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GB Virus C/Hepatitis G Virus Infection in Southern China

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The prevalence of GB virus C/hepatitis G virus (GBV-C/HGV) infection among intravenous drug users (IVDUs), patients with liver diseases, and blood donors in Nanning, southern China was studied. GBV-C/HGV RNA was detected by reverse transcription polymerase chain reaction with primers derived from the 5′-untranslated region. GBV-C/HGV RNA was detected in 64 of 85 IVDUs, 20 of 80 persons with liver disease, and 1 of 50 blood donors. Among IVDUs, GBV-C/HGV infection was associated with antibodies to hepatis C virus (HCV) and with hepatitis B surface antigen (HBsAg). Eleven nucleotide sequences were determined and analyzed by molecular evolutionary analysis. In a phylogenetic tree, the isolates were grouped in three clusters with GBV-C and HGV grouped in two clusters. These data indicate that GBV-C/HGV infection is common in China among IVDUs but uncommon among persons with liver disease without HBsAg or anti-HCV and that there is a new group of GBV-C/HGV.

Recently, two groups independently discovered a new hepatitis virus and called it GB virus C (GBV-C) and hepatitis G virus (HGV) [1, 2]. Comparison of the nucleotide sequences showed good homology. Thus, the isolates are probably from the same group of viruses [3, 4]. For simplicity, we shall call these viruses GBV-C/HGV [5]. Studies in blood product recipients have demonstrated the transmissibility of GBV-C/HGV [2]; however, the impact of GBV-C/HGV infection is not well established.

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All subjects gave informed consent, and all procedures were done according to the Declaration of Helsinki.

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The prevalence of chronic liver disease and hepatocellular carcinoma (HCC) in southern China is high [6]. Chronic hepatitis B virus (HBV) infection is very common, with 10%–15% of the population seropositive for HBV surface antigen (HBsAg), although the prevalence of hepatitis C virus (HCV) infection is low in this locality [6].

We examined the prevalence of GBV-C/HGV infection in Nanning, China. In addition, we used molecular evolutionary analysis to compare the nucleotide sequences of the 5′-untranslated subgenomic region (5′-UTR) of 11 isolates with known GBV-C and HGV sequences.

Materials and Methods

Serum samples were prospectively collected from 3 groups of Nanning residents (n = 215). Group 1 comprised 85 intravenous drug users (IVDUs) who attended IVDU clinics, group 2 consisted of 80 patients who attended liver clinics, and group 3 included 50

consecutive blood donors. So that group 2 had representatives of various stages of liver diseases, 20 consecutive patients with each of the following conditions were studied: acute hepatitis, chronic hepatitis, liver cirrhosis, and HCC.

All sera were tested by EIA (Dainabot, Tokyo) for HBsAg, antibodies to HCV (anti-HCV) and to human immunodeficiency virus (HIV), and GBV-C/HGV RNA. RNA was extracted from 100 μL of sera containing human placental RNase inhibitor (Toyobo, Osaka, Japan). cDNA was synthesized from the RNA samples by random priming using Moloney murine leukemia virus reverse transcriptase (GIBCO BRL, Gaithersburg, MD). One microliter of the cDNA was amplified by nested polymerase chain reaction (PCR) with the primers of 5′-UTR. First-round PCR was done with primer pair 5gf2 (sense, 5′-GGTTGGTAGGTCGTAAATCCCGGTCA-3′) and 5gr4 (antisense, 5′-GCGACGTGGACCGTACRTGGGCGT-3′, R = A + G). We used 1 μL of the first-round PCR product for the second-round PCR using the primer pair 5gf3 (sense, 5′-TGGTAGCCACTATAGGTGGGT-3′) and 5gr4. Amplified products were analyzed by electrophoresis on 3% agarose gels.

In 11 subjects positive for GBV-C/HGV RNA, the nested PCR products were cloned into a TA cloning vector (Invitrogen, San Diego), and one positive clone from each subject was sequenced bidirectionally using the dideoxy chain termination method with M13 primers.

The sequences obtained in this study have been deposited (DDBJ; National Institute of Genetics, Mishima, Japan). To clarify the relationship between the sequences we obtained and the known GBV-C, HGV, and HGV-R sequences based on 5′-UTR (nt 181–318, numbered according to HGV), a phylogenetic tree was constructed using the neighbor-joining method after estimating the numbers of the nucleotide substitutions at all positions by the six-parameter method of ODEN software [7–9].

Fisher's exact probability test was used for statistical determinations.

Results

All subjects were HIV-seronegative. The limited amount of serum precluded us from analyzing other HBV and HCV markers apart from HBsAg and anti-HCV. All 85 IVDUs were positive for anti-HCV, 16 were also positive for HBsAg, and GBV-C/HGV RNA was detected in 64 (75.3%). Similar proportions of IVDUs positive for HCV only and for HBsAg and anti-HCV were positive for GBV-C/HGV RNA: 52 (75.4%) of 69 and 12 (75%) of 16, respectively (table 1).

Among the 80 persons with liver disease, 60 were positive for HBsAg and negative for anti-HCV. None were negative for HBsAg and positive for anti-HCV, 7 (8.8%) were positive for both HBsAg and anti-HCV, and 13 (16.3%) were negative for both HBsAg and anti-HCV. Twenty patients were positive for GBV-C/HGV RNA. Of these 20, 2 had acute hepatitis, 5 each had chronic hepatitis and liver cirrhosis, and 8 had HCC. Among the 20 patients with GBV-C/HGV RNA, 15 were also positive for HBsAg, 2 were positive for both HBsAg and anti-HCV, and 3 (15%) were positive for GBV-C/HGV RNA alone. There was no association between the prevalence of GBV-C/HGV RNA and any diagnostic category (table 1).

Only 1 of the 50 blood donors was positive for GBV-C/HGV RNA, a significantly smaller proportion than among IVDUs and persons with liver disease ($P < .001$ for both). Only 1 blood donor was positive for HBsAg, and none was anti-HCV–positive (table 1), which is probably related to the region's blood donor screening program.

Eleven PCR amplicons were cloned and sequenced: 2 each from IVDUs and persons with chronic hepatitis and liver cirrhosis, 1 from a subject with acute hepatitis, 1 from a blood donor, and 3 from patients with HCC. When these sequences were aligned with known GBV-C, HGV, and HGV-R sequences, homologies with HGV, HGV-R, and GBV-C were 77.5%–100%.

To confirm that the sequences could be clustered, we made a phylogenetic tree using the molecular evolutionary method [7–9]. The tree showed that the clones could be divided into 3 groups. Group 1 consisted of the GBV-C sequence and CHNaLC14, group 2 included 5 sequences from China (CHNaBD16, CHNaAH40, CHHaIV6, CHNaIV8, CHNaCH2), and the remaining 5 were related to HGV and HGV-R (CHNaCH8, CHNaHCC3, CHNaHCC2, CHNaHCC59, CHNaLC89) (figure 1).

Discussion

This report is believed to be the first of GBV-C/HGV infection in southern China. We found the prevalence was significant among IVDUs and that 25% of the subjects with liver diseases were positive for GBV-C/HGV RNA and that most were also positive for HBsAg, anti-HCV, or both. Isolated GBV-C/HGV infection was uncommon. Finally, a phylogenetic tree showed that 5 of 11 GBV-C/HGV isolates clustered in a distinct phylogenetic branch different from the described GBV-C/HGV sequences, suggesting the presence of a new group of GBV-C/HGV in this locality.

GBV-C and HGV were first identified in non-A–E hepatitis patients in West Africa and the United States [1, 2]; however, a recent study detected GBV-C/HGV in 3 of 6 Japanese patients with fulminant hepatitis and in a high proportion of IVDUs [10–12]. The present study showed that GBV-C/HGV infection is also present in China. The high prevalence and coinfection with HCV or HBsAg among IVDUs suggests that GBV-C/HGV infection spread in China via the same route as HCV [13].

GBV-C/HGV infection was found in 25% of patients with liver diseases. This prevalence is much higher than among blood donors in the same geographic area. However, there may have been bias in this statistical inference, since blood donors who are found to be positive for HBsAg or for anti-HCV through screening programs are told not to donate blood. Thus, the low prevalence of GBV-C/HGV and of HBV infection may represent a selection bias. Of interest, most patients positive

Table 1. Prevalence of GBV-C/HGV RNA among intravenous drug users (IVDUs), patients with liver diseases, and blood donors in southern China.

Subject classification	No. tested	Positive for GBV-C/HGV RNA	GBV-C/HGV RNA positivity among subjects with			
			HBsAg only	anti-HCV only	Both HBsAg and anti-HCV	Negative for both HBsAg and anti-HCV
IVDUs	85	64	0	52/69	12/16	0
Liver disease	80	20	15/60	0	2/7	3/13
Acute hepatitis	20	2	2/13	0	0	0/7
Chronic hepatitis	20	5	4/14	0	1/6	0
Liver cirrhosis	20	5	4/18	0	0	1/2
Hepatocellular carcinoma	20	8	5/15	0	1/1	2/4
Blood donors	50	1	0/1	0	0	1/49
Total	215	85	15/61	52/69	14/23	4/62

NOTE. HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus.

for GBV-C/HGV were also positive for HBsAg, and HBV infection is endemic in China. GBV-C/HGV infection in the absence of HBV and HCV infection is uncommon in patients with liver diseases, which argues against an important role for GBV-C/HGV in the cause of liver disease in southern China. We found no correlation between disease severity in the 15 patients with HBsAg and GBV-C/HGV and the 45 cases with HBsAg alone. These data suggest little association between isolated GBV-C/HGV infection and manifestation of chronic liver disease, although chronic hepatitis and HCC are common in southern China.

The demonstration that all 11 clones sequenced from positive PCR amplicons had good homology with GBV-C, HGV, and HGV-R, but not with HCV or other flaviviruses or pestiviruses, confirmed the specificity of our PCR assay [3, 4]. However, it was obvious that there was significant heterogeneity within this region, suggesting that GBV-C/HGV is a heterogeneous virus. The 5′-UTR is usually quite well conserved among members of the Flaviviridae, since it commonly contains elements essential for viral replication and initiation of viral protein translation [14]. When the sequences were maximally aligned, it was obvious that CHNaLC14 had good homology with GBV-C, and

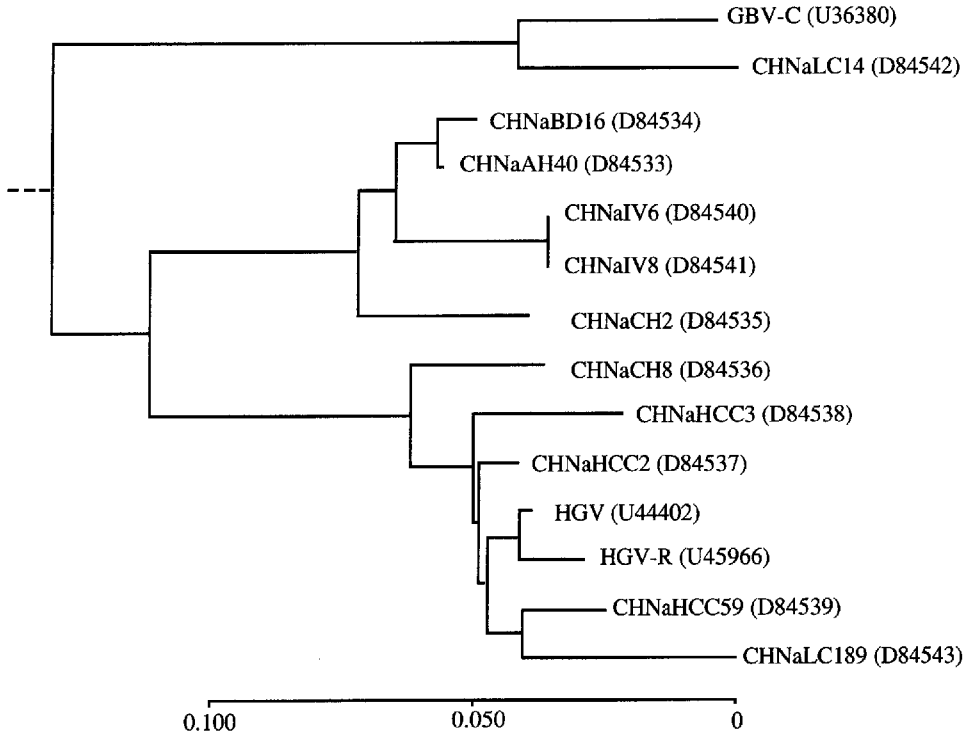

Figure 1. Phylogenetic tree of GBV-C/HGV 5′-untranslated subgenomic region. Isolates cluster into 3 phylogenetic branches: 1 clustered with GBV-C (accession no. U36380), 5 with HGV and HGV-R (accession nos. U44402 and U45966, respectively), and 5 at separate branch (CHNaBD16, CHNaAH40, CHNaIV6, CHNaIV8, CHNaCH2). Horizontal bar indicates no. of nt substitutions per site. Roots of neighbor-joining tree were tentatively taken as midpoint of longest path. Accession nos. in DDBJ/GenBank/EMBL are in parentheses.

that 5 other isolates had good homology with both HGV and HGV-R. However, GBV-C/HGV sequences from the other 5 subjects had significant differences from the HGV and GBV-C sequences. This was confirmed by construction of a phylogenetic tree, which showed that these 5 isolates clustered in a distinct phylogenetic branch. Whether these isolates belong to a different group or type requires further studies in two directions. First, other genomic areas of these isolates need to be sequenced to determine whether the phylogenetic distance occurs in other genomic regions. Second, the overall phylogenetic distance between the isolates needs to be evaluated to determine whether isolates from different phylogenetic branches should be called different subtypes or types on the basis of similar criteria for classification of HCV [15].

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