Molecular Epidemiology of Hepatitis B Virus

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Although safe and effective vaccines for hepatitis B virus (HBV) have been available for nearly three decades, this virus kills at least 600,000 people annually worldwide and remains the leading global cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Because the HBV reverse transcriptase lacks a proofreading function, many HBV genotypes, subgenotypes, mutants, and recombinants exist. At least 10 HBV genotypes (HBV-A through J) with distinct geographic distributions have been identified; by definition, their complete genomic sequences diverge by more than 8%. HBV genotype is increasingly becoming recognized as an important factor in the progression and clinical outcome of HBV-induced disease. Infections by HBV-C or -D are significantly more likely to lead to cirrhosis and hepatocellular carcinoma than are infections by HBV-A or -B. Additionally, the hepatitis B e antigen seroconversion response to standard or pegylated interferon is more favorable in patients with HBV-A or -B than in those with HBV-C or -D. However, therapeutic responses to nucleos(t)ide analogues are generally comparable among HBV genotypes. In conclusion, genotyping of HBV is useful in identifying chronic hepatitis B patients who are at increased risk of disease progression, thereby enabling physicians to optimize antiviral therapy for these patients.

Keywords: Antiviral agents; Genotype; Hepatitis B virus; Hepatocellular carcinoma; Molecular epidemiology

INTRODUCTION

Although safe and effective vaccines for hepatitis B virus (HBV) have been available for more than two decades, infection by this organism remains a burden to global public health, resulting in 600,000 to 1 million deaths per year worldwide [1]. Clinically, HBV infection can manifest in acute/fulminant hepatitis or in various chronic conditions, including an inactive carrier state, chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) [2]. Approximately 15-40% of HBV carriers who acquire the virus early in life eventually develop HBV-related cirrhosis or HCC [3].

Being a virus of the family Hepadnaviridae, HBV is the smallest human DNA virus, carrying a genome only 3,200 nucleotides in length [4]. The partially double-stranded circular DNA harbors four overlapping open reading frames encoding the S (surface), C (core), P (polymerase), and X genes (Fig. 1). Because the viral reverse transcriptase has a high error rate, the HBV genome evolves quickly over time, with an estimated nucleotide substitution rate of 1.4-3.2 × 10⁻⁵/site per year [4]. This unique replication strategy accounts for the majority of the point mutations, deletions, and insertions observed in the HBV genome. The lengthy evolution of HBV has led to the present existence of various genotypes, subgenotypes, mutants, recombinants, and even quasispecies of HBV [5].

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HBV is endemic in Asia and the Pacific islands, Africa, Southern Europe, and Latin America. The community 

prevalence of the HBV surface antigen (HBsAg) encoded 

by gene S ranges from 2 to 20% [1]. In Asian countries, 

the majority of HBV infections are acquired perinatally 

or in early childhood by the age of 2 years through verti - 

cal (mother-to-child) transmission; however, horizontal 

transmission is the main route of infection in African and 

Western countries [1].

Disease progression in patients with chronic hepatitis B 

varies widely. Several viral factors, including the HBV gen- 

otype, viral load, and specific viral mutations, have been 

documented to be strongly associated with disease pro - 

gression and outcomes (Table 1). The annual incidence of 

cirrhosis is estimated to be 2-6% in those who test positive 

for the HBV e antigen (HBeAg) and 8-10% in those who 

test negative. The annual incidence of HCC is less than 1% 

for patients without cirrhosis and 2-3% for patients with 

cirrhosis [2,3].

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Analyses of the divergence of HBV genomic sequences 

has led to the identification of 10 HBV genotypes (A 

through J) and several subtypes, where separate geno- 

types and subtypes are defined as strains exhibiting an 

entire genome sequence divergence of more than 8% or 

4-8%, respectively [6]. The geographic distribution of 

HBV genotypes A-H and their subtypes is shown in Table 

2. Genotype A is highly prevalent in sub-Saharan Africa 

(subtype A1), Northern Europe (subtype A2), and Western 

Africa (subtype A3). Genotypes B and C are prevalent in 

the Asian Pacific region. However, the prevalence rates of 

genotypes B and C vary among the different Asian coun- 

tries (Table 3) [7]. For example, HBV-C is more common 

than HBV-B in China, Japan, and Korea, whereas HBV-B 

is the more common genotype in Taiwan and Vietnam [8]. 

Of particular note is the fact that almost all chronic hepa- 

titis B patients in Korea are infected with HBV-C [7].

Genotype B has six subtypes, B1-B6. B1 is found in Ja -

apan; B2-B5 and B7 are found in East Asia, and B6 is found 

in Southeast Asia and the Western Pacific region [1].

**Table 1. Risk factors associated with disease progression in chronic hepatitis B patients**

| Viral factors                                      | Host factors       | Environmental factors                      |
|---------------------------------------------------|--------------------|-------------------------------------------|
| High viral load                                   | Advanced age       | Afatoxin exposure                         |
| Genotype (HBV-C > HBV-B; HBV-D > HBV-A)            | Male gender        | Alcohol consumption                       |
| Basal core promoter mutation                      | Genetic alterations | Cigarette smoking                         |
| Pre-S deletion                                    | Family history of HCC | Concurrent infection with hepatitis C or |
|                                                   | Ethnicity (Asian > Caucasian) | D virus or with HIV |

HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus.

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in indigenous populations living in Arctic regions, including Alaska, Northern Canada, and Greenland. Genotype C, which has subtypes C1-C5, is found primarily in East and Southeast Asia. Genotype D, which has subtypes D1-D5, is prevalent in Africa, Europe, the Mediterranean region, and India. Genotype E is restricted to West Africa. Genotype F, which has 4 subtypes (F1-F4), is found in Central and South America. Genotype G has been reported in France, Germany, and the United States. Genotype H is found in Central America [9,10]. Recently, genotype I was isolated in Vietnam and Laos [11], and genotype J was identified in Japan [12].

Table 2. Geographic distribution of HBV genotypes and subtypes

| Genotype | Subtype | Geographic location          |
|----------|---------|-----------------------------|
| A        | A1      | Sub-Saharan Africa          |
|          | A2      | Northern Europe             |
|          | A3      | Western Africa              |
| B        | B1      | Japan                       |
|          | B2-B5   | Taiwan, China, Indonesia, Vietnam, Philippines |
|          | B6      | Alaska, Northern Canada, Greenland |
| C        | C1-C3   | Taiwan, China, Japan, Korea, Southeast Asia |
|          | C4      | Australia                   |
|          | C5      | Philippines, Vietnam        |
| D        | D1-D5   | Africa, Europe, Mediterranean basin, India |
| E        |         | Restricted to West Africa   |
| F        | F1-F4   | Central and South America   |
| G        |         | France, Germany, United States |
| H        |         | Central America             |
| I        |         | Laos, Vietnam               |
| J        |         | Japan                       |

Table 3. Distribution of hepatitis B virus (HBV) genotypes in different Asian countries

| Country  | Prevalence (%) of HBV genotype |
|----------|-------------------------------|
|          | A | B | C | D | Others |
| China    | 2 | 41 | 53 | 1 | 1 |
| Hong Kong| 0 | 33 | 63 | 0 | 4 |
| Japan    | 2 | 12 | 85 | 0 | 1 |
| Korea    | 0 | 0 | 100 | 0 | 0 |
| Philippines | 51 | 22 | 27 | 0 | 0 |
| Solomon  | 0 | 0 | 55 | 45 | 0 |
| Taiwan   | 3 | 71 | 22 | 0 | 4 |
| Thailand | 1 | 12 | 87 | 0 | 2 |
| Vietnam  | 22 | 12 | 30 | 24 | 12 |

Current evidence for the impact of HBV genotype on the short- and long-term adverse outcomes of chronic HBV infection is summarized in Table 4. Most retrospective and case-control studies have indicated that patients infected with HBV-C have more severe liver disease, including cirrhosis and HCC, than those with infected with HBV-B [2,8,13]. In a recent community-based prospective cohort study of 2762 Taiwanese HBV carriers, HBV-C was associated with a higher risk of HCC than was HBV-B; the adjusted hazard ratio was 2.35 (95% confidence interval [CI], 1.68 to 3.30; p < 0.001) [14]. These findings confirm that HBV-C is associated with a higher risk of HCC development than HBV-B is.

HBV genotype also affects the clinicopathologic features of patients with resectable HCC. Of 193 patients with resectable HBV-related HCC in Taiwan, those infected with HBV-B had a higher rate of solitary tumor than did those infected with HBV-C (94% vs. 86%; p = 0.048), but they also had more satellite nodules (22% vs. 12%; p = 0.05). These features may contribute to the recurrence patterns and prognosis of HBV-related HCC patients infected with HBV-B or -C infection [15,16]. Death related to liver disease occurs more often in patients with HBV-D and -F than in those with HBV-A [17,18].

The importance of HBV genotype on the development of HCC has prompted the recent development of a clinical HBV scoring system, a nomogram including several independent risk predictors, including sex, age, family history of HCC, alcohol consumption, serum alanine aminotransferase (ALT) level, HBeAg status, serum HBV DNA level, and HBV genotype [19]. Based on clinical characteristics that can be determined noninvasively, this easy-to-use nomogram has been found to accurately predict HCC risk in HBV-infected persons and could facilitate communication between physicians and their patients, particularly in the Asian Pacific region.
The pathogenic differences among various HBV genotypes have been partially clarified. Intracellular expression levels of HBV DNA and HBV core antigen (HBcAg), as well as the extracellular levels of HBV DNA and HBeAg, have been found to be higher for HBV-B and -C genotypes than for HBV-A and -D. The intracellular accumulation of HBV DNA and viral antigens may play a role in inducing liver cell damage. Additionally, the higher replication capacity of the C genotype may explain why it is the genotype associated with the most severe HBV-induced liver disease [20, 21].

In recent in vitro studies, we found that; 1) the expression of intracellular HBV core protein increased in strains of the C genotype when mutations were made in the precore or basal core promoter regions affecting HBeAg expression; 2) the expression of intracellular HBV surface protein was lower for a precore wild-type HBV-C strain than for HBV-B; 3) the precore mutation was associated with a decreased level of extracellular HBV DNA; 4) less HBsAg was secreted by HBV-C than by HBV-B; and 5) less HBeAg was secreted by HBV-B than by HBV-C [22]. The in vivo and in vitro manifestations of HBV-B and -C are compared in Table 5.

### HBV Genotype and Anti-Viral Treatments

**Interferon-based therapy**

The therapeutic endpoints for the management of chronic hepatitis B include sustained suppression of the HBV DNA level, normalization of the serum ALT level, histologic improvement, HBeAg clearance or seroconversion (for HBeAg-positive patients), and HBsAg clearance or seroconversion [23]. Two types of drug therapy are in current use for HBV treatment: interferon (IFN)-α, which is used in both standard and pegylated forms, and nucleos(t)ide analogues, including lamivudine, telbivudine, entecavir, adefovir dipivoxil, and tenofovir disoproxil fumarate [6-8].

Recent studies have suggested that HBV genotype affects the therapeutic response to both IFN and nucleos(t)ide analogue-based agents [23, 24]. In brief, the incidence of sustained ALT normalization and HBeAg seroconversion 6-12 months after the cessation of interferon treatment is significantly higher in patients with HBV-A and -B than in those with HBV-C or -D [25, 26]. In Asian HBeAg-positive patients, those with HBV-B are susceptible to both standard and pegylated IFN, and those with HBV-C are more likely to respond favorably to pegylated IFN than to standard IFN [27-31]. Additionally, the incidence of durable HBeAg clearance 3 years after the cessation of pegylated IFN-a treatment is higher in patients with HBV-A or -B than in those with HBV-C or -D [32].

In the absence of a cure for chronic HBV infection, the desired endpoint in current management of this disease is the complete suppression of HBsAg expression. In a long-term follow-up study of HBeAg-negative patients treated with pegylated IFN, patients infected with HBV-A had a significantly higher mean incidence of HBsAg clearance (20%) than those infected with HBV-B, -C, or -D (mean clearance rates of 6, 9, and 6%, respectively) [33]. Additionally, the kinetics of HBsAg clearance during pegylated
IFN treatment varied among the different HBV genotypes. For example, at the end of treatment, the mean decline in HBsAg levels was greatest in patients with HBV-A, intermediate in those with HBV-B or -D, and least in those with HBV-C or -E; during the follow-up period, the serum HBsAg level continued to decrease in the patients with HBV-A or -D, whereas HBsAg rebound was observed in those with HBV-B, -C, or -E [34].

A recent meta-analysis confirmed that patients infected with HBV-A respond better to IFN-α treatment than do those infected with HBV-D, regardless of the patient’s HBeAg status, and that HBeAg-positive patients infected with HBV-B respond better to IFN-α treatment than do HBeAg-positive patients infected with HBV-C [35]. Therefore, patients with HBV-A or -B infection generally have a better response to IFN-α than do those with HBV-C or -D infection [36]. The data pooled from the two largest global trials of HBeAg-positive patients treated with pegylated IFN demonstrate that HBV-A patients with a higher ALT level or a lower HBV DNA level and HBV-B and -C patients with both a higher ALT level and a lower HBV DNA level have a high predicted probability of a sustained response and should be considered for IFN therapy. On the other hand, HBV-D patients have the lowest chance of sustained response, irrespective of their ALT or HBV DNA levels; IFN therapy is thus not recommended for these patients [37].

Therapy with nucleoside or nucleotide analogues

In most clinical studies, the therapeutic responses to lamivudine, adefovir, entecavir, and telbivudine have been similar among the different HBV genotypes [5,8,23,24], and a recent meta-analysis found no significant association between HBV genotype and the response to nucleos(t)ide analogues [35]. Although HBV genotypes seem to have little impact on the response to nucleos(t)ide analogue treatment [36], our retrospective analysis has suggested that genotype B is independently associated with earlier detection of lamivudine resistance. Additionally, we found that the development of lamivudine resistance within the first 12 months of lamivudine therapy had a significantly higher association with genotype B than with genotype C (odds ratio, 8.27; \( p = 0.004 \)) [38]. Therefore, more frequent monitoring of genotypic resistance might be needed for specific HBV genotypes during nucleos(t)ide analogue therapy.

Complete clearance of HBsAg is rare in chronic hepatitis B patients treated with oral agents. However, Marcellin et al. [39] recently reported that complete HBsAg clearance occurred in five of 158 HBeAg-positive patients after 48 weeks of treatment with tenofovir disoproxil. Of these five Caucasian patients, two were infected with HBV-A, and three were infected with HBV-D. Although the proportion of patients with HBsAg loss was too small to reach any definite conclusions, the association of HBV genotype with nucleos(t)ide analogue-induced HBsAg clearance is interesting and deserves further study.
ConClusIons

Investigations of the molecular epidemiology and clinical implications of HBV genotypes have resulted in clinically significant advances over the past decade, particularly with regards to genotypes found in the Asian Pacific region. Accumulating evidence [40] suggests that a patient with chronic HBV infection should receive HBV genotyping unless he or she lives in a country known to harbor only one HBV genotype. This measure will help practicing physicians to identify those patients who are at increased risk of disease progression to end-stage liver disease and those who can benefit most from interferon-based therapy [41,42].

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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