Molecular virology in chronic hepatitis B: genotypes

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Different hepatitis B virus genotypes have different geographical distributions, natural disease progression, risk of hepatocellular carcinoma and treatment responses. This article summarizes the recent literature in this area.

Chronic hepatitis B is a common problem worldwide. It may progress to liver cirrhosis with the subsequent development of complications such as ascites, hepatic encephalopathy, variceal haemorrhage and hepatocellular carcinoma. There has been substantial progress in the understanding of various aspects of hepatitis B virus (HBV) infection, including the virology, epidemiology, natural history of chronic infection and medical therapy. The objective of this article is to provide relevant clinical information regarding the different genotypes of HBV.

HBV, a member of the Hepadnaviridae family, is an enveloped, hepatotropic virus. It is a partially double-stranded DNA virus with 3200 base pairs in its genome. It replicates asymmetrically via reverse transcription of an RNA intermediate. This special genetic feature makes it more prone to mutations than other DNA viruses, but less prone than the RNA viruses. The higher mutation rate among RNA viruses is related to the lack of proofreading functions of RNA polymerases and reverse transcriptases.

HBV is classified into four major subtypes or serotypes (adr, adw, ayr, and ayw) based upon antigenic determinants of the hepatitis B surface antigen (HBsAg). HBV is also divided into 7 different genotypes (designated by capital letters A–G) according to the homogeneity of the viral sequence, which is based upon an inter-group divergence of 8% or more in the complete nucleotide sequence (Norder et al, 1994). More recently, a new genotype (H) of HBV, which is most similar to genotype F, has been reported in Central America and San Francisco (Aranz-Ruiz et al, 2002). Studies investigating the relationship between HBV serotypes and genotypes have yielded incomplete results because of the limited number of isolates analyzed. In addition, the same HBV serotype may be classified into different HBV genotypes. Many studies have been carried out concerning different genotypes of HBV, and their relationship to geographical distribution, ethnicity correlation, clinical behaviour and response to therapy will be discussed.

LABORATORY ASSAYS FOR HBV GENOTYPING

Although HBV genotyping is defined by the heterogeneity of the entire HBV genome, several signature sites can be identified at the HBV surface region (Norder et al, 1994). Using these genomic signatures, HBV genotype can be determined by either restriction fragment length polymorphism or short segment direct sequencing of the HBV surface gene (Lindh et al, 1997). HBV genotype can also be identified by a commercially available line probe assay using hybridization technology, and this gives a very high sensitivity to detect a mixed viral genotype.

GEOGRAPHICAL DISTRIBUTION OF DIFFERENT HBV GENOTYPES

The prevalence of different HBV genotypes varies geographically and is strongly associated with ethnicity (*Table 1*) (Lindh et al, 1997). Genotype A HBV is common among whites and African Americans, patients born in the United States, and those with sexually-acquired HBV infection. Genotypes B and C HBV are seen mostly in Asians, patients born outside the United States, and those with maternal-infant transmission of HBV infection. Genotype D HBV is most commonly found in the Mediterranean countries. Other HBV genotypes are less commonly found in

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different geographical locations. The difference in the geographical distribution of different HBV genotypes might partly relate to the observed difference in the natural history and disease pattern of chronic hepatitis B in different countries.

NATURAL HISTORY OF CHRONIC HEPATITIS B

Various studies have unequivocally demonstrated strong association between HBV genotype and the natural course of liver disease. Nonetheless, the basis for the difference in pathogenicity of the virus remains unclear. There is ample evidence to suggest that genotype C HBV is associated with more active and rapidly progressive liver disease than genotype B HBV. On longitudinal follow-up, genotype C HBV is associated with more aggressive disease particularly in the hepatitis Be antigen (HBeAg)-positive phase and delayed HBeAg seroconversion as compared to genotype B HBV (Chu et al, 2002; Chan et al, 2003a). Histological analyses have confirmed more severe liver damage and advanced liver fibrosis associated with genotype C HBV as compared to genotype B HBV (Chan et al, 2002; Sumi et al, 2003). Higher prevalence of liver cirrhosis is also found among patients infected with genotype C HBV *vs* those infected with genotype B HBV (Kao et al, 2000).

In a Spanish study with a prospective follow-up up to 180 months, genotype A HBV was associated with a higher rate of sustained biochemical remission, HBV DNA clearance and HBsAg clearance as compared to genotype D or genotype F HBV (Sanchez-Tapias et al, 2002). The rate of HBeAg seroconversion is not related to the HBV genotype (A, D or F). On the other hand, death related to liver disease occurs more often in patients infected with genotype F HBV than those infected with genotype A or D HBV. The clinical significance of genotype F HBV requires further validation because of the small number of patients infected with genotype F HBV in this study.

Owing to the difference in geographical distribution of HBV genotypes, there is no information on the direct comparison among HBV genotypes A, D and F *vs* genotypes B and C. There is currently a large nationwide study being conducted in the United States, including white Americans and African American as well as European and Asian immigrants. Preliminary analysis suggests that genotype B HBV is an independent factor associated with normal transaminase levels (Chu et al, 2003). Further results from follow up of these patients are awaited.

HEPATOCELLULAR CARCINOMA

Studies on the relationship between HBV genotypes and hepatocellular carcinoma have yielded conflicting results. A case-control study in Taiwan suggests that genotype B HBV is associated with development of hepatocellular carcinoma among younger patients whereas genotype C HBV is associated with hepatocellular carcinoma in older patients (Kao et al, 2000). The results of a cross-sectional controlled study in Japan as well as a prospective longitudinal study in Hong Kong suggest that genotype C HBV has higher risk of hepatocellular carcinoma than genotype B HBV (Fujie et al, 2001; Chan et al, 2004), but this association is not evident in other large cohort studies (Sumi et al, 2003). One possible explanation for the higher prevalence of hepatocellular carcinoma associated with genotype C HBV is its higher prevalence of basal core promoter mutation (A to T at nucleotide 1762 and G to A at nucleotide 1764), which is located on the X gene of the HBV genome.

Prognosis of hepatocellular carcinoma may also be related to HBV genotypes. In a Taiwanese study, patients with hepatocellular

TABLE 1.
Geographical distribution of hepatitis B virus genotypes

Hepatitis B virus genotypes	Areas of distribution
A	Northwest Europe, North America, Central Africa
B	Indonesia, China, Vietnam
C	East Asia, Korea, China, Japan, Polynesia, Vietnam
D	Mediterranean area, Middle East, India, America
E	Africa
F	American natives, Polynesia
G	United States, France
H	Central and West America

carcinoma infected with genotype C HBV had a higher tumour recurrence rate after curative resection of hepatocellular carcinoma compared with those infected with genotype B HBV (Chen et al, 2004). Nonetheless, more studies with longer follow-up are needed to clarify the impact of HBV genotype on the postoperative outcome of hepatocellular carcinoma. The relationship between HBV genotype and the outcomes of other modalities of treatment for hepatocellular carcinoma is also not certain.

RESPONSE TO ANTIVIRAL AGENTS

Interferon

Different HBV genotypes respond differently to interferon therapy. Most of the previous reports are limited by small sample size, and the results of different reports cannot be compared directly because of the heterogeneous regimens of interferon treatment in different studies. In Germany, the rate of interferon-induced HBeAg seroconversion is higher among patients infected with genotype A HBV than in those infected with genotype D HBV (37 *vs* 6%) (Erhardt et al, 2000). In Asian patients, there are consistent reports that the rate of HBeAg seroconversion is significantly higher among patients infected with genotype B HBV *vs* those infected with genotype C HBV (Wai et al, 2002). The relationship of HBV genotype and response to peginterferon, or combination treatment with peginterferon and nucleoside or nucleotide analogues awaits further studies.

Nucleoside and nucleotide analogues

Lamivudine is a nucleoside analogue with potent inhibitory effects on HBV polymerase/reverse transcriptase activity. All studies on HBV genotypes and lamivudine treatment are limited by the small number of patients and heterogeneity in duration of treatment. In general, there are controversial data on the association between HBV genotypes and treatment response. A small Italian study has revealed similar biochemical and virological responses between patients infected with genotype A and genotype D HBV (Buti et al, 2002), and no association on the sustained virological response has been found between genotype B and C HBV in another study in Hong Kong (Chan et al, 2003b).

In contrast, a Taiwanese study suggests that genotype C HBV is associated with a higher rate of post-treatment relapse than genotype B HBV among patients who have achieved lamivudine-related HBeAg seroconversion (Chien et al, 2003). Most of the existing data suggest that HBV genotypes do not have an important role in the selection of lamivudine-resistant mutants (Akuta et al, 2003), although one report suggests a difference in the mutational pattern between genotypes A and D HBV during lamivudine treatment (Zollner et al, 2004).

Adefovir dipivoxil is a prodrug of the nucleotide adefovir which has demonstrated potent antiviral efficacy in treatment-naïve patients and patients who carry the lamivudine-resistant HBV mutants. In a pooled data analysis of several phase III trials including 695 patients on adefovir dipivoxil treatment (Westland et al, 2003), the extent of HBV DNA reduction and rate of HBeAg seroconversion have been found to occur in similar proportions of patients infected with genotypes A to D HBV at the end of 48 weeks' treatment. This study does not provide any data on the durability of treatment response or the influence of HBV genotype on the development of adefovir resistance.

CONCLUSIONS

Different HBV genotypes predominate in different parts of the world and are related to ethnicity. There is growing evidence that HBV genotypes may influence the severity of liver disease and response to antiviral treatments. Further studies are warranted to confirm these observations. Currently, determination of HBV genotype is restricted as a research tool. In future, the determination of HBV genotype might be integrated into the protocol of clinical monitoring, hepatocellular carcinoma surveillance and/or pre-treatment assessment of chronic hepatitis B. HM

KEY POINTS

- Hepatitis B virus (HBV) can be divided into eight different genotypes (A–H) according to the genetic heterogeneity.
- Different HBV genotypes predominate in different geographical locations and different ethnic groups.
- Genotype C HBV is associated with more aggressive liver disease and delayed hepatitis Be antigen (HBeAg) seroconversion as compared to genotype B HBV.
- Genotypes D and F HBV are associated with more aggressive liver disease than genotype A HBV.
- The influence of HBV genotype on hepatocellular development is controversial.
- Patients infected with genotype B HBV tend to respond better to interferon treatment than those infected with genotype C HBV.
- Patients infected with genotype A HBV tend to respond better to interferon treatment than those infected with genotype D HBV.
- There is no clear association between HBV genotype and response to lamivudine and adefovir dipivoxil treatments or development of lamivudine resistance.

Conflict of interest: none.

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