Hepatitis B virus combo mutations improve the prediction and active prophylaxis of hepatocellular carcinoma: a clinic-based cohort study

Running title: Prediction and prophylaxis of HCC and death

Jianhua Yin,1 Junxue Wang,2 Rui Pu,1 Haiguang Xin,2 Zixiong Li,1 Xue Han,3 Yibo Ding,1 Yan Du,1 Wenbin Liu,1 Yang Deng,1 Xiaowei Ji,1 Ming Wu,4 Min Yu,5 Hongwei Zhang,1 Hongyang Wang,6 Timothy C. Thompson,7 Wu Ni,2 Guangwen Cao1

1 Department of Epidemiology, Second Military Medical University, Shanghai, China;

2 Department of Infectious Diseases, The 2nd Affiliated Hospital, Second Military Medical University, Shanghai, China;

3 Division of Chronic Diseases, Center for Disease Control and Prevention of Yangpu District, Shanghai, China;

4 Division of Chronic Diseases, Provincial Center for Disease Control and Prevention of Jiangsu, Nanjing, China;

5 Division of Chronic Diseases, Provincial Center for Disease Control and Prevention of Zhejiang, Hangzhou, China;
International Cooperation Laboratory on Signal Transduction, The 3rd Affiliated Hospital, Second Military Medical University, Shanghai, China;

Department of Genitourinary Medical Oncology-Research, University of Texas MD Anderson Cancer Center, Houston, Texas, USA.

The first 4 authors contributed equally to this work. Guangwen Cao and Wu Ni are equal senior authors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed by authors.

Grant support: This study was supported by the National Key Basic Research Program (2015CB554000 to G. Cao) and Key Project for Infectious Diseases (2012ZX10002-008 to G. Cao) from the Ministry of Science and Technology of China, National Natural Science Foundation of China (91129301 to G. Cao, 81025015 to G. Cao, 81302492 to Y. Du, 81221061 to H. Wang, 81373067 to J. Yin), and Outstanding Young Investigator Project from Shanghai Municipal Health and Family Planning Commission (XYQ2013072 to J. Yin).

Corresponding author: Guangwen Cao, Department of Epidemiology, Second Military Medical University, 800 Xiangyin Road, Shanghai, 200433, People's Republic of China. Phone: 86-21-81871060; Fax: 86-21-81871060; E-mail:
gcao@smmu.edu.cn.

**Word count:** Abstract 250; Main document 3417; References 39; Figures and Tables, 6; Supplementary Tables, 3; Supplementary Figures, 3.
Abstract

We aimed to evaluate if hepatitis B virus (HBV) mutations at the core promoter region could improve the prediction and specific prophylaxis of hepatocellular carcinoma (HCC) in chronic HBV-infected patients. A total of 2114 HBV-infected patients enrolled between August 1998 and December 2007 were followed-up for 18406 person-years. Of those, 612 received ≥48 week treatments with nucleos(t)ide analogue (NA) and/or interferon-α. Baseline HBV mutations were identified by sequencing. Propensity score matching was applied to reduce baseline differences between antiviral and control cohorts. Multivariate Cox regression analyses including baseline characteristics of 2114 patients showed that age, male, cirrhosis, and HBV mutations (C1653T, T1753V, and A1762T/G1764A) independently increased HCC risk. In control patients carrying A1762T/G1764A, addition of C1653T and/or T1753V significantly increased HCC risk (HR=1.57; \( P = 0.038 \)); combo mutations with C1653T, T1753V, and A1762T/G1764A improved the validity of HCC prediction by age, male, and cirrhosis (\( P = 0.002 \)). In the matched cohorts, antiviral treatment reduced HCC incidence (13.90/1000 vs. 7.70/1000 person-years, \( P = 0.005 \)); NA treatment for ≥60 months was required for the prophylaxis of HCC in cirrhotic patients (\( P = 0.03 \)); antiviral treatment reduced HCC risk in patients carrying A1762T/G1764A (HR=0.40; \( P = 0.002 \)) or C1653T (HR=0.45; \( P = 0.04 \)) and in those without T1753V (HR=0.42; \( P = 0.005 \)), but could not reduce HCC risk in patients without A1762T/G1764A or C1653T and in those with T1753V. In summary, HBV mutation
A1762T/G1764A, C1653T, and T1753V in combination improve HCC prediction in HBV-infected patients. To prevent HCC, patients infected with HBV carrying A1762T/G1764A or C1653T, but not T1753V, should be given priority of receiving antiviral treatments.

**Keywords:** hepatitis B virus; mutation; antiviral treatment; hepatocellular carcinoma; cohort study
Introduction

Primary liver cancer is the second leading cause of cancer-related death in men and the fifth causes of cancer death in women and hepatocellular carcinoma (HCC) accounts for 70% to 85% of the total liver cancer burden worldwide (1). Chronic infection with hepatitis B virus (HBV) contributes to more than half of global HCC cases. The lifetime (30-75 years) incidences of HCC for men and women positive for hepatitis B surface antigen (HBsAg) were 27.38% and 7.99%, respectively, in Taiwan (2), an area endemic for HBV genotypes B and C. Therefore, it is estimated that approximately 20 million HCC cases caused by chronic HBV infection will be diagnosed in next 50 years in Mainland China, an area with one-third of global HBV-infected subjects. HCC is a highly fatal malignancy, with a 5-year survival rate of 9.01% for patients without surgical treatment and 32.64% for those who received surgery in Shanghai, China (3). Much hope for controlling HCC is placed with active prophylaxis prior to its occurrence. Fortunately, the occurrence and recurrence of HBV-related HCC can be reduced via active prophylaxis using anti-HBV treatments with nucleos(t)ide analogue (NA) and/or interferon-α (IFN-α) (4-10). It is therefore important to identify the HBV-infected patients who are more likely to develop HCC, allowing timely intervention in those who will benefit most.

Epidemiological cohort studies have demonstrated that male gender, increasing age, cirrhosis, high viral load ($\geq 10^4$ copies/ml), hepatitis B e antigen (HBeAg) expression,
HBV genotype C2 (vs. genotype B2), low albumin, and elevated alanine aminotransferase (ALT) increase the risk of HCC in chronic HBV-infected patients (11-15). Some of these risk factors have been utilized to construct clinical scoring systems for the prediction of HCC (12-15). However, HBeAg sero-status and the levels of HBV DNA and ALT are usually changeable during the long-term HBV infection, and differ among the patients infected with different HBV genotypes, which might result in low discriminatory performance in different populations (16-21). Therefore, more robust biomarkers are needed to improve the prediction power of the current clinical scoring systems.

HBV demonstrates “mutation-selection-adaptation”, a viral evolutionary process involved in hepatocarcinogenesis. During this process, HBV accumulates HCC-risk mutations, predominantly in the core promoter region of HBV genome (22-26). Of these mutations, A1762T/G1764A has been prospectively shown to be an independent risk factor of HCC (15,26). A1762T/G1764A and A1762T/G1764A-based combo mutants are potentially selected by the immunocompromised microenvironment predisposed by genetic polymorphisms of key immune and proinflammatory molecules, and in turn promote an aggressive phenotype of HCC, as well as the recurrence of HCC after curative surgery (27-30). The objective of this study was to clarify if the baseline HBV mutations can predict the outcome of HBV-infected patients and if HCC risk contributed by the HBV mutations can be selectively reduced by antiviral treatment. This study should lay foundation for effective prediction and
specific prophylaxis of HCC in HBV-infected patients.

Materials and Methods

Cohort enrollment

This mixed cohort study was initiated in January 2008 and approved by the institutional review board of Second Military Medical University. From August 1998 to December 2007, 3304 consecutive chronic HBV-infected patients admitted to the Department of Infectious Diseases, the 2nd Affiliated Hospital of this university were invited to participate in this study. Patients were included in the study if (i) they were newly diagnosed as chronic hepatitis B; (ii) they provided sufficient cryopreserved sera from peripheral blood harvested within 2 weeks before antiviral treatment or during their initial visit; and (iii) they provided written informed consents. Patients were excluded from the study if (i) they had decompensated liver cirrhosis or HCC; (ii) they were co-infected with hepatitis A virus, hepatitis C virus, hepatitis D virus, hepatitis E virus, human immunodeficiency virus, and/or Treponema pallidum; (iii) they had autoimmune liver diseases; and (iv) they received antiviral treatment before enrollment. Chronic hepatitis B, liver cirrhosis, and HCC were diagnosed as previously described (25). Cirrhotic patients manifested with ascites, jaundice, variceal bleeding or hepatic encephalopathy were diagnosed as decompensated cirrhosis. A total of 2512 eligible patients were initially enrolled.
Collection of baseline demographic and clinical data

A structured questionnaire was designed to extract baseline data of study participants from their medical records. Data collected include sociodemographics, smoking, alcohol consumption, medical history, and biochemical parameters from blood tests such as liver function, antibodies, and platelet count within 2 weeks before antiviral treatment or at their first admission. Microbiological and biochemical parameters were tested as previously described (25).

HBV genotyping and mutation assay

HBV DNA was extracted from frozen sera, quantified twice using quantitative PCR, and genotyped as previously described (20). The core promoter region from nt.1626 to nt.2004 of HBV genome was amplified using nested PCR and directly sequenced or sequenced after cloning (25,31). The sequences were aligned and analyzed using MEGA5.0 and Bioedit 7.0 software packages and deposited in GenBank with accession numbers KF164837-KF166793. A total of 18 “hotspots” with a mutation frequency of >10% in this region as previously detailed (25) were evaluated.

Antiviral treatment
Antiviral treatment was administered according to disease indications as well as patients’ willingness, compliance, and cost. Generally, patients were suggested to receive antiviral treatment if they had high serum ALT (≥twice the upper limit of normal) and elevated HBV DNA titer (≥10^5 copies/mL for HBeAg-positive or ≥10^4 copies/mL for HBeAg-negative patients), or cirrhosis with detectable HBV DNA (32). For oral NA treatment, lamivudine (LAM, 100mg), adefovir dipivoxil (ADV, 10mg), entecavir (ETV, 0.5mg), or telbivudine (LdT, 600mg) was given daily for ≥48 weeks. Adding-on or switch-to ADV was adopted as a rescue therapy for patients with viral breakthrough and/or drug-resistant mutations under LAM, LdT or ETV treatment, and vice versa. For cirrhosis-free patients who were resistant to LAM, recombinant IFN-α1b was given if their liver function permitted. Intramuscular injection of recombinant IFN-α1b (5MU every other day) and subcutaneous administration of pegylated IFN-α (Peg-IFN-α2a, 180µg/week; Peg-IFN-α2b, 1.5µg/kg body weight/week) were adopted. A 48-week IFN-treatment course was essential for most patients. For those who had partial virological responses at week 24 of treatment, IFN-α course was prolonged to 72 weeks. Patients who did not respond to antiviral treatment mostly failed to complete antiviral treatment for ≥48 weeks and were excluded from the final analysis.

Follow-up

Participants received regular follow-up examinations at our outpatient clinics or at...
their local tertiary hospitals. We collected information including real-life regimens of antiviral treatment, liver function test results, and medical imaging findings by checking their medical records. Information regarding HCC occurrence and death before January 2008 was obtained through Shanghai Cancer Registry and Death Certification System. Information regarding HCC occurrence and death after January 2008 was obtained by conducting regular telephone interviews, and/or household visits. The follow-up was finished on May 31, 2013. Causes of death were determined according to the primary diagnosis of hospitalization within 3 months before death. The primary outcome was HCC occurrence. Death from HCC, decompensated cirrhosis, and/or liver failure was termed as liver death. All participants were self-reported Han Chinese.

Statistical analysis

Categorical variables were compared using chi-square test. Patients who survived or died of other causes were censored at their last follow-up visit. Kaplan-Meier method was applied to estimate the cumulative incidences of HCC and liver death, and log-rank test was performed to compare the survival curves. The hazard ratio (HR) and 95% confidence interval (CI) were calculated using the Cox proportional hazard model. The significant factors in the univariate Cox analysis were introduced into the multivariate Cox model to determine the factors independently contributing to HCC occurrence and liver death. To evaluate the prophylactic effect of antiviral treatments
on HCC and liver death, the propensity score (PS) matching method was used to balance baseline variables between antiviral and control cohorts. A PS was estimated for all patients with antiviral treatments using multiple logistic regression analysis. Variables used in the model were age, gender, cirrhosis, HBV genotype, HBeAg, HBV DNA, ALT, aspartate aminotransferase (AST), total bilirubin, direct bilirubin, albumin, and platelet count. We performed the nearest available matching on the PS, as described by Hosaka, et al (6). Demographic (gender, age, and cirrhosis) and all factors (gender, age, cirrhosis, C1653T, T1753V, and A1762T/G1764A) that independently increased HCC risk were introduced into the multivariate Cox model to re-calculate regression coefficient (β) of each variable, respectively. Projected HCC risk based on demographic and all independent risk factors were calculated using the equations: demographic=∑βi×Xi (Xi: gender, age, and cirrhosis), and all independent risk factors=∑βj×Xj (Xj: gender, age, cirrhosis, C1653T, T1753V, and A1762T/G1764A). Discrimination was analyzed using receiver operating characteristic (ROC). Area under ROC curve (AUC) was applied to evaluate if the HBV mutations could improve the prediction power of the demographic factors. All analyses were two-sided and conducted using SPSS version 18.0 for Windows (SPSS, Chicago, IL), R (http://www.r-project.org/), and medcalc (http://www.medcalc.org/). Significance was set as P<0.05.

Results
**Patient characteristics**

Of 2512 eligible patients, 398 were excluded (Supplementary Figure 1). There were no statistical differences in age, gender, cirrhosis, and major liver function parameters between the enrolled and excluded patients (data not shown). The remaining 2114 patients were followed-up for a mean of 8.92 years (IQR, 6.67-11.00 years). Table 1 shows baseline characteristics and follow-up data of the study patients. Of the 2114 patients, 209 developed HCC during 18406 person-years of follow-up (incidence: 11.36/1000 person-years); 361 died of HBV-related liver diseases (mortality: 19.61/1000 person-years). Of 612 patients with antiviral treatment, 153 were treated with IFN-α alone, 380 treated with NAs alone, and 79 treated with IFN-α plus NAs. Antiviral treatment significantly reduced HCC occurrence and liver death. HBV mono-genotype was identified in 1659 (78.48%) patients. HBV genotype B2 accounted for 26.94%; and genotype C2, 73.06%.

The PS matching with key baseline characteristics was applied to allow a common background for comparison between antiviral and control cohorts, resulting in a matched sample size with 521 patients in each cohort. In the antiviral cohort, 109 were treated with IFN-α alone, 347 treated with NAs alone, and 65 treated with IFN-α plus NAs. Antiviral treatment significantly reduced HCC incidence and liver death in the matched cohorts.
Factors affecting HCC occurrence and liver death

Univariate Cox analyses including the baseline characteristics of 2114 patients showed that male gender, increasing age, cirrhosis, HBV genotype C2, high direct bilirubin (>7 μmol/L), low albumin (<35 g/L), high AFP (>20 ng/mL), low platelet count (<100×10^9/L), and HBV mutations (C1653T, T1674CG, T1753V, and A1762T/G1764A) were significant risk factors for HCC development; whereas antiviral treatment was a protective factor of HCC. HBeAg and viral load were not statistically associated with HCC, even in 1502 control patients without antiviral treatment (data not shown). Multivariate Cox analysis indicated that male, age (≥40 yrs), cirrhosis, C1653T, T1753V, and A1762T/G1764A independently increased the risk of HCC (Table 2).

Univariate Cox analysis using the baseline characteristics of 2114 patients indicated that male, age (≥40 yrs), HBV DNA (≥10^6 copies/mL), AST (>37 U/L), total bilirubin (>20 μmol/L), direct bilirubin (>7 μmol/L), albumin (<35 g/L), AFP (>20 ng/mL), platelet count (<100×10^9/L), and the presence of cirrhosis, C1653T, T1674CG, T1753V, and A1762T/G1764A were significant risk factors, whereas antiviral treatment was a protective factor for liver death. Multivariate Cox analysis showed that male, age (≥40 yrs), albumin (<35g/L), and platelet count (<100×10^9/L) were independently risk factors, whereas antiviral treatment was an independent protective factor for liver death (Supplementary Table 1).
Baseline combo HBV mutations cumulatively increased the occurrences of HCC and liver death and significantly improved HCC prediction by demographic factors in control cohort

Patients with A1762T/G1764A had significantly higher cumulative risks of HCC and liver death compared to patients without A1762T/G1764A. Furthermore, in patients with A1762T/G1764A, addition of C1653T and/or T1753V significantly increased the risks of HCC (HR=1.57, 95% CI=1.03-2.41; \( P = 0.038 \)) and liver death (HR=1.56, 95% CI=1.02-2.40; \( P = 0.040 \)). In genotype C2 HBV-infected patients with A1762T/G1764A, addition of C1653T and/or T1753V significantly increased HCC risk (HR=1.77, 95% CI=1.09-2.87; \( P = 0.021 \)). However, this effect was not found in genotype B2 HBV-infected patients, possibly because of small sample size of patients with the two or more viral mutations (Figure 1).

We plotted ROC curves for the development of HCC in 919 antiviral-naïve patients (HCC, n=102) with baseline HBV mutation data. Combo HBV mutations (C1653T, T1753V, and A1762T/G1764A) significantly improved the validity of HCC prediction by age, gender, and cirrhosis (AUC: 0.727 vs. 0.676, \( P = 0.002 \)). Further stratification analyses indicated that the combo HBV mutations significant improved the validity of HCC prediction in genotype C2 HBV-infected patients (HCC incidence: 83/677), not in genotype B2 HBV-infected patients (HCC incidence: 18/232) (Figure 2).
Prophylactic effects of antiviral treatment in those with or without baseline cirrhosis in the PS matched cohorts

We first evaluated the prophylactic effects of antiviral treatment in 236 patients with cirrhosis. Compared to the matched controls, antiviral treatment did not affect HCC occurrence but significantly reduced death (HR=0.49, 95% CI=0.29-0.81; \( P=0.005 \)). The survival benefit was observed in those treated with NAs alone (n=98), but not in those treated with IFN-α (n=12) or with IFN-α plus NAs (n=8). NA treatment for 12-59 months (n=66) did not significantly reduce HCC occurrence and liver death; whereas NA treatment for \( \geq 60 \) months (n=32) had significant prophylactic effects on HCC occurrence and liver death. In 806 patients without cirrhosis, antiviral treatment significantly reduced HCC occurrence (HR=0.47, 96% CI=0.26-0.85; \( P=0.012 \)) and liver death (HR=0.50, 95% CI=0.32-0.77; \( P=0.002 \)); interestingly, these benefits were observed in patients treated with IFN-α alone (n=97) or IFN-α plus NAs (n=57), but not in those treated with NAs alone (n=249); NA treatment for \( \geq 60 \) months (n=77) did not affect HCC occurrence but significantly reduced liver death (Figure 3).

Impact of the HBV mutations on HCC occurrence and liver death were selectively repressed by antiviral treatment in the PS matched cohorts

We compared the effects of antiviral treatment on HCC occurrence and liver death of
patients with one or more mutations (C1653T, T1753V, and A1762T/G1764A) and patients without any of them. Antiviral treatment significantly reduced HCC occurrence in those with the HBV mutation(s), but did not reduce HCC risk in those without (Supplementary Figure 2). In patients with A1762T/G1764A, antiviral treatment significantly reduced HCC occurrence, whereas in patients without A1762T/G1764A, antiviral treatment did not reduce HCC occurrence; similar results were obtained in patients with or without C1653T; interestingly, in patients with T1753V, antiviral treatment did not reduce HCC occurrence, whereas in those without T1753V, antiviral treatment significantly reduced HCC occurrence (Figure 4).

Antiviral treatment significantly reduced liver death in patients with and without A1762T/G1764A or C1653T mutations. However, antiviral treatment did not affect liver death in patients with T1753V, but reduced liver death in those without T1753V (Supplementary Figure 3).

Factors affecting the prophylactic effect of antiviral treatments

We then assessed baseline factors affecting the prophylactic effect of antiviral treatments in 612 patients with antiviral treatment. Multivariate Cox analyses showed that age (≥60 yrs), male, and the presence of cirrhosis and T1753V independently predicted HCC development (Supplementary Table 2); while age (≥60 yrs), male, and T1753V independently predicted liver death (Supplementary Table 3).
Discussion

In this study, male, age, cirrhosis, C1653T, T1753V, and A1762T/G1764A were found to be independent risk factors of HCC in HBV-infected patients. High viral load, a well-established risk factor of HCC (11-14), did not significantly increase HCC risk in this study. The same was true for HBeAg, another risk factor of HCC (33). These inconsistencies were also reported in the constituents of the HCC scoring systems developed from hospital-based and community-based studies in East Asia (12-15), possibly because these covariates were collected at different time points during chronic HBV infection. In the natural history of chronic HBV infection, viral load can be high (10^6-10^{10} copies/mL) at HBeAg-positive stage, and then decreases dramatically (10^4-10^8 copies/mL) or fluctuates after HBeAg seroconversion (16). To determine the contribution of HBV replication to HCC risk in HBV-infected subjects, it may be better to determine the baseline HBV DNA level after HBeAg seroconversion. Furthermore, age, male, HBeAg, HBV genotype C, abnormal ALT, and high viral load can also predict the development of cirrhosis (14), a benign disease vastly different from HCC, indicating that the current scoring systems based on these demographic and clinical variables is not specific for HCC. During chronic HBV infection, the HCC-risk HBV mutations including A1762T/G1764A, C1653T, and T1753V accumulate consecutively prior to HCC occurrence. A1762T/G1764A is an earlier generated HCC-risk mutation, whereas C1653T and T1753V are later generated ones (25,31,34). Furthermore, HBV combo mutations have higher
specificities than single ones in indicating HCC (24,25). These HBV mutations accumulated during this evolutionary process should be more robust and more specific than those changeable clinical markers in predicting HCC occurrence. The HBV combo mutations significantly improved HCC prediction using the 3 significant demographic factors (Figure 2). Thus, the HBV combo mutations should be incorporated into the current risk prediction models to improve the prediction of HCC in HBV-infected patients.

HBV X protein (HBx) contributes centrally to HCC development at least partially via up-regulating HBx-induced cyclin D1 (35). HBV 1753/1762/1764 mutations at the C-terminal of HBx are associated with active viral replication in human hepatoma cell lines (36). It had been shown that A1762T/G1764A-based HBV combo mutants, rather than those with A1762T/G1764A alone, accelerate p21 degradation and stimulate expression of S-phase kinase-associated protein 2 in hepatocytes or HCC cells (29). These data may help in explaining why A1762T/G1764A-based combo mutation was more effective than A1762T/G1764A alone in predicting HCC and liver death.

Following the identification of high-risk HBV-infected patients, it becomes indispensable to develop an effective prophylactic option on HCC or liver death in patients with these viral mutations. We found, for the first time, that antiviral treatment significantly reduced HCC development in HBV-infected patients carrying
A1762T/G1764A or C1653T mutation. These data indicate that HBV with A1762T/G1764A and/or C1653T mutations has a more potent carcinogenic potential than its wild-type counterparts and also responds efficiently to standard antiviral treatment. Interestingly, the risk of HCC contributed by HBV T1753V mutation can not be significantly reduced by standard antiviral treatment. The reason remains unknown. We hypothesize that T1753V mutation introduces a novel drug-resistant capacity of HBV or some idiopathic drug-resistant mutations in HBV genome are synchronously generated with T1753V during this revolutionary process. Further studies are needed to address this issue. Therefore, HBV-infected patients with A1762T/G1764A or C1653T should be given priority of receiving antiviral treatment; while patients with T1753V should be monitored frequently to identify early, resectable HCC.

Our stratification analysis indicated that the combo mutations significantly improved HCC prediction in genotype C2 HBV-infected patients, but not in genotype B2 HBV-infected patients. A1762T/G1764A significantly increased HCC risk and liver death in genotype B2 HBV-infected patients (Figure 1C, 1D). Genotype B2 HBV has a relative weaker carcinogenic potential than does genotype C2, possibly because genotype B2 has less HCC-related mutations. A1762T/G1764A is more frequent in HBV genotypes C and D than in genotypes A and B (37). Although the HBV mutations increase with increasing age of HCC-free HBV-infected subjects, the HBV mutations such as A1762T/G1764A and T1753V are extremely higher in younger
HBV-HCC patients, compared to the age-matched HCC-free HBV-infected subjects (25). The HBV mutations independently predicted HCC occurrence (Table 2). Thus, the HBV mutations, whose patterns differ among HBV genotypes, are HCC-specific and the association of A1762T/G1764A-based HBV mutations with HCC risk may be generalized to populations with different HBV genotypes.

The minimum period for NA treatment required to reduce HCC occurrence in HBV-infected patients differs greatly among studies carried out in Taiwan (≥90 days), USA (45 months), Japan (≥48 weeks), and South Korea (≥24 weeks) (4-7). Our data indicated that NA treatment for ≥60 months was required for the prophylaxis of HCC in cirrhotic patients. IFN-α treatment independently suppressed the development of HCC in non-cirrhotic HBV-infected patients. This is supported by the fact that HBV genotype B2 is less apt to cause cirrhosis and more sensitive to IFN-α treatment than genotype C2 (38,39). Thus, these data are particularly important for the prophylaxis of HCC in chronic HBV-infected patients.

This study has several limitations. First, 701 patients meeting the current standard for antiviral treatment had not received antiviral treatment. Second, data concerning fibrosis stage, maternal HBsAg status, family history of HCC, and quantitative HBsAg were incomplete and therefore not included in the analyses.

In conclusion, baseline combo mutations in the core promoter region of HBV genome
are more effective than A1762T/G1764A alone in predicting HCC occurrence and liver death and significantly improve HCC prediction by well-established demographic factors. Antiviral treatment was extremely effective in reducing HCC occurrence in chronic HBV-infected patients carrying A1762T/G1764A or C1653T but not in those carrying T1753V mutation. These data are helpful for effective prediction and specific prophylaxis of HCC and liver death.
Acknowledgements

We gratefully acknowledge Drs. Hua Wang, Weiwei Gong, and experts responsible for the maintenance of Cancer Registry and Death Certification Systems of Shanghai for their great help in the follow-up study. We regret that some articles relevant to this study are not cited due to space limitations.
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FIGURE LEGENDS

**Figure 1.** Impact of baseline C1653T, T1753V, and A1762T/G1764A mutations on HCC occurrence and liver death in control patients. (A) HCC occurrence in patients with mutation data; (B) liver death in patients with mutation data; (C) HCC occurrence in genotype B2 HBV-infected patients; (D) liver death in genotype B2 HBV-infected patients. (E) HCC occurrence in genotype C2 HBV-infected patients; (F) liver death in genotype C2 HBV-infected patients. *P* values, log-rank test.

**Figure 2.** Comparison of HCC prediction by baseline demographic factors (age, gender, and cirrhosis) with and without baseline combo mutations of HBV in control patients. (A) All patients with mutation data; (B) Genotype B2 HBV-infected patients; (C) Genotype C2 HBV-infected patients.

**Figure 3.** The effects of antiviral treatment on HCC occurrence and liver death in patients with or without liver cirrhosis in the PS matched cohorts. (A) NA treatment on HCC occurrence in cirrhotic patients; (B) NA treatment on liver death in cirrhotic patients; (C) Antiviral treatment on HCC occurrence in non-cirrhotic patients; (D) Antiviral treatment on liver death in non-cirrhotic patients; (E) NA treatment on HCC occurrence in non-cirrhotic patients; and (F) NA treatment on liver death in non-cirrhotic patients. *P* values, log-rank test.
**Figure 4.** Effect of antiviral treatment on HCC occurrence in chronic HBV-infected patients with and without each of the HCC-risk mutations in the PS matched cohorts. (A) patients with A1762T/G1764A; (B) patients without A1762T/G1764A; (C) patients with C1653T; (D) patients without C1653T; (E) patients with T1753V; (F) patients without T1753V. *P* values, log-rank test.
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<td>597(39.7)</td>
<td>370(60.5)</td>
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<td><strong>Total bilirubin (μmol/L)</strong></td>
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<td>≤20</td>
<td>329(22.1)</td>
<td>180(30.0)</td>
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<tr>
<td>&gt;20</td>
<td>1160(77.9)</td>
<td>420(70.0)</td>
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<td><strong>Direct bilirubin (μmol/L)</strong></td>
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<tr>
<td>≤7</td>
<td>452(30.4)</td>
<td>239(39.8)</td>
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<td>1035(69.6)</td>
<td>361(60.2)</td>
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<td>≥35</td>
<td>&lt;35</td>
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<td>Albumin (g/L)</td>
<td>921(61.6)</td>
<td>414(67.8)</td>
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<td>Alanine aminotransferase (U/L)</td>
<td>575(38.4)</td>
<td>197(32.2)</td>
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<td>Aspartate aminotransferase (U/L)</td>
<td>381(25.7)</td>
<td>94(15.7)</td>
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<td>Platelet count (10⁹/L)</td>
<td>698(46.5)</td>
<td>306(50.0)</td>
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<td>Person-years of follow-up</td>
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<td>5121</td>
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<td>Follow-up duration (years)</td>
<td>9.2(7.0-11.3)</td>
<td>8.2(6.3-10.6)</td>
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<tr>
<td>Hepatocellular carcinoma</td>
<td>173(11.5)</td>
<td>36(5.9)</td>
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<tr>
<td>Incidence (/1000 person-years)</td>
<td>13.02</td>
<td>7.02</td>
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<td>Death from Liver Diseases</td>
<td>304(20.2)</td>
<td>57(9.3)</td>
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<tr>
<td>Fatality (/1000 person-years)</td>
<td>22.88</td>
<td>11.13</td>
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**NOTE:** a Data are number (%), unless otherwise indicated. Some percentages do not sum up to 100 because of rounding. b Median (IQR)
Table 2. Cox analysis of factors significantly affected hepatocellular carcinoma occurrence in patients with chronic HBV infection

<table>
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<tr>
<th>Variable</th>
<th>No. (% of participants)</th>
<th>Person-years of follow-up</th>
<th>No. of HCC</th>
<th>Incidence per 1000 person-years</th>
<th>Univariate analysis</th>
<th>P</th>
<th>Multivariate analysis</th>
<th>P</th>
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<td>Female</td>
<td>490 (23.2)</td>
<td>4364</td>
<td>24</td>
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<td>1.00</td>
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<tr>
<td>Male</td>
<td>1624 (76.8)</td>
<td>14042</td>
<td>185</td>
<td>13.17</td>
<td>2.91 (1.90-4.47)</td>
<td>&lt;0.001</td>
<td>3.10 (1.76-5.45)</td>
<td>&lt;0.001</td>
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<td>Age (years)</td>
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<td>&lt;40</td>
<td>912 (43.1)</td>
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<td>40-59</td>
<td>973 (46.0)</td>
<td>8123</td>
<td>123</td>
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<td>2.58 (1.87-3.55)</td>
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<td>2.09 (1.35-3.23)</td>
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<td>≥60</td>
<td>229 (10.8)</td>
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<td>32</td>
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<td>Antiviral therapy</td>
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<td>Albumin (g/L)</td>
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<td>HBV genotype</td>
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<td>C</td>
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<td>1.83(1.36-2.45)</td>
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<td>2.43(1.70-3.48)</td>
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<td>T</td>
<td>V (C or A or G)</td>
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<td>Mutation</td>
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<td>14.84</td>
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<td>2.89(1.83-4.56)</td>
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</table>

| **Mutation**     |         |                |
| A1762T/G1764A b  |         |                |
|                  | 1180(83.9) | 10530         |
|                  | 10530    | 96            |
|                  | 9.12     | 1.00          |
|                  | 1.00     | 1.00          |

**NOTE:** a Data are number (%). b HBV mutation. c Only included significant covariates in univariate analysis.
Figure 1

A

C1653T / T1753V / A1762TG1764A

HR 5.92 (95% CI 3.22 - 10.88), \( P < 0.001 \)

No. at risk

Follow-up time (months)

-/-/- 348 332 323 307 236 162 73 9

-/-/+ 276 250 239 216 172 114 62 9

+/-/+ or -/+/+ or +/+/+ 263 230 218 192 155 102 48 6

B

C1653T / T1753V / A1762TG1764A

HR 3.76 (95% CI 1.99 - 7.08), \( P < 0.001 \)

C

C1653T / T1753V / A1762TG1764A in genotype B

HR 6.21 (95% CI 1.98 - 19.51), \( P = 0.002 \)

No. at risk

Follow-up time (months)

-/-/- 136 125 122 117 86 56 27 4

-/-/+ 69 63 56 50 37 18 9 2

+/-/+ or -/+/+ or +/+/+ 20 17 16 14 14 10 3

D

C1653T / T1753V / A1762TG1764A in genotype B

HR 3.61 (95% CI 0.66 - 19.72), \( P = 0.138 \)

No. at risk

Follow-up time (months)

-/-/- 136 125 122 118 86 56 27 4

-/-/+ 69 63 57 51 39 21 9 2

+/-/+ or -/+/+ or +/+/+ 20 17 16 15 14 10 3

E

C1653T / T1753V / A1762TG1764A in genotype C

HR 5.37 (95% CI 2.63 - 10.94), \( P < 0.001 \)

No. at risk

Follow-up time (months)

-/-/- 208 203 198 187 147 103 44 5

-/-/+ 204 184 180 163 132 93 52 7

+/-/+ or -/+/+ or +/+/+ 241 231 200 147 140 91 44 5

F

C1653T / T1753V / A1762TG1764A in genotype C

HR 3.01 (95% CI 1.41 - 6.46), \( P = 0.005 \)

No. at risk

Follow-up time (months)

-/-/- 208 203 198 189 149 104 44 5

-/-/+ 204 186 182 167 139 95 55 7

+/-/+ or -/+/+ or +/+/+ 241 231 200 189 149 104 44 5

Reference

-/-/-, Reference

-/-/+ or -/+ or +/+ or +/++ or -/+ or +/+ or +/++

HR 3.76 (95% CI 2.22 - 10.88), \( P < 0.001 \)

HR 3.01 (95% CI 1.41 - 6.46), \( P = 0.005 \)

HR 2.64 (95% CI 1.82 - 3.83), \( P < 0.001 \)

HR 2.02 (95% CI 1.38 - 2.97), \( P < 0.001 \)

HR 2.64 (95% CI 1.82 - 3.83), \( P < 0.001 \)

Figure 1 for Cancer Research.
AUC (95% CI)
Demographics+mutations 0.727 (0.677 - 0.777)
Demographics 0.676 (0.623 - 0.730)

Sensitivity
1-Specificity
P = 0.002

AUC (95% CI)
Demographics 0.668 (0.604 - 0.729)
Demographics+mutations 0.746 (0.685 - 0.801)

Sensitivity
1-Specificity
P = 0.119

AUC (95% CI)
Demographics 0.677 (0.641 - 0.712)
Demographics+mutations 0.725 (0.689 - 0.758)

Sensitivity
1-Specificity
P = 0.005
Figure 3

A

Control vs. 12-59 mo.  \( P = 1.00 \)
Control vs. \( \geq 60 \) mo.  \( P = 0.03 \)
12-59 mo. vs. \( \geq 60 \) mo.  \( P = 0.04 \)

B

Control vs. 12-59 mo.  \( P = 0.11 \)
Control vs. \( \geq 60 \) mo.  \( P = 0.002 \)
12-59 mo. vs. \( \geq 60 \) mo.  \( P = 0.03 \)

C

Control vs. IFN  \( P = 0.01 \)
Control vs. NA  \( P = 0.30 \)
Control vs. IFN plus NA  \( P = 0.03 \)

D

Control vs. IFN  \( P = 0.01 \)
Control vs. NA  \( P = 0.12 \)
Control vs. IFN plus NA  \( P = 0.003 \)

E

Control vs. 12-59 mo.  \( P = 0.60 \)
Control vs. \( \geq 60 \) mo.  \( P = 0.23 \)
12-59 mo. vs. \( \geq 60 \) mo.  \( P = 0.37 \)

F

Control vs. 12-59 mo.  \( P = 0.63 \)
Control vs. \( \geq 60 \) mo.  \( P = 0.02 \)
12-59 mo. vs. \( \geq 60 \) mo.  \( P = 0.04 \)
Figure 4

A1762T/G1764A - positive

Control group

Antiviral treatment

HR 0.40 (95% CI 0.22 - 0.72),

P = 0.002

A

A1762T/G1764A - negative

Control group

Antiviral treatment

HR 1.47 (95% CI 0.51 - 4.23),

P = 0.479

B

C1653T - positive

Control group

Antiviral treatment

HR 0.45 (95% CI 0.21 - 0.98),

P = 0.04

C

C1653T - negative

Control group

Antiviral treatment

HR 0.57 (95% CI 0.30 - 1.07),

P = 0.08

D

T1753V - positive

Control group

Antiviral treatment

HR 1.06 (95% CI 0.44 - 2.56),

P = 0.89

E

T1753V - negative

Control group

Antiviral treatment

HR 0.42 (95% CI 0.23 - 0.77),

P = 0.005

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Hepatitis B virus combo mutations improve the prediction and active prophylaxis of hepatocellular carcinoma: a clinic-based cohort study

Jianhua Yin, Junxue Wang, Rui Pu, et al.


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