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

**ESPS Manuscript NO: 21084**

**Manuscript Type: Review**

**Clinical significance of hepatitis B surface antigen mutants**

Nicola C *et al*. HBsAg mutants

**Coppola Nicola, Onorato Lorenzo, Minichini Carmine, Di Caprio Giovanni, Starace Mario, Sagnelli Caterina, Sagnelli Evangelista**

**Coppola Nicola, Onorato Lorenzo, Minichini Carmine, Di Caprio Giovanni, Starace Mario, Sagnelli Evangelista,** Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, 80131 Naples, Italy

**Sagnelli Caterina,** Department of Clinical and Experimental Medicine and Surgery “F. Magrassi e A. Lanzara”, Second University of Naples, 80131 Naples, Italy

**Author contributions:** Coppola N has contributed to conception of the paper and draft the article; Onorato L has analyzed the role of HBsAg mutants associated with HCC development; Minichini C has analyzed the HBV virology and HBsAg structure; Di Caprio G has analyzed the role of HBsAg mutants associated with immune escape; Starace M has analyzed the HBV virology and HBsAg structure; Sagnelli C has analyzed the role of HBsAg mutants associated with failed HBsAg detection; Sagnelli E has contributed to conception of the paper and draft the article.

**Conflict-of-interest** **statement:** The authors declare no conflicts of interest regarding this manuscript.

**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:** **Nicola Coppola, MD, PhD,** Department of Mental Health and Public Medicine, Section of Infectious Diseases, Second University of Naples, Via: L. Armanni 5, 80131 Naples, Italy. [nicola.coppola@unina2.it](mailto:nicola.coppola@unina2.it)

**Telephone**: +39-8-15666719

**Fax**: +39-8-15666013

**Received:** June 30, 2015

**Peer-review started:** June 30, 2015

**First decision:** September 18, 2015

**Revised:** September 27, 2015

**Accepted:** November 13, 2015

**Article in press:**

**Published online:**

**Abstract**

Hepatitis B virus (HBV) infection is a major public health problem in many countries, with nearly 300 million people worldwide carrying HBV chronic infection and over 1 million deaths per year due to cirrhosisand liver cancer. Several hepatitis B surface antigen (HBsAg) mutations have been described, most frequently due to a single amino acid substitution and seldom to a nucleotide deletion. The majority of mutations are located in the S region, but they have also been found in the pre-S1 and pre-S2 regions. Single amino acid substitutions in the major hydrophilic region of HBsAg, called the “a” determinant, have been associated with immune escape and the consequent failure of HBV vaccination and HBsAg detection, whereas deletions in the pre-S1 or pre-S2 regions have been associated with the development of hepatocellular carcinoma. This review article will focus on the HBsAg mutants and their biological and clinical implications.

**Key words**: HBsAg mutants; Vaccine escape; Immune escape hepatocellular carcinoma; Hepatitis B virus infection

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

**Core tip:** Antibodies to the hepatitis B surface antigen (HBsAg) produced in response to hepatitis B virus infection or vaccination and those used in diagnostic assays to detect this antigen in serum are both directed against the ‘‘a’’ determinant region, common to all subtypes of the virus. Mutations occurring on the loops of the “a” determinant may be responsible for the lack of protection in immunized patients and in those individuals receiving hepatitis B immune globulin or for failed detection of HBsAg using commercial diagnostic assays. There is growing evidence in the last decade of the association between HBsAg mutations and the development of hepatocellular carcinoma (HCC), suggesting that the pre-S1 or pre-S2 large deletions are those prevalently associated with the development of HCC.This review article will focus on the clinical impact of the various HBsAg mutants.

Coppola N, Onorato L, Minichini C, Di Caprio G, Starace M, Sagnelli C, Sagnelli E. Clinical significance of hepatitis B surface antigen mutants. *World J Hepatol* 2015; In press

**INTRODUCTION**

Hepatitis B virus (HBV) infection is a major public health problem in most countries, with approximately 2 billion people worldwide showing exposure to the virus, nearly 300 million carrying HBV chronic infection and over 1 million deaths per year due to HBV-related end-stage liver disease, liver cirrhosisand liver cancer[1-5].

HBV is an enveloped Hepadnavirus with an incomplete double-stranded DNA genome of 3.2 Kb[6]. Eight genotypes, with a distinct geographical distribution, have been identified to date. Genotype A prevails in north-western Europe and in the United States, genotypes B and C in Asia, genotype D in the Mediterranean basin, the Middle East, and India, genotype E in western Africa, genotype F in South and Central America, genotype G in the United States and France, genotype H in northern Latin America[7], genotype I in Laos, Vietnam, eastern India[8,9] and north-western China[10] and genotype J in Japan[11,12].

The worldwide prevalence of chronic HBV infection in the general population borders 5%, but it differs widely from one geographical area to another, from 0.1%–2.0% in the United States and western Europe, from 2.0%–8.0% in eastern Mediterranean countries and Japan, and from 5.0%–20.0% in south-eastern Asia and sub-Saharan Africa[1,13].

Risk factors for HBV infection include transfusion of unscreened blood, renal dialysis, sexual promiscuity, sharing or re-using syringes among injection drug users, tattooing, piercing, working or residing in a health-care setting, living in a correctional facility and long-term household or intimate non-sexual contact with an HBsAg-positive individual. In highly endemic areas the majority of HBsAg chronic carriers acquire HBV infection at birth or in the first decade of life, whereas in countries with a low endemicity HBV transmission occurs mostly in adulthood due to unprotected sexual contact, syringe sharing or parenteral exposure to contaminated medical equipment[7,14-17].

A vaccine against HBV became available in 1982, and ten years later the World Health Organization recommended universal vaccination of newborn babies with the HBsAg produced by yeast cells into which the genetic code for HBsAg had been inserted. The complete vaccination schedule induces protective antibody levels in more than 95% of infants, children and young adults.

The emergence of single or multiple amino acid substitutions in the HBsAg region has been found in infants born to HBsAg-positive mothers who underwent passive/active immunoprophylaxis at the birth, in HBsAg-positive liver transplant recipients treated with hyperimmune anti-HBs immune globulin and in patients who experienced loss of HBsAg after anti-HBV therapy

This review article focuses on the impact of HBsAg mutants on vaccine escape, failure of diagnostic tests to detect HBsAg, and on the development of hepatocellular carcinoma (HCC).

**HBV VIROLOGY**

Human HBV is the prototype member of the Hepadnaviridae family, which includes a variety of avian and mammalian viruses sharing similar genomic organization, organ tropisms and a unique strategy of genome replication[14].

HBV is one of the smallest enveloped animal viruses with a diameter of 42nM consisting of an outer lipid envelope and an icosahedral nucleocapsid core composed of proteins. The nucleocapsid encloses the viral DNA and a DNA polymerase acting also as a reverse transcriptase[17]. The outer envelope contains the embedded proteins HBsAg, pre-S1 and pre-S2 involved in the viral binding of, and entry into susceptible cells. HBV is also called “Dane particle” after the name of the researcher who first observed it on electron microscopy together with filaments and 22 nmol/L spherical bodies in the serum of infected individuals[18]. HBV infects the hepatocytes, whereas the filaments and spherical bodies do not contain the viral DNA and do not infect the liver cells. These filaments and spherical bodies show the same HBsAg reactivity as the surface of HBV and are considered to be produced by HBsAg in excess during the life cycle of the virus[19].

The HBV genome consists of 3200 base pairs of partially double-stranded circular DNA containing four (P, C, S and X) overlapping open reading frames (ORF) with a nucleotide diversity of ≥ 8% in different genotypes[15,16]. The P gene codes for the viral polymerase/reverse transcriptase. It has four domains: a terminal domain, which serves as a protein primer for reverse transcription of pre-genomic viral RNA, a spacer region without no apparent function, the polymerase domain, which has reverse transcription activity, and the RNase H domain, responsible for the degradation of the RNA template during reverse transcription.

The core (C) gene codes for HBcAg, the major structural protein of the nucleocapsid. The preC/C ORF is transcribed into a precore/core fusion protein. During entry into the endoplasmic reticulum, 19 amino acids are cleaved from the N-terminal end of the precore protein by a signal peptidase. When transported into the Golgi compartment, additional amino acids are removed from the C-terminal end by intra-Golgi proteases to form the HBe antigen. This antigen, which is secreted into the serum, is used as a marker of active HBV replication in clinical practice. The possibility that the circulating HBe antigen may suppress the immune response and favor HBV replication has been hypothesized[14] but never proven, and the biological function of this protein, if any, remains unknown. The preS/S ORF encodes the envelope proteins HBsAg, pre-S1 and pre-S2. The X gene codes for potent transactivating factors of viral and cellular genes (HBxAg), some of which possibly correlated to the development of HCC[20,21].

**HBsAg STRUCTURE AND VARIANTS**

The preS/S ORF encodes three different structurally related envelope proteins, termed the large (L), middle-sized (M) and small (S) protein, that is synthesized from alternative initiation codons. The three proteins share the same carboxy-terminus but have different amino terminal extensions. In particular, the S protein corresponding to the HBsAg consists of 226 amino acids (aa), the M protein contains an extra N-terminal extension of 55 aa, and the L protein has a further N-terminal sequence of 108-119 aa compared with the M protein[22].The enhancer and basic core promoter regions of S region overlap with the X gene.

HBsAg is an envelope glycoprotein that is currently the primary element for diagnosis and target of immunoprophylaxis of HBV infection. The dominant epitopes of HBsAg, which are the targets of neutralizing B-cell responses, reside in the ‘‘a’’ determinant (aa 124–147) within the major hydrophilic region (MHR).

Several mutations in the S region have been described and those most frequently reported in the literature are listed in Table 1. In most cases, they were aa substitutions due to a single mutation, but nucleotide deletions have also been reported. The majority of mutations were located in the S region, but some mutations were also identified in the pre-S1 or pre-S2 regions. Mutations in the S region have been found in various HBV genotypes, while those in pre-S1 or pre-S2 have been frequently observed in patients with HBV genotype C (Table 1)[23-60].

**CLINICAL SIGNIFICANCE OF HBsAg MUTANTS**

Some HBsAg mutants have been associated with major biological or clinical events such as immune escape, failure to detect HBsAg and the development of HCC.

***HBsAg mutants associated with immune escape***

The MHR region, which is exposed to the outer surface of the virion, is situated between aa 99-169 of HBsAg. The antibodies produced after HBV vaccination and those used in diagnostic assays to detect serum HBsAg are both directed against this region and, specifically, to a cluster of B-cell epitopes, common to all subtypes of the virus, called ‘‘a’’ determinant and showing a two-loop structure of aa (124-147). Mutations may occur on both loops of the “a” determinant and may be responsible for a lack of protection and the occurrence of HBV infection in immunized patients (vaccine escape) or for failure to protect by the HBIG administered as a prophylactic measure or failure to detect HBsAg in diagnostic assays. Table 2 lists the studies suggesting the association of HBsAg mutants with vaccine escape or failed HBsAg detection.

In 1988 a follow-up Italian study[61] reported that children with a strong antibody response to HBV vaccine may still become infected with HBV. This observation was confirmed in other investigations and the conclusion on this point is that this phenomenon may involve nearly 2% of children born of HBsAg-positive mothers or with other HBsAg-positive household contacts[61,62].More detailed analysis identified an association of vaccine escape with a point mutation from glycine to arginine at position 145 (G145R)[61]. This G145R mutation is the vaccine-escape mutant most frequently detected[62-68], stable over time[61,69] and horizontally transmissible[70,71].

He *et al*[72] studied 176 restaurant employees before and one year after the HBV vaccination was completed. Six (3.4%) of the 176 became HBV-DNA positive after vaccination and four (2.3%) of the six showed a point mutation within the “a” determinant (Gly-145-Ala, and Ile/Thr-126-Asn/Ser).

Ngui *et al*[73] tested 17 HBV-infected mother/infant pairs because the infants became HBV-infected despite careful passive-active HBV immunoprophylaxis. Complete concordance in the S gene sequence was identified in 15 mother/infant pairs, while in the remaining two pairs the sequence of the S gene differed: one infant harbored three nucleic acid changes (P120Q, F134Y and D144A) and the other was carrying the I126N substitution, mutations that may interfere with HBsAg/anti-HBs binding. Mismatches in the HBV S gene were also observed in 16 of 41 HBV-infected mother/infant pairs in Singapore, of whom the infants acquired HBV infection despite HBV passive/active immunization[74].

***HBsAg mutants associated with failed HBsAg detection***

In 1999, Coleman *et al*[75] demonstrated that three commercial assays did not detect serum HBsAg in patients showing mutations including G145R in the “a” determinant. Subsequently, Zhang *et al*[76] prepared a panel containing four dilutions of an HBsAg wild-type serum, three recombinant mutants (G145R, K141E, and T131I) and one negative sample. This panel was tested for HBsAg reactivity by the laboratories of 85 blood banks using different assays. HBsAg reactivity was detected only in 19.4% of the assays in the presence of the aa substitution G145R and in 20% in the presence of the T131I or K141E mutants.

Sticchi *et al* [77] found G145A HBsAg mutants in 8 (3.1%) of 256 HBsAg chronic carriers, alone in 5 and with other HBsAg mutations in 3 (T126I, T131A, C139Y, E/D144G, T126I, M133L, P120Q or T126I). In the three patients with a multiple mutation, HBsAg was undetectable by 3 of 5 routine assays used in this study.

HBsAg mutants associated with failure to detect HBsAg have also been observed in patients with acute hepatitis B[78-80]. Laoi *et al*[78] studied 32 consecutive patients with acute hepatitis B and found a single or multiple amino acid substitution in 6 (18.5%) isolates. The G145A substitution along with the F134L were responsible for failure to detect HBsAg in one of these 6 and the D144E and S143L in another two isolates, whereas the other mutants identified (R113Thr, Ser114Pro, Thr118Val, Ala128Val) were of unclear significance.

***HBsAg mutations associated with HCC development***

The HBsAg mutations prevalently associated with the development of HCC are the large deletions involving the pre-S1 or pre-S2 regions[81]. These deletions naturally occur during the chronic phase of HBV infection and induce the synthesis of truncated variants of the large envelope protein, with important immunological and clinical consequences[82]. In fact, these variants present reduced antigenicity and, by altering the immune response, may favour the replicative activity of the virus[83]. In addition, the pre-S deletions decrease the expression of middle and small surface proteins, resulting in intracellular accumulation of viral particles that may induce stress in the endoplasmic reticulum, oxidative DNA damage and genomic instability, and possibly lead to a higher rate of neoplastic transformation[84]. The growing evidence on the association between HBsAg mutations and the development of HCC emerging in the last decade is shown in Table 3.

In 2003, Huy *et al*[85] conducted a multicenter cross-sectional study on 352 HBsAg-positive patients from 12 countries in five continents or subcontinents and demonstrated a higher prevalence of pre-S1 and/or pre-S2 deletions and pre-S2 start codon mutations in patients with HCC than in those without (35.7% *vs* 16.5%, *p*<0.05). In accordance with this, a correlation between a pre-S deletion and the presence of liver cirrhosis or HCC was described in an observational Japanese study[86]. Also, in a cross-sectional Italian study, the prevalence of pre-S2 deletions or start codon mutations was much higher in the 19 patients with HBV-related HCC than in 91 HBV carriers without HCC (84.2% *vs* 43.9%, *P* < 0.02)[88]. In 2006, Chen *et al*[89] found a higher prevalence of pre-S deletions in 50 Taiwanese patients with HBV-related hepatocellular carcinoma than in 102 HBV-infected individuals without HCC (52.0% *vs* 29.4, *P <* 0.0001). Similar data come from three studies performed in Taiwan[90], South Korea[91] and China[92], respectively. In addition, a South Korean study performed by Mun *et al*[93] demonstrated a correlation of both pre-S1 deletions and pre-S1 start codon mutations with the occurrence of HCC (*P* = 0.027 and *P* = 0.048, respectively); in this study the presence of pre-S2 deletions was also significantly associated with the development of liver cirrhosis (*P* = 0.001). The association of pre-S deletions and pre-S2 start codon mutations with the presence or the development of HCC was confirmed in other studies performed in southern Asia[94-96]. Moreover, a cross-sectional South Korean study on 119 HBsAg-positive patients[97] showed a higher prevalence of pre-S1 deletions in patients with HCC than in those without. In a case-control study[100] on 192 HBsAg-positive patients from Taiwan, the pre-S2, but not pre-S1 deletions, were associated with the occurrence of HCC, data endorsed by the results of a subsequent South Korean case-control study on 270 HBV-infected patients[101] that described a correlation between pre-S2 but not pre-S1 deletions or pre-S2 start codon mutations and HCC. The pre-S deletions were significantly associated with the development of HCC also in a study performed by Kao *et al*[102] on 168 HBV chronic patients from Taiwan.

A prospective study performed in South Korea investigated 195 patients with chronic HBV infection[103] and showed a higher incidence of HCC in those who tested positive for pre-S mutations. Two subsequent Chinese case-control studies on 317 and 193 HBsAg-positive patients, respectively, identified pre-S deletions[105,106] and pre-S2 start codon mutations[106] as independent predictors of HCC development. Abe *et al*[98] found a correlation between the presence of pre-S1 or pre-S2 deletions and the occurrence of HCC in a case-control study on 40 Asian children with chronic HBV infection, a finding confirmed in a retrospective study on 38 Taiwanese children[99] in which the presence of pre-S mutations was identified as an independent predictor of HCC development.

Instead, a cross-sectional study[104] enrolling 154 patients from Thailand failed to show an association between HCC and pre-S1 or pre-S2 deletions or start codon mutations. Likewise, a small case-control study showed no association between pre-S2 mutations and HCC in 35 patients from different countries[87].

The S region of the HBV genome may present point mutations that could alter HBsAg secretion. These point mutations were investigated by some Authors to identify a possible correlation between their presence and the development of HCC. Chen *et al*[96] found a correlation between the W4P/R mutation and the occurrence of HCC, an observation endorsed by the data from a cross-sectional study from South Korea[107] on 247 HBsAg-positive patients in which the prevalence of W4P/R mutants was higher in patients with cirrhosis or HCC than in those with a less severe liver illness. In addition, Qu *et al*[106] in a larger study confirmed the association of the T31C and T53C mutations with the occurrence of HCC previously demonstrated in a small cohort study[108] published in 2008. In Qu’s study the T766A mutant and HCC were not associated, whereas a case-control study carried out by Zhu *et al*[109] on 55 HBV-infected Chinese patients showed a significant association between the pre-S2 start codon (p=0.014), T53C (*P* = 0.004) and T766A (*P* = 0.043) mutations and the occurrence of HCC.

**CONCLUSION**

The association of single or multiple aa substitutions in the HBsAg region with failed protection in infants who received passive/active prophylaxis and in HBsAg-positive liver transplant patients undergoing continuous passive immunoprophylaxis should alert clinicians to the possible onset of acute hepatitis B or a reactivation of a previous HBV infection, respectively, in these cases.

Similarly, the possibility that some subjects resulting HBsAg-negative may harbor HBV infection because an aa substitution has made the presence of HBsAg undetectable with the commercially available assays should be taken into account by clinicians and healthcare personnel working in laboratories and blood banks.

Although several studies reported an association between HBsAg mutations and HCC, the data on this point are not conclusive because most of the studies were performed in south-eastern Asia, some of them were very small, most of them were cross-sectional and a few reported data contrasting with those from the majority of studies. A large worldwide study, planned on the basis of the data available, would almost certainly improve our knowledge on this topic.

**REFERENCES**

1 **Lavanchy D**. Worldwide epidemiology of HBV infection, disease burden, and vaccine prevention. *J Clin Virol* 2005; **34** Suppl 1: S1-S3 [PMID: 16461208 DOI: 10.1016/S1386-6532(05)00384-7]

2 **Sagnelli E**, Stroffolini T, Mele A, Imparato M, Sagnelli C, Coppola N, Almasio PL. Impact of comorbidities on the severity of chronic hepatitis B at presentation. *World J Gastroenterol* 2012; **18**: 1616-1621 [PMID: 22529690 DOI: 10.3748/wjg.v18.i14.1616]

3 **Sagnelli E**, Stroffolini T, Mele A, Imparato M, Almasio PL. Chronic hepatitis B in Italy: new features of an old disease--approaching the universal prevalence of hepatitis B e antigen-negative cases and the eradication of hepatitis D infection. *Clin Infect Dis* 2008; **46**: 110-113 [PMID: 18171224 DOI: 10.1086/524074]

4 **Coppola N**, Corvino AR, De Pascalis S, Signoriello G, Di Fiore E, Nienhaus A, Sagnelli E, Lamberti M. The long-term immunogenicity of recombinant hepatitis B virus (HBV) vaccine: contribution of universal HBV vaccination in Italy. *BMC Infect Dis* 2015; **15**: 149 [PMID: 25884719 DOI: 10.1186/s12879-015-0874-3]

5 **Borgia G**, Gentile I. Treating chronic hepatitis B: today and tomorrow. *Curr Med Chem* 2006; **13**: 2839-2855 [PMID: 17073632 DOI: 10.2174/092986706778521995]

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

7 **Arauz-Ruiz P**, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. *J Gen Virol* 2002; **83**: 2059-2073 [PMID: 12124470]

8 **Tran TT**, Trinh TN, Abe K. New complex recombinant genotype of hepatitis B virus identified in Vietnam. *J Virol* 2008; **82**: 5657-5663 [PMID: 18353958 DOI: 10.1128/jvi.02556-07]

9 **Olinger CM**, Jutavijittum P, Hübschen JM, Yousukh A, Samountry B, Thammavong T, Toriyama K, Muller CP. Possible new hepatitis B virus genotype, southeast Asia. *Emerg Infect Dis* 2008; **14**: 1777-1780 [PMID: 18976569 DOI: 10.3201/eid1411.080437]

10 **Yu H**, Yuan Q, Ge SX, Wang HY, Zhang YL, Chen QR, Zhang J, Chen PJ, Xia NS. Molecular and phylogenetic analyses suggest an additional hepatitis B virus genotype "I". *PLoS One* 2010; **5**: e9297 [PMID: 20174575 DOI: 10.1371/journal.pone.0009297]

11 **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]

12 **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]

13 **Sagnelli E**, Sagnelli C, Pisaturo M, Macera M, Coppola N. Epidemiology of acute and chronic hepatitis B and delta over the last 5 decades in Italy. *World J Gastroenterol* 2014; **20**: 7635-7643 [PMID: 24976701 DOI: 10.3748/wjg.v20.i24.7635]

14 **Ganem D**, Schneider R. Hepadnaviridae: the viruses and their replication. In: Knipe DM, Howley PM. Fields Virology. Philadelphia: Lippincott-Raven, 2001: 2923–2970

15 **Sagnelli C**, Ciccozzi M, Pisaturo M, Lo Presti A, Cella E, Coppola N, Sagnelli E. The impact of viral molecular diversity on the clinical presentation and outcome of acute hepatitis B in Italy. *New Microbiol* 2015; **38**: 137-147 [PMID: 25915056]

16 **Coppola N**, Tonziello G, Colombatto P, Pisaturo M, Messina V, Moriconi F, Alessio L, Sagnelli C, Cavallone D, Brunetto M, Sagnelli E. Lamivudine-resistant HBV strain rtM204V/I in acute hepatitis B. *J Infect* 2013; **67**: 322-328 [PMID: 23796869 DOI: pii: ]

17 **Coppola N**, Sagnelli C, Pisaturo M, Minichini C, Messina V, Alessio L, Starace M, Signoriello G, Gentile I, Filippini P, Sagnelli E. Clinical and virological characteristics associated with severe acute hepatitis B. *Clin Microbiol Infect* 2014; **20**: O991-O997 [PMID: 24930916 DOI: 10.1111/1469-0691.12720]

18 **Harrison T**. Desk Encyclopedia of General Virology. Boston: Academic Press, 2009: 455

19 **Howard CR**. The biology of hepadnaviruses. *J Gen Virol* 1986; **67** (Pt 7): 1215-1235 [PMID: 3014045 DOI: 10.1099/0022-1317-67-7-1215]

20 **Schaefer S**. Hepatitis B virus genotypes in Europe. *Hepatol Res* 2007; **37**: S20-S26 [PMID: 17627630 DOI: 10.1111/j.1872-034X.2007.00099.x]

21 **He B**, Fan Q, Yang F, Hu T, Qiu W, Feng Y, Li Z, Li Y, Zhang F, Guo H, Zou X, Tu C. Hepatitis virus in long-fingered bats, Myanmar. *Emerg Infect Dis* 2013; **19**: 638-640 [PMID: 23631923 DOI: 10.3201/eid1904.121655]

22 **Coppola N**, Loquercio G, Tonziello G, Azzaro R, Pisaturo M, Di Costanzo G, Starace M, Pasquale G, Cacciapuoti C, Petruzziello A. HBV transmission from an occult carrier with five mutations in the major hydrophilic region of HBsAg to an immunosuppressed plasma recipient. *J Clin Virol* 2013; **58**: 315-317 [PMID: 23856167 DOI: 10.1016/j.jcv.2013.06.020]

23 **Mirabelli C**, Surdo M, Van Hemert F, Lian Z, Salpini R, Cento V, Cortese MF, Aragri M, Pollicita M, Alteri C, Bertoli A, Berkhout B, Micheli V, Gubertini G, Santoro MM, Romano S, Visca M, Bernassola M, Longo R, De Sanctis GM, Trimoulet P, Fleury H, Marino N, Mazzotta F, Cappiello G, Spanò A, Sarrecchia C, Zhang JM, Andreoni M, Angelico M, Verheyen J, Perno CF, Svicher V. Specific mutations in the C-terminus domain of HBV surface antigen significantly correlate with low level of serum HBV-DNA in patients with chronic HBV infection. *J Infect* 2015; **70**: 288-298 [PMID: 25452041 DOI: 10.1016/j.jinf.2014.10.015]

24 **Martin CM**, Welge JA, Rouster SD, Shata MT, Sherman KE, Blackard JT. Mutations associated with occult hepatitis B virus infection result in decreased surface antigen expression in vitro. *J Viral Hepat* 2012; **19**: 716-723 [PMID: 22967103 DOI: 10.1111/j.1365-2893.2012.01595.x]

25 **Weinberger KM**, Bauer T, Böhm S, Jilg W. High genetic variability of the group-specific a-determinant of hepatitis B virus surface antigen (HBsAg) and the corresponding fragment of the viral polymerase in chronic virus carriers lacking detectable HBsAg in serum. *J Gen Virol* 2000; **81**: 1165-1174 [PMID: 10769057]

26 **Zheng X**, Weinberger KM, Gehrke R, Isogawa M, Hilken G, Kemper T, Xu Y, Yang D, Jilg W, Roggendorf M, Lu M. Mutant hepatitis B virus surface antigens (HBsAg) are immunogenic but may have a changed specificity. *Virology* 2004; **329**: 454-464 [PMID: 15518823 DOI: 10.1016/j.virol.2004.08.033]

27 **Olinger CM**, Weber B, Otegbayo JA, Ammerlaan W, van der Taelem-Brulé N, Muller CP. Hepatitis B virus genotype E surface antigen detection with different immunoassays and diagnostic impact of mutations in the preS/S gene. *Med Microbiol Immunol* 2007; **196**: 247-252 [PMID: 17503077 DOI: 10.1007/s00430-007-0050-5]

28 **Ly TD**, Servant-Delmas A, Bagot S, Gonzalo S, Férey MP, Ebel A, Dussaix E, Laperche S, Roque-Afonso AM. Sensitivities of four new commercial hepatitis B virus surface antigen (HBsAg) assays in detection of HBsAg mutant forms. *J Clin Microbiol* 2006; **44**: 2321-2326 [PMID: 16825343 DOI: 10.1128/JCM.00121-06]

29 **Hou J**, Wang Z, Cheng J, Lin Y, Lau GK, Sun J, Zhou F, Waters J, Karayiannis P, Luo K. Prevalence of naturally occurring surface gene variants of hepatitis B virus in nonimmunized surface antigen-negative Chinese carriers. *Hepatology* 2001; **34**: 1027-1034 [PMID: 11679975 DOI: 10.1053/jhep.2001.28708]

30 **Yao QQ**, Dong XL, Wang XC, Ge SX, Hu AQ, Liu HY, Wang YA, Yuan Q, Zheng YJ. Hepatitis B virus surface antigen (HBsAg)-positive and HBsAg-negative hepatitis B virus infection among mother-teenager pairs 13 years after neonatal hepatitis B virus vaccination. *Clin Vaccine Immunol* 2013; **20**: 269-275 [PMID: 23254298 DOI: 10.1128/CVI.00539-12]

31 **Yong-Lin Y**, Qiang F, Ming-Shun Z, Jie C, Gui-Ming M, Zu-Hu H, Xu-Bing C. Hepatitis B surface antigen variants in voluntary blood donors in Nanjing, China. *Virol J* 2012; **9**: 82 [PMID: 22500577 DOI: 10.1186/1743-422X-9-82]

32 **Pollicino T**, Raffa G, Costantino L, Lisa A, Campello C, Squadrito G, Levrero M, Raimondo G. Molecular and functional analysis of occult hepatitis B virus isolates from patients with hepatocellular carcinoma. *Hepatology* 2007; **45**: 277-285 [PMID: 17256766 DOI: 10.1002/hep.21529]

33 **Abdelnabi Z**, Saleh N, Baraghithi S, Glebe D, Azzeh M. Subgenotypes and mutations in the s and polymerase genes of hepatitis B virus carriers in the West Bank, palestine. *PLoS One* 2014; **9**: e113821 [PMID: 25503289 DOI: 10.1371/journal.pone.0113821]

34 **Darmawan E**, Turyadi KE, Nursanty NK, Thedja MD, Muljono DH. Seroepidemiology and occult hepatitis B virus infection in young adults in Banjarmasin, Indonesia. *J Med Virol* 2015; **87**: 199-207 [PMID: 25521058 DOI: 10.1002/jmv.24045]

35 **Romanò L**, Paladini S, Galli C, Raimondo G, Pollicino T, Zanetti AR. Hepatitis B vaccination. *Hum Vaccin Immunother* 2015; **11**: 53-57 [PMID: 25483515 DOI: 10.4161/hv.34306]

36 **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]

37 **Norouzi M**, Ghorashi S, Abedi F, Nejatizadeh A, Ataei B, Malekzadeh R, Alavian S, Judaki M, Ghamari S, Namazi A, Rahimnia R, Khedive A, Jazayeri S. Identification of Hepatitis B Virus Surface Antigen (HBsAg) Genotypes and Variations in Chronic Carriers from Isfahan Province, Iran. *Iran J Public Health* 2012; **41**: 104-111 [PMID: 23113154]

38 **Komatsu H**, Inui A, Sogo T, Konishi Y, Tateno A, Fujisawa T. Hepatitis B surface gene 145 mutant as a minor population in hepatitis B virus carriers. *BMC Res Notes* 2012; **5**: 22 [PMID: 22233650 DOI: 10.1186/1756-0500-5-22]

39 **Mele A**, Tancredi F, Romanò L, Giuseppone A, Colucci M, Sangiuolo A, Lecce R, Adamo B, Tosti ME, Taliani G, Zanetti AR. Effectiveness of hepatitis B vaccination in babies born to hepatitis B surface antigen-positive mothers in Italy. *J Infect Dis* 2001; **184**: 905-908 [PMID: 11509998 DOI: 10.1086/323396]

40 **Torresi J**, Earnest-Silveira L, Deliyannis G, Edgtton K, Zhuang H, Locarnini SA, Fyfe J, Sozzi T, Jackson DC. Reduced antigenicity of the hepatitis B virus HBsAg protein arising as a consequence of sequence changes in the overlapping polymerase gene that are selected by lamivudine therapy. *Virology* 2002; **293**: 305-313 [PMID: 11886250 DOI: 10.1006/viro.2001.1246]

41 **Zuckerman JN**, Zuckerman AJ. Mutations of the surface protein of hepatitis B virus. *Antiviral Res* 2003; **60**: 75-78 [PMID: 14638401 DOI: 10.1016/j.antiviral.2003.08.013]

42 **Margeridon-Thermet S**, Shulman NS, Ahmed A, Shahriar R, Liu T, Wang C, Holmes SP, Babrzadeh F, Gharizadeh B, Hanczaruk B, Simen BB, Egholm M, Shafer RW. Ultra-deep pyrosequencing of hepatitis B virus quasispecies from nucleoside and nucleotide reverse-transcriptase inhibitor (NRTI)-treated patients and NRTI-naive patients. *J Infect Dis* 2009; **199**: 1275-1285 [PMID: 19301976 DOI: 10.1086/597808]

43 **Araujo NM**, Vianna CO, Moraes MT, Gomes SA. Expression of Hepatitis B virus surface antigen (HBsAg) from genotypes A, D and F and influence of amino acid variations related or not to genotypes on HBsAg detection. *Braz J Infect Dis* 2009; **13**: 266-271 [PMID: 20231988 DOI: 10.1590/S1413-86702009000400005]

44 **Geretti AM**, Patel M, Sarfo FS, Chadwick D, Verheyen J, Fraune M, Garcia A, Phillips RO. Detection of highly prevalent hepatitis B virus coinfection among HIV-seropositive persons in Ghana. *J Clin Microbiol* 2010; **48**: 3223-3230 [PMID: 20631103 DOI: 10.1128/JCM.02231-09]

45 **Dunford L**, Carr MJ, Dean J, Nguyen LT, Ta Thi TH, Nguyen BT, Connell J, Coughlan S, Nguyen HT, Hall WW, Thi LA. A multicentre molecular analysis of hepatitis B and blood-borne virus coinfections in Viet Nam. *PLoS One* 2012; **7**: e39027 [PMID: 22720022 DOI: 10.1371/journal.pone.0039027]

46 **Kuzin SN**, Zabotina EE, Zabelin NN, Kudriavtseva EN, Samokhvalov EI, Borisova OV, Manina TA, Lisitsina EV, Kuzina LE, Malyshev NA, Lavrov VF. [Heterogeneity of hepatitis B virus and diagnostic potential of modern test systems for the detection of HBsAg]. *Zh Mikrobiol Epidemiol Immunobiol* 2012; **1**: 68-75 [PMID: 22442974]

47 **Lin YM**, Jow GM, Mu SC, Chen BF. Naturally occurring hepatitis B virus B-cell and T-cell epitope mutants in hepatitis B vaccinated children. *ScientificWorldJournal* 2013; **2013**: 571875 [PMID: 24379746 DOI: 10.1155/2013/571875]

48 **Bian T**, Yan H, Shen L, Wang F, Zhang S, Cao Y, Zhang S, Zhang Y, Bi S. Change in hepatitis B virus large surface antigen variant prevalence 13 years after implementation of a universal vaccination program in China. *J Virol* 2013; **87**: 12196-12206 [PMID: 24006443 DOI: 10.1128/JVI.02127-13]

49 **Magiorkinis E**, Paraskevis D, Pavlopoulou ID, Kantzanou M, Haida C, Hatzakis A, Boletis IN. Renal transplantation from hepatitis B surface antigen (HBsAg)-positive donors to HBsAg-negative recipients: a case of post-transplant fulminant hepatitis associated with an extensively mutated hepatitis B virus strain and review of the current literature. *Transpl Infect Dis* 2013; **15**: 393-399 [PMID: 23773581 DOI: 10.1111/tid.12094]

50 **Servant-Delmas A**, Mercier-Darty M, Ly TD, Wind F, Alloui C, Sureau C, Laperche S. Variable capacity of 13 hepatitis B virus surface antigen assays for the detection of HBsAg mutants in blood samples. *J Clin Virol* 2012; **53**: 338-345 [PMID: 22296790 DOI: 10.1016/j.jcv.2012.01.003]

51 **Wu C**, Shi H, Wang Y, Lu M, Xu Y, Chen X. A case of hepatitis B reactivation due to the hepatitis B virus escape mutant in a patient undergoing chemotherapy. *Virol Sin* 2012; **27**: 369-372 [PMID: 23180290 DOI: 10.1007/s12250-012-3284-3]

52 **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]

53 **Bahramali G**, Sadeghizadeh M, Amini-Bavil-Olyaee S, Alavian SM, Behzad-Behbahani A, Adeli A, Aghasadeghi MR, Amini S, Mahboudi F. Clinical, virologic and phylogenetic features of hepatitis B infection in Iranian patients. *World J Gastroenterol* 2008; **14**: 5448-5453 [PMID: 18803358 DOI: 10.3748/wjg.14.5448]

54 **Chamni N**, Louisirirotchanakul S, Oota S, Sakuldamrongpanish T, Saldanha J, Chongkolwatana V, Phikulsod S. Genetic characterization and genotyping of hepatitis B virus (HBV) isolates from donors with an occult HBV infection. *Vox Sang* 2014; **107**: 324-332 [PMID: 25040474 DOI: 10.1111/vox.12178]

55 **Youssef A**, Yano Y, El-Sayed Zaki M, Utsumi T, Hayashi Y. Characteristics of hepatitis viruses among Egyptian children with acute hepatitis. *Int J Oncol* 2013; **42**: 1459-1465 [PMID: 23404231 DOI: 10.3892/ijo.2013.1822]

56 **Hamkar R**, Aghakhani A, Soufian S, Banifazl M, Ghavami N, Nadri M, Sofian M, Ahmadi F, Razeghi E, Eslamifar A, Ramezani A. Surface gene mutations of hepatitis B virus among high-risk patients with occult hepatitis B virus infection. *Diagn Microbiol Infect Dis* 2010; **66**: 285-291 [PMID: 19903586 DOI: 10.1016/j.diagmicrobio.2009.10.006]

57 **Datta S**, Ghosh A, Dasgupta D, Ghosh A, Roychoudhury S, Roy G, Das S, Das K, Gupta S, Basu K, Basu A, Datta S, Chowdhury A, Banerjee S. Novel point and combo-mutations in the genome of hepatitis B virus-genotype D: characterization and impact on liver disease progression to hepatocellular carcinoma. *PLoS One* 2014; **9**: e110012 [PMID: 25333524 DOI: 10.1371/journal.pone.0110012]

58 **Yeung P**, Wong DK, Lai CL, Fung J, Seto WK, Yuen MF. Profile of pre-S deletions in the natural history of chronic hepatitis B infection. *J Med Virol* 2010; **82**: 1843-1849 [PMID: 20872710 DOI: 10.1002/jmv.21901]

59 **Kim H**, Lee SA, Kim DW, Lee SH, Kim BJ. Naturally occurring mutations in large surface genes related to occult infection of hepatitis B virus genotype C. *PLoS One* 2013; **8**: e54486 [PMID: 23349904 DOI: 10.1371/journal.pone.0054486]

60 **Zhang D**, Dong P, Zhang K, Deng L, Bach C, Chen W, Li F, Protzer U, Ding H, Zeng C. Whole genome HBV deletion profiles and the accumulation of preS deletion mutant during antiviral treatment. *BMC Microbiol* 2012; **12**: 307 [PMID: 23272650 DOI: 10.1186/1471-2180-12-307]

61 **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 DOI: 10.1016/S0140-6736(88)90541-7]

62 **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]

63 **Hsu HY**, Chang MH, Liaw SH, Ni YH, Chen HL. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999; **30**: 1312-1317 [PMID: 10534356 DOI: 10.1002/hep.510300511]

64 **François G**, Kew M, Van Damme P, Mphahlele MJ, Meheus A. Mutant hepatitis B viruses: a matter of academic interest only or a problem with far-reaching implications? *Vaccine* 2001; **19**: 3799-3815 [PMID: 11427251 DOI: 10.1016/S0264-]

65 **Ireland JH**, O'Donnell B, Basuni AA, Kean JD, Wallace LA, Lau GK, Carman WF. Reactivity of 13 in vitro expressed hepatitis B surface antigen variants in 7 commercial diagnostic assays. *Hepatology* 2000; **31**: 1176-1182 [PMID: 10796895 DOI: 10.1053/he.2000.6407]

66 **Lee KM**, Kim YS, Ko YY, Yoo BM, Lee KJ, Kim JH, Hahm KB, Cho SW. Emergence of vaccine-induced escape mutant of hepatitis B virus with multiple surface gene mutations in a Korean child. *J Korean Med Sci* 2001; **16**: 359-362 [PMID: 11410701]

67 **Moerman B**, Moons V, Sommer H, Schmitt Y, Stetter M. Evaluation of sensitivity for wild type and mutant forms of hepatitis B surface antigen by four commercial HBsAg assays. *Clin Lab* 2004; **50**: 159-162 [PMID: 15074469]

68 **Seddigh-Tonekaboni S**, Lim WL, Young B, Hou JL, Waters J, Luo KX, Thomas HC, Karayiannis P. Hepatitis B surface antigen variants in vaccinees, blood donors and an interferon-treated patient. *J Viral Hepat* 2001; **8**: 154-158 [PMID: 11264736 DOI: 10.1046/j.1365-2893.2001.00275.x]

69 **Zuckerman AJ**, Zuckerman JN. Molecular epidemiology of hepatitis B virus mutants. *J Med Virol* 1999; **58**: 193-195 [PMID: 10447411]

70 **Oon CJ**, Chen WN, Goo KS, Goh KT. Intra-familial evidence of horizontal transmission of hepatitis B virus surface antigen mutant G145R. *J Infect* 2000; **41**: 260-264 [PMID: 11120616 DOI: 10.1053/jinf.2000.0751]

71 **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]

72 **He C**, Nomura F, Itoga S, Isobe K, Nakai T. Prevalence of vaccine-induced escape mutants of hepatitis B virus in the adult population in China: a prospective study in 176 restaurant employees. *J Gastroenterol Hepatol* 2001; **16**: 1373-1377 [PMID: 11851835 DOI: 10.1046/j.1440-1746.2001.02654.x]

73 **Ngui SL**, O'Connell S, Eglin RP, Heptonstall J, Teo CG. Low detection rate and maternal provenance of hepatitis B virus S gene mutants in cases of failed postnatal immunoprophylaxis in England and Wales. *J Infect Dis* 1997; **176**: 1360-1365 [PMID: 9359739 DOI: 10.1086/514133]

74 **Oon CJ**, Lim GK, Ye Z, Goh KT, Tan KL, Yo SL, Hopes E, Harrison TJ, Zuckerman AJ. Molecular epidemiology of hepatitis B virus vaccine variants in Singapore. *Vaccine* 1995; **13**: 699-702 [PMID: 7483783]

75 **Coleman PF**, Chen YC, Mushahwar IK. Immunoassay detection of hepatitis B surface antigen mutants. *J Med Virol* 1999; **59**: 19-24 [PMID: 10440803 DOI: 10.1002/(SICI)1096-9071(199909)59: 1<19: : AID-JMV4>3.0.CO; 2-B]

76 **Zhang R**, Wang L, Li J. Hepatitis B virus transfusion risk in China: proficiency testing for the detection of hepatitis B surface antigen. *Transfus Med* 2010; **20**: 322-328 [PMID: 20409073 DOI: 10.1111/j.1365-3148.2010.01007.x]

77 **Sticchi L**, Caligiuri P, Cacciani R, Alicino C, Bruzzone B. Epidemiology of HBV S-gene mutants in the Liguria Region, Italy: Implications for surveillance and detection of new escape variants. *Hum Vaccin Immunother* 2013; **9**: 568-571 [PMID: 23296324 DOI: 10.4161/hv.23236]

78 **Laoi BN**, Crowley B. Molecular characterization of hepatitis B virus (HBV) isolates, including identification of a novel recombinant, in patients with acute HBV infection attending an Irish hospital. *J Med Virol* 2008; **80**: 1554-1564 [PMID: 18649329 DOI: 10.1002/jmv.21273]

79 **Luongo M**, Critelli R, Grottola A, Gitto S, Bernabucci V, Bevini M, Vecchi C, Montagnani G, Villa E. Acute hepatitis B caused by a vaccine-escape HBV strain in vaccinated subject: sequence analysis and therapeutic strategy. *J Clin Virol* 2015; **62**: 89-91 [PMID: 25542480 DOI: 10.1016/j.jcv.2014.11.029]

80 **Foy MC**, Thio CL, Hwang HS, Saulynas M, Hamilton JP, Fine DM, Atta MG. False-negative hepatitis B virus (HBV) surface antigen in a vaccinated dialysis patient with a high level of HBV DNA in the United States. *Clin Vaccine Immunol* 2012; **19**: 820-822 [PMID: 22441395 DOI: 10.1128/CVI.05696-11]

81 **Kay A**, Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; **127**: 164-176 [PMID: 17383765 DOI: 10.1016/j.virusres.2007.02.021]

82 **Fan YF**, Lu CC, Chen WC, Yao WJ, Wang HC, Chang TT, Lei HY, Shiau AL, Su IJ. Prevalence and significance of hepatitis B virus (HBV) pre-S mutants in serum and liver at different replicative stages of chronic HBV infection. *Hepatology* 2001; **33**: 277-286 [PMID: 11124846 DOI: 10.1053/jhep.2001.21163]

83 **Chisari FV**, Ferrari C. Hepatitis B virus immunopathogenesis. *Annu Rev Immunol* 1995; **13**: 29-60 [PMID: 7612225 DOI: 10.1146/annurev.iy.13.040195.000333]

84 **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]

85 **Huy TT**, Ushijima H, Win KM, Luengrojanakul P, Shrestha PK, Zhong ZH, Smirnov AV, Taltavull TC, Sata T, Abe K. High prevalence of hepatitis B virus pre-s mutant in countries where it is endemic and its relationship with genotype and chronicity. *J Clin Microbiol* 2003; **41**: 5449-5455 [PMID: 14662924 DOI: 10.1128/JCM.41.12.5449-5455.2003]

86 **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]

87 **Bläckberg J**, Kidd-Ljunggren K. Mutations within the hepatitis B virus genome among chronic hepatitis B patients with hepatocellular carcinoma. *J Med Virol* 2003; **71**: 18-23 [PMID: 12858404 DOI: 10.1002/jmv.10458]

88 **Raimondo G**, Costantino L, Caccamo G, Pollicino T, Squadrito G, Cacciola I, Brancatelli S. Non-sequencing molecular approaches to identify preS2-defective hepatitis B virus variants proved to be associated with severe liver diseases. *J Hepatol* 2004; **40**: 515-519 [PMID: 15123368 DOI: 10.1016/j.jhep.2003.11.025]

89 **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]

90 **Lin CL**, Liu CH, Chen W, Huang WL, Chen PJ, Lai MY, Chen DS, Kao JH. Association of pre-S deletion mutant of hepatitis B virus with risk of hepatocellular carcinoma. *J Gastroenterol Hepatol* 2007; **22**: 1098-1103 [PMID: 17608857 DOI: 10.1111/j.1440-1746.2006.04515.x]

91 **Choi MS**, Kim DY, Lee DH, Lee JH, Koh KC, Paik SW, Rhee JC, Yoo BC. Clinical significance of pre-S mutations in patients with genotype C hepatitis B virus infection. *J Viral Hepat* 2007; **14**: 161-168 [PMID: 17305881 DOI: 10.1111/j.1365-2893.2006.00784.x]

92 **Gao ZY**, Li T, Wang J, Du JM, Li YJ, Li J, Lu FM, Zhuang H. Mutations in preS genes of genotype C hepatitis B virus in patients with chronic hepatitis B and hepatocellular carcinoma. *J Gastroenterol* 2007; **42**: 761-768 [PMID: 17876546 DOI: 10.1007/s00535-007-2085-1]

93 **Mun HS**, Lee SA, Jee Y, Kim H, Park JH, Song BC, Yoon JH, Kim YJ, Lee HS, Hyun JW, Hwang ES, Kook YH, Kim BJ. The prevalence of hepatitis B virus preS deletions occurring naturally in Korean patients infected chronically with genotype C. *J Med Virol* 2008; **80**: 1189-1194 [PMID: 18461612 DOI: 10.1002/jmv.21208]

94 **Fang ZL**, Sabin CA, Dong BQ, Wei SC, Chen QY, Fang KX, Yang JY, Huang J, Wang XY, Harrison TJ. Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma: a matched nested case-control study. *J Gen Virol* 2008; **89**: 2882-2890 [PMID: 18931087 DOI: 10.1099/vir.0.2008/002824-0]

95 **Cao Z**, Bai X, Guo X, Jin Y, Qian G, Tu H. High prevalence of hepatitis B virus pre-S mutation and its association with hepatocellular carcinoma in Qidong, China. *Arch Virol* 2008; **153**: 1807-1812 [PMID: 18726170 DOI: 10.1007/s00705-008-0176-9]

96 **Chen CH**, Changchien CS, Lee CM, Hung CH, Hu TH, Wang JH, Wang JC, Lu SN. Combined mutations in pre-s/surface and core promoter/precore regions of hepatitis B virus increase the risk of hepatocellular carcinoma: a case-control study. *J Infect Dis* 2008; **198**: 1634-1642 [PMID: 18939932 DOI: 10.1086/592990]

97 **Jang JS**, Kim HS, Kim HJ, Shin WG, Kim KH, Lee JH, Kim HY, Kim DJ, Lee MS, Park CK, Jeong BH, Kim YS, Jang MK. Association of concurrent hepatitis B surface antigen and antibody to hepatitis B surface antigen with hepatocellular carcinoma in chronic hepatitis B virus infection. *J Med Virol* 2009; **81**: 1531-1538 [PMID: 19623669 DOI: 10.1002/jmv.21577]

98 **Abe K**, Thung SN, Wu HC, Tran TT, Le Hoang P, Truong KD, Inui A, Jang JJ, Su IJ. Pre-S2 deletion mutants of hepatitis B virus could have an important role in hepatocarcinogenesis in Asian children. *Cancer Sci* 2009; **100**: 2249-2254 [PMID: 19719772 DOI: 10.1111/j.1349-7006.2009.01309.x]

99 **Huang HP**, Hsu HY, Chen CL, Ni YH, Wang HY, Tsuei DJ, Chiang CL, Tsai YC, Chen HL, Chang MH. Pre-S2 deletions of hepatitis B virus and hepatocellular carcinoma in children. *Pediatr Res* 2010; **67**: 90-94 [PMID: 19816238 DOI: 10.1203/PDR.0b013e3181c1b0b7]

100 **Yeung P**, Wong DK, Lai CL, Fung J, Seto WK, Yuen MF. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in chronic hepatitis B. *J Infect Dis* 2011; **203**: 646-654 [PMID: 21227916 DOI: 10.1093/infdis/jiq096]

101 **Lee MH**, Kim do Y, Kim JK, Chang HY, Kang SH, Ryu HJ, Ju HL, Kim SU, Lee JM, Park JY, Han KH, Chon CY, Ahn SH. Combination of preS deletions and A1762T/G1764A mutations in HBV subgenotype C2 increases the risk of developing HCC. *Intervirology* 2012; **55**: 296-302 [PMID: 21865669 DOI: 10.1159/000329941]

102 **Kao JH**, Liu CJ, Jow GM, Chen PJ, Chen DS, Chen BF. Fine mapping of hepatitis B virus pre-S deletion and its association with hepatocellular carcinoma. *Liver Int* 2012; **32**: 1373-1381 [PMID: 22676233 DOI: 10.1111/j.1478-3231.2012.02826.x]

103 **Sinn DH**, Choi MS, Gwak GY, Paik YH, Lee JH, Koh KC, Paik SW, Yoo BC. Pre-s mutation is a significant risk factor for hepatocellular carcinoma development: a long-term retrospective cohort study. *Dig Dis Sci* 2013; **58**: 751-758 [PMID: 23053886 DOI: 10.1007/s10620-012-2408-9]

104 **Thongbai C**, Sa-nguanmoo P, Kranokpiruk P, Poovorawan K, Poovorawan Y, Tangkijvanich P. Hepatitis B virus genetic variation and TP53 R249S mutation in patients with hepatocellular carcinoma in Thailand. *Asian Pac J Cancer Prev* 2013; **14**: 3555-3559 [PMID: 23886144 DOI: 10.7314/APJCP.2013.14.6.3555]

105 **Zhao ZM**, Jin Y, Gan Y, Zhu Y, Chen TY, Wang JB, Sun Y, Cao ZG, Qian GS, Tu H. Novel approach to identifying the hepatitis B virus pre-S deletions associated with hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 13573-13581 [PMID: 25309088 DOI: 10.3748/wjg.v20.i37.13573]

106 **Qu LS**, Liu JX, Liu TT, Shen XZ, Chen TY, Ni ZP, Lu CH. Association of hepatitis B virus pre-S deletions with the development of hepatocellular carcinoma in Qidong, China. *PLoS One* 2014; **9**: e98257 [PMID: 24849936 DOI: 10.1371/journal.pone.0098257]

107 **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]

108 **Sung JJ**, Tsui SK, Tse CH, Ng EY, Leung KS, Lee KH, Mok TS, Bartholomeusz A, Au TC, Tsoi KK, Locarnini S, Chan HL. Genotype-specific genomic markers associated with primary hepatomas, based on complete genomic sequencing of hepatitis B virus. *J Virol* 2008; **82**: 3604-3611 [PMID: 18216102 DOI: 10.1128/JVI.01197-07]

109 **Zhu Y**, Jin Y, Guo X, Bai X, Chen T, Wang J, Qian G, Groopman JD, Gu J, Li J, Tu H. Comparison study on the complete sequence of hepatitis B virus identifies new mutations in core gene associated with hepatocellular carcinoma. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2623-2630 [PMID: 20699378 DOI: 10.1158/1055-9965.EPI-10-0469]

110 **Hung CH**, Chen CH, Lee CM, Hu TH, Lu SN, Wang JH, Huang CM. Role of viral genotypes and hepatitis B viral mutants in the risk of hepatocellular carcinoma associated with hepatitis B and C dual infection. *Intervirology* 2013; **56**: 316-324 [PMID: 23838434 DOI: 10.1159/000350738]

**P-Reviewer:** He JY, Horvat R **S-Editor:** Qiu S **L-Editor: E-Editor:**

**Table 1** **Mutations reported in the hepatitis B surface antigen regions**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Codon** | **Type Mutation** | **Mutation** | **Phenotypic consequence** | **HBV**  **Genotype** | **Ref.** |
| Wt1 atg  Mt1 acg  Wt2/3 tac  Mt2 tgc  Mt3 cac  Wt4 ttt  Mt4 ttg | AAS | M197T1,  Y206C2/ H3, F220L4 | Low serum HBV DNA | D | [1] |
| Wt1 gga  Mt1 gca | AAS | G145A1 | Immune escape1  Lamivudine resistance1 | A-B-C-D | [2,9,10,28-31] |
|  | AAS | G145R | Immune escape | A,B-C-D | [6,14,15,22,23,29,31-34] |
| Wt tgc  Mt tgg | AAS | C121W | Immune escape | A | [3] |
| Wr tgc  Mt tgg | AAS | C147W | Immune escape | D | [3,4] |
| Wt act  Mt att | AAS | T189I | Immune escape, reducing HBsAg detection signal | E-A | [5,6,12,24] |
| WT act  Mt aat | AAS | I126N | Immune escape | C | [7] |
| WtCaa  Mt cga | AAS | Q129R | Vaccine escape | B | [8,13] |
| Wt ttc  Mt tac | AAS | F161Y | Immune escape | C | [9,35] |
| Wt atg  Mt atc | AAS | M103I | Immune escape | D | [10,36] |
| Wt ttg  Mt tcg | AAS | L94S | HCC | D | [11] |
| WT gac  Mt gaa | AAS | D144E | Immune escape | D,C,A | [6,12,22,36] |
| Wt cct  Mt Tct | AAS | P127S | Immune escape | B,D | [12,20,13] |
| Wt act  Mt agt | AAS | T126S | Immune escape | B | [12,13,19] |
| WT act  MT atc | AAS | T126I | Immune escape | C,D | [6,13,37] |
| WT act  Mt agc | AAS | T143S | Immune escape | C,A | [13,37] |
| WTcca  Mt aca | AAS | P120T | Immune escape | B,D | [13,37] |
| Wt gca  Mt Gta | AAS | A184V | Immune escape | E | [16] |
| Wt gta  Mt gca | AAS | V184A | Immune escape | E | [16] |
| Wt Tcg  Mt acg | AAS | S143t | Immune escape | E | [16,21,37] |
| Wt tgt  Mt ttc | AAS | C76F | Immune escape | E | [16] |
| Wt cct  Mt act | AAS | P70T | Immune escape | E | [16] |
| Wt ata  Mt act | AAS | I82T | Immune escape | E | [16] |
| Wt att  Mt ctt | AAS | I110L | Immune escape | A,C | [18,37] |
| Wt tat  Mt ttt | AAS | Y134F | Immune escape | D | [21,39] |
| Wt agt  Mt aac | AAS | S207N | Immune escape | D | [21,40] |
| Wt tat  Mt cat | AAS | Y134H | Immune escape | D | [36] |
| Wt acg  Mt aat | AAS | T125N | Increased HBsAg reactivity in immunological diagnostic assays | D | [26] |
| Wt atg  Mt atc  WT aag  Mt agg | AAS | M103I- K122R | Immune escape | A-C-D | [28] |
| Wt tat  Mt tgt | AAS | Y100C | Immune escape | B-C | [29] |
| Wt ccc  Mt ctc  MtQ caa  MtS tcg  Mtt acc | AAS | P120L/Q/S/T | Immune escape | B-C | [29] |
| Wt tcg  Mt cta | AAS | S143L | Immune escape | D | [36] |
| Wt ctt  Mt cct | AAS | L127P | Immune escape | E | [16] |
| Wt cct  Mt ctt | AAS | P127L | Immune escape | A | [16] |
| Mutations in pre-S1 region | | | | | |
| Wt tct  Mt aca | AAS | S98T | Significant association with disease progression (LF, LC, HCC) | D | [41] |
| Wt aac  Mt act | AAS | N48T | Reduced HBsAg detection signals | C | [16] |
| Wt cag  Mt cct | AAS | Q82P | Reduced HBsAg detection signals | C | [16] |
| Wt acc  Mt aat | AAS | T97N | HBsAg not detected | C | [16] |
| Wt aat  Mt acc | AAS | N97T | HBsAg not detected | E | [16] |
| Wt cct  Mt cag | AAS | P93Q | HBsAg not detected | E,C | [16] |
| Deletion size (bp) 39 | D | Region (nt)  3046–3084 | Progression to advanced liver disease | C | [42] |
| Deletion size (bp)108 | D | Region (nt)  2959–3066 | Progression to advanced liver disease | C | [42] |
| Deletion size (bp) 39 | D | Region (nt)3046–3084 | Progression to advanced liver disease | C | [42] |
| Deletion size (bp)108 | D | Region 2959–3066 | Progression to advanced liver disease | C | [42] |
| 104th codon | AAS | Q104Stop | HCC development and immune escape | C | [43] |
| preS1 start | D | Not specified | HCC development and immune escape | C | [43] |
| Wt ucc  Mt gcc | AAS | S17A | Immune escape | C | [43] |
| Wt cct  Mt ctt | AAS | P32L | Immune escape | C | [43] |
| Wt tgg  Mt ctg  Mt agg | AAS | W43L/R | Immune escape | C | [43] |
| 104th codon | AAS | Q104Stop | HCC development and immune escape | C | [43] |
| Mutations in pre-S2 region | | | | | |
| Not specified | AAS | preS2-W3Stop | Immune escape | C | [43] |
| From 8th codon to 23rd codon | D |  | Immune escape | C | [43] |

AAS: Amino acid substitution; D: Deletion; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HBsAg: Hepatitis B surface antigen.

**Table 2** **Studies on hepatitis B surface antigen mutations associated with immune escape and failure to detect hepatitis B surface antigen**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Ref.** | **Country** | **No. of**  **patients** | **Type of Study** | **Patients with HBsAg mutation,**  **n° (%)** | **Clinical significance** | **HBsAg mutation** |
| Sticchi *et al*[77] | Italy | 256 | Cross-sectional | 8 (3.1) | Detection failure;  Vaccine escape | G145R ,T126I |
| Luongo *et al*[79] | Italy | 1 | Case report | 1 | Vaccine escape | M125T, T127P Q129H |
| Lee *et al*[66] | Korea | 1 | Case report | 1 | Vaccine escape | G145R, P120Q,  I126T |
| Seddigh *et al*[68] | UK | 4 |  | 2 | Vaccine escape | P142S, G145R, G145A |
| Ngui *et al*[73] | England, Wales | 17 | Cross-sectional | 2 (12%) | Vaccine escape | P120Q, F134Y, D144A, I126N |
| Carman *et al*[62] | multinational | 32 | Cross-sectional | 1 | Vaccine escape | G145R |
| Laoi *et al*[78] | Ireland | 32 | Cross-sectional | 6 (18.5%) | Vaccine escape, detection failure | G145A,F134L, D144E,S143L |
| Foy *et al*[80] | United States | 1 | Case report | 1 | Immune escape | D144E |

HBsAg: Hepatitis B surface antigen.

**Table 3** **Clinical significance of hepatitis B surface antigen mutations in chronic hepatitis B**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Ref.** | **Country** | **No. of**  **patients** | **Type of Study** | **Patients with HBsAg mutation,**  **n° (%)** | **Clinical significance** |
| Abe *et al*[98] | Japan | 40 | Case-control | 27/30 (90) in HCC+  0/10 (0) in HCC- | Correlation between PreS1-S2 deletion and HCC (*P* < 0.001) |
| Blackberg *et al*[87] | Sweden | 35 | Case-control | 8/16 (50) in HCC+  4/19 (21) in HCC- | No correlation between Pre-S2 mutations and HCC (*P* > 0.05) |
| Cao *et al*[95] | China | 97 | Case-control | 34/47 (72.3) in HCC+  13/50 (26) in HCC- | Correlation between Pre-S deletion or Pre-S2 start codon mutation and HCC (*P* < 0.001) |
| Chen *et al*[89] | Taiwan | 152 | Cross-sectional | 26/50 (52.0) in HCC+  30/102 (29.4) in HCC- | Correlation between Pre-S deletion and HCC (*P* < 0001) |
| Chen *et al*[96] | Taiwan | 240 | Case-control | Pre-S deletion: 28/80 (35) in HCC+ *vs*  27/160 (16.9) in HCC-  W4P/R: 10/80 (12.5) in HCC+ *vs* 7/160 (4.4) in HCC- M1V/I/A: 23/80 (28.8) in HCC+ *vs* 24/160 (15) in HCC- | Correlation between Pre-S deletion (*P* = 0.002), W4P/R (*P* = 0.021) and M1V/I/A mutations (*P* = 0.011) and HCC |
| Choi *et al*[91] | South Korea | 300 | Cross-sectional | 31/72 (43.1) in HCC+  51/228 (22.4) in HCC- | Correlation between Pre-S deletion or Pre-S2 start codon mutation and HCC (*P* < 0.001) |
| Fang *et al*[94] | China | 66 | Case-control | 15/33 (45.5) in HCC+  6/33 (18.2) in HCC- | Correlation between Pre-S deletion and HCC (*P* < 0.01) |
| Gao *et al*[92] | China | 79 | Cross-sectional | 10/26 (38.5) in HCC+  3/53 (5.7) in HCC- | Correlation between Pre-S deletion and HCC  (*P* = 0.001) |
| Huang *et al*[99] | Taiwan | 38 | Case-control | 9/19 (47.4) in HCC+  1/19 (5.3) in HCC- | Correlation between Pre-S deletion and HCC  (*P* = 0.008) |
| Hung *et al*[110] | Taiwan | 313 | Cross-sectional | 41/146 (40) in HCC+  5/167 (3.0) in HCC- | Correlation between Pre-S deletion and HCC  (*P* < 0.001) |
| Huy *et al*[85] | 12 countries | 352 | Cross-sectional | 17/49 (34.7) in HCC+  50/303 (16.5) in HCC- | Correlation between Pre-S1/S2 deletion and Pre-S2 start codon mutations and HCC (*P* < 0.05) |
| Jang *et al*[97] | South Korea | 119 | Cross-sectional | 17/48 (35.4) in HCC+  13/71 (18.3) in HCC- | Correlation between Pre-S deletion and HCC  (*P* < 0.05) |
| Kao *et al*[102] | Taiwan | 168 | Case-control | 56/112 (50.0) in HCC+  4/56 (7.1) in HCC- | Correlation between Pre-S deletion and HCC  (*P* < 0.001) |
| Lee *et al*[101] | South Korea | 270 | Case-control | 28/135 (18.5) in HCC+  6/135 (4.4) in HCC- | Correlation between Pre-S2 deletion and HCC  (*P* < 0.001) |
| Lee *et al*[107] | South Korea | 247 | Cross-sectional | 19/153 (12.4) in advanced liver disease (LC or HCC)  1/94 (1.1) in non-advanced liver disease | Correlation between W4P/R mutation and HCC or cirrhosis (*P* < 0.05) |
| Lin *et al*[90] | Taiwan | 266 | Cross-sectional | 19/64 (29.7) in HCC+  25/202 (12.4) in HCC- | Correlation between Pre-S deletion and HCC  (*P* = 0.02) |
| Mun *et al*[93] | South Korea | 120 | Cross-sectional | Pre-S1: 13/40 (32.5) in HCC+ *vs* 11/80 (13.7) in HCC-  Pre-S1 start codon: 9/40 (22.5) in HCC+ *vs* 4/80 (5.0) in HCC- Pre-S2: 8/21 (38.1) in LC+ *vs* 4/59 (6.8) in LC- | Correlation between Pre-S1 (*P* = 0.027) and Pre-S1 start codon mutations (*P* = 0.048) and HCC. Correlation between Pre-S2 deletions and cirrhosis (*P* = 0.001) |
| Qu *et al*[106] | China | 193 | Case-control | Pre-S deletion:  28/96 (29.2) *vs*  11/97 (11.3) , Pre-S2 start codon: 17/96 (17.7) *vs* 7/97 (7.2), T31C: 23/96 (24.0) *vs* 37/97 (38.1), T53C: 36/96 (37.5) *vs* 23/97 (23.7), T766A: 13/96 (13.5) *vs* 14/97 (14.4) in HCC+ *vs* HCC- | Correlation between Pre-S deletion (*P* = 0.003), Pre-S2 start codon (*P* = 0.027), T31C (*P* = 0.044), T53C (*P* = 0.027) but not T766A  mutation (*P* = 0.966) and HCC |
| Raimondo *et al*[88] | Italy | 110 | Cross-sectional | 16/19 (84.2) in HCC  40/91 (43.9) in HCC- | Correlation between Pre-S2 deletion or start codon mutation and HCC (*P* < 0.02) |
| Sinn *et al*[103] | South Korea | 195 | Cohort | 13/24 (54.2) in HCC+  31/171 (18.1) in HCC- | Correlation between Pre-S mutation and HCC (*P* < 0.001) |
| Sugauchi *et al*[86] | Japan | 160 | Cross-sectional | 20/58 (34.5) in advanced liver disease (LC or HCC)  17/102 (16.7) in non-advanced liver disease | Correlation between Pre-S deletion and HCC or cirrhosis (*P* < 0.05) |
| Sung *et al*[108] | Hong Kong | 26 | Case-control | T31C: 6/16 (37.5) in HCC+ *vs* 0/10 (0.0) in HCC- T53C: 6/16 (37.5) in HCC+ *vs* 1/10 (10.0) in HCC- | Correlation between T31C and T53C mutations and HCC (*P* < 0.05) |
| Thongbai *et al*[104] | Thailand | 154 | Cross-sectional | 24/65 (36.9) in HCC+  34/89 (38.2) in HCC- | No correlation between Pre-S1/S2/S deletion or start codon mutation and HCC (*P* > 0.1) |
| Yeung *et al*[100] | Taiwan | 192 | Case-control | 28/96 (29.2) in HCC+  14/96 (14.6) in HCC- | Correlation between Pre-S deletion and HCC  (*P* = 0.015) |
| Zhao *et al*[105] | China | 317 | Case-control | 74/157 (47.1) in HCC+  45/160 (28.1) in HCC- | Correlation between Pre-S deletion and HCC  (*P* < 0.001) |
| Zhu *et al*[109] | China | 55 | Case-control | 4/20 (20.0) with Pre-S2 start codon, 5/20 (25.0) with T53C and 3/20 with T766A in HCC+ *vs* 0/20 in HCC- | Correlation between Pre-S2 start codon (*P* = 0.014), T53C (*P* = 0.004) and T766A mutation (*P* = 0.043) and HCC |

HBV: Hepatitis B virus; HBIG: Hepatitis B immune globulin; HCC: Hepatocellular carcinoma; HBsAg: Hepatitis B surface antigen; AAS: Amino acid substitution; D: Deletion.