World Journal of Gastroenterology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.3748/wjg.v22.i1.145

World J Gastroenterol 2016 January 7; 22(1): 145-154
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

2016 Hepatitis B virus: Global view

Overview of hepatitis B virus mutations and their implications in the management of infection

Patrizia Caligiuri, Rita Cerruti, Giancarlo Icardi, Bianca Bruzzone

Patrizia Caligiuri, Giancarlo Icardi, Department of Health Sciences, University of Genoa, 16132 Genoa, Italy

Rita Cerruti, Giancarlo Icardi, Bianca Bruzzone, Hygiene Unit, I.R.C.C.S. A.O.U. San Martino-IST, 16132 Genoa, Italy

Author contributions: Caligiuri P, Cerruti R, Icardi G and Bruzzone B analyzed the literature and wrote this review.

Conflict-of-interest statement: The authors have no conflict of interest regarding this review.

Correspondence to: Dr. Patrizia Caligiuri, Department of Health Sciences, University of Genoa, Largo R. Benzi 10, 16132 Genoa, Italy. patrizia.caligiuri@libero.it
Telephone: +39-10-5600591
Fax: +39-10-5600912

Received: May 29, 2015
Peer-review started: June 3, 2015
First decision: July 14, 2015
Revised: August 19, 2015
Accepted: December 1, 2015
Article in press: December 1, 2015
Published online: January 7, 2016

Abstract

Hepatitis B virus (HBV) affects approximately two billion people worldwide and more than 240 million people in the world are currently chronic carrier that could develop serious complications in the future, like liver cirrhosis and hepatocellular carcinoma. Although an extended HBV immunization program is being carried out since the early '80s, representing effective preventive measure, leading to a dramatic reduction of HBV hepatitis incidence, globally HBV infection still represents a major public health problem. The HBV virus is a DNA virus belongs to the *Hepadnaviridae* family. The HBV-DNA is a circular, partial double strand genome. All coding information is on the minus DNA strand and it is organized into four open reading frames. Despite hepatitis B virus is a DNA virus, it has a high mutation rate due to its replicative strategy, that leads to the production of many non-identical variants at each cycle of replication. In fact, it contains a polymerase without the proofreading activity, and uses an RNA intermediate (pgRNA) during its replication, so error frequencies are comparable to those seen in retroviruses and other RNA viruses rather than in more stable DNA viruses. Due to the low fidelity of the polymerase, the high replication rate and the overlapping reading frames, mutations occur throughout the genome and they have been identified both in the structural and not structural gene. The arise of mutations being to develop of a whole of viral variants called "quasi-species" and the prevalent population, which favors virus replication, was selected by viral fitness, host's immune pressure and external pressure, *i.e.*, vaccination or antiviral therapy. Naturally occurring mutations were found both in acute and chronic subjects. In the present review we examine and discuss the most recent available data about HBV genetic variability and its significance.

Key words: Hepatitis B virus; Mutations; Open reading frames; Molecular biology tools; Liver disease

Core tip: Hepatitis B virus (HBV) is a global health problem, with almost 2 billion infected persons, many

of whom deemed to develop chronic carrier state and eventually die from cirrhosis or liver cancer. Unlike in other DNA viruses, its high mutation rate and replicative capability arise considerable genetic variability, recently analyzed by molecular biology tools. HBV mutations occur in all four overlapping open reading frames encoding viral polymerase, surface antigen, core and X protein. Understanding the correlation between mutations and liver disease progression is crucial for an effective clinical management in HBV patients with resistance to antiviral drugs, hepatitis B surface antigen escape mutant, "occult" hepatitis and hepatocellular carcinoma.

Caligiuri P, Cerruti R, Icardi G, Bruzzone B. Overview of hepatitis B virus mutations and their implications in the management of infection. *World J Gastroenterol* 2016; 22(1): 145-154 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i1/145.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i1.145

INTRODUCTION

Hepatitis B virus (HBV) mutations have been found both in acute and chronic patients and in all the four HBV open reading frames (ORFs - preS/S, polymerase, preCore/core, and X).

The preS/S ORF codes for three different surface molecules that form the surface antigen (HBsAg). This is the main antigen recognized by the immune system, responsible for the attachment of the virus to hepatocytes and the epitope binding the neutralizing antibodies. Point mutations, deletions and also genetic recombinations have been found within the preS/S ORF, which is recognized as the part of HBV genome with the highest heterogeneity. Genetic changes in this region are driven by viral fitness and polymerase infidelity, but also, due to the strict relationships of the products of these genes with the immune system, by host's immune pressure[1,2].

The pol ORF codes for the reverse transcriptase (RT) domain of HBV polymerase that represents the target of the new antiviral agents belonging to the nucleoside/nucleotide analogues and to the acyclic nucleotide analogs (NAs) classes. Under the NAs selective pressure, mutations, collected during the replicative cycles, are selected and confer resistance to NAs. In addition to the high mutation rate, due to the HBV replicative strategy, other factors (viral fitness, potency and genetic barrier of the drugs) are associated to the development of resistance. NAs with high potency and high genetic barrier could prevent resistance generation and should be preferred in HBV therapy. Moreover, due to the overlapping S reading frame, mutations arising in the RT domain cause the appearance of mutations in the preS/S ORF (escape mutants)[1,2].

PreCore/core ORF codes for the core nucleocapsid (HBcAg) and the e antigen (HBeAg) synthesis. Mutations in these sites mainly cause the well-known HBeAg negative hepatitis. The A1762T and the G1764A, responsible for the decreased preCore (PC) mRNA synthesis, were detected in the specific basal core promoter (BCP) and described in patients with HBeAg negative hepatitis. The G1896A mutation caused by a G to A switch is the most prevalent and produces a translation stop codon at amino acid position 28 in the HBeAg sequence, with inhibition of HBeAg synthesis. Moreover, both BCP and stop codons are often associated and recent reports suggest their association with a more severe outcome of hepatitis[1,2].

X ORF encodes for a multifunctional nonstructural protein, originally defined X protein because its functions were unknown and are still unclear. It has been proposed a function in the establishment of infection and viral replication. Furthermore, a role of gene X in the HBV carcinogenesis has been recently hypothesized[1,2].

In the present review we examine and discuss the most recent available data about HBV genetic variability and its significance.

EPIDEMIOLOGY

The virus is transmitted by contact with blood or other body fluids from an infected person. Hepatitis B virus is endemic worldwide and hyper-endemic in many parts of the world.

The prevalence of HBV carriers varies from 0.1% to 2% in low prevalence areas (United States and Canada, Western Europe, Australia and New Zealand), from 3% to 5% in intermediate prevalence areas (Mediterranean countries, Japan, Central Asia, Middle East, and Latin and South America), from 10% to 20% in high prevalence areas (Southeast Asia, China, sub-Saharan Africa). A systematic review focusing on data in the United States estimated that there are 2.2 million individuals with chronic HBV, two-thirds of whom were foreign born[3].

The wide range in HBV carrier rate in different parts of the world is largely related to differences in the age of infection, which is inversely related to the risk of chronicity. The rate of progression from acute to chronic HBV infection is approximately 90% for perinatally acquired infection, from 20% to 50% for infections acquired at 1 to 5 years of age and less than 5% for adult acquired infection[4].

With regard to Europe in 2012, 17329 cases of hepatitis B were reported in 29 countries (no data from Belgium and Liechtenstein), resulting in an overall crude rate of 3.5 per 100000 inhabitants. Of these cases, 2798 (16.1%) were classified as acute infection, 12306 (71.0%) as chronic infection and 1865 (10.8%) as unknown. Three hundred and sixty cases (2.1%) could not be classified in any of these groups. However, due to the differences in surveillance

systems across Europe, these figures are known to be an underestimation of the true situation[5,6].

Ten genotypes have been identified (A-J) on the base of a sequence difference greater than 8% in the entire HBV genome or 4% in the S region. Each genotype is further divided into sub-genotypes when differences in nucleotide sequences are major than 4% but minor than 8%. Interestingly, both genotypes and sub-genotypes are related to clinical course, geographical distribution and mode of transmission. Hyper-endemic areas and at high incidence of hepatocellular carcinoma (HCC) were found in East Asia (genotypes B and C) and in sub-Saharan Africa (genotype A). Moreover, genotypes A and D are prevalent in countries where horizontal transmission is common, *i.e.*, sub-Saharan Africa, Mediterranean, Middle East and India, whilst genotypes B and C are prevalent in countries where vertical transmission is common, *i.e.,* East Asia[7-9].

MORPHOLOGY AND VIRAL GENOME

HBV is a partially double-stranded circular DNA virus that belongs to the *Hepadnaviridae* family. The virus consists of the HBcAg, which contains circular DNA molecule approximately of 3.2 kb, and an outer envelope containing the HBsAg. One of the two strands is incomplete and associated with a DNA polymerase able to complete the strand. This virus is unique among human viral pathogens, since it is a DNA virus that replicates by reverse transcription of an RNA intermediate. The longer strand of HBV DNA (L strand) is a complete circle, whereas the complementary strand is shorter (minus strand). Minus strand DNA is the template for the synthesis of the viral mRNA transcripts. HBV DNA has a very compact coding organization with four partially overlapping ORFs that are translated into seven known proteins: polymerase protein (Pol gene); HBcAg and HBeAg (both from the C gene); large, medium, and small HBsAg (S gene); and the X regulatory protein (X gene). The overlap in the ORFs does not seem to limit variability since all HBV genes have variants. Noncoding regions are not present[10-12].

The first step in the HBV life cycle is its attachment to the hepatocyte through the interaction of its envelope proteins (pre-S1 region) with the host cell receptors.

Then it penetrates in the hepatocyte, uncoating, and the viral genome, organized as relaxed circular partially double stranded DNA (rc DNA), is sent to the nucleus and converted into covalently closed circular DNA (ccc DNA). The cccDNA acts as template for transcription of four co-terminal mRNAs: 3.5 kb pre-core (pre-C) and progenomic RNA (pgRNA), 2.4 kb large surface mRNA, 2.1 kb middle and small surface mRNA and 0,7 kb X mRNA. pgRNA serves as template for the reverse transcriptase and, after being transported to the cytoplasm, encodes viral capside protein and viral polymerase, thus playing an important role in viral genome amplification and replication[1,2].

The latter is transcripted into viral RNA gene products: HBV surface protein, structural core protein, non-structural core protein (secreted HBeAg), X protein and viral polymerase.

After this step the viral assembly occurs (encapsidation by the core protein to form the viral nucleocapsid), followed by the virion secretion or the recycle of the newly generated nucleocapsid into the nucleus for conversion to cccDNA.

The permanence of cccDNA into the hepatocyte nucleus is a basic factor for viral persistence, because it allows for viral replication to restart, either during the antiviral therapy (resistance) or after the antiviral therapy is stopped (reactivation)[13,14].

HBV S-GENE MUTANTS

The pre-S1/S2/S ORFs encode three envelope proteins (large, middle and small) which are determinant for virus assembly and virus attachment to hepatocytes. L protein (pre-S1 domain) is the substrate for viral receptor attachment; M protein (pre-S2 domain) function is not well understood and, finally, S protein (S domain) is commonly referred to as the HBsAg or Australian antigen. The small, the middle and the large proteins are detected as HBsAg. HBsAg protein contains the major B cell epitope, the "a" determinant (121-149 aa)[1].

HBsAg is the surface antigen that is targeted by the antibodies present in vaccinated people and by the antibodies binding to HBsAg in serological immunoassays. It is the major envelop protein, formed by 226 amino acids, it is highly heterogenic, but within the protein there are conserved areas defining the genotype.

The amino acid positions between 99 and 169 are called the major hydrophilic region (MHR), in which the "a" determinant is located (comprising two loops of amino acids, 124-147), that is the main target of neutralizing B cell responses[15,16].

Mutations causing a conformational change within the "a" determinant could affect the antigenicity of HBsAg, essential for inducing protective antibody, and be responsible for escaping vaccine induced immunity, escaping anti HBV immunoglobulin therapy and providing false negative results in serological tests[17-19].

In 1988 HBV S-gene mutants were observed in Italian vaccinated children's sera with the presence of both HBs antigen and anti-HBs antibodies. These children acquired infection from the mother and their S-gene sequences revealed glycine (G) to arginine (A) substitution at position 145, within the *a*-determinant of S-gene, causing conformational changes that allowed for the virus to escape the vaccine-induced response[20]. G145R is the major vaccine-induced immune escape mutation and in the last years an increase of G145R detection has been reported by

several studies, mainly in countries with high rate of endemicity and universal immunization program. Nevertheless, it has been recently demonstrated that the risk of acquiring HBV infection is extremely low in a vaccinated subject. Other mutations were later found in "a" determinant T116N, P120S/E, I/T126A/N/I/S, Q129H/R, M133L, K141EP142S, D144A/E and considered as "immune escape" as well[15].

Similar mutations were also detected in immune-compromised patients and were considered responsible for HBV reactivation by immune escape in previously anti-HBs immune persons. These mutations in the HBsAg can result crucial in failing virus detection in the routine screening.

In recent studies it has been observed that during immunosuppression, some patients, with resolved infection, showed HBV reactivation that in some cases could lead to severe acute hepatitis, synthetic dysfunction, fulminant liver failure and death. In a very recent report, Salpini *et al*[21] showed, that 75.9% of HBV reactivated patients were carriers of more than one HBsAg mutations. 8/13 mutations were located in the major hydrophilic loop (M103I-L109I-T118K-P120A-Y134H-S143L-D144E-S171F) and 5/13 in T cell epitopes belonging to class Ⅰ (C48G-V96A-L175S-G185E-V190A).

In recent years, occult HBV infection (OBI) has been widely investigated. OBI is identified as the persistence of HBV-DNA in HBsAg negative patient's liver with or without other serological markers of previous HBV infection. To explain this phenomenon three mechanisms have been proposed. For two of these, the common factor is the change in the steric configuration in HBsAg molecule, determined by mutations located within the "a" determinant. These modified HBsAg molecules, most commonly, either cannot be detected by commercial available assays or are actually very weakly exposed in the surface of hepatocytes due to a poor recognition by the immune system. Finally, several authors suggest that host immune surveillance and epigenetic mechanisms are probably involved[22].

Some studies report that several and different mutations are correlated with OBI depending on subtypes and also sub-subtypes, or even that OBI associated mutations are unique for each subtype[23,24]. Cassini *et al*[25] suggest that a change in the C695T nucleotide leads to a stop codon in the 181 amino-acid that could be responsible for the strongly reduction of HBsAg production.

Finally, also deletions in the S-region seem to be involved in OBI development, in fact, they can influence the expression, synthesis and secretion of HBsAg[26,27].

According to some authors, mutations in this region might contribute to hepato-carcinogenesis. Lee *et al*[28] discovered the W4P/R pre-S1 mutations. They may be associated with disease severity in male patients chronically infected with HBV genotype C. These W4P/R mutants were significantly related to severe liver diseases [HCC and liver cirrhosis (LC) (12.4%, 19/153 patients) *vs* chronic hepatitis and carrier (1.1%, 1/94 patients), $P < 0.001$]. Interestingly, all the W4P/R mutants were found only in the male gender, not in the female gender, which may in part provide a likely explanation for the relatively high male to female ratio in the incidence of HCC generation in Korean HBV chronic patients.

Other mutations, that usually occur in Pre-S/S region, seem to play an important role in inactivation of the preS2/S region promoter, resulting in interference with HBsAg secretion. As in this region there is the hepatocyte binding site they are associated with occult HBV status as well[29]. Several studies dispute about the important role of pre-S deletions on the progression of liver disease. Above all, it seems that a set of deletions or mutations in different genes is associated with the progression of liver disease. The regions involved are: pre-S, BCP and PC; moreover, it seems that the PC mutations precede the appearance of the others. Pre-S deletions, observed both in pre-S1 and pre-S2 regions, cause a decrease in the synthesis and secretion of small surface antigen which tends to accumulate in the hepatocytes and especially in the endoplasmic reticulum (ER). This supposedly causes an ER stress which in turn causes an oxidative DNA damage that induces mutagenesis and finally HCC. Several other hypotheses have been formulated[30,31]. Wang *et al*[32] suggest that the conspicuous increase of the cyclin A, implicated in the DNA synthesis and centrosome duplication, observed in the HCC tissues and mainly in patients with the pre-S2 deletions, could be activated by the ER stress and could be responsible of the development of HCC. Finally, a study of Yang *et al*[33] demonstrated that, in chronic HBV patients, the pre-S mutants, besides causing ER stress and DNA damage, also cause a vascular endothelial growth factor-A (VEGF-A) overexpression on the ground glass hepatocytes (GGHs). This could be implicated in the preneoplastic GGHs progression to HCC through the activation of Akt/mTOR (mammalian target of rapamycin).

POL-GENE MUTANTS

The goal of treatment in patients with chronic hepatitis B (CHB) is to eliminate the virus, thus reducing the risk of progressive liver damage that leads to the development of complications such as cirrhosis and HCC. However, due to the persistence of cccDNA forms in the infected hepatocytes nucleus, a complete and definitive virus eradication is not achievable.

The currently available drugs, approved for treatment of CHB in many parts of the world, are 2 immuno-modulators (inteferon α-2a and peginteferon α-2a) and 5 antiviral agents belonging to the NAs: lamivudine (LAM-3TC), telbivudine (LdT), entecavir (ETV) and the acyclic nucleotide analogues adefovir

Table 1 Cross-resistance data for the most frequent resistant hepatitis B virus variant

HBV variants	Level of susceptibility				
	Lamivudine	Telbivudine	Entecavir	Adefovir	Tenofovir
Wild-tipe	S	S	S	S	S
M204V	R	S	I	I	S
M204I	R	R	I	I	S
L180M + M204V	R	R	I	I	S
A181A/T	I	S	S	R	S
N236T	S	S	S	R	I
L180M + M204V/I ± I196T ± V173L ± M250V	R	R	R	S	S
L180M + M204V/I ± T184G ± S202I/G	R	R	R	S	S

The amino-acid substitution profiles are shown in the left column and the level or susceptibility is given for each drugs: S (sensitive), I (intermediate/reduced susceptibility), R (resistant). EASL Clinical Practice Guidelines 2012.

dipivoxil (ADV) and tenofovir disoproxil fumarate (TDF)[34]. These last five drugs are inhibitors of RT domain of HBV polymerase.

The viral polymerase/RT is encoded by the largest ORF. This protein arises from the translation product of the 3.5 kb pre-core mRNA and pgRNA, that serves as template for reverse transcriptional synthesis of viral DNA.

Due to the absence of proofreading activity, the HBV polymerases/RT, as already mentioned, leads to the introduction of random mutations into HBV genome. The error rate of HBV polymerase is approximately 1×10^5 to 10^7 base syntheses, as result of the highly error-prone nature of the HBV RT[34,35].

Under the selective pressure by means of the administration of antiviral agents, quasi species of HBV converge on a dominant HBV mutant that can escape selection pressure, creating a drug-resistant HBV strain.

Earlier researches have suggested that LAM is the major cause of YMDD (tyrosine-methionine-aspartate-aspartate) mutations (M204I/V) in the catalytic sites (C domain) within HBV P-ORF[36].

The mutations rtM204I/V (domain C), rtL180M (domain B) and rtA181T/V (domain B) confer resistance to LAM and LdT (Table 1).

M204I/V are often associated with compensatory mutations in other domains such as rtL80V/I, 58 rtI169T,59 rtV173L, rtL180M, rtT184S/G, rtS202I, and rtQ215S which increase viral replication[36-38]. Other proposed compensatory mutations are rtV84M, rt214, rtL217P, rtL229M, rtI233V and rtN238H[37]. Among them, the rtL217P substitution, known to confer replicative advantage if emerging in a wild-type virus, in the context of LAM resistance likely represents a compensatory mutation to boost replication[39] (Table 1).

In fact, compensatory mutations emerge because the selection of resistance-associated changes in the viral polymerase is usually associated with some cost in replication fitness for the virus; these compensatory mutations are important in the setting of antiviral resistance because they "fix" the discriminatory primary drug-resistant mutations into the genetic archive of viral cccDNA, thus providing a "quasi species memory"[38].

ADV resistance is associated with two primary resistant mutations (belonging to the pathway for alkyl phosphonates) in the B and D domain, the rtA181T and the rtN236T. Furthermore, rtI233V is another mutation that has been identified in ADF-resistant HBV variants; its true significance remains contradictory since some authors have confirmed and some have denied its capability to confer resistance[40,41] (Table 1).

Mutations in the B domain of RT, the rtA181T/V, were shown to confer resistance to LAM, LdT and ADV. The rtA181T mutation also encodes a stop codon in the overlapping S reading frame (sW172*) thus resulting in the truncation of the HBsAg proteins. As Warner and Locarnini emphasized, the rtA181T/sW172* variant has a secretory defect and exerts a dominant negative effect on the wild-type HBV virion secretion. This mutation is often present in patients with primary HCC[42-44].

Due to high genetic barrier, ETV and TDF are considered the most potent antiviral agents and at low risk of developing resistance. Indeed, they result to have a mutation incidence rate of 1.2% and 0%, respectively[45]. Long-term monitoring shows HBV resistance to ETV in nucleoside-naive patients is rare through 5 years of therapy. Multiple mutations are required to obtain high-level resistance to ETV. Those usually involved in ETV resistance are rtL180M + M204V and another among rtI169T, rt184G/S, rtS202I/G and rtM250V; actually, ETV resistance appears in LAM treated patients in which the rtL180M and M204V mutations were formerly present[38] (Table 1).

To date, there have been no confirmed reports of resistance selection during treatment of CHB with TDF in mono-infected individuals. Kitrinos *et al*[46] in their study report that TDF mono-therapy maintained effective viral suppression over up to 6 years of continuous therapy without selecting TDF resistance. Recently, a complex TDF-resistance associated mutation pattern, including the rtR192PR substitution, very close to the site of the rtA194T mutation which has been found to confer TDF resistance *in vitro,* has been reported in a HIV-HBV co-infected individual failing TDF[47].

Table 2 Impact of drug resistant mutations in the the hepatitis B virus Pol on the hepatitis B surface antigen

Antiviral drugs	Resistance mutations	HBsAg corresponding changes
Lamivudine (LAM[2])	rtL180M	No change
Tebivudine (LdT[2])	rtM204V	sI195M
	rtM204I[2]	sW196[1]/S/L
Adefovir (AdV)	rtA181T[2]	sW172[1]
Tenofovir (TDF)	rtA181T[2]	sW172L
LAM[2]	rtA181V[2]	sL173F
	rtN236T	After end of HBs open reading frame
Entecavir (ETV)	rtI169T	sF161H/L
	rtT184A	No change
	rtT184C	sL175F + sL176V
	rtT184I	No change
	rtT184G	sL176V
	rtT184S	sL175F
	rtT184M	sL176[1]
	rt184L	sL175F
	rtS202C	No change/sS193F
	rtS202I	sV194F/S
	rtS202G	No change/sS193L
	rtM250I	After end of HBs open reading frame
	rtM250V	After end of HBs open reading frame

[1]Stop codon; [2]Cross-resistance. HBsAg: Hepatitis B surface antigen. Modified from Zoulim *et al*[38].

The common mutations that confer resistance to LAM and LdT (*e.g*., rtM204V/I, rtL180M) give cross-resistance to other L-nucleosides and reduce sensitivity to ETV but not to ADV or TDF. Conversely, mutations causing resistance to ADV (rtA181T/V, rtN236T) and TDF generally do not give rise to resistance to L-nucleosides and ETV. Both the L-nucleosides (LMV and LdT) and the alkyl phosphonates (ADV and TDF) also select the mutation rtA181T/V, thereby making it a marker for multidrug resistance[36-38] (Table 1).

Further research has revealed that strains with YMDD mutations also exist in patients with chronic HBV infection not previously treated with lamivudine[48,49].

A recent research showed that spontaneous YMDD mutations were detected in LC and HCC patients. Moreover, it has been demonstrated that in genotype C, HCC patients had a significantly higher spontaneous YMDD mutation rate than LC patients, and that genotype C was associated with a higher risk for the development of HBV-related HCC than patients infected by other HBV genotype (P = 0.013, 95%CI: 1.540-39.264). This may have been caused by different genotype strains having different biological properties, pathogenicity and carcinogenicity[50].

Furthermore, the rate of viral breakthrough tended to be lower in patients without natural YMDD mutations than in those with natural YMDD mutations. Naturally occurring YMDD mutations are found in a large proportion of CHB patients who have not undergone anti-viral therapy. The incidence of YMDD mutations may be correlated with the HBeAg status and the HBV DNA level. These results also suggest that LAM therapy improved the clinical course in HBV patients with natural YMDD mutations[51].

The HBV polymerase (Pol) gene overlaps the HBsAg in a frame-shifted manner with the result that drug resistant mutations in the HBV Pol can directly impact on the HBsAg and its function. Therefore, drug resistance mutations in the polymerase gene may result in the production of mutations and stop codons in the envelope gene leading to modified viral secretion, infectivity and creating both viral escape to anti-HBs antibodies[38] and modified HBsAg molecule not detected by screening tests (Table 2). About this last topic, the study of Hsu *et al*[52] found that the P120A mutation in the HBsAg gene, selected during LAM therapy in 6/11 samples patient, was responsible for HBsAg detection failure, misinterpreted as HBsAg clearance.

Through a molecular analysis performed in HIV-HBV co-infected and HBsAg-negative patients, Amini-Bavil-Olyaee in 2009 revealed an unusual HBV polymerase mutation (rtV191I), during TDF therapy, conferring simultaneously immune escape by HBsAg negativity and resistance to LAM, but not TDF. Due to the overlapping surface antigen the rtV191I mutation also created a stop-codon in sW182s, deleting the last 44 amino acids of the HBsAg, which resulted HBsAg negative in diagnostic serum assays[53].

Interestingly, neither the ADV-associated resistance mutation rtN236T nor the TDF-associated resistance mutation rtA194T, selected only *in vitro*, cause changes in the HBV surface gene[54].

HBV mutants carrying drug and vaccine resistance may represent a considerable individual risk and public health concern.

With regard to the best treatment strategy after HBV resistance, the international practice guidelines recommend the use of a nucleoside/tide analogue with high antiviral potency and high genetic barrier, such as ETV or TDF. Nevertheless, incomplete response to ETV therapy has been reported[55].

PRE CORE/CORE MUTANTS

Pre-Core/Core region encodes for two proteins, one structural, the HBcAg, that forms the nucleocapsid, and the HBeAg that is a secretion protein[2-56].

HBeAg is the marker of HBV replication and infectivity. In the natural course of HBV chronic infection, the loss of HBeAg expression and the appearance of antibodies directed against it (anti-HBe) usually represent the end of viral replication and the resolution of hepatitis. Mutations in the pre-core and core regions cause HBeAg-negative chronic hepatitis B with presence of anti-HBe, in which replicative infection continues and HBV-DNA remains detectable (> 2000 IU/mL)[2,15,56].

HBeAg negativity is due to basal core promoter

(BCP) and precore (PC) mutations that respectively modulate HBeAg secretion during transcription and stop HBeAg production[2,15,56].

Recently, in Korea, Lee *et al*[57] described 36 prospectively enrolled patients with acute hepatitis B, 20 of which, infected with HBV genotype C, showed detectable HBV DNA. Among them, 4 patients had BCP mutations, and two had PC mutations. Platelet counts were significantly lower in the 4 patients with PC/BCP mutations compared to those with wild type. The A1762T and the G1764A, responsible for the decreased PC mRNA synthesis, were the typical specific BCP mutations detected and described, mainly together, in patients with HBeAg negative hepatitis. These two mutations were first found in a study of Baptista *et al*[58] aimed at investigating the presence of mutations responsible for the HBeAg negativity and their possible role in hepato-carcinogenesis in the HBeAg negative patients. This study showed that these two mutations produced a decrease in the HBeAg secretion and had a significant role in hepato-carcinogenesis[59]. The increased risk of HCC in patients harboring a virus with the A1762T and the G1764A was confirmed by several studies but the mechanism of oncogenesis remains unknown[59-63]. Furthermore, Yang *et al*[64] investigated the risk of HCC considering, in addition to BCP mutations, also HBV genotypes and PC mutations. They proved that the highest risk of HCC development depends on genotype (mostly genotype C), and on the presence of the A1762T and G1764A BCP mutations and of the G1896A PC mutation.

In addition to the A1762T and G1764A mutations, other BCP mutations have been identified: the T1753C, and the C1766T. Basically, these mutations reduce the HBeAg synthesis and enhance viral replications in liver cells, often in association with more severe and advanced liver disease[65].

Some of these mutations (T1753C, A1762T and 1764A), together with A1752G, A1846T, G1896A and G1899A, were significantly correlated with HBeAg seroconversion; in a recent work the authors showed significant differences between HBeAg positive and HBeAg negative child patients groups. But the frequencies of the mutations in HBeAg-negative child patients were significant lower than in HBeAg negative adult patients, because the role of BCP/PC mutations is less important in the early phases of HBeAg seroconversion[66].

The main prevalent PC region mutations collected over the years in various works are the G1896A and the G1899A, alone or associated. The G1896A mutation is due to a G to A switch that produce a translation stop codon at amino acid position 28 in the HBeAg sequence, with inhibition of its synthesis. This mutation was often found in non-A genotypes associated with the mutation C1858T, whose onset is eased by typical viral structure of certain genotypes (B, D, E, C, F). Also these mutations have been first found in Mediterranean Countries, where the majority of patients are genotype D carriers.

In a longitudinal study on 99 HBV- DNA positive patients, all genotype D, HBeAg negative and with PC G1896A mutation, Besharat observed that they still had a detectable HBV-DNA even after 7 years of monitoring, unlike the patients with the wild type PC sequence[67].

X-GENE MUTANTS

Gene X encodes for a multifunctional nonstructural protein, originally defined X protein because its function was unknown and even now are unclear. It has been proposed a function in the establishment of infection and viral replication. Furthermore, a role of gene X in the HBV carcinogenesis has been recently hypothesized[68].

The HBX gene overlaps with the core promoter region and mutations here in this gene may alter the functions of the HBX protein, playing an important role in HBV replication and hepato-carcinogenesis. According to Yan *et al*[69], the HBX mutants linked with core promoter mutations may regulate p53 through a S-phase kinase associated protein 2 (SKp2), promoting or preventing cellular transformation and proliferation.

In HBX region, twelve mutations were associated with hepato-carcinogenesis, suppression of HBeAg secretion and increase of viral DNA synthesis[70].

CONCLUSION

In the last decade, mainly due to molecular biology studies, a lot of information about HBV life cycle, genetic variability and pathogenesis has been achieved. HBV genomic sequencing, back in 1988, allowed Zanetti *et al*[20] to discover the G145R mutation within the "a" determinant of S gene, the first escape mutant identified. Other escape mutants have been detected afterwards and the relevant role of other mutations has been established in immune compromised patients and in OBI infection. Sequencing of *pol* gene, especially performed to drive clinicians to the better treatment, not only has allowed to achieve knowledge on the mutations able to confer resistance to the new NAs, some of which are often related with hepato-carcinogenesis, but, considering the overlapping of the *pol* gene with the S gene, also to discover other escape mutants or stop codons in this site. Sequencing also allowed to identify mutations responsible of HBeAg-negative chronic hepatitis B and finally to identify mutations, deletions and insertion in X gene probably associated with hepato-carcinogenesis, suppression of HBeAg secretion and increase of viral DNA synthesis. Nevertheless, further studies are needed in the field of HBV genetic variability, especially to investigate on the role of X gene, about which there are still too few data which also need to be confirmed. Finally,

further studies are also needed to understand whether and how much genotypes and sub-genotypes could influence the response to treatment, the appearance of viral variants and the risk of cirrhosis and HCC. It is possible to hypothesize that additional knowledge above viral variants, genotypes and sub-genotypes could be considered into clinical decision.

REFERENCES

1 **Datta S**, Chatterjee S, Veer V, Chakravarty R. Molecular biology of the hepatitis B virus for clinicians. *J Clin Exp Hepatol* 2012; **2**: 353-365 [PMID: 25755457 DOI: 10.1016/j.jceh.2012.10.003]

2 **Croagh CM**, Desmond PV, Bell SJ. Genotypes and viral variants in chronic hepatitis B: A review of epidemiology and clinical relevance. *World J Hepatol* 2015; **7**: 289-303 [PMID: 25848459 DOI: 10.4254/wjh.v7.i3.289]

3 **Kowdley KV**, Wang CC, Welch S, Roberts H, Brosgart CL. Prevalence of chronic hepatitis B among foreign-born persons living in the United States by country of origin. *Hepatology* 2012; **56**: 422-433 [PMID: 22105832 DOI: 10.1002/hep.24804]

4 **World Health Organization**. Hepatitis B - Fact sheet N° 204. Geneva: WHO, 2015

5 **European Centre for Disease Prevention and Control**. Hepatitis B and C surveillance in Europe. Europe: ECDC, 2012

6 **European Centre for Disease Prevention and Control**. Hepatitis B and C in the EU neighbourhood: prevalence, burden of disease and screening policies. EU neighbourhood: ECDC, 2010

7 **Zehender G**, Ebranati E, Gabanelli E, Sorrentino C, Lo Presti A, Tanzi E, Ciccozzi M, Galli M. Enigmatic origin of hepatitis B virus: an ancient travelling companion or a recent encounter? *World J Gastroenterol* 2014; **20**: 7622-7634 [PMID: 24976700 DOI: 10.3748/wjg.v20.i24.7622]

8 **Norder H**, Couroucé AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO. Genetic diversity of hepatitis B virus strains derived worldwide: genotypes, subgenotypes, and HBsAg subtypes. *Intervirology* 2004; **47**: 289-309 [PMID: 15564741 DOI: 10.1159/000080872]

9 **Cao GW**. Clinical relevance and public health significance of hepatitis B virus genomic variations. *World J Gastroenterol* 2009; **15**: 5761-5769 [PMID: 19998495 DOI: 10.3748/wjg.15.5761]

10 **Harrison TJ**. Hepatitis B virus: molecular virology and common mutants. *Semin Liver Dis* 2006; **26**: 87-96 [PMID: 16673287 DOI: 10.1055/s-2006-939754]

11 **Baumert TF**, Thimme R, von Weizsäcker F. Pathogenesis of hepatitis B virus infection. *World J Gastroenterol* 2007; **13**: 82-90 [PMID: 17206757 DOI: 10.3748/wjg.v13.i1.82]

12 **Hollinger FB**. Hepatitis B virus genetic diversity and its impact on diagnostic assays. *J Viral Hepat* 2007; **14** Suppl 1: 11-15 [PMID: 17958637]

13 **Churin Y**, Roderfeld M, Roeb E. Hepatitis B virus large surface protein: function and fame. *Hepatobiliary Surg Nutr* 2015; **4**: 1-10 [PMID: 25713800 DOI: 10.3978/j.issn.2304-3881.2014.12.08]

14 **Wang XY**, Chen HS. Emerging antivirals for the treatment of hepatitis B. *World J Gastroenterol* 2014; **20**: 7707-7717 [PMID: 24976708 DOI: 10.3748/wjg.v20.i24.7707]

15 **Lazarevic I**. Clinical implications of hepatitis B virus mutations: recent advances. *World J Gastroenterol* 2014; **20**: 7653-7664 [PMID: 24976703 DOI: 10.3748/wjg.v20.i24.7653]

16 **Petit MA**, Maillard P, Capel F, Pillot J. Immunochemical structure of the hepatitis B surface antigen vaccine--II. Analysis of antibody responses in human sera against the envelope proteins. *Mol Immunol* 1986; **23**: 511-523 [PMID: 3748012]

17 **Chisari FV**. Hepatitis B virus biology and pathogenesis. *Mol Genet Med* 1992; **2**: 67-104 [PMID: 1333869]

18 **Gerlich WH**, Glebe D, Schüttler CG. Deficiencies in the standardization and sensitivity of diagnostic tests for hepatitis B virus. *J Viral Hepat* 2007; **14** Suppl 1: 16-21 [PMID: 17958638]

19 **Echevarría JM**, Avellón A. Improved detection of natural hepatitis B virus surface antigen (HBsAg) mutants by a new version of the VITROS HBsAg assay. *J Med Virol* 2008; **80**: 598-602 [PMID: 18297712 DOI: 10.1002/jmv.21146]

20 **Zanetti AR**, Tanzi E, Manzillo G, Maio G, Sbreglia C, Caporaso N, Thomas H, Zuckerman AJ. Hepatitis B variant in Europe. *Lancet* 1988; **2**: 1132-1133 [PMID: 2460710]

21 **Salpini R**, Colagrossi L, Bellocchi MC, Surdo M, Becker C, Alteri C, Aragri M, Ricciardi A, Armenia D, Pollicita M, Di Santo F, Carioti L, Louzoun Y, Mastroianni CM, Lichtner M, Paoloni M, Esposito M, D'Amore C, Marrone A, Marignani M, Sarrecchia C, Sarmati L, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Hepatitis B surface antigen genetic elements critical for immune escape correlate with hepatitis B virus reactivation upon immunosuppression. *Hepatology* 2015; **61**: 823-833 [PMID: 25418031 DOI: 10.1002/hep.27604]

22 **Cento V**, Van Hemert F, Neumann-Fraune M, Mirabelli C, Di Maio VC, Salpini R, Bertoli A, Micheli V, Gubertini G, Romano S, Visca M, De Sanctis GM, Berkhout B, Marino N, Mazzotta F, Cappiello G, Spanò A, Sarrecchia C, Ceccherini-Silberstein F, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Anti-HBV treatment induces novel reverse transcriptase mutations with reflective effect on HBV S antigen. *J Infect* 2013; **67**: 303-312 [PMID: 23796863 DOI: 10.1016/j.jinf.2013.05.008]

23 **Svicher V**, Cento V, Bernassola M, Neumann-Fraune M, Van Hemert F, Chen M, Salpini R, Liu C, Longo R, Visca M, Romano S, Micheli V, Bertoli A, Gori C, Ceccherini-Silberstein F, Sarrecchia C, Andreoni M, Angelico M, Ursitti A, Spanò A, Zhang JM, Verheyen J, Cappiello G, Perno CF. Novel HBsAg markers tightly correlate with occult HBV infection and strongly affect HBsAg detection. *Antiviral Res* 2012; **93**: 86-93 [PMID: 22086128 DOI: 10.1016/j.antiviral.2011.10.022]

24 **Yuan Q**, Ou SH, Chen CR, Ge SX, Pei B, Chen QR, Yan Q, Lin YC, Ni HY, Huang CH, Yeo AE, Shih JW, Zhang J, Xia NS. Molecular characteristics of occult hepatitis B virus from blood donors in southeast China. *J Clin Microbiol* 2010; **48**: 357-362 [PMID: 19940057 DOI: 10.1128/JCM.01781-09]

25 **Cassini R**, De Mitri MS, Gibellini D, Urbinati L, Bagaglio S, Morsica G, Domenicali M, Verucchi G, Bernardi M. A novel stop codon mutation within the hepatitis B surface gene is detected in the liver but not in the peripheral blood mononuclear cells of HIV-infected individuals with occult HBV infection. *J Viral Hepat* 2013; **20**: 42-49 [PMID: 23231083 DOI: 10.1111/j.1365-2893.2012.01623.x]

26 **Huang CH**, Yuan Q, Chen PJ, Zhang YL, Chen CR, Zheng QB, Yeh SH, Yu H, Xue Y, Chen YX, Liu PG, Ge SX, Zhang J, Xia NS. Influence of mutations in hepatitis B virus surface protein on viral antigenicity and phenotype in occult HBV strains from blood donors. *J Hepatol* 2012; **57**: 720-729 [PMID: 22634131 DOI: 10.1016/j.jhep.2012.05.009]

27 **Chen SJ**, Zhao YX, Fang Y, Xu WZ, Ma YX, Song ZW, Teng X, Gu HX. Viral deletions among healthy young Chinese adults with occult hepatitis B virus infection. *Virus Res* 2012; **163**: 197-201 [PMID: 21963662]

28 **Lee SA**, Kim KJ, Kim DW, Kim BJ. Male-specific W4P/R mutation in the pre-S1 region of hepatitis B virus, increasing the risk of progression of liver diseases in chronic patients. *J Clin Microbiol* 2013; **51**: 3928-3936 [PMID: 24025913 DOI: 10.1128/JCM.01505-13]

29 **Besharat S**, Katoonizadeh A, Moradi A. Potential mutations associated with occult hepatitis B virus status. *Hepat Mon* 2014; **14**: e15275 [PMID: 24829588 DOI: 10.5812/hepatmon.15275]

30 **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]

31 **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]

32 **Wang HC**, Huang W, Lai MD, Su IJ. Hepatitis B virus pre-S mutants, endoplasmic reticulum stress and hepatocarcinogenesis. *Cancer Sci* 2006; **97**: 683-688 [PMID: 16863502 DOI: 10.1111/j.1349-7006.2006.00235.x]

33 **Yang JC**, Teng CF, Wu HC, Tsai HW, Chuang HC, Tsai TF, Hsu YH, Huang W, Wu LW, Su IJ. Enhanced expression of vascular endothelial growth factor-A in ground glass hepatocytes and its implication in hepatitis B virus hepatocarcinogenesis. *Hepatology* 2009; **49**: 1962-1971 [PMID: 19475690 DOI: 10.1002/hep.22889]

34 **Kim JH**, Park YK, Park ES, Kim KH. Molecular diagnosis and treatment of drug-resistant hepatitis B virus. *World J Gastroenterol* 2014; **20**: 5708-5720 [PMID: 24914332 DOI: 10.3748/wjg.v20.i19.5708]

35 **Girones R**, Miller RH. Mutation rate of the hepadnavirus genome. *Virology* 1989; **170**: 595-597 [PMID: 2728351]

36 **Bartholomeusz A**, Locarnini S. Hepatitis B virus mutations associated with antiviral therapy. *J Med Virol* 2006; **78** Suppl 1: S52-S55 [PMID: 16622878]

37 **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]

38 **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]

39 **Ji D**, Liu Y, Si LL, Li L, Chen GF, Xin SJ, Zhao JM, Xu D. Variable influence of mutational patterns in reverse-transcriptase domain on replication capacity of hepatitis B virus isolates from antiviral-experienced patients. *Clin Chim Acta* 2011; **412**: 305-313 [PMID: 21056552 DOI: 10.1016/j.cca.2010.10.028]

40 **Curtis M**, Zhu Y, Borroto-Esoda K. Hepatitis B virus containing the I233V mutation in the polymerase reverse-transcriptase domain remains sensitive to inhibition by adefovir. *J Infect Dis* 2007; **196**: 1483-1486 [PMID: 18008227 DOI: 10.1086/522521]

41 **Ismail AM**, Sharma OP, Kumar MS, Kannangai R, Abraham P. Impact of rtI233V mutation in hepatitis B virus polymerase protein and adefovir efficacy: Homology modeling and molecular docking studies. *Bioinformation* 2013; **9**: 121-125 [PMID: 23423477 DOI: 10.6026/97320630009121]

42 **Warner N**, Locarnini S. The antiviral drug selected hepatitis B virus rtA181T/sW172* mutant has a dominant negative secretion defect and alters the typical profile of viral rebound. *Hepatology* 2008; **48**: 88-98 [PMID: 18537180]

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

44 **Yeh CT**, Chen T, Hsu CW, Chen YC, Lai MW, Liang KH, Chen TC. Emergence of the rtA181T/sW172* mutant increased the risk of hepatoma occurrence in patients with lamivudine-resistant chronic hepatitis B. *BMC Cancer* 2011; **11**: 398 [PMID: 21933446 DOI: 10.1186/1471-2407-11-398]

45 **Tenney DJ**, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503-1514 [PMID: 19280622 DOI: 10.1002/hep.22841]

46 **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]

47 **Mikulska M**, Taramasso L, Giacobbe DR, Caligiuri P, Bruzzone B, Di Biagio A, Viscoli C. Case report: management and HBV sequencing in a patient co-infected with HBV and HIV failing tenofovir. *J Med Virol* 2012; **84**: 1340-1343 [PMID: 22825811 DOI: 10.1002/jmv.23338]

48 **Matsuda M**, Suzuki F, Suzuki Y, Tsubota A, Akuta N, Hosaka T, Someya T, Kobayashi M, Saitoh S, Arase Y, Satoh J, Takagi K, Kobayashi M, Ikeda K, Kumada H. Low rate of YMDD motif mutations in polymerase gene of hepatitis B virus in chronically infected patients not treated with lamivudine. *J Gastroenterol* 2004; **39**: 34-40 [PMID: 14767732 DOI: 10.1007/s00535-003-1242]

49 **Yang JH**, Zhang H, Chen XB, Chen G, Wang X. Relationship between hepatocellular carcinoma and hepatitis B virus genotype with spontaneous YMDD mutations. *World J Gastroenterol* 2013; **19**: 3861-3865 [PMID: 23840126 DOI: 10.3748/wjg.v19.i24.3861]

50 **Yang HC**, Chen CL, Shen YC, Peng CY, Liu CJ, Tseng TC, Su TH, Chuang WL, Yu ML, Dai CY, Liu CH, Chen PJ, Chen DS, Kao JH. Distinct evolution and predictive value of hepatitis B virus precore and basal core promoter mutations in interferon-induced hepatitis B e antigen seroconversion. *Hepatology* 2013; **57**: 934-943 [PMID: 23112104 DOI: 10.1002/hep.26121]

51 **Shen T**, Yan XM. Hepatitis B virus genetic mutations and evolution in liver diseases. *World J Gastroenterol* 2014; **20**: 5435-5441 [PMID: 24833874 DOI: 10.3748/wjg.v20.i18.5435]

52 **Hsu CW**, Yeh CT, Chang ML, Liaw YF. Identification of a hepatitis B virus S gene mutant in lamivudine-treated patients experiencing HBsAg seroclearance. *Gastroenterology* 2007; **132**: 543-550 [PMID: 17258721 DOI: 10.1053/j.gastro.2006.12.001]

53 **Amini-Bavil-Olyaee S**, Sheldon J, Lutz T, Trautwein C, Tacke F. Molecular analysis of an HBsAg-negative hepatitis B virus mutant selected in a tenofovir-treated HIV-hepatitis B virus co-infected patient. *AIDS* 2009; **23**: 268-272 [PMID: 19098499 DOI: 10.1097/QAD.0b013e3283224316]

54 **Sheldon J**, Soriano V. Hepatitis B virus escape mutants induced by antiviral therapy. *J Antimicrob Chemother* 2008; **61**: 766-768 [PMID: 18218641 DOI: 10.1093/jac/dkn014]

55 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]

56 **Schödel F**, Peterson D, Zheng J, Jones JE, Hughes JL, Milich DR. Structure of hepatitis B virus core and e-antigen. A single precore amino acid prevents nucleocapsid assembly. *J Biol Chem* 1993; **268**: 1332-1337 [PMID: 8419335]

57 **Lee JH**, Hong SP, Jang ES, Park SJ, Hwang SG, Kang SK, Jeong SH. Analysis of HBV genotype, drug resistant mutations, and pre-core/basal core promoter mutations in Korean patients with acute hepatitis B. *J Med Virol* 2015; **87**: 993-998 [PMID: 25712861 DOI: 10.1002/jmv.24148]

58 **Baptista M**, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 1999; **29**: 946-953 [PMID: 10051502]

59 **Kao JH**, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003; **124**: 327-334 [PMID: 12557138 DOI: 10.1053/gast.2003.50053]

60 **Chou YC**, Yu MW, Wu CF, Yang SY, Lin CL, Liu CJ, Shih WL, Chen PJ, Liaw YF, Chen CJ. Temporal relationship between hepatitis B virus enhancer II/basal core promoter sequence variation and risk of hepatocellular carcinoma. *Gut* 2008; **57**: 91-97 [PMID: 17502344 DOI: 10.1136/gut.2006.114066]

61 **Fang ZL**, Sabin CA, Dong BQ, Ge LY, Wei SC, Chen QY, Fang KX, Yang JY, Wang XY, Harrison TJ. HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. *Am J Gastroenterol* 2008; **103**: 2254-2262 [PMID: 18844615 DOI: 10.1111/j.1572-0241.2008.01974.x]

62 **Wu CF**, Yu MW, Lin CL, Liu CJ, Shih WL, Tsai KS, Chen CJ. Long-term tracking of hepatitis B viral load and the relationship with risk for hepatocellular carcinoma in men. *Carcinogenesis* 2008; **29**: 106-112 [PMID: 17999990 DOI: 10.1093/carcin/bgm252]

63 **Yuan JM**, Ambinder A, Fan Y, Gao YT, Yu MC, Groopman JD. Prospective evaluation of hepatitis B 1762(T)/1764(A) mutations on hepatocellular carcinoma development in Shanghai, China. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 590-594 [PMID: 19190166 DOI: 10.1158/1055-9965.EPI-08-0966]

64 **Yang HI**, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, Wang LY, Lu SN, You SL, Chen DS, Liaw YF, Chen CJ. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; **100**: 1134-1143 [PMID: 18695135 DOI: 10.1093/jnci/djn243]

65 **Kramvis A**, Kew MC. The core promoter of hepatitis B virus. *J Viral Hepat* 1999; **6**: 415-427 [PMID: 10607259 DOI: 10.1046/j.1365-2893.1999.00189.x]

66 **Huang Y**, Deng H, Shan X, Gong X, Li X, Tu Z, Long Q, Huang A. Lower mutation frequency of BCP/precore regions in e antigen-negative chronic HBV-infected children instead of adults patients. *PLoS One* 2015; **10**: e0120733 [PMID: 25822176 DOI: 10.1371/journal.pone.0120733]

67 **Besharat S**, Poustchi H, Mohamadkhani A, Katoonizadeh A, Moradi A, Roshandel G, Freedman ND, Malekzadeh R. Association of Mutations in the Basal Core Promoter and Pre-core Regions of the Hepatitis B Viral Genome and Longitudinal Changes in HBV Level in HBeAg Negative Individuals: Results From a Cohort Study in Northern Iran. *Hepat Mon* 2015; **15**: e23875 [PMID: 25788956 DOI: 10.5812/hepatmon.23875]

68 **Bouchard MJ**, Schneider RJ. The enigmatic X gene of hepatitis B virus. *J Virol* 2004; **78**: 12725-12734 [PMID: 15542625 DOI: 10.1128/JVI.78.23.12725-12734.2004]

69 **Yan J**, Yao Z, Hu K, Zhong Y, Li M, Xiong Z, Deng M. Hepatitis B Virus Core Promoter A1762T/G1764A (TA)/T1753A/T1768A Mutations Contribute to Hepatocarcinogenesis by Deregulating Skp2 and P53. *Dig Dis Sci* 2015; **60**: 1315-1324 [PMID: 25567052 DOI: 10.1007/s10620-014-3492-9]

70 **Xie Y**, Liu S, Zhao Y, Guo Z, Xu J. X protein mutations in hepatitis B virus DNA predict postoperative survival in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 10325-10331 [PMID: 25034530 DOI: 10.1007/s13277-014-2331-0]

P- Reviewer: Tsai WL **S- Editor**: Yu J **L- Editor**: A
E- Editor: Wang CH

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com

ISSN 1007-9327