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TOPIC HIGHLIGHT

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Hepatitis B virus genetic mutations and evolution in liver diseases

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Abstract

Hepatitis B virus (HBV) belongs to the genus *Orthohepadnavirus* of the *Hepadnaviridae* family and is approximately 3.2 kb in length. Owing to a lack of proofreading capacity during reverse transcription and a high replication rate, HBV exhibits as quasispecies. To detect the genetic mutations of HBV, many methods with different sensitivities and throughputs were developed. According to documentary records, HBV mutation and evolution were important vial parameters in predicting disease progression and therapeutic outcome. In this review, we separately discussed the correlation between HBV genomic mutations in four open reading frames and liver disease progression. Since some of the results were controversial from different laboratories, it remains to be seen whether functional analyses will confirm their role in modifying the course of infection.

Key words: Hepatitis B virus; Mutation; Genotype; Liver disease; Risk markers

Core tip: Understanding the characteristics of hepatitis B virus (HBV) is crucial for early diagnosis and optimized treatment. In this review, we reviewed the technologies being used in the evolutionary and mutational analysis of HBV and introduced a high throughput method of deep sequencing for HBV (ultra-high-throughput next generation sequencing technology). And then, we separately discussed the correlation between HBV genomic mutations in four open reading frames and liver disease progression. Since some of the results were controversial from different laboratories, it remains to be seen whether functional analyses will confirm their role in modifying the course of infection.

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INTRODUCTION

Hepatitis B virus (HBV) belongs to the genus *Orthohepadnavirus* of the *Hepadnaviridae* family and is approximately 3.2 kb in length with four overlapping open reading

frames (ORFs) encoding the polymerase (P), core (C), surface antigen (S), and X protein. Based on ⩾ 8% intergenotype divergences in the entire genome, HBV has been classified into at least ten different genotypes, named A-J[1]. In addition, subgroups have been reported in different genotypes of HBV[2-6]. As documented in many studies, HBV exhibits a mutation rate more than 10-fold higher than that of other DNA viruses and exists as quasispecies, owing to a lack of proofreading capacity during reverse transcription and a high replication rate.

METHODS FOR DETECTION HBV GENETIC MUTATIONS

HBV DNA was almost simultaneously cloned and sequenced in 1978 by three pioneers[7-10]. From then on, several methods have been developed to determine the HBV genome and its genetic mutations[11-13], including polymerase chain reaction (PCR) amplification and direct Sanger sequencing[14,15] or pyrosequencing[16], restriction fragment length polymorphism[17], line probe assay[18,19], enzyme-linked immunoassay[20], clone-based sequencing (CBS)[21,22], real-time PCR (RT-PCR) assay, fluorescence resonance energy transfer (FRET)-based RT-PCR assay[23], and hybridization-fluorescence polarization assay[24]. Among these methods, direct PCR sequencing detects mutations present in > 20% of the circulating virus population (on average). Clone-based sequencing has a higher sensitivity for detecting low-prevalence HBV mutations and has been commonly used for detecting HBV heterogeneity. However, its throughput limitation and time consuming nature can not be satisfied with the growing need for HBV complexity and diversity analysis. In recent years, ultra-high-throughput next generation sequencing (NGS) technology is used in the HBV heterogeneity analysis[25,26]. It is more sensitive and efficient in terms of low abundant variation detection (< 20% minority variants) than that by CBS method[27], and can simultaneously detect mutations in different HBV gene regions[28], thus sheds light on the future clinical application of NGS in HBV quasispecies studies.

HBV GENOTYPING IN LIVER DISEASES

HBV genotype is an important viral parameter in predicting disease progression and therapeutic outcome[29-33]. Many population-based or community-based long-term cohort studies showed that genotype is one of the high risk factors for liver disease progression. More than a decade followed-up studies revealed that persons in the inactive phase of hepatitis B with genotype B were at a high risk of reactivation[34], and HIV-infected patients with HBV genotype B were more likely to experience acute exacerbations of hepatitis and liver disease-related death than those with genotype C coinfection[35]. Other cohort studies revealed that compared with genotypes A and B cases, HBV genotypes C and D infection is associated with higher prevalence of basal core promoter mutation and a higher risk of hepatocellular carcinoma (HCC)[36-38]. These observations suggest pathogenic differences between HBV genotypes[1].

Several studies of standard interferon therapy showed that genotypes A and B were associated with better response to Peg-IFN-α-2a therapy and higher rates of HBeAg seroconversion compared to genotypes C and D[31,33,39-42], and HBV genotype B was an independent factor for HBeAg clearance[43]. Interestingly, other studies of pegIFN-α reported that genotypes A and D but not genotype B were associated with a higher rate of HBeAg seroconversion[44,45]. These discrepant results may be due to several intrinsic features and weaknesses in the majority of clinical trials conducted, such as different ethnicities and different patient enrollment criteria. Thus, guidelines from three regional bodies-AASLD, APASL and EASL - all stop short of recommending genotyping as part of the management of chronic hepatitis B[46-49]. Still, additional multicenter data on the relation between HBV genotypes and treatment response are needed before testing for HBV genotypes in clinical practice is recommended.

HBV GENETIC MUTATIONS AND EVOLUTION IN LIVER DISEASES

Many investigations demonstrated that during the progression of liver diseases, genetic mutations and evolution were observed in the HBV gene-coding regions, and some of them could be risk markers for liver injury (Table 1).

PreS1/S2/S ORF

The HBV S-ORF is composed of three forms of HBV surface genes: pre-S1, preS2, and S domain. The pre-S domain is the essential binding site for hepatocyte receptors and contains several epitopes for T or B cells. Mutations at this region may directly influence HBV infection and liver disease progression. Pre-S deletion was observed in chronic hepatitis B infection, fulminant hepatitis B, acute hepatitis B and HCC[49-58]. Several cross-sectional studies have shown an association between pre-S mutation and HCC[59-61]. Longitudinal observations demonstrated a gradual combination of pre-S deletion during the development of HCC, and patients with pre-S mutations had significantly higher 5-year cumulative incidences of HCC than those without (26.5% *vs* 5.7%, $P < 0.001$)[60,61]. Variation and deletion in the 3' terminus of pre-S1 are also associated with occult HBV infection[62]. Besides deletion variation, a novel preS1 mutation, W4P/R was observed with the progression of liver diseases and male predominance from a Korean chronic cohort through a molecular epidemiologic study. These W4P/R mutants were significantly related to severe liver diseases [HCC and liver cirrhosis (12.4%, 19/153 patients) *vs* chronic hepatitis and carrier (1.1%, 1/94 patients), $P < 0.001$]. Interestingly, all of the W4P/R mutants were found only in the male gender, not in the female gender, which may in part provide the likely explanation for the relatively high ratio of male to female incidence in HCC

Table 1 Possible risk markers for liver injury

ORF	Major Mutations	Clinical status	Ref.
PreS1/S2/S			
	preS deletion	CHB	[49-51]
		FHB	[52,53]
		AHB	[54,55]
		HCC	[56-61,78]
		Occult infection	[62]
	W4P/R	male predominace HCC and LC	[23]
S			
	T207A	LC	[63]
	T770C	HCC	GenBank no.AY206393
	C695	Occult infection	[64]
	T207A	HBsAg(-)	[65-67]
X			
	A1762T/G1764A	LC,HCC	[68,69,71,75-79]
		mild liver histology	[72]
	C1653T	LC,HCC	[75,77-79]
	T1753V	LC,HCC	[75,77-80]
	G1386M	LC,HCC	
	B1499	LC,HCC	[75]
	G1613A	LC,HCC	[77,79]
	A1727G	LC,HCC	[76,77]
	G1757A	LC,HCC	[76,77]
	C1766T	LC,HCC	[76-78]
	T1768A	LC,HCC	[77,78]
	A1727G	HCC	[76]
	C1773T	HCC	[76]
preC/C			
	A1896T	FHB,HCC	[37,80]
	G1899A	HCC	[37]
	C1913A or C1914T	HCC	[37]
	A2149C/T	HCC	[37]
	A2188T/C	HCC	[37]
	C2198A	HCC	[37]
	C2444A/T	HCC	[37]
	core antigen internal deletions	CHB	[82,83]
		HCC	[84]
		immune-suppressed patients	[85]
P			
	G741H	HCC	[89,93]
		CHB	[90-93]

ORF: Opening reading frame; HCC: Hepatocellular carcinoma; CHB: Chronic hepatitis B; FHB: Fulminant hepatitis B; AHB: Acute hepatitis B; LC: Liver cirrhosis.

generation in Korean chronic patients[23].

Hepatitis B surface antigen (HBsAg) is a main serological marker for diagnosis of HBV infection. Mutations like T207A and T770C mutations in the small S region were observed in cirrhosis[63] and HCC patients (Genbank number AY206393), respectively. Recently, a C695 mutation in the S gene was found in liver tissue of occult HBV infection patients[64], which could be responsible for reduced production leading to undetectability of HBsAg. Besides these mutations, a high degree of the quasispecies was observed in the HBV infection patients, which was probably linked to the severity of the infection[65-67].

X ORF

HBV-X protein is associated with the pathogenesis of HBV related diseases, especially hepatocellular carcinoma in chronic patients. Genetic variability of the X gene includes genotypic specific variations and mutations emerging during chronic infection. The double mutation of nucleotide A1762T/G1764A in basal core promoter (BCP) is frequently observed in HBV sequences isolated from patients with chronic HBV infection, fulminant hepatitis, HCC, and in reactivation of HBV with a fulminant course[68,69], which results in mutations at two codons in the carboxyl functional region of X protein (K130M and V131I). At present, there are conflicting opinions regarding 1762T/1764A hotspot mutations. Some studies suggest that these mutations decrease HBeAg expression and slightly increase viral DNA replication, and are mostly found in patients who seroconvert to anti-HBe and develop HCC[70-72]. By contrast, other studies indicate that these mutations are not associated with HBeAg/anti-HBe status or HBV DNA or HCC[73,74]. Other mutations including M1386, T1485, B1499, A1613, T1653, G1727, A1757/T1764/G1766, T1773, G or C1753, and T1766/A1768 mutations have been reported to be associated with the development of HCC[74-79], which are alone and/or in combination to be the predictive markers for hepatocarcinogenesis.

PreCore/core ORF

HBV precore/core ORF encoding proteins, hepatitis B e antigen (HBeAg) and core antigen (HBcAg), are two indicators of active viral replication. Mutations in the C region were mainly distributed in MHC restricted region. In particular, mutations in the MHC class II restricted region (in M2RR) were found to be significantly related to HCC. Six (preC-W28*, C-P5H/L/T, C-E83D, C-I97F/L, C-L100I and C-Q182K/*) and seven types (preC-W28*, preC-G29D, C-D32N/H, C-E43K, C-P50A/H/Y, C-A131G/N/P and C-S181H/P) of mutations in the preC/C region were found to be related to HCC and to affect the HBeAg serostatus, respectively[37,80], However, children with HBV infection in the immune-tolerance phase had not have pre-w28* mutation, suggests that this mutation may be the result of life-long chronic HBV infection[81]. Also, a heterogeneous population of core antigen internal deletions (CID) has been found to be highly prevalent in chronic HBV carriers[51,82,83], HCC patients[84] and immunosuppressed patients[85], suggesting that the host immune pressure against T cells is the major driving force of preC/C mutations[86-88].

Polymerase ORF

Mutations in the reverse transcriptase domain and different HBV genotypes may result in changes in amino acid sequence and protein configuration of HBV polymerase. Prior research has suggested that lamivudine is the major cause of YMDD mutations in HBV P-ORF,

and lamivudine-related YMDD mutation is an independent risk factor for HCC[89]. However, the mechanism remains unclear. Further research has revealed that strains with YMDD mutations also exist in patients with chronic HBV infection not previously treated with lamivudine[90-93]. A recent research showed that spontaneous YMDD mutations were detected in LC and HCC patients, and genotype C strains in HCC patients had a significantly higher spontaneous YMDD mutation rate than in LC patients, and was associated with a 7.775-fold higher risk for the development of HBV-related HCC than patients infected by other type HBV strains (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[94].

In all, understanding the characteristics of hepatitis B virus is crucial for early diagnosis and optimized treatment. There is great need to develop methodologies that take into account both factors from the host and the pathogen. Many of candidate mutations seem unexpected given our current knowledge of the molecular genetics of HBV. Thus, it remains to be seen whether functional analyses will confirm their role in modifying the course of infection[95].

REFERENCES

1 **Okamoto H**, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. Typing hepatitis B virus by homology in nucleotide sequence: comparison of surface antigen subtypes. *J Gen Virol* 1988; **69** (Pt 10): 2575-2583 [PMID: 3171552]

2 **Schaefer S**. Hepatitis B virus taxonomy and hepatitis B virus genotypes. *World J Gastroenterol* 2007; **13**: 14-21 [PMID: 17206751]

3 **Shi W**, Zhang Z, Ling C, Zheng W, Zhu C, Carr MJ, Higgins DG. Hepatitis B virus subgenotyping: history, effects of recombination, misclassifications, and corrections. *Infect Genet Evol* 2013; **16**: 355-361 [PMID: 23538336 DOI: 10.1016/j.meegid.2013.03.021]

4 **Liu WC**, Phiet PH, Chiang TY, Sun KT, Hung KH, Young KC, Wu IC, Cheng PN, Chang TT. Five subgenotypes of hepatitis B virus genotype B with distinct geographic and virological characteristics. *Virus Res* 2007; **129**: 212-223 [PMID: 17825452]

5 **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]

6 **Shi W**, Zhu C, Zheng W, Zheng W, Ling C, Carr MJ, Higgins DG, Zhang Z. Subgenotyping of genotype C hepatitis B virus: correcting misclassifications and identifying a novel subgenotype. *PLoS One* 2012; **7**: e47271 [PMID: 23077582 DOI: 10.1371/journal.pone.0047271]

7 **Gerlich WH**. Medical virology of hepatitis B: how it began and where we are now. *Virol J* 2013; **10**: 239 [PMID: 23870415 DOI: 10.1186/1743-422X-10-239]

8 **Charnay P**, Pourcel C, Louise A, Fritsch A, Tiollais P. Cloning in Escherichia coli and physical structure of hepatitis B virion DNA. *Proc Natl Acad Sci USA* 1979; **76**: 2222-2226 [PMID: 377294]

9 **Valenzuela P**, Gray P, Quiroga M, Zaldivar J, Goodman HM, Rutter WJ. Nucleotide sequence of the gene coding for the major protein of hepatitis B virus surface antigen. *Nature* 1979; **280**: 815-819 [PMID: 471053]

10 **Pasek M**, Goto T, Gilbert W, Zink B, Schaller H, MacKay P, Leadbetter G, Murray K. Hepatitis B virus genes and their expression in E. coli. *Nature* 1979; **282**: 575-579 [PMID: 399329]

11 **Ohba K**, Mizokami M, Ohno T, Suzuki K, Orito E, Lau JY, Ina Y, Ikeo K, Gojobori T. Relationships between serotypes and genotypes of hepatitis B virus: genetic classification of HBV by use of surface genes. *Virus Res* 1995; **39**: 25-34 [PMID: 8607280]

12 **Nagasaki F**, Niitsuma H, Cervantes JG, Chiba M, Hong S, Ojima T, Ueno Y, Bondoc E, Kobayashi K, Ishii M, Shimosegawa T. Analysis of the entire nucleotide sequence of hepatitis B virus genotype B in the Philippines reveals a new subgenotype of genotype B. *J Gen Virol* 2006; **87**: 1175-1180 [PMID: 16603518]

13 **Zumbika E**, Ruan B, Xu CH, Ni Q, Hou W, Chen Z, Liu KZ. HBV genotype characterization and distribution in patients with HBV-related liver diseases in Zhejiang Province, P.R. China: possible association of co-infection with disease prevalence and severity. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 535-543 [PMID: 16286258]

14 **Werle B**, Cinquin K, Marcellin P, Pol S, Maynard M, Trépo C, Zoulim F. Evolution of hepatitis B viral load and viral genome sequence during adefovir dipivoxil therapy. *J Viral Hepat* 2004; **11**: 74-83 [PMID: 14738561]

15 **Singla B**, Chakraborti A, Sharma BK, Kapil S, Chawla YK, Arora SK, Das A, Dhiman RK, Duseja A. Hepatitis B virus reverse transcriptase mutations in treatment Naïve chronic hepatitis B patients. *J Med Virol* 2013; **85**: 1155-1162 [PMID: 23918533 DOI: 10.1002/jmv.23608]

16 **Lindström A**, Odeberg J, Albert J. Pyrosequencing for detection of lamivudine-resistant hepatitis B virus. *J Clin Microbiol* 2004; **42**: 4788-4795 [PMID: 15472342 DOI: 10.1128/JCM.42.10.4788-4795.2004]

17 **Mizokami M**, Nakano T, Orito E, Tanaka Y, Sakugawa H, Mukaide M, Robertson BH. Hepatitis B virus genotype assignment using restriction fragment length polymorphism patterns. *FEBS Lett* 1999; **450**: 66-71 [PMID: 10350059]

18 **Ghabeshi S**, Sharifi Z, Hosseini SM, Mahmoodian Shooshtari M. Correlation between viral load of HBV in chronic hepatitis B patients and precore and Basal core promoter mutations. *Hepat Mon* 2013; **13**: e7415 [PMID: 23599717 DOI: 10.5812/hepatmon.7415]

19 **van Bömmel F**, Trojan J, Deterding K, Wedemeyer H, Wasmuth HE, Hüppe D, Möller B, Bock FJ, Feucht HH, Berg T. Evolution of adefovir-resistant HBV polymerase gene variants after switching to tenofovir disoproxil fumarate monotherapy. *Antivir Ther* 2012; **17**: 1049-1058 [PMID: 22892524 DOI: 10.3851/IMP2307]

20 **Usuda S**, Okamoto H, Iwanari H, Baba K, Tsuda F, Miyakawa Y, Mayumi M. Serological detection of hepatitis B virus genotypes by ELISA with monoclonal antibodies to type-specific epitopes in the preS2-region product. *J Virol Methods* 1999; **80**: 97-112 [PMID: 10403681]

21 **Suzuki F**, Hosaka T, Suzuki Y, Akuta N, Sezaki H, Hara T, Kawamura Y, Kobayashi M, Saitoh S, Arase Y, Ikeda K, Kobayashi M, Watahiki S, Mineta R, Kumada H. Long-term efficacy and emergence of multidrug resistance in patients with lamivudine-refractory chronic hepatitis B treated by combination therapy with adefovir plus lamivudine. *J Gastroenterol* 2013 Aug 9; Epub ahead of print [PMID: 23929069]

22 **Chen L**, Zheng CX, Lin MH, Huang ZX, Chen RH, Li QG, Li Q, Chen P. Distinct quasispecies characteristics and positive selection within precore/core gene in hepatitis B virus HBV associated acute-on-chronic liver failure. *J Gastroenterol Hepatol* 2013; **28**: 1040-1046 [PMID: 23278564 DOI: 10.1111/jgh.12109]

23 **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]
24 **Li D**, Cheng H, Gong W, Jiang Y, Liang P, Zhang J. Detection of primary YMDD mutations in HBV-related hepatocellular carcinoma using hybridization-fluorescence polarization. *J Virol Methods* 2013; **187**: 259-263 [PMID: 23178585 DOI: 10.1016/j.jviromet.2012.11.017]
25 **Lin KT**, Shann YJ, Chau GY, Hsu CN, Huang CY. Identification of latent biomarkers in hepatocellular carcinoma by ultra-deep whole-transcriptome sequencing. *Oncogene* 2013 Oct 21; Epub ahead of print [PMID: 24141781 DOI: 10.1038/onc.2013.424]
26 **Gong L**, Han Y, Chen L, Liu F, Hao P, Sheng J, Li XH, Yu DM, Gong QM, Tian F, Guo XK, Zhang XX. Comparison of next-generation sequencing and clone-based sequencing in analysis of hepatitis B virus reverse transcriptase quasispecies heterogeneity. *J Clin Microbiol* 2013; **51**: 4087-4094 [PMID: 24088859]
27 **Sede M**, Ojeda D, Cassino L, Westergaard G, Vazquez M, Benetti S, Fay F, Tanno H, Quarleri J. Long-term monitoring drug resistance by ultra-deep pyrosequencing in a chronic hepatitis B virus (HBV)-infected patient exposed to several unsuccessful therapy schemes. *Antiviral Res* 2012; **94**: 184-187 [PMID: 22453135 DOI: 10.1016/j.antiviral.2012.03.003]
28 **Homs M**, Buti M, Quer J, Jardí R, Schaper M, Tabernero D, Ortega I, Sanchez A, Esteban R, Rodriguez-Frias F. Ultra-deep pyrosequencing analysis of the hepatitis B virus preCore region and main catalytic motif of the viral polymerase in the same viral genome. *Nucleic Acids Res* 2011; **39**: 8457-8471 [PMID: 21742757 DOI: 10.1093/nar/gkr451]
29 **Kao JH**, Chen PJ, Lai MY, Chen DS. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. *J Clin Microbiol* 2002; **40**: 1207-1209 [PMID: 11923332]
30 **Kao JH**, Chen PJ, Lai MY, Chen DS. Hepatitis B virus genotypes and spontaneous hepatitis B e antigen seroconversion in Taiwanese hepatitis B carriers. *J Med Virol* 2004; **72**: 363-369 [PMID: 14748059]
31 **Kao JH**, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; **33**: 998-1002 [PMID: 11131465]
32 **Liu CJ**, Kao JH, Chen DS. Therapeutic implications of hepatitis B virus genotypes. *Liver Int* 2005; **25**: 1097-1107 [PMID: 16343058]
33 **Su TH**, Liu CJ, Tseng TC, Liu CH, Yang HC, Chen CL, Chen PJ, Kao JH, Chen DS. Longitudinal change of HBsAg in HBeAg-negative patients with genotype B or C infection. *PLoS One* 2013; **8**: e55916 [PMID: 23437072 DOI: 10.1371/journal.pone.0055916]
34 **Tohme RA**, Bulkow L, Homan CE, Negus S, McMahon BJ. Rates and risk factors for hepatitis B reactivation in a cohort of persons in the inactive phase of chronic hepatitis B-Alaska, 2001-2010. *J Clin Virol* 2013; **58**: 396-400 [PMID: 24001884 DOI: 10.1016/j.jcv.2013.08.012]
35 **Sheng WH**, Hung CC, Chang SY, Liu CJ, Chen MY, Hsieh SM, Kao JH, Chen PJ, Chang SC. Differential clinical and virologic impact of hepatitis B virus genotypes B and C on HIV-coinfected patients receiving lamivudine-containing highly active antiretroviral therapy. *Clin Infect Dis* 2012; **54**: 548-555 [PMID: 22156858 DOI: 10.1093/cid/cir851]
36 **Lin CL**, Kao JH. Risk stratification for hepatitis B virus related hepatocellular carcinoma. *J Gastroenterol Hepatol* 2013; **28**: 10-17 [PMID: 23094699 DOI: 10.1111/jgh.12010]
37 **Kim DW**, Lee SA, Hwang ES, Kook YH, Kim BJ. Naturally occurring precore/core region mutations of hepatitis B virus genotype C related to hepatocellular carcinoma. *PLoS One* 2012; **7**: e47372 [PMID: 23071796 DOI: 10.1371/journal.pone.0047372]
38 **Milosevic I**, Delic D, Lazarevic I, Pavlovic IP, Korac M, Bojovic K, Jevtovic D. The significance of hepatitis B virus (HBV) genotypes for the disease and treatment outcome among patients with chronic hepatitis B in Serbia. *J Clin Virol* 2013; **58**: 54-58 [PMID: 23838671 DOI: 10.1016/j.jcv.2013.06.017]
39 **Wai CT**, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002; **36**: 1425-1430 [PMID: 12447868]
40 **Erhardt A**, Blondin D, Hauck K, Sagir A, Kohnle T, Heintges T, Häussinger D. Response to interferon alfa is hepatitis B virus genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* 2005; **54**: 1009-1013 [PMID: 15951551]
41 **Janssen HL**, van Zonneveld M, Senturk H, Zeuzem S, Akarca US, Cakaloglu Y, Simon C, So TM, Gerken G, de Man RA, Niesters HG, Zondervan P, Hansen B, Schalm SW; HBV 99-01 Study Group; Rotterdam Foundation for Liver Research. Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; **365**: 123-129 [PMID: 15639293]
42 **Fan HB**, Guo YB, Zhu YF, Chen AS, Zhou MX, Li Z, Xu LT, Ma XJ, Yan FM. Hepatitis B Virus Genotype B and High Expression of Interferon Alpha Receptor β Subunit are Associated With Better Response to Pegylated Interferon Alpha 2a in Chinese Patients With Chronic Hepatitis B Infection. *Hepat Mon* 2012; **12**: 333-338 [PMID: 22783345 DOI: 10.5812/hepatmon.6173]
43 **Chen CH**, Lee CM, Hung CH, Hu TH, Wang JH, Wang JC, Lu SN, Changchien CS. Clinical significance and evolution of core promoter and precore mutations in HBeAg-positive patients with HBV genotype B and C: a longitudinal study. *Liver Int* 2007; **27**: 806-815 [PMID: 17617124]
44 **Lau GK**, Piratvisuth T, Luo KX, Marcellin P, Thongsawat S, Cooksley G, Gane E, Fried MW, Chow WC, Paik SW, Chang WY, Berg T, Flisiak R, McCloud P, Pluck N. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; **352**: 2682-2695 [PMID: 15987917]
45 **Boxall E**, Sira J, Kaskar S, Workman J, Kelly D. Does genotype predict response to treatment in children infected with hepatitis B perinatally? *J Med Virol* 2012; **84**: 1535-1540 [PMID: 22930499 DOI: 10.1002/jmv.23308]
46 **Cooksley WG**. Do we need to determine viral genotype in treating chronic hepatitis B? *J Viral Hepat* 2010; **17**: 601-610 [PMID: 20529201 DOI: 10.1111/j.1365-2893.2010.01326.x]
47 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539 [PMID: 17256718]
48 **European Association For The Study Of The Liver**. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009; **50**: 227-242 [PMID: 19054588 DOI: 10.1016/j.jhep.2008.10.001]
49 **Liaw YF**, Leung N, Kao JH, Piratvisuth T, Gane E, Han KH, Guan R, Lau GK, Locarnini S; Chronic Hepatitis B Guideline Working Party of the Asian-Pacific Association for the Study of the Liver. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2008 update. *Hepatol Int* 2008; **2**: 263-283 [PMID: 19669255 DOI: 10.1007/s12072-008-9080-3]
50 **Shen T**, Yan XM, Zhang JP, Wang JL, Zuo RX, Li L, Wang LP. Evolution of Hepatitis B Virus in a Chronic HBV-Infected Patient over 2 Years. *Hepat Res Treat* 2011; **2011**: 939148 [PMID: 21785721 DOI: 10.1155/2011/939148]
51 **Shen T**, Yan XM, Zou YL, Gao JM, Dong H. Virologic characteristics of hepatitis B virus in patients infected via maternal-fetal transmission. *World J Gastroenterol* 2008; **14**: 5674-5682 [PMID: 18837083]
52 **Garfein RS**, Bower WA, Loney CM, Hutin YJ, Xia GL, Jawanda J, Groom AV, Nainan OV, Murphy JS, Bell BP. Factors associated with fulminant liver failure during an outbreak among injection drug users with acute hepatitis B. *Hepatol-*

ogy 2004; **40**: 865-873 [PMID: 15382123]
53 **Owiredu WK**, Kramvis A, Kew MC. Molecular analysis of hepatitis B virus genomes isolated from black African patients with fulminant hepatitis B. *J Med Virol* 2001; **65**: 485-492 [PMID: 11596083]
54 **Bowyer SM**, van Staden L, Kew MC, Sim JG. A unique segment of the hepatitis B virus group A genotype identified in isolates from South Africa. *J Gen Virol* 1997; **78** (Pt 7): 1719-1729 [PMID: 9225049]
55 **Chirara MM**, Chetsanga CJ. Variant of hepatitis B virus isolated in Zimbabwe. *J Med Virol* 1994; **42**: 73-78 [PMID: 8308523]
56 **Shen FC**, Su IJ, Wu HC, Hsieh YH, Yao WJ, Young KC, Chang TC, Hsieh HC, Tsai HN, Huang W. A pre-S gene chip to detect pre-S deletions in hepatitis B virus large surface antigen as a predictive marker for hepatoma risk in chronic hepatitis B virus carriers. *J Biomed Sci* 2009; **16**: 84 [PMID: 19751529]
57 **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]
58 **Heo NY**, Lee HC, Park YK, Park JW, Lim YS, Kim KM, Shim JH, Lee YJ. Lack of association between hepatitis B virus pre-S mutations and recurrence after surgical resection in hepatocellular carcinoma. *J Med Virol* 2013; **85**: 589-596 [PMID: 23296476 DOI: 10.1002/jmv.23502]
59 **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]
60 **Qu L**, Kuai X, Liu T, Chen T, Ni Z, Shen X. Pre-S deletion and complex mutations of hepatitis B virus related to young age hepatocellular carcinoma in Qidong, China. *PLoS One* 2013; **8**: e59583 [PMID: 23555717 DOI: 10.1371/journal.pone.0059583]
61 **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]
62 **Mu SC**, Lin YM, Jow GM, Chen BF. Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan. *J Hepatol* 2009; **50**: 264-272 [PMID: 19070923 DOI: 10.1016/j.jhep.2008.09.017]
63 **Veazjalali M**, Norder H, Magnius L, Jazayeri SM, Alavian SM, Mokhtari-Azad T. A new core promoter mutation and premature stop codon in the S gene in HBV strains from Iranian patients with cirrhosis. *J Viral Hepat* 2009; **16**: 259-264 [PMID: 19222745 DOI: 10.1111/j.1365-2893.2009.01069.x]
64 **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]
65 **Mathet VL**, Feld M, Espínola L, Sánchez DO, Ruiz V, Mandó O, Carballal G, Quarleri JF, D'Mello F, Howard CR, Oubiña JR. Hepatitis B virus S gene mutants in a patient with chronic active hepatitis with circulating Anti-HBs antibodies. *J Med Virol* 2003; **69**: 18-26 [PMID: 12436473]
66 **Datta S**, Banerjee A, Chandra PK, Chakraborty S, Basu SK, Chakravarty R. Detection of a premature stop codon in the surface gene of hepatitis B virus from an HBsAg and antiHBc negative blood donor. *J Clin Virol* 2007; **40**: 255-258 [PMID: 17869170]
67 **Panigrahi R**, Biswas A, Datta S, Banerjee A, Chandra PK, Mahapatra PK, Patnaik B, Chakrabarti S, Chakravarty R. Anti-hepatitis B core antigen testing with detection and characterization of occult hepatitis B virus by an in-house nucleic acid testing among blood donors in Behrampur, Ganjam, Orissa in southeastern India: implications for transfusion. *Virol J* 2010; **7**: 204 [PMID: 20799931 DOI: 10.1186/1743-422X-7-204]
68 **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]
69 **Yuen MF**, Tanaka Y, Fong DY, Fung J, Wong DK, Yuen JC, But DY, Chan AO, Wong BC, Mizokami M, Lai CL. Independent risk factors and predictive score for the development of hepatocellular carcinoma in chronic hepatitis B. *J Hepatol* 2009; **50**: 80-88 [PMID: 18977053 DOI: 10.1016/j.jhep.2008.07.023]
70 **Yu H**, Zhu R, Zhu YZ, Chen Q, Zhu HG. Effects of mutations in the X gene of hepatitis B virus on the virus replication. *Acta Virol* 2012; **56**: 101-110 [PMID: 22720699]
71 **Ochwoto M**, Chauhan R, Gopalakrishnan D, Chen CY, Ng'ang'a Z, Okoth F, Kioko H, Kimotho J, Kaiguri P, Kramvis A. Genotyping and molecular characterization of hepatitis B virus in liver disease patients in Kenya. *Infect Genet Evol* 2013; **20**: 103-110 [PMID: 23978387 DOI: 10.1016/j.meegid.2013.08.013]
72 **Barbini L**, Tadey L, Fernandez S, Bouzas B, Campos R. Molecular characterization of hepatitis B virus X gene in chronic hepatitis B patients. *Virol J* 2012; **9**: 131 [PMID: 22769058 DOI: 10.1186/1743-422X-9-131]
73 **Sayed SK**, Kobeisy MA. The relationship between core promoter mutation of hepatitis B virus, viral load and hepatitis B e antigen status in chronic hepatitis B patients. *Cell Immunol* 2012; **276**: 35-41 [PMID: 22551558 DOI: 10.1016/j.cellimm.2012.03.003]
74 **Chui SH**, Chen JH, Szeto YT, Yam WC. Prevalence of hepatitis B genotype and viral basic core promoter and precore mutations among teenagers in Macao: relationship with hepatocellular carcinoma development. *Br J Biomed Sci* 2011; **68**: 143-146 [PMID: 21950207]
75 **Choi CS**, Cho EY, Park R, Kim SJ, Cho JH, Kim HC. X gene mutations in hepatitis B patients with cirrhosis, with and without hepatocellular carcinoma. *J Med Virol* 2009; **81**: 1721-1725 [PMID: 19697408 DOI: 10.1002/jmv.21591]
76 **Khan A**, Al Balwi MA, Tanaka Y, Hajeer A, Sanai FM, Al Abdulkarim I, Al Ayyar L, Badri M, Saudi D, Tamimi W, Mizokami M, Al Knawy B. Novel point mutations and mutational complexes in the enhancer II, core promoter and precore regions of hepatitis B virus genotype D1 associated with hepatocellular carcinoma in Saudi Arabia. *Int J Cancer* 2013; **133**: 2864-2871 [PMID: 23740667 DOI: 10.1002/ijc.28307]
77 **Kitab B**, Essaid El Feydi A, Afifi R, Trepo C, Benazzouz M, Essamri W, Zoulim F, Chemin I, Alj HS, Ezzikouri S, Benjelloun S. Variability in the precore and core promoter regions of HBV strains in Morocco: characterization and impact on liver disease progression. *PLoS One* 2012; **7**: e42891 [PMID: 22905181 DOI: 10.1371/journal.pone.0042891]
78 **Qu LS**, Liu TT, Jin F, Guo YM, Chen TY, Ni ZP, Shen XZ. Combined pre-S deletion and core promoter mutations related to hepatocellular carcinoma: A nested case-control study in China. *Hepatol Res* 2011; **41**: 54-63 [PMID: 20973883 DOI: 10.1111/j.1872-034X.2010.00732.x]
79 **Park YM**, Jang JW, Yoo SH, Kim SH, Oh IM, Park SJ, Jang YS, Lee SJ. Combinations of eight key mutations in the X/preC region and genomic activity of hepatitis B virus are associated with hepatocellular carcinoma. *J Viral Hepat* 2014; **21**: 171-177 [PMID: 24344773 DOI: 10.1111/jvh.12134]
80 **Malik A**, Singhal DK, Albanyan A, Husain SA, Kar P. Hepatitis B virus gene mutations in liver diseases: a report from

New Delhi. *PLoS One* 2012; **7**: e39028 [PMID: 22720023 DOI: 10.1371/journal.pone.0039028]
81 **Kang HS**, Kang KS, Song BC. Precore and core promoter mutations of the hepatitis B virus gene in chronic genotype C-infected children. *J Korean Med Sci* 2011; **26**: 546-550 [PMID: 21468263 DOI: 10.3346/jkms.2011.26.4.546]
82 **Okamoto H**, Tsuda F, Mayumi M. Defective mutants of hepatitis B virus in the circulation of symptom-free carriers. *Jpn J Exp Med* 1987; **57**: 217-221 [PMID: 3430799]
83 **Melegari M**, Scaglioni PP, Wands JR. The small envelope protein is required for secretion of a naturally occurring hepatitis B virus mutant with pre-S1 deleted. *J Virol* 1997; **71**: 5449-5454 [PMID: 9188617]
84 **Hosono S**, Tai PC, Wang W, Ambrose M, Hwang DG, Yuan TT, Peng BH, Yang CS, Lee CS, Shih C. Core antigen mutations of human hepatitis B virus in hepatomas accumulate in MHC class II-restricted T cell epitopes. *Virology* 1995; **212**: 151-162 [PMID: 7545853]
85 **Günther S**, Li BC, Miska S, Krüger DH, Meisel H, Will H. A novel method for efficient amplification of whole hepatitis B virus genomes permits rapid functional analysis and reveals deletion mutants in immunosuppressed patients. *J Virol* 1995; **69**: 5437-5444 [PMID: 7636989]
86 **Thimme R**, Chang KM, Pemberton J, Sette A, Chisari FV. Degenerate immunogenicity of an HLA-A2-restricted hepatitis B virus nucleocapsid cytotoxic T-lymphocyte epitope that is also presented by HLA-B51. *J Virol* 2001; **75**: 3984-3987 [PMID: 11264388]
87 **Ferrari C**, Penna A, Giuberti T, Tong MJ, Ribera E, Fiaccadori F, Chisari FV. Intrahepatic, nucleocapsid antigen-specific T cells in chronic active hepatitis B. *J Immunol* 1987; **139**: 2050-2058 [PMID: 2957446]
88 **Milich DR**, McLachlan A. The nucleocapsid of hepatitis B virus is both a T-cell-independent and a T-cell-dependent antigen. *Science* 1986; **234**: 1398-1401 [PMID: 3491425]
89 **Hosaka T**, Suzuki F, Kobayashi M, Hirakawa M, Kawamura Y, Yastuji H, Sezaki H, Akuta N, Suzuki Y, Saitoh S, Arase Y, Ikeda K, Miyakawa Y, Kumada H. Development of HCC in patients receiving adefovir dipivoxil for lamivudine-resistant hepatitis B virus mutants. *Hepatol Res* 2010; **40**: 145-152 [PMID: 19788684 DOI: 10.1111/j.1872-034X.2009.00582.xHEP582]
90 **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]
91 **Li D**, Gu HX, Zhang SY, Zhong ZH, Zhuang M, Hattori T. YMDD mutations and genotypes of hepatitis B virus in northern China. *Jpn J Infect Dis* 2006; **59**: 42-45 [PMID: 16495633]
92 **Ou Z**, Zhang Y, Zhang R, He Y, He X. Relationship between Mutation of HBV YMDD Motif and Pathogenesis of Hepatocellular Carcinoma. *Redai Yixve Zazhi* 2008; **6**: 525-528 [DOI: 10.3969/j.issn.1672-3619.2008.06.002]
93 **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]
94 **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]
95 **Szmaragd C**, Foster GR, Manica A, Bartholomeusz A, Nichols RA, Balloux F. Genome-wide characterisation of hepatitis B mutations involved in clinical outcome. *Heredity* (Edinb) 2006; **97**: 389-397 [PMID: 16896341]

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