

Genetic Diversity of Hepatitis B Virus as an Important Factor Associated with Differences in Clinical Outcomes

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(See the article by Livingston et al., on pages 5–11.)

Approximately 350 million people worldwide are persistently infected with hepatitis B virus (HBV) [1], which has been classified into 8 genotypes (A–H) by means of molecular evolutionary analyses [2–4]. HBV genotypes have different geographic distributions [5]. Genotype D is ubiquitous and scattered worldwide, whereas genotype A is prevalent in sub-Saharan Africa, North America, and Europe and genotypes B and C are common in Asia. Genotype E is mainly restricted to western Africa, and genotype F is considered to be indigenous to aboriginal populations in Central and South America [6]. In addition, a number of genotype F–specific phylogenetic clusters have been attributed to local populations in different geographic areas [7, 8], such as Argentina [9], Venezuela [10], and Central America [11], as well as in the drainage area of the Yukon and Kuskokwim Rivers in southwestern Alaska [12]. Genotype F also has

been detected sporadically in Brazil [13], North America [6, 14], Europe [8], and Polynesia [15]. Genotype H has been found in Mexico and Central America [4, 6]. Genotype G has been detected infrequently, and its epidemiologic profile is unclear.

Genotypes are further subdivided into subgenotypes, on the basis of phylogenetic relationships [16]. Subgenotype Aa/A1 has been identified in South Africa and Asia, whereas subgenotype Ae/A2 has been detected in Europe and the United States [17, 18]. Subgenotype Ac/A3 has been detected in central and western Africa [19]. Suguchi et al. [20] identified 2 subgenotypes within genotype B in Asian countries. One subgenotype (Bj/B1) is the authentic genotype B and is indigenous in Japan, whereas another subgenotype (Ba/B2) is predominant in Asian countries other than Japan and exhibits a recombination with genotype C over the precore region and core gene [20, 21]. Recently, subgenotypes have also been recognized in genotypes C and D [16, 22–24].

There is increasing evidence regarding the influence of HBV genotypes/subgenotypes on liver disease, in both acute and chronic HBV infections [5, 25]. Owing to the geographic distribution of genotype prevalence, comparative analyses have been restricted mainly to the predominant genotypes—namely, genotype B versus genotype C in some Asian countries and ge-

notype A versus genotype D in Europe and India [26–28]—although a few multinational studies also have compared >2 genotypes [29, 30]. In Asian cohorts, genotype C has been associated with a higher frequency of cirrhosis or hepatocellular carcinoma (HCC) and a weaker patient response to interferon- α -based treatment, compared with genotype B [31]. Similarly, genotype-related differences have been reported for long-term outcomes of chronic infection with HBV genotype A, D, or F [32]: rates of sustained biochemical remission and clearance of HBV DNA and hepatitis B surface antigen were significantly higher among patients infected with genotype A than among patients infected with genotype D or F. In addition, the frequency of death related to liver disease has been reported to be higher for genotype F than for genotype A or D [32].

In this issue of the *Journal*, Livingston et al. [12] report that genotype F strains were found to be significantly associated with the occurrence of HCC among Alaska Native people, compared with genotype A, B, C, or D. In a multiple logistic regression analysis of region-, sex-, and birth-adjusted cohorts, this association was still significant ($P < .001$; odds ratio, 8.9 [95% confidence interval, 4.4–17.8]). In addition, Alaska Native people infected with genotype F were younger at the time of diagnosis of HCC: the median age at diagnosis of HCC was lower for patients

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infected with genotype F than for patients infected with other genotypes (22.5 vs. 60 years, respectively; $P = .002$). These novel findings should be of interest to clinicians and researchers, especially in regions where genotype F predominates.

Differences in age at diagnosis of HCC between genotypes/subgenotypes have also been reported in Asia: younger patients in Taiwan were more likely to be infected with genotype B (Ba) than C, and subgenotype Ba was associated with the development of HCC in young patients who did not have cirrhosis [33]. Similarly, in South Africa, a 4.5-fold increased risk for HCC and a younger age at diagnosis (by 6 years) was reported among HBV carriers infected with genotype A (Aa), compared with those infected with non-A genotypes [34].

Mutations of the HBV genome in the basic core promoter (BCP) region (T1762/A1764) and a stop codon-generating mutation in the precore region (A1896) have been found to be associated with HBeAg seroconversion and with viral replication [35]. Both the BCP and precore stop codon mutations are often found in patients with advanced liver disease, such as HCC [36–38]. Beyond these mutations, a C→T mutation in the upstream regulatory sequence (C1653T) has been associated with fulminant hepatitis [39], possibly because of its location in the α box, which is a strong activating element of both enhancer II and core promoter [40]. Takahashi et al. [41] reported that the C1653T and T1753V (not T) mutations were associated with the progression of liver disease from chronic hepatitis to cirrhosis and/or HCC, and further studies have indicated that the T1653 mutation is found frequently in patients with HCC and genotype C infection [42, 43]. Associations for these mutations are being actively investigated within the context of different genotypes, which could suggest different mechanisms for genotypes A, D, and C and even their subgenotypes [42–44]. Virologic differences between genotypes and

subgenotypes have been confirmed by recent in vitro studies [45].

In Costa Rica, where HBV genotype F is the most prevalent (96%), a BCP double mutation was detected in carriers with chronic infection (41.4%) but not in patients who recovered from acute infection [46]. Livingston et al. [12] showed no association between the BCP mutation and HCC among Alaska Native people infected with genotype F; in Alaska, the prevalence of the BCP mutation among patients with HCC and genotype F infection (41%), versus that in control patients with genotype F infection but without HCC (70% including mixture), was higher than that previously reported for blood donors with genotype F infection in Argentina (33%) [9], suggesting possible differences in genotype F between different population groups. On the other hand, for genotypes A, C, and D, a significantly higher frequency of BCP mutations was observed among patients with HCC, versus that among the genotype-matched control patients. Thus, this relationship between BCP mutations and HCC may be valid for some HBV genotypes but not for genotype F. Different mechanisms and pathways might play a role in different genotypes, and this issue is far from being completely understood.

A clear association between genotype F and the 1896A precore stop codon mutation was found among Argentinean blood donors (36/48 [75%]; $P < .05$) [9]. This association was described in a previous report on HBV-infected patients of Hispanic origin in Central America [47], where most genotype F strains contain T1858. Among Alaska Native people, however, a relatively low prevalence of the precore mutation and a similar frequency of the mutation in patients with HCC (22%) and control patients (40% including mixture) were found [12]. Differences in the viral sequences of HBV genotype F in patients with HCC and those without HCC would be important to investigate, as would the search for the genetic features

of genotype F that are potentially associated with carcinogenesis.

The risk of development of chronic HBV infection is inversely related to age at time of infection, that is, to the transmission route. The risk is highest when the infection is acquired perinatally (vertical transmission) or in early childhood and decreases with age [48, 49]. This is likely to be associated with different host immunological responses to HBV infection at different ages. Different transmission patterns predominate in different countries, and little is known regarding whether these differences are associated with the specific behavior of different genotypes, particularly genotypes F and D in Alaska.

Environmental influences could also play an important role in disease outcome. Although an association with aflatoxin B₁, previously found to be associated with HCC in Africa, was not found in a study of traditional food in Alaska [50], another study has reported that at least 13% of Alaska Native people with HCC appear to have chronic hepatitis not related to HBV or hepatitis C virus infection, suggesting the possibility of some form of unrecognized chronic liver disease predisposing to HCC [51]. Additional studies are needed to investigate the possible overlap of such factors among young patients with HCC who are infected with genotype F. Furthermore, to help develop more-effective therapies, the focus of hepatitis B research should concentrate on viral, host, and environmental factors that determine clinical outcomes in patients with chronic HBV infection.

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