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Partial sequencing of 5'non-coding region of 7 HGV strains isolated from different areas of China

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INTRODUCTION

Although sensitive tests for detection of known hepatitis viruses are available, the etiology of 10%-15% post-transfusion and community-acquired hepatitis cases has remained undefined. It suggests the existence of unknown causative agents associated with the disease. GBV-C and HGV were newly discovered as putative non-A to E hepatitis viruses reported by Simons[1] and Linnen[2] independently. However, the sequence homology analysis of the two strains revealed that they are different isolates of the same virus. HGV is a positive-strand RNA virus with an entire genome of 10kb which contains a continuous open reading frame (ORF) encoding a viral polyprotein. The structural region (C, E1 and E2) is located at the N-terminal, while the non-structural region (NS2, NS3, NS4A/B, NS5A/5B) is situated at the C-terminal. The long ORF is preceded by a 5' untranslated sequence and followed by a 3' untranslated sequence. Our previous report has confirmed the existence of HGV infection in China[3]. There is evidence that the gene of hepatitis C virus (HCV) is hypervariable in different areas[4-8]. The variability of HCV is also found in the same strain of the virus. HGV and HCV are classified in the same genus of the flaviviridae family. So it is of great significance to clarify the geographical distribution of HGV genotypes in the world[9]. In this study, the partial sequences of 5' non-coding region of 7 HGV strains isolated from different areas of China were analyzed and compared with GBV-C (U36380) and HGV (U44402) reported from the United States.

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MATERIALS AND METHODS

Subjects

Seven HGV RNA positive sera tested by RT-PCR were collected from blood donors of Beijing, Jiangsu, Anhui, Liaoning, Hebei Provinces, and Guangxi Zhuang and Xin jiang Uighur Autonomous Regions.

Primers

According to the nucleotide sequence of 5' non-coding region of a Chinese HGV strain, the primers for RT nPCR were designed using the software of OLIGO 5.0. They were as follows: S1 5' GGT GGT GGA TGG GTG ATGAC 3'; A1 5' CCG AAG GAT TCT TGG GCT AC 3'; S2 5' GCT GGT AGG TCG TAA ATC 3'; A2 5 ' ACT GGT CCT TGT CAA CTC 3'.

Detection of HGV RNA and nucleotide sequencing

HGV RNA extraction, HGV cDNA synthesis and PCR procedure were performed by the methods described previously[3]. All the PCR products were cloned into the pGEMT vector (Promega, Madison, WI), and positive clones were identified. The PCR products were purified and sequenced bidirectionally using the dideoxynucleotide chain termination method. The HGV cDNA sequences were analyzed with a DNA sequencer (ABI PRISM 377 DNA Sequencer, Perkin-Elmer Cetus).

RESULTS

Detection of HGV RNA

The positive rates of anti-HGV varied from 1.2% (35/2916) to 5.4% (49/907) in blood donors and 42.9% (15/35) 75.5% (37/49) of anti-HGV positive sera were also HGV RNA positive.

Partial sequencing of 7 Chinese HGV strains

The partial nucleotide sequences of the 5' non-coding region of 7 HGV strains isolated from blood donors of Beijing, Jiangsu, Anhui, Liaoning, Hebei Provinces, and Guangxi Zhuang and Xinjiang Uighur Autonomous Regions, China were analyzed and compared with GBV-C (U36380) and HGV (U44401) (Figure 1).

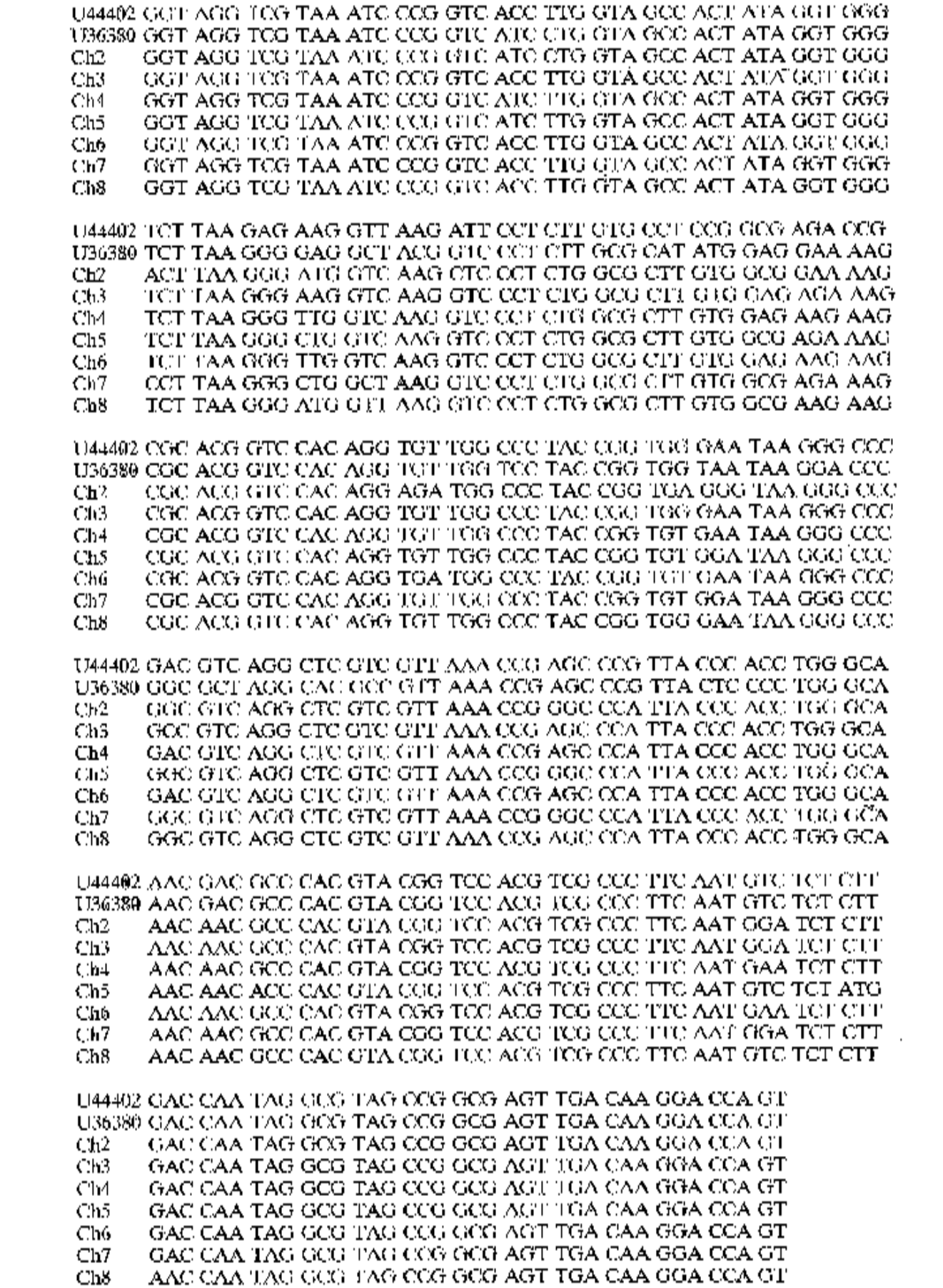

Figure 1 Comparison of partial nucleotide sequences of the 5'non-coding region of 7 HGV strains isolated from different regions of China. Ch2: Beijing; Ch3: Jiangsu; Ch4: Anhui; Ch5: Liaoning; Ch6: Guangxi; Ch7: Xinjiang; Ch8: Hebei

Table 1 Comparison of the partial nucleotides of 7 Chinese strains o f HGV with reported strains

HGV strains	Homology of the nucleotides (%)								
	U36380	U44402	Ch2	Ch3	Ch4	Ch5	Ch6	Ch7	Ch8
U36380	100.0								
U44402	87.23	100.0							
Ch2	85.92	86.85	100.0						
Ch3	88.26	92.02	93.42	100.0					
Ch4	88.26	86.67	92.96	96.24	100.0				
Ch5	85.54	89.20	92.96	93.90	94.37	100.0			
Ch6	86.85	89.67	92.96	96.24	99.06	93.43	100.0		
Ch7	85.92	89.67	94.84	97.18	95.31	96.71	95.31	100.0	
Ch8	88.26	91.55	92.02	95.30	95.31	93.70	95.31	94.37	100.0

Homology of 7 Chinese HGV strains

The nucleotide homology of the 5' non-coding region of 7 Chinese HGV strains was 85.92%, 88.26%, 88.26%, 85.45%, 86.85%, 85.92% and 88.26%, respectively, as compared with the African strain GBV-C (U36380). It was 86.85%, 92. 02%, 86.67%, 89.02%, 89.67% and 91.55%, respectively, as compared with the A merican strain HGV (U44402). The homology of nucleotide sequences was 92.02 % - 97.18 % among the 7 Chinese HGV strains (Table 1).

DISCUSSION

HGV is transmitted parenterally, and the infection seems not to cause significant hepatic damage as hepatitis viruses A E do. Although transmission through blood or parenteral exposure is well documented for HGV, little is known about its prevalence in blood donors of China. This study shows that the prevalence rate o f anti-HGV ranged from 1.2% to 5.4% in the population of different areas of C hina. The data indicate that the HGV infection is widely spread in the different areas of China. The nucleotide homology of the 5' non-coding region among the 7 Chinese HGV strains was 92.0%-97.2%. However, the identity of these 7-Chinese strains was 85.9 % - 92.0 % at the nucleotide level as compared with the African strain of GBV-C (U36380) and the American HGV strain (U44402). The data suggest that the Chinese HGV isolates belong to a new group which is different from the African and American strains reported by Simons[1] and Linnen[2]. The divergence of nucleotide sequences among Chinese HGV strains shows the correlation between HGV variation and the geographical locations.

The homology of NS3 nucleotide sequences of the 3 Chinese HGV strains reported previously by our group[3] was 92.48%, 89.09% and 85.34%, respectively with GBV-C (U36380), and 89.09%, 85.34% and 85.34% with HGV (U44402). It is very close to the homology of the 5'non-coding region of 7 Chinese HGV strains with GBV-C (U36380) and HGV (U44402), indicating that the NS3 region may not be the site of immune selection.

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