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Name of Journal: *World Journal of Hepatology*

Manuscript NO: 69861

Manuscript Type: MINIREVIEWS

Review on hepatitis B virus precore/core promoter mutations and their correlation with genotypes and liver disease severity

Kumar R. HBV preC/C mutations and liver disease

Abstract

350 million people worldwide are chronically infected with the hepatitis B virus (HBV) and are at risk of developing cirrhosis and hepatocellular carcinoma (HCC) later in life. HBV is the most diverse DNA virus, and its genome is composed of four open reading frames: The presurface antigen/surface (preS/S) antigen gene, the precore/core (preC/C) gene, the polymerase (P) gene, and the X gene. HBV produces quasispecies naturally or antiviral-associated because of the absence of proofreading activity amid reverse transcription and a high replication rate. The virus has 10 genotypes (A to J) with different geographical distributions. There are various HBV mutations in the HBV genome, including preC/C promoter mutations, preS/S deletion mutations, P gene mutations, and X gene mutations. The core promoter region plays a vital part in the replication, morphogenesis, and pathogenesis of the virus. The precore region also plays a crucial role in viral replication. Both core promoter and precore region mutations make the virus rescue from host immune surveillance and result in the formation of mutated strain which may have altered pathogenicity. Further, preC/C promoter mutations are associated with liver disease progression. Pre-core mutations stop hepatitis B e antigen (HBeAg) production and basal core promoter mutations downregulate HBeAg production. Mutations in the basal core promoter are also associated with increased HBV replication and an increased incidence of advanced liver disease such as cirrhosis and HCC. The emergence of antiviral-resistant mutation is the main reason for treatment failure. In this review, we focus mainly on preC/C promoter mutations and their correlation with genotypes and liver disease severity. Thorough perception and know-how of HBV genetic variety and mutants could be vital to discover the techniques for the prognosis and control of HBV infection.

Key Words: Hepatitis B virus; Hepatitis B virus e antigen; Hepatocellular carcinoma; Basal core promoter; Core promoter region; Pre-core region; Fulminant hepatitis; Acute hepatitis

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Core Tip: Worldwide, 350 million people are chronically infected with hepatitis B virus and are at risk for cirrhosis and hepatocellular carcinoma. Both core promoter and precore region mutations help the virus to rescue from host immune surveillance and result in the formation of mutated strain which may have altered pathogenicity. Further, precore/core (preC/C) promoter mutations are associated with advanced liver disease. We discuss mainly preC/C promoter mutations and their correlation with genotypes and liver disease severity.

INTRODUCTION

The hepatitis B virus (HBV) epidemic exists as a leading public health issue worldwide even with the accessibility of effective vaccines^[1,2]. Worldwide almost 350 million people are chronically infected with HBV and it accounts for more than 1 million deaths as a result of progressive liver diseases like cirrhosis and hepatocellular carcinoma (HCC)^[3]. Persistent HBV infection leads to various clinical manifestations like inactive carrier state, chronic hepatitis, cirrhosis, and HCC^[3].

HBV is an enveloped virus of the *Hepadnaviridae* family. The genome of this virus has partially double-stranded circular DNA of around 3200 nucleotides long. There are four overlapping open reading frames (ORFs) that make up the polymerase (P), core (C), surface antigen (S), and X protein^[4-7].

Based on an intergroup divergence of more than 7.5% in its complete genome, this virus can be divided into nine genotypes (A to I)^[8,9], and based on nucleotide divergence of more than 4%, these genotypes are further subdivided into subgenotypes^[8,9]. Genotypes A to H has long been established as individual genotypes. Two new genotypes (I and J) were suggested more recently^[10,11]. Evidence support that HBV genotypes play a significant role in deciding HBV disease severity. Several findings reveal that patients infected with genotype A evolve too speedily than

genotype D, which causes challenges for treatment^[12]. In addition, genotype C infected patients have the more advanced liver disease than genotype B infected patients^[13]. Further, genotype F infected patients have more death rate than genotype A or D infected patients^[14]. In an Indian study, genotype D is related to more advanced liver disease than other genotypes^[15]. In a study from the United States, genotype D acts as an independent risk factor for fulminant hepatitis (FH)^[16]. In another study, genotype F infected patients have a high rate of liver-related death than genotype D infected patients^[17]. Generally, patients infected with genotype C and D are more associated with liver disease severity (HCC) than patients infected with genotype A and B^[18].

Replication in HBV occurs by the reverse transcription of RNA intermediate. This virus has a high error rate during reverse transcription leading to various mutations in the HBV genome^[19]. These mutations have clinical and epidemiological significance. Various mutations (preC/C promoter and preS/S deletion), have been related to advanced liver disease and the possibility of HCC^[19]. The basal core promoter (BCP) and precore mutations are the frequently appearing natural variants of HBV. In this review, we discuss various preC/C promoter mutations reported till now and their correlation with genotypes and severity of the liver disease.

HBV CORE PROMOTER MUTATIONS AND RELATEDNESS WITH GENOTYPE AND ADVANCEMENT OF LIVER DISEASE

Core promoter region

The CP region (nt. 1575-1849) of the virus genome plays a vital part in the replication and morphogenesis of the virus^[20]. CP region controls transcriptional initiation for the synthesis of the precore mRNA and pregenomic RNA (pgRNA). The CP region includes BCP (nt. 1743-1849) and upper regulatory region (URR, nt. 1613-1742). BCP has a crucial role in the replication of virus by promoting the formation of precore and pgRNAs^[20]. The URR contains positive and negative regulatory elements which regulate the activity of the promoter^[20]. Hence, core promoter mutations may have an impact on virus gene expression and/or replication, and contribute to viral pathogenesis.

Now, we will discuss various core promoter mutations and their correlation with genotypes and the advancement of liver disease.

A1762T/G1764A double mutation

A1762T/G1764A double mutation is the most commonly observed core promoter mutation and it is associated with hepatitis B e antigen (HBeAg) negativity and one of the causes of advanced liver disease. There are various observations associated with this double mutation described below.

² Kim *et al*^[21] observed the role played by the precore mutation G1896A and the BCP double mutation, A1762T/G1764A in the progression of HBV-related liver diseases.¹⁴ BCP double mutation, A1762T/G1764A may prevent the formation of HBeAg by inserting a premature stop codon into the concerned ORF or may enhance the pgRNA transcription by evacuating of the nuclear receptor-binding motif and led to the feeble immune response that caused HCC^[22,23]. From transfection studies, it was observed that HBeAg negativity caused by BCP double mutation, A1762T/G1764A had been linked with serious liver disease^[24].

BCP mutant may hike the host immune response towards HBV infected hepatocytes by reducing circulating HBeAg, leading to apoptosis and regeneration of hepatocytes, which contributed to liver injury^[22,25]. In an *in vivo* study, A1762T/G1764A mutant was related with cytoplasmic positioning of intracellular HBcAg, which were strongly linked with active necroinflammation of hepatocytes^[26]. The A1762T/G1764A double mutation was found to influence the amino acid sequence of X gene of HBV, up-regulating S-phase kinase-associated protein 2 and down-regulating its target, cyclin kinase inhibitor p21. The combined action of the above-mentioned events may lead to the inhibition of precore mRNA and a hike in pgRNA transcription, followed by increased viral replication, which may finally lead to HCC^[27].

In a report, it was found that A1762T/G1764A mutation usually appeared almost ten years before the identification of HCC and could be an early episode in hepatocarcinogenesis^[28]. In a study, the BCP double mutation, A1762T/G1764A was

found to be related to more chances of progressing HCC^[29] independently of HBV genotype^[24,30] and viral load^[30,31]. A study revealed that mutations (A1762T, G1764A, and A1762T/G1764A) played a vital part in the progression of chronic HBV infection to HCC^[25,32,33]. In a study from East Kalimantan, Indonesia, BCP mutations, mainly the double mutation A1762T/G1764A and T1753V, were linked with the risk of advanced liver disease (ALD)^[34].

HCC patients with genotype C had more chances of carrying the A1762T/G1764A double mutation than those with HCC patients with genotype B^[35]. In China, A1762T/G1764A mutation was the familiar mutation and found generally in HBeAg-negative chronic hepatitis B patients with genotype C and may be related to the progression of chronic HBV infection^[36]. In a study on HBV-infected children in China, the frequencies of BCP mutations in genotype C samples were significantly higher than that of genotype B samples^[37]. From a Taiwanese study, genotype C and A1762T/G1764A mutations may be the cause of liver cirrhosis and could act as molecular markers for the diagnosis of the clinical symptoms of chronic HBV disease^[38]. From a North Indian study, genotype D infected patients had more advanced liver disease and more frequency of BCP mutations, A1762T/G1764A than patients infected with genotype A^[39].

A1762T/G1764A mutation also acted as a significant viral marker for cirrhosis development^[40]. From a study of chronic HBV infection, it was observed that A1762T/G1764A mutation was an independent threat sign for advancement to cirrhosis instead of HCC^[41].

Table 1 showed various qualitative and quantitative factors associated with HBV-related HCC.

1753 mutation

1753V (V represents C/A/G) is one of core promoter mutations that is associated with advanced liver diseases like cirrhosis and HCC. The following observations prove this observation.

In a study from East Kalimantan, Indonesia, BCP mutations mainly the double mutation A1762T/G1764A and T1753V were linked with the risk of ALD^[34]. In a study, it was found that BCP T1753V mutation was associated with an increased risk of HCC^[28]. The T1753C mutation was treated as one of the 'hot spot' mutations of protein-x encoding gene^[28] and was proclaimed to increase the transactivation and antiproliferation activity of protein-x in HBV genotype D and led to carcinogenesis^[42]. In a study on HBeAg-positive genotype C1 carriers, 1753 mutation was detected as a prognosis factor for HCC^[28].

In a study from China, T1753V mutation in combination with BCP A1762T/G1764A double mutation was assumed to be related to HCC progression notably in HBV/C1 infected patients^[43]. Further from the Mongolian study, patients infected with HBV/D, C1752, and/or T1753V mutation were found to be related to HCC^[42]. In a study from Southern Guangxi, China, BCP mutations were associated with HCC risk and the add-on impacts of A1762T/G1764A double mutations and T1753V mutations along with dietary aflatoxin B1 exposure for HCC risk^[44]. In a study from Chiba, Japan, preC mutation (A1896) and CP mutations (T1753V and 1754) were observed more often in FH patients than in acute self-limited hepatitis patients^[45]. In a study from Taiwan, the frequency of genotype C, frequency of various mutations (A1762T/ G1764A, T1753C, T1766/A1768, and A1896) increased and the frequency of various mutations (T/G1752, T1773, G1799, and C1858) declined with advancing liver diseases^[38].

In a study, T to C/A mutation at nt. 1753 in the BCP and G to A mutation at nt. 1899 in the precore region were more frequently found in liver cirrhosis patients than in the other clinical statuses^[46]. From another study, 1653T/1753C mutation may be observed as a marker for hepatic failure^[47].

1757 mutation

1757 mutation is one of core promoter mutations. It is associated with the severity of liver disease as described below in various studies.

In a study, G1757 mutation along with BCP A1762T/G1764A double mutation was directly linked with the high level of serum HBV DNA^[48]. Various CP mutations (M1386, T1485, B1499, A1613, T1653, G1727, A1757/T1764/G1766, G or C1753, T1773, and T1766/A1768) were related to the progression to HCC. These mutations individually and/or in combination were the prognostic markers of HCC^[49-51].

1764/1766 mutation

1764/1766 mutation is one of the core promoter mutations. It is associated with patients with severe liver disease which are described below in many studies.

Experiments revealed that 1762/1764/1766 and 1753/1762/1764/1766 mutations showed increased viral replication and decreased HBeAg expression than 1762/1764 mutations alone; however, 1753/1762/1764 mutant showed only a little decrease in HBeAg expression like that of 1762/1764 mutant. So, core promoter mutations other than mutation (1762 and 1764) can have a vital influence on virus DNA replication and HBeAg expression^[52].

1809-1812 mutation

1809-1812 mutations (Kozak region mutations) are responsible for HBeAg negativity and are found mainly in genotype A; subgenotype A1 as described below.

Several mutations can reduce HBeAg expression: 1762T1764A mutation at the transcriptional level; 1809-1812 mutations at the translational level and 1862T mutation at the post-translational level. The existence of A1762T/G1764A mutation along with 1809-1812 mutations cumulatively reduced HBeAg expression^[53]. In a study from Kenya, mutations in the Kozak region (1809-1812) or precore start codon (1814-1816) were responsible for HBeAg-negativity in patients with subgenotype A1^[54].

Table 2 shows the clinical potency of frequently found BCP mutations.

HBV PRECORE MUTATIONS AND RELATEDNESS WITH GENOTYPE AND ADVANCEMENT OF LIVER DISEASE

Precore region

The precore gene spans the length from nt. 1814 to 1901. This region has the start site for synthesis of pgRNA, direct repeat 1, and RNA encapsidation signal. The precore region plays a crucial role in viral replication. The presence of mutations in this region disrupts viral replication and results in the formation of defective replicative intermediates. These replicative intermediates (which are not encapsidated) in hepatocytes, increase the chances for integration of HBV DNA into chromosomal DNA and lead to advanced liver disease like HCC^[55].

1896 mutation

1896 mutation is the one of most important precore mutations. It causes HBeAg negativity and advanced liver diseases like HCC. These observations are described below in various studies.

Moreover, the precore mutation G1896A along with the BCP double mutation, A1762T/G1764A played a significant part in the progression of HBV-related liver diseases^[21]. The precore region mutation, G1896A was one of the most frequently observed mutations in ALD and chronic HBV patients^[56].

The G1896A mutation gives rise to a premature stop codon and reduces HBeAg synthesis. This mutation stops HBeAg formation by checking the translation of HBeAg and the existence of a stop codon mutation may be a cause of immune evasion^[56,57]. The most commonly observed precore mutations, G1896A stop HBeAg formation by substitution of a tryptophan residue at amino acid position 28 with a premature stop codon^[58]. This mutation was observed in more than 50% of individuals with chronic hepatitis B in Asia and the Mediterranean area^[58]. This mutation suppresses HBeAg production, thus causing increased HBV replication and liver disease progression^[21,59,60]. Precore 1858-1896 mutations were related to high serum HBV DNA levels^[61].

In a meta-analytic study among Asians, G1896A mutation was found to be related to more chances of HCC and liver disease severity^[62]. Various mutations and

combinations, notably at nt. 1762/1764 and nt. 1896/1899 in BCP-preC region were observed to induce HBeAg negativity, liver function impairment, and HCC progression^[63].

In some of the studies given below G1896A mutation is associated with less severity of the liver disease. In a study from Taiwan, the G1896A mutation was found to be linked with a low HCC risk^[64].

1858 mutation

1858 mutation is one of the precore mutations. 1858 mutation is related to more liver disease severity as described below in various studies.

In a study from Vietnam, genotype C infected patients contained a high rate of C-1858 mutation (70%) and it could play a decisive role in generating severe chronic liver disease^[65].

1858C mutation was found to be related to A, F, and H genotypes and 1858T mutation with B, D, and E genotypes^[66]. C-1858 strains may be more infectious, but more investigations are required to find the pathogenesis of this strain^[67]. According to a study, high serum HBV DNA level was related to precore 1858-1896 mutations. These BCP/precore mutations may act as a prognostic marker for HBV DNA rise^[61]. A study reported that TCC at positions 1856 to 1858 of the precore region of HBV might serve as HBV strain related with alarming liver disease than other HBV strains with genotype C^[68].

The existence of C rather than T at nt. 1858 prevents G1896A mutation because the existence of the above-mentioned mutations would diminish ¹³ the stem-loop structure of the RNA encapsidation signal. So, the CCC polymorphism and the 1896 mutation were mutually exclusive^[69]. HBV-infected patients with C at position 1858 were having more prevalence of core promoter mutations, while patients infected with T at position 1858 were having only precore stop codon mutations^[70].

1862 mutation

1862 mutation is one of the precore mutations. This mutation is also one of the leading causes of HBeAg negativity and advanced liver diseases as described below in various studies.

In a study on Chinese patients, G1862T mutation caused an amino acid change in codon 17 of the precore protein, a part of the signal peptidase recognition motif. G1862T mutations were found only in genotype B infected patients. These variants have reduced capacity to form HBeAg and the absence or reduced HBeAg may be a causing factor in the fulminant disease. G1862 mutation was observed more in FH patients than in chronic carriers^[71].

G1862 mutation was mainly found in HBV genotype A (sub-genotype A1). G1862T mutation was associated with HBeAg negativity and low viremia level in genotype A infected patients^[21]. Saha *et al*^[72] reported in an East India study that all HBV/A1 isolates had G1862T mutation regardless of HBeAg status. So, the G1862T variant might present a natural mutation in HBV/A1 isolates.

1888 mutation

1888 mutation is one of the precore mutations. This mutation is found only in subgenotype A1 and causes HBeAg negative serostatus.

The linked loci 1809-1812, 1862, and 1888 were observed regularly in subgenotype A1 and they caused inhibition of HBeAg expression^[53]. The 1888A mutation was related to HBV subgenotype A1 and it expressed a start codon upstream from the core open reading frame and can influence the core protein expression resulting in lowering of viral level^[73].

1915 mutation

1915 mutation is one of the precore mutations. It is reported as one of the HCC survival mutations.

Xie *et al*^[74] observed five different mutations in the preC/C region (1915, 2134, 2221, 2245, and 2288) and these mutations act as independent predictors of HCC survival.

8
Various newly reported preC/C promoter mutations (1690A, 1695A/T/G, 1700A/C, 1703C, 1850A, and 1915A/G) were observed in HBV-infected isolates of Punjab state (North India)^[75].

Scanty/rare mutations

Some rare mutations in preC/C promoter region are described below which contribute to the severity of the liver disease.

Various studies reported that mutations in the enhancer II/BCP regions (C1653T, T1674C/G, T1753V, A1762T/G1764A, and C1766T/T1768A) and mutations in the precore region (G1899A, C2002T, A2159G, A2189C, and G2203A/T) were significantly associated with an increased risk of HCC^[76]. Various newly reported individual/combo mutations of the X/precore region in HBV genotype D1 acted as markers of HCC^[50]. These mutations were T1673/G1679, G1727, C1741, C1761, A1757/T1764/G1766, T1773, T1773/G1775, and C1909^[50]. Numerous core promoter mutations, 1653T, 1753V, A1762T, G1764A, 1766T, and 1768A had been observed regularly in HCC patients^[77].

In an *in vitro* study, individual mutations in the BCP/precore region, T1753V (C/A/G), C1766T, T1768A, G1862T, and G1899A were linked with enhanced viral replication and/or reduced HBeAg expression and related with acute liver failure in some instances^[16,52,71,78]. Ren *et al*^[79] observed that individual mutations, T1753V (C/A/G), A1762T, G1764A, G1896A, and G1899A were more regularly detected in hepatitis B-related acute-on-chronic liver failure (ACLF) than chronic hepatitis B (CHB) patients. Patients having precore mutation had more risk of a fatal outcome.

Tsai *et al*^[80] reported that triple BCP mutation, 1762/1764/1766 was related to the maximum increase in pgRNA transcription while 1762/1764 double mutation was not so effective. As reported in a recent study, T1846 and A/G1913 mutations were linked with ACLF in genotypes B and C infected HBV patients^[81].

In Table 3 various precore/CP mutations and their correlation with clinical outcomes are shown.

DISCUSSION AND FUTURE PERSPECTIVES

HBV genotypes and viral mutations have a fair connection³ for various clinical conditions of CHB and this information should be utilized to predict likely clinical course, *e.g.*, lengthy duration of HBeAg phase and more risk of progression to cirrhosis in genotype C and increased risk of HCC in genotypes C and F. Evidences also confirmed the role of genotypes and sub-genotypes in pathogenesis and clinical symptoms of HBV infection. Further, HBeAg seroconversion is delayed in patients infected with genotype C compared to other genotypes^[86]. So, more research is needed about the pathogenicity of genotypes E, F, and H.

In addition, preS/S region mutations are related to vaccine failure, immune escape, occult HBV infection, and HCC occurrence. P region mutations may create drug resistance to NA antivirals. Mutations in the preC/C region are associated with HBeAg negativity, immune escape, and persistent hepatitis. Mutations in the X region play a vital part in developing HCC^[86]. Mutations in various parts of the HBV genome could be the reason for unwanted clinical outcomes or evasion of detection by various diagnostic procedures, thus it becomes necessary to detect these mutations for proper evaluation of patients.

Revill and Locarnini^[87] observed and recommended that in genotype B and C infected patients, BCP mutation is a significant viral biomarker of the risk of cirrhosis. So, identification and quantification of BCP mutants should be done and used for proper treatment in Asian CHB patients. More work on the importance of these mutations in other genotypes is suggested.

HBV genotypes and mutations act as useful viral biomarkers for the prognosis of disease progression and also assist clinicians to diagnose patients who can be treated mostly from interferon therapy. In the future, clinical trials classified by different genotypes/mutants should be adopted and it is essential to apply individualized treatment regimes for chronic hepatitis B patients.

For population health prospects, proper observing of the pervasiveness³ of vaccine escape and S escape mutations is needed and more research will be required into vaccines that stand effective against mutant strains of HBV. Identification of resistance profile is important in deciding the exact antiviral agent to start therapy.

CONCLUSION

⁹ HBV has more genomic diversity and various HBV mutants are significantly related with response to antiviral therapy, vaccine escape, diagnostic failure, liver fibrosis, liver cirrhosis, and HCC development. Understanding the correlation between various mutations and the clinical manifestations of HBV infection should lead to advances in diagnostic strategies and therapeutic directions.

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