Prevalence and Genotype of Hepatitis G Virus in Chinese Professional Blood Donors and Hepatitis Patients

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Prevalence of hepatitis G virus (HGV) was determined in a cohort of Chinese blood donors and hepatitis patients by the detection of viral RNA via reverse transcription–polymerase chain reaction. While HGV RNA was detected in only 1 of 150 healthy volunteers, the detection rate among professional blood donors was surprisingly high (21/265, 7.9%), and plasmapheresis was identified as a significant risk factor in this population. It was also shown that an elevated serum alanine aminotransferase level is not a reliable marker for HGV infection. Prevalences of HGV in patients with hepatitis C, with non-A–E hepatitis, and with hepatocellular carcinoma were relatively low (8.2%, 16.7%, and 6.1%, respectively). Striking sequence homology (>90%) shared by 5 HGV cDNA clones implicated that they belonged to the same genotype. Phylogenetic analysis of a 446-bp NS3 cDNA confirmed that this genotype was closely related to the prototype viruses.

The GB virus C (GBV-C) and hepatitis G virus (HGV) are 2 closely related isolates of a human flavivirus associated with non-A–E hepatitis [1, 2]. Hereafter, we will use the HGV nomenclature for clarity.

Phylogenetic analysis of DNA sequences has demonstrated the distinction of HGV from hepatitis C virus (HCV) [1], and prevalence studies have confirmed the disease association of this newly identified virus [2–4]. However, the transmission and clinical implications of HGV are still incompletely understood [5]. In particular, it is not clear whether the distribution of HGV infection would display unique patterns in developing countries in which other hepatitis viruses (A–E) are hyper endemic or with a large population of professional blood donors or both. Neither is it known whether the sequence of HGV isolates from these areas would display unique features. Besides, recent reports have suggested that HGV would probably account for at best a minority of non-A–E hepatitis [5, 6].

To shed light on the epidemiologic and molecular characteristics of Chinese HGV strains, we performed an HGV prevalence study in Chinese professional blood donors and hepatitis patients and a phylogenetic analysis based on the 446-bp NS3 sequence from a Chinese HGV isolate.

Materials and Methods

Patients. Serum samples were collected from 150 healthy volunteers, 265 professional blood donors, and 379 patients with hepatitis or hepatocellular carcinoma (HCC).

Volunteer and professional donors who donated their blood in 1995 to five blood banks in different geographic areas (Beijing, Henan, Hunan, and Guangdong) were selected for serum collection without a notable bias. Healthy volunteers were mainly college students and soldiers (mean age, 21 ± 3 years; many of them were from rural areas). Professional donors are mainly young peasants (mean age, 30 ± 6 years) who were paid for blood donation at least three times within 4 months. All blood donors were negative for hepatitis B virus (HBV) and HCV in a previous screen. Patient sera were collected from all available patients with hepatitis or HCC in three hospitals in Beijing, Xi’an, and Hunan.

Serum samples were stored at −70°C and shipped on dry ice to the investigators. Repeated freezing and thawing was avoided.

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Human experimentation guidelines of the People’s Republic of China Ministry of Health were followed.

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Detection of hepatitis A virus (HAV), HBV, HCV, or hepatitis E virus (HEV) infection relied on commercially available immunoas- say kits (Abbott Laboratories, Abbott Park, IL). HCC was diag- nosed by liver biopsy. Acute hepatitis was diagnosed by symptoms and blood alanine aminotransferase (ALT) level (>$200 U/L). The diagnosis of chronic hepatitis was based on persistently elevated serum ALT level (>$100 U/L) for at least 6 months.

Reverse transcription–polymerase chain reaction (RT-PCR).
HGV RNA was isolated from 200 µL of sera by use of a previously described method [4]. Reverse transcription was primed by random hexamers and catalyzed by Superscript reverse transcriptase (Life Technologies GIBCO BRL, Gaithersburg, MD). cDNA was amplified by nested primer sets HGNS3-1 and HGNS3-2. HGNS3-1 is the outer primer set with the following sequence: 5'-GACGTT- GTGAGATCCCTT-3' (antisense) and 5'-CGAATTTTCCGTT- GTACCC-3' (antisense). The sequence of the inner primer set HGNS3-2 is 5'-GGATCCCCCTTTATGGGATGCG-3' (sense) and 5'-ATGGATCCCCGAAAGGC-3' (antisense). The primer set HGNS3-3 was used for cloning of a 486-bp cDNA. The sequence of HGNS3-3 is 5'-TCGACGTGTTGAGATCCCTC-3' (sense) and 5'-CATTCTTACGAGCTAC-3' (antisense). All primers were designed to amplify sequences from the NS3 region of HGV genome. Both rounds of PCR included 35 cycles of denaturation (94°C for 45 s), annealing (52°C for 45 s), and extension (72°C for 45 s). PCR products were separated by 2% agarose gel electrophoresis, and the ethidium bromide–stained gel was visualized under UV light. Extreme caution was observed to avoid contamination and product carryover. This included separate cabinets for PCR reaction and product analysis, as well as other procedures as suggested by Roche Molecular Systems (Branchburg, NJ).

Statistical analysis. The χ² test was used for comparison of the detection rates of HGV RNA between different groups.

DNA sequencing. Double-stranded template was sequenced on both strands by the dyeoxy method by use of Sequenase 2.0 (United States Biochemicals, Cleveland) as per manufacturer’s protocol.

Phylogenetic analysis. A distance matrix tree was generated by programs in the Wisconsin package 8.1 (Genetic Computer Group, Madison, WI). The tree reconstruction was performed by use of a UPGMA algorithm.

Results

A DNA band of expected size (236 bp) was amplified from 49 samples in the first round of PCR when HGNS3-1 was used as the primer. The anticipated 217-bp product was obtained from all 49 samples and from none of the others in the second-round PCR, which used primer set HGNS3-2. The distribution of the 49 HGV-positive subjects in different populations is summarized in table 1.

To our surprise, 21 (7.9%) of 265 professional blood donors were found to be positive for HGV RNA. This rate is exceed- ingly high and merits a retrospective survey. Among the 21 HGV-positive subjects (mean age, 38 ± 13 years), 18 (85.7%) had histories of plasmapheresis. In a comparison of 82 plasmapheresis donors (mean age, 35 ± 10 years) with 183 non-

![Table 1. Prevalence of HGV viremia in blood donors and patients.](image)

<table>
<thead>
<tr>
<th>Condition</th>
<th>No. of patients</th>
<th>No. HGV RNA–positive (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy volunteer</td>
<td>150</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Professional blood donor</td>
<td>265</td>
<td>21 (7.9)</td>
</tr>
<tr>
<td>Plasmapheresis</td>
<td>82</td>
<td>18 (22.0)</td>
</tr>
<tr>
<td>Non-plasmapheresis</td>
<td>183</td>
<td>3 (1.64)</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>48</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>73</td>
<td>6 (8.2)</td>
</tr>
<tr>
<td>Hepatitis E</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Hepatitis non-A–E</td>
<td>108</td>
<td>18 (16.7)</td>
</tr>
<tr>
<td>Acute</td>
<td>41</td>
<td>6 (14.6)</td>
</tr>
<tr>
<td>Chronic</td>
<td>67</td>
<td>12 (17.9)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>49</td>
<td>3 (6.1)</td>
</tr>
</tbody>
</table>

plasmapheresis donors (mean age, 44 ± 12 years), plasmapheresis was identified as a significant risk factor for HGV infection (22.0%, P < .001). The increased risk is probably due to nosocomial transmission during plasmapheresis. No other known risk factors, such as intravenous drug use [7], sexual promiscuity, or hemodialysis [8], were identified in this popula- tion. Also noteworthy is that only 2 of 21 HGV-positive donors had slight elevations in serum ALT levels (105 and 81 U/L).

In the present study, no HGV RNA was detected in any of the patients with HAV, HBV, or HEV infection. This result is different from a previous report in which 7 (9.7%) of 72 chronic HBV patients were shown to be coinfected by HGV [2]. The difference is not accounted for by the chronicity of the disease, since all patients in both studies had chronic HBV infection. The detection rate of HGV RNA in HCV-positive persons in the present study was 8.2% (6/73), considerably lower (P = .0025) than that (18.8%, 18/96) in a previous report [2]. The positivity of HGV RNA in non-A–E hepatitis patients was 16.7%, and no significant difference was found between acutely and chronically infected patients (P > .05). This is comparable to the rate described by Linnen et al. [2] but significantly lower (P = .0029) than that in the Italian data (18/49, 36.7%) [3]. The prevalence of HGV in patients with HCC was 6.1% (3/ 49). This is remarkably lower (P < .01) than the prevalence of HBV or HCV, either in the same group (51.2% [25/49] or 30.7% [15/49]) or in similar populations described elsewhere (>40%) [9, 10]. Notably, no coinfection either by HGV and HCV or by HGV and HBV was found here, while coinfection by HBV and HCV was common (10.2%, 5/49).

We randomly selected 5 samples from 49 positive sera and cloned the 217-bp PCR products amplified from these samples. These 5 clones were definitely derived from 5 different patients, and these patients were from different geographic locations. The sequences of these cDNA clones derived from the NS3 region of the HGV genome were then determined and compared. An alignment of amino acid sequence deduced from the 5 cDNA clones is shown in
Figure 1. A, Alignment of amino acid sequence derived from 5 NS3 cDNA clones of Chinese HGV isolates (CN/WJ1–CN/WJ5). Dashes indicate identity. B, Sequence of 446-bp NS3 cDNA from 1 of Chinese HGV isolates (CN/WJ1). C, Phylogenetic tree relating 446-bp NS3 sequence from Chinese HGV isolate (designated as hgv-cn) to corresponding regions in other HGV/GBV-C, HCV, GBV-A, or GBV-B isolates. GenBank accession numbers of sequence being analyzed: hgv-cn, U67782; hgv-r10291, U45966; hgv-pnf2161, U44402; gbv-c, U36380; gbv-a, U22303; hcv-cn, S73758; gbv-b, U22304.

The homology between the different clones was 88.9%–92.1% at the DNA level and 95.8%–98.6% at the peptide level. This striking homology suggests that all of these isolates would belong to the same genotype. It would be of interest to determine the relatedness of the Chinese HGV genotype to the American prototype viruses, but our concern is that the 174-bp NS3 sequence deprived of primers is too short and would be inappropriate for phylogenetic analysis. In this regard, the phylogenetic relationship of Taiwanese, African, and American HGV isolates presented
in a previous study from Taiwan is statistically uncertain, because only a 118-bp sequence was analyzed [4].

With this in mind, we redesigned our RT-PCR to amplify a 486-bp NS3 fragment by using primer set HGN53-3. The 486-bp NS3 cDNA derived from 1 of the 5 selected positive sera was cloned and sequenced (figure 1B; GenBank accession number U67782). A phylogenetic analysis was then performed to relate the 446-bp sequence (deprived of primers) representing the Chinese HGV isolate (designated as CNWJ1) to the corresponding NS3 sequence in all GBV-C or HGV and some representative HCV, GBV-A, or GBV-B isolates deposited in the current GenBank database. As shown in the phylogram (figure 1C), the Chinese HGV genotype is more closely related to the American prototype viruses (HGV-PNF2161 and -R10291 isolates). The DNA sequence homology to HGV-PNF2161 (GenBank U44402) is 86.8% (387/446), and the peptide sequence homology is 98.0% (146/149). The DNA homology to HGV-R10291 (GenBank U45966) is 85.4% (381/446), and the peptide homology is 97.3% (145/149). The level of homology is similar to that between the HGV and GBV-C isolates (see figure 1C) [2] but much higher than that reported for the Italian and Taiwanese isolates [3, 4]. It is notable that most (>93%) changes in nucleotide sequence are silent.

Discussion

The HGV prevalence data presented here show that HGV infection was rather common in some Chinese paid blood donors. Retrospective surveys identified plasmapheresis as a significant risk factor in this population. Previous studies have also noted the increased risk for HCV infection in Chinese and Cuban plasma donors [11, 12]. This is in sharp contrast to a very low infectious risk of plasma from the United States [13] or plasma from well-developed areas in China [14], indicating that the situation would have been greatly improved if more effort had been made. Also shown in our data is that the prevalence of HGV in Chinese healthy volunteers (<1%) and even in hepatitis patients is slightly lower than those reported for the Western countries. Hence, a switch from professional to volunteer donors would be helpful to block HGV transmission.

Our results also confirm that coinfection by HCV and HGV is common. This suggests that HCV and HGV may share not only sequence homology but also epidemiologic features. However, we were unable to show coinfection with HBV and HGV, implying that either the transmission or the immune clearance of these two viruses is different, at least in certain populations.

The low incidence of active hepatitis in HGV-positive blood donors would render blood screening more difficult. It is evident from our data that blood ALT levels are an unreliable indicator of HGV infection. This also implicates that HGV infection is asymptomatic or mild but persistent. In this sense, the outcome of HGV and HCV viremia would be different. A low prevalence of HGV in HCC patients described in the present and a previous study [2] corroborates this notion. However, further investigations are required to compare the different pathogenic and oncogenic potentials of HCV and HGV and to derive some insights into the molecular mechanisms underlying this difference.

Compared with that in HCV isolates, the sequence variation between the different Chinese HGV isolates is relatively low. Also low is the divergence of the Chinese HGV sequence from that of the American viruses. On the basis of the NS3 sequence data presented here, we propose that all 4 HGV isolates (HGV-PNF2161, HGV-R10291, HGV-CNWJ1, GBV-C) belong to the same genotype. We believe that this would be the predominant or major genotype. A sequence comparison of the NS4 and NS5 cDNAs from these 4 HGV isolates also showed a high level (~90%) of homology (unpublished data). This lends further support to our proposal. The striking homology shared by HGV isolates from different geographic origins indicates that HGV is less variable than HCV [15]. The high frequency of silent changes in the HGV nucleotide sequence also suggests that the intrinsic variability of its RNA genome is physically constrained by the stable functions of its proteins. It would be of great interest to see whether the reduced variability of HGV would link to changes in pathogenesis, attenuation of virulence, or both.

Taken together, our HGV prevalence and phylogeny studies suggest that HGV infection is frequently found in certain populations in China and that the Chinese HGV isolates are less variable than HCV and are closely related to the American prototype. Our data also raise the possibility that HGV infection might be milder and less oncogenic than infection with HCV.

References

Two Major Strains of Type 1 Wild Poliovirus Circulating in Indochina

Hiromu Yoshida, Jie Li,* Tetsuo Yoneyama, Kumiko Yoshii, Hiroyuki Shimizu, Nguyen Thi Hien Thanh, Kohei Toda,* Nguyen Thanh Long, Phan Van Tu, Tatsuo Miyamura, and Akio Hagiwara

Two hundred ninety-four isolates from 329 patients with acute flaccid paralysis in Cambodia and Vietnam during 1992–1995 were identified as type 1 wild polioviruses. Among these isolates, 85 were selected as geographic representatives and were examined by determining the nucleotide sequences of their genome in the VP1 region. The phylogenetic analysis revealed that all of the isolates examined were classified into groups A and B. Isolates belonging to group A had been found only in northern Vietnam until 1993 but not in 1994 and 1995. Group B isolates were located in both northern and southern Vietnam and Cambodia. In 1994 and 1995, however, only group B isolates were found in the Mekong Delta area in southern Vietnam and Cambodia. Isolates of groups A and B were genetically different from strains previously isolated in other Asian countries. One of the two indigenous wild polioviruses still remains to be eliminated in this area.

The number of cases of poliomyelitis in the world has been drastically reduced by the extensive polio eradication program of the World Health Organization. No wild type poliovirus has been isolated since 1991 in either the North or South American continent [1]. In the Western Pacific Region of the World Health Organization, the reported number of clinically confirmed polio cases decreased from 1918 in 1992 to 426 in 1995 [2]. About 58% of the cases in 1995 were located in Vietnam and Cambodia. Strengthening of the routine immunization and adoption of nationwide extensive supplementary vaccinations (national immunization days), which had been held in China, Vietnam, Cambodia, Laos, Mongolia, and the Philippines, played a key role in this drastic reduction of poliomyelitis in this region. It appears that the eradication program is going well.

However, those data were mostly based on clinical diagnosis, and not all of the cases were confirmed by wild poliovirus isolation. According to the World Health Organization [2], 397 isolates of wild type poliovirus were reported in the Western Pacific Region during 1992–1995. Most of them were found in Cambodia, southern Vietnam, and China. Most were type 1 poliovirus, and only 17 isolates were of type 3.

Polymerase chain reaction (PCR)–based molecular epidemiology is useful for studying the circulation of poliovirus. The poliovirus genome shows the highest variation in the capsid protein region [3]. Sequence analysis of this region of the poliovirus isolates can consequently provide important clues to define the transmission of these viruses in different areas in which the virus is epidemic [4–9].

In this study, we analyzed the nucleotide sequences in the VP1 region of the genome of type 1 wild poliovirus isolates from Indochina (Vietnam and Cambodia). We then compared those sequences with those of isolates from neighboring countries in the area.

Materials and Methods

Cells and viruses. 2HEP-2 and RD cells were used for virus isolation and identification. Polioviruses were isolated from fecal samples collected from patients with acute flaccid paralysis (AFP) in Vietnam and Cambodia. All isolates were identified by the microplate method using poliovirus type–specific polyclonal anti-