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Natural history of acute and chronic hepatitis B: The role of HBV genotypes and mutants

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A B S T R A C T

Molecular epidemiologic studies reveal remarkable differences in the geographical distribution of hepatitis B virus (HBV) genotypes. The frequency of mutants among HBV genotypes also varies. The role of HBV genotypes/mutants in the pathogenesis of HBV infection and natural history of HBV infection has been extensively investigated. The distribution of HBV genotypes in acute hepatitis B patients reflects the predominant genotypes in a given geographic area. In chronic hepatitis B patients, genotype C and D have a higher frequency of basal core promoter A1762T/G1764A mutations than genotype A and B. HBV genotypes C, D and F carry a higher lifetime risk of cirrhosis and HCC development than genotype A and B. HBV pre-S/S gene mutations were associated with immune escape of hepatitis B immunoglobulin or vaccine-induced immunity. Mutations in the pre-S, core promoter and X regions correlate with an increased risk of cirrhosis and HCC. In summary, HBV genotypes and mutants are associated with the disease progression and long-term outcome of HBV infection. They may serve as viral genetic markers for risk stratification of chronic hepatitis B patients in clinical practice.

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Introduction

HBV infection is endemic in Asia and the Pacific islands, Africa, Southern Europe and Latin America [1]. Most patients with chronic HBV infection acquire the virus early in life [2]. On the basis of immune interactions between virus and host, HBV infection has diverse clinical manifestations, including acute hepatitis, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) [1]. HBV, a genome of 3,200 base pairs [3], is transcribed through reverse transcription in the cell nucleus from a covalently closed circular DNA (cccDNA) template, then fully double-stranded DNA through DNA polymerase within the nucleocapsid. DNA-containing nucleocapsids can be either recirculated into the nucleus to form additional cccDNA or can be enveloped for secretion [4].

Because the absence of proofreading activity for the spontaneous error of viral reverse transcriptase, nucleotide mutations of HBV genome, the long-term evolution of HBV leads to the occurrence of various genotypes, subtypes, mutants, recombinants, and even quasispecies [3,5–7].

HBV genotypes have distinct geographical distributions, and ample evidence reveals that HBV genotype is associated with HBV endemicity, transmission modes, as well as clinical outcomes [6–10]. In addition, several naturally occurring HBV mutants, including precore, core promoter mutations and pre-S/S deletion mutations, bear clinical and epidemiological implications [10–12]. In this article, recent advances regarding the impact of HBV genotypes and mutants on the natural history of acute and chronic HBV infection will be reviewed and discussed.

Molecular epidemiology and global distribution of HBV genotypes

According to the genetic divergence of the entire HBV genomic sequences, at least 10 HBV genotypes (A to J) and several subtypes have been identified [6–10]. The major genotypes vary in geographical distributions. Genotype A is highly prevalent in Africa,

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Europe, India and America. Genotypes B and C are the most common in Asia–Pacific region. Genotype D is prevalent in Africa, Europe, the Mediterranean region and India. Genotype E to L seem to be more restricted geographically. Genotype E is restricted to West and Central Africa and Saudi Arabia. Genotype F is found in Central and South America. Genotype G has been reported in France, Germany and the Americas. Genotype H is found in Central America, Mexico. Genotype I is isolated in Vietnam and Laos. Genotype J is identified in Japan. In addition, more than 30 subtypes have been determined to date. The geographical distribution of HBV genotypes and subtypes are shown in Table 1.

Because of the quasispecies nature of HBV [13], mixed genotypes infection and intergenotypic recombination are not uncommon and of great virological and clinical interest [14]. Previous large cohort studies in Taiwan showed the prevalence of mixed HBV genotype infections was 16.3% and 34.4% in HBsAg-positive and intravenous drug users with occult HBV infection, respectively [15,16]. Intergenotypic recombination of HBV strains has been widely reported. Genotypes A and C have a higher recombination tendency than other genotypes do [17]. HBV genotype B2 is a recombination of HBV genotype B and C [18]. HBV genotype C/D recombinant identified in Southwest China has a higher prevalence in Tibetan patients than in other populations [19–21]. HBV genotype I is a novel intergenotypic recombination of genotypes A, C, and G [22,23]. Intergenotypic recombination may be an important strategy for HBV evolution. Further studies are required to clarify the clinical significance of these HBV recombinants.

Three modes of HBV transmission, including perinatal, sexual and blood transmission, have long been recognized [24]. The specific global distribution of HBV genotypes is associated with different transmission modes [1]. For example, genotype B and C are prevalent in highly endemic areas, such as Asian countries, where perinatal or mother-to-infant transmission plays an important role in spreading HBV. Whereas the remaining genotypes are frequently found in areas where sexual and blood transmissions between adults are the most common route of HBV infection. Thus, distinct modes of HBV transmission may be associated with a specific geographical distribution of the HBV genotypes. In Taiwan, the prevalence of HBsAg carriage in children from families clustering with HBV carriers was significantly higher than that in the general population (77.8% vs. 15%). Possible intrafamilial modes of transmission were determined by identifying the concordant HBV genotype between carrier children and their parents [25]. The modes of transmission may also influence the distribution of HBV in a given country where universal hepatitis B vaccination has not yet been launched. In a nation-wide survey before the implementation of universal hepatitis B vaccination, Matsuura et al. found that the prevalence of HBV genotype A in chronic hepatitis B patients in Japan increased from 1.7% in 2000 to 3.5 % in 2006 [26]. Therefore, HBV genotyping can serve as an epidemiologic marker to determine the correlation of HBV genotype distribution with modes of transmission, implying an important social behavioral change.

Table 1
Geographic distribution of hepatitis B virus genotypes and subtypes.

Genotypes	Subtypes	Geographic location
A	A1	Sub-Saharan Africa, India
	A2	Northern Europe, India
	A3	Western Africa
	A4–A7	Gambia, Nigeria
B	B1	Japan
	B2–B5	East Asia, Taiwan, China, Indonesia, Vietnam, Philippines
	B6	Alaska, Northern Canada, Greenland
C	C1-C3	Taiwan, China, Korea, Japan and Southeast Asia.
	C4	Australia
	C5	Philippines, Vietnam
	C6-C11	Indonesia
D	D1–D7	Africa, Europe, Mediterranean countries, India, Indonesia, Australia
E		West and central African, Saudi Arabia
F	F1–F4	Central and South America
G		France, Germany and the United States
H		Central America
I		Vietnam and Laos
J		Japan

HBV genotypes/mutants and pathogenesis of HBV infection

HBV genotypes and naturally occurring HBV mutants may play a critical role in viral pathogenesis, including the change of host immune recognition, the enhanced virulence with increased HBV replication, the facilitation of cell attachment or penetration and the association with hepatocarcinogenesis (Fig. 1) [10,12]. HBV precore nucleotide 1896 stop codon mutation and basal core promoter A1762T/G1764A mutations are responsible for HBV replication in the absence of HBeAg [27]. Persistent viremia leads to prolongation of chronic hepatic inflammation [28,29]. In an in vitro study, intracellular expression of HBV DNA was higher for genotypes C than B and genotypes D than A [30]. Our previous in vivo study revealed that the secretion of HBeAg in genotype B was lower than in genotype C. Intracellular core protein expression was increased when was introduced in the genotype C with basal core promoter A1762T/G1764A mutations [31]. HBV basal core promoter A1762T/G1764A mutations were also significantly associated with cytoplasmic localization of intracellular HBcAg, which elicited host immune response [32]. In our cohort study, genotype C patients had a higher prevalence of basal core promoter A1762T/G1764A mutations than genotype B patients [33]. Similarly, genotype D patients had a higher prevalence of basal core promoter A1762T/G1764A mutations than those with genotype A patients [34]. Therefore, basal core promoter mutations may contribute to HBV genotypes-specific immunopathogenesis.

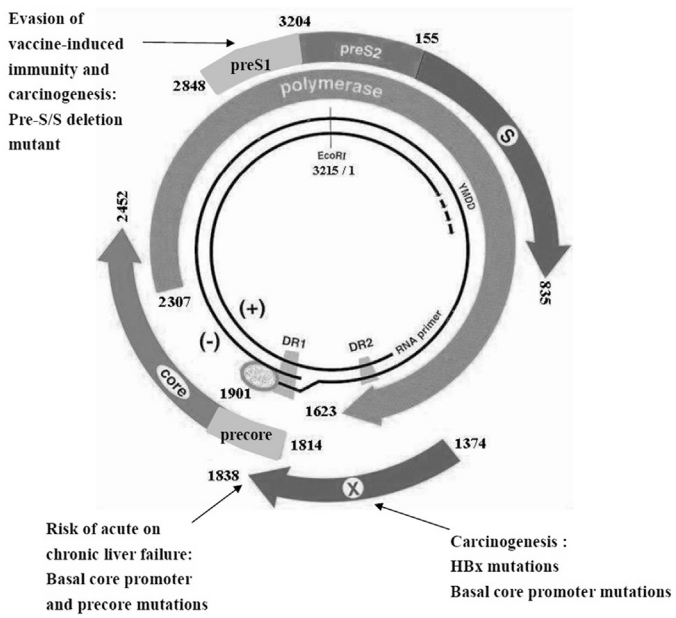

Fig. 1. Naturally occurring hepatitis B viral mutations in precore, core promoter, pre-S, S and X regions have been reported to be associated with the pathogenesis of progressive liver disease. Adapted from Kao JH. Ref. [12].

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Clinical impact of HBV genotypes and mutants

HBV genotypes/mutants and hepatitis B vaccine failure

Although the prevalence of HBsAg in children in HBV endemic regions decreases dramatically after the implementation of universal hepatitis B vaccination [35,36], breakthrough infections by HBV mutants have been reported in children with complete immunoprophylaxis [37–39]. A previous report from Gambia showed that breakthrough HBV infection in vaccinated children was mainly caused by the wild-type genotype E strain [37]. On the contrary, in HBV genotype B/C prevalent region, S gene mutations were prominent among vaccinated children with breakthrough HBV infection [38]. In addition, HBV infection with mutations in the S gene in vaccinated individuals is increased after adolescence [39]. The relationship between HBV genotypes/mutants and vaccine escape remains largely unknown and better preventive strategies are required in the future.

HBV genotypes and acute hepatitis B

On the contrary to chronic hepatitis B, there were only few reports available regarding the relation of HBV genotypes and acute hepatitis B. Our previous report showed that genotype B was predominant in Taiwanese patients with acute hepatitis B [40]. The distribution of the HBV genotypes in acute hepatitis B patients is similar to that in HBsAg-positive blood donors [41] and chronic hepatitis B patients in Taiwan [42]. Through promiscuous sexual contacts, HBV genotype A is prevalent in patients with acute hepatitis B in Japan [43], and the percentage increased gradually in recent years [44]. Regarding clinical severity, a higher peak level of HBV DNA and a lower peak of alanine aminotransferase levels were characteristics in acute hepatitis B patients with genotype A infection [45].

HBV genotype D was the dominant genotype in acute hepatitis B in India, where sexual promiscuity emerged as the predominant mode of HBV infection [46]. A retrospective study from Argentina revealed that 65.2% and 4.2% of acute hepatitis B were caused by genotype HBV F and D, respectively [47].

Mixed HBV genotype infections are not common in acute infection. Our previous study reported that genotype B took over genotype C as the predominant strain in the course of an acute hepatitis B patient with genotypes B and C coinfection, and a transient recombination within the pre-S region was observed [48]. Recent study from China also found that the prevalence of intergenotypic recombinant of genotypes B/C and C/D was significantly increased in acute hepatitis B patients. Interestingly, serum HBV DNA levels were significantly lower in patients with intergenotypic recombinants than those without intergenotypic recombinants [49]. Collectively, the distribution of HBV genotypes in acute hepatitis B patients is associated with the mode of transmission and may reflect the predominance of certain genotypes in a given geographic area.

HBV genotypes and tendency of chronicity after acute HBV infection

The relationship of HBV genotypes and the tendency of chronicity of HBV infection has been partially elucidated. In a nationwide multicenter study of 212 patients with acute hepatitis B in Japan, the persistence of HBsAg positivity for more than 6 months after acute hepatitis B was higher in patients with genotype A infection (23.4%) than those with non-A genotype (8.6%) infection ($P = 0.003$). Multivariate logistic regression analysis revealed that genotype A was independently associated with viral persistence following acute hepatitis B [45]. In another study from India, HBV genotypes were compared between patients with acute and chronic HBV infections. The percentage of genotype C was significantly high in chronic hepatitis B patients as compared to acute hepatitis B. However, there was no significant difference in the frequency of genotype A in acute and chronic patients [46]. Taken together, the persistence of HBV infection after acute infection may be attributable to the variable strength of host–viral interactions, the modes of transmission as well as the varying distribution of genotypes.

HBV genotypes/mutants and the milestones of chronic hepatitis B

The milestones in the natural history of chronic HBV infection include HBeAg seroconversion and HBsAg seroclearance. The impact HBV genotype on these milestones and long-term adverse outcomes of HBV infection has been explored.

HBeAg seroconversion and HBsAg seroclearance

In the natural history of chronic HBV infection, persistent HBeAg seropositivity with hepatitis activity may accelerate the progression of chronic hepatitis leading to cirrhosis [50]. In addition, among HBeAg seroconverters, persistent HBsAg positivity and detectable HBV DNA is still associated with risk of HCC after HBeAg seroconversion [51]. Therefore, seroconversion of HBeAg and seroclearance of HBsAg are important events which reflect the host immuno-control of HBV infection. In our cohort study on 272 Taiwanese patients with chronic HBV infection, genotype C patients have prolong HBeAg-positive period despite multiple hepatitis flares than do those with genotype B infection [52,53]. Genotype C patients had significantly lower rates of spontaneous HBeAg seroconversion than genotype B patients. The estimated annual rates of HBeAg seroconversion in genotype B and C infections were 15.5% and 7.9%, respectively [54]. Accordingly, genotype C patients endure delayed HBeAg seroconversion and thus have a longer duration of high viral load than genotype B patients. With long-term immunologic response, genotype C patients are correspondingly more prone to develop advanced fibrosis, cirrhosis and HCC than genotype B patients. However, the intensity of host immune responses during HBeAg positivity may lead to HBsAg clearance after HBeAg seroconversion. Our recent study in 2121 HBeAg-negative patients revealed that genotype C patients have higher lifetime chance of HBsAg loss than genotype B patients, with hazard ratio of 1.8 (95% confidence interval: 1.4–2.4) [55]. Genotype C infection seems to be associated with both disease progression and remission.

The possible influence of other genotypes or mutants on HBeAg/HBsAg seroconversion remains limited. One study of Spanish patients with chronic HBV infection did not reveal any differences in the probability of HBeAg seroconversion between patients infected with genotype A and D. However, the rate of HBsAg clearance was higher in genotype A than in genotype D [56]. In West and Central Africa, where genotype E is predominant, the annual rate of HBeAg and HBsAg seroclearance were 7.4% and 1.0%, respectively [57]. Our recent study in HBsAg-negative patients with detectable HBV DNA reported that one viral mutation (preS1T68I), which decreased S promoter activity, was identified to be associated with the seroclearance of HBsAg in patients with occult HBV infection [58]. Collectively, further study is warranted to clarify the certain genotypes and mutants of HBV and host interactions in the pathogenesis of HBV infection.

Risk of cirrhosis and HCC

The risk factors associated with the development of HCC include chronic infection with either HBV or HCV, the presence of cirrhosis,

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carcinogen exposure especially aflatoxin, cigarette smoking, alcohol abuse, obesity, and male gender. Among these risk factors, chronic hepatitis viral infections, particularly those with cirrhosis, have the strongest association with the development of HCC in Asian countries. Recently, several HBV viral factors, including viral load, HBsAg level, HBV genotype, HBV genome mutations have been reported to be associated with different risks of liver disease progression (Fig. 2). HBV genotype-specific virological characteristics are significantly associated with progression of cirrhosis and HCC. In a prospective study with 4841 Taiwanese male HBV-infected patients without HCC at enrollment, Yu et al. found that HBV viral load was higher in genotype C than genotype B patients, while genotype C-infected patients who also had very high viral load had a 26-fold higher risk of HCC than those with genotype B and low viral load [59]. In HCC patients, Wu et al. also reported that liver inflammation activity was higher in HBV genotype C patients than in genotype B patients, and more genotype C patients tended to have a high viral load than genotype B patients [60]. Both community-based and hospital-based prospective cohort study demonstrated that HBV genotype C was associated with an increased risk of HCC than genotype B [61,62]. Of interesting, several reports showed HBV genotype B, especially subtype B2, is associated with risk of early-onset HCC (less than 30 years old) [42,53,63,64]. Another prospective study from Hong Kong showed that subtypes C1/C2 have a higher risk of HCC compared to genotype B [65]. In addition, subtype C4, was associated with more rapid liver disease progression and risk of HCC [66]. Genotype B2 (recombination of genotype B and C) and C4 (recombination of genotype C and J) have been shown to be recombinants with other genotypes which may play an important role in pathogenesis [66,67].

The relationship between other HBV genotypes and the risk of HCC remains unclear. Earlier study demonstrated that HCC was more frequent in patients with HBV genotype D infection than those with genotype A infection [68]. Later studies from Alaska revealed that HCC incidence was significantly higher for patients with genotype F1 infection compared with genotype A2 and D [69,70]. A retrospective study from Argentina revealed that genotype F tended to display more severe histological activity than genotype A and D [47]. HBV genotype H was predominant in patients with occult HBV infection in Mexico, a low endemic area of HBV infection [71]. The low incidence of cirrhosis and HCC in Mexico may be reasoned by the virulence of genotype H is different from other HBV genotypes in high endemic areas [72].

Of the various naturally occurring HBV mutants, available evidence supports a critical role of HBV X gene mutations in the carcinogenesis of HBV-related HCC, irrespective of genotype or geographical distribution [73–78]. Previous studies indicated that 3′-end X gene deletion is frequently found and leads to a C-terminal truncated HBx protein [79,80]. C-terminal truncated HBx protein occurs in nearly 80% of HCC tissues, and may contribute to hepatocarcinogenesis via loss of proapoptotic ability of full gene and activation of cell transformation and tumor promotion [76].

Basal core promoter A1762T/G1764A mutations are consistently shown to be associated with an increased risk of liver disease progression and HCC development in several cohort and case-control studies [33,81–84]. A large-scale long-term follow-up study of Taiwanese patients and a meta-analysis further confirmed that basal core promoter A1762T/G1764A mutations is an independent risk factor of HCC development [61,85]. The increased proportion of basal core promoter mutant strain was reported to correlate with cirrhosis in genotype B or C HBV carriers. Quantitative analysis using pyrosequencing revealed that risk of cirrhosis was higher in patients with basal core promoter A1762T/G1764A mutations $\geq$45% compared to <45% (OR: 2.81; 95% CI: 1.40 to 5.67; P = 0.004) [86].

Mutations in enhancer II (C1653T) and in the basal core promoter (T1753V) have also been found to be associated with HCC development [86–91]. The combined effect of multiple mutations in the X/precore regions have also been associated with HCC. In Korean with HBV genotype C2 infection, eight key mutations comprise G1613A, C1653T, T1753V, A1762T, G1764A, A1846T, G1896A and G1899A that are significantly associated with HCC [92,93].

Through increasing active HBV replication in the absence of HBeAg, mutations in basal core promoter and precore region may associated with acute on chronic liver failure. Recent meta-analyses revealed that alone or combination of T1753V, A1762T, G1764A, C1766T, T1768A, A1846T, G1896A and G1899A mutations are correlated with an increased risk of acute on chronic liver failure [94].

Several studies have indicated that the pre-S gene deletion mutations were significantly associated with the development of cirrhosis and HCC [95–98]. In our case-control study, the presence of pre-S deletion was an independent risk factor associated with HCC development. The frequency of pre-S deletion was significantly higher in genotype C patients than genotype B patients [96]. The mapping study of pre-S region suggested that all the deletion

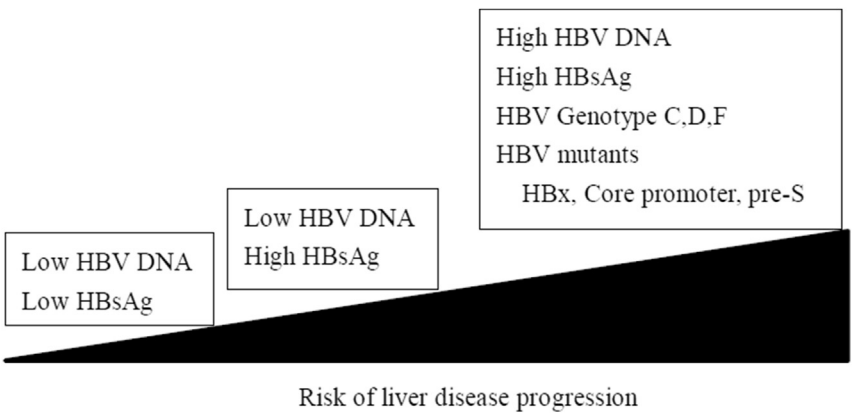

Fig. 2. Risk of liver cirrhosis and hepatocellular carcinoma for chronic hepatitis B patients stratify by hepatitis B viral factors.

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regions encompassed T- and B-cell epitopes, including the polymerized human serum albumin-binding site and nucleocapsid-binding site. These deletion mutations may lead to defective immunity against HBV and contribute directly to hepatocarcinogenesis [84]. Recently, pre-S2 mutant large surface protein is recognized as an oncoprotein. The accumulation of pre-S2 mutant protein in hepatocyte induced endoplasmic reticulum stress and cause oxidative DNA damage [99]. Subsequent investigation has demonstrated that pre-S2 mutant large surface protein inhibits hepatocyte DNA double-strand break repair and leads to genomic instability. Thus, pre-S2 mutant large surface protein may be the major viral oncoprotein in HBV-infected hepatocytes, leading to HCC tumorigenesis [100,101].

Conclusions and perspectives

Accumulating lines of evidence have clarified the clinical implications of HBV genotypes and mutants over the past decade. In summary, tendency of chronicity is higher in genotype A patients. Genotype C, D and F patients have a higher risk of cirrhosis and HCC than other genotypes, leading to a poorer clinical outcome. Mutations in core promoter and the pre-S regions are also associated with an increased risk of HCC. The emergence of pre-S/S gene mutations is a challenge to the success immunity of universal hepatitis B vaccination. HBV genotypes and mutants could be responsible to clinical outcomes, and have potential to be useful viral biomarkers to predict disease progression. In the foreseeable future, identification HBV genotypes and monitor mutants in certain regions of HBV genome are recommended to implement individualized management for patients with HBV infection.

Practice points

- Ten HBV genotypes (A to J) with geographical distributions have been identified.
- HBV genotypes C, D and F carry a higher lifetime risk of cirrhosis and HCC than other genotypes.
- HBV pre-S/S gene mutations are associated with evasion of vaccine-induced immunity.
- Mutations in the pre-S, core promoter and X regions correlate with an increased risk of cirrhosis and HCC.

Research agenda

- The relationship between HBV genotypes/mutants and vaccine escape remains largely unknown and better preventive strategies are required in the future.
- The certain genotypes of HBV and host interactions in the pathogenesis of HBV infection need to further clarify.
- Intergenotypic recombination may play an important role in HBV pathogenesis.

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Conflict of interest

All authors have no conflict of interest.

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