Role of hepatitis B virus genotypes Ba and C, core promoter and precore mutations on hepatocellular carcinoma: a case control study

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The role of hepatitis B virus (HBV) genotypes, core promoter (CP) and precore mutants on hepatocellular carcinoma (HCC) is still controversial. We aimed to determine their role on the development and clinical features of HCC. HBV genotypes and CP/precore mutations were determined in 90 HCC patients and 180 matched control patients. In the 90 HCC patients, 22 (24.4%) and 68 (75.6%) had subtype Ba and genotype C, respectively. The prevalence of genotype C and CP mutations was significantly higher in HCC patients compared with controls (75.6 versus 57.8%, P = 0.004; 90.9 versus 74.8%, respectively, P = 0.007). Among carriers of genotype C, 91.8% of the HCC patients and 88.8% of controls had CP mutations. Among carriers of subtype Ba, HCC patients had a higher prevalence of CP mutations compared with controls (88.2 versus 54.5%, respectively, P = 0.02). By logistic regression analysis, the only factor associated with HCC was a mutation of the CP region (P = 0.032). There were no differences in the clinical features on presentation, the chance of receiving treatment and the cumulative survival rate for chemoembolization-treated patients between patients with subtype Ba and genotype C. There was too small a number of CP wild-type to do a similar comparison with CP mutants. In conclusion, there was a significantly higher prevalence of both genotype C and CP mutations in patients with HCC. The association between HBV genotype C and HCC was probably not genuine but was due to the high percentage of CP mutations in patients with genotype C.

Introduction

Hepatitis B virus (HBV) genotypes have been shown to influence the disease profile of chronic hepatitis B infection in some recent studies (1–9). In Asia where HBV genotypes B and C are commonly found, the natural history of patients with genotype B differs from those with genotype C. The former had a higher rate and earlier age of hepatitis B e antigen (HBeAg) seroconversion, less serious liver diseases and better interferon treatment response compared with the latter (1–7,10,11).

However, the exact role of these two genotypes on the development of hepatocellular carcinoma (HCC) is still controversial. According to an early Taiwan study, patients with genotype B are associated with the development of HCC at young age (< 50 years old) (12). Subsequent studies conducted in Japan, however show contradictory findings. HBV genotype C is more commonly found in HCC patients below age 50 (13,14). According to Orito and his colleagues, the mean age of the HCC patients with genotype B is older than that of patients with genotype C (15). Another study conducted by Sumi and his colleagues report that although patients with genotype C compared with patients with genotype B are associated with a slower development of HCC, there is no difference in the life-time risk of development of HCC (2). The role of HBV genotypes in the development of HCC therefore remains uncertain.

Patients with core promoter (CP) mutants compared with those with wild-type have also been shown to have a higher risk of development of HCC (16–18). It has been demonstrated further that HBV genotype C is associated with CP mutations (1). It is important to elucidate the interplay between HBV genotypes and CP mutations since both are associated with the development of HCC.

Concerning the clinical features of HCC, a previous study examining nine patients (four with genotype B, five with genotype C) with HCC shows that patients with genotype B have a better response to embolization therapy and better survival compared with patients with genotype C (19). Large-scale studies are required to determine whether there are any differences in the clinical presentation, chance of receiving treatment and outcome of the HCC between patients with genotypes B and C.

The aims of the present study are to elucidate the exact role of HBV genotypes, CP and precore mutations on the development of HCC and to determine whether there were any differences between HCC patients with genotypes B and C in terms of the clinical features, the chance of receiving treatment and survival.

Materials and methods

Between January 1999 and December 2002, all patients (n = 128) presented with HCC to the Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong were recruited in the present study. The diagnosis of HCC was made in 100 patients by the elevated alpha-fetoprotein (AFP) with typical features of HCC by imaging studies (computerized tomography or magnetic resonance imaging). For the 28 patients with normal AFP level, HCCs were diagnosed by ultrasound guided fine needle aspiration. Of the 128 patients, 26 were excluded because of non-HBV etiology of HCC (10 had chronic hepatitis C infection [all were negative for hepatitis B surface antigen (HBsAg) and six were positive for antibody to HBsAg], 12 had

Abbreviations: AFP, alpha-fetoprotein; CP, core promoter; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; TACE, transarterial chemoembolization.
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alcoholic liver disease, four had cryptogenic cirrhosis]. The remaining 102 patients were positive for HBsAg and negative for anti-HDV. Of these, 90 patients in whom the HBV genotype results were available were recruited for analyses in the present study. The remaining 12 patients obtained no PCR amplification for HBV genotyping.

Blood was taken for measurement of complete blood count, liver biochemistry, prothrombin time, AFP. HBV serological markers including HBsAg, HBeAg and antibody to HBeAg (anti-HBe) [measured by the micro-particle enzyme immunoassay (MEIA), Abbott Laboratories, Chicago, IL]. Serum HBV DNA levels were measured by Digene Hybrid Capture assay, Digene Corporation, Gaithersburg, MD (lower limit of detection 0.14 × 10⁶ copies/ml).

All patients were assessed for the feasibility of surgical resection. For patients in whom surgical resections were not possible, they were assessed for the possibility of transarterial chemoembolization (TACE) treatment, which was performed every 8–12 weeks continuously. TACE would be terminated in patients with progressive disease after five treatment courses. Tumor responses were assessed by the difference in the largest dimensions of the index tumor at the first and the last sessions of TACE at the time of writing. If TACE was not feasible, patients were treated conservatively.

To examine the role of HBV genotypes B and C in the development of HCC, every patient with HCC was matched for gender, age and HBeAg status with two HBsAg-positive patients without HCC. Therefore, the total number of disease controls was 180. These control patients are consecutive patients with chronic hepatitis B infection without HCC, being followed up during the period of recruitment of the present study in our Hepatitis Clinic, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong.

HBV genotyping for the 90 patients with HCC and 180 patients without HCC (controls) was determined by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies against seven distinct epitopes (b, m, k, s, u, f, and g) on the preS2-region products of HBsAg (20). HBV genotype B subtypes were determined by a PCR-restriction fragment length polymorphism method as described in our previous study (21).

The CP and precore mutations were determined by direct sequencing. Briefly, serum HBV DNA, in a final volume of 200 μl, was extracted from 200 μl of serum, using the QIAGEN DNA blood mini kit (QIAGEN GmbH, Germany). The HBV genome at nucleotides (nt) 1653–1974 containing the precore and CP regions was amplified by PCR using the primers HBV1 (5'-CATAAAGGACTCTTGGACT-3') and HBV2 (5'-GGAAAGAGTGCA-GAGGC-3') in a GeneAmp 9700 PCR system (Applied Biosystems, USA) with the following conditions: 95°C for 10 min, followed by 94°C for 30 s, 54°C for 30 s and 72°C for 1 min up to 40 cycles and a final extension 72°C for 10 min. The 322 bp PCR products were purified by ethanol precipitation with ammonium acetate in a final volume of 20 ml. Two microliters of the purified amplified DNA products after PCR were used in a thermocycle sequencing with the DYE terminer ET Terminator Cycle Sequencing Kit as directed (Amersham Biosciences, Uppsala, Sweden) using HBV2 as the primer. Automated sequencing was performed with an ABI prism 3700 DNA Analyzer. For CP region, the wild-type (nt 1762T/1764A) and the mutants (nt 1762A/1764A, nt 1762A/1764T, nt 1762T/1764A) were determined. For the precore region, the wild-type (nt 1896G) and the mutant (nt 1896A) were determined.

Statistical analysis

All statistical analyses were performed using the Statistical Program for Social Sciences (SPSS 10.0 for windows, SPSS, Chicago, IL). Continuous variables with skewed distribution were tested by Mann–Whitney U test. Categorical variables were tested by χ² test with Yates’ correction or Fisher’s exact test. Logistic regression analysis was used to test whether factors were independently associated with the development of HCC. The cumulative survival was examined by Kaplan–Meier analysis using log rank test. Values of <0.05 were considered as statistically significant.

Results

Demographics

The demographic data of the 90 patients with HCC are listed in Table 1.

Prevalence of HBV genotypes in HCC patients

Twenty-two patients (24.4%) had genotype B. All were of subtype Ba. The remaining 68 patients (75.6%) had genotype C. The distribution of subtype Ba and genotype C in patients with HCC [median age 61.1 (range 32.8–81.2) years] and control patients [median age 59.7 (range 33.3–81) years, P = 0.59] is illustrated in Figure 1. Patients with HCC had a higher prevalence of genotype C compared with control patients [75.6 versus 57.8% (104 out of 180), respectively, P = 0.004, odds ratio (OR) 2.3, 95% confidence interval (CI) 1.3–4.0]. Patients with young onset of HCC (≤50 years old) had a higher prevalence of genotype C compared with control patients (age ≤50 years old) (84.4 versus 54.2%, respectively, P = 0.014, OR 5.4, 95% CI 1.4–20.5). There was a trend for patients with late onset of HCC (>50 years old) to have a higher prevalence of genotype C compared with control patients (age >50 years old) (72.1 versus 59.1%, respectively, P = 0.07). However, there was no difference in the median age of development of HCC between patients with subtype Ba and genotype C [60.1 (range 32.8–81.2) versus 63.0 (range 40.4–79.2) years, respectively, P = 0.35].

CP and precore mutations and HCC

Sixty-six HCC patients and 135 control patients were available for analyses of CP and precore mutations (the unavailability of the remaining samples were due to the absence of the amplified DNA products after PCR). All the CP mutations identified in the HCC patients and control patients had double mutations, i.e. nt 1762T/1764A. All the precore mutations identified in HCC patients and control patients were nt 1896A. There was a significantly higher percentage of patients with CP mutations in the HCC group (60 out of 66, 90.9%) compared with that of the control group (101 out of 135, 74.8%; P = 0.007, OR 3.4, 95% CI 1.3–8.5). There was no difference in the percentage of

| Table 1. Demographic data of the 90 patients with HCC |
|------------------|-------------|-------------|
| Number of patients | 90          |             |
| Sex M:F            | 75:15       |             |
| Median age (range), years | 61.1 (32.8–81.2) | 23:67 |
| HBsAg:anti-HBe     |             |             |
| HBV DNA level, ×10⁶ copies/ml (range) | 0.5 (<0.142–593.6) |         |
| Liver biochemistry, median (range) |             |             |
| Albumin, g/l       | 37 (16–47)  |             |
| Bilirubin, μmol/l  | 19 (6–196)  |             |
|Alanine aminotransferase, U/l | 49.5 (4–1154) | 54 (6–109) |
| Median prothrombin time, s | 13.5 (10.2–30.7) | 117.5 (20–626) |
| Median platelet count, ×10⁹/l |             |             |
| Child-Pugh grade   |             |             |
| A (%)              | 46 (51.1)   |             |
| B (%)              | 38 (42.2)   |             |
| C (%)              | 6 (6.7)     |             |
| Features of HCC    |             |             |
| AFP, ng/ml         | 83 (3–1 060 000) | 45:45 |
| Unifocal:multifocal |             |             |
| Median largest dimension of the index tumor (range), cm | 3.1 (0.7–19) |
| Portal vein thrombosis (%) | 9 (10) |
| CLIP score         |             |             |
| Median (range)     | 2 (0–6)     |             |
| 0–3 (%)            | 66 (73.3)   |             |
| 4–6 (%)            | 24 (26.7)   |             |
| Okuda stage        |             |             |
| I (%)              | 40 (44.4)   |             |
| II (%)             | 46 (51.1)   |             |
| III (%)            | 4 (4.4)     |             |
| Median follow-up (range), months | 15.6 (0.2–108.7) |

HBsAg, hepatitis B e antigen; anti-HBe, antibody to HBsAg; CLIP, Cancer of the Liver Italian Program.
patients with precore mutations between the HCC group and the control group (18 out of 66, 27.3% versus 45 out of 90, 33.3%, respectively, \( P = 0.38 \)).

**HBV genotypes, CP mutations and HCC**

By using logistic regression analysis, the presence of CP mutations was the only independent factor associated with development of HCC \( (P = 0.032, \text{OR} \ 2.8, 95\% \ CI \ 1.1-7.4) \). HBV genotype was not a significant independent factor.

The role of CP mutations in HCC was further examined separately in patients with subtype Ba and genotype C. Of the 66 HCC patients with available results of CP sequences, 17 had subtype Ba and 49 had genotype C. CP mutations were found in 15 HCC patients (88.2%) with subtype Ba and in 45 HCC patients (91.8%) with genotype C. There was no difference in the prevalence of CP mutations between HCC and control patients with genotype C (45 out of 49, 91.8% versus 71 out of 80, 88.8%, respectively; \( P = 0.77 \)). Interestingly, there was a significantly higher prevalence of CP mutations in HCC patients compared with control patients with subtype Ba (15 out of 17, 88.2% versus 30 out of 55, 54.5%, respectively; \( P = 0.02, \text{OR} \ 6.3, 95\% \ CI \ 1.3-30.0 \)).

**Clinical features of HCC patients with genotypes Ba and C**

Since only six out of 66 HCC patients had CP wild-type, comparison of the clinical features between patients with CP mutants and those with wild-type could not be performed due to type II errors. The various comparisons between patients with subtype Ba and those with genotype C were analyzed below.

**Liver function tests, HBV DNA levels and tumor characteristics**

The demographic data, prevalence of CP and precore mutations, liver biochemistry, prothrombin time, platelet counts and HBV DNA levels of HCC patients with genotypes Ba and C are listed in Table II. There were no differences in any of the parameters. The characteristics of HCC in patients with genotypes Ba and C are also listed in Table II. There were also no differences in any tumor parameters between the two groups of patients.

**Treatment of HCC**

Of the 90 patients with HCC, 14 patients (15.6%) were treated with surgical resection and 46 patients (51.1%) were treated with TACE. There were no significant differences in the chances of receiving surgical resection [5/22 (22.7%) versus 9/68 (13.2%), \( P = 0.32 \)] and TACE treatment [10/22 (45.5%) versus 36/68 (52.9%), \( P = 0.72 \)] between patients with genotypes Ba and C, respectively.

For TACE treatment, tumor responses defined as a 50% reduction of the largest dimension were observed in three patients (30%) with subtype Ba and 12 patients (38.7%, \( P = 1.0 \)) with genotype C. There was no difference in the percentage of patients with a reduction of AFP level, which was defined as a 50% reduction for those with an elevated AFP level at baseline, between patients with subtype Ba and those with genotype C [1/5 (20%) versus 4/23 (17.4%), respectively, \( P = 1.0 \)].

**Survival**

The cumulative survival rate was examined in 46 patients who received TACE (10 with subtype Ba, 36 with genotype C) (Figure 2). There was no difference in the cumulative survival rate between patients with subtype Ba and genotype C \( (P = 0.65) \). The median survival time was 45 and 47.5 months for patients with genotypes Ba and C, respectively. The number of patients receiving surgical resection or conservative treatment was too small for meaningful statistical analysis.

**Discussion**

All the patients with genotype B in the present study from Hong Kong were of subtype Ba. This supports the phenomenon observed by Sugauchi et al. that HBV genotype Bj is exclusively found in Japan and Ba in the rest of the countries of Asia (21,22). Any differences in the disease profile of HCC...
between patients with subtypes Bj and Ba would require comparative studies of patients from different countries in Asia.

In the present study, HBV genotype C and CP mutants had a higher chance of development of HCC when compared with HBV subtype Ba and CP wild-type, respectively. However, the only independent factor that was associated with HCC was CP mutations. HBV genotype C was not an independent factor. It has been shown that HBV genotypes are associated with the presence or absence of CP mutations (1,2,5,15). The apparent association between genotype C and HCC as demonstrated initially in the present study as well as other previous studies (13,14) might be in fact due to the close association between HBV genotype C and CP mutations, with CP mutations being the real risk factor for HCC. However, when genotype B (mainly subtype Bj) was compared with genotype C in a previous Japanese study, carriers of genotype C were independently associated with advanced liver diseases such as HCC (14). This might depend on the incidence of CP mutations among subtypes Bj, Ba and genotype C. A recent case-control study indicated that the double mutation in the CP occurred significantly less often in carriers of HBV subtype Bj than those of subtype Ba (15 versus 33%, \( P < 0.05 \)) or genotype C (15 versus 63%, \( P < 0.001 \)) (21). It is currently believed that subtype Ba is derived from the recombination of subtype Bj with genotype C in the precore and core regions (22), spanning nucleotide position from 1740--1838 to 2443--2485 (including the cytotoxic T-cell epitopes). Thus, subtype Ba is similar to genotype C in having a higher disease inducing capacity than subtype Bj. Additionally, even in HCC patients with subtype Ba, the prevalence of CP mutations was extraordinarily high when compared with that of controls.

| Patients with subtype Ba \( (n = 22) \) | Patients with genotype C \( (n = 68) \) | \( P \) value |
|---|---|---|
| Age | 60.1 (32.8-81.2) | 63.0 (40.4-79.2) | 0.35 |
| Sex M:F | 19:3 | 56:12 | 1.0 |
| HBsAg:anti-HBc | 3:19 | 20:48 | 0.17 |
| CP mutations | 15/17 (88.2%) | 45/49 (91.8%) | 0.64 |
| Precore mutations | 8/17 (47.1%) | 10/49 (20.4%) | 0.07 |

### Liver Biochemistry

| | Patients with subtype Ba \( \times 10^6 \) | Patients with genotype C \( \times 10^6 \) | \( P \) value |
|---|---|---|---|
| Albumin, g/l | 35.5 (19-47) | 37 (16-47) | 0.87 |
| Bilirubin, \( \mu \text{mol/l} \) | 17.5 (6-88) | 20.5 (6-196) | 0.10 |
| ALT, U/l | 39.5 (16-506) | 52.5 (4-1154) | 0.15 |
| ALP, U/l | 123 (62-511) | 113.5 (55-511) | 0.72 |
| Platelet count, \( \times 10^9 /l \) | 116 (56-441) | 118.5 (20-626) | 0.95 |
| Prothrombin time, s | 13.4 (11.5-17.5) | 13.6 (10.2-30.7) | 0.97 |

| | Patients with subtype Ba | Patients with genotype C | \( P \) value |
|---|---|---|---|
| Child-Pugh grade A (%) | 11 (50) | 35 (51.5) | 0.87 |
| B (%) | 10 (45.5) | 28 (42.1) | 0.61 |
| C (%) | 1 (4.5) | 5 (7.4) | 0.46 |
| HBV DNA level, \( \times 10^6 \) copies/ml (range) | 0.48 (<0.142-517.3) | 0.56 (<0.142-593.6) | 0.71 |
| Median AFP, ng/ml | 55 (3-464 000) | 100 (3-1 060 000) | 0.43 |

### Tumor Parameters

| | Patients with subtype Ba | Patients with genotype C | \( P \) value |
|---|---|---|---|
| Unifocal-multifocal (%) | 13.9 (59:140:9) | 32:36 (47:1:52:9) | 0.44 |
| Median largest dimension of the index tumor (range), cm | 3.3 (0.7-10.2) | 3.1 (0.8-19) | 1.0 |
| Portal vein thrombosis (%) | 2 (0-5) | 8 (11.8) | 0.44 |
| Distant metastasis (%) | 2 (0-5) | 3 (4.4) | 0.26 |
| CLIP score | 5.0 (0-15) | 5.0 (0-15) | 0.39 |
| Median (range) | 2 (0-5) | 2 (0-6) | 0.26 |
| 0-3 (%) | 18 (81.8) | 48 (70.6) | 0.44 |
| 4-6 (%) | 4 (18.2) | 20 (29.4) | 0.43 |

### Okuda stage

| | Patients with subtype Ba | Patients with genotype C | \( P \) value |
|---|---|---|---|
| I (%) | 12 (54.5) | 28 (41.2) | 0.53 |
| II (%) | 9 (40.9) | 37 (54.4) | 0.43 |
| III (%) | 1 (4.5) | 3 (4.4) | 0.43 |

Continuous variables are expressed in median (range). M, male; F, female; ALT, alanine aminotransferase; ALP, alkaline phosphatase; CLIP, Cancer of the Liver Italian Program.

Fig. 2. The cumulative survival of patients with subtype Ba (solid line) and C (dotted line) who were treated with transarterial chemoembolization.
(88.2 versus 54.5%, respectively, \( P = 0.02 \)). However, in view of the relatively small number of HCC patients with subtype Ba in the present study, larger series are required to confirm this finding. Nevertheless, similar results have been shown in a recent study conducted by Kao et al. (16) that the risk of HCC for patients with genotype B (mainly of subtype Ba\(^2\)) and C is high if they have CP mutations. Although the exact mechanism for the development of HCC with CP mutations remains unclear, the CP mutations have been shown to increase viral replication at least by two ways. First, CP mutations by causing a shift change of the viral pre-genomic secondary structure, can lead to an enhanced viral replication (23). Secondly, the transcription of the pre-genomic RNA may also be increased by the CP mutations through the removal of the nuclear receptor-binding site and at the same time creating a binding site for hepatocyte nuclear factor (24). This combination of changes increases the core RNA transcription (enhances core protein, DNA polymerase, pre-genomic RNA synthesis) but suppresses the precore RNA transcription (normally decreases the pre-genomic RNA packaging) (25). Therefore, the end result will be an increase in viral replication. However, this enhanced viral replication has not been confirmed from other cross-sectional studies (7,26,27). Longitudinal studies monitoring viral load continuously are required to demonstrate the effects of CP mutations on the viral replication in chronic hepatitis B infection, which is a longstanding disease. Furthermore, the CP mutations (T1762/A1764) may induce not only amino acid change in X protein but also an alteration of HBV gene expression. The alteration of X protein might play a role in hepatocarcinogenesis, because its coding sequence overlaps regions of crucial importance for viral replication such as enhancer II and the CP (18,28). Besides, CP mutations may give rise to HCC through other unknown pathways that also require further investigations.

Many previous studies (1–7) show that patients with genotype B, compared with patients with genotype C have less aggressive disease as reflected by the higher HBsAg seroconversion and better histology. Further studies are required to determine whether these favorable parameters observed in patients with genotype B are due to the lower chance of having CP mutations. But, as far as the risk for HCC is concerned, the present study showed that patients with subtype Ba with concomitant CP mutations had a higher risk of development of HCC compared with that of patients with subtype Ba without CP mutations (OR 6.3, 95% CI 1.3–30.0). There was no difference in the risk for HCC between patients with subtype Ba and genotype C when the confounding factor, i.e. CP mutations, was taken into consideration.

In addition, the present study showed that there were no differences in the liver function, the tumor characteristics, chance of receiving treatment and survival between patients with subtype Ba and genotype C. It was unfortunate that similar comparisons between patients with CP mutants and wild-type could not be carried out because the overwhelming majority of HCC patients, 91.0%, had CP mutations.

In conclusion, there was a significantly higher prevalence of both genotype C and CP mutations in patients with HCC. The association between HBV genotype C and HCC was probably not genuine but was due to the high percentage of CP mutations. Concomitant CP mutations in patients with subtype Ba increased the risk of development of HCC. There were no differences in clinical features, chance of receiving treatment and survival between HCC patients with subtype Ba and genotype C.

References

1. Yuen,M.F., Sablon,E., Yuan,H.J., Wong,D.K.H., Hui,C.K., Wong,B.C.Y., Chan,A.O.O. and Lai,C.L. (2003) The significance of hepatitis B genotype in acute exacerbation, HBeAg seroconversion, cirrhosis-related complications and hepatocellular carcinoma. Hepatology, 37, 562–567.
2. Suni,H., Yokosuka,O., Seki,N., Araki,M., Imazeki,F., Kurihara,T., Kanda,T., Fukai,K., Kato,M. and Saisho,H. (2003) Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. Hepatology, 37, 10–26.
3. Chu,C.J., Hussain,M. and Lok,A.S.F. (2002) Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. Gastroenterology, 122, 1756–1762.
4. Chan,H.L., Tsang,S.W., Liew,C.T., Tse,C.H., Wong,M.L., Ching,J.Y., Leung,N.W., Tam,J.S.L. and Sung,J.Y.Y. (2002) Viral genotype and hepatitis B virus DNA levels are correlated with histological liver damage in HBeAg-negative chronic hepatitis B virus infection. Am. J. Gastroenterol., 97, 406–412.
5. Ding,X., Mizokami,M., Ge,X., Orito,E., Iino,S., Ueda,R. and Nakanishi,M. (2002) Different hepatitis B virus genotype distributions among asymptomatic carriers and patients with liver diseases in Nanning, southern China. Hepatol. Res., 22, 37–44.
6. Shiina,S., Fujino,H., Uta,Y., Tagawa,K., Unuma,Y., Yoneyama,M., Ohmori,T., Suzuki,S., Kurita,M. and Ohashil,Y. (1991) Relationship of HBsAg subtypes with HBeAg/anti-HBe status and chronic liver disease Part I: analysis of 1744 HBsAg carriers. Am. J. Gastroenterol., 86, 866–871.
7. Lindh,M., Hannoun,C., Dhillon,A.P., Norkrans,G. and Horal,P. (1999) Core promoter mutations and genotypes in relation to viral replication and liver damage in East Asian hepatitis B virus carriers. J. Infect. Dis., 179, 775–782.
8. Sanchez-Tapias,J.M., Costa,J., Mas,A., Bruguera,M. and Rodes,J. (2002) Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. Gastroenterology, 123, 845–856.
9. Kao,J.H., Chen,P.J., Lai,M.Y. and Chen,D.S. (2002) Genotypes and clinical phenotypes of hepatitis B virus patients in chronic hepatitis B virus infection. J. Clin. Microbiol., 40, 1207–1209.
10. Kao,J.H., Wu,N.H., Chen,P.J., Lai,M.Y. and Chen,D.S. (2000) Hepatitis B genotypes and the response to interferon therapy. J. Hepatol., 33, 998–1002.
11. Wai,C.Y., Chu,C.J., Hussain,M. and Lok,A.S. (2002) HBV genotype B is associated with a higher response rate to interferon therapy than genotype C. Hepatology, 36, 1425–1430.
12. Kao,J.H., Chen,P.J., Lai,M.Y. and Chen,D.S. (2000) Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology, 118, 554–559.
13. Fuji,H., Moriya,K., Shintani,Y., Totsuyanagi,H., Iino,S., Kimura,S. and Koike,K. (2001) Hepatitis B genotypes and hepatocellular carcinoma in Japan. Gastroenterology, 120, 1564–1565.
14. Sakugawa,H., Nakaseo,H., Nakayoshi,T., Orito,E., Mizokami,M., Yamashiro,T., Maeshiro,T., Kinjo,F., Saito,A. and Miyagi,Y. (2002) Preponderance of hepatitis B virus genotype B contributes to a better prognosis of chronic HBV infection in Okinawa, Japan. J. Med. Virol., 67, 484–489.
15. Orito,E., Mizokami,M., Sakugawa,H., Michitaka,K., Ishikawa,K., Ichida,T., Okanouchi,T., Totsuyanagi,H. and Iino,S. (2001) A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C in Japan HBV genotype research group. Hepatology, 33, 218–223.
16. Kao,J.H., Chen,P.J., Lai,M.Y. and Chen,D.S. (2003) Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology, 124, 327–334.
17. Fang,Z.L., Ling,R., Wang,S.S., Nong,J., Huang,C.S. and Harrison,T.J. (1998) HBV core promoter mutations prevail in patients with hepatocellular carcinoma from Guangxi, China. J. Med. Virol., 56, 18–24.
18. Baptista,M., Kramvis,A. and Kew,M.C. (1999) High prevalence of 1762T/1764A mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. Hepatology, 29, 946–953.
19. Tsubota, A., Arase, Y., Ren, F., Tanaka, H., Ikeda, K. and Kumada, H. (2001) Genotype may correlate with liver carcinogenesis and tumor characteristics in cirrhotic patients infected with hepatitis B virus subtype adw. *J. Med. Virol.*, **65**, 257–265.

20. Usuda, S., Okamoto, H., Iwanari, H., Baba, K., Tsuda, F., Miyakawa, Y. and Mayami, M. (1999) Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. *J. Virol. Methods*, **80**, 97–112.

21. Sugauchi, F., Orito, E., Ichida, T., Kato, H., Chutaputti, A., Lai, C.L., Gish, R., Ueda, R., Miyakawa, Y. and Mizokami, M. (2003) Epidemiologic and virologic characteristics of hepatitis B virus genotype B having the recombination with genotype C. *Gastroenterology*, **124**, 925–932.

22. Sugauchi, F., Orito, E., Ichida, T. et al. (2002) Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. *J. Virol.*, **76**, 5985–5992.

23. Kidd, A.H. and Kidd-Ljunggren, K. (1996) A revised secondary structure model for the 3'-end of hepatitis B virus pregenomic RNA. *Nucleic Acid Res.*, **24**, 3295–3301.

24. Li, J., Buckwold, V.E., Hon, M.W. and Ou, J.H. (1999) Mechanism of suppression of hepatitis B virus precore RNA transcription by a frequent double mutation. *J. Virol.*, **73**, 1239–1244.

25. Buckwold, V.E., Xu, Z., Chen, M., Yen, T.S.B. and Ou, J.H. (1996) Effects of a naturally occurred mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J. Virol.*, **70**, 5845–5851.

26. Kurosaki, M., Enomoto, N., Asahina, Y., Sakuma, L., Ikeda, T., Tozuka, S., Iwami, N., Marumo, F. and Sato, C. (1996) Mutations in the core promoter region of hepatitis B virus in patients with chronic hepatitis B. *J. Med. Virol.*, **49**, 115–123.

27. Yuen, M.F., Sablon, E., Yuan, H.J., Hui, C.K., Wong, D.K.H., Doutreloigne, J., Wong, B.C.Y., Chan, A.O.O. and Lai, C.L. (2002) The relationship between the development of precore and core promoter mutations and HBsAg seroconversion in chronic hepatitis B. *J. Infect. Dis.*, **186**, 1335–1338.

28. Kidd-Ljunggren, K., Oberg, M. and Kidd, A.H. (1995) The hepatitis B virus X gene: analysis of functional domain variation and gene phylogeny using multiple sequences. *J. Gen. Virol.*, **76** (Pt 9), 2119–2130.

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