Classification of Hepatitis B Virus Genotype B into 2 Major Types Based on Characterization of a Novel Subgenotype in Arctic Indigenous Populations

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Hepatitis B virus genotype B (HBV/B) has been classified into 5 subgenotypes. Except for Bj/B1 in Japan, the subgenotypes (Ba/B2–B5) have undergone recombination with HBV/C in the core promoter/precore/core genomic region. Phylogenetic analyses of complete sequences show that the Arctic strains belong to a novel subgenotype (HBV/B6) without the recombination, analogous to what is seen with Bj/B1. Comparison of 50 HBV/B6 carriers from the Arctic versus 50 Bj and 50 Ba age- and sex-matched carriers from Asia revealed that clinical characteristics of HBV/B6 carriers were similar to those of Bj/B1 carriers in Japan. The results suggest that HBV/B may be classified into nonrecombinant (Bj/B1 and B6) and recombinant (Ba/B2–B5) types.

Hepatitis B virus (HBV) infection affects >350 million people and is one of the major causes of acute and chronic liver diseases in the world. The majority of acute HBV infections are self-limiting, whereas chronic HBV infection can cause chronic hepatitis (CH), liver cirrhosis (LC), or hepatocellular carcinoma (HCC). Eight major genotypes (and their subgenotypes) of HBV have been classified by molecular evolutionary analyses and have been designated “A”–“H” [1, 2].

HBV genotype B (HBV/B) can be divided into 2 major geographically distinct groups, provisionally designated “Bj” (“j” for “Japan”) and “Ba” (“a” for “Asia”) [3, 4]. Our previous molecular evolutionary analyses have shown that HBV/Ba includes, first, 3 subgenotypes B2–B4 [3, 5] and, later, a fourth, B5 [4]. Distinguishing themselves from the HBV/Bj/B1 subgenotype, the HBV/Ba/B2–B5 subgenotypes demonstrate evidence of intergenotypic recombination with HBV/C, in a genome part corresponding to the core promoter/precore/core (Cp/preC/C) genomic region [5]. Clinically, HBV/Ba infection has been found, in many Asian countries, such as Taiwan, to be associated with a higher risk of development of HCC in HBV carriers [6]. By contrast, in Japan, HCC has less commonly been associated with subgenotype Bj [7]. In addition, the prevalence of hepatitis B e antigen (HBeAg) in persons infected with HBV/Bj has been found to be lower than that in persons infected with HBV/C or HBV/Ba [7]. With regard to the geographical distribution of HBV/Bj without recombination, studies to determine whether this subtype occurs outside Japan have not been conducted.

Studies performed in several Arctic regions and countries, including Alaska, Canada, and Greenland, have indicated that HBV is also endemic in their indigenous populations. High rates of HCC have been found in Yupik Eskimos and Inuit, in both Alaska and Greenland [8]. A recent molecular epidemiological study of HBV in indigenous Alaskans has identified HBV/B in Yupik Eskimos in southwestern Alaska [9]. However, thus far there are no reports describing HBV/B-associated HCC in indigenous Alaskans. Two independent groups have identified HBV/B in hepatitis B surface antigen (HBsAg)–positive Inuits from Baker Lake, Canada, and from Greenland and have reported a low prevalence of HBeAg and low levels of HBV DNA in these HBV carriers [10]. Assuming that it is possible that HBV/B was carried from Asia to the Arctic during the time of human migration, we sought, in the present study, to determine whether the HBV/B strains found in the Arctic were similar to those endemic to Japan. Furthermore, we sought to compare clinical and virological features of the HBV/B strains circulating in the Arctic and Asia.

Materials and methods. During 2005, 31 HBV/B-infected carriers were enrolled in Alaska, 11 HBV/B carriers were en-
rolled at Sisimiut in western Greenland, and 8 HBV/B carriers were enrolled at Baker Lake, Nunavut (Canada). All samples from Alaska and Baker Lake were obtained through a serosurvey for HBV and were from participants who had an average of 20 years of follow-up. The samples from Greenland were from a serum bank created during a large population serosurvey in 1998 in Sisimiut in western Greenland. All participants were members of the indigenous populations in their respective regions. The
participants were classified into 3 clinical groups: (1) asymptomatic carriers, (2) patients with chronic liver disease, and (3) patients with LC or HCC, all of whom were diagnosed on the basis of clinical findings and diagnostic imaging. None of the participants was coinfected with either hepatitis C virus or human immunodeficiency virus. No patients had received antiviral treatment. The study protocol was approved by the ethics committees of the participating institutions, in accordance with the 1975 Declaration of Helsinki, and informed consent was obtained from each participant before they underwent any study-related procedures.

The complete sequences of HBV/B, as well as clinical data on the 8 carriers in the indigenous population of Baker Lake, have been reported elsewhere [10]. A total of 16 complete genomes (12 B6, 2 Bj/B1, and 2 Ba/B2) and 30 partial HBV genes bearing the enhancer II (EnhII)/Cp/preC/C genomic regions (nt 1611–2009) were amplified by polymerase chain reaction (PCR) using several primers sets [3]. The sequences reported herein have been deposited in the GenBank/DDBJ/EMBL databases, under the following accession numbers: Alaska433 (AB287314), Alaska419 (AB287315), Alaska427 (AB287316), Alaska416 (AB287317), Alaska2 (AB287318), AlaskaG23 (AB287319),
Greenland-1 (AB287320), Greenland-2 (AB287321), Greenland-3 (AB287322), Greenland-4 (AB287323), Greenland-5 (AB287324), Greenland-6 (AB287325), JPN Bj A44 (AB287326), JPN Bj A53 (AB287327), JPN Ba 51 (AB287328), and JPN Ba A55 (AB287329).

Available clinical and serological data on participants persistently infected with HBV/Bj and HBV/Ba (50 participants for each) were included in the study. The control specimens represent a randomized selection from samples previously investigated in our laboratory, to provide samples from individuals who are age and sex matched to the 50 HBV/B carriers from the Arctic. All HBV/Bj and 10 HBV/Ba samples were obtained from hepatitis B carriers living in Japan [11]; the remaining 40 HBV/Ba carriers were from Hong Kong and China [5, 12].

Statistical differences were evaluated by the Mann-Whitney nonparametric test, Fisher’s exact probability test, χ² test with Yates’s correction, and Student’s t test, as appropriate. P < .05 was considered to be statistically significant.

Results and discussion. A total of 42 HBV/B strains, including 31 from Alaska and 11 from Greenland, were successfully amplified in their EnhII/Cp/preC/C genomic regions, spanning 398 bp, and were compared with genotype B strains previously deposited in GenBank/DDBJ and with other selected references for other genotypes. All of these 42 Arctic HBV/B strains formed a common phylogenetic cluster along with the 8 strains from Baker Lake, with a 100% bootstrap index (data not shown). The complete genome sequences were obtained from 12 of the 42 HBV/B strains (6 of the 31 Alaskan strains and 6 of the 11 Greenland strains). As shown in figure 1a, a phylogenetic analysis of the complete genome sequences from the samples obtained from the Arctic and of those from Japan and Asia revealed 6 distinct clusters within HBV/B that were supported by a 100% bootstrap resampling index; 5 of these clusters were previously designated as “Bj/B1,” “Ba/B2,” “Ba/B3,” “Ba/B4” and “Ba/B5,” according to recent proposals regarding HBV nomenclature [2], and the sixth (unclassified) cluster consisted of the 6 HBV/B strains from Alaska, 6 HBV/B strains from Greenland, and 8 HBV/B strains from Baker Lake. This sixth cluster was tentatively designated “B6” and was closely related to HBV/Bj/B1, having bootstrap values exceeding 75%, and had a branch more distant from the ancestral point, suggesting that the group has diverged from the other subgenotypes B (i.e., B1–B5) and has evolved independently.

The most significant virological feature of the HBV/Ba/B2-B5 strains is the presence of recombination with genotype C in part of the Cp/preC/C genomic regions, whereas HBV/Bj/B1 strains have no evidence of this recombination [3]. To test for the presence of the possible intergenotypic recombination, bootscan analyses were performed with the complete genome sequences. No evidence of recombination was observed in any of the B6 strains, in contrast to what was observed for the HBV/Ba/B2-B5 strains (data not shown). The phylogenetic tree for all of the HBV strains is shown in figure 1 and was reconstructed by use of the partial genomic region corresponding to the Cp/preC/C regions (nt 1740–2443) (figure 1b). In this region, HBV/B6 strains were phylogenetically very similar to HBV/Bj strains. A clear separation of HBV/B6 and HBV/Bj/B1 strains from the HBV/Ba/B2-B5 strains, which grouped with HBV/C strains, was observed in the tree, therefore suggesting that all of the HBV/B strains may be classified into 2 major forms: “nonrecombinant” and “recombinant.” This is the first report of a novel HBV/B subgenotype without recombination that has been discovered outside Japan.

According to previous reports, HBV/Ba is associated with development of HCC in persons of younger age [6], in striking contrast with HBV/Bj in Japan. The prevalence of HBeAg has previously been demonstrated to be significantly lower in carriers of HBV/Bj than in carriers of HBV/Ba [5]. To clarify the clinical characteristics of HBV/B6, we performed an age- and sex-matched control study to examine clinical and virological differences between the 50 HBV/B6 carriers and the control subjects (consisting of 50 HBV/Bj carriers and 50 HBV/Ba carriers) [5, 11, 12]. Table 1 summarizes comparative data on HBeAg status, levels of HBV DNA, levels of alanine transaminase (ALT), and clinical findings. In the present study, the level of HBV DNA was defined as high when it was >5 log IU/mL, because this value is known to be predictive for disease progression. The prevalence of HBeAg was significantly lower in carriers of HBV/B6 or HBV/Bj than in carriers of HBV/Ba (P = .0026 and P = .0143, respectively), whereas the levels of HBV DNA and of ALT were significantly higher in carriers of HBV/Ba than in carriers of HBV/B6 or HBV/Bj (P < .001). Seroconversion, at an older age, from positivity for HBeAg to the presence of antibodies against HBeAg; high levels of DNA; and high levels of ALT may be related to the risk of more-severe liver disease [13]. Indeed, when we compared the clinical findings for the 3 HBV groups (HBV/B6 vs. Bj vs. Ba), in whom the mean age was 48 years, the proportion of asymptomatic carriers was significantly higher in the HBV/B6 group than in the HBV/Ba group (P = .0001), whereas patients with LC and/or HCC were more prevalent in the HBV/Ba group than in the HBV/B6 group or the HBV/Bj group (P < .0001 and P = .0214, respectively). These results support previous reports that HBV/Ba infection may be a risk factor for the development of HCC in Taiwanese HBV/Ba carriers <50 years of age [6]. In contrast, HBV/Bj was rarely found in Japanese patients with HCC, and HBV/Bj-associated HCC was observed mainly in elderly persons (mean age, 67 years) [5, 14].

In the participants infected with HBV/B6, we looked for mutations, particularly the basal core-promoter mutation (T1762/A1764), which previously had been found to be associated with HCC [15]. It has been reported that, in Japan, this mutation is found less frequently in HBV/Bj/B1 strains (13%) than in HBV/Ba strains (33%) [5]. In Taiwan and Hong Kong, the frequency of the T1762/A1764 mutation in HBV/Ba carriers has
Japan might be an ancestor of the novel HBV/B6 strain in the land. These results suggest that the original HBV/Bj/B1 strain in ward from northern Alaska to inhabit Arctic Canada and Greenland 1000 years ago the forefathers of present-day Inuit spread east- via the Bering Land Bridge llian ancestry. Historically, the first Eskimos migrated from Siberia to Alaska via the Bering Land Bridge many genetic characteristics with Asians and, like the Japanese indigenous population. These native peoples of the Arctic share individuals living in each of these regions are members of its same cluster, with significant bootstrap values. The majority of the Arctic and that it might have been carried by indigenous groups when they migrated to North America and Greenland.

In conclusion, we have found a novel subgenotype, “B6,” of HBV/B in indigenous Arctic populations with chronic liver disease. Our results demonstrate that clinical and virological characteristics of the HBV/B6 strain are similar to those of the HBV/Bj/B1 strain but different from those of the Ba/B2-B5 strains. We believe that the presence of the intergenotypic recombination affecting basal core-promoter and precore/core genes is a key element in these differences. Thus, we recommend that HBV/B be considered to be a genotype present in 2 major forms: a non-recombinant type (Bj/B1 and B6) and a recombinant type (Ba/B2-B5). Further prospective studies and case-control studies in the Arctic, with larger cohorts of patients infected with HBV/B6, should be designed to further evaluate the clinical manifestations of HBV/B6 infection.

Table 1. Results of age- and sex-matched case-control study of clinical differences between HBV/Bj, HBV/Ba, and HBV/B6.

<table>
<thead>
<tr>
<th>Feature</th>
<th>HBV/B6 (n = 50)</th>
<th>HBV/Bj (n = 50)</th>
<th>HBV/Ba (n = 50)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>33 (66)</td>
<td>34 (68)</td>
<td>36 (72)</td>
<td>Matched</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>48.1 ± 19.6</td>
<td>48.1 ± 16.9</td>
<td>47.9 ± 13.1</td>
<td>Matched</td>
</tr>
<tr>
<td>Hepatitis B e antigen</td>
<td>6 (12)</td>
<td>8 (16)</td>
<td>20 (40)</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>DNA &gt;5 log copies/mL</td>
<td>9 (18%)</td>
<td>18 (36)</td>
<td>36 (72)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Alanine transaminase</td>
<td>40.3 ± 36.3</td>
<td>43.1 ± 33.4</td>
<td>94.0 ± 94.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Clinical state</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asymptomatic</td>
<td>35 (61)d</td>
<td>22 (44)</td>
<td>15 (30)</td>
<td>&lt;.02</td>
</tr>
<tr>
<td>Chronic hepatitis</td>
<td>15 (30)</td>
<td>24 (50)</td>
<td>21 (42)</td>
<td>NS</td>
</tr>
<tr>
<td>Liver cirrhosis/hepatocellular carcinoma</td>
<td>0</td>
<td>4 (8)</td>
<td>14 (28)c</td>
<td>&lt;.03</td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of participants, unless otherwise indicated. HBeAg, hepatitis B e antigen; NS, no significant difference.

a For B6 vs. Ba, P = .0026; for Bj vs. Ba, P = .0143.
b For B6 vs. Ba, P < .0001; for Bj vs. Ba, P = .0006.
c For B6 vs. Ba, P = .0006; for Bj vs. Ba, P = .0005.
d For B6 vs. Ba, P = .0001; for B6 vs. Bj, P = .0154.
e For B6 vs. Ba, P < .0001; for Bj vs. Ba, P = .0214.

been reported to be 29% and 46%, respectively [15]. The present study has revealed that the prevalence of the T1762/A1764 mutation is quite low (4.0%) in HBV/B6 carriers, occurring at a frequency similar to that in age- and sex-matched HBV/Bj/B1 carriers.

The precore stop mutation (A1896) that inhibits translation of the HBeAg precursor and induces an HBeAg-negative phenotype has been reported more frequently in HBV/Bj/B1 carriers [5]. In the present study, the prevalence of the A1896 mutation was very high (88%) in HBV/B6 carriers. Thus, within the Cp/preC regions, the virological features of HBV/B6 strains were found to be similar to those of HBV/Bj/B1 strains, providing further evidence of the relatedness of these strains.

In the phylogenetic analyses, HBV/B6 was found to have 3 independent clusters, each of which was associated with 1 of the 3 groups of infected indigenous participants from the 3 geographical regions—Yupik Eskimos from Alaska in the United States, Inuit from Baker Lake in Canada, and Inuit from Sisimiut in western Greenland—but they all were found to belong to the same cluster, with significant bootstrap values. The majority of individuals living in each of these regions are members of its indigenous population. These native peoples of the Arctic share many genetic characteristics with Asians and, like the Japanese people, are believed to have originated from peoples of Mongol- an ancestry. Historically, the first Eskimos migrated from Sib-eria to Alaska via the Bering Land Bridge ~10,000 years ago, and 1000 years ago the forefathers of present-day Inuit spread eastward from northern Alaska to inhabit Arctic Canada and Greenland. These results suggest that the original HBV/Bj/B1 strain in Japan might be an ancestor of the novel HBV/B6 strain in the

References


