

# Sequence Analysis of Hepatitis C Virus Isolated From a Fulminant Hepatitis Patient

Takanobu Kato,<sup>1</sup> Akihiro Furusaka,<sup>1,2</sup> Michiko Miyamoto,<sup>1</sup> Tomoko Date,<sup>1</sup> Kotaro Yasui,<sup>1</sup> Jun Hiramoto,<sup>2</sup> Kazuo Nagayama,<sup>2</sup> Teruji Tanaka,<sup>2</sup> and Takaji Wakita<sup>1\*</sup>

<sup>1</sup>Department of Microbiology, Tokyo Metropolitan Institute of Neuroscience, Tokyo, Japan

<sup>2</sup>Department of Internal Medicine, Jikei University School of Medicine (Daisan), Tokyo, Japan

Although hepatitis C virus (HCV) is a major cause of non-A non-B hepatitis, its pathogenic role in fulminant hepatitis remains controversial. A 32-year-old man contracted hepatitis. Serum ALT concentration was reached to 6,970 IU/L, the lowest prothrombin time value was 16% and jaundice and stage II encephalopathy were developed. HCV RNA was detected in this patient by reverse transcription polymerase chain reaction in sera at the acute phase, and it was undetectable during the remission phase when anti-HCV was found. The entire genome of infected HCV was recovered, cloned, and sequenced from this patient, and compared with the clones of six other chronic hepatitis patients. Phylogenetic analysis revealed a clustering around genotype 2a and a deviation from the other 2a chronic hepatitis strains. Calculating the genetic distance in each subgenomic region revealed that the 5'untranslated region (5'UTR), core, nonstructural (NS) 3, and NS5A were severely deviated. Of 20 clones of the hypervariable region (HVR), 17 showed an identical sequence with the others showing a difference of only one amino acid. HCV was isolated from a fulminant hepatitis patient and its entire genome was recovered; a clustering around genotype 2a was observed, but the sequence deviated especially in 5'UTR, core, NS3, and NS5A; and monoclonality of the HVR sequence was found not only in the fulminant hepatitis patient but in a certain percentage of chronic hepatitis patients. **J. Med. Virol. 64:334–339, 2001.**

© 2001 Wiley-Liss, Inc.

**KEY WORDS:** HCV; genotype 2a; 5'UTR; core; NS3; NS5A

## INTRODUCTION

Fulminant hepatitis is defined as an occurrence of hepatic encephalopathy within 8 weeks of the initial symptoms of hepatitis [Trey and Davidson, 1970].

Various etiologies are known to cause fulminant hepatitis. Hepatotropic viruses, hepatitis A virus (HAV), and hepatitis B virus (HBV) are some of the most common causes of fulminant hepatitis [Lee, 1993; O'Grady et al., 1993]. HBV has been implicated in fulminant hepatitis in a number of studies, such as those investigating genomic mutations in the precore and core promoter regions [Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Sato et al., 1995]. Although the discovery that hepatitis C virus (HCV) as a main cause of acute and chronic non-A non-B hepatitis, its pathogenic role in fulminant hepatitis remains controversial. HCV was not detected in fulminant hepatitis in some studies [Wright et al., 1991; Liang et al., 1993; Fukai et al., 1998], whereas others suggested substantial etiological involvement of HCV in fulminant hepatitis [Yoshida et al., 1994; Gordon et al., 1995; Villamil et al., 1995]. For a possible explanation of this discrepancy, some viral characteristics of HCV should be considered, such as the differential distribution of genotypes or strains with specific genomic mutations in partial regions. Farci et al. [1996] described the clinical, virological, and histological profile of a patient with HCV associated fulminant hepatitis. They reported subsequently on another case of fulminant hepatitis with HCV whose serum was inoculated experimentally into a chimpanzee [Farci et al., 1999]. The chimpanzee developed unusually severe acute hepatitis, suggesting that a virulent HCV strain may be implicated in the pathogenesis of fulminant hepatitis.

Recently, a case of fulminant hepatitis in which HCV RNA that was detected in sera was diagnosed. To elucidate the role of strain specific viral characteristics of HCV in fulminant hepatitis, the entire nucleotide

The nucleotide sequences in this paper will appear in the DDBJ/EMBL/GenBank with the following accession numbers: AB047639 (JFH-1) and AB047640-AB047645 (JCH-1-6).

Grant sponsor: Ministry of Education, Science and Culture of Japan; Grant Sponsor: Tokyo Metropolitan government.

\*Correspondence to: Dr. Takaji Wakita, The Tokyo Metropolitan Institute for Neuroscience, Department of Microbiology, 2-6 Musashidai, Fuchu, Tokyo, Japan. E-mail: wakita@tmin.ac.jp

Accepted 8 September 2000

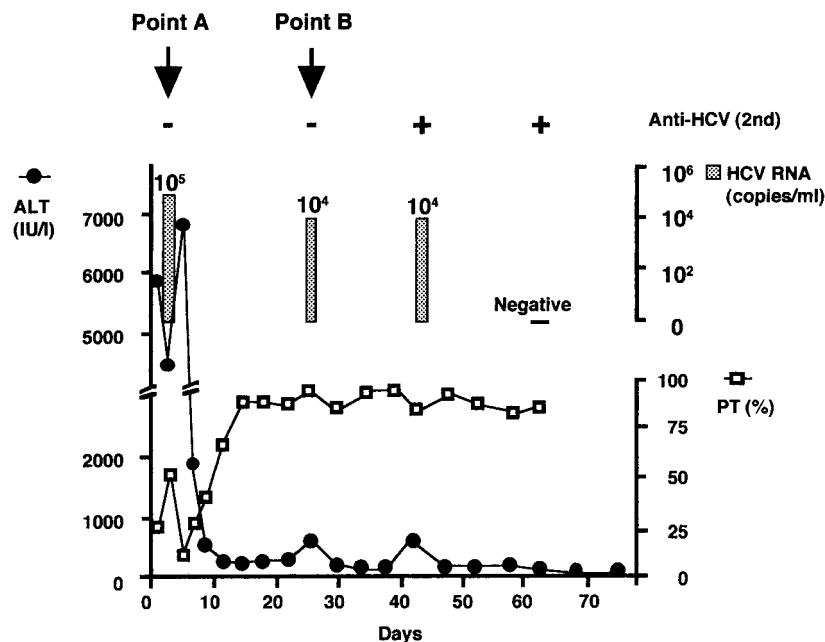


Fig. 1. Clinical course of the fulminant hepatitis patient with HCV. Time points A and B indicate the points at which serum samples were obtained. ALT, alanine aminotransferase; PT, prothrombin time value.

sequence of HCV isolated from his sera was determined. For a comparison, the entire nucleotide sequences of six other strains isolated from chronic hepatitis patients were also determined.

## MATERIALS AND METHODS

### Patients

A 32-year-old man was admitted with a 5-day history of general fatigue, high-grade fever, and liver dysfunction. No evidence of prior liver diseases was found, and the patient had no history of drug-taking or alcohol consumption. He had not received any blood transfusions, taken any intravenous drugs, undergone acupuncture, nor had sexual contact with a known hepatitis virus carrier in the previous 6 months. Physical examination revealed slight conjunctival jaundice. After admission, serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) concentrations were 9,160 IU/l and 6,970 IU/l, respectively. The minimum prothrombin time value was 16%. Stage II encephalopathy developed 5 days after admission. HCV RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) in sera at the acute phase. Serum HCV RNA was quantified by using an Amplicor Monitor HCV test (Roche Diagnostic Systems, NJ). The titre was 10<sup>5</sup> copies/ml at admission, 10<sup>4</sup> copies/ml 25 days after admission, and undetectable during the remission phase. Anti-HCV antibody was positive at this point (second generation enzyme linked immunosorbent assay, Ortho Diagnostics, Tokyo, Japan) (Fig. 1). The genotype of this HCV strain was 2a. All viral markers of the other hepatitis viruses, anti-HAV antibodies

(IgG and IgM), HBV markers (HBsAg, anti-HBs, HBeAg, anti-HBe, anti-HBc, and HBV DNA), and GB virus -C/hepatitis G virus RNA, were negative. Analysis of antibodies to the EB virus and cytomegalovirus revealed a past history of infection. Following treatment, including plasma exchange and administration of prednisolone, his condition improved and he is now in good overall health. Serum samples at two points during the acute phase and recovered HCV RNA were collected (points A and B, Fig. 1).

For a comparison, six chronic hepatitis patients with HCV genotype 2a were selected randomly and serum samples were taken (patient CH 1-6, CH1, a 59-year-old woman; CH2, a 47-year-old man; CH3, a 49-year-old man; CH4, a 59-year-old woman; CH5, a 39-year-old man; and CH6, a 45-year-old man). All patients were followed for at least 6 months and diagnosed as chronic hepatitis by biochemical liver function tests, ultrasonography, computerised tomography, and histology of biopsy specimens. All samples were stored at 80°C until use.

### RNA Extraction and cDNA Synthesis

Total RNA was extracted from 250 µl of serum by using the acid-guanidiniumisothiocyanate-phenol-chloroform method (ISOGEN-LS, Nippon gene Co., Ltd., Tokyo, Japan), precipitated with isopropanol, washed with ethanol, and dissolved in 20 µl of distilled water with diethylpyrocarbonate. A 10 µl aliquot of the RNA solution was subjected to reverse transcription with random hexamer and moloney murine leukemia virus reverse transcriptase (Superscript II, Life Technologies, Rockville, MD) at 37°C for 1 hour.

### Isolation of HCV

PCR primers of 20-mer designed based on the sequence of HC-J6 (accession number is D00944) were used to amplify 12 fragments of HCV cDNA (nt 64-466, nt 337-829, nt 6371303, nt 1158-2348, nt 2305-3491, nt 3489-4648, nt 4566-5951, nt 5902-6983, nt 6967-8015, nt 7972-8872, nt 8700-9262, and nt 9251-9613; nucleotide numbers were according to HC-J6 and not including primer sequence) to cover the entire HCV genome. One microlitre of the cDNA was subjected to PCR with TaKaRa LA Taq polymerase (Takara Biochemicals, Kyoto, Japan), and PCR conditions consisted of 40 cycles each of denaturing at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. To determine the terminal 5' end sequence, cDNA was synthesised with a 5'UTR primer (antisense), tailed with terminal deoxynucleotidyl transferase and dCTP homopolymer, and then amplified by PCR (5' RACE System for Rapid Amplification of cDNA Ends Version 2.0, Life Technologies). To determine the terminal 3' end sequence, extracted RNAs were polyadenylated using poly A polymerase (Takara Biochemicals), converted to cDNA with 38-mer oligonucleotid containing (T)<sub>33</sub>, and amplified with a 3'UTR primer and primer used on reverse transcription. Amplified products were separated by agarose gel electrophoresis, and then cloned into pGEM-T EASY vector (Promega, Madison, WI), sequenced with Big Dye Terminator Mix and an automated DNA sequencer model 310 (PE Biosystems, Fostercity, CA). The consensus sequence of five isolates was adopted in each region. To obtain comparative sequences, the entire genome of HCV from six chronic hepatitis patients was determined in the same manner as the strain from the fulminant hepatitis patient.

### Computer Analysis

To elucidate the relationship between the entire genome sequence of HCV isolated from the fulminant hepatitis patient and the reference sequences from the other chronic hepatitis patients and strain HC-J6 described previously, a phylogenetic tree was constructed. The number of nucleotide substitutions per site at each nucleotide position was estimated by the six-parameter method [Gojobori et al., 1982]. Based on the estimate, a phylogenetic tree was drawn using the neighbour-joining method [Saitou and Nei, 1987]. In order to clarify the diversity in each subgenomic region, genetic distances were calculated between all possible pairs of HCV 2a strains isolated in this study and HC-J6, and between the strain from the fulminant hepatitis patient and the other patients by the six-parameter method (nucleotide) and Kimura's two-parameter method (amino acid) [Kimura, 1980] using the molecular evolutionary software system ODN version 1.1.1 [Ina, 1994]. The ratio of these two values (mean genetic distance between JFH-1 and other 2a strains/mean genetic distance among all 2a strains) were compared.

### Population of HVR Sequences

To assess the complexity of infected HCV in each patient, HVR sequences of 20 clones from the fulminant hepatitis patient and of 9–10 clones from the other six chronic hepatitis patients were determined.

## RESULTS

### HCV Isolated From Patient

The entire genome of HCV was recovered from the serum of the patient with fulminant hepatitis during the acute phase of hepatitis at point A shown in Figure 1. This strain (tentatively named as JFH-1) has a 9,678 bp genome and contains a long open reading frame spanning nt 341-9439 and coding 3033 aa, as does HC-J6. Strains isolated from the six chronic hepatitis patients (CH1–6) were composed of 9681, 9677, 9678, 9676, 9691, and 9686 bp, respectively, and were coding 3032–3033 aa (tentatively named as JCH-1–6, respectively). By phylogenetic analysis, these strains were clustered into genotype 2a, whereas JFH-1 was seen to deviate from the other strains, JCH-1–6 and HC-J6 (Fig. 2).

### Genetic Distances in Each Subgenomic Region

To determine the degree of deviation in each subgenomic region, the ratios of mean genetic distances (mean genetic distance between JFH-1 and other 2a strains/mean genetic distance among all 2a strains) were calculated. In the nucleotide, the mean genetic distance of the entire genome between JFH-1 and the other 2a strains and among all 2a strains were  $0.1136 \pm 0.0073$  and  $0.0969 \pm 0.0140$ , respectively, and

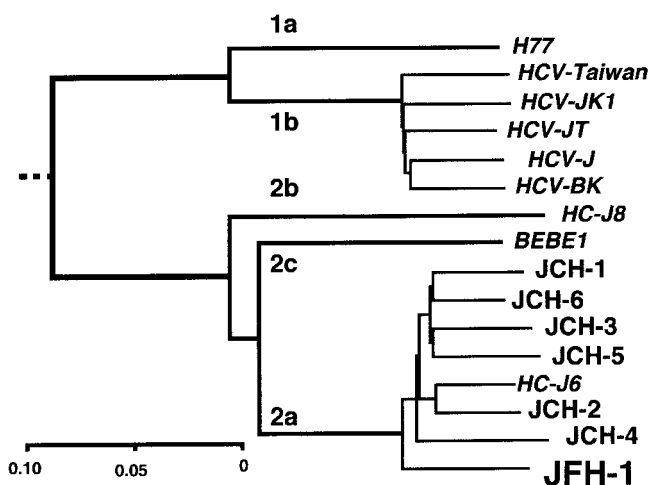


Fig. 2. Phylogenetic tree drawn of the entire genome of HCV including strains isolated in this study and strains which have been reported entire genome (HC-J6; accession number is D00944, HC-J8; D10988, and BEBE1; D50409) in the branch of genotype 2 and representative strains which have been reported entire genome (H77; AF009606, HCV-Taiwan; M84754, HCV-JK1; X61596, HCV-JT; D11168 and HCV-BK; M58335) in the branch of genotype 1. The root of the neighbor-joining tree was tentatively taken as the midpoint of the longest path. The length of the horizontal bar indicates the number of nucleotide substitutions per site.

TABLE I. Ratio of Mean Genetic Distances Between Pairs Among JFH-1 and Genotype 2a Strains in Each Subgenomic Region\*

Region	Nucleotide			Amino Acid		
	JFH-1 <sup>a</sup> (mean±SD)	Genotype 2a <sup>b</sup> (mean±SD)	Ratio	JFH-1 <sup>a</sup> (mean±SD)	Genotype 2a <sup>b</sup> (mean±SD)	Ratio
5'UTR	0.0130±0.0039	0.0094±0.0048	1.387	NA		
core	0.0744±0.0075	0.0595±0.0119	1.251	0.0741±0.0129	0.0475±0.0225	1.560
E1	0.1182±0.0104	0.1199±0.0173	0.986	0.1023±0.0196	0.189±0.0231	0.940
E2	0.1580±0.0162	0.1428±0.0233	1.107	0.1399±0.0130	0.1313±0.0155	1.066
NS2	0.1498±0.0098	0.1205±0.0256	1.243	0.1413±0.0157	0.1088±0.0307	1.298
NS3	0.1145±0.0091	0.0980±0.0142	1.168	0.0657±0.0050	0.0449±0.0136	1.464
NS4A	0.1407±0.0166	0.1127±0.0275	1.249	0.0456±0.0104	0.0437±0.0182	1.044
NS4B	0.0949±0.0081	0.0806±0.0117	1.178	0.0239±0.0053	0.0196±0.0065	1.223
NS5A	0.1122±0.0081	0.0918±0.0155	1.222	0.1616±0.0100	0.1013±0.0378	1.596
NS5B	0.0835±0.0072	0.0688±0.0122	1.213	0.0555±0.0074	0.0460±0.0108	1.208
3'UTR <sup>c</sup>	0.0791±0.0230	0.0799±0.0266	0.989	NA		
Entire genome	0.1136±0.0073	0.0969±0.0140	1.173	0.0918±0.0052	0.0716±0.0139	1.282

\*NA, not available; UTR; untranslated region; E, envelope; NS, nonstructural.

<sup>a</sup>Mean genetic distances are calculated between JFH-1 and the other genotype 2a strains.

<sup>b</sup>Mean genetic distances are calculated between all possible pairs of genotype 2a strains including JFH-1.

<sup>c</sup>These data are not including HC-J6.

the ratio of these two values was 1.173. Among the subgenomic regions, the ratio of 5' untranslated region (UTR) showed the greatest diversity: 1.387. In the amino acids, mean genetic distances of the entire genome between JFH-1 and the other 2a strains and among all 2a strains were 0.0918±0.0052 and 0.0716±0.0139, respectively, and the ratio of these two values was 1.282. Among the subgenomic regions, the ratios of core, nonstructural (NS) 3, and NS5A indicated greater diversity were 1.560, 1.464, and 1.596, respectively (Table I). In the envelope region,

mean genetic distances were greater both between JFH-1 and the other 2a strains and among all 2a strains. Therefore a lower ratio was found.

### Population of HVR Sequence

Quasispecies distributions of the hypervariable region (HVR) were analysed in the fulminant hepatitis patient and chronic hepatitis patients. Amino acid sequences and their frequencies in each patient are shown in Table II. At point A in the fulminant hepatitis

TABLE II. Population of HVR Sequence Among Patients\*

Patient	HVR sequence	Number	
FH	point A	:GTTTVGGAVARSTNVIAGVFSHGPPQN	17
		:.....P.....	1
		:.....I.....	1
		:.....S.....	1
		point B	:.....
CH-1		:YTTTTGSAAGRTTSSLASAFSPGARQN	6
		:.....A.S.....V.A.....	3
CH-2		:GTAVGGSTAHTTKGIASFFSPGARQN	2
		:H . RT . A . . . . AAQ . LTGL . TQ . . . .	2
		:H . RT . A . . . . AAQSLTGL . TQ	1
		:Q . RT . . AAGR . AHSF . L . A . . . . Q . . .	1
		:Q . RT . . AAGR . AIAF . L . A . . . . Q . . .	1
		:Q . RT . A . . . . RAAQSFTGL . TQ . . . . .	1
		:S . RT . . . . V . . A . LS . L . T . . . . .	1
CH-3		:D . . . . . V . RAAQSFTGL . TQ . . . . .	1
		:HTVTVGGSAGFTTSSLTALFVRGPQQK	8
		:.....A.....	1
CH-4		:.....D.....	1
		:TQ-TIFSSAAHNAYGLARVFTAGAKQN	9
CH-5		:.....S.....	1
		:GTHTTGAVAASNARGLASLFTSGPKQN	5
		:R . M . . GA . GRS . FSITT . . . . SR . SQ . K	3
		:N . M . . G . . RA . S . F . . . . SF . SH . K	1
CH-6		:N . M . . G . . RA . S . F . . . . SF . SH . E	1
		:HTRT-GSSVGATSGIVGLFTSGPKQN	10

\*HVR, hypervariable region; FH, fulminant hepatitis patient; CH, chronic hepatitis patient.

patient, 17/20 clones of HVR sequences were identical, and the other three clones differed by only one amino acid. At point B, all (20/20) clones were identical. On the other hand, in the chronic hepatitis patients, varied populations of the HVR sequences were demonstrated in each case. In CH-2 and 5, one or two predominant strains and other minor strains were isolated, and a higher complexity was observed. In contrast, in CH-4 and 6, most clones were identical and the population of the HVR sequences appeared to be monoclonal. In the other patients, CH-1 and 3, the complexity of the HVR population was intermediate.

## DISCUSSION

In the present case, the diagnosis of fulminant hepatitis was based on an assessment of clinical, biochemical, and virological parameters. HCV was detected during the acute phase of hepatitis and became negative at the remission phase when anti-HCV was detected. All other causative agents, hepatitis viruses including HAV and HBV, drugs, and alcohol were ruled out. These findings suggested strongly that the hepatitis in this case was caused by HCV infection. Therefore, the entire genome of HCV from this patient was recovered and compared with the strains isolated from six chronic hepatitis patients. Based on the data, it was concluded that 1) the HCV strain from this patient (JFH-1) was clustered around genotype 2a, but some deviation from the other strains, especially in 5'UTR, core, NS3, and NS5A were observed, and 2) monoclonality of the HVR sequence was observed not only in the fulminant hepatitis patient but in a certain percentage of the chronic hepatitis patients.

HCV is believed to be noncytopathic and hepatitis is mediated by host immune responses. Vento et al. [1996] reported two patients infected with HCV, who developed fulminant hepatitis upon withdrawal of chemotherapy. Thus, host immune status may be an important factor in the development of fulminant hepatitis. In the case of HBV, a particular viral strain was shown to cause an outbreak of fulminant hepatitis from a single HBV carrier [Oren et al., 1989]. Similarly, a specific HCV strain has also been suggested to cause severe acute hepatitis in the chimpanzee [Farci et al., 1999]. Therefore, some HCV strains or genotypes may be involved in the pathogenesis of fulminant hepatitis. In terms of genotype, cases of fulminant hepatitis with HCV genotypes 1a, 1b, and 2a have been reported [Chu et al., 1994; Farci et al., 1996, 1999; Vento et al., 1996]. In these reports, no apparent correlation was observed between a certain genotype of HCV and fulminant hepatitis. The prevalence of HCV genotypes is known to differ among the various geographic regions worldwide. Genotype 2a is relatively common in Asian areas, such as Japan, Taiwan, China, and Indonesia, and relatively rare in Western countries [Okamoto et al., 1992; Ohno et al., 1997]. Likewise, in Asian countries, the involvement of HCV in fulminant hepatitis is often suggested. If the particular strain of HCV genotype 2a

shows a greater tendency to cause fulminant hepatitis than the other genotypes, this hypothesis may explain the discrepancy between the prevalence of HCV in fulminant hepatitis patients in Western and Asian countries.

Among other RNA viruses, such as poliovirus or Japanese encephalitis virus, point mutations are known to be important determinants of virulence and other viral phenotypes [Nomoto, 1993; Sumiyoshi et al., 1995]. All of the ten clones of full length HCV genome of genotype 2a that had been deposited in the DDBJ/EMBL/GenBank showed clustering with our clones from chronic hepatitis patients (JCH-1-6) but not with the clone from fulminant hepatitis (JFH-1) (data not shown). This strain (JFH-1) showed deviation from the other 2a strains, especially in 5'UTR, core, NS3, and NS5A (Table I). The regions, 5'UTR, NS3, and NS5A, appeared to be associated with viral replication by some investigators [Tsukiyama-Kohara et al., 1992; Grakoui et al., 1993; Kim et al., 1995]. Mutations in these regions may affect the efficacy of viral replication, although the motifs of serine protease, helicase and polymerase of HCV or predicted secondary structures of internal ribosome entry site were conserved in all strains isolated in this study. Acceleration of viral replication may play a pivotal role in the development of fulminant hepatitis. On the other hand, the core region encodes the viral capsid protein and is considered to have multiple functions in the regulation of cellular genes at the transcriptional level [Ray et al., 1995, 1996a,b]. The influence on cell death in hepatitis of core proteins may be related to the development of fulminant hepatitis. It is necessary to determine which regions are important in causing fulminant hepatitis by constructing infectious RNA and experimental animal models in future studies. The biological characteristics of this clone need to be studied before any conclusion can be drawn about the causal relationship between this clone and fulminant hepatitis.

In a previous report, monoclonality of the HVR sequence was demonstrated in a fulminant hepatitis patient [Farci et al., 1996]. With the present data, a lower degree of diversity was observed in the HCV populations in HVR sequences of our fulminant hepatitis patient. Monoclonality of the HCV population at the time of infection or during hepatitis may be essential in the development of fulminant hepatitis. However, this monoclonality was also observed in a certain percentage of the other chronic hepatitis patients. Thus, a lower degree of diversity of HCV does not always lead to severe hepatitis. In other words, monoclonality of the viral population might be important but not sufficient for the development of fulminant hepatitis. The lower degree of diversity of the viral population might contribute to the development of specific immune responses and elimination of HCV infection, given that this patient showed an unusually recovery from fulminant hepatitis. In any event, to clarify the association between viral characteristics of HCV and the development of fulminant hepatitis,

further studies are needed to determine the biological characters of JFH-1 and other HCV clones isolated from fulminant hepatitis.

In summary, an entire genome of HCV was recovered from a fulminant hepatitis patient, and this strain was clustered around genotype 2a, but some deviation was observed from other strains isolated from chronic hepatitis patients, especially in 5'UTR, core, NS3, and NS5A.

## REFERENCES

- Chu CM, Sheen IS, Liaw YF. 1994. The role of hepatitis C virus in fulminant viral hepatitis in an area with endemic hepatitis A and B. *Gastroenterology* 107:189–195.
- Farci P, Alter HJ, Shimoda A, Govindarajan S, Cheung LC, Melpolder JC, Sacher RA, Shih JW, Purcell RH. 1996. Hepatitis C virus-associated fulminant hepatic failure. *N Engl J Med* 335:631–634.
- Farci P, Munoz SJ, Shimoda A, Govindarajan S, Wong DC, Coiana A, Peddis G, Rubin R, Purcell RH. 1999. Experimental transmission of hepatitis C virus-associated fulminant hepatitis to a chimpanzee. *J Infect Dis* 179:1007–1011.
- Fukai K, Yokosuka O, Fujiwara K, Tagawa M, Imazeki F, Saisho H, Omata M. 1998. Etiologic considerations of fulminant non-A, non-B viral hepatitis in Japan: analyses by nucleic acid amplification method. *J Infect Dis* 178:325–333.
- Gojobori T, Ishii K, Nei M. 1982. Estimation of average number of nucleotide substitutions when the rate of substitution varies with nucleotide. *J Mol Evol* 18:414–423.
- Gordon FD, Anastopoulos H, Khettry U, Loda M, Jenkins RL, Lewis WD, Trey C. 1995. Hepatitis C infection: a rare cause of fulminant hepatic failure. *Am J Gastroenterol* 90:1171–120.
- Grakoui A, McCourt DW, Wychowski C, Feinstone SM, Rice CM. 1993. Characterization of the hepatitis C virus-encoded serine proteinase: determination of proteinase-dependent polyprotein cleavage sites. *J Virol* 67:2832–2843.
- Ina Y. 1994. ODN: a program package for molecular evolutionary analysis and database search of DNA and amino acid sequences. *Comput Appl Biosci* 10:11–12.
- Kim DW, Gwack Y, Han JH, Choe J. 1995. C-terminal domain of the hepatitis C virus NS3 protein contains an RNA helicase activity. *Biochem Biophys Res Commun* 215:160–166.
- Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequence. *J Mol Evol* 16:111–120.
- Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshida M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. 1991. Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. *Gastroenterology* 100:1087–1094.
- Lee WM. 1993. Acute liver failure. *N Engl J Med* 329:1862–1872.
- Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. 1991. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 324:1705–1709.
- Liang TJ, Jeffers L, Reddy RK, Silva MO, Cheinquer H, Findor A, De Medina M, Yarbough PO, Reyes GR, Schiff ER. 1993. Fulminant or subfulminant non-A, non-B viral hepatitis: the role of hepatitis C and E viruses. *Gastroenterology* 104:556–562.
- Nomoto A. 1993. Recombinant polioviruses as candidates for oral live poliovaccines. *Microbiol Immunol* 37:169–174.
- O'Grady JG, Schalm SW, Williams R. 1993. Acute liver failure: redefining the syndromes. *Lancet* 342:273–275.
- Ohno O, Mizokami M, Wu RR, Saleh MG, Ohba K, Orito E, Mukaide M, Williams R, Lau JY. 1997. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J Clin Microbiol* 35:201–207.
- Okamoto H, Sugiyama Y, Okada S, Kurai K, Akahane Y, Sugai Y, Tanaka T, Sato K, Tsuda F, Miyakawa Y, Mayumi M. 1992. Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol* 73:673–679.
- Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. 1991. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. *N Engl J Med* 324:1699–1704.
- Oren I, Hershov RC, Ben-Porath E, Krivoy N, Goldstein N, Rishpon S, Shouval D, Hadler SC, Alter MJ, Maynard JE, Alroy G. 1989. A common-source outbreak of fulminant hepatitis B in a hospital. *Ann Intern Med* 110:691–698.
- Ray RB, Lagging LM, Meyer K, Steele R, Ray R. 1995. Transcriptional regulation of cellular and viral promoters by the hepatitis C virus core protein. *Virus Res* 37:209–220.
- Ray RB, Lagging LM, Meyer K, Ray R. 1996a. Hepatitis C virus core protein cooperates with ras and transforms primary rat embryo fibroblasts to tumorigenic phenotype. *J Virol* 70:4438–4443.
- Ray RB, Meyer K, Ray R. 1996b. Suppression of apoptotic cell death by hepatitis C virus core protein. *Virology* 226:176–182.
- Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425.
- Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. 1995. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. *Ann Intern Med* 122:241–248.
- Sumiyoshi H, Tignor GH, Shope RE. 1995. Characterization of a highly attenuated Japanese encephalitis virus generated from molecularly cloned cDNA. *J Infect Dis* 171:1144–1151.
- Trey C, Davidson CS. 1970. The management of fulminant hepatic failure. *Prog Liver Dis* 3:282–298.
- Tsukiyama-Kohara K, Iizuka N, Kohara M, Nomoto A. 1992. Internal ribosome entry site within hepatitis C virus RNA. *J Virol* 66:1476–1483.
- Vento S, Cainelli F, Mirandola F, Cosco L, Di Perri G, Solbiati M, Ferraro T, Concia E. 1996. Fulminant hepatitis on withdrawal of chemotherapy in carriers of hepatitis C virus. *Lancet* 347:92–93.
- Villamil FG, Hu KQ, Yu CH, Lee CH, Rojter SE, Podesta LG, Makowka L, Geller SA, Vierling JM. 1995. Detection of hepatitis C virus with RNA polymerase chain reaction in fulminant hepatic failure. *Hepatology* 22:1379–1386.
- Wright TL, Hsu H, Donegan E, Feinstone S, Greenberg H, Read A, Ascher NL, Roberts JP, Lake JR. 1991. Hepatitis C virus not found in fulminant non-A, non-B hepatitis. *Ann Intern Med* 115:111–112.
- Yoshida M, Dehara K, Inoue K, Okamoto H, Mayumi M. 1994. Contribution of hepatitis C virus to non-A, non-B fulminant hepatitis in Japan. *Hepatology* 19:829–835.