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Genetic Diversity of Hepatitis C Virus in Korea

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=국문초록=

한국내 C형 간염바이러스의 유전적 다양성

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C형 간염바이러스 (HCV)는 각 개체간에 뉴클레오티드 서열상의 다양성을 나타내고, 이러한 유전적 다양성이 임상병리적 증상과 밀접한 연관이 있을 것으로 고려되어 왔다. 본 연구에서는 HCV E1과 NS5B 부위의 염기서열 분석을 통해 한국의 C형 간염바이러스의 분포와 다양성에 관해 분석하고, 발생계통도를 그려 HCV간의 진화적 거리를 확인하였다. 염기서열분석은 서울대학교 병원과 충남대학교 병원으로부터 얻은 56개의 HCV-양성 혈청을 대상으로 RT-PCR과 PCR 과정을 통해 얻은 유전자 산물을 클로닝하여 수행되었다. 56개의 혈청중 53개의 샘플에서 HCV RNA가 검출되었다. 이들 53개 샘플에 대한 분석결과, 유전형 1a, 1b, 2a, 2b, 7a가 각각 5.7, 45.3, 45.3, 1.9, 1.9%로 분포하고 있고, 1b형과 2a형이 한국에서의 주요한 HCV 유전형으로 밝혀졌다. 본 연구는 염기서열 분석을 통해 한국에서 1b형과 마찬가지로 2a형도 높은 빈도로 분포하고 있고, 비록 분포 빈도는 낮지만 1a형과 7a형도 존재하고 있음을 밝힌 최초의 보고이다.

Key Words : Hepatitis C virus, RT-PCR, sequencing, HCV typing.

INTRODUCTION

Hepatitis C virus (HCV) is a member of *Flaviviridae* family but is placed in its own genus and has a highly extensive sequence variation. Enomoto *et al.* [1] were the first group to have typed HCV into 4 groups as PT, K1, K 2a, and K2b according to nucleotide sequence homology in the 340 bp of NS5B. Okamoto *et al.* [2] also divided HCV into 4 groups, I (PT), II (K1), III (K2a), IV (K2b) by PCR typing based on variation in nucleotide sequence within core gene. Mori *et al.* [3] additionally found V and VI types from HCV patients in Thailand. Bukh *et al.* [4] analyzed nucleotide and amino acid sequences of E1 gene from anti-HCV sera collected worldwide and classified into 12 genotypes, 1a, 1b, 2a, 2b, 2c, 3a, 4a, 4b, 4c, 4d, 5a, and 6a. Simmonds *et al.*[5] made nucleotide sequence analysis for 222 bp of NS5B and found that all HCV could be clearly grouped into three categories, that is, type, subtype and isolate after pairwise comparisons of the 222 bp sequences of NS5 region. Their classification was the following: 1a, 1b, 1c, 2a, 2b, 2c, 3a, 3b, 4a, 5a, and 6a. Simmonds *et al.* [6,7] afterwards revealed that the classification of Bukh *et al.* [4] is well in accord with that of their own and suggested a system for the nomenclature of HCV genotypes. According to the system, HCV types

should be numbered in order of discovery with arabic numerals and subtypes should be identified by lower-case alphabet letter, again in order of discovery. Analyzing more HCV, Tokita *et al.* [8] revealed that the NS5B-222 bp of Simmonds *et al.* [6] and the E1-576 bp of Bukh *et al.*[4] are not always appropriate for clear typing because of overlapping between types and subtypes and suggested the larger region of NS5B (1093 bp: nt 7939-9031) could solve such overlapping problems between type and subtype. As shown in Table 1, there are 9 major types and a total of 28 genotypes identified to date [3, 4, 5, 8, 9, and 10]. Their worldwide distributions appear region-specific, though some types like 1, 2, and 3 are spread around the world. Type 1a is prevalent in Europe and America and 1b in Europe and America as well as in Asia. Type 2 is prevailing in Asia and Europe and 2c is mostly found in Argentina. Type 3 is a major type in South East Asia, Australia, and East Europe. In Africa and neighboring Middle East, type 4 is exclusively rampant, although 4d is found only in Denmark. Type 5 is a major in South Africa and is also found in Australia, Belgium, and U.S.A. Type 6 is common in Hong Kong, Macau, and Vietnam. And type 7, 8, and 9 are found only in Vietnam.

It has been revealed that degrees of liver disease and responses to antiviral therapy correlated to HCV genotypes are clearly different [11-14]. However, correlation between HCV genotypes and their differences still remains to be studied further since additional HCV genotypes are being discovered. Therefore, it is necessary to study their correlation for a development of efficient anti-HCV systems such as vaccines and other antiviral agents. Such requirement is enough to stir up endeavors to investigate diversity of HCV in a region or around the world since HCV is spread all over the world and efficient anti-HCV systems still seem to be far away.

In this study, we carried out the nucleotide sequence analysis for the NS5-222 bp of Simmonds *et al.* [6] and additionally for the E1-576 bp of Bukh *et al.* [4] from Korean hepatitis patients. The 222 bp of NS5B and the 576 bp of E1 were suitable for typing Korean HCV. According to the two reports [6,10], for the 222 bp of NS5B HCV's with more than 88% homology of nucleotide sequence were classified as the same type (isolate), those with 72.1 - 86% subtype, and those with 50.1-76. 1% different type (type). Likewise, for the 576 bp of E1 HCV's with more than 83.7% homology of nucleotide sequence were classified as the same type, those with 67.5-78.6% subtype, and those with 52.8-72% different type.

MATERIALS AND METHODS

Anti-HCV positive serum samples

Of a total of fifty-six anti-HCV positive serum samples 46 samples were collected from Seoul National University School of Medicine (SNUSM thereafter) and 10 samples were from ChoongBook University School of Medicine (CBUSM thereafter). Of forty-six sera from SNUSM numbered from SU-1 to SU-46, thirty-five samples (from SU-1 to SU-35) had already been typed by SNUSM using a PCR typing method [15] and were compared with results of a sequencing method in this study. The remaining 11 had been untypeable by the PCR typing. Ten sera from CBUSM were numbered from CU-1 to CU-10 and had not ever been typed.

RT-PCR primers

The positions of RT-PCR primers are depicted in Figure 1. For the NS5B-222 bp nested PCR, RT primer JCK 14: 5'-CTGGTCATAGCCTCCGTGAA-3', external sense JCK 12: 5'-ATGGGBTTCTCGTATGACAC-3' {B=(C,G,T)}, external antisense JCK 14 also used as RT primer, internal sense JCK 13: 5'-TTTGACTCAACCGTCACTGA-3', internal antisense JCK 15: 5'-ACGACCAGRTCGTCK-

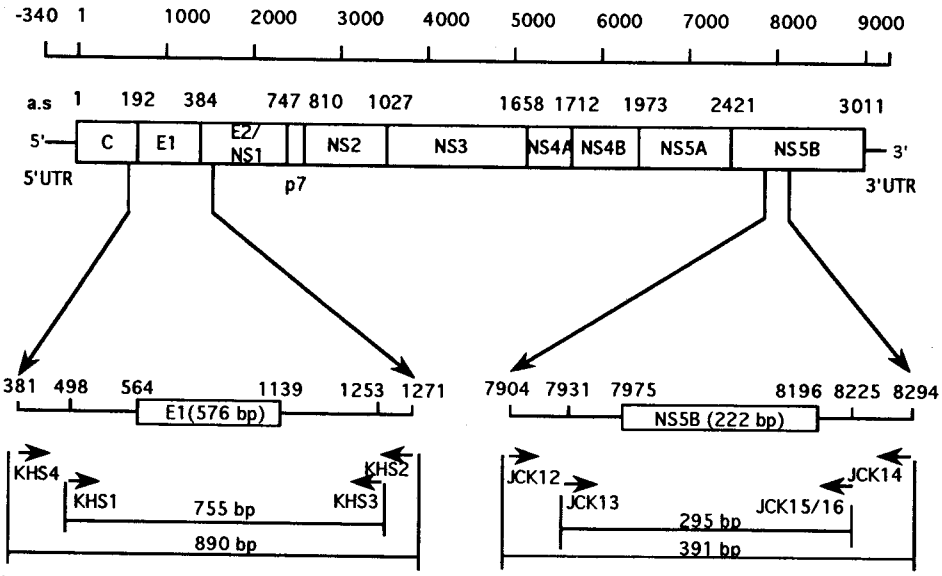


Fig. 1. Primers and regions used for RT-PCR of HCV. It shows the positions of the PCR primers for the 222 bp of NS5B and the 576 bp of E1 for sequence analysis.

CCRCA-3' {R=(A,G), K=(G,T)}, and another internal antisense JCK 16: 5'-ACAACMAR-GTCRTCGCCGCA-3' {M=(A,C)} were manufactured. For the E1-576 bp nested PCR, there were also made RT primer and external antisense KHS 2: 5'-YARGGCVGTSCTRTT-GAKGT-3' {Y=(C,T), V=(A,C,G), S=(C,G)}, external sense KHS 4: 5'-GCCGAYCTC-ATGGGGTA-3', internal sense KHS 1: 5'-CCYGGT TGCTCYTTYTCTAT-3', and internal antisense KHS 3: 5'-GTGCCAN-CTGCCRTTKGW RT-3' {N=(A,C,G,T), W=(A,T)}. As all the PCR primers used here were designed from as many sequence data published to date as possible, any new HCV types were expected to be captured.

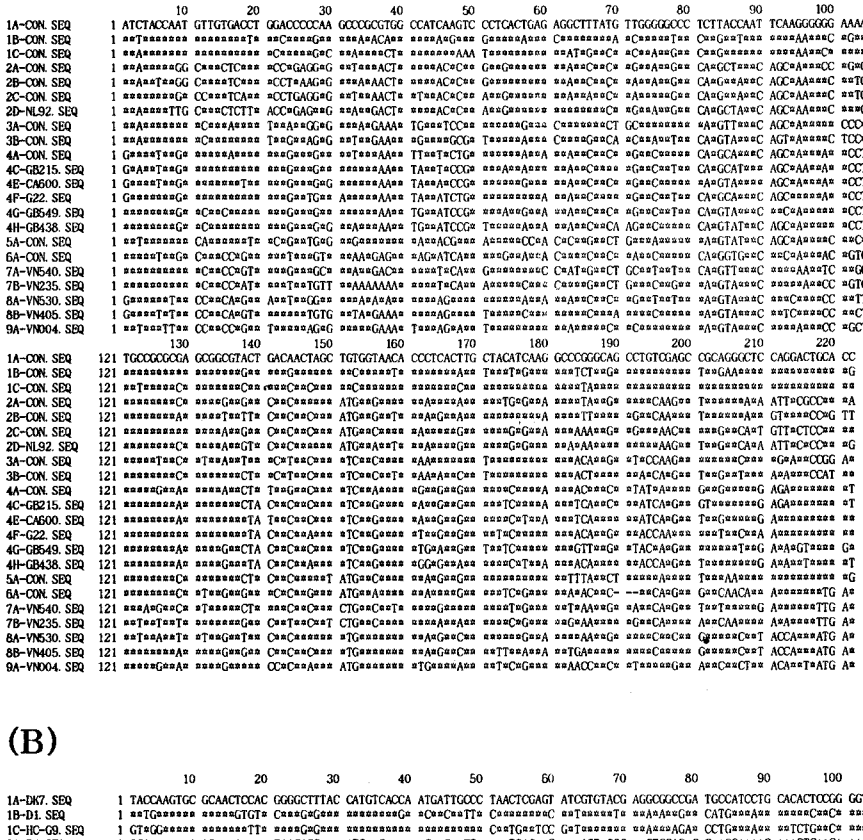
RNA extraction from sera

RNA was extracted from anti-HCV-positive sera by the user's manual of RNA STAT-60 kit (Tel-Test"B") or the single-step method [16]. The RNA pellet was air-dried and was dissolved in 5-10 μl of 0.1% DEPC-treated D. W at 50℃ for about 15 min. Finally, 1 μl of that RNA extract was used for RT and the rest was kept at -70℃.

RT-PCR

RT-PCR was conducted as reported by Kim *et al.* [17]. Reverse transcription proceeded at 37℃ for over 1.5 hr with M-MLV reverse transcriptase (BRL GIBCO). RT-primers were JCK 14 for the NS5B and KHS 2 for the E1. PCR was operated with the automatic thermocycler (Ericom, Inc) as follows - the first cycle: 3 min. at 94℃; 1 min. at 45℃; and 1.5 min. at 72℃, the rest 40 cycles: 0.5-1 min. at 94℃; 0.5-1 min. at 53℃; 1 min. at 72℃, and the last 10 min. at 72℃. For the second PCR, one-tenth volume of the first PCR solution was mixed with the second PCR buffer solution. External sense and antisense primers were JCK 12/14 for the NS5B and KHS 4/2 for the E1. Internal sense and antisense primers were JCK 13/15+16 for the NS5B and KHS 1/3 for the E1. The second PCR of a total 50 μl was operated in the same program with the first. Negative controls at each step (RT, the first, and the second PCR) were used to confirm 'no contamination' and 'hot start' was used to increase specificity for the E1-PCR.

(A)



(B)

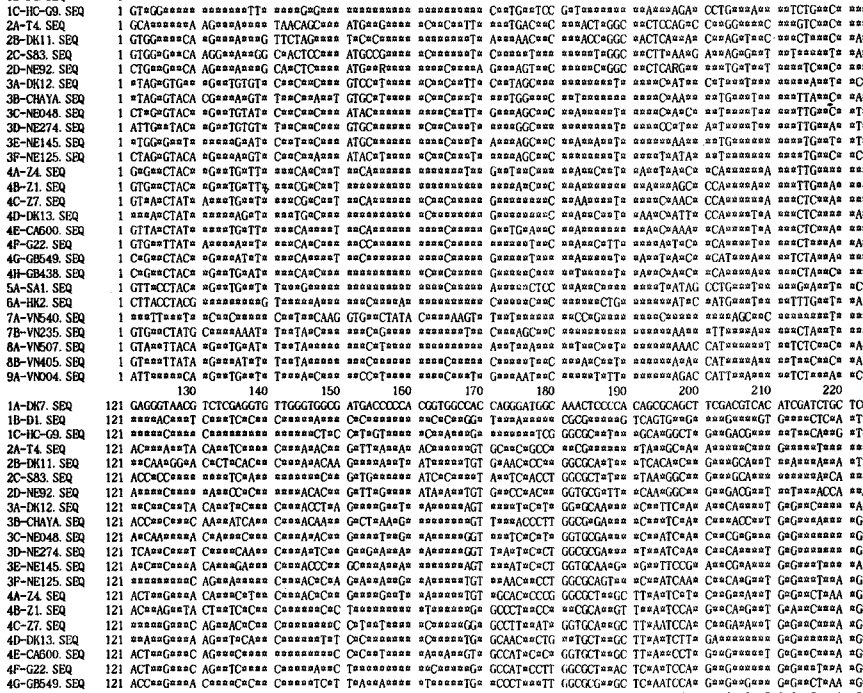


Fig. 2AB.

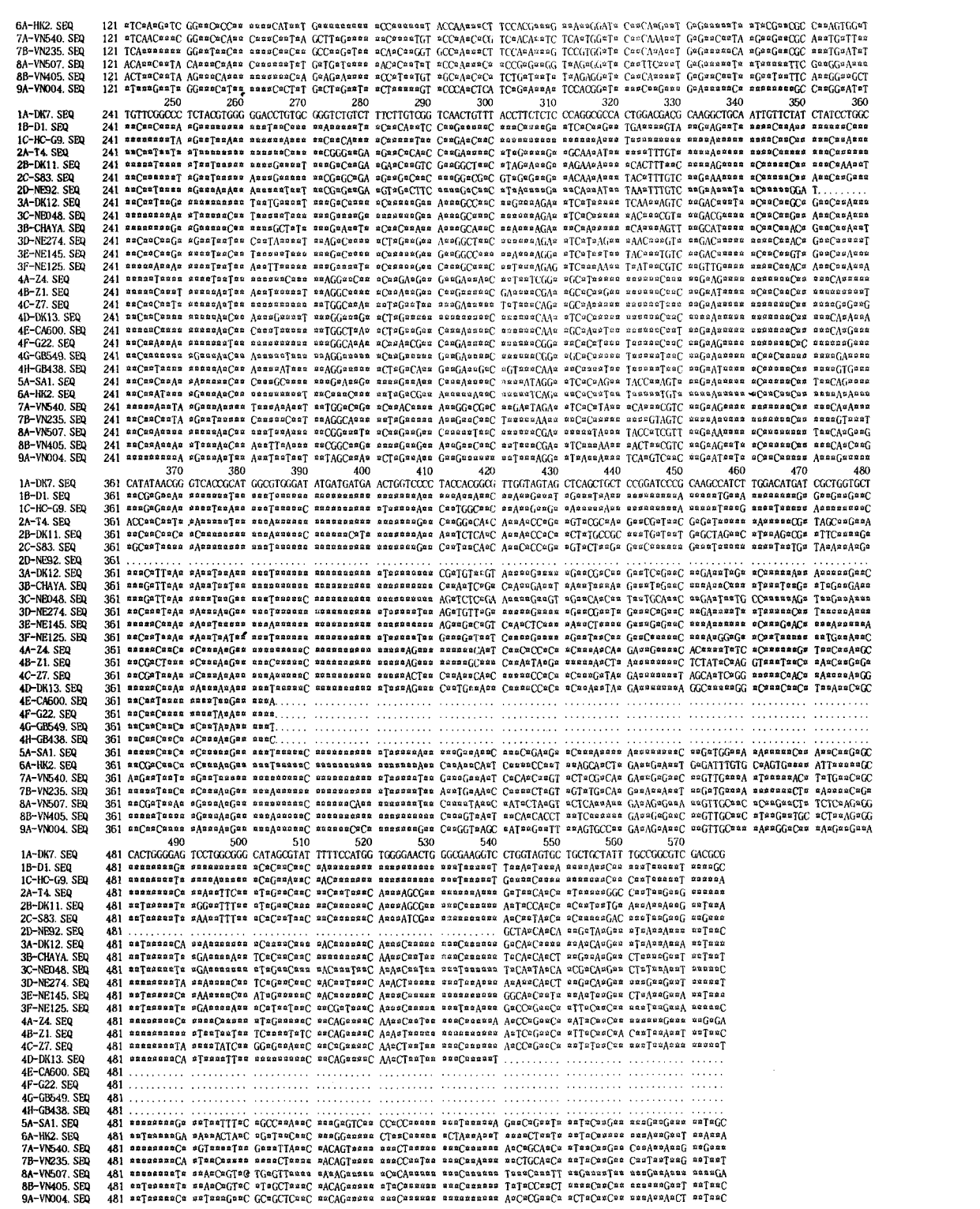


Fig. 2. Nucleotide sequence data of HCV types identified to date (A) for the 222 bp of NS5B and (B) for the 576 bp of E1. They were from GenBank or EMBL. Their accession numbers are in Materials and Methods.

Detection, Purification, and Cloning of PCR product

One-tenth volume of the second PCR product was loaded on 1.5-2% agarose gel and stained with ethidium bromide to visualize PCR bands of the expected size. For cloning, one-three μl of the second PCR product was directly mixed with T4 DNA ligase solution as follows: 2-3 μl of pUC19/HincII or pBluescript II SK/SmaI (10 ng/μl); 1.5 μl of 10x T4 ligase buffer; 1 μl of 10 mM ATP (final 0.5-1 mM); 5-20 U of T4 ligase; and D.W to be a final 10-15 μl of ligation solution. The solution was then incubated at 16-20℃ for over 12 hr. and transformation to competent cells of E.coli JM109 was conducted. Transformed cells were spread on McConkey agar plates mixed with ampicilin and incubated for 24 hr. White colonies were picked up and cultured overnight in 2 ml of LB media containing ampicilin. Plasmids were purified either by the alkaline lysis or by the boiling method [18] and were digested with *Hind*III and *Bam*HI the restriction sites of which are not on the PCR products of anytype-NS5B to identify positive clones. Purification of the second PCR product toed the line of the Geneclean method. Purified products were used in ligation or direct sequencing.

DNA Sequencing

Double strand DNA sequencing was conducted with T7 sequenase 2.0 (USB)' using universal primers of pUC19 or pBluescript II SK, -40 (forward) and -21 (reverse) ones. Direct sequencing was conducted by using the second PCR primers as sequencing primers, JCK 13/15+16 for the NS5B and KHS 1/KHS 3 for the E1 [19].

E1 and NS5B nucleotide sequence data for comparison of homology

E1 and NS5B sequence data were from GenBank and EMBL (Fig. 2). For the 222 bp of NS5B the accession numbers are the followings: L23435-L23475; U14281-U14320; D10078-D10081, for the E1: U14194-U14223; M74804-M74815; L16628-L16678; D11443; D16616; D16612; D16620; D16618; D16614, and for both E1 and the 222 bp of NS5B: D14853; D26556; D17763; L29574-L29635; D14204; D14205; D14207-D14209; D14211; D17470-D17509; D21315-D21328; D30796; and D30797. Nucleotide sequence homology analysis was made by DNASIS 7.0 (Hitachi).

Phylogenetic tree

The phylogenetic tree was constructed by CLUSTAL W program using the neighbor-joining method and visualized by TreeTool program.

RESULTS

1. Characteristics of RT-PCR product

In order to get RT-PCR product for cloning, RT-PCR was carried for the HCV RNA which was isolated from patients. We designed universal primers, which would hybridize to any known HCV types. Fig. 3 shows partial results of RT-PCR products for the NS5B-222 bp region and the E1-576 bp region. For the NS5B-222 bp region, all the samples except SU-39 and SU-41 gave PCR products of expected 295 bp or unexpected 325 bp (Fig. 4A). PCR bands of unexpected 325 bp were from samples such as SU-1, SU-2, SU-3, SU-11, SU-13, SU-14, SU-17, SU-19, SU-22, SU-46, CU-2, and CU-4. They were considered to be HCV-BK-type HCV's according to the sequencing results [17]. In the HCV-BK-like HCV's, mismatch in the binding site of 3'-end of antisense primers, JCK15 and JCK16, was occurred and this is the reason why primers could not bind to the expected site and instead they bound 30 bases downstream from expected site. These HCV's, therefore, were considered to be type 1b like HCV-BK, which was confirmed by sequencing (Fig. 4). SU-39

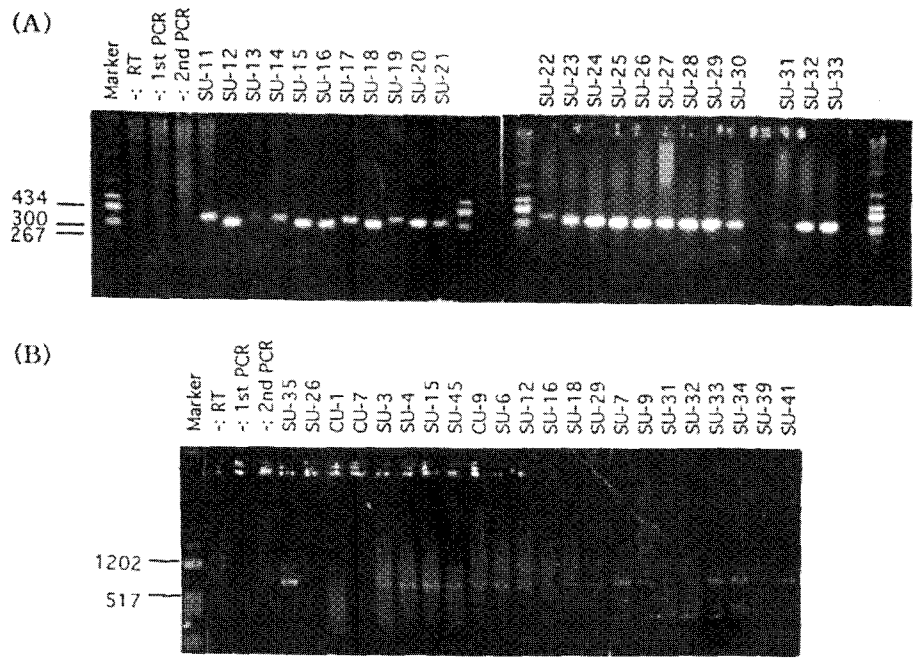


Fig. 3. Parts of results for RT-nested PCR and Cloning. (A) shows the NS5B-222 bp RT-nested PCR products. Such products as SU-11, SU-13, SU-14, SU-17, SU-19 and SU-22 appeared a bit larger than the others. They were revealed to have the same origin as HCV-BK (type 1b), a Japanese type (Ref. 17). (B) shows the E1-576 bp RT-nested PCR products.

did not show PCR products for both the NS 5B and the E1 region. So SU-39 may be anti-HCV Ab-positive but not HCV RNA-positive. On the other hand, SU-41 showed the specific PCR product for the E1 region, but not for the NS5B region. It might happen because the HCV of SU-41 sample had a or some mutations in the primer binding site of the NS5B. Some samples such as SU-13, SU-14, SU-19, SU-22, SU-31, and SU-40 showed comparatively-dim PCR bands. They may have a low titre of HCV. SU-40 gave too little amount of PCR product to identify its type. For the E1-576 bp region, SU-31, SU-32, and SU-39 did not show PCR bands. SU-26, CU-1, and CU-7 showed dim PCR bands. The reason why PCR for the E1-576 bp was more fastidious than the NS5B-222 bp may be due to more variability of nucleotide sequence in E1 than in NS5B. RT-PCR products of some samples were cloned into *Hinc*II site of pUC19 or *Sma*I site of pBluescript II SK plasmid and their nucleotide sequences were determined. RT-PCR products of other samples were directly sequenced.

2. Genotype distribution determined by sequence analysis

Figure 4 shows nucleotide sequences of HCV types studied in this paper: (A) for the 222 bp of NS5B (nt: 7975-8196) and (B) for the 576 bp of E1. For the NS5B, samples except SU-39, SU-40, and SU-41 gave good sequence data to identify HCV types. SU-22#5 and SU-24#3 did not provide the full sequence of 222 bp. It did not, however, make any problem for sequence analysis and type-identification. In case of SU-44, only 79 bases were determined and their sequence analysis showed that SU-44 may belong to a new genotype. Further sequence on the upsteam 24 bases from the shown sequence of SU-44 re-

(A)

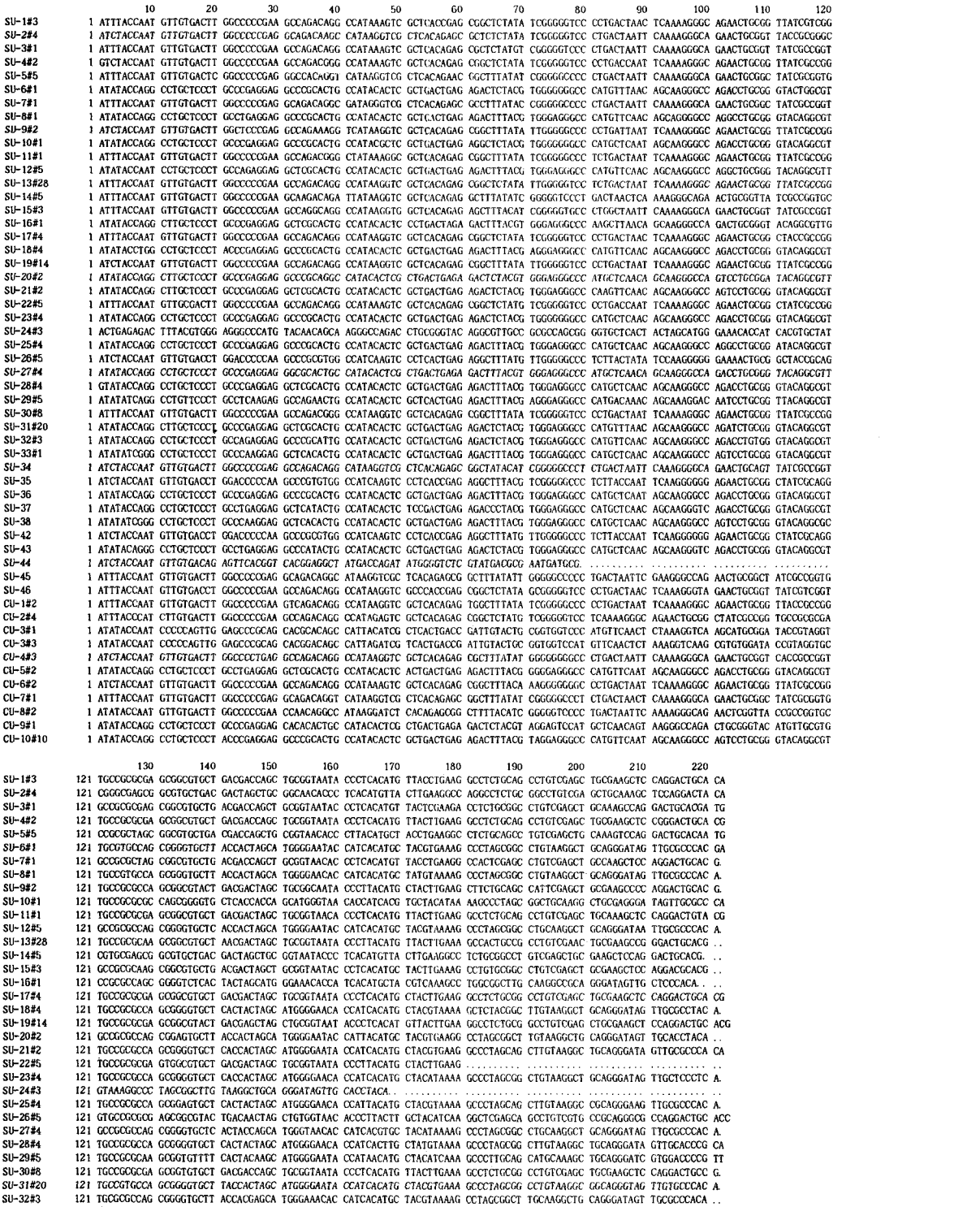


Fig. 4.

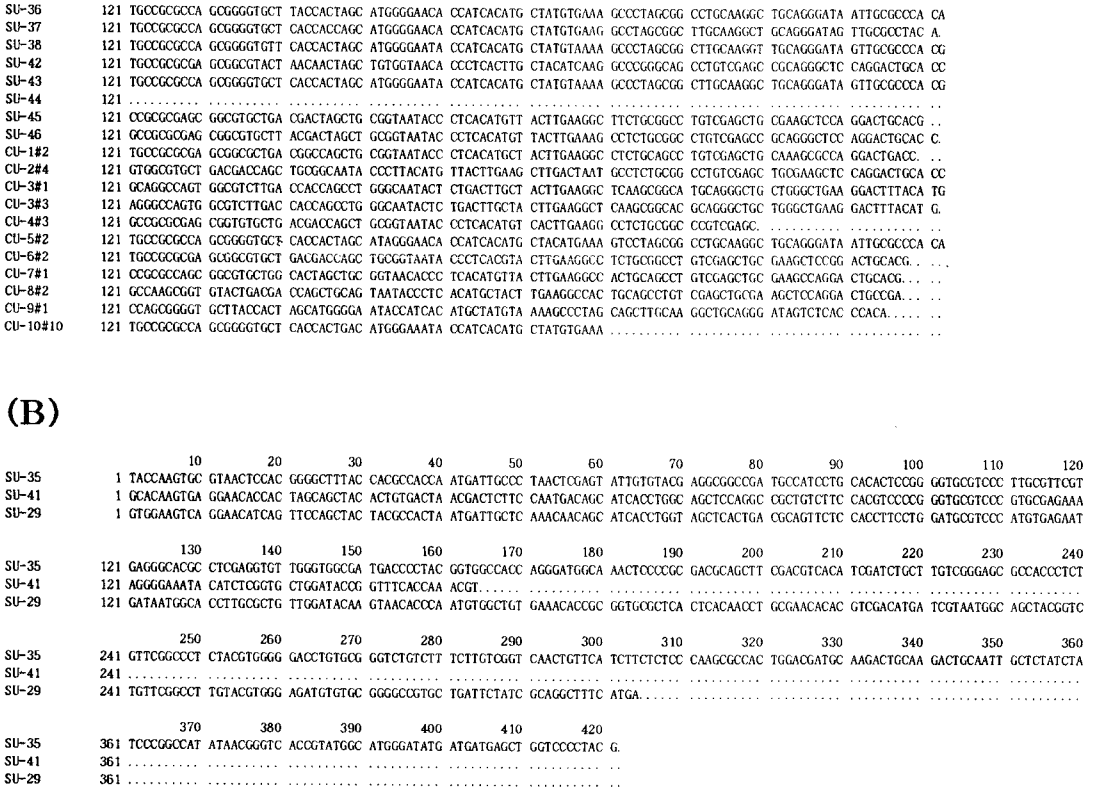


Fig. 4. Nucleotide sequence data revealed here (A) for the 222 bp of NS5B and (B) for the 576 bp of E1. In (A), SU-22, SU-24 and SU-44 did not provide the full 222 bp. SU-22 and SU-24, however, did not make any problem in sequence analysis. In case of SU-44, sequence data were not enough for sequence analysis. In (B), though the full 576 bp was not obtained for each sequenced sample, there was no any ambiguous overlapping during analysis. RT-PCR product of SU-41 was not available for NS5B but available for E1 and its E1-sequence data were given.

vealed SU-44 has a high homology to type 1. But the rest of the 222 bp should be revealed to identity what is the real type of SU-44. For the E1, sequencing was conducted by direct sequencing for SU-41 whose PCR product was obtainable only in the E1 region. Direct sequencing of SU-35 was also conducted and SU-35 was identified as type 1a, which is the first report in Korea. Direct sequencing was also conducted for SU-29 and CU-3 which are a rare type 2b and an unexpected type 7a, respectively. Type 7a was only reported in Vietnam[10]. Sequence analysis of SU-29 and SU-35 for the E1 is well consistent with those for the NS5B and SU-41 was belonged to type 2a by the E1 analysis.

3. Construction of phylogenetic tree

Figure 5 is the phylogenetic trees constructed based on the NS5B-222 bp sequences. The tree clearly shows nine major genotypes, a total of 28 genotypes, and grouping of all the samples identified here. From these data, it was revealed that of a total of 56 anti-HCV positive sera fifty-three were typeable, three were untypeable. Of the typeable 53 sera, three samples belong to type 1a, twenty-four type 1b, twenty-four type 2a, one type 2b, and one type 7a. Because thirty-three samples from SU-1 to SU-35 had already been classified by PCR typing based on the NS5B (nt: 7890-8261), even though some sera like SU-7, SU-9,

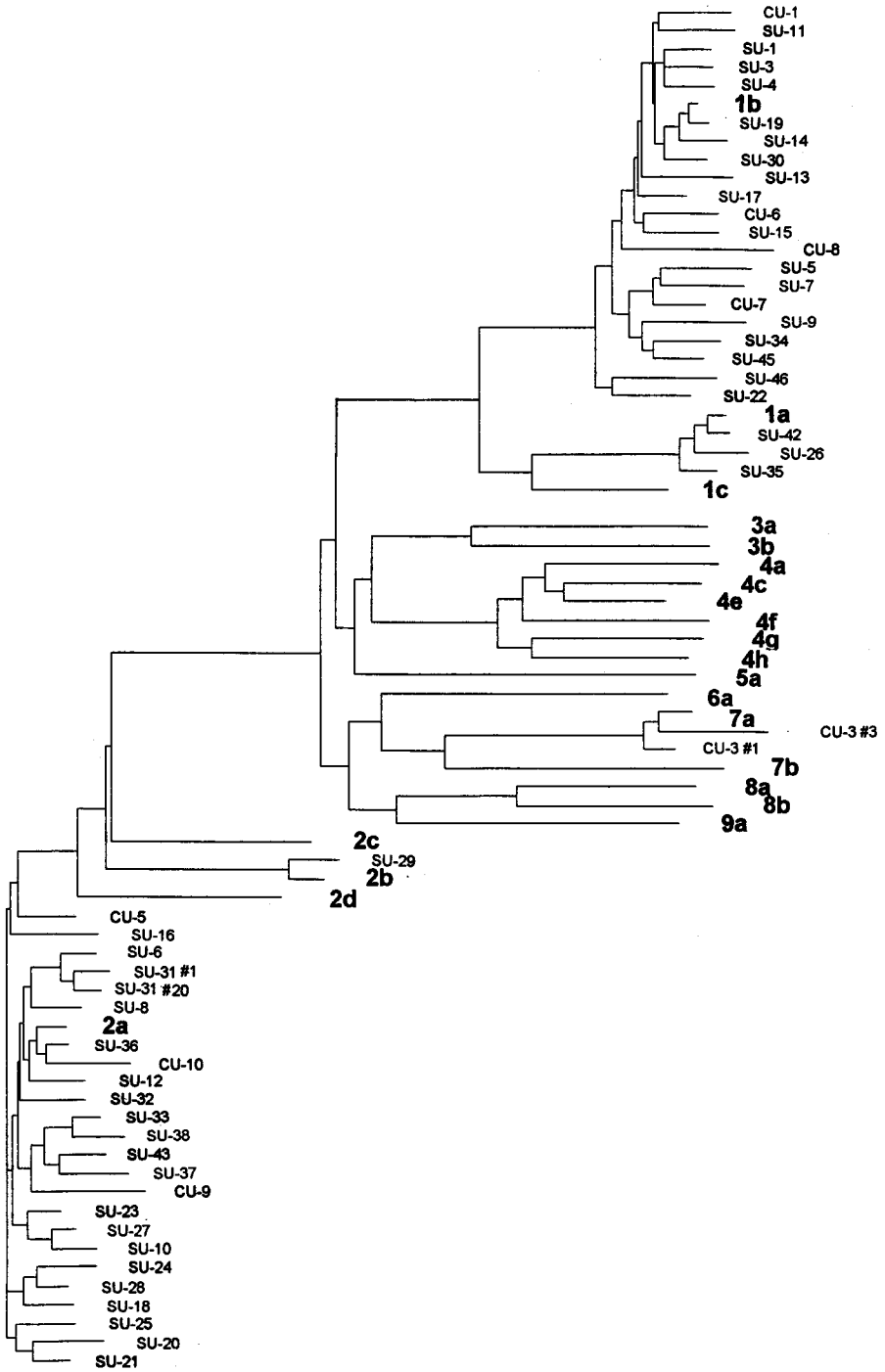


Fig. 5. Phylogenetic tree based on the 222 bp of NS5B. The number 0.1 and the line just below represent the unit of nucleotide substitutions per site, the evolutionary distance. It shows clear separation of each genotype and unequivocal groupings of samples sequenced in this study. In particular, CU-3 #1 and #3 are shown to be a member of type 7a. The bold typed are genotype-representatives (Fig. 2.).

and SU-34 were not consistent with results by sequencing, we could not count them for the distribution of HCV types in Korea. So when, of the rest 21 samples never typed before, typeable 18 were analyzed, the distribution is as the following: 1a-1; 1b-8; 2a-8; 7a-1. In particular, it is very interesting that type 1a and type 7a have been identified in Korea for the first time.

DISCUSSION

It is very important to investigate the distribution of HCV types in Korea for therapy as well as research. Despite a total of twenty-eight HCV genotypes were revealed to date, their differences in a clinical meaning as well as in a sense of molecular and biochemical features, if any, were little studied, even though type 1b showed relatedness with chronic hepatitis and stronger resistance to interferon [11-13]. Recently, there was a very interesting report that 5'-half of cDNA for NS3 (having a serine proteinase function) transformed NIH 3T3 cells [20]. If that was true, HCV would be an oncogenic virus and molecular mechanism of hepatocellular carcinoma by HCV might be revealed. Development of vaccines or antiviral therapeutic agents without weighing differences among different types is limited and of little importance. And why any type which exists few in a certain area can not be neglected is because it can be a major type in other areas, as shown in Table 1. Infection routes should be further studied, though parental routes as in transfusion or drug abuse were believed to be the major one. Also there were reports that saliva, seminal fluid, urine, and ascites of HCV patients contained HCV RNA [21, 22].

There were several reports for determining HCV types in Korea. Kwag *et al.* [23] investigated nucleotide and amino acid sequences of the E1 (576 bp) from 22 anti-HCV sera and suggested a possible existence of a unique Korean HCV type existing. But according to the analysis of Tokita *et al.* [8], the presumed unique Korean HCV type is revealed to be a member of type 1b. Yoon *et al.* [24] reported from the experiment using PCR typing based on NS5B (nt: 7900-8290) that among 70 anti-HCV positive sera 43 could be PCR-typeable, and of the 43 samples type 1b was 75% and type 2a 25%, and in the analysis of liver biopsy from hepatitis patients 1b was 63%, 2a 3.7%, and coinfection of 1b and 2a 18.5%. However, since only three type-specific primers (1a, 1b, and 2a) were used, existence of other types could not be revealed in that experiment.

Some problems for PCR typing were suggested [17]. As shown in Table 2, for SU-7, SU-9, and SU-34 results of PCR typing were different from those of sequencing. So sequencing is suggested for precise typing. In this study, sequencing method was used for typing and it was concurrently expected that other genotypes which had not been found by PCR typing or new genotypes which had not been identified as yet might be found.

For sequence analysis, the 222 bp of NS5B was selected because the target size for sequencing was relatively short, many sequence data for that region had been amassed, and there was unequivocal separation among types by sequence analysis [5, 14]. Later, this region was not always appropriate for sequence analysis because of overlapping between subtypes and types and Tokita et al [13] suggested that much larger region (1093 bp: nt 7939-9031) would be more appropriate for nonoverlapping sequence analysis. Nonetheless, the 222 bp of NS5B is still of use for quick sequence analysis, which is confirmed in this study, that is, there were no unclear overlapping samples. To design universal primers which can bind any HCV type, as many sequence data revealed to date as possible were analyzed and their efficiency proved good in that, for the NS5B, fifty-four

Table 1. HCV genotypes and their worldwide distribution

Region	Country	HCV genotype																												
		1			2				3						4								5	6	7		8		9	
		a	b	c	a	b	c	d	a	b	c	d	e	f	a	b	c	d	e	f	g	h	a	a	a	b	a	b	a	
Asia	Japan	F	M		SM	F			F	F																				
	*Korea	F	M		M	F																			F					
	Hong Kong	F	M			F			F															SM						
	Taiwan	F	M		SM	F																								
	China	F	M		F																									
	Macau	F	M		F	F			F															SM						
	Thailand	F	SM						M	F																				
	Philippines	M	SM						F																					
	Singapore	F	M		F				SM	F																				
	Malaysia	SM	SM						M																					
	Indonesia		M	F	F																									
	Vietnam	M	M		F																			SM	F	F	F	F	F	
	India	F	M						F																					
	Nepal	F	M						F	F	F	F	F	F																
	SaudiArabia														M															
	Turkey	M																												
	Lebanon			M																										
	Bahrain														M															
	Yemen														M															
Australia	Australia	M	SM		F	F			M															F						
Europe	Italia	M			F				F						F															
	Spain	M																												
	Portugal	M																												
	France	SM	M	F			F								F															
	Germany		M																											
	Denmark	M	SM			SM			F										F											
	Sweden	M	SM			SM																								
	Scotland	SM	F		SM	F			M																					
	Finland	SM	F			M			M																					
	Netherlands	SM	M		SM	F		F	SM	F																				
	Belgium																							F						
	Hungary	SM	M												F															
	UnitedKingdom	M			SM				SM																					
Africa	Egypt														M															
	Zaire														F	F	M													
	Cameroon																		M	M										
	Gabon																M		SM		SM	SM								
	South Africa	F	SM		F				F						F									M						
America	U.S.A	M	SM		F	F			F															F						
	Dominican Rep.	M	SM																											
	Canada	M	SM		F	F																								
	Peru		M																											
	Argentina		SM				M																							

*Data for Korea are from the present study plus ref. (Greene et al., 1995)
M: major; SM: sub-major; F: few

Table 2. Comparison of each typing method and HCV Distribution

Serum	PCR typing	Sequencing		$ Reverse dot blot	Serum	PCR typing	Sequencing		$ Reverse dot blot
		#NS5B	E1				NS5B	#E1	
SU-1	1b	1b (95)			SU-29	2b	2b (95)	2b (89)	x
SU-2	1b	1b (92)			SU-30	1b	1b (97)		
SU-3	1b	1b (95)		1b	SU-31		2a (93)		
SU-4	1b	1b (95)		1b	SU-32		2a (93)		
SU-5	1b	1b (91)			SU-33		2a (94)		2a
SU-6	2a	s1 (93)		2a	SU-34	2a	1b (93)		1a+1b
SU-7	2a	1b (92)		1b	SU-35	1a	1a (96)	1a (93)	1a+1b
SU-8	2a	2a (94)			SU-36		2a (97)		2a
SU-9	2a	1b (90)		1b	SU-37		2a (94)		x
SU-10	2a	2a (92)			SU-38		2a (93)		
SU-11	1b	1b (96)			SU-39				
SU-12	2a	2a (95)		2a	SU-40				
SU-13	1b	1b (94)			SU-41			2a (92)	2a
SU-14	1b	1b (95)			SU-42		1a (98)		x
SU-15	1b	1b (94)		1b	SU-43		2a (94)		2a
SU-16	2a	2a (92)		2a	SU-44		?		x
SU-17	1b	1b (97)			SU-45		1b (94)		1b
SU-18	2a	2a (92)		2a	SU-46		1b (94)		x
SU-19	1b	1b (98)			CU-1		1b (90)		x
SU-20	2a	2a (92)		2a	CU-2		1b (94)		
SU-21	2a	2a (95)			CU-3		7a (95)		x
SU-22	1b	1b (95)			CU-4		1b (92)		
SU-23	2a	2a (95)			CU-5		2a (94)		
SU-24	2a	2a (91)			CU-6		1b (94)		
SU-25	2a	2a (92)			CU-7		1b (92)		1b
SU-26	1b	1b (96)		x	CU-8		1b (90)		
SU-27	2a	2a (94)			CU-9		2a (89)		2a
SU-28	2a	2a (95)			CU-10		2a (89)		

*** HCV Distribution in Korea revealed by sequencing and reverse dot blot except PCR typing***

A. Of a totla of 56 anti-HCV sera
la: 3, 1b: 24, 2a: 24, 2b: 1, 7a: 1, untypeable: 3
(among them, two were 1a+1b-coinfected)

B. Of 18 sera except untypeable three ones from SU-36 to CU-10
1a: 1, 1b: 8, 2a: 8, 7a: 1

#. Parentheses means percentages of nucleotide sequence homology.
$. x means the reverse dot blot was not successful.

among a total of fifty-six sera were PCR-positive, even though SU-40 was too weakly PCR-positive to be cloned and sequenced. Moreover, of eleven samples (from SU-36 to SU-46) untypeable by PCR typing, eight samples were RT-nested PCR-positive in this study. There are very intriguing cases after the NS5B-222 bp sequence analysis. One of them is an occurrence of type 1a (SU-26, SU-35, and SU-42). Their nucleotide sequences were presented in Fig. 4B. SU-26 was obtained from a patient with a past medical history of a major abdominal surgery and multiple blood transfusion in the United States 15 years ago, therefore, type 1a of SU-26 was considered to be originated from the United States. In comparison, SU-35 and SU-42 were obtained from patients without any past medical history of blood transfusion and travelling abroad; thus we do not know the origin of HCV type in

those patients. In Far East Asia countries where type 1a exists sparse and is strongly considered as an imported one, the presumed Korea-native type 1a is enough to arouse our interests. It still remains to be studied, however, whether the type 1a is a real Korea-native or not.

It was surprising that type 7a was found among 10 sera from CBUSM. How the patient who already died of hepatitis gained the type 7a was unknown. It is probably possible to infer that the patient may be a Vietnam war veteran or may have contacted any Vietnamese hepatitis patient. In the Phylogenetic tree of Fig. 5A, type 6a, 7a, 7b, 8a, 8b, and 9a are shown more related to each other than to other genotypes and have been found only in Far East (Hong Kong, Macau, Korea) and South East Asia (Vietnam) (Table 1). Disease severity or other profound clinical meanings for type 6a, 7a, 7b, 8a, 8b, and 9a have not been revealed. Since the interrelationship between Korea and Vietnam grows up, such Vietnamese type of HCVs are anticipated to spread more in the future in Korea. Further studies are required for these types including broader screening of anti-HCV positive sera.

In this study, HCV distribution in Korea is as the following: type 1b and 2a are major types and others in order of 1a, 2b/7a occupy the next (Table 2). Knowing that type 2a in most Far East Asia countries are sub-major or few, it is very interesting that type 2a is a major type in Korea. Type 3 may exist in Korea because it is found in our neighbours like Japan, Hong Kong, and Macau and it is prevailing in South East and Central Asia (Table 1). Existence of a unique Korean HCV can not also be excluded.

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