BCP mutations (A1762T/G1764A) were reported to be associated with the development of HCC.[41,42] However, other studies have reported that BCP mutations do not predict HCC development and that genotype C HBV infection is an independent risk factor for HCC development.[43,44] In this study, all patients were infected with HBV genotype C, and A1762T/G1764A mutations were more common for the HBV genotype C than genotype B,[45] therefore A1762T/G1764A mutations have higher positive rates in both the non-HCC and HCC groups. Although the G1764A mutation differed significantly only at the beginning of the follow-up between the non-HCC and the HCC group, A1762T/G1764A mutations showed a higher positive rate in the HCC group than in the non-HCC group at both the beginning of follow-up and the end of follow-up.

Although several factors differed significantly, according to univariate analysis, between the HCC and non-HCC groups at the beginning of follow-up (Table 7), multivariate analysis showed that only the G1317A mutation and HBeAg negativity were associated with the development of HCC. Since pre-S2 deletions and T1341C/A/G overlapped with each other or with other variables such as A1762T and G1764A mutants, they may be not independent predictors of the development of HCC.

In conclusion, some substitution and deletion mutations may be predictive factors for the development of HCC. G1317A, T1341C/A/G mutations in the X promoter region and pre-S2 deletion in chronic HBV carriers may be useful markers for predicting the risk of developing HCC.

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References

1 Lok AS, McMahon BJ. Chronic hepatitis B: update of recommendations. *Hepatology* 2004; **39**: 857–61.
2 Waris G, Siddiqui A. Regulatory mechanisms of viral hepatitis B and C. *J Biosci* 2003; **28**: 311–21.
3 Koike K. Hepatitis B virus HBx gene and hepatocarcinogenesis. *Intervirology* 1995; **38**: 134–42.
4 Levrero M, Balsano C, Avantaggiati ML *et al.* Hepatitis B virus and hepatocellular carcinoma: a possible role for the viral transactivators. *Ital J Gastroenterol* 1991; **23**: 576–83.
5 Kekule AS, Lauer U, Meyer M *et al.* The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. *Nature* 1990; **343**: 457–61.
6 Lauer U, Weiss L, Hofschneider PH *et al.* The hepatitis B virus pre-S/S (t) transactivator is generated by 3′ truncations within a defined region of the S gene. *J Virol* 1992; **66**: 5284–9.
7 Pollicino T, Campo S, Raimondo G. PreS and core gene heterogeneity in hepatitis B virus (HBV) genomes isolated from patients with long-lasting HBV chronic infection. *Virology* 1995; **208**: 672–7.
8 Pollicino T, Zanetti AR, Cacciola I *et al.* Pre-S2 defective hepatitis B virus infection in patients with fulminant hepatitis. *Hepatology* 1997; **26**: 495–9.
9 Kajiya Y, Hamasaki K, Nakata K *et al.* Full-length sequence and functional analysis of hepatitis B virus genome in a virus carrier: a case report suggesting the impact of pre-S and core promoter mutations on the progression of the disease. *J Viral Hepat* 2002; **9**: 149–56.
10 Takahashi K, Akahane Y, Hino K *et al.* Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients: comparative analysis of 40 full-length isolates. *Arch Virol* 1998; **143**: 2313–26.
11 Sumi H, Yokosuka O, Seki N *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37**: 19–26.
12 Gunther S, Li BC, Miska S *et al.* A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. *J Virol* 1995; **69**: 5437–44.
13 Stuyver LJ, Locarnini SA, Lok A *et al.* Nomenclature for antiviral-resistant human hepatitis B virus mutations in the polymerase region. *Hepatology* 2001; **33**: 751–7.
14 Kobayashi M, Koike K. Complete nucleotide sequence of hepatitis B virus DNA of subtype adr and its conserved gene organization. *Gene* 1984; **30**: 227–32.
15 Huy TT, Ushijima H, Quang VX *et al.* Genotype C of hepatitis B virus can be classified into at least two subgroups. *J Gen Virol* 2004; **85**: 283–92.
16 Norder H, Courouce AM, Coursaget P *et al.* Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289–309.
17 Tanaka Y, Orito E, Yuen MF *et al.* Two subtypes (subgenotypes) of hepatitis B virus genotype C: a novel subtyping assay based on restriction fragment length polymorphism. *Hepatol Res* 2005; **33**: 216–24.
18 Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980; **16**: 111–20.
19 Mizokami M, Orito E, Ohba K *et al.* Constrained evolution with respect to gene overlap of hepatitis B virus. *J Mol Evol* 1997; **44**: S83–90.
20 Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29–60.
21 Sirma H, Giannini C, Poussin K *et al.* Hepatitis B virus X mutants, present in hepatocellular carcinoma tissue abrogate both the antiproliferative and transactivation effects of HBx. *Oncogene* 1999; **18**: 48 448–59.
22 Yen TS. Hepadnaviral X protein: Review of recent progress. *J Biomed Sci* 1996; **3**: 20–30.
23 Urashima T, Saigo K, Kobayashi S *et al.* Identification of hepatitis B virus integration in hepatitis C virus-infected hepatocellular carcinoma tissues. *J Hepatol* 1997; **26**: 771–8.
24 Nakamura I, Koike K. Identification of a binding protein to the X gene promoter region of hepatitis B virus. *Virology* 1992; **191**: 533–40.
25 Tokusumi Y, Zhou S, Takada S. Nuclear respiratory factor 1 plays an essential role in transcriptional initiation from the hepatitis B virus gene promoter. *J Virol* 2004; **78**: 10 856–64.
26 Guo WT, Bell KD, Ou JH. Characterization of the hepatitis B virus Enh1 Enhancer and X promoter complex. *J Virol* 1991; **65**: 6686–92.
27 Raimondo G, Campo S, Smedile V *et al.* Hepatitis B virus variant, with a deletion in the preS2 and two translational stop codons in the precore regions, in a patient with hepatocellular carcinoma. *J Hepatol* 1991; **13**: S74–7.
28 Santantonio T, Jung MC, Schneider R *et al.* Hepatitis B virus genomes that cannot synthesize pre-S2 proteins occur frequently and as dominant virus populations in chronic carriers in Italy. *Virology* 1992; **188**: 948–52.
29 Huy TT, Ushijima H, Win KM *et al.* High prevalence of hepatitis B virus pre-s mutant in countries where it is endemic and its relationship with genotype and chronicity. *J Clin Microbiol* 2003; **41**: 5449–55.
30 Pontisso P, Schiavon E, Fraiese A *et al.* Antibody to the hepatitis B virus receptor for polymerized albumin in acute infection and in hepatitis B vaccine recipients. *J Hepatol* 1986; **3**: 393–8.
31 Fan YF, Lu CC, Chen WC *et al.* Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. *Hepatology* 2001; **33**: 277–86.
32 Tai PC, Suk FM, Gerlich WH *et al.* Hypermodification and immune escape of an internally deleted middle-envelope (M) protein of frequent and predominant hepatitis B virus variants. *Virology* 2002; **292**: 44–58.
33 Gerken G, Paterlini P, Manns M *et al.* Assay of hepatitis B virus DNA by polymerase chain reaction and its relationship to pre-S- and S-encoded viral surface antigens. *Hepatology* 1991; **13**: 158–66.
34 Chen BF, Liu CJ, Jow GM *et al.* High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; **130**: 1153–68.
35 Wang HC, Wu HC, Chen CF *et al.* Different types of ground glass hepatocytes in chronic hepatitis B virus infection contain specific pre-S mutants that may induce endoplasmic reticulum stress. *Am J Pathol* 2003; **163**: 2441–9.
36 Hsieh YH, Su IJ, Wang HC *et al.* Pre-S mutant surface antigens in chronic hepatitis B virus infection induce oxidative stress and DNA damage. *Carcinogenesis* 2004; **25**: 2023–32.
37 Caselmann WH, Meyer M, Kekule AS *et al.* A trans-activator function is generated by integration of hepatitis B virus preS/S sequences in human hepatocellular carcinoma DNA. *Proc Natl Acad Sci USA* 1990; **87**: 2970–4.
38 McMahon BJ, Holck P, Bulkow L *et al.* Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med* 2001; **135**: 759–68.
39 Fattovich G, Giustina G, Schalm SW *et al.* Occurrence of hepatocellular carcinoma and decompensation in western European patients with cirrhosis

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type B. The EUROHEP Study Group on Hepatitis B Virus and Cirrhosis. *Hepatology* 1995; **21**: 77–82.
40 Yang HI, Lu SN, Liaw YF *et al.* Hepatitis B e antigen and the risk of hepatocellular carcinoma. *N Engl J Med* 2002; **347**: 168–74.
41 Kao JH. Hepatitis B virus genotypes and hepatocellular carcinoma in Taiwan. *Intervirology* 2003; **46**: 400–7.
42 Baptista M, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 1999; **29**: 946–53.
43 Chan HL, Tsang SW, Wong ML *et al.* Genotype B hepatitis B virus is associated with severe icteric flare-up of chronic hepatitis B virus infection in Hong Kong. *Am J Gastroenterol* 2002; **97**: 2629–33.
44 Chan HL, Hui AY, Wong ML *et al.* Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; **53**: 1494–8.
45 Kao JH, Chen PJ, Lai MY *et al.* Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003; **124**: 327–34.