RELATIONSHIP OF GENOTYPE RATHER THAN RACE TO HEPATITIS B VIRUS PATHOGENICITY: A STUDY OF JAPANESE AND SOLOMON ISLANDERS

NORIHIRO FURUSYO, NORIHIKO KUBO, HISASHI NAKASHIMA, KENICHIRO KASHIWAGI, AND JUN HAYASHI

Department of General Medicine, Kyushu University Hospital, Fukuoka, Japan; Department of Environmental Medicine and Infectious Disease, Faculty of Medical Sciences, Kyushu University, Fukuoka, Japan

Abstract. The aim of this study was to determine the predominant hepatitis B virus (HBV) genotype in the Solomon Islands and determine if there is any racial correlation between genotype and hepatitis B e antigen (HBeAg) production in Japanese and Melanesian individuals. A total of 403 serum samples from 206 Melanesian HBV carriers in the Solomon Islands and 197 Japanese carriers from Fukuoka (n = 106) and Okinawa (n = 91) living in Japan in 2001 were tested. The HBV genotypes of 206 Melanesian subjects were 114 with genotype C (55.3%) and 92 with genotype D (44.7%). The HBV genotypes of 197 Japanese subjects were 74 with genotype B (37.6%) and 123 with genotype C (62.4%). The total HBeAg prevalence of subjects in Fukuoka (36.8%) was significantly higher than that of subjects in Okinawa (14.3%) (P < 0.0001) and subjects in the Solomon Islands (35.0%; P = 0.0014, by the Mantel-Haenszel test). The genotype C prevalences were significantly different, ranging from 24.2% in Okinawa, to 54.4% in the Solomon Islands, to 95.3% in Fukuoka (all P < 0.0001, by chi-square test). The prevalence of HBeAg positivity was significantly higher in Melanesian genotype C subjects (42.0%) than Melanesian genotype D subjects (26.6%) (P = 0.0310). Similarly, the prevalence of HBeAg positivity was significantly higher in Japanese genotype C subjects (36.6%) than Japanese genotype B subjects (9.5%) (P < 0.0001). These findings indicate that HBV was of genotypes C and D in the Solomon Islands, and that the pathogenesis of HBV-infected patients is related to HBV genotype rather than race.

INTRODUCTION

Hepatitis B virus (HBV) infection is a global public health problem. More than 300 million HBV carriers are estimated worldwide, of whom one million die annually from HBV-related liver disease.1 Asia and the southwestern Pacific area, including Japan and the Solomon Islands, are reported to be hyperendemic for HBV infection.2–4 This virus has long been characterized into different antigenic subtypes and more recently into nucleotide divergence-based genotypes. Traditionally, HBV has been classified into four subtypes (adr, adw, ayr, and ayw) based on antigenic determinants of the hepatitis B surface antigen (HBsAg). The HBsAg subtypes show distinct geographic distribution.5 Early studies reported HBsAg subtype-related clinical differences in HBV infection.6–8 Recently, in place of the HBsAg subtype classification, HBV genotype has often been used in analysis of the clinical features of HBV infection.8–11 After the start of phylogenetic analyses based on intergroup divergence of 8% or more over the complete HBV nucleotide sequence, seven different genotypes, arbitrarily designed A-G, have been recognized.9–11 Several reports have shown geographic distribution of the genotypes, with genotypes A and D predominant in western Europe, B and C in the Far East, and F in South America.9–13 Moreover, genotype C has been reported to cause more severe liver damage and to have lower rates of hepatitis B e antigen (HBeAg) clearance, which usually indicates cessation of HBV replication and represents a later stage of chronic HBV infection, than genotype B in Japanese and Chinese patients.14–16 Reports from Spain and France found the prevalence of genotype A to be significantly higher in HBeAg-positive patients, with genotype D more prevalent in patients positive for antibody to HBeAg (anti-HBe).17,18 We previously demonstrated that the rates and ages of seroconversion from HBeAg to anti-HBe were higher and younger in patients with HBsAg subtype adw (typically genotype B) than in those with adr (typically genotype C) in Japanese patients with chronic HBV infection, suggesting that the HBeAg secretion and HBV replication were higher in genotype C than in genotype B, and that this may be the cause of the more severe pathogenesis of genotype C than genotype B.7,8,16,19

We have also reported HBV infection to be highly endemic in the Solomon Islands, which are located in the southwestern Pacific Ocean, probably due to high prevalence of HBeAg.3 The HBsAg subtype adr has been reported as being predominant in Melanesians, who account for approximately 90% of the original islander population.2,4 Although there is increasing evidence of clinical differences among HBV genotypes, most studies have compared genotype A with D or genotype B with C. No studies have compared the same genotype in different areas, the present study was done to determine the predominant HBV genotype in the Solomon Islands, and to determine if there is any racial correlation between genotype and HBeAg production in Japanese and Melanesian individuals.

MATERIALS AND METHODS

Serum samples. A total of 403 serum samples from 206 Melanesian and 197 Japanese HBV carriers who were without serologic markers of infection with hepatitis C virus or human immunodeficiency virus type 1 in 2001 were tested. The samples were from 110 male and 96 female healthy Melanesian volunteer blood donors in the Solomon Islands (age range = 16–43 years, mean ± SD = 26.0 ± 6.7 years) and 115 Japanese males (60 in Fukuoka and 55 in Okinawa) and 82 females (46 in Fukuoka and 36 in Okinawa), all residents of Fukuoka and Okinawa Prefectures, Japan (106 subjects, age range = 22–66 years, mean ± SD = 39.5 ± 13.2
years in Fukuoka and 91 subjects, age range = 10–62 years, mean ± SD = 32.5 ± 15.5 years in Okinawa). All were born and raised in these areas and voluntarily participated in the present study in response to our announcement of free health examinations (Table 1). The study protocol was reviewed and approved by the Ethics Committee of Kyushu University, and informed consent to participate in the study was obtained from all adult participants and from the patients or legal guardians of minors.

The Solomon Islands carrier group includes 206 islanders of Melanesian ancestry from Guadalcanal Island, which is principal one in the Solomon Islands. The Solomon Islands, located in the tropical southwestern Pacific Ocean (5–12°S, 156–170°E), are part of the great island chain that extends eastward from southeast Asia north of Australia to New Caledonia and are comprised of seven provinces made of the principal islands and islands groups. Ethnographic and demographic data were determined by interviews conducted in the native language or in Melanesian pidgin, as described previously.4

The Japanese carrier group includes 106 participants from Fukuoka Prefecture and 91 from the Yaeyama District of Okinawa Prefecture. Fukuoka Prefecture is located in the northern part of Kyushu Island, the most southern of the main islands of Japan.16 Okinawa is located in the subtropical zone approximately 1,000 km south of the main islands of Japan, which lie in the temperate zone. The Yaeyama District, with nine inhabited islands, is situated in the southwest part of Okinawa and is close to Taiwan.8,16,19,21

**Serologic assays.** All serum samples were separated and stored at -20°C until testing for HBsAg, HBeAg, HBsAg subtype, and HBV genotype. The HBsAg subtype was determined by enzyme immunoassay (ELISA) using monoclonal antibodies (HBsAg Subtype EIA; Institute of Immunology, Tokyo, Japan). The presence of HBeAg was determined by an enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies labeled with horseradish peroxidase using commercial kits (HBV Genotype EIA, Institute of Immunology, Tokyo, Japan).22,23

**Genotyping of HBV.** The HBV genotypes were determined by an enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies (mAb) against epitope sequences in the hepatitis B virus (HBV) preS2, core, and X) and two enhancer elements (Enh I and Enh II). The mAbs were raised against genotype-specific epitopes in the preS2 region of HBV and labeled with horseradish peroxidase using commercial kits (HBV Genotype EIA, Institute of Immunology, Tokyo, Japan).22,23

**TABLE 1**

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>No. tested</th>
<th>Melanese (Solomon Islands)</th>
<th>No. tested</th>
<th>Japanese (Fukuoka)</th>
<th>No. tested</th>
<th>Japanese (Okinawa)</th>
<th>No. tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–19</td>
<td>42</td>
<td></td>
<td>0</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20–29</td>
<td>99</td>
<td></td>
<td>16</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30–39</td>
<td>56</td>
<td></td>
<td>24</td>
<td>23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>9</td>
<td></td>
<td>28</td>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–59</td>
<td>0</td>
<td></td>
<td>29</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>0</td>
<td></td>
<td>9</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td></td>
<td>106</td>
<td>91</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* HBV = hepatitis B virus.

Briefly, HBsAg in sera was captured in wells of a microtiter plate coated with mAbs 3207 and 5124A, both directed to the common determinant epitope a, and tested for binding with genotype-specific monoclonal antibodies labeled with horseradish peroxidase (mAb 5520, epitope b; T2741, m; K0610A, k; 4408, s; 3465, u; 5142A, f; and 5156, g) (Figure 1). From the amino acid sequence found by reaction with mAbs, genotypes A to F were determined: b, and for genotype A; b and m for B; b, k, and s for C; b, k, s, and u for D, b, k, s, u, f, and g for E; and b, k, and f for F. Genotype G was determined by the combination of the above preS2-based ELISA genotype kits for genotype D and HBsAg subtype adw.24

**Statistical analysis.** Statistical analysis was done with BMDP statistical software for the IBM (Yorktown Heights, NY) 3090 system computer (BMDP Statistical Software, Inc., Saugus, MA). Continuous data were expressed as the mean ± SD. An unpaired t-test and the Mann-Whitney U test were used to compare the means of samples between the two groups. The chi-square test or Fisher’s exact test was used for categorical variables for comparisons between the two groups. Because there were significant differences in age between the subjects of the studied areas, we used the Mantel-Haenszel test for differences in the HBeAg prevalences of the HBV carriers from these different areas. A P value < 0.05 was considered statistically significant.

**RESULTS**

**Age-specific HBeAg prevalences by geographic region.** The age-specific prevalence of HBeAg of all 403 studied individuals is shown in Figure 2. The HBeAg prevalences by age in the Solomon Islands subjects were 10–19 years old, 45.2% (19 of 42); 20–29 years old, 38.4% (38 of 99); 30–39 years old, 23.2% (13 of 56); and 40–49 years old, 22.2% (2 of 9). The Okinawa prevalences were 10–19 years old, 66.7% (4 of 6); 20–29 years old, 23.5% (3 of 12); 30–39 years old, 13.0% (3 of 23); 40–49 years old, 9.1% (2 of 22); 50–59 years old, 5.0% (1 of 20); and ≥ 60 years old, 0% (0 of 8). The Fukuoka prevalences were 20–29 years old, 62.5% (10 of 16); 30–39 years old, 58.3% (14 of 24); 40–49 years old, 32.1% (9 of 28); 50–59 years old, 20.7% (6 of 29); and ≥ 60 years old, 0% (0 of 9). The HBeAg prevalences decreased with age in each region. Because the mean ± SD ages were different in the Solomon Islands (26.0 ± 6.7 years), Fukuoka (39.5 ± 13.2 years), and Okinawa (32.5 ± 15.5 years), we used the Mantel-Haenszel test to analyze the differences of the total HBeAg

**FIGURE 1.** Relationship between genotype-determined epitopes and the amino acid sequence of the hepatitis B virus (HBV) preS2 region product.
the Solomon Islands and Japan (Fukuoka and Okinawa). Dashed line = HBV carriers in the Solomon Islands; solid line with closed circles = HBV carriers in Fukuoka; solid line with open circles = HBV carriers in Okinawa.

prevalence of these areas. The total HBBeAg prevalence of Fukuoka subjects (39 of 106, 36.8%) was significantly higher than that of Okinawa subjects (13 of 91, 14.3%) ($P < 0.0001$) and the Solomon Islands subjects (72 of 206, 35.0%) ($P = 0.0014$). There was no statistically significant difference in prevalence between Okinawa and the Solomon Islands ($P = 0.3925$). No significant difference was found in sex-specific prevalence of HBBeAg in each region.

Genotype distribution of HBV. The distribution of HBV genotype in the Solomon Islands and Japan is shown in Table 2. The HBV genotypes of 206 Melanesian subjects from the Solomon Islands were 114 with genotype C (55.3%) and 92 with genotype D (44.7%). No other genotypes were found in our Melanesian patients. The HBV genotypes of 197 Japanese subjects were 74 with genotype B (37.6%) and 123 with genotype C (62.4%): 5 with genotype B (4.7%) and 101 with genotype C (95.3%) in 106 Fukuoka subjects and 69 with genotype B (75.8%) and 22 with genotype C (24.2%) in 91 Okinawa subjects. The vast majority were genotype B in Okinawa and genotype C in Fukuoka. No other genotypes were found in our Japanese subjects. The genotype C prevalences were significantly different, ranging from 24.2% in Okinawa, to 54.4% in the Solomon Islands, to 95.3% in Fukuoka (Okinawa versus Solomon Islands, Solomon Islands versus Fukuoka, Okinawa versus Fukuoka; all $P < 0.0001$, by chi-square test), which reflected the HBBeAg prevalence differences by region.

Differences between Japanese patients in Okinawa and Fukuoka by HBV genotype. Among 91 Okinawa subjects, genotype B subjects had a significantly lower rate of HBBeAg positivity (7 of 69, 10.1%) than genotype C subjects (7 of 22, 31.7%) ($P = 0.0359$). The 106 Fukuoka subjects had similar characteristics to those of Okinawa; HBBeAg positivity was 0% (0 of 5) in those with genotype B versus 37.6% (38 of 101) in those with genotype C. No significant difference was observed in male-to-female ratio or mean age between the genotype-classified groups between Okinawa and Fukuoka. These findings showed similar clinical outcome for subjects with the same HBV genotype in Okinawa and Fukuoka.

Prevalence of HBBeAg classified by HBV genotype. The characteristics of all 403 subjects with HBV infection are shown in Table 3. As reported earlier in this study, the clinical state in Japanese subjects with chronic HBV infection depended mainly on HBV genotype, not on any geographic difference. Therefore, we pooled the two Japanese populations (Okinawa and Fukuoka) in Table 3. No significant difference was observed in male-to-female ratio or mean age between the genotype-classified groups of Japanese or in Melanesians. The major HBsAg subtype was adw (63.5%) in the Japanese genotype B group, adr (80.5% and 81.3%) in the Japanese and Melanesian genotype C groups, and ayr (91.5%) in Melanesian genotype D group. Several HBsAg subtypes and an undetermined subtype were found in each genotype group in both the Japanese and Melanesians individuals. The prevalence of HBBeAg positivity was significantly higher in Melanesian genotype C subjects (42.0%) than in Melanesian genotype D subjects (26.6%) ($P = 0.0310$, by chi-square test). Similarly, the prevalence of HBBeAg positivity was significantly higher in Japanese genotype C subjects (36.6%) than in Japanese genotype B subjects (9.5%) ($P < 0.0001$, by chi-square test).

Differences in the preS2 epitope reaction in the HBV genotype C between subjects in the Solomon Islands and Japan. As mentioned in the Materials and Methods, the HBV genotype is determined from the pattern of reaction with mAb (epitope) in the preS2 region. The pattern of preS2 epitope reaction in genotype C subjects in the Solomon Islands and Japan is shown in Table 4. The distribution of preS2 epitope pattern was 13 bks (11.6%), 25 bks-fg (22.3%), 73 bks-f (65.2%), and 1 bks-g (0.9%) in the Solomon Islanders with genotype C, and 5 bks (4.1%), 109 bks-fg (88.6%), 8 bks-f (6.5%), and 1 bks-g (0.9%) in Japanese with genotype C. The epitope pattern differed between Melanesians and Japanese infected with genotype C. The epitope reaction for bks-f was found most frequently in the Solomon Islands subjects, whereas the bks-fg epitope combination was found most frequently in Japanese subjects. When classified by the combination of preS2 epitope, the prevalences of HBBeAg were 7.7% (1 of 13) in bks, 56.0% (14 of 25) in bks-fg, 42.5% (31 of 73) in bks-f, and 0% (0 of 1) in bks-g among the Solomon Islands subjects, and 20.0% (1 of 5) in bks, 38.6% (42 of 109) in bks-fg, 37.5% (3 of 8) in bks-f, and 0% (0 of 1) in bks-g among the Japanese subjects. There was no significant difference in HBBeAg prevalence by preS2 epitope reaction between the Solomon Islands and Japan.

**DISCUSSION**

We previously reported that HBV infection was highly endemic in the Solomon Islands population, the major HBsAg subtype was adw, and that HBBeAg positivity rate was markedly high, suggesting a correlation between HBV endemicity...
and HBsAg subtype. Although HBsAg subtypes adw and adr are generally associated with HBV genotypes B and C, respectively, the subtypes do not necessarily parallel the genotypes. In addition, some cases of undetermined subtype found in HBV infection. In Asian patients, including Japanese, genotype C is more likely to result in HBeAg positivity and severe liver damage than genotype B. Genotypes C and D were found in the Solomon Islands. No racial differences in the pathogenesis of genotype C were found in a comparison of the Melanesian and Japanese subjects in the present study.

Due to the geographic distribution pattern, HBV genotypes B and C are commonly observed in south Asia and the Far East. These two genotypes are found in Japanese HBV-infected patients, with genotype D found infrequently in Japan. The distribution of HBV genotypes in the southwestern Pacific Ocean countries is unknown. The present study showed that genotypes C and D were observed in the Solomon Islanders, although this was expected. Data of our previous report showed the major HBsAg subtypes to be adw and ayw. The population of the Solomon Islands is a mixture of ethnic groups: Melanesian, Polynesian, and Micronesian. Our recent survey showed that a majority (92.9%) of the Solomon Islanders and Japanese, we cannot thoroughly address the validity of conclusions that can be drawn from such samples. However, to the best of our knowledge, there have been no reports, such as ours, regarding the investigation of the relationship between HBV genotype and HBeAg prevalences comparing such different areas and races.

Recently, data has been accumulating regarding the characterization of specific viral variants and their clinical significance. For example, Sugauchi and others reported two subgroups of the same HBV genotype B, one of which possessed the recombination with genotype C over the precore region plus core gene and was more prevalent in HBsAg, and the other that did not. The former was found in non-Japanese Asians, and the latter only in Japanese individuals. In the present study, HBV genotyping was done with a serologic ELISA with mAbs against seven genotypic epitopes in the preS2 gene product of HBsAg. Genotypes deduced from serotypes, determined by the assay, are consistent with genotypes defined by nucleotide sequences. We found differences of preS2 epitope reaction in genotype C between subjects in the Solomon Islands and Japan, suggesting the presence of nucleotide sequence differences. Parts of the preS region contribute the most variable part of the HBV genome. This region is important for virus attachment and cell entry. To determine the correlations between race and genome differences, further study is needed to investigate different HBsAg

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Melanesian genotype (n = 112)</th>
<th>Japanese genotype (n = 74)</th>
<th>Combined (n = 186)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, no. (%)</td>
<td>64 (57.1)</td>
<td>45 (61.5)</td>
<td>109 (58.1)</td>
</tr>
<tr>
<td>Age (mean ± SD years)</td>
<td>27.7 ± 7.1</td>
<td>24.9 ± 6.2</td>
<td>26.5 ± 6.8</td>
</tr>
<tr>
<td>HBsAg subtype, no. (%)</td>
<td>adr 91 (81.3)</td>
<td>0</td>
<td>91 (48.6)</td>
</tr>
<tr>
<td></td>
<td>adw 0</td>
<td>47 (63.5)</td>
<td>47 (63.5)</td>
</tr>
<tr>
<td></td>
<td>adwr 3 (2.7)</td>
<td>0</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td></td>
<td>adyr 3 (2.7)</td>
<td>0</td>
<td>3 (2.7)</td>
</tr>
<tr>
<td></td>
<td>aydw 5 (4.5)</td>
<td>0</td>
<td>5 (4.5)</td>
</tr>
<tr>
<td></td>
<td>ayr 1 (0.9)</td>
<td>0</td>
<td>1 (0.9)</td>
</tr>
<tr>
<td></td>
<td>ayw 0</td>
<td>86 (91.5)</td>
<td>86 (91.5)</td>
</tr>
<tr>
<td></td>
<td>aywr 0</td>
<td>4 (4.3)</td>
<td>4 (4.3)</td>
</tr>
<tr>
<td></td>
<td>Undetermined 9 (8.0)</td>
<td>3 (3.2)</td>
<td>12 (6.5)</td>
</tr>
<tr>
<td>HBeAg positivity no. (%)</td>
<td>47 (42.0)*</td>
<td>25 (26.6)*</td>
<td>72 (38.5)</td>
</tr>
</tbody>
</table>

* HBV = hepatitis B virus.

**Table 4**

Differences in the preS2 epitope combination in HBV genotype C between subjects in the Solomon Islands and Japan

- **Table 3**

Characteristics of 206 Melanesians and 197 Japanese infected with HBV and classified by HBV genotype

Comparison of the Melanesian and Japanese subjects in the present study showed that a majority (94.9%) of the Solomon Islanders and Japanese were Melanesians. These two genotypes are found in Japanese, genotype C is more likely to result in HBeAg positivity and severe liver damage than genotype B. Genotypes deduced from nucleotide sequence differences, further study is needed to investigate differences.
ferences in clinical features based on large numbers of patients infected with some subgroups of genotype C.

The most important findings of the present study were that HBV was of genotypes C and D in the Solomon Islands, and that the pathogenesis of HBV-infected patients is related to HBV genotype rather than race.

Received June 4, 2003. Accepted for publication October 12, 2003.

Acknowledgments: We thank Sadaka Usuda and Shunju Mishiro (Department of Medical Sciences, Toshiba General Hospital, Tokyo, Japan) and Ricky Eddie (Pathology Division, Central Hospital, Honiara, Solomon Islands) for their constant interest in and support of this work. We also thank Naoko Kimkawa for providing advice on the statistical assessments.

Authors’ addresses: Norihiro Furusyo and Jun Hayashi, Department of General Medicine, Kyushu University Hospital, Higashi-Ku, Fukuoka, 812-8582, Japan. Norihiko Kubo, Hisashi Nakashima, and, of General Medicine, Kyushu University Hospital, Higashi-Ku, Fukuoka, 812-8582, Japan.

Reprint requests: Norihiro Furusyo, Department of General Medicine, Kyushu University Hospital, Higashi-Ku, Fukuoka, 812-8582, Japan.

REFERENCES