosis, subgenotype C2 (OR=2.033, 95%CI=1.246-3.323, *P*=0.003 for LC *vs* CHB; OR=3.247, 95%CI=1.742-6.096, *P*=0.001 for HCC with cirrhosis *vs* CHB; respectively), and the mutations (OR=1.914, 95%CI=1.188-3.085, *P*=0.005 for LC *vs* CHB; OR=2.996, 95%CI=1.683-5.353, *P*=0.002 for HCC with cirrhosis *vs* CHB; respectively) (Table 4). The risks of

LC and HCC with cirrhosis for subgenotype C2 infection were similar to that for the mutations in the patients with CHB, but the risks of advanced liver diseases for these two variants in combination (OR=2.662, 95%CI=1.593-4.460, *P*=0.001 for LC *vs* CHB; OR=3.837, 95%CI=2.131-6.930, *P*=0.001 for HCC with cirrhosis *vs* CHB; respectively) were seemed to higher than that for univariant (Table 4). Interestingly, no differences in frequencies of these two variants, alone or in combination between LC and HCC with cirrhosis groups were found.

Table 4: Association analysis of HBV subgenotype C2, A1762T/G1764A mutations and advanced liver disease

Variants	CHB (*n*=160)	LC (*n*=150)	HCC with cirrhosis (*n*=90)	OR (95%CI)[a]	*P*[a]	OR(95%CI)[b]	*P*[b]	OR(95%CI)[c]	*P*[c]
Subgenotype C2	83 (51.88)	103 (68.67)	70 (77.78)	2.033 (1.246-3.323)	0.003	3.247 (1.742-6.096)	0.001	1.597 (0.838-3.062)	0.128
A1762T/G1764A mutations	66 (41.25)	86 (57.33)	61 (67.78)	1.914 (1.188-3.085)	0.005	2.996 (1.683-5.353)	0.002	1.565 (0.874-2.812)	0.108
Subgenotype C2 + A1762T/G1764A mutations	38 (23.75)	68 (45.33)	49 (54.44)	2.662 (1.593-4.460)	0.001	3.837 (2.131-6.930)	0.001	1.444 (0.824-2.521)	0.172

Data was presented as number (%).

[a], LC *vs*. CHB ; [b], HCC with cirrhosis *vs*. CHB ; [c], HCC with cirrhosis *vs*. LC

CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence intervals

Discussion

Infection with HBV causes a various clinical manifestation, ranging from asymptomatic infection to acute self-limiting or fulminate hepatitis, or chronic infection. Chronically infected patients may eventually develop to liver failure, cirrhosis or HCC. However, its pathogenesis remains uncertain. It is believed that both the host genetic background and the biological characteristics of HBV are important to its clinical outcomes. The virological factors such as HBV DNA level, HBeAg status, genotypes/subgenotypes and mutations were considered as variants that affected the prognosis of HBV infection (1, 4, 5, 8).

Ten HBV genotypes (A-J) and 34 subgenotypes had been identified (13). Among them, genotypes B and C are predominant in China, but the prevalence of subgenotypes is still unclear, especially in Southeast China. HBV genotype C infection may be related with HCC in general description in current literatures. HBV A1762T/G1764A mutations were first identified by Okamato and Sato et al. from Japanese patients, which may be related to cirrhosis, HCC and acute liver failure (14). Following studies (15-18) suggested that HBV genotype C infection could induce the mutations more frequently than genotype B infection. Nevertheless, some clinical researches (7-9) reported there were no obvious association between HBV genotype /subgenotype C2 infection, the mutations and HCC development, and there is little data to discriminate the differences in risks between HCC with and without cirrhosis for these two variants.

Now it is accepted there are positive correlation between serum HBeAg expression and HBV DNA level usually, which are the indicators of HBV replication. Some prospective studies suggested that the risk of HCC increased with increasing baseline serum HBV DNA levels, which was an independent risk factor for HCC (4, 8, 13-15). In this study, we analyzed 466 patients and did not find any difference in HBeAg status among CHB, LC and HCC patients, but HBV

DNA level showed obviously higher in the patients with advanced liver disease. Our study revealed HBV genotypes B and C were predominant, and B2 and C2 were major subgenotypes in Southeast China. We found the frequencies of both subgenotype C2 and A1762T/G1764A mutations were higher in LC or HCC group than in CHB group, but the differences of both variants did not be found between LC and HCC groups. It indicated preliminarily that subgenotype C2 infection and the mutations were risks of LC and HCC development in patients with CHB, but not the risks of HCC development in patients with cirrhosis. In addition, our data supported that the mutations were closely related with subgenotype C2 infection and occurrence of HBeAg seroconversion. A1762T/G1764A mutations in CHB and LC patients were up to 41.25% (66/160) and 57.33% (86/150), respectively, which suggested it were an early event in HCC development in patients with chronic HBV infection.

Since a large proportion of *HCC in Southeast China* usually developed from *cirrhosis during chronic HBV infection,* we collected clinicopathological data from 156 HCC patients which consisted of four important parameters. The results showed subgenotype C2 infection and A1762T/G1764A mutations were not related to tumor stage, vascular invasion or lymph node metastasis and tumor size, but were both significant difference in distribution frequencies between HCC with cirrhosis and without cirrhosis(P=0.016 for subgenotype C2; P=0.009 for the mutations; respectively). Through association analysis, we found that subgenotype C2 infection and the mutations showed the similar risks of LC or HCC with cirrhosis development in patients with CHB, and the odds ratios of LC and HCC with cirrhosis for two variants in combination seemed to higher than that for uni-variant. Interestingly, these two variants alone or in combination all could not predict the risk of HCC development in patients with cirrhosis. Therefore, there are probably other mutations and cirrhosis are critical in HCC development in LC patients. Meanwhile, we speculated that HCC development in patients with chronic HBV subgenotype C2 infection was related to A1762T/G1764A mutations which contributed to cirrhosis in Chinese.

So far, the mechanisms of HBV genotype C infection and A1762T/G1764A mutations developing to advanced liver disease are still vague. In this study, subgenotype C2 was closely related to the mutations. Some researches indicated the mutations could down-regulate the transcription of pre-C mRNA of HBV through reducing the binding activity of liver-specific transcription factors, and decrease about 70% of HBeAg expression, but up-regulate pregenome RNA resulting in enhancement of virus replication in finally. Since some of the same epitopes presenting in HBeAg and HBcAg, the reduction of HBeAg expression could trigger stronger immune response to HBcAg in the liver cell (16-20). HBV BCP region overlaps partially with HBx coding sequence, so A1762T/G1764A mutations result in coding changes at codons 130 and 131 in the HBX protein changing lysine to methionine and valine to isoleucine, respectively, which affect the transactivation capability of HBX protein (21-23). The mutations locate in p53 binding domains, can affect the multiple functions of p53 including tumor suppression, DNA damage repair and cell cycle regulation, etc (24). In addition, the mutations also can promote liver cell apoptosis, which is an important pathophysiological basis of hepatic fibrosis (25).

Some reports suggested that HBV genotype C infection and A1762T/G1764A mutations were not related to LC and HCC (7- 9, 26). The biases of these studies probably resulted from the number of participants, study design, statistical methods using and population race. A cross-sectional case-control study, which compared the frequencies of HBV genotypes/subgenotypes and the mutations among CHB patients, LC patients, and HCC patients, should be reliable for analyzing the risk factors of advanced liver disease. In conclusion, HBV subgenotype C2 infection and A1762T/G1764A mutations are both risk factors of LC and HCC with cirrhosis development in CHB patients in Southeast China, which may be useful biomarkers alone or in combination for

predicting these diseases, but all no helpful for predicting HCC development in LC patients.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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Abbreviations

HBV: hepatitis B virus
BCP: basal core promoter region
CHB: chronic hepatitis B
LC: liver cirrhosis
HCC: hepatocellular carcinoma
ALT: alanine aminotransferase
AST: aspartate aminotransferase
TBIL: total bilirubin
HbeAg: HBV e antigen
AFP: α fetoprotein
FQ-PCR: fluorescence quantitative polymerase chain reaction
OR: odds ratio
CI: confidence intervals

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