

## Clinical Significance of Hepatitis B Virus Genotypes

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**T**raditionally, hepatitis B virus (HBV) is classified into 4 subtypes or serotypes (adr, adw, ayr, and ayw) based on antigenic determinants of the hepatitis B surface antigen.<sup>1</sup> These subtypes can be further classified into 9 serotypes (ayw1, ayw2, ayw3, ayw4, ayr, adw2, adw4, adr<sup>+</sup>, and adr<sup>-</sup>).<sup>2</sup> Epidemiologic studies found that the prevalence of these serotypes varies in different parts of the world. In addition, antibody to the common determinant, "a," confers protection against all serotypes. To date, there has been very little data on the clinical significance of HBV serotypes.

Advances in molecular biology techniques revealed significant diversities in sequences of HBV isolates, accounting for the allelic differences among the 4 major HBV serotypes. Based on an intergroup divergence of 8% or more in the complete nucleotide sequence, HBV can be classified into 7 genotypes A-G.<sup>3-5</sup> However, genotyping can be accomplished based on a partial sequence of the HBV genome such as the pre-S or S gene. Several methods have been used for HBV genotyping including direct sequencing, restriction fragment length polymorphism, line probe assay, and enzyme-linked immunoassay.

Contrary to hepatitis C virus genotyping, HBV genotyping is a research tool that is only beginning to gain popularity among researchers in hepatitis B. Whether HBV genotyping will constitute part of the clinical evaluation of hepatitis B patients depends on the availability of simple and inexpensive tests and the relevance of the information gained. Currently, restriction fragment length polymorphism is the most commonly used method for HBV genotyping. A line probe assay similar to that used for hepatitis C virus genotyping is also available. These assays can be easily applied in clinical diagnostic laboratories. The key issue is, does knowledge of the HBV

genotype help in patient management? The specific questions include, (1) Is there a correlation between HBV genotype and HBV replication, activity of liver disease, clinical outcome, and treatment response? (2) What is the predominant HBV genotype in each country? Is the geographical distribution of HBV genotypes related to the endemicity of HBV infection? (3) Is there a correlation between HBV genotype and risk of progression to chronic infection? (4) Does infection with one HBV genotype confer protection against infection with other HBV genotypes?

Answers to some of the questions raised are beginning to emerge but many of the answers are based on a few studies in selected patient populations. Current information on the geographical distribution of HBV genotypes is summarized in Table 1. However, existing information is incomplete. As an example, earlier studies suggested that HBV genotype A is predominant in the United States. A recent study indicated that HBV genotype G is also prevalent because it was present in 11 of 82 patients from the state of Georgia.<sup>5</sup> However, in an ongoing study involving 17 liver centers across the United States, we found all 7 HBV genotypes: A (33%), B (21%), C (34%), D (9%), E (1%), F (1%), and G (1%) (personal observations). HBV genotype A was more common among whites and African Americans, whereas genotypes B and C were predominantly found in Asian Americans.

The high prevalence of HBV genotypes B and C among Asians raise the possibility that HBV genotype may be related to the endemicity of HBV infection. To date, there has been no study on the relationship between HBV genotype and mode of transmission. One study in Switzerland found that genotype A was more common among patients with chronic hepatitis B, whereas genotype D was more prevalent among patients with resolving acute hepatitis B suggesting that HBV genotype A was associated with a higher rate of chronic HBV infection.<sup>6</sup> However, this study involved a total of 65 patients only and confounding factors such as age at infection, gender, mode of transmission, and coinfection with other hepatitis virus or human immunodeficiency virus were not analyzed.

HBV genotypes may contribute to the wide range in prevalence of HBV infection in different parts of the world through differences in rates of replication and abilities to evade immune clearance, but studies comparing

Abbreviations: HBV, hepatitis B virus; HBeAg, hepatitis B e antigen.

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**Table 1. Geographic Distribution of HBV Genotypes and Serotypes**

Genotypes	Serotypes	Distribution
A	adw2, ayw1	NW Europe, N America, Central Africa
B	adw2, ayw1	SE Asia, China, Japan
C	ayr, adr <sup>q</sup> +, adr <sup>q</sup> -, adw2	SE Asia, China, Japan
D	ayw2, ayw3	S Europe, Middle East, India
E	ayw4	Africa
F	adw4q-	American natives, Polynesia, Central and South America
G	adw2	United States, France

the replication capacity and immune response of the various HBV genotypes have not been performed. However, many studies have shown a strong relationship between HBV genotypes and mutations in the precore and core promoter regions that abolish or diminish the production of hepatitis B e antigen (HBeAg).<sup>7-10</sup> Thus, the most common precore mutation, a G to A substitution at nucleotide 1896 (G<sub>1896</sub>A), which creates a premature stop codon (eW28X) is found in association with HBV genotypes B, C, and D but not genotype A. This accounts for the preponderance of HBeAg-negative chronic hepatitis B in Southern Europe and Asia. The basis for the genotype-dependent selection of the precore G<sub>1896</sub>A mutation is related to the need to maintain base pairing of the stem-loop structure of the pregenome encapsidation sequence ( $\epsilon$ ).<sup>11,12</sup> HBV genotypes B, C, and D frequently have a T at nucleotide 1858, which is directly opposite nucleotide 1896 in the stem of  $\epsilon$ , whereas HBV genotype A usually has a C at nucleotide 1858, which forms a more stable bond with the wild type (G) rather than the variant sequence (A).<sup>13</sup>

In the last issue of HEPATOLOGY, Kato et al. explored the mechanism by which HBV genotype G, which has 2 stop codons in the precore region, maintains HBeAg production.<sup>14</sup> They found that all 4 patients with HBV genotype G, who were HBeAg positive, were also coinfecting with HBV genotype A. In one patient who seroconverted from HBeAg to hepatitis B e antibody, a shift from predominant HBV genotype A to predominant HBV genotype G was shown. Based on partial sequencing of a few clones, the investigators suggested that there is evidence of recombination between the two genotypes, but more studies are needed to confirm these findings. It would also be important to determine if patients infected with one HBV genotype can be superinfected with other genotypes and if infection with multiple HBV genotypes results in more severe liver disease.

Several studies reported a correlation between HBV genotype and HBeAg clearance. These studies, all of Asian patients, found that the prevalence of HBeAg was

higher in patients with genotype C compared to those with genotype B suggesting that HBeAg clearance occurred at higher rates among patients with genotype B.<sup>8,10,15</sup> One study of 466 Japanese patients found that HBeAg was present in 53% of genotype C versus 16% of genotype B patients.<sup>10</sup> This difference was maintained after matching for gender, age, and liver disease in the two groups. In a recent study of 269 Chinese patients, we found that spontaneous HBeAg seroconversion occurred approximately one decade earlier among patients with HBV genotype B.<sup>16</sup> We also showed that patients with genotype B were more likely to have a sustained biochemical remission after spontaneous HBeAg seroconversion.

A correlation between HBV genotype and liver disease has also been found in several studies from Asia. One study in Japan found that liver dysfunction (defined as abnormal aminotransferase levels) was observed less frequently in hepatitis B carriers with adw serotype (mainly genotype B) compared to those with adr serotype (mainly genotype C).<sup>17</sup> Another study found that hepatitis B surface antigen carriers with genotype B had lower histologic activity scores.<sup>8</sup> Two other studies involving a total of 490 Chinese patients with chronic HBV infection found that genotype C was more prevalent in patients with cirrhosis.<sup>15,18</sup> It is possible that a longer duration of high levels of HBV replication may contribute to more active liver disease and, in turn, a higher rate of progression to cirrhosis among patients with HBV genotype C. The relationship between HBV genotypes and hepatocellular carcinoma is inconclusive. One study found that HBV genotype B is associated with development of hepatocellular carcinoma at an earlier age,<sup>18</sup> but this finding was not confirmed by other studies.<sup>15,19,20</sup> The relationship between HBV genotypes and liver disease in other ethnic populations has not been examined.

HBV genotype has also been related to response to interferon therapy. One study of 64 German patients found that the rate of interferon-induced HBeAg seroconversion was higher among patients with genotype A than in those with genotype D (37% vs. 6%).<sup>21</sup> Another report involving 58 patients in Taiwan found that the rate of HBeAg loss was significantly higher in patients with genotype B compared to those with genotype C (41% vs. 15%).<sup>22</sup> A third study in 35 HBeAg-negative patients found that patients infected with HBV genotype A responded better than those with genotype D/E (70% vs. 40%).<sup>23</sup> The correlation between HBV genotype and response to other antiviral therapy (such as lamivudine) remains to be determined. One study based on 26 patients reported that patients with adw serotype were more likely to develop resistance to lamivudine than those with

ayw serotype but the correlation between serotype and response was not mentioned.<sup>24</sup>

In summary, there is growing evidence that HBV genotypes may influence HBeAg seroconversion rates, mutational patterns in the precore and core promoter regions, and the severity of liver disease. In addition, different HBV genotypes predominate in various parts of the world. Thus, the heterogeneity in disease manifestations and response to antiviral treatment among patients with chronic hepatitis B in different parts of the world may, at least in part, be attributed to differences in HBV genotypes. Further studies are needed to confirm these observations to determine if HBV genotyping should be included in the clinical evaluation of patients with chronic HBV infection and if treatment should be tailored accordingly.

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