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Phylogenetic Analysis of Hepatitis G Virus by Group-Specific Sequences in the 5'-Untranslated Region

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Abstract

The nucleotide sequences of the 5'-untranslated region(5'-UTR) of Hepatitis G virus(HGV) from sera of Korean patients were determined. When compared to the previously reported isolates, the Korean isolates have higher sequence homology with the Japanese isolates indicating the geographic distribution of HGV variants. Interestingly, three discrete regions which are highly conserved among HGV isolates from the same geographical area, thus could be applied to distinguish HGV isolates from the different areas were noticed in the 5'-UTR. Based on the sequences of these group-specific regions, twenty four different HGV isolates could be classified into 5 groups. By using the group-specific regions, inconsistency in HGV typing when based on the different regions of HGV could be solved.

Key words : *GBV-C, group-specific sequences, HGV, phylogenetic analysis, 5'-UTR*

Introduction

Hepatitis G virus(HGV) is a newly identified positive-strand RNA virus belonging to the *Flaviviridae* family.[1-2] Although its genomic organization is similar to HCV, it could be clearly distinguished from HCV because it does not appear to encode a nucleocapsid or capsid protein at the 5' end of the genome. The virus infects humans and is parenterally and vertically transmitted.[3-4] The clinical significance of infection with HGV is poorly understood and its association with hepatitis remains controversial. Although relatively high frequency of HGV infection was detected in the HBV and/or HCV positive population,[5] coinfection of HGV with HBV or HCV does not seem to aggravate the clinical condition of hepatitis patients compared with HBV or HCV infection alone.[6]

Phylognetic analysis of HGV isolates based on the sequences of 5'-untranslated region(5'-UTR) demonstrated the presence of five groups which correlated with their geographic origin.[7] Similar observations were made by Fukushi *et al.*(1996),[8] who compared 5'-UTR sequences from six Japanese isolates with those from the previously known HGV isolates. Furthermore, the grouping of HGV variants based on the analysis of the entire 5'-UTR can be replaced by analysis of the shorter, 374-nucleotide region from the 5'-UTR.[9] In contrast, independent analysis of the E2, NS3, or NS5b region sequences does not identify groups of HGV variants that correlate with geographic origin, although longer coding regions can produce HGV groupings that are similar to that determined from 5'-UTR sequence analysis.[7]

In this study, we have determined the nucleotide sequences of the HGV 5'-UTR isolates from two Korean

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patients and their genotypes by comparing their sequences to those of the previously reported isolates. Also, we found that three group-specific regions which can be explored for genotyping of HGV are present in the 5' UTR of viral genome.

Materials and methods

1. Isolation of the HGV viral RNA.

The HGV RNA was isolated from sera of Korean patients with chronic infection of HCV by proteinase K/phenol-chloroform extraction method.[10]

2. RT-PCR.

Total RNA extracted from 200$\mu\ell$ of serum was reverse transcribed [1×reverse transcriptase buffer(Promega), 0.5 mM dNTP, 100 pmole antisense UTR primer(UTR-A), 250 unit of M-MLV reverse transcriptase (Promega)] in a total volume of 25$\mu\ell$ at 37 ℃ for 60 min and then inactivated at 99 ℃ for 5 min. The entire cDNA synthesis product was used in PCR. A pair of primers, 5'-UTRS(5'-ACCGACGCCTATTTAAAC-3) and 5'-UTRA(5'-CGGAGCT-GGGTGGCCCCATG-3') were designed from the conserved regions of the Japanese isolates.[8]

3. Cloning and sequencing.

PCR products were cloned into pGEM-T using a TA cloning kit(Promega) and the sequence of clones was determined using the Sequenase version 2.0 kit(Amersham) by Sanger's dideoxy chain termination method.[11] At least two clones from the same RT-PCR product were sequenced to exclude the sequence variation by PCR artifacts. For sequence homology comparison, nucleotide sequences of the clones were submitted to European Bioinformatics Institute(EBI)'s world wide web site for Fasta homology search[12] with EMBL nucleotide sequence database.

Results and discussion

To obtain Korean HGV isolates, sera from patients with HCV positive hepatitis were subjected to a RT-PCR procedure using primers derived from the conserved regions of HGV 5'-UTR.[8] We could obtain two isolates from forty specimens and determined their nucleotide sequences. The nucleotide sequences of the two Korean isolates have been deposited in the GeneBank nucleotide sequence database and indicated by their accession numbers AF030337 and AF030338, respectively. According to Muerhoff *et al.*(1996),[6] HGV isolates could be separated into five groups or genotypes by comparison of nucleotide sequences of the 5'-UTR. Based on this scheme, both Korean isolates could be assigned to Group 3 whose members were found mainly in Japan (Fig. 1). Both of the Korean isolates have above 94% sequence homology with a member of group 3 but only 85-92% with other isolates belong to groups 1a, 1b, 2a, and 3(Table 1).

Although several deletions/additions and substitutions of nucleotides are observed in the 5'-UTR of HGV, the sequence heterogeneity was confined mainly to three regions(Fig. 1). Interestingly, sequences of these regions are conserved among isolates from the same geographic areas. Therefore, these regions could be explored for HGV typing because several reports suggested that different genotypes of HGV are correlated with geographic separation.[7, 9, 13-14] Furthermore, several distinct nucleotides which could be considered as group-specific nucleotides are exist in these regions(Fig. 2). Based on the sequences of the group-specific regions, twenty four isolates including those described in this manuscript could be successfully assigned into a distinct group(Table 2). Consistently to the phylogenetic analysis of HGV isolates based on the 597 nucleotide sequences in the 5'-UTR,[7] groups 1a and 1b were found predominantly in Western Africa, while groups 2a and 2b were found in the United States and Europe. Group 3 isolates were found mainly in Asia.

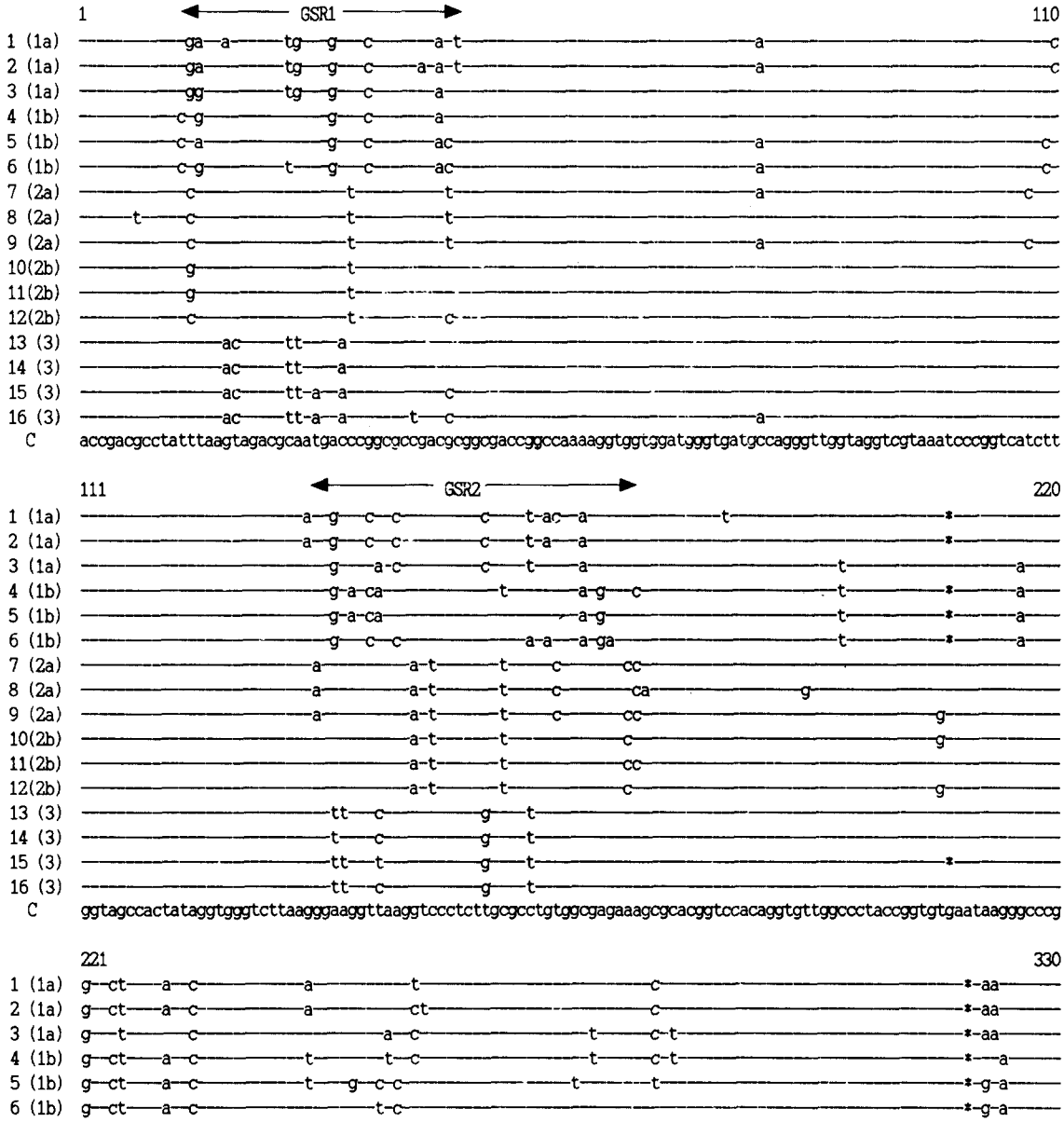

Fig. 1. Alignment of representative HGV 5'-UTR sequences. The number to the left of each sequence corresponds to the isolate described in Table 2. The genotype is shown to the right of the isolate number. The consensus sequence(C) was determined by the base that occurs most frequently at that position. Dashes(-) represent bases identical to the consensus sequence while deletions are indicated by asterisks(*). Bases are shown only at those positions in the positions that differ from the consensus. Positions of group-specific region(GSR) are indicated by arrows.

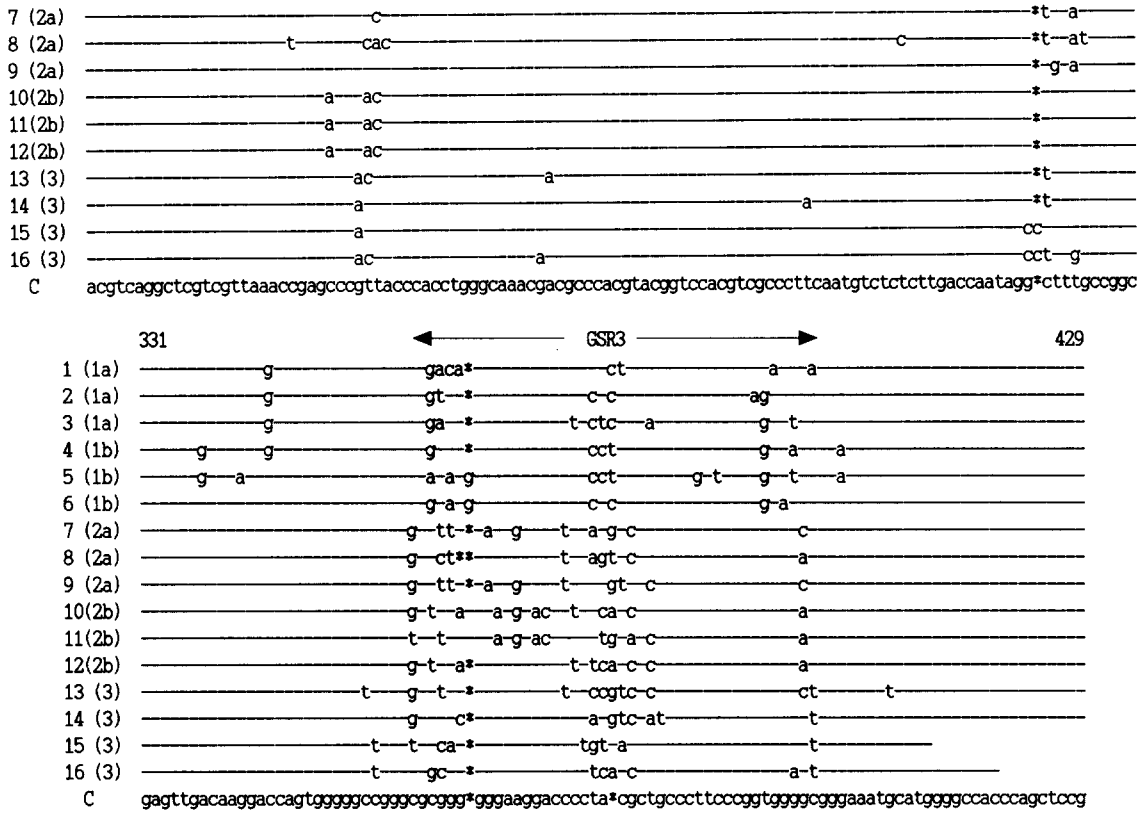

Fig. 1 계속

Table 1. Primary sequence identity(%) of the Korean isolates with various HGV isolates

Isolate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	16
15	87.4	87.9	88.8	87.7	85.3	87.0	91.1	90.2	91.4	89.7	90.9	91.6	94.2	94.8	96.7
16	89.0	88.3	89.5	87.4	86.0	88.1	91.6	90.4	90.9	91.1	91.1	92.1	94.7	94.5	

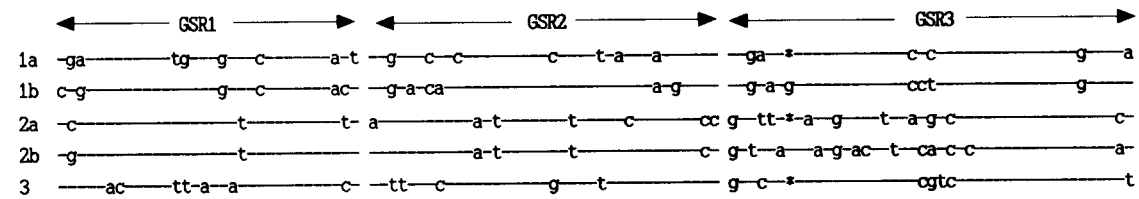

Fig. 2. The characteristic sequences of group-specific regions. The bases which can represent each genotype are indicated.

Table 2. HGV isolates, geographic origin, and grouping

Isolate	Accession number	Origin	Group	Reference
1	U59543	Ghana	1a	7
2	U59544	Ghana	1a	7
3	U59540	Ghana	1a	7
4	U59555	Ghana	1b	7
5	U59556	Ghana	1b	7
6	U36380	Ghana	1b	15
7	D87255	Japan	2a	16
8	U59518	USA	2a	7
9	U44402	USA	2a	4
10	U59533	Greece	2b	7
11	U59529	E. Africa	2b	7
12	U63715	E. Africa	2b	7
13	AF30337	Korea	3	this study
14	AF30338	Korea	3	this study
15	D87252	Japan	3	16
16	U76892	Taiwan	3	13
17	U45966	USA	2a	4
18	U75356	China	3	17
19	D87255	Japan	2a	16
20	U76894	Taiwan	3	13
21	D90601	Japan	3	18
22	D90600	Japan	2a	18
23	U59519	USA	2a	7
24	U59557	Ghana	1b	7

As several phylogenetic analysis of HGV isolates worldwide supported the geographic separation of HGV groups, it may be important to find out the geographically distinct sequences to facilitate HGV grouping. Muerhoff *et al.*[7, 9] demonstrated that HGV isolates could be classified into 5 geographic groups by their entire or shorter, 374-nucleotide region from the 5'-UTR. According to our study, sequence heterogeneity among HGV isolates from different geographic areas is confined mainly to the specific regions and HGV grouping based on these shorter sequences is consistent to that based on the entire 5'-UTR sequence. Therefore, they should be ideal sequences for genotyping of HGV. Furthermore, difficulties in HGV grouping derived from the low sequence heterogeneity among HGV isolates could be solved by using the short group-specific regions for HGV typing. The presence of group-specific sequences in the 5'-UTR may have a significant evolutionary importance. It may represent susceptible regions for mutations but cannot be tolerated by random mutations. Recent reports suggesting that parts of these regions could form specific hairpin structures composing the internal ribosome entry site(IRES)[15] imply the importance of these regions for viral replication.

References

1. Leary, T. P., Muerhoff, A. S., Simons, J. N., Pilot-Matias, T. J., Erker, J. C., Chalmers, M. L., Schlauder, G. G., Dawson, G. J., Desai, S. M., Mushahwar, I. K. : Sequence and genomic organization of GBV-C : a novel member of the falviviridae associated with human non-A-E hepatitis, *J. Med. Virol.*, **48**, 60-67(1996).
2. Simons, J. N., Leary, T. P., Dawson, G. J., Pilot-Matias, T. J., Muerhoff, A. S., Schlauder, G. G., Desai, S. M., and Mushahwar, I. K., Isolation of novel virus-like sequences associated with human hepatitis, *Nat. Med.*, **1**, 564-569(1995).
3. Feucht, H.-H., Zollner, B., Polywka, S., and Laufs, R., Vertical transmission of hepatitis G virus, *Lancet*, **347**, 615-616(1996).
4. Linnen, J., Wages, J., Zhang-Keck, Z. Y., Fry, K. E., Krawczynski, K., Alter, H., Koonin, E., Gallagher, M., Alter, M., Hadziyannis, S., Karayiannis, P., Fung, K., Naskatsuji, Y., Shih, W. K., Young, L., Piatak, M. Jr., Hoover, C., Fernandez, J., Chen, S., Zou, J, C., Morris, T., Hyams, K. C., Ismay, S., Lifson, J. D., Hess, G., Foung, S. K. H., Thomas, H., Bradley, D., Margolis, H., Kim, J. P. : Molecular cloning and disease association of hepatitis G virus : a transfusion-transmissible agent, *Science*, **271**, 505-508(1996).
5. Sugai, Y., Nakayama, H., Fukuda, M., Sawada, N., Tanaka, T., Tsuda, F., Okamoto, H., Miyakawa, Y., Mayumi, M. : Infection with GB virus C in patients with chronic liver disease, *J. Med. Virol.*, **51**, 175-181(1997).

6. Berg, T., Dirla, U., Naumann, U., Heuft, H. G., Kuther, S., Lobeck, H., Schreier, E., and Hopf, U., Responsiveness to intergerone alpha treatment in patients with chronic hepatitis C coinfected with hepatitis G virus, *J. Hepatol.*, **25**, 763-768(1996).
7. Muerhoff, A. S., Simmons, J. N., Leary, T. P., Erker, J. C., Chalmers, M. L., Pilot-Matias, T. J., Dawson, G. J., Desai, S. M., and Mushahwar, I. K., Sequence heterogeneity within the 5'-terminal region of the hepatitis GB virus C genome and evidence for genotypes, *J. Hepatol.*, **25**, 379-384(1996).
8. Fukushi, S., Kurihara, C., Ishiyama, N., Okamura, H., Hoshino, B. F., Oya, A., and Katayama, K., Nucleotide sequences of the 5' noncoding region of hepatitis G virus isolated from Japanese patients : comparison with reported isolates, *Biochem. Biophys. Res. Commun.*, **226**, 314-318(1996).
9. Muerhoff, A. S., Smith, D. B., Leary, T. P., Erker, J. C., Desai, S. M., and Mushahwar, I. A., Identification of GB virus C variants by phylogenetic analysis of 5'-untranslated and coding region sequences, *J. Virol.*, **71**, 6501-6508(1997).
10. Okamoto, H., Okada, S., Sugiyama, Y., Yotsumoto, S., Tanaka, T., Yoshizawa, H., Tsuda, F., Miyakawa, Y., and Mayumi, M., The 5'-terminal sequence of the hepatitis C virus genome, *Japan. J. Exp. Med.*, **60**, 167-177(1990).
11. Sanger, F., Nicklen, S., Coulson, A. R. : DNA sequencing with chain-terminating inhibitors, *Proc. Natl. Acad. Sci. U. S. A.*, **74**, 5463(1977).
12. Pearson W. R. and Lipman D. J., Improved tools for biological sequence comparison, *Proc. Natl. Acad. Sci. U. S. A.*, **85**, 2444-2448(1988).
13. Hsieh, S. Y., Yang, P. Y., Chen, H. C., Liaw, Y. F. : Cloning and characterization of the extreme 5'-terminal sequences of the RNA genomes of GB virus C/hepatitis G virus, *Proc. Natl. Acal. Aci. U. S. A.*, **94**, 3206-3210(1997).
14. Erker, J. C., Simons, J. N., Muerhoff, A. S., Leary, T. P., Chalmers, M. L., Desai, S. M., Mushahwar, I. K. : Molecular cloning and characterization of a GB virus C isolate from a patient with non-A%E hepatitis, *J. Gen. Virol.*, **77**, 2713-2720(1996).
15. Simons, J. N., Desai, S. M., Schultz, D. E., Lemon, M., Mushahwar, I. K. : Translation initation in GB viruses A and C : evidence for internal ribosome entry and implications for genome organization, *J. Virol.*, **70**, 6126-6135(1996).
16. Shao, L., Shinzawa, H., Ishikawa, K., Zhang, X., Ishibashi, M., Misawa, H., Yamada, N., Togashi, H., Takahashi, T. : Sequence of hepatitis G virus genome isolated from a Japanese patient with non-A-E-hepatitis : amplification and cloning by long reverse transcription-PCR, *Biochem. Biophys. Res. Commun.*, **228**, 785-791(1996).
17. Zhou, Y. S., Chen, W., Zhao, H. L., Zhang, J. S., Xu, J., Wang, H. T. : cDNA cloning and sequencing of HGV genome from chinese, *Bull. Acad. Mil. Med. Sci.*, **20**, 249-253(1996).
18. Okamoto, H., Nakao, H., Inoue, T., Fukuda, M., Kishimoto, J., Lizuka, H., Tsuda, F., Miyakawa, Y., Mayumi, M. : The entire nucleotide sequences of two GB virus C/hepatitis G virus isolates of distinct genotypes from Japan, *J. Gen. Virol.*, **78**, 737-745(1997).

초록 : 5'-UTR 영역의 그룹특이적 염기서열에 의한 HGV의 계통분석

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한국인 환자의 혈청에서 분리한 HGV 5'-UTR영역의 염기서열을 결정하였다. 이들 염기서열을 이미 보고된 서열들과 비교한 결과, 한국 분리주들은 일본 분리주들과 더 높은 상동성을 나타내어 지리적 격리에 의해 HGV의 염기서열의 변이가 축적되었음을 알 수 있다. 흥미롭게도 동일 지역에서 분리된 HGV 분리주들 간에는 고도로 보존되어 있어 HGV의 분류에 이용가능한 세개의 영역이 5'-UTR에서 발견되었다. 이들 그룹-특이적 영역에 기초하여, 24 HGV 분리주들을 5개의 그룹으로 분류할 수 있었다.