GB VIRUS C/HEPATITIS G VIRUS INFECTION AMONG COLOMBIAN NATIVE INDIANS

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Abstract. To elucidate the prevalence of GB virus C/hepatitis G virus (GBV-C/HGV) infection in Colombian native Indians, serum GBV-C/HGV RNA was assayed in 163 native Indians and 67 members of the general population in Colombia. The native Indians (males : females = 40:123) and the members of the general population (males : females = 20:47) were tested by reverse transcription–semi-nested polymerase chain reaction. Of the 163 native Indians, 10 (6.1%) were positive for GBV-C/HGV RNA, compared with one (1.5%) of 67 from the general population. All Indians were negative for hepatitis B surface antigen and antibody to hepatitis C virus. Of 10 Indians with GBV-C/HGV RNA, the genotype of nine subjects was the Asian type. These data indicated that 1) the prevalence of GBV-C/HGV RNA in Colombian native Indians is high, and 2) GBV-C/HGV was probably brought from Asia and inherited for generations in some native Indian groups.

Recently, two flavivirus-like RNA viruses were identified independently from the patients with chronic hepatitis and were designated GB virus C (GBV-C) and hepatitis G virus (HGV), respectively. These viruses show 85.5% nucleotide identity and 100% acid identity in the nonstructural protein 3 (NS3) region. Thus, GBV-C and HGV are designated as GB virus C (GBV-C) and hepatitis G virus (HGV) in this study, according to the suggestion by Zuckermann. They were detected frequently among patients with hepatitis B virus (HBV) and/or hepatitis C virus (HCV) infections, intravenous drug users, hemodialysis patients, and also at a low incidence in volunteer blood donors.

However, there is little information on the prevalence of GBV-C/HGV, and the origin and etiology of GBV-C/HGV have been unclear.

On the other hand, in Colombian native Indians, a high prevalence of human T cell lymphotropic virus type 1 (HTLV-1) or HTLV-2 has been found and these viruses might have been brought from Asia to Colombia by the ancestors of the Indians. However, the influx of hepatitis viruses to Colombia has been unclear. We investigated the prevalence of HBV, HCV, and GBV-C/HGV infections in three separated Colombian native Indian groups, the Inga, Kamsa, and Wayuu Indians, compared with the general population in Colombia. Furthermore, molecular evolutionary analysis was performed to elucidate the origin of GBV-C/HGV.

Detection of GBV-C/HGV RNA. Serum samples from all patients were stored at −80°C until assayed. Serum RNA was extracted from 100μl of serum using the Sepa GeneRVR kit (Sanko, Tokyo, Japan), precipitated with isopropanol, and washed with ethanol. Complementary DNA (cDNA) was synthesized from the RNA samples at 37°C for 1 hr using Moloney murine leukemia virus reverse transcriptase (Gibco-BRL, Gaithersburg, MD). The GBV-C/HGV RNA was detected by a semi-nested polymerase chain reaction (PCR) with primers derived from the 5′-untranslated region (5′-UTR), as previously described with slight modifications. The primers used in this study were confirmed to be specific and highly sensitive for the sequences of all strains of GBV-C/HGV isolated worldwide.

Briefly, the first round of the PCR was performed with sense primer 5gf2 (5′-GGTGGTAGGCTGATACCTCCGGTTCA-3′) and antisense primer 5gr4-2 (5′-GCCAGCTGGACCGTACTGGCGC-3′) for 9 min at 96°C, followed by 40 cycles, consisting of denaturation for 1 min at 94°C, annealing for 1 min at 55°C, and extension for 1 min at 72°C, using a 96-well cycler (GeneAmp 9600: Perkin-Elmer Cetus, Norwalk, CT). The second round of the PCR was performed with

Subjects, Materials, and Methods

Subjects. Sixty-nine serum samples were consecutively collected, after obtaining informed consent, from three different ethnic groups in the Andes highlands of Colombia (15 samples from Inga Indians and 54 samples from Kamsa Indians), and 94 samples from another ethnic group on the Atlantic coast of Colombia (Wayuu Indians) (Figure 1). As controls excluding native Indians, 67 samples from the general population (the Mestizo of mixed blood populations) were also collected. These samples were taken in 1990 for the study of HTLV-1 and HTLV-2, and HLA-DRB1 and DBQ1 haplotypes.

FIGURE 1. Map showing the location of the three native Indian groups in Colombia.
sense primer 5gf3 (5'-TGGTAGCCACTATAGGGTGGGT-3') and anti-sense primer 5gr4-2 for 35 cycles using the same conditions as in the first round of the PCR. The amplicons were analyzed by electrophoresis on 3% agarose gels, stained with ethidium bromide, and observed under ultraviolet light.

Specificity was confirmed by direct sequencing of the amplified products using fluorescence labeled primers with a violet light.

**Measurements of HBV- and HCV-related markers.** All serum samples were analyzed for hepatitis B surface antigen (HBsAg) (AUSSRIA II; Abbott Laboratories, North Chicago, IL), antibody to hepatitis B surface antigen (anti-HBs) (Fujiirebio, Tokyo, Japan), and antibody to HCV (anti-HCV) (EIA-2, Ortho, Raritan, NJ).

**Molecular evolutionary analysis.** Molecular evolutionary analysis was performed to elucidate the relationship between the isolates in this study and previously reported GBV-C/HGV strains. The analyses were performed with version 1.1.1 of the computer program ODEN. The number of nucleotide substitutions per site between all possible pairs of these isolates were estimated by the six-parameter method. Based on these values, a phylogenetic tree was constructed by the neighbor-joining (N-J) method.

There are three major GBV-C/HGV genotypes determined by molecular evolutionary analysis of the sequences of many strains isolated from all over the world. The three major GBV-C/HGV genotypes were designated as GB type, HG type, and a new type (Asian type) in our previous study, corresponding to genotypes 1, 2, and 3, respectively, according to Muerhoff and others. We used the markers of genotypes in the tree as U36380 (GBV-C), U44402 (HGV PNF216 1), U45966 (HGV-R10291), U63715, D87255, D90600, and D90601. The isolates that were obtained from the DNA Data Bank of Japan (DDBJ), European Molecular Biology Laboratory (EMBL), and Genbank DNA databases, and the sequences obtained in this study are represented as COL36, 72, 84, 87, 92, 156, 191, 192, 195, and 200.

**Statistical analysis.** Data were analyzed by Fisher's exact test, the Mann-Whitney U test, the Kruskal-Wallis test, or analysis of variance. All tests of significance were two-tailed, with a $P$ value $< 0.05$ considered to indicate statistical significance.

**RESULTS**

The mean ± SD age of the native Indians was 31.6 ± 15.2 years, ranging from eight to 80 years old, compared with 34.0 ± 14.5 years in the general population group. The male:female ratio was 40:123, compared with 20:47, respectively (Table 1). There were no significant differences in the age and gender between these groups. Among three

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### Table 1

Characteristics and prevalence of GB virus C/hepatitis G virus (GBV-C/HGV) RNA in Colombian subjects

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Inga</th>
<th>Kamsa</th>
<th>Wayuu</th>
<th>Total</th>
<th>General population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>15</td>
<td>54</td>
<td>94</td>
<td>163</td>
<td>67</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>3:12</td>
<td>7:47</td>
<td>30:64</td>
<td>40:123</td>
<td>20:47</td>
</tr>
<tr>
<td>Mean ± SD age (years)</td>
<td>35.4 ± 14.6</td>
<td>30.9 ± 12.2</td>
<td>31.5 ± 16.9</td>
<td>31.6 ± 15.2</td>
<td>34.0 ± 14.5</td>
</tr>
<tr>
<td>HBsAg (+)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>Anti-HBs (+)</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>6 (3.7%)</td>
<td>2 (3.0%)</td>
</tr>
<tr>
<td>Anti-HCV (+)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td>GBV-C/HGV RNA (+)</td>
<td>1 (6.7%)</td>
<td>3 (5.6%)</td>
<td>6 (6.4%)</td>
<td>10 (6.1%)</td>
<td>1 (1.5%)</td>
</tr>
<tr>
<td>ALT &gt;25 (U/L)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>4 (2.5%)</td>
<td>2 (3.0%)</td>
</tr>
</tbody>
</table>

* Inga, Kamsa, and Wayuu are Colombian native Indian groups. NS = not significant; HBsAg = hepatitis B surface antigen; anti-HBs = antibody to hepatitis B surface antigen; anti-HCV = antibody to hepatitis C virus; ALT = alanine aminotransferase. $P$ value was estimated between native Indians and the general population.

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### Table 2

Profiles of GB virus C/hepatitis G virus (GBV-C/HGV) RNA-positive subjects

<table>
<thead>
<tr>
<th>Identification number</th>
<th>Ethnic group</th>
<th>Age (years)</th>
<th>Gender</th>
<th>GBV-C/HGV RNA</th>
<th>GBV-C/HGV genotype</th>
<th>HBsAg</th>
<th>Anti-HBs</th>
<th>Anti-HCV</th>
<th>ALT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>COL200</td>
<td>Inga</td>
<td>34</td>
<td>M</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>COL192</td>
<td>Kamsa</td>
<td>32</td>
<td>F</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>COL191</td>
<td>Kamsa</td>
<td>34</td>
<td>F</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>11</td>
</tr>
<tr>
<td>COL195</td>
<td>Kamsa</td>
<td>34</td>
<td>F</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>COL87</td>
<td>Wayuu</td>
<td>14</td>
<td>F</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td>COL85</td>
<td>Wayuu</td>
<td>15</td>
<td>M</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>COL84</td>
<td>Wayuu</td>
<td>16</td>
<td>M</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>8</td>
</tr>
<tr>
<td>COL36</td>
<td>Wayuu</td>
<td>19</td>
<td>M</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
</tr>
<tr>
<td>COL72</td>
<td>Wayuu</td>
<td>19</td>
<td>F</td>
<td>+</td>
<td>Asian(3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6</td>
</tr>
<tr>
<td>COL92</td>
<td>Wayuu</td>
<td>50</td>
<td>F</td>
<td>+</td>
<td>HG(2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>12</td>
</tr>
<tr>
<td>COL156</td>
<td>Mestizo</td>
<td>32</td>
<td>F</td>
<td>+</td>
<td>HG(2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>9</td>
</tr>
</tbody>
</table>

* Inga, Kamsa, and Wayuu are Colombian native Indian groups. Mestizo is the general population in Colombia. GBV-C/HGV genotype was detected by the molecular evolutionary analysis.

(1) = GBV-C/HGV genotypes designated by Muerhoff and others. HBsAg = hepatitis B surface antigen; anti-HBs = antibody to hepatitis B surface antigen; anti-HCV = antibody to hepatitis C virus; ALT = alanine aminotransferase.
Comparison of subjects with and without GB virus C/hepatitis G virus (GBV-C/HGV) RNA*

<table>
<thead>
<tr>
<th>Positive for GBV-C/HGV RNA</th>
<th>Negative for GBV-C/HGV RNA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>11</td>
<td>219</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>4:7</td>
<td>63:156</td>
</tr>
<tr>
<td>Mean ± SD age (years)</td>
<td>27.2 ± 11.3</td>
<td>32.8 ± 15.1</td>
</tr>
<tr>
<td>ALT &gt;25 (U/L)</td>
<td>0 (0%)</td>
<td>6 (2.7%)</td>
</tr>
<tr>
<td>HBsAg (+)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Anti-HBs (+)</td>
<td>0 (0%)</td>
<td>9 (4.1%)</td>
</tr>
<tr>
<td>Anti-HCV (+)</td>
<td>0 (0%)</td>
<td>1 (0.5%)</td>
</tr>
</tbody>
</table>

*NS = not significant; ALT = alanine aminotransferase; HBsAg = hepatitis B surface antigen; anti-HBs = antibody to hepatitis B surface antigen; anti-HCV = antibody to hepatitis C virus.

Figure 2. Alignment of the GB virus C/hepatitis G virus (GBV-C/HGV) 5'-untranslated region sequences. COL36, 72, 84, 85, 87, 92, 191, 192, 195 and 200 refer to the strains isolated from Colombian native Indians with GBV-C/HGV RNA in this study. COL156 was sequenced from the general population of Colombia. U36380 (GBV-C), U44402 (HGV PNF2161), U45966 (HGV-R 1029 1), D87255, and D90601 sequences were obtained from the DDBJ, EMBL, and GenBank databases. Dots indicate identical nucleotides. Stars represent consensus sequences.

Of the 163 native Indians, 10 (6.1%) were positive for GBV-C/HGV RNA (1 [6.7%] of 15 Inga Indians, 3 [5.6%] of 54 Kamsa Indians, and 6 [6.4%] of 94 Wayuu Indians) compared to one (1.5%) of 67 in the control group. The incidence of GBV-C/HGV infection in native Indians tended to be high, compared with that of the general population. However, all native Indians were negative for both HBsAg and anti-HCV, but six (3.7%) were positive for anti-HBs. In the general population group, only one was positive for anti-HCV, two (3.0%) for anti-HBs, and none for HBsAg (Table 1). Profiles of GBV-C/HGV RNA-positive subjects are shown in Tables 2 and 3. Their mean ± SD age was 27.2 ± 11.3 years, ranging from 14 to 50 years old, and the male : female ratio was 4:7. The alanine aminotransferase (ALT)
concentrations of these subjects were all normal. The GBV-C/HGV nucleotide sequences from these subjects in the 5'-UTR showed high homology, 81.5–86.1% with GBV-C (U36380) from West Africa, 88.3–94.2% with HGV (U44402) from the United States, and 90.5–97.8% with D90601 from Japan (Figure 2).

The GBV-C/HGV genotypes of these 11 subjects were obtained from phylogenetic analysis using the N-J method. As previously reported, the isolates were separated clearly into three distinct phylogenetic branches (or genotypes), tentatively named GB type (genotype 1), HG type (2), and Asian type (3) by Mukaide and others (corresponding to Muerhoff and others). Of nine native Indians with GBV-C/HGV RNA, the GBV-C/HGV genotype of eight subjects was the Asian type and one was the HG type (Table 2). One (COL156) in the general population was the HG type (Figure 3). Furthermore, the GBV-C/HGV sequences of three GBV-C/HGV RNA-positive subjects in the Kamsa Indians were very similar and were clustered by molecular evolutionary analysis (Figure 3). Furthermore, these subjects were female and their ages were similar, ranging from 32 to 34 years old.

When the subjects were stratified into two groups, those with and without GBV-C/HGV RNA, there were no significant differences in the prevalence of HBsAg/anti-HBs/anti-HCV, mean age, gender, or ALT concentrations (Table 3).

DISCUSSION

Recently, two flavivirus-like genomes designated GBV-C and HGV, which cause human hepatitis and are different from GBV-A and GBV-B, were cloned. There may be little or no association between chronic GBV-C/HGV infection and chronic liver disease, but fulminant hepatitis caused by acute GBV-C/HGV infection has been reported. Furthermore, two reports described detection of GBV-C/HGV in hepatitis-associated aplastic anemia patients. These findings indicate that GBV-C/HGV is associated not only with hepatitis but also with other serious diseases such as aplastic anemia.

It is generally believed that Colombian native Indians migrated from Asia to Colombia approximately 12,000 years ago and were isolated from other people for religious reasons. On the other hand, since HTLV-1 or HTLV-2 may have been brought from Asia to Colombia with Mongoloid migrants, GBV-C/HGV may also have been brought with them. To elucidate this hypothesis, we investigated the prevalence of GBV-C/HGV RNA in Colombia in three separated native Indian groups and phylogenetic analysis was performed.

There were several important findings in this study. First, the prevalence of GBV-C/HGV RNA in Colombian native Indians was higher than that previously reported in the gen-
eral population, but we could not find any significant differences in the prevalence of GBV-C/HGV RNA among the Colombian native Indians and the general Colombian population in this study because of a comparison among a small number of individuals. Furthermore, the genotype of most Colombian native Indians was the Asian type, which is frequently found in Asian countries. It was reported previously that the prevalence of GBV-C/HGV infection was very high in Mongolia and China, so an outbreak of GBV-C/HGV infection might have occurred among the Colombian native Indians when Mongoloid migrants migrated from Asia to Colombia. Also supportive of this hypothesis is the fact that the rates of nucleotide substitutions for GBV-C/HGV were slower than those of the other hepatitis viruses. Interestingly, the other viral markers such as HBsAg and anti-HCV were not found in these groups. These data indicate that GBV-C/HGV has been independently transmitted to the geographically separated native Indians.

Second, GBV-C/HGV infection was distributed among young people in the native Indians. It is probable that some subjects become infected with GBV-C/HGV in childhood, and some may have recovered from their infections as they aged. Furthermore, the ages of the native Indians with GBV-C/HGV RNA in each groups were similar, and the genotype determined by molecular evolutionary analysis was classified as the Asian type except in one subject (HG type). The nucleotide sequences of three subjects, especially in the Kamsa Indians, were clustered by the phylogenetic tree, so it was indicated that there might be horizontal infections of GBV-C/HGV, including contact with the same infected carrier or infection with the same contaminated parenteral source. For the detection of past GBV-C/HGV infections, a specific and sensitive antibody assay system is required. If such an antibody assay system becomes available, it will be possible to further study the natural course of infection and the possibility of intrafamilial spread, from both mother to infant and spouse to spouse.

Finally, in this study ALT concentrations of all GBV-C/HGV positive native Indians were normal (ranging from 2 to 12 U/L). The causal association between GBV-C/HGV infection and clinical hepatitis C in patients on maintenance hemodialysis. N Engl J Med 334: 1485–1490.

All nucleotide sequence data reported in this paper have now been submitted to the DDBJ, EMBL and GenBank nucleotide sequence databases.

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number of nucleotide substitutions when the rate of substitution varies with nucleotide. *J Mol Evol* 18: 414–423.


