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Hepatitis B Virus Genotypes and Variants

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At least 10 hepatitis B virus (HBV) genotypes (A to J) with distinct geographic distributions and several HBV mutants, including precore/core promoter mutations and pre-S/S deletion mutations, have been recognized to be not only predictive of liver disease progression but also associated with response to antiviral therapy. HBV genotype–specific pathogenesis may contribute to heterogeneous clinical outcomes in chronic hepatitis B patients across the world. For example, patients with HBV genotypes C and D infection have a lower rate of spontaneous HBeAg seroconversion. In addition, HBV genotypes C and D have a higher frequency of core promoter and pre-S mutations than genotypes A and B. Genotypes C and D also carry a higher lifetime risk of cirrhosis and HCC development than genotypes A and B. Core promoter and pre-S mutations also correlate with an increased risk of hepatocellular carcinoma (HCC). Therapeutically, genotypes A and B patients have a better response to interferon-based therapy than genotypes C and D patients, but the response to nucleos(t)ide analogs is comparable across different HBV genotypes. In conclusion, HBV genotypes and variants may serve as viral genetic markers to predict disease progression as well as help practicing physicians optimize individualized antiviral therapy in clinical practice.

Hepatitis B virus (HBV) is one of the most common viral infections in humans, which is endemic in Asia, Pacific Islands, Africa, Southern Europe and Latin America. The prevalence of chronic HBV infection in the general population of different countries ranges from 2% to 20% (Kao and Chen 2002). Persistent HBV infection has a wide spectrum of clinical manifestations, including inactive carrier state, chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (Kao and Chen 2002). Eventually, 15%–40% of HBV carriers have a lifetime risk to develop cirrhosis, liver failure, or HCC (Fattovich et al. 2008).

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HBV is the smallest human DNA virus with a genome of 3200 bp (Lau and Wright 1993). Through reverse transcription, pregenomic RNA can be transcribed from covalently closed circular DNA (cccDNA) to serve as the template of negative-strand DNA and then fully double-stranded DNA through DNA polymerase within the nucleocapsid, finally with the assembly of envelope protein to form mature HBV virions (Beck and Nassal 2007). Because of the spontaneous error rate of viral reverse transcriptase, HBV genome evolves with an estimated rate of nucleotide substitution at $1.4–3.2 \times 10^{-5}$/sites/yr (Lau and Wright 1993). This unique replication strategy leads to the occurrence of various genotypes, subtypes, mutants, recombinants, and even quasispecies in the context of the long-term evolution of HBV (Hunt et al. 2000; Kao 2002, 2003; Kao and Chen 2006).

On the basis of recent advances in molecular biology techniques, genotypic classifications of HBV and their geographic/ethnic distributions have been increasingly recognized. Ample evidence reveals that HBV genotype is associated with HBV endemicity, transmission mode, as well as clinical outcomes (Kao 2002, 2003; Kao and Chen 2006; Lin and Kao 2011, 2013c). In addition, naturally occurring variations in the HBV genome also have clinical and epidemiological implications. Several HBV mutants, including precore/core promoter mutations and pre-S/S deletion mutations, have been reported to be associated with progressive liver disease and risk of HCC development (Fig. 1) (Chotiyaputta and Lok 2009; Kao et al. 2010). In this article, recent advances regarding the impact of HBV genotypes and variants on the disease progression, risk of HCC development, and

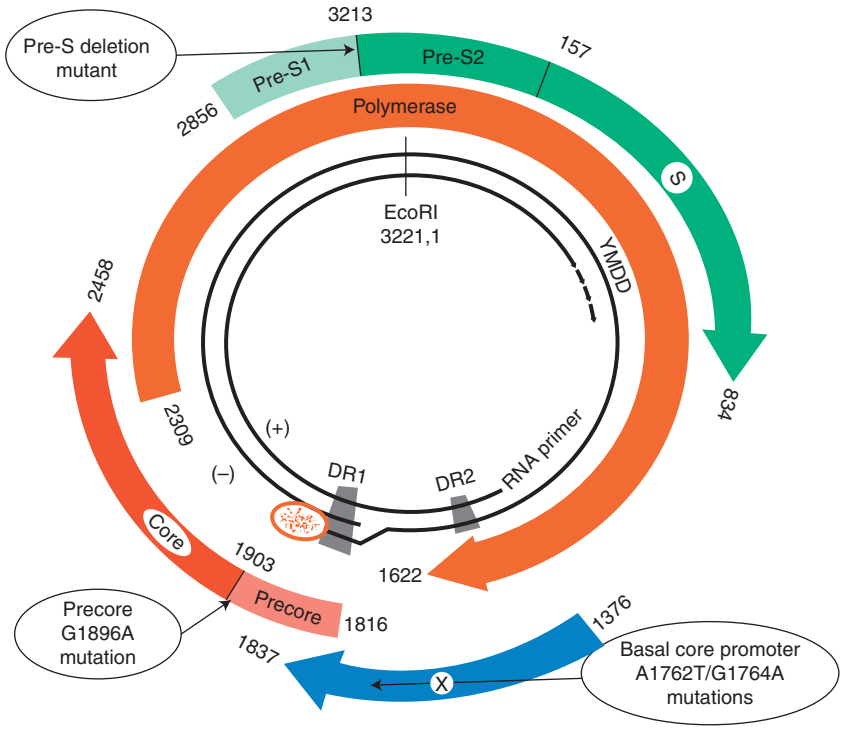

Figure 1. The partially double-stranded circular DNA of hepatitis B virus encodes four overlapping open reading frames: S for the surface or envelope gene; C for the core gene; P for the polymerase gene; and X for the X gene. Naturally occurring mutant strains including mutations in precore, core promoter, and deletion mutation in pre-S genes have been reported to be associated with the pathogenesis of progressive liver disease and risk of hepatocellular carcinoma (HCC) development. (From Kao et al. 2010; adapted, with permission, Elsevier © 2010.)

responses to antiviral treatments in chronic hepatitis B (CHB) patients will be reviewed and discussed.

GLOBAL DISTRIBUTION OF HBV GENOTYPES AND SUBTYPES

The hepatitis B surface antigen (HBsAg) derived from different HBV strains carries serologically defined group-specific antigenic determinants. Based on different serological reactivities of HBsAg, HBV can be classified into four serotypes, adw, adr, ayw, and ayr (Le Bouvier et al. 1972; Tiollais et al.1985), with specific geographical distributions (Nishioka et al. 1975; Sung and Chen 1977). In addition, according to the homogeneity of the virus sequence, >8% or 4–8% genetic divergence, at least 10 HBV genotypes (A to J) and several subtypes have been identified, also with specific geographical distributions (Datta 2008; Cao 2009; McMahon 2009; Kurbanov et al. 2010). For example, genotype A is highly prevalent in Africa, Europe, India, and America. Genotypes B and C are common in the Asia-Pacific region. Genotype D is prevalent in Africa, Europe, the Mediterranean region, and India. Genotype E is restricted to West Africa. 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 and Mexico. Genotype I was isolated in Vietnam and Laos (Tran et al. 2008; Phung et al. 2010). The newest HBV genotype, J, was identified in Japan (Tatematsu et al. 2009). The correlation and geographical distributions of HBV serotypes, genotypes, and subtypes are shown in Table 1. A molecular epidemiologic study showed that, in Taiwan, adw and adr account for 70% and 30% of HBV strains, respectively. All adr strains are genotype C, whereas 81% and 12% of the adw strains are genotypes B and C, respectively (Liu et al. 2002). Another study from Korea revealed extraordinary predominance of HBV genotype C2 in CHB patients; 92.3% and 5.7% were adr and adw serotypes, respectively (Kim et al. 2007). Similar to the specific global distribution of HBV genotypes, different transmission modes of HBV are shown (Kao and Chen 2002). For example, genotypes 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

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

Genotypes	Serotypes	Subtypes	Geographic location
A	adw	A1	Sub-Saharan Africa and India
		A2	Northern Europe and India
		A3	Western Africa
B	adw, ayw	B1	Japan
		B2–5	East Asia, Taiwan, China, Indonesia, Vietnam, and the Philippines
		B6	Alaska, Northern Canada, and Greenland
C	adw, ayr, adr	C1–3	Taiwan, China, Korea, and Southeast Asia
		C4	Australia
		C5	The Philippines and Vietnam
		C6–11	Indonesia
D	ayw	D1–6	Africa, Europe, Mediterranean countries, India, and Indonesia
E	ayw		Restricted to West Africa
F	adw	F1–4	Central and South America
G	adw		France, Germany, and the United States
H	adw		Central America
I	adw		Vietnam and Laos
J			Japan

found in areas in which horizontal transmission (close personal contact between young children, blood, or sexual interactions between adults) is the most common route of HBV infection. Thus, distinct modes of HBV transmission have been associated with a specific geographical distribution of the HBV genotypes. In Taiwan, HBV genotyping has been applied to investigate the modes of intrafamilial HBV transmission (Lin et al. 2005); 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 (Lin et al. 2005). The modes of transmission may also influence the distribution of HBV in a given country in which universal hepatitis B vaccination has not yet been launched. For example, through promiscuous sexual contacts, HBV genotype A is prevalent in patients with acute hepatitis B in Japan (Yotsuyanagi et al. 2005). In a nationwide survey, Matsuura et al. further found that the prevalence of HBV genotype A in chronic hepatitis B patients in Japan increased from 1.7% in year 2000 to 3.5% in 2006 (Matsuura et al. 2009). Therefore, HBV genotyping can serve as an epidemiologic marker to determine the correlation of HBV genotype distribution with modes of transmission, linked to important social behavioral changes.

Intergenotypic Recombination

The quasi-species nature of HBV infection has been documented (Ngui and Teo 1997). In addition, high prevalence of more than one dominant genotype in a certain region is common, especially for genotypes B and C in Asian-Pacific regions, or genotypes A and D in Western countries. Therefore, mixed infection with different HBV genotypes is not uncommon and of great virological and clinical interest. For example, previous large cohort studies in Taiwan showed that the prevalence of mixed HBV genotype infections was 16.3% and 34.4%, respectively, in HBsAg-positive and occult HBV–infected intravenous drug users (Chen et al. 2004a; Lin et al. 2007a). Coinfection with different HBV genotypes further leads to recombination of different viral strains (Locarnini et al. 2013). Intergenotypic recombination of HBV strains has been widely reported. Most of the recombinants were genotypes B/C or A/D hybrids. Genotypes A and C showed a higher recombination tendency than did other genotypes (Yang et al. 2006). For example, HBV genotype B2 is a recombinant, in which the majority of the genetic framework is HBV genotype B, and the precore/core region is from genotype C (Sugauchi et al. 2002). HBV genotypes C/D recombinant is dominant in the Qinghai-Tibet plateau in Southwest China (Wang et al. 2005; Zhou et al. 2011; Shen et al. 2014). HBV genotype I, a novel intergenotypic recombination among genotypes A, C, and G was isolated in Vietnam and Laos (Tran et al. 2008; Phung et al. 2010). The newest HBV genotype, J, was identified in the Ryukyu Islands in Japan, and this genotype has a close relationship with gibbon genotypes and human genotype C (Tatematsu et al. 2009). Recombination is important for HBV evolution. Further studies are required to clarify the mechanisms of intergenotypic recombination and the clinical significance of these HBV recombinants.

THE IMPACT OF HBV GENOTYPES AND VARIANTS ON THE NATURAL COURSE OF CHB

As a noncytopathic virus, the immunopathogenesis of HBV infection is mainly mediated by cellular responses to epitopes of HBV proteins expressed on the surface of hepatocytes with consequent liver injury (Chisari and Ferrari 1995; Ganem and Prince 2004; Rehermann 2013). Persistent HBV replication may trigger strong and continued immune responses against the virus and result in severe liver damage (Perrillo 2001). The relationship of HBV genotypes and the tendency of chronic infection has been elucidated. Ito et al. reported 212 adult patients with acute hepatitis B recruited from 2005 to 2010 in Japan. The persistence of HBV infection was higher in patients with genotype A infection (23.4%) than those with non-A genotype (8.6%) infection ($P = 0.003$). In addition, mul-

tivariate logistic regression analysis revealed that only genotype A was independently associated with viral persistence following acute hepatitis B (Ito et al. 2014). These data suggest that infection with genotype A prevails in patients with acute hepatitis B in Japan where genotypes B and C are common, and tends to persist. In a European study of 65 patients, genotype D was more prevalent in patients with acute, self-limited hepatitis B as compared with genotype A (80% vs. 10%, $P < 0.01$). In contrast, genotype A predominated over genotype D in patients with chronic HBV infection (80% vs. 11%, $P < 0.01$) (Mayerat et al. 1999). In general, the rate of chronicity following acute genotypes A and D infection were reported to be high compared with genotypes B and C (Heijtink et al. 1999; Mayerat et al. 1999; Kobayashi et al. 2004; Leblebicioglu and Eroglu 2004; Suzuki et al. 2005; Ito et al. 2014). 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.

In the natural history of chronic HBV infection, seroconversion of hepatitis B e antigen (HBeAg) and seroclearance of HBsAg are landmark events in the control of HBV infection. Earlier HBeAg seroconversion with subsequent low viral load (<2000 IU/mL) usually confers a favorable clinical outcome (Liu et al. 2013a), whereas late or absent HBeAg seroconversion after multiple hepatitis flares may accelerate the progression of chronic hepatitis leading to cirrhosis; it, therefore, has a poor clinical outcome (Chen et al. 2002; Hsu et al. 2002; Yang et al. 2002; Chu and Liaw 2007; Liu et al. 2013a). The pathogenic differences among various HBV genotypes have been partially clarified and influence the clinical outcomes of HBV infection (Table 2). In our cohort study on 272 Taiwanese patients with chronic HBV infection, genotype

Table 2. HBV genotype–specific implications in patients with chronic hepatitis B

	B versus C		A versus D	
Genotypes compared[a]	B	C	A	D
Virological implications				
Serum HBV DNA level	Lower	Higher	ND[b]	ND
Frequency of precore A1896 mutation	Higher	Lower	Lower	Higher
Frequency of basal core promoter A1762T/G1764A mutation	Lower	Higher	Lower	Higher
Frequency of pre-S deletion mutation	Lower	Higher	ND	ND
Intracellular expression of HBV DNA	Lower	Higher	Lower	Higher
Secretion of HBeAg	Lower	Higher	ND	ND
Immunologic implications				
Tendency of chronic infection	Higher	Lower	Lower	Lower
HBeAg seroconversion	Earlier	Later	Earlier	Later
HBsAg seroclearance	More	Less	More	Less
Histologic activity	Lower	Higher	Lower	Higher
Clinical implications				
Modes of transmission	Perinatal/vertical	Perinatal/vertical	Horizontal	Horizontal
Incidence of progression to cirrhosis and hepatocellular carcinoma (HCC)	Lower	Higher	Lower	Higher
Response to interferon-based therapy	Higher	Lower	Higher	Lower
Response to nucleos(t)ide analogs	[c]	[c]	[c]	[c]

[a]Because of the unique distribution of HBV genotypes in Asian and Western countries, sufficient data for meaningful comparisons are available only for comparisons between genotypes B and C or between genotypes A and D.

[b]ND, no available data.

[c]No significant difference between genotypes A to D.

C patients were more likely to have HBeAg-positive chronic hepatitis B despite multiple hepatitis flares (Kao et al. 2002a). In addition, genotype C infection was associated with lower rates of spontaneous HBeAg seroconversion than genotype B (27% vs. 47%, $P < 0.025$) during the follow-up. The estimated annual rates of HBeAg seroconversion in genotypes B and C infections were 15.5% and 7.9%, respectively (Kao et al. 2004). Furthermore, a long-term follow-up study with 460 Taiwanese HBV chronically infected children indicated that the positive rate of HBeAg after 20 years of follow-up was 70% in genotype C and 40% in genotype B carriers (Ni et al. 2004). Accordingly, genotype C patients experience delayed HBeAg seroconversion and, thus, have a longer duration of high HBV replication than genotype B patients.

Regarding genotypes A and D, one prospective study of Spanish patients with chronic HBV infection did not reveal any differences in the probability of HBeAg seroconversion between patients infected with genotypes A and D. However, the rate of sustained remission after HBeAg seroconversion was higher in genotype A patients (55% vs. 32%, $P < 0.01$) (Sanchez-Tapias et al. 2002). In addition, compared with genotypes C and D patients, genotypes A and B patients had a higher rate of spontaneous HBsAg seroclearance (Sanchez-Tapias et al. 2002; Yuen et al. 2004a). Taking these lines of evidence together, genotypes C and D patients, compared with genotypes A and B patients, have late or absent HBeAg seroconversion after multiple hepatitis flares that may accelerate the progression of chronic hepatitis, thereby conferring a worse clinical outcome.

The possible influence of other genotypes on the natural history of HBV infection remains limited. A previous study on vaccinated children in the Gambia showed that breakthrough HBV infection in these children was mainly caused by the wild-type genoytype E strain and that immune escape mutants were uncommon (Mendy et al. 2008). On the contrary, among vaccinated Taiwanese children (genotypes B and C infection are common) with breakthrough HBV infection, S gene mutations have become prominent (Chang 2010). Further investigations are awaited to determine the relationship between HBV genotype and vaccine response.

A recent retrospective study from Argentina revealed that 62% of acute hepatitis B and 37% of chronic hepatitis B were caused by genotype F HBV. Among chronic cases, genotype F tended to display higher histological activity indexes (Pezzano et al. 2011). Roman et al. (2010) further reported a high prevalence (14%) of occult HBV infection in Mexico natives, a low endemic area of HBV infection. In addition, HBV genotype H was predominant in those with occult HBV infection in Mexico (Panduro et al. 2013). 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 (Roman et al. 2009).

Naturally occurring HBV mutants can display important variation in epitopes that are important in host immune recognition, enhance virulence with increased levels of HBV replication, or facilitate cell attachment/penetration, thereby having basic and clinical significance (Fig. 1) (Hunt et al. 2000; Kao et al. 2010). These mutant strains, including the HBV precore nucleotide 1896 stop codon mutation and basal core promoter (BCP) A1762T/G1764Avariants, are known to be responsible for ongoing HBV DNA replication even after HBeAg seroconversion and lead to prolongation of chronic hepatic inflammation (Lin and Kao 2008; Mansourian et al. 2013). These findings suggest that hepatitis B viral characteristics may play an important role in HBV genotype–specific pathogenesis. For example, it has been shown that different HBV genotypes have different HBV replication capacities, both in vitro and in vivo. In an in vitro study, intracellular expression of HBV DNA was higher for genotypes C than B and genotypes D than A (Sugiyama et al. 2006). Furthermore, the secretion of HBeAg in genotype B was lower than that in genotype C (Liu et al. 2011). The intracellular accumulation of HBV DNA may, thus, induce liver cell damage. In addition, the higher replication capacity of genotype C explains why this genotype is associated with more severe liver disease than others. Further investigation revealed an increase of intracellular core protein expression when BCP A1762T/

G1764A variants were introduced in the genotype C strains (Liu et al. 2011). Our in vivo study also revealed that HBV BCP A1762T/G1764A variants were significantly associated with cytoplasmic localization of intracellular HBcAg, which were closely related to active necroinflammation of hepatocytes (Liu et al. 2009a). Similarly, genotype D–infected patients who had more progressive liver disease showed a higher prevalence of BCP A1762T/G1764A variants than those with genotype A infection (Sharma et al. 2010).

In summary, the specific virological manifestations of HBV genotypes and variants include (1) genotype C shows a higher frequency of BCP A1762T/G1764A variants and pre-S deletion mutations than genotype B; (2) serum HBV viral load is higher in genotype C than genotype B; (3) the expression of intracellular HBV DNA increases in genotype C; (4) the expression of intracellular core protein increases in genotype C with BCP A1762T/G1764A variants; and (5) more HBeAg is secreted by genotype C than by genotype B. These findings may partly explain why genotype C is associated with more severe liver disease than other genotypes (Kao 2011; Vutien et al. 2013).

HBV GENOTYPES AND RISK OF HCC DEVELOPMENT

As already highlighted, most retrospective or case-control studies indicated that patients with genotype C infection have more severe liver disease, including cirrhosis and HCC, than those with genotype B (Kao et al. 2000a, 2003; Chan et al. 2004; Yuen et al. 2004b). A community-based prospective cohort study on 2762 Taiwanese HBV carriers showed that HBV genotype C was associated with an increased risk of HCC than genotype B; the adjusted hazard ratio (HR) was 2.35 (95% confidence interval [CI]: 1.68–3.30; $P < 0.001$) (Yang et al. 2008). In a prospective study with 4841 Taiwanese, male, and HBV-infected patients without HCC at enrollment, Yu et al. found that HBV viral load was higher in genotype C than genotype B patients, whereas genotype C–infected patients who also had very high viral load had a 26-fold higher risk of HCC than those with other genotypes and low or undetectable viral loads (Yu et al. 2005). Our recent hospital-based cohort study on 2688 Taiwanese HBsAg-positive patients without evidence of cirrhosis for a mean time period of 14.7 years (elucidation of risk factors for disease control or advancement in Taiwanese hepatitis B carriers [ERADICATE-B] cohort) also showed that genotype C patients have a higher annual incidence rate of HCC than genotype B patients by univariate analysis (Tseng et al. 2012a). These findings confirm that genotype C correlates with a higher risk of HCC development. Of interest, several reports showed HBV genotype B is associated with risk of HCC development at a young age, whereas genotype C is associated with HCC development at an older age (Kao et al. 2000a; Ni et al. 2004; Yin et al. 2008).

HBV genotype also influences the clinicopathological features of patients with resectable HCC. In Taiwan, among 193 HBV-related HCC patients receiving surgery, genotype B patients had a higher rate of solitary tumor (94% vs. 86%, $P = 0.048$) but more satellite nodules (22% vs. 12%, $P = 0.05$) than genotype C patients (Chen et al. 2004b; Lin et al. 2007b). Wu et al. (2009) also reported that in HCC patients, liver inflammation activity was higher in HBV genotype C patients than in genotype B patients ($P = 0.023$), and more genotype C patients tended to have a higher viral load ($>10^6$ copies/ml) than genotype B patients (52.3% vs. 37.6%, $P = 0.067$). These characteristics may contribute to the recurrence patterns and prognosis of HBV-related HCC patients with genotypes B or C infection.

Compared with HBV genotypes, the clinical significance of HBV subtypes remains less clear. A study analyzed the distribution of HBV subtypes in 296 HBV-related HCC patients collected from all across Japan (Orito et al. 2005). They found HBV subtype B2 in 4.4%, B1 in 7.4%, and genotype C in 86.5%. Interestingly, in the Tohoku district and Okinawa, subtypes B2, B1, and genotype C were found in 6.7%, 40.0%, and 48.9%, respectively, compared with 4.0%, 1.6%, and 93.2% in the other districts in Japan. These data suggest that HBV subtype B1 may run a more indolent course than subtype B2. A

study of 242 Taiwanese HBsAg carriers revealed that there was no significant difference in the distribution of the HBV genotype C subtypes among patients with different stages of liver disease, suggesting that subtypes of genotype C may have minimal impact on liver disease progression of chronic hepatitis B in Taiwan (Tseng et al. 2007). However, another prospective study on 1006 CHB patients with a median follow-up of 7.7 years from Hong Kong showed that subtype C2 has the highest risk of HCC (HR: 2.75; 95% CI: 1.66–4.56; $P < 0.0001$) and subtype C1 has intermediate risk (HR: 1.70; 95% CI: 1.09–2.64; $P = 0.020$) compared with genotype B (Chan et al. 2008). In addition, multiple mutations in subtype C4 were associated with more rapid liver disease progression and an increased risk of HCC in indigenous Northern Australian populations (Littlejohn et al. 2014). Genotypes B2 and C4 have been shown to be recombinants with other genotypes, which may play an important role in natural history and pathogenesis. Further studies from different parts of world with detailed characterization of the strains, are needed to confirm the association between HBV subtypes and risk of HCC development.

Although limited studies have examined the relationship between other HBV genotypes and the risk of HCC development, HCC is more frequent in patients with HBV genotypes D and F infection than those with genotype A infection (Thakur et al. 2002; Livingston et al. 2007). A prospective study with Alaska native HBV carriers revealed that the risk of HCC development was significantly higher for genotype F than genotypes A–D ($P < .001$; OR: 7.73; 95% CI: 3.69–16.4; $P < 0.001$) (Livingston et al. 2007).

HBV VARIANTS AND RISK OF HCC DEVELOPMENT

Of the various naturally occurring HBV mutants, several mutations in the X gene of the HBV genome are frequently found in patients with HCC (Yeh et al. 2000; Chen et al. 2005; Guo et al. 2008; Ma et al. 2008), suggesting that these mutants may play a significant role in the malignant potential of HBV. Several studies showed that 3′-end X gene is frequently deleted in HCC cells, leading to a carboxy-terminal truncated HBx protein (Iavarone et al. 2003; Wang et al. 2004). Subsequent studies showed carboxy-terminal truncated HBx in ~80% of HCC tissues, and may contribute to hepatocarcinogenesis via the activation of cell proliferation and loss of proapoptotic ability (Ma et al. 2008).

In a cohort study of genotypes B– or C–infected HBV carriers, genotype C had a higher prevalence of BCP A1762T/G1764A variants than genotype B (odds ratio [OR]: 5.18; 95% CI: 2.59–10.37; $P < 0.001$). Patients with BCP A1762T/G1764A variants were more significantly associated with the development of HCC than those without (HR: 10.6; 95% CI: 4.92–22.86; $P < 0.001$) (Kao et al. 2003). These findings were further confirmed by a long-term follow-up study involving 1526 HBV carriers (risk evaluation of viral load elevation and associated liver disease/cancer-hepatitis B virus [REVEAL-HBV] study), revealing the presence of BCP A1762T/G1764A variants as an independent predictor for progression to HCC (HR: 1.73; 95% CI: 1.13–2.67; $P = 0.013$) (Yang et al. 2008). In addition, a meta-analysis produced a summary odds ratio of HCC for BCP A1762T/G1764A variants that was 3.79 (95% CI: 2.71–5.29) (Liu et al. 2009b). Recently, BCP A1762T/G1764A variants were determined both qualitatively and quantitatively to correlate with cirrhosis in Taiwanese HBV carriers with genotypes B or C infection. BCP A1762T/G1764A variants served as an independent risk factor for cirrhosis development (HR: 4.26; 95% CI: 1.32–13.77). Quantitative analysis using pyrosequencing revealed that risk of cirrhosis was higher in patients with BCP A1762T/G1764A variants $\geq$45% compared with <45% (adjusted OR: 2.81; 95% CI: 1.40–5.67; $P = 0.004$) (Tseng et al. 2014). Finally, the BCP A1762T/G1764A variants also affect the carboxy-terminal codons 130 and 131 of the X protein (K130M and V131I) because of the overlapping nature of the HBV genome. Taking these lines of evidence together, BCP A1762T/G1764A variants may select out amino acid changes in the X protein, which increase the fibrogenic activity and promote hepatocarcinogenesis, regardless of HBV genotypes.

Mutations in enhancer II (C1653T) and elsewhere in the basal core promoter (T1753V) have also been found to be associated with HCC development. A case-control study from Hong Kong revealed that patients with C1653T mutation had a significantly higher risk of HCC than those without (OR: 2.43; 95% CI: 1.08–5.54; $P = 0.028$) (Yuen et al. 2008). Another cohort study from Taiwan indicated that patients with the T1753V mutation had a significantly higher risk for developing HCC than those without this change (OR: 2.43; 95% CI: 1.33–4.44; $P = 0.028$) (Chou et al. 2008).

Previous reports also showed that the deletion mutations within the pre-S gene were significantly associated with the development of cirrhosis and HCC (Chen et al. 2007; Lin et al. 2007c; Fang et al. 2008). The proposed mechanism is that the endoplasmic reticulum stress associated with pre-S accumulation induced oxidative DNA damage and so, pre-S gene deletion mutation variants may lead to mutagenesis of the host genome, thereby contributing to hepatocarcinogenesis (Hsieh et al. 2004). In our case-control study, the presence of pre-S deletion was an independent risk factor associated with HCC development (OR: 3.72; 95% CI: 1.44–9.65; $P = 0.007$). In addition, the frequency of pre-S deletion was significantly higher in genotype C patients than genotype B patients (Lin et al. 2007c). A meta-analysis further confirmed that the OR of HCC for pre-S deletion was 3.77 (95% CI: 2.57–5.52). Of particular note, the summary OR for pre-S deletion was higher in genotype C patients than genotype B patients (Liu et al. 2009b). Our previous mapping study of pre-S regions suggested that all the deletion regions encompassed T- and B-cell epitopes, and most of them lost one or more functional sites, including the polymerized human serum albumin-binding site and nucleocapsid-binding site. Therefore, HBV pre-S deletion mutations may also lead to defective immunity against HBV and contribute directly to progressive liver cell damage and finally hepatocarcinogenesis (Chen et al. 2006). The odds ratios of HCC for HBV carriers by mutations in core promoter and pre-S regions are shown in Figure 2. Furthermore, subsequent investigations have shown that the combination of pre-S deletion and core promoter mutations rather than either single mutation profile was strongly associated with disease progression and development of HCC (Chen et al. 2006, 2007; Yuen et al. 2008; Liu et al. 2009b).

In addition to the potential hepatocarcinogenesis, HBV variants also influence the postoperative prognosis of patients with HCC. In a study of 185 liver samples obtained from the noncancerous part of surgically removed HBV-associated HCC tissues, the presence of the BCP mutation independently predicted disease-free survival (adjusted HR: 2.075; 95% CI: 1.203–3.579). In addition, short stretch (<100 bp) pre-S deletions were significantly associated with poorer disease-free survival ($P = 0.005$) (Yeh et al. 2010).

CLINICAL SIGNIFICANCE OF QUANTITATIVE HBsAg AND ITS CORRELATION WITH HBV GENOTYPES AND VARIANTS

Recently, quantitative hepatitis B surface antigen (qHBsAg) has been increasingly recognized to be a promising biomarker to predict both favorable and adverse outcomes of HBV carriers (Tseng et al. 2011a, 2012a,b, 2013; Lin and Kao 2013a). An Italian study showed that the combination of qHBsAg <1000 IU/ml and serum HBV DNA level <2000 IU/ml can predict the inactive HBV carrier state in HBV genotype D patients (Brunetto et al. 2010). Our recent study further showed that low serum levels of HBsAg (<100 IU/ml) at 1 year after HBeAg seroconversion could predict HBsAg loss in patients with HBV genotypes B or C infection (Tseng et al. 2011a). In addition, qHBsAg was better than serum HBV DNA level for the prediction of spontaneous HBsAg loss in HBeAg-negative carriers with a low viral load (<2000 IU/mL). Low serum HBsAg level was the strongest predictor of spontaneous HBsAg seroclearance in patients with a low viral load (Tseng et al. 2012b; Liu et al. 2013b). In the recent update of REVEAL-HBV study with more than 3000 HBV carriers, qHBsAg, HBV DNA levels, and HBV genotype C were all independent predic-

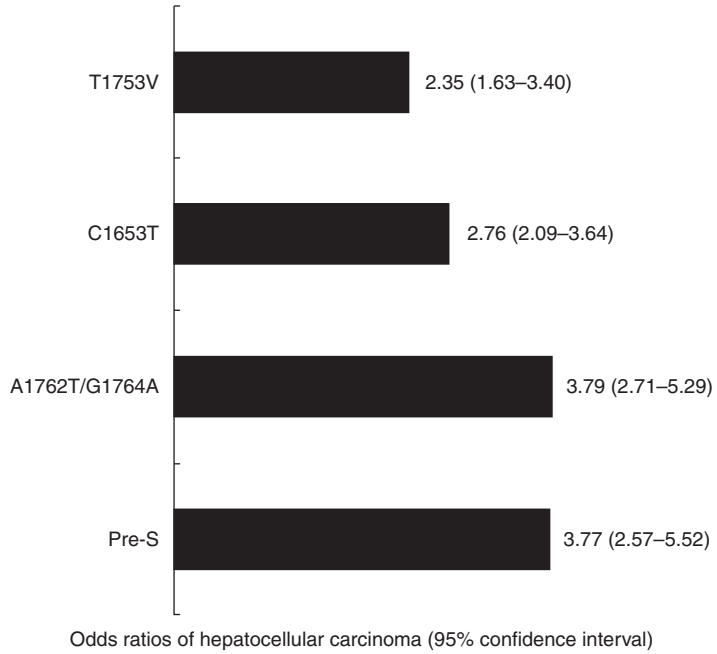

Figure 2. Odds ratios of hepatocellular carcinoma for HBV carriers by mutations in core promoter and pre-S regions in a meta-analysis. (Imaged created from data in Liu et al. 2009b.)

tors of HCC development. Of particular note, qHBsAg was significantly associated with cirrhosis and HCC in a dose-response manner (P for trend <0.001) in the HBeAg-negative patients with low serum HBV DNA levels ($<$200,000 IU/mL) (Lee et al. 2013). Our hospital-based ERADICATE-B cohort study also showed similar findings. A total of 2688 noncirrhotic Taiwanese chronic hepatitis B patients were followed for a mean of 14.7 years HCC risk increased when patients had increased HBV DNA levels (HR: 4.7; 95% CI: 2.2–10.0), increased qHBsAg (HR: 7.2; 95% CI: 1.8–28.6), and elevated ALT levels (HR: 6.6; 95% CI: 2.2–19.8). In addition, for patients with low serum HBV DNA levels ($<$2000 IU/mL), qHBsAg $\geq$ 1000 IU/mL was an independent risk factor for HCC development (HR: 13.7; 95% CI: 4.8–39.3) (Tseng et al. 2012a). A recent study evaluated the correlation between HBV DNA and HBsAg levels according to HBV genotype. These investigators found that serum HBsAg level tended to correlate with HBV DNA level for genotype A, but did not reach significant for genotype D (Tuaillon et al. 2012). We recently followed 187 patients with chronic HBV infection for a median of 8 years. We found that inactive carriers had a significantly lower qHBsAg at baseline and during follow-up compared with patients with elevated serum HBV DNA levels. However, the longitudinal qHBsAg change was independent of genotypes B or C, the most common genotypes in Taiwan (Su et al. 2013). More studies are definitely warranted to clarify the relationship between HBV genotype and qHBsAg level in different clinical situations as well as other geographical locations.

THE IMPACT OF HBV GENOTYPES ON RESPONSE TO ANTIVIRAL THERAPY

The therapeutic endpoints for chronic hepatitis B treatment include sustained suppression of HBV replication to below the detection limit of real-time PCR assays, biochemical remission, histological improvement, HBeAg loss, or HBeAg seroconversion for HBeAg-positive patients and, ideally, HBsAg loss or even HBsAg

seroconversion (Lok and McMahon 2009; EASL 2012; Liaw et al. 2012). Currently, two types of therapy are recommended: standard interferon (IFN) or pegylated interferon (PEG-IFN) and nucleos(t)ide analogs (NUCs), including lamivudine, telbivudine, entecavir, adefovir dipivoxil, and tenofovir disoproxil (Lok and McMahon 2009; EASL 2012; Liaw et al. 2012). The impact of HBV genotype on therapeutic responses to both IFN-based and NUCs has been increasingly recognized (Liu et al. 2005; Liu and Kao 2008; Lin and Kao 2013b,c). Because patients infected with genotypes E–J are less common, their responses to antiviral therapy remain largely unknown. Therefore, the influences of HBV genotypes on response to antiviral therapy can only be reliably shown for genotypes A, B, C, and D (Table 2).

Interferon-Based Therapy

In HBeAg-positive patients treated with standard IFN, patients with genotypes A and B had a significantly higher rate of HBeAg seroconversion posttreatment than those with genotypes C and D (Kao et al. 2000b; Hou et al. 2001; Wai et al. 2002; Erhardt et al. 2005). For the HBeAg-positive Asian population, genotype B HBV–infected patients show higher response rates with IFN-based therapies, regardless of pegylated or standard type IFN products, whereas genotype C–infected patients have a higher likelihood of response to PEG-IFN compared with standard IFN (Cooksley et al. 2003; Lau et al. 2005). Furthermore, HBV genotype B infection and younger age were independent factors associated with sustained response of low-dose, 24-wk IFN regimen in HBeAg-positive Chinese patients (Zhao et al. 2007). Another multicenter study on PEG-IFN for HBeAg-positive patients revealed that the rate of HBeAg clearance also differed according to the infecting HBV genotype: genotype A, 47%; genotype B, 44%; genotype C, 28%; and genotype D, 25% (Janssen et al. 2005). Subsequent analysis consistently showed a higher rate of HBsAg clearance in genotype A compared with other genotypes in both HBeAg-positive and HBeAg-negative CHB patients (Flink et al. 2006). In addition, compared with genotypes C and D patients, durable loss of HBeAg at 3 yr after PEG-IFN treatment was higher in genotypes A and B patients (Buster et al. 2008). Among HBeAg-negative patients treated with PEG-IFN, HBsAg clearance was significantly higher in genotype A (20%) than genotype B (6%), genotype C (9%), and genotype D (6%) (Marcellin et al. 2009). On the basis of existing data, a meta-analysis further confirmed that HBV genotypes are informative concerning responses to IFN-based therapy. HBV genotype A has a better response to IFN treatment than genotype D patients, regardless of HBeAg status. HBV genotype B has a higher response rate to IFN treatment than genotype C in HBeAg-positive patients (Wiegand et al. 2008). Recent pooled data from two largest global trials of HBeAg-positive patients with PEG-IFN treatment showed that higher levels of ALT and lower levels of HBV DNA predicted a sustained response to PEG-IFN therapy for genotypes A–, B–, and C–infected patients. On the contrary, genotype D–infected patients had the lowest chance of sustained response, irrespective of ALT or HBVDNA levels (Buster et al. 2009).

More on-treatment decline of qHBsAg has been shown in patients with IFN-based therapy than in those with NUC therapy (Su et al. 2010). Recent data further revealed the correlation between the declines of qHBsAg and HBV genotype in patients receiving IFN-based therapy (Tseng and Kao 2013). The on-treatment decline of qHBsAg level has proven a useful predictor of response for IFN-based therapy. For HBeAg-positive genotypes B and C patients, HBeAg seroconversion was significantly associated with serum HBsAg level $<$1500 IU/mL at wk 12 of PEG-IFN treatment, whereas patients with serum HBsAg level $>$20,000 IU/mL at wk 12 of PEG-IFN treatment did not respond (Liaw et al. 2011; Sonneveld et al. 2013).

Similarly, for HBeAg-positive genotypes A and D patients, no change in the serum HBsAg level at wk 12 of PEG-IFN predicted a poor response of HBeAg loss at 26 wk after treatment (Sonneveld et al. 2010). Recently, a futility rule has been developed in which no HBsAg decline and $<$2 log copies/ml decline in HBV DNA at wk 12 of therapy with PEG-IFN has been pro-

posed. This has been validated in genotype D HBeAg–negative patients (Rijckborst et al. 2010, 2012). The HBsAg kinetics during PEG-IFN treatment also varied among different HBV genotypes. For example, at the end of treatment, the mean decrease of HBsAg level was highest with genotype A infection, intermediate in genotypes B and D, and lowest in genotypes C and E. During follow-up, serum HBsAg levels continued to decrease in genotypes A and D, whereas rebound was observed in genotypes B, C, and E (Moucari et al. 2009). A recent study further revealed that high positive predictive values for long-term virological response at 5-yr post-PEG-IFN treatment could be obtained by applying end-of-treatment genotype-specific qHBsAg cutoff levels: 75%, 47%, 71%, and 75% for genotypes A (<400 IU/ml), B (<50 IU/ml), C (<75 IU/ml), and D (<1000 IU/ml), respectively (Brunetto et al. 2013). Therefore, monitoring HBV genotype–specific qHBsAg may improve response-guided treatment of HBeAg-negative chronic hepatitis B (Fig. 3).

NUCLEOSIDE AND NUCLEOTIDE ANALOGS

In contrast to IFN-based therapy, the therapeutic responses to NUCs as well as the development of resistance were comparable among patients with different genotypes (Kao et al. 2002b; Chan et al. 2003; Westland et al. 2003; Yuen et al. 2003; Hou et al. 2008; Wiegand et al. 2008; Chen et al. 2013). Although HBV genotypes seem not to have an impact on the response and resistance to NUC treatment, our retrospective study found that HBV genotype B was independently associated with an earlier detection of lamivudine-resistant strains. In addition, genotype B was significantly associated with development of lamivudine resistance within the first 12 mo of lamivudine therapy compared with genotype C (OR: 8.27; $P = 0.004$) (Hsieh et al. 2009). Therefore, more frequent monitoring of genotypic resistance might be needed for particular HBV genotypes during NUC therapy.

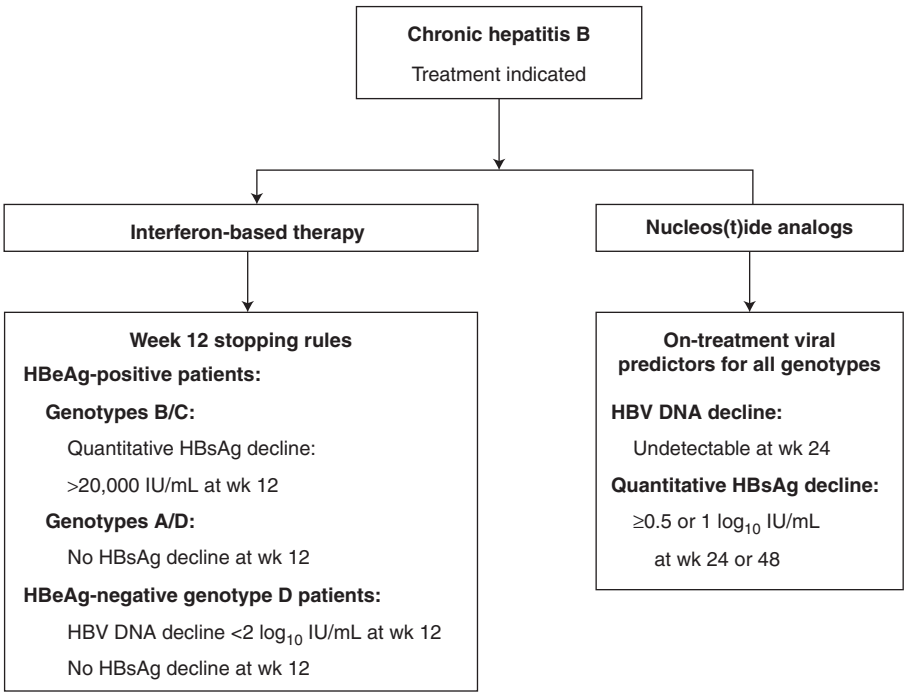

Figure 3. Hypothetical algorithm for HBV genotype–specific antiviral treatment in patients with chronic hepatitis B.

Clearance of HBsAg has been reported in patients treated with NUCs. HBsAg loss was achieved in 8% of HBeAg-positive patients after 3 yr of tenofovir therapy (Heathcote et al. 2011). Similarly, the HBsAg loss rates for HBeAg-positive patients treated with entecavir or lamivudine for 2 yr were 5% and 3%, respectively (Gish et al. 2010). More specifically, most patients with NUC-associated HBsAg loss had HBV genotypes A or D infection (Gish et al. 2010; Wursthorn et al. 2010; Heathcote et al. 2011), and the clearance of HBsAg by NUCs seems very rare in Asian patients with genotypes B or C infection. Although the proportion of patients with HBsAg loss is too small to reach any conclusion, the association between HBV genotype and NUC-induced HBsAg loss deserve further study.

THE IMPACT OF HBV VARIANTS ON RESPONSE TO ANTIVIRAL THERAPY

The impact of HBV variants on HBV treatment remains largely unknown. Previous studies have shown that the precore G1896A mutation or BCP A1762T/G1764A variants have been associated with response to IFN-α or NUC therapy (Tseng et al. 2010). Our previous study on 116 HBeAg-positive CHB patients receiving short-term lamivudine therapy revealed that precore G1896A mutation, compared with wild-type (75% vs. 52%, $P = 0.045$), correlated with the loss of HBeAg at the end of therapy (Tseng et al. 2009). Similarly, a recent study on 137 HBeAg-positive NUC-treated CHB patients revealed that the chance of HBeAg seroconversion was higher in patients with mixed populations of precore and/or BCP mutants ($P = 0.01$) (Zoutendijk et al. 2013). Another study on 115 HBeAg-positive patients receiving PEG-IFN-α-2a for 6 mo indicated that the presence of BCP A1762T/G1764A variants was associated with a combined response defined as HBeAg seroconversion, HBV DNA levels less than 20,000 IU/mL, as well as ALT normalization at 6 mo off therapy (OR: 8.04; 95% CI: 2.00–32.28) (Tseng et al. 2011b). On the contrary, recent analysis on 214 HBeAg-positive patients receiving PEG-IFN-α with or without lamivudine for 1 yr revealed that patients without presence of precore and BCP mutants at baseline were more likely to achieve HBeAg loss with HBV DNA <10,000 copies/mL and HBsAg clearance at 6 mo off therapy than patients with presence of precore or BCP mutants. The wild type of precore and BCP region of virus at baseline was an independent predictor of response (OR: 2.90; 95% CI: 1.15–7.31; $P = 0.023$) and HBsAg clearance (OR: 5.58; 95% CI: 1.26–24.63; $P = 0.013$) (Sonneveld et al. 2012). Using a new assay employing pyrosequencing to quantify the precore G1896A and BCP A1762T/G1764A variants percentages, a correlation between dynamic changes of these mutants and IFN-associated HBeAg seroconversion was determined in 203 HBeAg-positive CHB patients (Yang et al. 2013). The chance of HBeAg seroconversion increased by 2.2% (OR: 1.022; 95% CI: 1.009–1.034; $P = 0.001$) and 2.3% (OR: 1.023; 95% CI: 1.010–1.037; $P = 0.001$) per 1% increase of the pretreatment precore G1896A and BCP A1762T/G1764A variants percentages, respectively (Yang et al. 2013). Confirmatory data are limited and, therefore, further large-scale studies are required to explore the association of common HBV variants with treatment response to currently available antiviral agents.

CONCLUSIONS AND PERSPECTIVES

Over the past decade, ample evidence in molecular studies has clarified the clinical implications of HBV genotypes and variants. In brief, compared with genotypes A and B patients, genotypes C and D patients have a higher risk of cirrhosis and HCC, leading to a poorer clinical outcome. Mutations in core promoter and the pre-S regions are also associated with an increased risk of HCC. In addition, genotypes A and B patients have a better response to IFN-based therapy than genotypes C and D patients. However, the association between HBV genotypes/variants and therapeutic response to NUCs seems minimal. Despite numerous lines of evidence connecting HBV genotypes/variants and the disease progression as well as responses to antiviral therapy (Chen 2011), HBV

genotyping is still not recommended as a part of the management of chronic hepatitis B in the recent update guidelines for the management of HBV infection (Lok and McMahon 2009; EASL 2012; Liaw et al. 2012). Nevertheless, HBV genotypes and variants have shown potential to be useful viral biomarkers for the prediction of disease progression as well as help practicing clinicians identify patients who can benefit most from IFN-based therapy. In the foreseeable future, clinical trials stratified by different genotypes/variants and treatment regimens are mandatory to implement individualized therapies for chronic hepatitis B patients.

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