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News and Perspectives

Clinical Implications of Hepatitis B Virus Variants

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Hepatitis B virus (HBV) is a global public health problem and the leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) worldwide.[1] As the smallest human DNA virus with a genome of 3200 bp,[2,3] the partially double-stranded circular HBV DNA encodes four overlapping open reading frames: S gene for the surface or envelope protein, C gene for the core protein, P gene for the DNA polymerase, and X gene for multifunctional nonstructural protein.[4,5] The S and C genes also have upstream regions designated pre-S and pre-C. The pre-S region contains pre-S1 and pre-S2 domains (Figure 1).[6] During HBV DNA replication, DNA polymerase provides reverse transcription at the intermediate stage. As a result of the lack of proofreading function of viral reverse transcriptase, HBV genome evolves with an estimated rate of nucleotide substitution at $1.4–3.2 \times 10^{-5}$ per sites a year.[7] This unique replication strategy accounts for the majority of point mutations and deletions or insertions observed in the HBV genome. The long-term evolution of HBV therefore leads to the occurrence of various genotypes, subgenotypes, mutants, recombinants, and even quasispecies.[5,8]

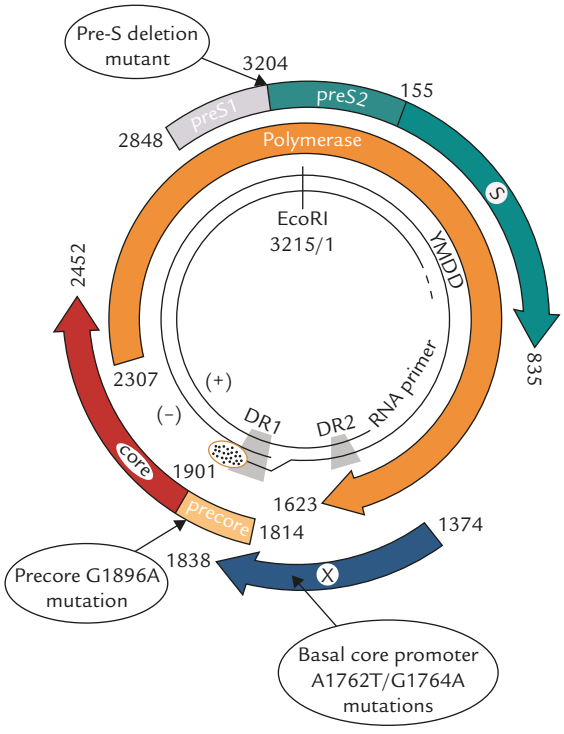

Figure 1. The partially double-stranded circular DNA of hepatitis B virus encodes four overlapping open reading frames: Naturally occurring mutant strains including mutations in precore and core promoter and deletion mutation in pre-S genes are associated with the pathogenesis of progressive liver disease. Adapted and modified from Kao.[6]

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On the basis of divergence in the entire HBV genomic sequence of greater than 8%, at least eight HBV genotypes (A–H) with distinct geographical and ethnic distribution have been identified.[9] The influence of HBV genotype on disease progression and clinical outcomes has been increasingly recognized. For example, patients with genotype C or D infection are significantly more likely to develop HCC than those with genotype A or B infection.[8,9] In addition, naturally occurring variations in the HBV genome also have implications at the clinical and epidemiological levels. Several HBV mutants, including precore/core promoter mutations and pre-S/S deletion mutations, have been reported to be associated with progressive liver disease (Figure 1).[10] In this article, the latest advances in our understanding of the effect of HBV variants on long-term clinical outcomes are discussed.

Hepatitis B Precore/Core Promoter Mutants

Hepatitis B e antigen (HBeAg) is a circulating peptide that usually serves as a marker of active viral replication.[11] During the natural course of chronic HBV infection, patients with HBeAg seroconversion are usually in the low replication phase or inactive carrier state, with low or undetectable serum HBV DNA level (< 2000 IU/mL) and normal serum alanine aminotransferase level. However, a significant proportion of patients continue to have a moderate level of HBV replication (> 2000 IU/mL) and active liver disease that is designated as HBeAg-negative chronic hepatitis B.[12] The clinical spectrum of HBeAg-negative chronic HBV infection ranges from inactive carrier to aggressive chronic hepatitis with or without cirrhosis.[13,14] Several HBeAg-negative viral mutant strains are known to be responsible for the continuous HBV DNA replication after HBeAg seroconversion.[15] HBV precore nucleotide 1896 mutation from G to A (precore G1896A) as well as changes of two nucleotides, A to T transversion at nucleotide 1762, together with a G to A transition at nucleotide 1764 within the basal core promoter (BCP A1762T/G1764A), lead to active HBV replication in the absence of HBeAg.[5,16–18] Several cohort and case-control studies have suggested the controversial association between precore G1896A mutation and HCC development.[19–23] A recent meta-analysis has shown that precore G1896A mutation is not significantly associated with HCC risk [odds ratio (OR) = 1.15, 95% confidence interval (CI) = 0.83–1.60).[24] Therefore, the appearance of precore G1896A mutation alone might be an innocent bystander and play a minimal role in the pathogenesis of chronic HBV infection. However, BCP A1762T/G1764A mutations have consistently been shown to be associated with an increased risk of liver disease progression and HCC development for genotypes B and C infection.[19–23] In a cohort study, we have investigated the prevalence of BCP A1762T/G1764A mutations in 250 genotype B- or C-infected HBV carriers with different stages of liver disease. The results have shown that genotype C has a higher prevalence of BCP A1762T/G1764A mutations than genotype B (OR = 5.18, 95% CI = 2.59–10.37; $p < 0.001$). Patients with BCP A1762T/G1764A mutations are significantly more associated with the development of HCC than those without (OR = 10.6, 95% CI = 4.92–22.86; $p < 0.001$).[19] These findings have been confirmed in a long-term follow-up study of 1526 HBV carriers, which has shown the presence of BCP A1762T/G1764A mutations is an independent predictor for progression to HCC (OR = 1.73, 95% CI = 1.13–2.67; $p = 0.013$).[25] In addition, a meta-analysis has yielded a summary OR of 3.79 (95% CI = 2.71–5.29) for development of HCC in patients with BCP A1762T/G1764A mutations.[24] Taking these lines of evidence together, BCP A1762T/G1764A mutation plays an important role in liver disease progression in HBV carriers, regardless of HBV genotype.

Mutations in enhancer II (C1653T) and elsewhere in the BCP (T1753V) are associated with HCC development. A case-control study from Hong Kong has revealed that patients with C1653T mutation have a significantly higher risk

of HCC than those without (OR = 2.43, 95% CI = 1.08–5.54; $p = 0.028$).[26] Another cohort study from Taiwan has indicated that patients with T1753V mutation have a significantly higher risk of HCC than those without (OR = 2.43, 95% CI = 1.33–4.44; $p = 0.028$).[27]

Pre-S Gene Deletion Mutations

Deletion mutations in the pre-S gene of the HBV genome frequently occur in chronic HBV infection.[28,29] Deletion of the pre-S gene might affect the expression of middle and small surface proteins, which results in intracellular accumulation of a large surface protein,[30] and might contribute to progressive liver cell damage and hepatocarcinogenesis.[31,32] In our case-control study, the frequency of pre-S deletion mutations was significantly higher in genotype C than genotype B patients (33.8% *vs.* 11.6%, $p = 0.01$). The presence of pre-S deletion mutations is an independent risk factor associated with hepatitis activity (OR = 3.91, 95% CI = 1.57–9.76; $p = 0.003$) as well as with development of HCC (OR = 3.72, 95% CI = 1.44–9.65; $p = 0.007$).[33,34] Similarly, a longitudinal study from Southern Taiwan has also shown that pre-S deletion mutations are significantly associated with the development of liver cirrhosis and HCC over time.[35] In addition, a matched nested case-control study from China has shown that pre-S deletion mutations constitute an independent risk factor for HCC, and their emergence and effect on HCC are independent of BCP mutations.[36] A meta-analysis has indicated that the OR of HCC for pre-S deletion mutations was 3.77 (95% CI = 2.57–5.52).[24] Our previous mapping study of the pre-S region has suggested that all the deletion regions encompass T- and B-cell epitopes, and most of them lose one or more functional sites, including those for polymerized human serum albumin and nucleocapsid binding. Therefore, HBV pre-S deletion mutations could lead to defective immunity against HBV and contribute to more progressive liver cell damage and finally hepatocarcinogenesis.[23]

Complex Viral Mutations

HBV mutations in precore, core promoter and pre-S genes accumulate during the course of chronic HBV infection; therefore, the emergence of complex HBV mutants is anticipated. In a cross-sectional study to investigate the interactions among precore G1896A mutation, BCP A1762T/G1764A mutations and pre-S deletion mutations in chronic hepatitis B patients with various stages of liver disease,[23] we found that a combination of pre-S deletion and BCP A1762T/G1764A mutations rather than single mutations is associated with the development of progressive liver disease. These results have been confirmed by a longitudinal study, which has shown that two or three combinations of pre-S deletion and BCP A1762T/G1764A and C1766T and/or T1768A mutations, rather than a single mutation are significantly associated with cirrhosis.[35] In Hong Kong, Yuen et al also reported that patients with BCP A1762T/G1764A mutations and C1653T mutation have a 9.9-fold increased risk of HCC compared to patients with wild-type sequences for both regions.[26] These findings suggest that accumulation of complex viral mutations in the core promoter and pre-S region synergistically affect the long-term outcomes of HBV carriers.

The pattern of complex viral mutations is not only an important risk factor associated with cirrhosis and HCC, but also a potential biomarker for the prediction of HCC development. In a meta-analysis of 43 studies, Liu et al found that three combinations of C1653T, T1753V and BCP A1762T/G1764A mutations have a high specificity (93.9%; 95% CI = 90.5–97.2) for the prediction of HCC.[24]

Conclusions and Perspective

On the basis of existing lines of evidence, several hepatitis B viral factors predictive of clinical outcomes have been identified, including high HBV viral load, genotype C, and core promoter and pre-S deletion mutations. Among these, persistently

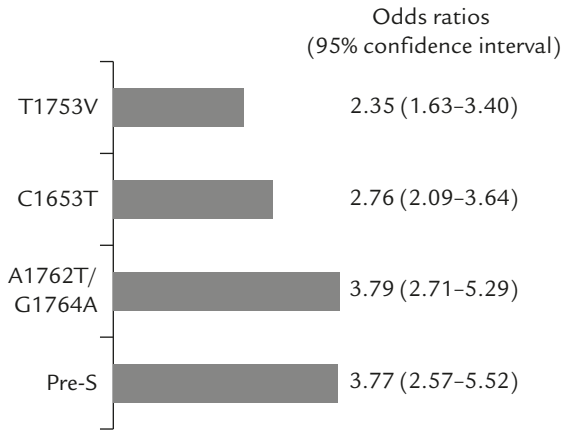

Figure 2. Odds ratio for development of hepatocellular carcinoma for hepatitis B virus carriers with mutations in the core promoter and pre-S regions in a meta-analysis. Adapted and modified from the meta-analysis of Liu et al.[24]

high serum HBV DNA level is the best predictor of adverse outcomes (cirrhosis, HCC and death from liver disease) in adult HBV carriers.[6,10] In addition, mutations in core promoter and pre-S regions are also associated with an increased risk of HCC (Figure 2).[24] Thus these mutations might serve as potential viral genetic markers to predict disease progression, as well as help clinicians identify patients who most need antiviral treatment. However, molecular mechanisms involved in the pathogenesis of these complex HBV mutations remain largely unknown, and further studies are required to address this important issue.

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