**Name of journal: *World Journal of Hepatology***

**ESPS Manuscript NO: 15526**

**Columns: REVIEW**

**Clinical relevance of hepatitis B virus variants**

GaoS*et al*. HBV mutants

Shan Gao, Zhong-Ping Duan, Carla S Coffin

**Shan Gao, Zhong-Ping Duan,** Artificial Liver Center, Beijing You’an Hospital, Capital Medical University, Beijing 100000, China

**Shan Gao, Carla S Coffin,** Liver Unit, Division of Gastroenterology and Hepatology, Department of Medicine, University of Calgary, Alberta T2N 4Z6, Canada

**Author contributions:** Gao S wrote the paper first draft; Duan ZP provided feedback and edited the manuscript; Coffin CS designed the paper, suggested topics for writing and literature to review and provided feedback on manuscript draft.

**Supported by** the National Science and Technology Key Project of China on “Major Infectious Diseases such as HIV/AIDS, Viral Hepatitis Prevention and Treatment”, No. 2013-ZX10002002-006 (Duan ZP); speaker, advisory board and/or consulting fees from Boehringer ingelheim, GlaxoSmithKline, Janssen Pharmaceuticals, Bristol Myers Squibb, Roche Pharmaceuticals and Gilead Sciences (Coffin CS); and the Canadian Institutes for Health Research (Coffin CS).

**Conflict-of-interest:** There are no disclosures relating to this body of work.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to: Carla S Coffin, MD, MSc, FRCPC,** Liver Unit, Division of Gastroenterology and Hepatology, Department of Medicine, University of Calgary, 2500 University Drive Northwest, Calgary, Alberta T2N 4Z6, Canada. cscoffin@ucalgary.ca

**Telephone:** +1-403-5925049

**Fax:** +1-403-5925090

**Received:** November 28, 2014

**Peer-review started:** December 2, 2014

**First decision:** January 8, 2015

**Revised:** January 28, 2015

**Accepted:** February 10, 2015

**Article in press:**

**Published online:**

**Abstract**
The hepatitis B virus (HBV) is a global public health problem with more than 240 million people chronically infected worldwide, who are at risk for end-stage liver disease and hepatocellular carcinoma. There are an estimated 600000 deaths annually from complications of HBV-related liver disease. Antiviral therapy with nucleos/tide analogs (NA) targeting the HBV polymerase (P) can inhibit disease progression by long-term suppression of HBV replication. However, treatment may fail with first generation NA therapy due to the emergence of drug-resistant mutants, as well as incomplete medication adherence. The HBV replicates *via* an error-prone reverse transcriptase leading to quasispecies. Due to overlapping open reading frames mutations within the HBV P can cause concomitant changes in the HBV surface gene (*S*) and vice versa. HBV quasispecies diversity is associated with response to antiviral therapy, disease severity and long-term clinical outcomes. Specific mutants have been associated with antiviral drug resistance, immune escape, liver fibrosis development and tumorgenesis. An understanding of HBV variants and their clinical relevance may be important for monitoring chronic hepatitis B disease progression and treatment response. In this review, we will discuss HBV molecular virology, mechanism of variant development, and their potential clinical impact.

**Key words:** Hepatitis B virus; Molecular virology; Genetic heterogeneity; Quasispecies; Drug resistance; Immune escape; Viral lymphotropism

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:** The hepatitis B virus (HBV) has significant genomic diversity and some HBV variants are associated with antiviral therapy response, vaccine escape, diagnostic failure, liver fibrosis progression and hepatocellular carcinoma development. Understanding HBV molecular epidemiology as well as the clinical and pathological relevence of HBV variants during different disease phases may enable more accurate risk-stratification of individual patients at risk for serious sequelae of chronic hepatitis B infection.

GaoS*,* Duan ZP, Coffin CS. Clinical relevance of hepatitis B virus variants. *World J Hepatol* 2015; In press

**EPIDEMIOLOGY OF CHRONIC HEPATITIS B**

Chronic HBV infection (CHB) is a serious global public health problem. There are an estimated 600000 deaths annually from complications of HBV-related liver disease. For over 3 decades, there has been a safe and effective HBV vaccine consisting of recombinant HBV surface (S) (*i.e.,* envelope) protein that has reduced infection rates in countries with widespread immunization programs[1]. The HBV is transmitted parenterally by contact with blood or body fluids of an infected person. In highly endemic areas, such as China, the incidence of HBV infection is greater than 8%, and is often acquired at birth or in early childhood from exposure to HBV infected mothers or family members. About 90% of unvaccinated infants born to mothers with CHB will became chronic carriers, and the risk of CHB is up to 30% in children infected at 1-4 years of age[2]. Despite implementation of widespread childhood vaccination programs, the incidence and mortality of HBV-related cirrhosis and HCC continues to increase due to the enormous burden of chronically infected carriers worldwide.

**NATURAL HISTORY OF CHB INFECTION**

The HBV is a non-cytopathic virus and liver cell injury is due to a host immune mediated antiviral response to an infected cell. CHB is a dynamic disease, and the interplay between the virus and the host immune system influences disease course. In clinical practice, CHB is divided into four disease phases: immune tolerant, immune clearance, inactive, and reactivation phase[3]. The immune tolerant phase is characterized by persistently normal serum alanine aminotransferase (ALT) levels, high HBV DNA levels and presence of HBV e antigen (HBeAg), but with no evidence of liver injury. The immune clearance phase is characterized by presence of HBeAg, persistently high ALT and HBV DNA levels with some degree of liver inflammation. HBeAg seroconversion may occur at the late stage of the immune clearance phase. Thereafter, patients are likely to progress to the immune inactive phase characterized by normal ALT level, low/undetectable HBV DNA (< 2000 IU/mL or < 104 virus copies/mL), absence of HBeAg and presence of anti-HBe, as well as no/minimal histological injury. HBV reactivation can occur in some and is characterized by rebound viremia, presence of anti-HBe, elevated ALT levels and liver inflammation. This so-called “reactivation phase” may also occur due to the presence of preC/BCP mutations that abolish or downregulate HBeAg production leading to HBeAg negative CHB. There is recent data challenging the classification of these clinical phases. Imunological characterization of apparent immune-tolerant HBV-infected adolescents did not reveal any tolerogenic T-cell pattern[4]. Further, histologically active disease has been reported in CHB children considered to be immune tolerant[5,6]. Finally, analysis of HBV quasispecies (QS) in children with an immune tolerant clinical profile showed significant HBV diversity, which may be due to immune selective pressure[7].

 In general antiviral therapy for CHB is recommended in patients with advanced liver disease (*i.e.,* cirrhosis) or prolonged immune active disease flares due to the risk of liver fibrosis progression. The currently approved anti-HBV therapies include interferon (*i.e.,* pegylated-interferon, Peg-IFN), which has non-specific antiviral and immunomudulatory effects and nucleos/tide analogs (NA) targeting the HBV polymerase/reverse transcriptase (P/RT) region. There are five currently available NAs: lamivudine (LMV), telbivudine (LdT), entecavir (ETV), adefovir (ADF) and tenofovir (TDF). The second generation NA’s (*i.e.,* TDF and ETV) are potent with a high genetic barrier to resistance and persistently suppress HBV replication. These drugs have a low reported risk of drug resistance or treatment failure despite years of sustained therapy[8,9]. In contrast older generation NA has an increased risk of treatment failure with long-term use due to drug resistance (see Section 5.4)[10]. NA are very effective at reducing liver disease risk but must be used for prolonged periods as they do not offer a cure for CHB due to its unique replication strategy.

**OVERVIEW OF HBV REPLICATION AND TISSUE TROPISM**

The HBV is the prototype member of the *Hepadnaviridae* family which includes various avian and mammalian viruses sharing similar genome structure and organism trophisms [11]. It is a small DNA virus with ~3.2 Kb partially double stranded relaxed circular (rcDNA) genome within a nucleocapsid surrounded by a lipid envelope. The full-length virus negative-strand has a ~7-9 nucleotide redundancy and the complementary positive-strand is ~50%-70% full genome length. The HBV genome consists of 4 overlapping open reading frame (ORF) encoding the polymerase gene (*P*), pre-S1/pre-S2/S gene (*preS1*/*preS2*/*S*), precore/core gene (*preC/C*) and *X* gene. Viral entry occurs after binding of the viral pre-S1 protein to its specific functional receptor, the recently identified sodium taurocholate cotransporting polypeptide[12]. The intact virion or “Dane particle” uncoats in the cytoplasm and the rcDNA genome is transported into the nucleus and repaired to covalently closed circular DNA (cccDNA) by host and viral polymerases. The presence of cccDNA indicates successful establishement of HBV infection[13]. The cccDNA is transcribed to a 3.5 Kb pregenomic (pg)-RNA molecule with a unique stem-loop epsilon structure located at its 5' end. Thus, HBV cccDNA is the “master” template for HBV negative-strand synthesis via reverse transcription, as well as hepatitis B core antigen (HBcAg) or nucleocapsid protein and P/RT translation[14]. Additionally, the cccDNA is the template for four subgenomic mRNAs, which are translated into soluble or secreted HBeAg (from 3.5 kb precore mRNA), subviral S or envelope particles (2.4 kb and 2.1 kb mRNA) and X (0.8 kb mRNA). The HBV pgRNA is transported to the cytoplasm and binding of the viral polymerase to its 5’ end epsilon structure initiates encapsidation by HBV core particles[15]. Following encapsidation, the pgRNA is reverse-transcribed and is gradually degraded by viral polymerase ribonuclease H (RNase H). The positive-strand DNA is then synthesized from the newly transcribed negative-strand DNA template[11,16]. Once the relaxed circular (rc) HBV genome synthesis is complete, the nucleocapsid interacts with envelope protein in endoplasmic reticulum to form mature virions and they are secreted from the host cell. Alternatively, The rcDNA genome within the nucleocapsid core particles may also recycle to the cell nucleus to replenish the nuclear cccDNA pool. In summary, the HBV is a DNA virus but utilizes reverse transcription of an RNA intermediate to replicate its genome similar to retroviruses. This error-prone replication strategy combined with high viral replication rate (~1012 virus/d) leads to significant viral variability or quasispecies (QS). The HBV genomic mutation rate occurring at each nucleotide of the HBV genome is estimated at ~10-5 base/site/cycle[13]. The long half-life of hepatocytes and cccDNA template play an important role in archiving spontaneously occurring and antiviral drug-associated mutants[17].

Although the HBV is predominantly a hepatotropic virus, there is increasing evidence documenting that the immune (lymphoid) system is also an important site for maintaining viral persistence[18]. In the closely related woodchuck animal model of HBV, woodchuck hepatitis virus (WHV) infection can be completely restricted to the lymphoid system and WHV invasion of lymphoid cells is related to the viral load[19,20]. In human studies, HBV genomes are detectable in peripheral blood mononuclear cells (PBMC) from chronically infected patients despite long-term suppressive anti-HBV nucleos/tide analog (NA) therapy[21], in patients after resolution of acute hepatitis B with HBV surface antigen (HBsAg) clearance[22,23], and in circulating transplacental PBMC from HBV positive mothers possibly leading to *in utero* infection of the neonate[24]. HBV antigens, messenger RNA (mRNA), covalently closed circular DNA (cccDNA) and integrated forms have been detected in PBMC and extrahepatic tissues such as, bone marrow cells, spleen, and lymphoblastoid cell lines[25,26]. Additionally, upregulation of HBV replication in PBMC occurs following *ex-vivo* mitogen stimulation and the release of viral particles capable of further infection and replication from these HBV infected PBMC[27]. HBV genomes and viral proteins have been detected within a variety of immune cell subpopulations and, in some reports the virus appears to specifically target B cells and monocytes[28-31].

**OVERVIEW OF HBV GENOTYPES**

There are nine major HBV genotypes (A-I) worldwide, which are identified by greater than 7.5% divergence across the HBV full genome between each genotype[32]. There is also a tenth putative genotype “J” isolated from a Japanese individual[33]. In addition to HBV genotypes, at least 35 subgenotypes (*i.e.,* within genotype A, B, C, D, F, H, but not in genotype E, G) have been identified. The HBV genotypes/subgenotypes are ethnically and geographically distributed. For instance, genotype B and C are prevalent in Asia, while genotype A and D are most frequently seen in Europe, the Mediterranean region and the Middle East[34]. Certain genotypes may exhibit different mutations. The common HBV pre-core (pre-C) mutation more frequently exists in genotype B, C, and D than in genotype A[35,36]; genotype C tends to carry more mutations compare to genotype B[37]. In addition, genotypes are also linked to the natural history of CHB leading to distinct clinical outcomes and responses to therapy[38-40]. For instance, the cumulative rate of spontaneous HBeAg seroconversion with genotype B is higher than patients with genotype C infection[41,42]. Others report genotype-specific differences in NA response, resistance to older generation NA (*i.e.,* LMV or ADF) and, durability of HBeAg seroconversion (138.) Whilst this has less clinical relevance with the newer potent NA (*i.e.,* TDF and ETV), alternative therapy endpoints such as HBsAg loss and HCC potential may be identified. The role of genotypes in CHB management has been extensively reviewed[43-45]. In summary, clinically relevant features of HBV genotypes include: the rate and durability of HBeAg loss/seroconversion (A and D > B and C), the risk of developing aggressive HBeAg (-) CHB (C and D > A), spontaneous HBeAg loss (B > C), cirrhosis (C), HCC (C in Asians, F in Alaska Natives), and response to antivirals (A and B > C and D).

**OVERVIEW OF HBV QUASISPECIES AND CLINICALLY RELEVANT HBV VARIANTS**

***HBV Quasispecies***

The HBV replicates via an error-prone RT leading to non-identical but a genetically closely related variants pool, which is known as quasispecies (QS). Both the wildtype and HBV QS are archived in the hepatocytes reservoir. In the process of Darwinian evolution, QS that survive selective pressue (*i.e.,* host immune response and/or NA therapy) may predominate. Thus, the HBV QS diversity may reflect host humoral response. It was reported that less HBV variants were found in patients in the immune tolerant phase compared to the immune active phase[46]. Recent studies have found that HBeAg seroconverstion was associated with dynamic changes in the HBV QS pool years before viral load drop, hence HBeAg seroconversion may be a slow process rather than a sudden immunological event[47]. In other studies, NA-associated HBV mutations were commonly found in CHB patients as minor populations even before the initiation of antiviral therapy[48]. It has been reported that NA treatment experienced patients, even without carrying a specific drug resistant mutation (*i.e.,* LMV-R), still demonstrate a high possibility to develop cross- resistance to a related drug[49]. Thus, it is possible that LMV-R mutations may pre-exist as a minor HBV QS strain. Further, HBV QS diversity and/or complexity 4 wk after initiation of antiviral therapy has been associated with response to treatment[50,51]. Due to the sensitivity of direct sequencing assays, some minor variants may not be detected, especially when the mutation proportion is less than 20%[52,53]. However, clonal sequencing and next generation sequencing assays can overcome these limitations and detect even minor QS variants.

HBV variants have been shown to be relevant to disease progression, development of HCC, reliability of diagnostic assay detection, vaccine failures and response to antiviral therapy[54,55]. We will summarize how specific mutations can impact the major functions of the 4 HBV gene products, highlighting variants associated with liver disease development (Table 1).

***HBV PRES/S variants (immune escape, diagnostic assay detection, and occult HBV infection)***

The HBV envelope protein is encoded by *preS1*/*preS2*/*S* gene in a frame-shift manner generating three different envelope proteins: large (L), middle (M) and small (S). Detection of either the secreted or virion associated HBsAg for greater than 6 months in serum confirms chronic infection. The HBsAg pre-S1 is involved in attachment to host cell receptor and neutralizing antibody binding. The antibodies predominantly target the hydrophilic region of major HBsAg protein, known as the “a-determinant”, located at amino acid positon 99-170. Thus, “a-determinant” mutations may affect HBsAg antigenicity, leading to vaccine escape, false-negative results by diagnostic HBsAg detection assays, and hepatitis B immunoglobulin (HBIG) treatment failures[56].

The transmission of HBV vaccine escape variants to susceptible individuals may have significant public health care implications[57]. The sG145R point mutation is the most widely reported “vaccine escape” mutant, which can infect anti-HBs positive individuals by reduced anti-HBs binding. The sG145R mutant is stable and can be transmitted horizontally in presence of high titer anti-HBs[58]. Furthermore, G145R mutant along with an insertion between 122 and 123 in the “a” determinant was reported in patients with fulminant reactivation of hepatitis B[59]. In addition to sG145R, the K141E, T131I variant, and insertion of three amino acids between 123 and 124 can significantly affect the structure of HBsAg[60]. More recently, other a-determinant substitutions were reported in association with vaccine escape (*i.e.,* T116N, P120S/E, I/T126A/N/I/S, Q129H/R, M133L, K141E, P142S and D144A/E). Although vaccine-escape mutations appear to be more common in endemic areas with universal immunization programs, to date these mutants have not caused any negative effect on global immunization programs since they appear to develop slowly[61].

Due to the overlapping ORF of the HBV *S* gene and *P* gene, NA targeting the HBV RT/ *P* gene and induced antiviral mutations may lead to corresponding *S* gene mutation (and vice-versa), or so called antiviral-drug-associated *S* gene mutations (ADASM)[62]. The ADASM may influence clinical outcome by altering envelope protein antigenicity, viral fitness and oncogenic potential. For example, the *S* gene premature stop codon at position 172 (W172\*), with a 55 amino acids missing at 3’-terminus, might result from the rtA181T mutation in the overlapping *P* gene. The W172\* was shown to be associated with liver cirrhosis and HCC[63].

Occult HBV infection (OBI) is characterized by negative HBsAg in serum but with persistent HBV DNA in liver. According to the Taormina consensus conference definition, OBI is usually due to the presence of low-level replication competent virus in which viral HBsAg cannot be detected by standard commercial assays[64]. The viral DNA is only detectable in liver, serum, as well as PBMC but the viral load is usually very low (< 200 virus copes/mL). However, HBsAg negativity with ongoing moderate to high-level viral replication may be due to infection with HBsAg mutants that produce a modified HBsAg that cannot be detected by current commercial assays. Further, based on our groups studies it is speculated that during OBI, the HBV preferentially infects PBMC (compared to liver), especially at very low viral load suggesting a specifc selective mechanism involved in the course of OBI infection of the host immune system[21,65,66].

***HBV PRES1/PRES2 deletion mutations***

The *preS* gene represents the highest heterogeneity of the HBV genome[67]. The preS region mediates virus binding with hepatocytes, and interaction with B cells and T cells indicating that it plays an important role in the host immune response against HBV infection[68-71]. Thus immune pressure from vaccination as well as immunotherapy may induce the preS region mutation. Previous researchers have reported that the preS gene mutation can affect immune response, virus expression, synthesis and secretion[71-74]. It was found that preS deletion mutants often exist in CHB, especially in patients with HBV genotype C infection[75]. The *preS* deletion mutant strongly correlates with liver disease progression, possibly due to defective secretion, accumulation of HBsAg in the hepatocyte ER, leading to ER-induced cell stress. The cell cytotoxicity can contribute to oncogenesis[76]. It was suggested that the preS deletion mutation together with another S point mutation is correlated with coexistence of HBsAg and anti-HBs, indicating specific immune selection pressure[77]. Additionally, the preS deletion mutation has been associated with the occurrence of HCC in several studies, which reported a 52%-62% incidence of *preS* deletion in patients who developed HCC[71,76,78-80]. The HBV genome can also integrate into human chromosome and play an oncogenic role. For instance, *preS2*/*S* genes were found with a 3’ end truncation from integrated HBV DNA in HCC tissue. The truncated proteins may have transcriptional/transactivation potential leading to HCC development[81, 82].

***HBV P variants and drug-resistant mutations***

The HBV P has 4 functional domains: a priming region, a spacer region, a catalytic region that plays a RNA-dependent RNA polymerase/DNA polymerase function, and a carboxy terminal region that has ribonuclease H activity. There are 7 domains in P/RT region: A-G. The YMDD (tyrosine, methionine, aspartate, aspartate) motif locates in catalytic site in the domain C. It is highly conserved in all genotypes and plays an essential catalytic role in HBV replication. Thus, YMDD mutations, such as YVDD (rtM204V, methionine to valine mutation) and YIDD (rtM204I, methionine to isoleucine mutation) mutations could lead to antiviral resistance and defective viral replication. As noted, NAs inhibit the HBV P/RT and both plus and minus strand HBV DNA synthesis. The NAs have a similar structure to natural nucleotides with a modified sugar ring or base group that competes with the natural nucleotides in binding to the HBV P, leading to chain termination. Compared to IFN, NAs are more commonly used due to their more favorable side effect profile. However they require prolonged treatment as they have minimal effect on the cccDNA pool. The molecular mechanism of drug-resistance is specific to the NA sugar ring structure. To date, four major drug resistance pathways have been identified[83]: (1) L-nucleosides pathway which is characterized by rtM204V/I mutation resulting in resistance to LAM and LdT; (2) acyclic/alkyl phosphonate sugar pathway which is identified by presence of rtN236T substitution leading to resistance to ADF and reduced susceptibility of TDF; (3) the pathway which is shared by both L-nucleosides (LMV, LdT, reduced sensitivity to TDF) and acyclic/alkyl phosphonates (ADF) by emergence of rtA181T/V; (4) the D-cyclopentante pathway which is characterized by presence of rtL180M and rtM204V/I mutations plus at least one substitution in one of the rtT184, rt202 and rtM250 amino acid (aa) positions. LMV has the worst resistance profile with an annual resistance rate of 15%-25% and > 80% after 5 years treatment[84]. The rtM204V/I mutant, which is located at position 204 of YMDD motif, can result in LMV and LdT resistance and is often accompanied with compensatory mutations (*i.e.,* rtL80V/I, rtI169T, rtV173L, rtL180M, rtT184S/G, rtS202I and rtQ215S)[85]. The compensatory mutations are able to restore HBV replication activity to near wild type levels. In addition, YMDD variants were also found in patients without prior NA exposure[86]. In recent study, the spontaneous YMDD variants were reported more frequently occurred in HCC patients with HBV genotype C, which might be the cause of greater oncogenesis of genotype C compare to genotype B[87]. Thus, it is important to monitor YMDD mutations in patients on NA therapy in order to adjust treatment regimen in time. The resistance rate to ADF is ~30% after 5 years treatment but may be higher in patients with pre-existing NAs-associated mutations[88]. Two primary mutations induced by ADF and TDF (rtA181T and rtN236T) belong to the acyclic/alkyl phosphonates pathway. The rtA194T variant has been reported to be associated with partial TDF resistance, and confer reduced HBV replication *in vitro*[89]. In clinical practice, however, TDF resistance and virological breakthrough has not been reported in patients after more than six years of treatment [8]. Similarly, rtP177G and rtF249A have also been shown to impact HBV replication and enhance resistance to TDF both *in vitro* and *in vivo*[90]. ETV also has a very high genetic barrier to the development of drug-resistant mutations; the rate of resistance occurrence is 1.2% after 5 years in treatment naïve patients[91]. The resistance to D-cyclopentante group (ETV) occurs only when at least three mutations are present: rtL180M + rtM204V and either rtT184G/S or rtS202I/G or rtM250V[17]. However, due to cross-resistance, the presence of LMV-resistant mutations can lead to ETV resistance and treatment failure. Of note the rtA181T/V mutation in domain B of HBV P, was reported to confer resistance to both L-nucleosides and acyclic/alkyl phosphonates[92]. Further, the rtA181T also encodes a stop codon at aa172 in the overlapping S region (sW172\*) in a frame-shift manner, which leads to truncated S protein production. The rtA181T/sW172\* mutation can cause defective secretion of HBV S and may play an oncogenic role leading to HCC by transactivation of cellular promoters[93].

Due to the overlapping ORF of HBV *P/S* gene, HBV P drug-resistance variants selected by NAs may lead to HBsAg amino acid change and altered antigenicity. Conversely, immune pressure on HBsAg is able to introduce variants that correspond with primary or compensatory drug-resistant mutations in the *P* gene[94], as noted above.

***PREC/BCP mutations (HCC associated)***

The HBV *preC/C* gene encodes both the HBV precore and core protein with distinct start codon (*i.e.,* preC initiates from the first start codon while core protein from the second). The preC protein encodes soluble HBeAg. It has an additional 29 aa at the N-teminus end, which serves as a signal to transport the pre-core protein to the cellular ER, the first 16 aa is cleaved, and the viral protein secreted from the cell as a soluble HBeAg antigen. HBeAg is believed to play an important role in immune tolerance and viral persistence. The HBeAg-negative CHB phase with active hepatitis occurs in association with a precore and BCP region variant[95,96]. The most prevalent mutation in preC region is G1896A, which generates a premature stop codon at aa 28 in the sequence of HBeAg, which affects the trafficking of the precore to the ER and subsequent HBeAg secretion. This mutation is significantly associated with HBV genotypes harboring a T nucleotide (genotypes B, D, E and part of genotypes C and F) rather than C nucleotide at positon 1858[95]. This is because this variant affects the stability of the pregenomic episilon structure, and the pregenomic encapsidaton signal (see Section 3.0). The preC mutation is more often observed in genotype D HBV infection (65%) compared to HBV genotype A infections (9%). It was found that the preC deletion mutation is often associated with more severe liver disease, but has also been found in inactive HBV carriers. In addition, the preC and BCP mutations are also related to response to IFN therapy: e.g., the G1896A mutation was showed to be associated with poor response to IFN therapy independent of HBeAg status[97] while the presence of less mutations in BCP region are associated with a better treatment response[98].

The HBV BCP is located upstream of the *preC* gene, hence mutations that occur in the BCP region can downregulate preC mRNA transcription and inhibit HBeAg synthesis. The A1762T/G1764A double mutation in the BCP region, leads to preC mRNA reduction resulting in HBeAg seroconversion and a ~50% reduction of HBeAg levels[99,100]. Similar to preC mutations, BCP mutations also show genotype specific prevalence, and are more often seen in HBV genotype C and D infections[101]. One study demonstrated a significant temporal correlation between the relative increase in mutant concentration and HBeAg seroconversion. In HBeAg-negative hepatitis patients, viral load is usually several log lower compared to HBeAg-positive patients but the HBV replication capacity may be partially restored by BCP mutant, especially if accompanied with any of 3 additional BCP mutations (T1753C, C1766T, T1768A). The increased HBV replication may be associated with disease progression[102]. The preC stop codon mutation and BCP mutations often appear together. Recent studies demonstrated that the combination of BCP and preC mutations and preS1, preS2 deletion mutants could lead to more severe liver disease including fulminant hepatitis and HCC. It is now believed that the development of HBV-induced HCC involves various factors in the interaction between HBV and the host. Multiple HBV mutations existing in different regions were shown to play an important role in HBV associated oncogenesis. For example, the BCP A1762T/G1764A double mutations and preC mutations are prone to HCC generation compared to patients with wild type HBV infection[37,103]. A recent meta-analysis concluded that HBV carriers, especially Asians, were significantly more likely to develop to HCC and severe liver disease with the presence of G1896A mutations. The other mutations in preC and BCP regions, such as G1899A, T1753V and C1653T are also associated with an increased risk of HCC development[104].

***HBV X variants***

The *HBV X* is the smallest gene, with an N-terminal negative regulatory/anti-apoptotic domain and a C-terminal transactivation/pro-apoptotic domain. The HBx is an unique regulatory viral protein since it does not bind to either viral or host DNA, however, it is able to activate transcription of viral and cellular genes by direct or indirect interaction with a variety of targets[105]. Thus, it is required for HBV persistence. Additionally, it can modulate various cellular functions, including active humoral and cellular immune responses which may ultimately result in HBV-associated hepatocarcinogenesis[106]. It was demonstrated that the HBx was a nuclear coactivator or could stimulated signal transduction by several pathways, such as NF-κB signaling pathway. The NF-κB pathway was reported stimulated by HBx though direct acting on NF-κB itself, stimulating phosphorylation of IκB or interaction with upstream signal transduction pathway[107,108]. NF-κB is necessary for cell growth and viability; recent study showed the activation of NF-κB could prevent apoptosis. Thus, the HBx-induced NF-κB pathway activation may promote the survival of infected and mutated cells that favors the hepatocarcinogenesis[109,110]. Several *X* gene mutants and deletions have been reported in HCC patients. For instance, the existence of HBx130+HBX131 double mutation and HBx5+HBx130+HBx131 triple mutation showed a significant risk for HCC development[111]. This was suggested due to the increasing activity of NF-κB by double HBx mutation and increased cell burden of triple HBx mutation and its potential influence on structure and NF-κB activity[111]. The HBV DNA integrates into host cellular chromosomes often with 3’-end deletion that may play an important role in HBV oncogenesis. Integrated HBV *X* gene sequences were found in liver tissue of most CHB patients and ~86% of HBV-related HCC patients[112].

**CONCLUSION**

The HBV has significant genomic diversity and some HBV variants are associated with antiviral therapy response, vaccine escape, diagnostic failure, liver fibrosis progression and HCC development. Understanding HBV molecular epidemiology as well as the clinical and pathological relevence of HBV variants during different disease phases may enable more accurate risk-stratification of individual patients at risk for serious sequelae of chronic hepatitis B infection.

**REFERENCES**

1 **FitzSimons D**, Hendrickx G, Vorsters A, Van Damme P. Hepatitis B vaccination: a completed schedule enough to control HBV lifelong? Milan, Italy, 17-18 November 2011. *Vaccine* 2013; **31**: 584-590 [PMID: 23142301 DOI: 10.1016/j.vaccine.2012.10.101]

2 **Hyams KC**. Risks of chronicity following acute hepatitis B virus infection: a review. *Clin Infect Dis* 1995; **20**: 992-1000 [PMID: 7795104 DOI: 10.1093/clinids/20.4.992]

3 **Weinbaum CM,** Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW; Centers for Disease Control and Prevention (CDC). Recommendations for identification and public health management of persons with chronic hepatitis b virus infection. *MMWR Recomm Rep* 2008; **57**: 1-20 [PMID: 18802412]

4 **Kennedy PT**, Sandalova E, Jo J, Gill U, Ushiro-Lumb I, Tan AT, Naik S, Foster GR, Bertoletti A. Preserved T-cell function in children and young adults with immune-tolerant chronic hepatitis B. *Gastroenterology* 2012; **143**: 637-645 [PMID: 22710188 DOI: 10.1053/j.gastro.2012.06.009]

5 **Chang MH**, Hwang LY, Hsu HC, Lee CY, Beasley RP. Prospective study of asymptomatic HBsAg carrier children infected in the perinatal period: clinical and liver histologic studies. *Hepatology* 1988; **8**: 374-377 [PMID: 3356419 DOI: 10.1002/hep.1840080231]

6 **Bertoletti A,** Kennedy PT. The immune tolerant phase of chronic HBV infection: new perspectives on an old concept. *Cell Mol Immunol* 2014; Epub ahead of print [PMID: 25176526 DOI: 10.1038/cmi.2014.79]

7 **Wang HY**, Chien MH, Huang HP, Chang HC, Wu CC, Chen PJ, Chang MH, Chen DS. Distinct hepatitis B virus dynamics in the immunotolerant and early immunoclearance phases. *J Virol* 2010; **84**: 3454-3463 [PMID: 20089644 DOI: 10.1128/JVI.02164-09]

8 **Kitrinos KM**, Corsa A, Liu Y, Flaherty J, Snow-Lampart A, Marcellin P, Borroto-Esoda K, Miller MD. No detectable resistance to tenofovir disoproxil fumarate after 6 years of therapy in patients with chronic hepatitis B. *Hepatology* 2014; **59**: 434-442 [PMID: 23939953 DOI: 10.1002/hep.26686]

9 **Colonno RJ**, Rose R, Baldick CJ, Levine S, Pokornowski K, Yu CF, Walsh A, Fang J, Hsu M, Mazzucco C, Eggers B, Zhang S, Plym M, Klesczewski K, Tenney DJ. Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. *Hepatology* 2006; **44**: 1656-1665 [PMID: 17133475 DOI: 10.1002/hep.21422]

10 **Papatheodoridis GV**, Manolakopoulos S, Dusheiko G, Archimandritis AJ. Therapeutic strategies in the management of patients with chronic hepatitis B virus infection. *Lancet Infect Dis* 2008; **8**: 167-178 [PMID: 18053766 DOI: 10.1016/S1473-3099(07)70264-5]

11 **Seeger C,** Mason WS. 36 hepadnavirus. New York: Cold Spring Harbor Monograph Archive 2006: 729-744 [DOI: 10.1101/087969766.47.729]

12 **Yan H,** Zhong G, Xu G, He W, Jing Z, Gao Z, Huang Y, Qi Y, Peng B, Wang H, Fu L, Song M, Chen P, Gao W, Ren B, Sun Y, Cai T, Feng X, Sui J, Li W. Sodium taurocholate cotransporting polypeptide is a functional receptor for human hepatitis b and d virus. *Elife* 2012; **1**: e00049 [PMID: 23150796 DOI: 10.7554/eLife.00049]

13 **Locarnini S**, Zoulim F. Molecular genetics of HBV infection. *Antivir Ther* 2010; **15** Suppl 3: 3-14 [PMID: 21041899 DOI: 10.3851/imp1619]

14 **Summers J**, Mason WS. Replication of the genome of a hepatitis B--like virus by reverse transcription of an RNA intermediate. *Cell* 1982; **29**: 403-415 [PMID: 6180831 DOI: 10.1016/0092-8674(82)90157-X]

15 **Bartenschlager R**, Schaller H. Hepadnaviral assembly is initiated by polymerase binding to the encapsidation signal in the viral RNA genome. *EMBO J* 1992; **11**: 3413-3420 [PMID: 1380455]

16 **Will H**, Reiser W, Weimer T, Pfaff E, Büscher M, Sprengel R, Cattaneo R, Schaller H. Replication strategy of human hepatitis B virus. *J Virol* 1987; **61**: 904-911 [PMID: 3806799]

17 **Zoulim F**, Locarnini S. Hepatitis B virus resistance to nucleos(t)ide analogues. *Gastroenterology* 2009; **137**: 1593-1608.e1-2 [PMID: 19737565 DOI: 10.1053/j.gastro.2009.08.063]

18 **Pontisso P**, Vidalino L, Quarta S, Gatta A. Biological and clinical implications of HBV infection in peripheral blood mononuclear cells. *Autoimmun Rev* 2008; **8**: 13-17 [PMID: 18706529 DOI: 10.1016/j.autrev.2008.07.016]

19 **Coffin CS**, Michalak TI. Persistence of infectious hepadnavirus in the offspring of woodchuck mothers recovered from viral hepatitis. *J Clin Invest* 1999; **104**: 203-212 [PMID: 10411550 DOI: 10.1172/JCI5048]

20 **Michalak TI**, Mulrooney PM, Coffin CS. Low doses of hepadnavirus induce infection of the lymphatic system that does not engage the liver. *J Virol* 2004; **78**: 1730-1738 [PMID: 14747538 DOI: 10.1128/JVI.78.4.1730-1738.2004]

21 **Coffin CS**, Mulrooney-Cousins PM, Peters MG, van Marle G, Roberts JP, Michalak TI, Terrault NA. Molecular characterization of intrahepatic and extrahepatic hepatitis B virus (HBV) reservoirs in patients on suppressive antiviral therapy. *J Viral Hepat* 2011; **18**: 415-423 [PMID: 20626626 DOI: 10.1111/j.1365-2893.2010.01321.x]

22 **Michalak TI**, Pasquinelli C, Guilhot S, Chisari FV. Hepatitis B virus persistence after recovery from acute viral hepatitis. *J Clin Invest* 1994; **93**: 230-239 [PMID: 8282792 DOI: 10.1172/JCI116950]

23 **Cabrerizo M**, Bartolomé J, Caramelo C, Barril G, Carreno V. Molecular analysis of hepatitis B virus DNA in serum and peripheral blood mononuclear cells from hepatitis B surface antigen-negative cases. *Hepatology* 2000; **32**: 116-123 [PMID: 10869298 DOI: 10.1053/jhep.2000.8541]

24 **Shao Q**, Zhao X, Yao Li MD. Role of peripheral blood mononuclear cell transportation from mother to baby in HBV intrauterine infection. *Arch Gynecol Obstet* 2013; **288**: 1257-1261 [PMID: 23708388 DOI: 10.1007/s00404-013-2893-x]

25 **Mason A**, Wick M, White H, Perrillo R. Hepatitis B virus replication in diverse cell types during chronic hepatitis B virus infection. *Hepatology* 1993; **18**: 781-789 [PMID: 8406351 DOI: 10.1002/hep.1840180406]

26 **Stoll-Becker S**, Repp R, Glebe D, Schaefer S, Kreuder J, Kann M, Lampert F, Gerlich WH. Transcription of hepatitis B virus in peripheral blood mononuclear cells from persistently infected patients. *J Virol* 1997; **71**: 5399-5407 [PMID: 9188611]

27 **Bouffard P**, Lamelin JP, Zoulim F, Lepot D, Trepo C. Phytohemagglutinin and concanavalin A activate hepatitis B virus in peripheral blood mononuclear cells of patients with chronic hepatitis B virus infection. *J Med Virol* 1992; **37**: 255-262 [PMID: 1402824 DOI: 10.1002/jmv.1890370404]

28 **Yoffe B**, Noonan CA, Melnick JL, Hollinger FB. Hepatitis B virus DNA in mononuclear cells and analysis of cell subsets for the presence of replicative intermediates of viral DNA. *J Infect Dis* 1986; **153**: 471-477 [PMID: 3005423 DOI: 10.1093/infdis/153.3.471]

29 **Trippler M**, Meyer zum Büschenfelde KH, Gerken G. HBV viral load within subpopulations of peripheral blood mononuclear cells in HBV infection using limiting dilution PCR. *J Virol Methods* 1999; **78**: 129-147 [PMID: 10204703 DOI: 10.1016/S0166-0934(98)00172-4]

30 **Chemin I,** Vermot-Desroches C, Baginski I, Saurin JC, Laurent F, Zoulim F, Bernaud J, Lamelin JP, Hantz O, Rigal D. Selective detection of human hepatitis b virus surface and core antigens in peripheral blood mononuclear cell subsets by flow cytometry. *J Viral Hepat* 1994; **1**: 39-44 [PMID: 8790558 DOI: 10.1111/j.1365-2893.1994.tb00060.x]

31 **Bouffard P**, Lamelin JP, Zoulim F, Pichoud C, Trepo C. Different forms of hepatitis B virus DNA and expression of HBV antigens in peripheral blood mononuclear cells in chronic hepatitis B. *J Med Virol* 1990; **31**: 312-317 [PMID: 2269882 DOI: 10.1002/jmv.1890310413]

32 **Kramvis A**, Arakawa K, Yu MC, Nogueira R, Stram DO, Kew MC. Relationship of serological subtype, basic core promoter and precore mutations to genotypes/subgenotypes of hepatitis B virus. *J Med Virol* 2008; **80**: 27-46 [PMID: 18041043 DOI: 10.1002/jmv.21049]

33 **Tatematsu K**, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, Nakayoshi T, Wakuta M, Miyakawa Y, Mizokami M. A genetic variant of hepatitis B virus divergent from known human and ape genotypes isolated from a Japanese patient and provisionally assigned to new genotype J. *J Virol* 2009; **83**: 10538-10547 [PMID: 19640977 DOI: 10.1128/JVI.00462-09]

34 **Kramvis A**, Kew M, François G. Hepatitis B virus genotypes. *Vaccine* 2005; **23**: 2409-2423 [PMID: 15752827 DOI: 10.1016/j.vaccine.2004.10.045]

35 **Lindh M**, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus--large-scale analysis using a new genotyping method. *J Infect Dis* 1997; **175**: 1285-1293 [PMID: 9180165 DOI: 10.1086/516458]

36 **Chu CJ**, Keeffe EB, Han SH, Perrillo RP, Min AD, Soldevila-Pico C, Carey W, Brown RS, Luketic VA, Terrault N, Lok AS. Hepatitis B virus genotypes in the United States: results of a nationwide study. *Gastroenterology* 2003; **125**: 444-451 [PMID: 12891547 DOI: 10.1016/S0016-5085(03)00895-3]

37 **Liu S**, Zhang H, Gu C, Yin J, He Y, Xie J, Cao G. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. *J Natl Cancer Inst* 2009; **101**: 1066-1082 [PMID: 19574418 DOI: 10.1093/jnci/djp180]

38 **Kramvis A**, Kew MC. Relationship of genotypes of hepatitis B virus to mutations, disease progression and response to antiviral therapy. *J Viral Hepat* 2005; **12**: 456-464 [PMID: 16108759 DOI: 10.1111/j.1365-2893.2005.00624.x]

39 **Liaw YF**, Brunetto MR, Hadziyannis S. The natural history of chronic HBV infection and geographical differences. *Antivir Ther* 2010; **15** Suppl 3: 25-33 [PMID: 21041901 DOI: 10.3851/imp1621]

40 **McMahon BJ**. The influence of hepatitis B virus genotype and subgenotype on the natural history of chronic hepatitis B. *Hepatol Int* 2009; **3**: 334-342 [PMID: 19669359 DOI: 10.1007/s12072-008-9112-z]

41 **Chu CJ**, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002; **122**: 1756-1762 [PMID: 12055581 DOI: 10.1053/gast.2002.33588]

42 **Sumi H**, Yokosuka O, Seki N, Arai M, Imazeki F, Kurihara T, Kanda T, Fukai K, Kato M, Saisho H. Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; **37**: 19-26 [PMID: 12500184 DOI: 10.1053/jhep.2003.50036]

43 **Kim BK**, Revill PA, Ahn SH. HBV genotypes: relevance to natural history, pathogenesis and treatment of chronic hepatitis B. *Antivir Ther* 2011; **16**: 1169-1186 [PMID: 22155900 DOI: 10.3851/IMP1982]

44 **Cooksley WG**. Do we need to determine viral genotype in treating chronic hepatitis B? *J Viral Hepat* 2010; **17**: 601-610 [PMID: 20529201 DOI: 10.1111/j.1365-2893.2010.01326.x]

45 **Congly SE**, Wong P, Al-Busafi SA, Doucette K, Fung SK, Ghali P, Fonseca K, Myers RP, Osiowy C, Coffin CS. Characterization of hepatitis B virus genotypes and quantitative hepatitis B surface antigen titres in North American tertiary referral liver centres. *Liver Int* 2013; **33**: 1363-1369 [PMID: 23763288 DOI: 10.1111/liv.12222]

46 **Tedder RS**, Bissett SL, Myers R, Ijaz S. The 'Red Queen' dilemma--running to stay in the same place: reflections on the evolutionary vector of HBV in humans. *Antivir Ther* 2013; **18**: 489-496 [PMID: 23792884 DOI: 10.3851/imp2655]

47 **Lim SG**, Cheng Y, Guindon S, Seet BL, Lee LY, Hu P, Wasser S, Peter FJ, Tan T, Goode M, Rodrigo AG. Viral quasi-species evolution during hepatitis Be antigen seroconversion. *Gastroenterology* 2007; **133**: 951-958 [PMID: 17854598 DOI: 10.1053/j.gastro.2007.06.011]

48 **Kim D,** Ahn S, Chang H, Shim H, Heo J, Cho M, Moon B, Moon Y, Paik Y, Lee K. 563 hepatitis b virus quasispecies in the polymerase gene in treatment-naive chronic hepatitis b patients. *J Hepatol* 2008; **48**: S211 [DOI: 10.1016/S0168-8278(08)60565-6]

49 **Lee JH**, Cho Y, Lee DH, Lee M, Yoo JJ, Choi WM, Cho YY, Lee YB, Yu SJ, Yoon JH, Lee HS, Kim YJ. Prior exposure to lamivudine increases entecavir resistance risk in chronic hepatitis B Patients without detectable lamivudine resistance. *Antimicrob Agents Chemother* 2014; **58**: 1730-1737 [PMID: 24395227 DOI: 10.1128/AAC.02483-13]

50 **Peveling-Oberhag J**, Herrmann E, Kronenberger B, Farnik H, Susser S, Sarrazin C, Zeuzem S, Hofmann WP. Dynamics of hepatitis B virus quasispecies heterogeneity and virologic response in patients receiving low-to-moderate genetic barrier nucleoside analogs. *J Viral Hepat* 2013; **20**: 234-239 [PMID: 23490367 DOI: 10.1111/jvh.12013]

51 **Chen L**, Zhang Q, Yu DM, Wan MB, Zhang XX. Early changes of hepatitis B virus quasispecies during lamivudine treatment and the correlation with antiviral efficacy. *J Hepatol* 2009; **50**: 895-905 [PMID: 19304333 DOI: 10.1016/j.jhep.2008.12.018]

52 **Lok AS**, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, Liaw YF, Mizokami M, Kuiken C. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; **46**: 254-265 [PMID: 17596850 DOI: 10.1002/hep.21698]

53 **Kao JH**. Diagnosis of hepatitis B virus infection through serological and virological markers. *Expert Rev Gastroenterol Hepatol* 2008; **2**: 553-562 [PMID: 19072403 DOI: 10.1586/17474124.2.4.553]

54 **Lai MW**, Huang SF, Hsu CW, Chang MH, Liaw YF, Yeh CT. Identification of nonsense mutations in hepatitis B virus S gene in patients with hepatocellular carcinoma developed after lamivudine therapy. *Antivir Ther* 2009; **14**: 249-261 [PMID: 19430100]

55 **Locarnini SA**. Hepatitis B virus surface antigen and polymerase gene variants: potential virological and clinical significance. *Hepatology* 1998; **27**: 294-297 [PMID: 9425951 DOI: 10.1002/hep.510270144]

56 **Carman WF**, Zanetti AR, Karayiannis P, Waters J, Manzillo G, Tanzi E, Zuckerman AJ, Thomas HC. Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; **336**: 325-329 [PMID: 1697396 DOI: 10.1016/0140-6736(90)91874-A]

57 **Thakur V**, Kazim SN, Guptan RC, Hasnain SE, Bartholomeusz A, Malhotra V, Sarin SK. Transmission of G145R mutant of HBV to an unrelated contact. *J Med Virol* 2005; **76**: 40-46 [PMID: 15778957 DOI: 10.1002/jmv.20321]

58 **Chakravarty R**, Neogi M, Roychowdhury S, Panda CK. Presence of hepatitis B surface antigen mutant G145R DNA in the peripheral blood leukocytes of the family members of an asymptomatic carrier and evidence of its horizontal transmission. *Virus Res* 2002; **90**: 133-141 [PMID: 12457969 DOI: 10.1016/S0168-1702(02)00147-8]

59 **Carman WF**, Korula J, Wallace L, MacPhee R, Mimms L, Decker R. Fulminant reactivation of hepatitis B due to envelope protein mutant that escaped detection by monoclonal HBsAg ELISA. *Lancet* 1995; **345**: 1406-1407 [PMID: 7539089 DOI: 10.1016/S0140-6736(95)92599-6]

60 **Seddigh-Tonekaboni S**, Waters JA, Jeffers S, Gehrke R, Ofenloch B, Horsch A, Hess G, Thomas HC, Karayiannis P. Effect of variation in the common "a" determinant on the antigenicity of hepatitis B surface antigen. *J Med Virol* 2000; **60**: 113-121 [PMID: 10596008 DOI: 10.1002/(SICI)1096-9071(200002)60: 2<113: : AID-JMV2>3.0.CO; 2-0]

61 **Basuni AA**, Butterworth L, Cooksley G, Locarnini S, Carman WF. Prevalence of HBsAg mutants and impact of hepatitis B infant immunisation in four Pacific Island countries. *Vaccine* 2004; **22**: 2791-2799 [PMID: 15246613 DOI: 10.1016/j.vaccine.2004.01.046]

62 **Kamili S**, Sozzi V, Thompson G, Campbell K, Walker CM, Locarnini S, Krawczynski K. Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model. *Hepatology* 2009; **49**: 1483-1491 [PMID: 19274751 DOI: 10.1002/hep.22796]

63 **Lee SA**, Kim K, Kim H, Kim BJ. Nucleotide change of codon 182 in the surface gene of hepatitis B virus genotype C leading to truncated surface protein is associated with progression of liver diseases. *J Hepatol* 2012; **56**: 63-69 [PMID: 21827734 DOI: 10.1016/j.jhep.2011.06.028]

64 **Raimondo G**, Pollicino T, Romanò L, Zanetti AR. A 2010 update on occult hepatitis B infection. *Pathol Biol* (Paris) 2010; **58**: 254-257 [PMID: 20303674 DOI: 10.1016/j.patbio.2010.02.003]

65 **Coffin CS**, Mulrooney-Cousins PM, van Marle G, Roberts JP, Michalak TI, Terrault NA. Hepatitis B virus quasispecies in hepatic and extrahepatic viral reservoirs in liver transplant recipients on prophylactic therapy. *Liver Transpl* 2011; **17**: 955-962 [PMID: 21462295 DOI: 10.1002/lt.22312]

66 **Coffin CS**, Mulrooney-Cousins PM, Osiowy C, van der Meer F, Nishikawa S, Michalak TI, van Marle G, Gill MJ. Virological characteristics of occult hepatitis B virus in a North American cohort of human immunodeficiency virus type 1-positive patients on dual active anti-HBV/HIV therapy. *J Clin Virol* 2014; **60**: 347-353 [PMID: 24881491 DOI: 10.1016/j.jcv.2014.04.021]

67 **Sterneck M,** Will H. Naturally occurring variants of hepatitis b virus. *Adv Virus Res* 1999; **52**: 25 [PMID: 103842354 DOI: 10.1016/S0065-3527(08)60298-5]

68 **Kuroki K**, Floreani M, Mimms LT, Ganem D. Epitope mapping of the PreS1 domain of the hepatitis B virus large surface protein. *Virology* 1990; **176**: 620-624 [PMID: 1693249 DOI: 10.1016/0042-6822(90)90032-M]

69 **Maeng CY,** Ryu CJ, Gripon P, Guguen-Guillouzo C, Hong HJ. Fine mapping of virus-neutralizing epitopes on hepatitis b virus pres1. *Virology* 2000; **270**: 9-16 [PMID: 10772975 DOI: 10.1006/viro.2000.0250]

70 **Park JH**, Cho EW, Lee YJ, Shin SY, Kim KL. Determination of the protective effects of neutralizing anti-hepatitis B virus (HBV) immunoglobulins by epitope mapping with recombinant HBV surface-antigen proteins. *Microbiol Immunol* 2000; **44**: 703-710 [PMID: 11021401 DOI: 10.1111/j.1348-0421.2000.tb02552.x]

71 **Chen BF**, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; **130**: 1153-1168 [PMID: 16618410 DOI: 10.1053/j.gastro.2006.01.011]

72 **Kim HS**, Kim YK, Ryu SE, Hong HJ. Production of hepatitis B virus preS polypeptide in Escherichia coli by mutation of the 5'-end coding sequence and its purification and characterization. *Gene* 1996; **177**: 173-177 [PMID: 8921864 DOI: 10.1016/0378-1119(96)00296-X]

73 **Bruss V**. A short linear sequence in the pre-S domain of the large hepatitis B virus envelope protein required for virion formation. *J Virol* 1997; **71**: 9350-9357 [PMID: 9371594]

74 **Chaudhuri V**, Tayal R, Nayak B, Acharya SK, Panda SK. Occult hepatitis B virus infection in chronic liver disease: full-length genome and analysis of mutant surface promoter. *Gastroenterology* 2004; **127**: 1356-1371 [PMID: 15521005 DOI: 10.1053/j.gastro.2004.08.003]

75 **Sugauchi F**, Ohno T, Orito E, Sakugawa H, Ichida T, Komatsu M, Kuramitsu T, Ueda R, Miyakawa Y, Mizokami M. Influence of hepatitis B virus genotypes on the development of preS deletions and advanced liver disease. *J Med Virol* 2003; **70**: 537-544 [PMID: 12794715 DOI: 10.1002/jmv.10428]

76 **Chen CH**, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, Lu SN, Changchien CS. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007; **133**: 1466-1474 [PMID: 17915220 DOI: 10.1053/j.gastro.2007.09.002]

77 **Huang X**, Qin Y, Zhang P, Tang G, Shi Q, Xu J, Qi F, Shen Q. PreS deletion mutations of hepatitis B virus in chronically infected patients with simultaneous seropositivity for hepatitis-B surface antigen and anti-HBS antibodies. *J Med Virol* 2010; **82**: 23-31 [PMID: 19950231 DOI: 10.1002/jmv.21669]

78 **Hsieh YH**, Su IJ, Wang HC, Tsai JH, Huang YJ, Chang WW, Lai MD, Lei HY, Huang W. Hepatitis B virus pre-S2 mutant surface antigen induces degradation of cyclin-dependent kinase inhibitor p27Kip1 through c-Jun activation domain-binding protein 1. *Mol Cancer Res* 2007; **5**: 1063-1072 [PMID: 17951406 DOI: 10.1158/1541-7786.mcr-07-0098]

79 **Ito K**, Tanaka Y, Kato M, Fujiwara K, Sugauchi F, Sakamoto T, Shinkai N, Orito E, Mizokami M. Comparison of complete sequences of hepatitis B virus genotype C between inactive carriers and hepatocellular carcinoma patients before and after seroconversion. *J Gastroenterol* 2007; **42**: 837-844 [PMID: 17940837 DOI: 10.1007/s00535-007-2100-6]

80 **Kajiya Y**, Hamasaki K, Nakata K, Nakagawa Y, Miyazoe S, Takeda Y, Ohkubo K, Ichikawa T, Nakao K, Kato Y, Eguchi K. 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**: 149-156 [PMID: 11876799 DOI: 10.1046/j.1365-2893.2002.00335.x]

81 **Caselmann WH**, Meyer M, Kekulé AS, Lauer U, Hofschneider PH, Koshy R. A trans-activator function is generated by integration of hepatitis B virus preS/S sequences in human hepatocellular carcinoma DNA. *Proc Natl Acad Sci U S A* 1990; **87**: 2970-2974 [PMID: 2158099 DOI: 10.1073/pnas.87.8.2970]

82 **Kekulé AS**, Lauer U, Meyer M, Caselmann WH, Hofschneider PH, Koshy R. The preS2/S region of integrated hepatitis B virus DNA encodes a transcriptional transactivator. *Nature* 1990; **343**: 457-461 [PMID: 2153938 DOI: 10.1038/343457a0]

83 **Locarnini S**. Primary resistance, multidrug resistance, and cross-resistance pathways in HBV as a consequence of treatment failure. *Hepatol Int* 2008; **2**: 147-151 [PMID: 19669299]

84 **Lai CL**, Dienstag J, Schiff E, Leung NW, Atkins M, Hunt C, Brown N, Woessner M, Boehme R, Condreay L. Prevalence and clinical correlates of YMDD variants during lamivudine therapy for patients with chronic hepatitis B. *Clin Infect Dis* 2003; **36**: 687-696 [PMID: 12627352 DOI: 10.1086/368083]

85 **Bartholomeusz A**, Locarnini SA. Antiviral drug resistance: clinical consequences and molecular aspects. *Semin Liver Dis* 2006; **26**: 162-170 [PMID: 16673294 DOI: 10.1055/s-2006-939758]

86 **Bréchot C**, Thiers V, Kremsdorf D, Nalpas B, Pol S, Paterlini-Bréchot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; **34**: 194-203 [PMID: 11431751]

87 **Yang J**, Chen X, Zhang H, Chen G. HBV genotype C strains with spontaneous YMDD mutations may be a risk factor for hepatocellular carcinoma. *J Med Virol* 2014; **86**: 913-917 [PMID: 24615989 DOI: 10.1002/jmv.23895]

88 **Marcellin P**, Chang TT, Lim SG, Sievert W, Tong M, Arterburn S, Borroto-Esoda K, Frederick D, Rousseau F. Long-term efficacy and safety of adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2008; **48**: 750-758 [PMID: 18752330 DOI: 10.1002/hep.22414]

89 **Amini-Bavil-Olyaee S**, Herbers U, Sheldon J, Luedde T, Trautwein C, Tacke F. The rtA194T polymerase mutation impacts viral replication and susceptibility to tenofovir in hepatitis B e antigen-positive and hepatitis B e antigen-negative hepatitis B virus strains. *Hepatology* 2009; **49**: 1158-1165 [PMID: 19263474 DOI: 10.1002/hep.22790]

90 **Qin B**, Budeus B, Cao L, Wu C, Wang Y, Zhang X, Rayner S, Hoffmann D, Lu M, Chen X. The amino acid substitutions rtP177G and rtF249A in the reverse transcriptase domain of hepatitis B virus polymerase reduce the susceptibility to tenofovir. *Antiviral Res* 2013; **97**: 93-100 [PMID: 23261845 DOI: 10.1016/j.antiviral.2012.12.007]

91 **Chang TT**, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, Poordad F, Halota W, Horsmans Y, Tsai N, Zhang H, Tenney DJ, Tamez R, Iloeje U. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010; **51**: 422-430 [PMID: 20049753 DOI: 10.1002/hep.23327]

92 **Villet S**, Pichoud C, Billioud G, Barraud L, Durantel S, Trépo C, Zoulim F. Impact of hepatitis B virus rtA181V/T mutants on hepatitis B treatment failure. *J Hepatol* 2008; **48**: 747-755 [PMID: 18331765 DOI: 10.1016/j.jhep.2008.01.027]

93 **Lai MW**, Yeh CT. The oncogenic potential of hepatitis B virus rtA181T/ surface truncation mutant. *Antivir Ther* 2008; **13**: 875-879 [PMID: 19043921]

94 **Torresi J**, Earnest-Silveira L, Civitico G, Walters TE, Lewin SR, Fyfe J, Locarnini SA, Manns M, Trautwein C, Bock TC. Restoration of replication phenotype of lamivudine-resistant hepatitis B virus mutants by compensatory changes in the "fingers" subdomain of the viral polymerase selected as a consequence of mutations in the overlapping S gene. *Virology* 2002; **299**: 88-99 [PMID: 12167344 DOI: 10.1006/viro.2002.1448]

95 **Akahane Y**, Yamanaka T, Suzuki H, Sugai Y, Tsuda F, Yotsumoto S, Omi S, Okamoto H, Miyakawa Y, Mayumi M. Chronic active hepatitis with hepatitis B virus DNA and antibody against e antigen in the serum. Disturbed synthesis and secretion of e antigen from hepatocytes due to a point mutation in the precore region. *Gastroenterology* 1990; **99**: 1113-1119 [PMID: 2394332]

96 **Brunetto MR**, Giarin MM, Oliveri F, Chiaberge E, Baldi M, Alfarano A, Serra A, Saracco G, Verme G, Will H. Wild-type and e antigen-minus hepatitis B viruses and course of chronic hepatitis. *Proc Natl Acad Sci U S A* 1991; **88**: 4186-4190 [PMID: 2034663 DOI: 10.1073/pnas.88.10.4186]

97 **Fattovich G**, McIntyre G, Thursz M, Colman K, Giuliano G, Alberti A, Thomas HC, Carman WF. Hepatitis B virus precore/core variation and interferon therapy. *Hepatology* 1995; **22**: 1355-1362 [PMID: 7590647 DOI: 10.1002/hep.1840220503]

98 **Lok AS**, Hussain M, Cursano C, Margotti M, Gramenzi A, Grazi GL, Jovine E, Benardi M, Andreone P. Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. *Hepatology* 2000; **32**: 1145-1153 [PMID: 11050068 DOI: 10.1053/jhep.2000.19622]

99 **Buckwold VE**, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J Virol* 1996; **70**: 5845-5851 [PMID: 8709203]

100 **Scaglioni PP**, Melegari M, Wands JR. Biologic properties of hepatitis B viral genomes with mutations in the precore promoter and precore open reading frame. *Virology* 1997; **233**: 374-381 [PMID: 9217060 DOI: 10.1006/viro.1997.8594]

101 **Chan HL**, Hussain M, Lok AS. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. *Hepatology* 1999; **29**: 976-984 [PMID: 10051506 DOI: 10.1002/hep.510290352]

102 **Baumert TF,** Rogers SA, Hasegawa K, Liang TJ. Two core promotor mutations identified in a hepatitis b virus strain associated with fulminant hepatitis result in enhanced viral replication. *J Clin Invest* 1996; **98**: 2268 [PMID: 8941643 DOI: 10.1172/JCI119037]

103 **Yin J**, Xie J, Liu S, Zhang H, Han L, Lu W, Shen Q, Xu G, Dong H, Shen J, Zhang J, Han J, Wang L, Liu Y, Wang F, Zhao J, Zhang Q, Ni W, Wang H, Cao G. Association between the various mutations in viral core promoter region to different stages of hepatitis B, ranging of asymptomatic carrier state to hepatocellular carcinoma. *Am J Gastroenterol* 2011; **106**: 81-92 [PMID: 20959817 DOI: 10.1038/ajg.2010.399]

104 **Liao Y,** Hu X, Chen J, Cai B, Tang J, Ying B, Wang H, Wang L. Precore mutation of hepatitis b virus may contribute to hepatocellular carcinoma risk: Evidence from an updated meta-analysis. *PloS one* 2012; **7**: e38394 [PMID: 22675557 DOI: 10.1371/journal.pone.0038394]

105 **Twu JS**, Schloemer RH. Transcriptional trans-activating function of hepatitis B virus. *J Virol* 1987; **61**: 3448-3453 [PMID: 2822953]

106 **Su Q**, Schröder CH, Hofmann WJ, Otto G, Pichlmayr R, Bannasch P. Expression of hepatitis B virus X protein in HBV-infected human livers and hepatocellular carcinomas. *Hepatology* 1998; **27**: 1109-1120 [PMID: 9537452 DOI: 10.1371/journal.pone.0038394]

107 **Weil R**, Sirma H, Giannini C, Kremsdorf D, Bessia C, Dargemont C, Bréchot C, Israël A. Direct association and nuclear import of the hepatitis B virus X protein with the NF-kappaB inhibitor IkappaBalpha. *Mol Cell Biol* 1999; **19**: 6345-6354 [PMID: 10454581]

108 **Su F**, Schneider RJ. Hepatitis B virus HBx protein activates transcription factor NF-kappaB by acting on multiple cytoplasmic inhibitors of rel-related proteins. *J Virol* 1996; **70**: 4558-4566 [PMID: 8676482]

109 **Beg AA**, Sha WC, Bronson RT, Ghosh S, Baltimore D. Embryonic lethality and liver degeneration in mice lacking the RelA component of NF-kappa B. *Nature* 1995; **376**: 167-170 [PMID: 7603567 DOI: 10.1038/376167a0]

110 **Murakami S**, Cheong J, Ohno S, Matsushima K, Kaneko S. Transactivation of human hepatitis B virus X protein, HBx, operates through a mechanism distinct from protein kinase C and okadaic acid activation pathways. *Virology* 1994; **199**: 243-246 [PMID: 8116251 DOI: 10.1006/viro.1994.1119]

111 **Lee JH**, Han KH, Lee JM, Park JH, Kim HS. Impact of hepatitis B virus (HBV) x gene mutations on hepatocellular carcinoma development in chronic HBV infection. *Clin Vaccine Immunol* 2011; **18**: 914-921 [PMID: 21490166 DOI: 10.1128/cvi.00474-10]

112 **Sung WK**, Zheng H, Li S, Chen R, Liu X, Li Y, Lee NP, Lee WH, Ariyaratne PN, Tennakoon C, Mulawadi FH, Wong KF, Liu AM, Poon RT, Fan ST, Chan KL, Gong Z, Hu Y, Lin Z, Wang G, Zhang Q, Barber TD, Chou WC, Aggarwal A, Hao K, Zhou W, Zhang C, Hardwick J, Buser C, Xu J, Kan Z, Dai H, Mao M, Reinhard C, Wang J, Luk JM. Genome-wide survey of recurrent HBV integration in hepatocellular carcinoma. *Nat Genet* 2012; **44**: 765-769 [PMID: 22634754 DOI: 10.1038/ng.2295]

**P-Reviewer:** Apostolou KG, Chetty R, Lesmana RA, Odenthal M

 **S-Editor:** Tian YL **L-Editor: E-Editor:**

**Table 1 Summary of clinically revelant hepatitis B virus variants**

|  |  |  |
| --- | --- | --- |
| Location | Amino Acid or Nucleotide Substitution (Associated Overlapping Gene Mutation) | Clinical Impact |
| P (RT-A) | rtI169T (sF161L) | ETV resistance |
| P (RT-B) | rtL180M (sE164D) | ETV resistance |
| P (RT-B) | rtA181T/V | LAM, LdT, ADF/TDF resistance |
| P (RT-B) | rtT184S/A/I/L/G/CM | ETV resistance |
| P (RT-C) | rtS202C/G/I | ETV resistance |
| P (RT-C) | rtM204V/I | LAM resistance |
| P (RT-C) | rtM204I (sW196S) | LdT resistance |
| P (RT-C) | rtM204V (sI195M) | ETV resistance |
| P (RT-D) | rtN236T | ADF/TDF resistance |
| P (RT-E) | rtM250I/V | ETV resistance |
| P (RT-A) | rtL80V/I | Poor antiviral response to ADF with prior LMV resistant variants |
| P (RT-B) | rtF166L (sF158Y) | LMV-associated, compensatory |
| P (RT-B) | rtV173L (sE164D) | Compensatory mutation associated with LMV resistance (enhanced replication) |
| P (RT-B) | rtA194T | TDF resistance |
| S (‘a’ determinant) | sG145R (rtW153Q) | Antibody-associated escape mutation; reduced HBsAg level; restore LMV resistant HBV replication |
| S (‘a’ determinant) | sD144E/G145R (rtG153E) | Antibody-associated escape mutation |
| S (‘a’ determinant) | sP120T (rtT128N) | Reduced HBsAg level |
| EnhII | C1653T | HCC development (genotype C) |
| BCP | T1753V | HCC development (genotype B) |
| BCP | A1762T/G1764A | HBeAg production reduced by 50%; HBeAg seroconversion; escape anti-HBe immunity  |
| Pre-C | G1896A | HBeAg seroconverstion; escape anti-HBe immunity; more severe course of disease; HCC development |
| S  | W172\* (rtA181T) | Cirrhosis and HCC development |
| Pre-S1/Pre-S2  | Pre-S1/pre-S2 deletion (preS2 start codon and/or deletions in the 5'-terminal half of the preS2 region and preS1 3'-terminal half of the preS1 region) | More common in genotype C; progressive liver diseases; HCC development |
| Pre-S  | pre-S1 promoter mutation pre-S2 promoter mutation  | HCC development  |
| X  | K130M+V131I (double) | HCC development |
| X  | V5M/L+K130M+V131I (triple) | HCC development |

HCC: Hepatocellular carcinoma; BCP: Basal core promoter; HBeAg: HBV e antigen; HBV: Hepatitis B virus; ADF: Adefovir; TDF: Tenofovir; LMV: Lamivudine; HBsAg: Hepatitis B surface (S) antigen; LdT: Telbivudine.