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Change of Hepatitis B Virus Genotypes in Acute and Chronic Infections in Japan

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During 35 years from 1971 to 2005, 153 patients with acute and 4,277 with chronic HBV infection visited the Toranomon Hospital in Tokyo, Japan. They were grouped into seven 5-year periods, and HBV genotypes/subgenotypes were determined. Patients with acute HBV infection were younger ($P=0.046$), predominantly male ($P=0.004$), possessed higher alanine aminotransferase levels ($P<0.001$), positive more frequently for HBeAg ($P<0.001$), and had lower HBV DNA loads ($P=0.014$) than those with chronic infection. Sexual transmission was more frequent in patients with acute than chronic HBV infection (67% vs. 3%, $P<0.001$). The number of patients with acute infection increased throughout 1971–2005. Patients with chronic infection increased since 1971, peaked in 1986–1990 and then decreased. The number of patients increased since 1990–2000 again, however, reflecting recent boost of acute HBV infection. The distribution of HBV genotypes was considerably different between patients with acute and chronic infections (A, B, and C: 28.6%, 10.3%, and 59.5% vs. 3.0%, 12.3%, and 84.5%, respectively, $P<0.001$). Since 1991, genotype A foreign to Japan started to increase sharply in patients with acute infection, and gradually in those with chronic infection. There was a trend for the foreign subgenotype B2/Ba to increase recently ($P<0.05$). Despite immunoprophylaxis of high-risk babies born to carrier mothers with hepatitis B e antigen, implemented nationally since 1986, acute and chronic infections with HBV have been increasing in Japan. Based on genotypes/subgenotypes changing with time, the resurgence of hepatitis B could be attributed to infections, with foreign HBV genotypes/subgenotypes, spreading swiftly by sexual contact. ***J. Med. Virol. 80: 1880–1884, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: acute hepatitis; chronic hepatitis; genotypes; hepatitis B virus: subgenotypes

INTRODUCTION

Worldwide, an estimated 350 million people are infected persistently with hepatitis B virus (HBV), and approximately a third develop serious liver disease such as decompensated cirrhosis and hepatocellular carcinoma (HCC) during the lifetime [Lee, 1997]. Universal vaccination of newborns has been implemented successfully in Taiwan [Ni et al., 2001], the United States [MMWR, 2002] and elsewhere. Catch-up vaccination is extended to children and adults for preventing HBV infection further.

Japan is unique in that, since 1986, passive and active immunopropylaxis has been performed annually on some 4,000 babies born to mothers who are infected with HBV and have hepatitis B e antigen (HBeAg) in the serum [Koyama et al., 2003; Noto et al., 2003]. This policy is based on a high risk of such babies to develop persistent HBV infection, in contrast to a low risk of babies born to carrier mothers with antibody to HBeAg (anti-HBe) [Okada et al., 1976]. As a result, the prevalence of HBV infection has been decreasing in Japan during past decades, which is reflected in the age-specific frequency of hepatitis B surface antigen (HBsAg) among first-time blood donors. The prevalence of HBsAg is low in blood donors born after 1981 at 0.23%, in remarkable contrast to 1.5% in those born between 1941 and 1950 [Tanaka et al., 2004].

The high incidence of HBV infection in men aged 20 years or older has been noted in the United States since 1999 [MMWR, 2004], forecasting continued new

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infections in the next two decades. There is a possibility that Japan, as well as any country in the world, will suffer from resurgent HBV infection that might be inapparent in the general population. During 35 years from 1971 to 2005, a city hospital in the Metropolitan Tokyo was visited by 4,430 patients infected with HBV. Patients with acute and chronic infections increased since 1996, thereby indicating that HBV infection has not been controlled efficiently in Japan.

MATERIALS AND METHODS

Patients

During 35 years from 1971 through 2005, 4,430 patients with HBV infection visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo, including 153 with acute and 4,277 with chronic HBV infection. Genotypes were A in 158 (3.6%) patients, B in 521 (11.8%), C in 3,564 (80.5%), D in 7 (0.2%), F in 3 (0.06%), H in 2 (0.04%) and not typeable in the remaining 175 (3.9%) patients. The median age of the patients was 37 years (range: 0.1–83) at the presentation, and included 3,210 (72.1%) men. Acute infection was diagnosed by high-titered antibody to hepatitis B core antigen of the IgM class and/or the development of HBsAg in previously seronegative individuals. Chronic hepatitis was diagnosed by liver biopsy carried out by laparoscopy and/or ultrasonic images, and liver cirrhosis by liver biopsy and/or ultrasonographic images plus laparoscopic findings. The number of patients with acute and chronic hepatitis B changed through 35 years, and the genotypes/subgenotypes were surveyed for predicting future trends of HBV infection in Japan. The study design conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the institution. Every patient gave an informed consent for this study.

Markers of HBV Infection

HBsAg and the corresponding antibody (anti-HBs) were determined by hemagglutination (MyCell, Institute of Immunology Co., Ltd., Tokyo, Japan), and HBeAg by enzyme-linked immunosorbent assay (F-HBe, Sysmex, Kobe, Japan). HBV DNA was determined by the polymerase chain reaction (PCR) followed by hybridization (Amplicor HBV Monitor, Roche Molecular Systems, Inc., Branchburg, NJ) and the results were expressed in log copies/ml over a range from 2.6 to 7.6. HBV genotypes (A–H) were determined by enzyme-linked immunosorbent assay (HBV GENOTYPE EIA, Institute of Immunology) [Usuda et al., 1999, 2000] and PCR-Invader assay with genotype-specific probes [Tadokoro et al., 2006]. Subgenotypes of A, B and C were determined by sequence analysis, restriction fragment length polymorphism [Sugauchi et al., 2004a, 2004b; Tanaka et al., 2005] and PCR-Invader assay [Tadokoro et al., 2006].

Statistical Analysis

Frequencies were compared between groups by the Chi-squared test and Fisher's exact test, and medians by the Mann–Whitney's *U*-test. Analysis of data was conducted with the computer program SPSS ver.11.0 (SPSS Inc., Chicago, IL). The trend of subgenotypes B1/Bj and B2/Ba was analyzed by the Cochran-Armitage trend test with SAS version 9.1.3 software (SAS Institute, Inc., Cary, NC). A P value less than 0.05 was considered significant.

RESULTS

Patients With HBV Infection During 35 Years (1971–2005)

During 35 years from 1971 through 2005, the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo was visited by 4,430 patients infected with HBV, including 153 with acute and 4,277 with chronic infection. Table I compares the demographic, clinical and virological characteristics between the patients with acute and chronic HBV infection at the baseline. Patients with acute HBV infection were younger ($P = 0.046$), predominantly male ($P = 0.004$), had higher alanine aminotransferase levels ($P < 0.001$),

TABLE I. Baseline Characteristics of Patients Infected With HBV Who Visited Toranomon Hospital During 35 Years (1971–2005)

Features[a]	Acute infection (n = 153)	Chronic infection (n = 4,277)	Differences (P value)
Age in years	34 (19–69)	38 (0.1–83)	0.046
≤39	99 (65%)	2,358 (55%)	
40–59	49 (32%)	1,642 (38%)	
≥60	5 (3%)	277 (7%)	
Men	125 (82%)	3,067 (72%)	0.004
ALT (IU/L)	1,460 (19–6,876)	58 (12–3,520)	<0.001
Sexual transmission	102 (67%)	129 (3%)	<0.001
Liver disease			
Symptom-free	0	1,035 (24%)	
Chronic hepatitis	0	2,617 (61%)	
Cirrhosis	0	405 (10%)	
Hepatocellular carcinoma	0	220 (5%)	
HBeAg	100 (65%)	2,131 (50%)	<0.001
HBV DNA (log copies/ml)	5.9 (<2.6 to >7.6)	6.4 (<2.6 to >7.6)	0.014

[a]Data are expressed in number of patients with percentage in parentheses or the median value with a range in parentheses.

were positive more frequently for HBeAg ($P < 0.001$), and had lower HBV DNA loads ($P = 0.014$) than those with chronic infection. Sexual transmission was more frequent in patients with acute than chronic HBV infection (67% vs. 3%, $P < 0.001$).

The number of new patients presenting with acute and chronic HBV infections during a 5-year period was compared during 1971 through 2005 (Fig. 1). In the initial four 5-year periods (1971–1990), both patients with acute and chronic HBV infections increased linearly. In the fifth 5-year period (1991–1995), however, patients with acute or chronic HBV infection decreased to less than those in the previous 5-year period (1986–1990). In the next 5-year period (1996–2000), nevertheless, patients with acute HBV infection began to increase while a decrease in chronic HBV infection was observed. In the seventh 5-year period (2001–2005), patients with acute HBV infection kept increasing. In addition, there was a small but appreciable increase of patients with chronic HBV infection in comparison with the previous 5-year period (1996–2000). Taken altogether, acute HBV infection resurged since 1991 accompanied by an increase in chronic HBV infection since 2001.

HBV Genotypes in Patients Infected With HBV

HBV was typeable in 126 of the 153 (82.4%) patients with acute and 4,121 of the 4,277 (96.4%) with chronic HBV infection (Table II). Genotype A, foreign to Japan, was more frequent in acute than chronic HBV infection (28.6% vs. 3.0%, $P < 0.001$). There were no differences in the distribution of endemic genotypes B and C; combined, they accounted for 69.8% and 96.8%, respectively, in patients with acute and chronic HBV infections. Foreign genotypes other than A (D–H) were detected in 2 (1.6%) and 10 (0.24%) patients with acute and chronic HBV infections, respectively. One each genotype D and H were found in patients with acute HBV infection; and 6 with genotype D, 3 genotype F and 1 genotype H in those chronic infection. Among patients with chronic HBV infection, genotype B was more frequent (566/3,481 [16.3%] vs. 28/508 [5.5%], $P < 0.001$), while genotype C was less common (2,915/3,481 [83.7%] vs. 480/508 [94.5%], $P < 0.001$), in those with chronic hepatitis than cirrhosis and/or HCC.

TABLE II. Distribution of Genotypes in Patients With Acute and Chronic HBV Infections

Genotypes[a]	Acute (n = 126)	Chronic (n = 4,129)	Differences (P value)
A	36 (28.6%)	122 (3.0%)	<0.001
B	13 (10.3%)	508 (12.3%)	NS
C	75 (59.5%)	3,489 (84.5%)	NS
D	1 (0.8%)	6 (0.1%)	NS
E	0	0	NS
F	0	1 (0.02%)	NS
G	0	0	NS
H	1 (0.8%)	3 (0.07)	NS

[a]Data are expressed in number of patients with percentage in parentheses.

Subgenotypes of HBV

Subgenotypes of A, B, and C were determined in patients with HBV infection. Of the 158 patients infected with genotype A, 15 (9.5%) were classified into subgenotype A1/Aa and 121 (76.6%) into A2/Ae; the remaining 22 (13.9%) were not typeable. Likewise, of the 521 patients with genotype B, 388 (74.5%) were infected with the domestic subgenotype B1/Bj and 102 (19.6%) with foreign subgenotype B2/Ba; subgenotypes in the remaining 31 (6%) patients could not be determined. Figure 2 compares the proportion of these subgenotypes among the seven 5-year periods. By the trend analysis, subgenotype B2/Ba was increasing recently ($P < 0.05$). Subgenotypes of C were domestic C2/Cs in all the 1,610 HBV isolates tested. The foreign subgenotype C1/Ce was not detected in any patient infected with HBV genotype C.

Change in the Distribution of Genotypes in Patients Infected With HBV

Figure 3 illustrates distributions of genotypes A–C in patients with acute and chronic HBV infection during

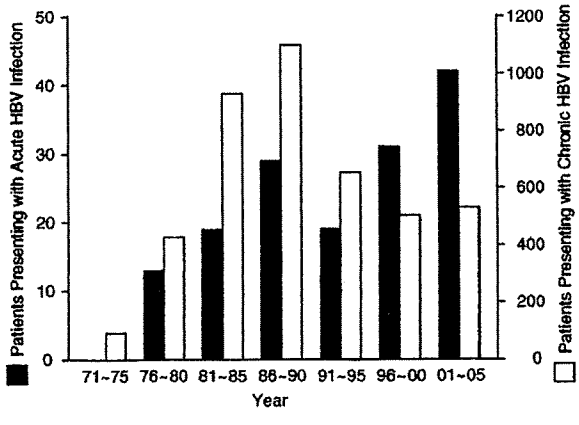

Fig. 1. Patients with acute and chronic HBV Infection who visited Toranomon Hospital during 35 years from 1971 to 2005. Numbers are indicated in different scales for patients with acute and chronic HBV infections for seven 5-year periods.

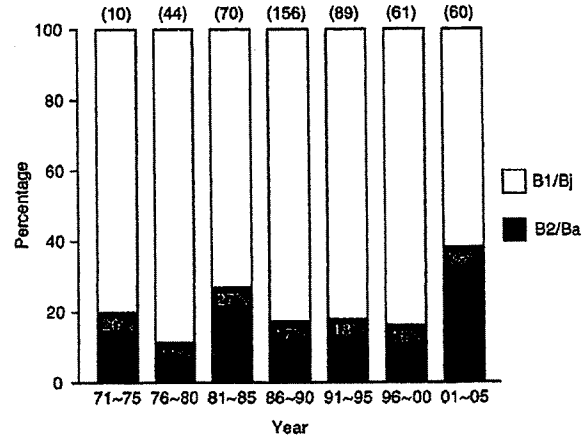

Fig. 2. Distribution of subgenotypes of genotype B shifting during 1971 through 2005. The number of patients is shown in parentheses for each seven 5-year period.

seven 5-year periods. The genotype distribution in patients with acute HBV infection changed through seven 5-year periods. The proportion of genotype A started to increase sharply in the fifth 5-year period (1996–1995) and accounted for 43% and 40%, respectively, in the sixth (1996–2000) and seventh (2001–2005) 5-year periods. The proportion of genotype A during the fifth and seventh 5-year periods (1991–2005) was significantly higher than that during the second through fourth 5-year periods (1976–1990) (39% [33/84] vs. 8% [3/40], $P < 0.001$). Before 1995, genotype A was detected in men <35 years but not found in those >36 years (7/21 [33%] vs. 0/25 [0%]). However, genotype A became comparably frequent since 1996 (15/29 [52%] vs. 14/32 [44%]).

By remarkable contrast, the distribution of genotypes in patients with chronic HBV infection remained fairly constant, although the proportion of genotypes A kept increasing constantly. Thus the proportion of genotype A during the fifth through seventh 5-year periods (1991–2005) was greater than that during the first to fourth 5-year periods (1971–1990) (4.3% [70/1,638] vs. 2.4% [51/2,128], $P < 0.001$).

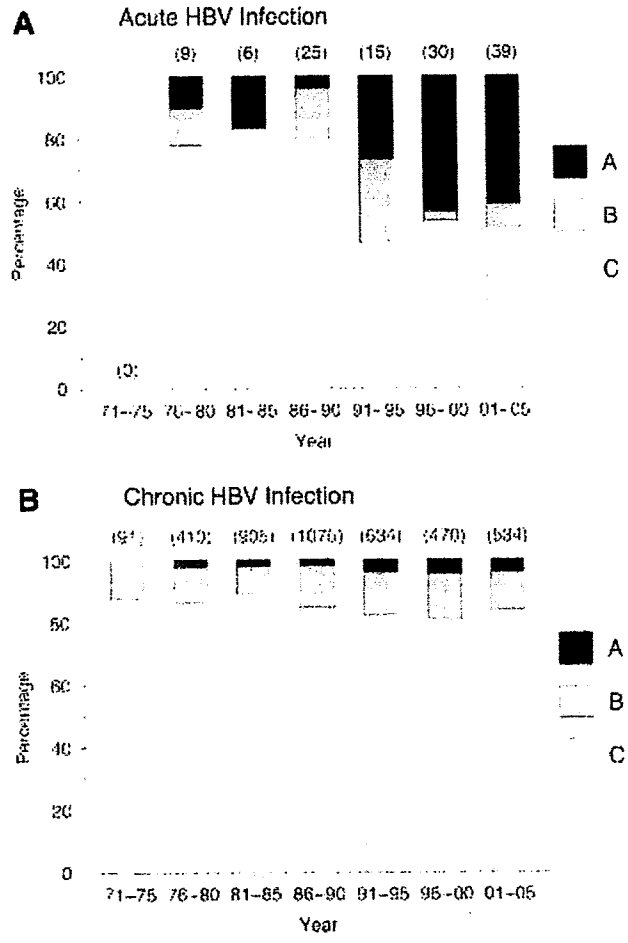

Fig. 3. Time-dependent distribution of HBV genotypes in patients with acute and chronic HBV infections during 1971 through 2005. Distribution of genotypes A–C in patients with acute HBV infection (A) and those with chronic HBV infection (B) are shown for seven 5-year periods. The number of patients is shown in parentheses for each seven 5-year period.

DISCUSSION

The Department of Hepatology at the Toranomon Hospital was visited by 153 with acute and 4,277 patients with chronic HBV infection during 35 years from 1971 through 2005. Patients with acute HBV infection were younger, more commonly male and had been infected by sexual contact more frequently than those with chronic infection. Patients were grouped by the year when they visited the department, and they were compared among seven 5-year periods spanning 1971–2005, for the purpose of estimating time-dependent trends of acute and chronic HBV infections in Japan.

Remarkably, patients presenting with acute HBV infection increased during the past 35 years (Fig. 1). Patients with chronic HBV infection peaked in 1986–1990 and then decreased until 1996–2000. They did not decrease further, but instead, increased slightly in the 21st century. Such a recent increase in chronic HBV infection would reflect resurgence of acute infection, which is supported by the analysis of genotypes.

The distribution of HBV genotypes was much different between patients with acute and chronic infections. Of note, infection with genotype A was much more frequent in acute than chronic infection (28.6% vs. 3%, $P < 0.001$). HBV genotypes have distinct geographic distribution [Miyakawa and Mizokami, 2003; Fung and Lok, 2004; Norder et al., 2004]. The Japanese have been infected with genotypes B and C since the prehistoric era [Yamashita et al., 1975], and foreign genotypes represented by A (both subgenotypes A1/Aa and A2/Ae) were introduced by travelers and immigrants after the end of World War II. Since 1991, foreign genotypes have been increasing in acute HBV infection in Japan [Sugauchi et al., 2006]. As for chronic HBV infection, genotype C was more prevalent in patients with cirrhosis and/or HCC than in those with chronic hepatitis (480/508 [94.5%], vs. 2,915/3,481 [83.7%], $P < 0.001$), standing in corroboration with previous studies [Kao et al., 2000; Orito et al., 2001].

There was a dramatic change in the distribution of HBV genotypes in patients with acute HBV infection during the past 35 years. This change is attributed to ever increasing infection with genotype A in them. It accounted for only 8.1% before 1990, in marked contrast to 39.3% after 1991 ($P < 0.001$). The recent resurgence of acute infection in Japan could be due to increase in the transmission with HBV of foreign genotypes. The gradual increase of genotype A, in patients with chronic HBV infection since 2001, would be accounted for by an increase of acute infection with this genotype in Japan. In support of this view, infection with genotype A tends to persist, infection even in adulthood, and becomes chronic in 10% of infected adults [Suzuki et al., 2005; Kobayashi et al., 2006]. In an outbreak transmitted by a surgeon, 5 of the 16 (31%) patients infected with genotype A became HBV carriers [Harpaz et al., 1996].

There are two types of risk for exposure to HBV. One is avoidable and mediated by promiscuous sexual contacts and the use of illicit intravenous drugs. The other is not preventable and can involve citizens without high-risk behaviors. For instance, HBV can be transmitted from patient to patient in dental care [Redd et al., 2007]. HBV can spread from carrier surgeons who are negative for serum HBeAg [Perry et al., 2006]. In 2002, the largest outbreak of HBV involving 38 patients occurred in a physician's office in New York City by multidose vials contaminated with HBV [Samandari et al., 2005]. There is a pressing need to investigate and determine the risk of HBV transmission in the health care setting [Allos and Schaffner, 2007]. Fortunately, risks of HBV infection can be avoided by vaccination. Mass vaccination of newborns and catch-up vaccination, such as those conducted in the United States [MMWR, 2002], Taiwan [Ni et al., 2001] and elsewhere, would need to be considered in Japan. The ultimate national protection would be universal vaccination of all age groups.

In conclusion, acute HBV infection is increasing in Japan in spite of immunoprophylaxis of high-risk babies implemented nationally since 1986. Based on genotypes/subgenotypes changing with time, the increase may be attributed to infections with HBV of foreign genotypes/subgenotypes predominantly by sexual contact. Since HBV genotype A, with a high propensity to persist, prevailed in acute infection, chronic infection would increase in the foreseeable future. Effective measures have to be taken for preventing HBV transmission among young men at high risk in Japan.

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ORIGINAL ARTICLE

Poor Response to Pegylated Interferon and Ribavirin in Older Women Infected with Hepatitis C Virus of Genotype 1b in High Viral Loads

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Abstract *Background* Response to treatment in patients with chronic hepatitis C, with reference to age and gender, has not been examined fully. *Aim* The influence of gender and age on treatment with pegylated interferon (PEG-IFN) and ribavirin was evaluated in a retrospective study. *Methods* PEG-IFN and ribavirin were given for 48 weeks to 179 men and 121 women infected with hepatitis C virus (HCV) of genotype 1b in high viral loads (>100 kIU/ml). *Results* Sustained virological response at 24 weeks after treatment was poorer in women than men who were aged ≥50 years (22% vs 53%, $P < 0.001$). Among the patients aged ≥50 years who had received ≥80% of the doses of PEG-IFN, ribavirin, or both, women responded less often than men (26% vs 64%, $P < 0.001$; 33% vs 61%, $P = 0.022$; and 32% vs 63%, $P = 0.016$; respectively). In multivariate analysis, male gender, retention of indocyanine green, ribavirin dose and compliance with therapy increased sustained virological response. *Conclusions* Response to combined PEG-IFN and ribavirin is poorer in female than male patients with hepatitis C who are aged ≥50 years, irrespective of compliance with treatment. Low estrogen levels in older women could be responsible for their impaired response to PEG-IFN and ribavirin.

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Keywords Aging · Women · Chronic hepatitis C · Genotypes · Interferon · Ribavirin

Introduction

There are an estimated 170 million people worldwide that are chronically infected with hepatitis C virus (HCV) [1]. HCV can persist in 70–80% of individuals who have been exposed to it, and it can induce chronic liver disease, through cirrhosis to hepatocellular carcinoma (HCC) in approximately 30% of them until 30–40 years after they were infected [2–4]. A number of viral and host factors influence the velocity of fibrosis progression in chronic hepatitis C. Thus, stage and grade of hepatitis are more severe in patients who are infected with HCV genotype 1 in high viral loads [5–7]. Male gender, age and intake of alcohol accelerate fibrosis, as well [8–10].

Interferon (IFN) combined with ribavirin has been the most effective and favored treatment of chronic hepatitis C to date. The combined treatment with the standard IFN can terminate HCV-1 infection with high viral loads in approximately 20% [11], and that with pegylated IFN (PEG-IFN) in >40% [12]. Owing to hemolytic side effects, however, women are less tolerant to ribavirin [13]. Although the response to combined treatment has been shown to be better in women than in men in previous studies, there remains a possibility that it could be influenced by age. Hence, there is a need for the comparison of the response between men and women in different age groups.

Virological response to PEG-IFN and ribavirin at the end of a 48-week treatment (ETR), as well as sustained virological response (SVR) 24 weeks after the completion of therapy, was compared between 179 men and 121

women who were infected with HCV-1b in high viral loads. In patients aged ≥50 years, both ETR and SVR were poorer in women than in men, irrespective of the total dose of IFN, ribavirin or both.

Methods

Study Population

From December 2001 to February 2006, 490 consecutive patients with chronic hepatitis C received combination therapy with PEG-IFN and ribavirin at the Department of Hepatology in the Toranomon Hospital in Metropolitan Tokyo. The following inclusion criteria were met by 300 (61%) patients: they were (1) positive test results for antibodies to HCV (anti-HCV) and for HCV RNA genotype 1b by qualitative methods, and not co-infected with HCV of other genotypes; (2) negative test results for hepatitis B surface antigen or antibodies to human immunodeficiency virus type-1 (HIV-1); (3) confirmed findings of high HCV RNA levels ≥100 kIU/ml, which is the Japanese definition of high viral loads [14, 15], within the past 2 months; (4) no cirrhosis diagnosed by laparoscopy and ultrasonography, and with platelet counts $>80 \times 10^3/mm^3$; (5) body weight ≥40 kg and not pregnant or lactating; (6) total alcohol intake <500 kg in the past; (7) no HCC, hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic hepatitis or autoimmune hepatitis; (8) no treatment with antivirals or immunosuppressants during the previous 3 months; and (9) with the wish to comply with the treatment protocol for 48 weeks. None of them received growth factors before or during the study period.

The 300 patients, comprising 179 men and 121 women, received PEG-IFN and ribavirin for 48 weeks and were followed for at least 24 weeks after completion of this combination therapy. Informed consent was obtained from each patient, and the study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Serum Markers of HCV Infection

Anti-HCV was determined by third-generation enzyme-linked immunosorbent assay (ELISA) by commercial kits (Ortho HCV Ab ELISA Test 3; Chiron Cooperation, Emeryville, CA, USA). HCV RNA was determined quantitatively by polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor ver. 2.0, Roche Diagnostics, Tokyo, Japan) in serum diluted tenfold at the baseline, as well as at least monthly during and after treatment; it has a dynamic range between 5 kIU/ml and 5,000 kIU/ml. Sera negative for HCV RNA (<5 kIU/ml) by quantitative assay were tested by qualitative PCR (Amplicor, Roche Molecular Systems, Inc., Branchburg, NJ, USA) with a detection limit at 100 copies/ml.

Combined PEG-IFN and Ribavirin Therapy

Patients underwent subcutaneous administration of PEG-IFN-α2b (PEG-Intron, Schering-Plough Corp, Kenilworth, NJ, USA), weekly, at a median dose of 1.4 μg/kg (range 0.8–1.9 μg/kg), together with ribavirin orally, at a median daily dose of 11 mg/kg (range 3.7–14.2 mg/kg) for 48 weeks. The dose of ribavirin was adjusted by body weight: 600 mg for patients weighing ≤60 kg; 800 mg for those between >60 kg and <80 kg; and 1,000 mg for those ≥80 kg. It was tapered in the 99 (33%) patients in whom hemoglobin levels decreased below 10 g/dl during the combination therapy.

Statistical Analysis

Variables were compared between groups by the chi-square test, Fisher's exact probability test and the Mann–Whitney U test. Differences in the loss of HCV RNA from the serum between groups was evaluated with the Kaplan–Meier life table with use of the log rank test. The influence of various factors on the response to PEG-IFN/ribavirin was evaluated by logistic regression in univariate and multivariate analyses. Analysis of all data was performed with the computer program SPSS software (SPSS Inc., Chicago, IL, USA), and a P value less than 0.05 was considered significant.

Results

Baseline Characteristics of Male and Female Patients Infected with HCV-1b in High Loads

PEG-IFN and ribavirin were given for 48 weeks to 179 men and 121 women who had been infected with HCV-1b in high viral loads (>100 kIU/ml). Table 1 compares baseline characteristics between them. Women were older, had lower hemoglobin values and platelet counts, and lower levels of albumin, gamma-glutamyl-transpeptidase (γ-GTP) and ferritin, than men. The stage of fibrosis was lower in women than in men, although their alanine aminotransferase (ALT) levels were comparable. Three months before the start of combination therapy, IFN had been given to 75 (42%) of the 179 male patients, comparably frequently to 40 of the 121 (33%) female patients. Age distribution for men and women is shown in Fig. 1. The proportion of patients ≥60 years was higher in women than in men (39% vs 19%, $P < 0.001$).

Table 1 Baseline characteristics of 300 patients with chronic hepatitis with high-titers of HCV-1b RNA who had received PEG-IFN and ribavirin for 48 weeks and were followed for 48 weeks or longer

Characteristic[a]	Men (n = 179)	Women (n = 121)	Differences P
Age (years)	50 (19–66)	57 (30–69)	<0.001
Previous IFN treatment	75 (42%)	40 (33%)	0.146
Hemoglobin (g/dl)	15.2 (11.5–17.8)	13.5 (11.2–15.1)	<0.001
Platelets ($\times 10^3/mm^3$)	176 (88–366)	165 (91–331)	0.025
Albumin (g/dl)	3.9 (3.2–4.6)	3.8 (3.0–4.6)	0.004
ALT (IU/l)	77 (23–504)	68 (19–391)	0.078
γ-GTP (IU/l)	78 (14–409)	37 (11–171)	0.011
LDL (mg/dl)	98 (50–176)	99 (57–168)	0.920
Ferritin (mg/l)	186 (<10–1,327)	95 (<10–4 42)	<0.001
ICG_{15} (%)	14 (4–41)	13 (2–31)	0.969
Stage (F0-1/F2-3)	80/66 (50 unknown)	42/55 (57 unknown)	0.050

ALT alanine aminotransferase, *γ-GTP* gamma-glutamyl transpeptidase, *LDL* low density lipoprotein, ICG_{15}, retention of indocyanine green at 15 min

[a] The means (ranges) are given

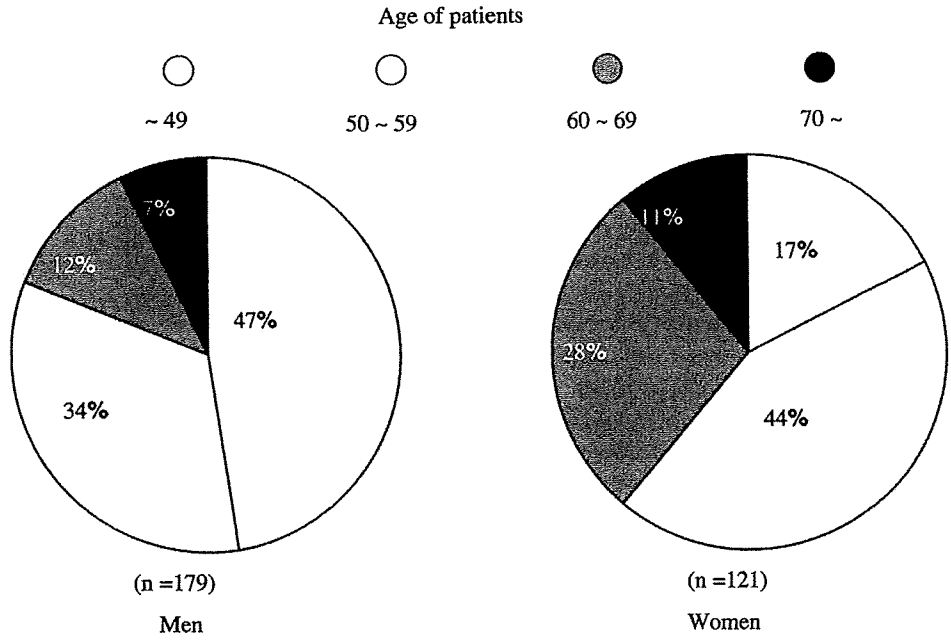

Fig. 1 Distribution of ages in the male and female patients with chronic hepatitis C who were infected with HCV-1b in high loads

Virological Response During the 48-Week Treatment with PEG-IFN and Ribavirin

On-treatment response to the combined treatment is compared between men and women in Fig. 2. Through 48 weeks on treatment, women gained a virological response less frequently than did men. ETR was achieved by 58% of women as against 83% of men ($P < 0.001$). Restricted to the patients who gained ETR, women lost HCV RNA from serum later than men did [median (range) 24.1 (2.0–36.4) vs 13.0 (2.0–48.0) weeks, $P < 0.001$]. Figure 3 depicts the on-treatment virological response in patients <50 years and those ≥50 years separately. The virological response was no different between men and women <50 years. However, it was poorer in women than

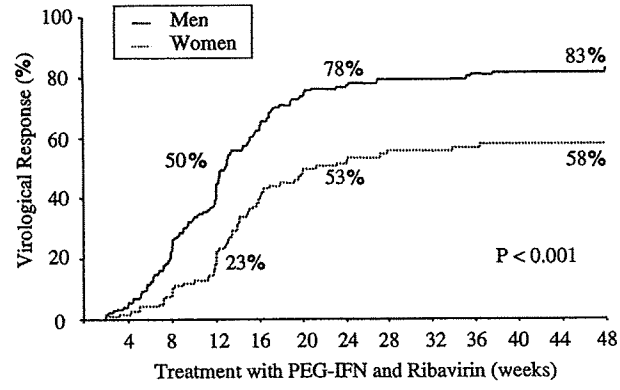

Fig. 2 On-treatment virological responses to PEG-IFN and ribavirin in male and female patients infected with HCV-1b in high viral loads

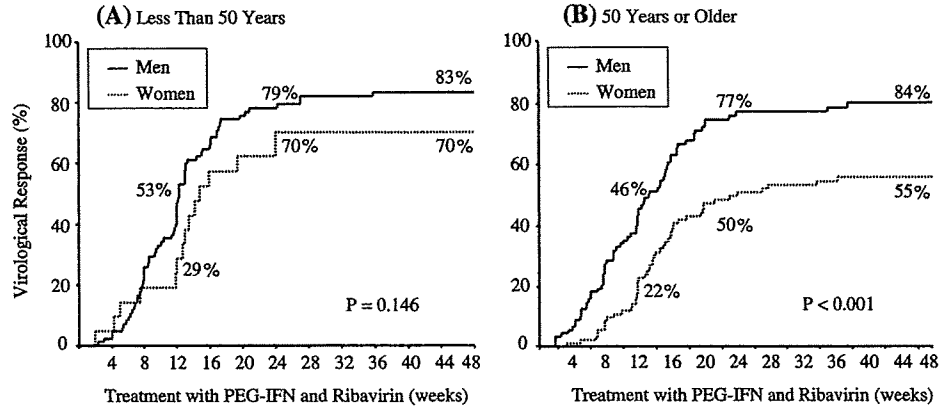

Fig. 3 On-treatment virological responses to combined IFN and ribavirin in male and female patients infected with HCV-1b in high viral loads who were less than 50 years (**a**) or 50 years or older (**b**)

in men ≥50 years. Differences between men and women in total (Fig. 2), therefore, were attributed to a poorer response of women ≥50 years to the combined treatment.

Sustained Virological Response to the 48-Week Treatment with PEG-IFN and Ribavirin

Sustained virological response 24 weeks after the completion of combined treatment was accomplished much less frequently in women than in men [33/121 (27%) vs 105/179 (59%), $P < 0.001$]. SVR was influenced by age both in men and in women (Fig. 4). It was found significantly less often in women than men who were 50 years or older.

Figure 5 illustrates the relationship between the earliest on-treatment virological response and SVR in men and women. Patients with a virological response at 4 weeks gained SVR invariably. However, in the patients with virological response in later weeks, SVR was achieved less frequently in women than in men. In the patients who had lost HCV RNA from the serum at 12 weeks, in particular, SVR was achieved significantly less often in women than in men (63% vs 88%, $P = 0.012$). The relationship

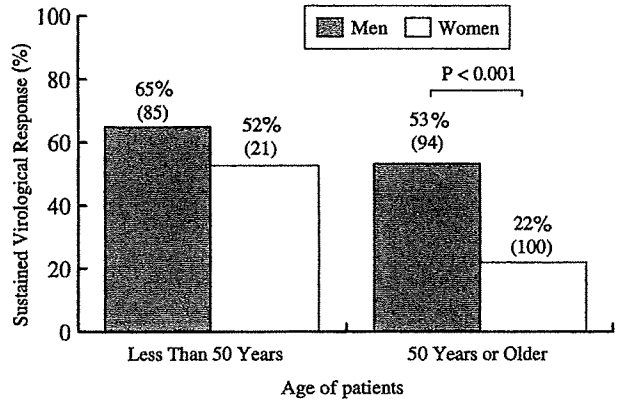

Fig. 4 Sustained virological response to PEG-IFN and ribavirin in male and female patients stratified by age. The number of patients is indicated in parentheses in each column

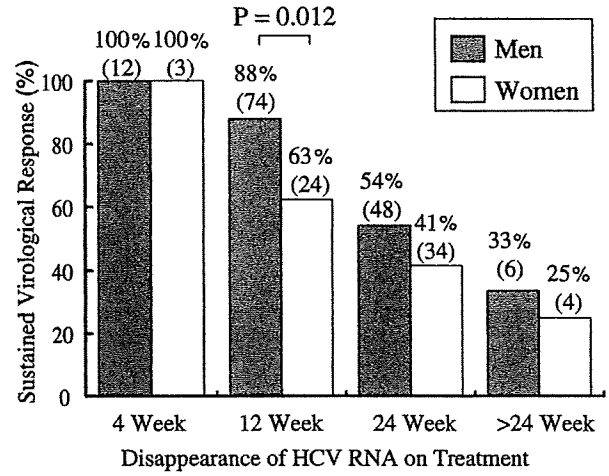

Fig. 5 Sustained virological response in male and female patients who lost HCV RNA from the serum at various weeks on treatment with PEG-IFN and ribavirin

between on-treatment virological response and SVR was compared among women in different age groups (Fig. 6). In the patients with the earliest virological response at 12 weeks and 24 weeks, SVR was achieved less frequently in women aged ≥50 years than in those <50 years, but the difference fell short of being significant due to the small numbers of patients in the comparison.

SVR and Compliance with PEG-IFN Therapy, Ribavirin Therapy, or Both

Table 2 compares compliance with the combined treatment between men and women. Either or both of PEG-IFN and ribavirin were tolerated to a lesser extent by women than by men. Thus, doses ≥80% were reached less frequently in women than in men for PEG-IFN or ribavirin, or both. The initial dose of ribavirin was no different between men and women.

SVR was achieved less frequently in women than in men who had received ≥80% of the dose of PEG-IFN

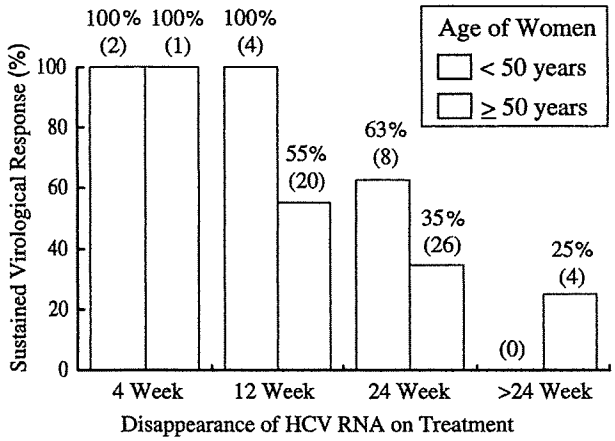

Fig. 6 Sustained virological response to PEG-IFN and ribavirin in female patients stratified by age who lost HCV RNA from serum at various weeks on treatment

Table 2 PEG-IFN and ribavirin received by patients with chronic hepatitis with high-titers of HCV-1b RNA

PEG-IFN and ribavirin	Men ($n = 179$)	Women ($n = 121$)	Differences P
Initial ribavirin dose (mg/kg body weight)	11.1 (5.0–14.1)	11.2 (3.7–14.3)	0.735
Total dose			
PEG-IFN ≥ 80%	139 (78%)	94 (53%)	<0.001
Ribavirin ≥ 80%	117 (65%)	42 (35%)	<0.001
Both ≥ 80%	110 (61%)	32 (27%)	<0.001
Withdrawn	28 (16%)	28 (23%)	0.131

[20/64 (31%) vs 92/138 (67%), $P < 0.001$], ribavirin [19/42 (45%) vs 79/120 (66%), $P = 0.027$] or both [13/32 (41%) vs 72/109 (66%), $P = 0.013$]. Again, differences were observed only in patients ≥50 years (Fig. 7). In the patients <50 years, in contrast, the rate of SVR was no different between women and men who had received ≥80% of the dose of PEG-IFN, ribavirin, or both.

The influence of age was compared between male and female patients in different age groups. SVR was achieved significantly more frequently in the men aged ≥60 years [88/145 (61%) vs 17/74 (37%), $P = 0.001$] and <60 years [17/34 (50%) vs 6/48 (13%), $P < 0.001$]. Likewise, SVR was more common in male than female patients aged 50–59 years [33/60 (55%) vs 16/53 (30%), $P = 0.013$].

Multivariate Analysis for Factors Accelerating the Response to PEG-IFN and Ribavirin Therapy

In univariate analysis, age, gender, hemoglobin, albumin, ICG_{15}, ribavirin dose and compliance with PEG-IFN therapy, ribavirin therapy, or both, influenced SVR. In multivariate analysis, only male gender, ICG_{15}, ribavirin dose and compliance with PEG-IFN, as well as both PEG-IFN and ribavirin, accelerated the chance of SVR (Table 3).

Discussion

In a retrospective study, response to PEG-IFN and ribavirin for 48 weeks was compared between 179 men and 121 women with chronic hepatitis C who had been infected with HCV-1b in high viral loads by the Japanese definition (>100 kIU/ml) [14, 15]. Loss of HCV RNA from serum occurred less often in women than in men throughout the 48 weeks of treatment. Both ETR (55% vs 83%, $P < 0.001$) and SVR (27% vs 59%, $P < 0.001$) were achieved significantly less frequently in women than in men. The observed low response to PEG-IFN and ribavirin stands at odds with the better response to antiviral treatments and slow progression of fibrosis in women than in men [9, 16, 17]. There are, however, viral and host factors other than gender that can influence the course of chronic hepatitis C and, by inference, the response to antiviral treatments.

Viral factors such as HCV genotypes and infection load affect the course of chronic hepatitis C. Thus, hepatitis is

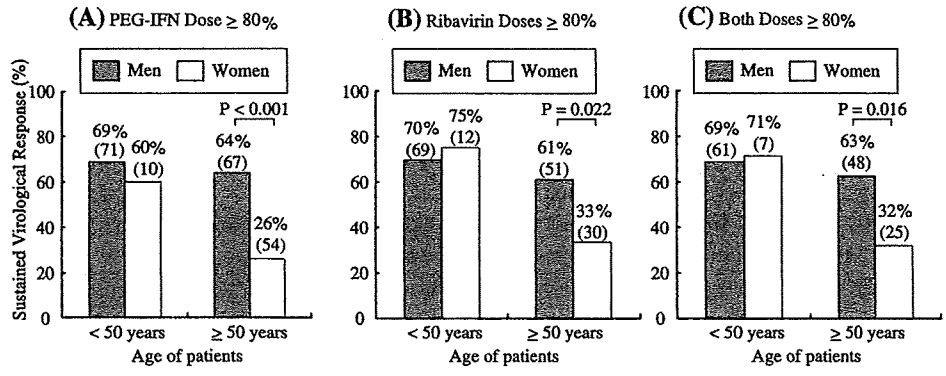

Fig. 7 Sustained virological response to PEG-IFN and ribavirin in patients who had received 80% or more of the dose of IFN (**a**), ribavirin (**b**) or both of them (**c**). Results are shown for men and women in two age groups

Table 3 Factors promoting the response to PEG-IFN and ribavirin in multivariate analysis

Factors	Odds ratio	95% Confidence interval	*P*
Male gender	3.50	1.71–7.17	0.001
$ICG_{15} \leq 13.5\%$	2.09	1.07–4.08	0.031
Ribavirin $\geq$ 11.1 mg/kg per day	2.17	1.11–4.25	0.024
Total PEG-IFN $\geq$ 80%	6.96	2.26–21.4	0.001
PEG-IFN/ribavirin $\geq$ 80%	12.66	2.32–71.4	0.003

more severe and less responsive to IFN in patients infected with HCV genotypes 1 and 4 than in those with HCV genotypes 2, 3 and 6 [18–22]. Likewise, high viral loads are associated with rapid progression of liver disease and poor response to IFN [23–25]. In our study, such viral factors were excluded in comparing the response to PEG-IFN and ribavirin between women and men. All the patients were infected with HCV genotype 1b in high viral loads (>100 kIU/ml).

Age influences the severity of chronic hepatitis C [9, 26], and disease progresses faster and response to antiviral therapy is poorer in older patients [23]. There were significant differences in age between female and male patients in our study. The women were older than the men [mean (range) 57 (30–69) years vs 50 (19–66) years, $P < 0.001$], and the proportion of patients $\geq$60 years was higher in women than in men (39% vs 19%, $P < 0.001$). Hence, the response to PEG-IFN and ribavirin was evaluated in patients aged $\geq$50 years and <50 years separately. There were no differences in the response between female and male patients <50 years, during and at the end of the 48-week treatment, as well as 24 weeks thereafter. However, ETR (55% vs 84%, $P < 0.001$) and SVR (22% vs 53%, $P < 0.001$) were gained significantly less often in women than men who were aged $\geq$50 years.

The influence of gender was observed, also, in patients aged $\geq$60 years and those aged 50–60 years. Hence, women would become less responsive than men to PEG-IFN and ribavirin after they had entered their fifties.

From a therapeutic notion, compliance with treatment can alter the response. Since ribavirin accumulates in erythrocytes and induces hemolysis, it is less tolerated in women who tend to be anemic than men without such an inclination [27]. At the baseline, women had lower levels of hemoglobin and ferritin than men. These would have been responsible for the lower tolerance to PEG-IFN and ribavirin in women than men in our study. In fact, $\geq$80% of the dose of PEG-IFN, ribavirin, or both, was tolerated less frequently in women than men ($P < 0.001$ for each). Even in the patients who had received $\geq$80% of the dose, however, the response to PEG-IFN and ribavirin was gained less frequently in women than in men. Again, the difference was due to a significantly lower response in female patients than in male patients aged $\geq$50 years, while the response was no different between those <50 years of age.

Taken altogether, the poorer response to PEG-IFN and ribavirin in women than in men was attributable to impaired response in the female patients aged $\geq$50 years. Older women with chronic hepatitis C, therefore, would be less responsive to the combined treatment with PEG-IFN and ribavirin currently in use. In support of this view, the response to human lymphoblastoid IFN for 24 weeks is dependent on gender and age [28]. The greatest physiological change precipitated in women by aging is a decreased serum concentration of bioavailable estrogen after they enter the menopause [29]. Estrogen has been shown to have an antifibrotic potential in both experimental and clinical studies. In experimental cirrhosis induced by dimethylnitrosamine in rats, administration of neutralizing antibodies to estradiol and ovariectomy enhanced fibrogenesis in female rats [30]. Hepatocytes have the receptor to estrogen [31], and myofibroblastic transformation in hepatic stellate cells of rats is inhibited in culture supplemented with this hormone [32]. Consequently, hepatic fibrosis progresses faster in menopausal women with chronic hepatitis C, and hormone replacement therapy may be able to prevent it [33]. Furthermore, in women aged $\geq$50 years, the number of estrogen receptor in hepatocytes decreases to one-half of that in those aged <50 years. This would stand in further support of the notion that the antifibrotic effects of decreased estrogen levels in patients aged $\geq$50 years with chronic hepatitis C would produce a lesser response to PEG-IFN and ribavirin.

Favorable effects of female sex hormones on hepatitis have long been suggested. Chronic hepatitis C is mild in menstruating women [34]; its activity is suppressed during pregnancy and enhanced after delivery [35]. The velocity of fibrosis progression is extremely low in young women exposed to HCV through mass-administration of immunoglobulin-D. Only two of 184 (1.2%) and four of 1,018 (0.4%) developed cirrhosis over 24 years and 20 years, respectively, in Irish and German studies [36, 37]. It does need to be pointed out, however, that the majority of women in those studies had not been followed beyond the menopause. There is a possibility that chronic hepatitis C may progress at a faster speed during their next few decades. Continued observations of them would be necessary to evaluate the validity of such an assumption.

Although decreased levels of estrogen can explain the enhanced activity of chronic hepatitis C in older women, as well as their concomitant resistance to PEG-IFN and ribavirin, it does not give an account of the better response in men than women who were aged $\geq$50 years. Feminization represented by gynecomastia is common in men

who have developed cirrhosis, and it can increase even in healthy men with age [38]. Possibly in the background of this phenomenon, circulating levels of free estrogen in men exceed those in women, after they enter their fifties, with margins widening with age [29]. It is tempting to speculate that elevated estrogen levels in men with chronic hepatitis C are responsible for their better response to the combination therapy than women who were aged $\geq$50 years. Whether or not such a speculation would hold would have to be evaluated by a comparison of estrogen levels between older men and women with chronic hepatitis C.

Although osteoporosis is an extrahepatic manifestation of chronic hepatitis C [39], hormone replacement therapy has been withheld for fear of potential hepatotoxicity. There is evidence, however, that oral contraceptives inhibit the progression of fibrosis in women [33]. It may lead to the possibility that the response to antiviral treatment in older women with chronic hepatitis C would be improve by substituting estrogen in them. The merit of hormone replacement therapy for them, of course, would need to be balanced against any harmful effects associated with it.

There are limitations in this study. All the patients were infected with genotype 1b in high viral loads. Hence, the results obtained may or may not be extended to patients with chronic hepatitis C who are infected with HCV of other genotypes in low viral loads. The influence of sex hormones needs to be substantiated by their determination in correlation with SVR. These limitations notwithstanding, the results obtained warrant a special caution in the treatment of women older than 50 years due to their lesser responsiveness to PEG-IFN and ribavirin.

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Sustained Virological Response Reduces Incidence of Onset of Type 2 Diabetes in Chronic Hepatitis C

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Diabetes is present in patients with chronic hepatitis C virus infection. The aim of this retrospective cohort study was to assess the cumulative development incidence and predictive factors for type 2 diabetes after the termination of interferon therapy in Japanese patients positive for hepatitis C virus (HCV). A total of 2,842 HCV-positive patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin were enrolled. The mean observation period was 6.4 years. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses during follow-up. The primary goal was the onset of type 2 diabetes. Evaluation was performed by using the Kaplan-Meier method and Cox proportional hazard analysis. Of 2,842 HCV patients, 143 patients developed type 2 diabetes. The cumulative development rate of type 2 diabetes was 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years. Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), the patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was ≥50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). *Conclusion:* Our results indicate sustained virological response causes a two-thirds reduction in the risk of type 2 diabetes development in HCV-positive patients treated with IFN. (HEPATOLOGY 2009;49:000-000.)

Hepatitis C virus (HCV) is one of the more common causes of chronic liver disease in world. Chronic hepatitis C is an insidiously progressive form of liver disease that relentlessly but silently progresses to cirrhosis in 20% to 50% of cases over a period of 10 to 30 years.[1-3] In addition, HCV is a major risk for hepatocellular carcinoma (HCC).[4-8] Moreover, chronic HCV infection has been associated with a variety of extrahepatic complications such as essential mixed cryoglobulinemia, porphyria cutanea tarda, membranoproliferative glomerulonephritis, autoimmune thyroiditis, sialadenitis, and cardiomyopathy.[9-13] Lately, data supporting a link between type 2 diabetes mellitus (T2DM) and chronic hepatitis C infection have been reported.[14,15]

Although there is growing evidence to support the concept that HCV infection is a risk factor for developing T2DM, there have been a few interventional studies confirming this issue. This issue needs to be confirmed with a long-term follow-up of patients with high risk of developing diabetes. Thus, prospective studies including metabolic evaluations are clearly needed to clarify these issues.

With this background in mind, the cohort study was initiated to investigate the cumulative incidence and risk factors of T2DM after prolonged follow-up in HCV-infected patients treated with interferon (IFN) monotherapy or combination therapy with IFN and ribavirin. The strengths of the current study are the large numbers of patients included and the long-term follow-up of patients.

Patients and Methods

Patients. There were 5,890 patients diagnosed with chronic HCV infection and treated with IFN mono-

Abbreviations: CI, confidence interval; FPG, fasting plasma glucose; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; SVR, sustained virological response; T2DM, type 2 diabetes mellitus.

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therapy or combination IFN + ribavirin therapy between September 1990 and March 2007 in the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 2,842 patients satisfied the following criteria: (1) no evidence of diabetes mellitus for 3 months after the termination of IFN (plasma glucose concentration <126 mg/dL [6.9 mmol/L] in the fasting state, <200 mg/dL [11.0 mmol/L] in casual state and/or 2 hours after a 75-g oral glucose load); (2) features of chronic hepatitis or cirrhosis diagnosed via laparoscopy and/or liver biopsy before the initiation of IFN therapy; (3) positivity for serum HCV RNA before the initiation of IFN therapy; (4) period of ≤1 year of IFN therapy; (5) negativity for hepatitis B surface antigen (HBsAg), antinuclear antibodies, or antimitochondrial antibodies in serum, as determined via radioimmunoassay or spot hybridization; (6) no evidence of HCC nodules as shown on ultrasonography and/or computed tomography; and (7) no underlying systemic disease, such as systemic lupus erythmatosus or rheumatic arthritis.

Patients who were taking medications known to alter glucose tolerance or had illnesses that could seriously reduce their life expectancy or their ability to participate in the trial were excluded from the study. Patients were classified as having normal glucose or pre-diabetes based on fasting plasma glucose (FPG), casual plasma glucose, or 2-hour plasma glucose. The normal glucose group was regarded as having an FPG of <100 mg/dL, casual plasma glucose of <140 mg/dL, and/or 2-hour plasma glucose of <140 mg/dL. The pre-diabetes group was regarded as having an FPG of 100-125 mg/dL, casual plasma glucose of 140-200 mg/dL, and/or 2-hour plasma glucose of 140-200 mg/dL.[16]

Next, we assessed predictive factors for T2DM in chronic hepatitis C patients treated with IFN. The physicians in charge explained the purpose and method of this clinical trial to each patient and/or the patient's family. Informed consent was obtained from all living patients included in the present cohort study. The study was approved by the Institutional Review Board of our hospital.

Outcome Measures. The primary outcome was T2DM, diagnosed by the use of the 2003 criteria of the American Diabetes Association.[16] These criteria include (1) casual plasma glucose ≥200 mg/dL; (2) FPG ≥126 mg/dL; (3) 2-hour post-glucose (oral glucose tolerance test) ≥200 mg/dL.

Laboratory Investigation. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II; Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, version 2.0; Roche, Tokyo, Japan). Hepatitis B surface antigen was tested via radioimmunoassay (Abbott Laboratories, Detroit, MI). The used serum samples were stored at −80°C at the first consultation. Diagnosis of HCV infection was based on detection of serum HCV antibody and positive RNA. Height and weight were recorded at baseline, and the body mass index was calculated as weight (in kg)/height (in m^2).

Evaluation of Liver Cirrhosis. Liver status of the 2,842 patients was mainly determined via peritoneoscopy and/or liver biopsy. Liver biopsy specimens were obtained using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University, Kakinuma Factory, Tokyo, Japan), fixed in 10% formalin, and stained with hematoxylin-eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff after diastase digestion. The size of specimens for examination was more than six portal areas.[17]

Follow-up. The starting time of follow-up was 3 months after the termination of IFN therapy. After that, patients were followed up monthly to tri-monthly in our hospital. Physical examination and biochemical tests were conducted at each examination together with regular check-up. An overnight (12-hour) fasting blood sample or a casual blood sample was taken for routine analyses. These included aminotransferase activities, total cholesterol, platelet counts, and serum HCV RNA level. Three hundred twenty-four patients were lost to follow-up; because the appearance of T2DM and death was not identified in these patients, they were considered as censored data in the statistical analysis.[18] Moreover, patients retreated with antiviral agents were regarded as withdrawals at the time of starting the retreatment of antiviral agents.

Statistical Analysis. The cumulative appearance rate of T2DM was calculated from 3 months after the termination of IFN treatment to the appearance of T2DM using the Kaplan-Meier method. Differences in the development of T2DM were tested using the log rank test. Independent factors associated with the incidence rate of T2DM were analyzed by the Cox proportional hazard model. The following 11 variables were analyzed for potential covariates for incidence of T2DM at the time of termination of IFN therapy at our hospital: age, sex, state of liver disease (chronic hepatitis or liver cirrhosis), body mass index, glucose level, aspartate aminotransferase level, alanine aminotransferase level, type of IFN, total dose of IFN, efficacy of IFN therapy, hypertension, triglyceride level, and total cholesterol level. A P value of less than 0.05 was considered significant. Data analysis was performed using SPSS 11.5 for Windows (SPSS, Chicago, IL).

Results

Patient Characteristics. Table 1 shows the characteristics of the 2,842 HCV-positive patients treated with

Table 1. Patient Characteristics

N	2,842
Sex (male/female)	1,778/1,064
Age (years)	51.8 ± 9.0
Height (cm)	163.8 ± 9.1
Body weight (kg)	62.7 ± 11.7
Body mass index	23.3 ± 3.2
Blood pressure (systolic/diastolic, mm Hg)	128 ± 18/77 ± 12
HCV genotype (1b/2a/2b/other)	744/752/290/56
HCV RNA level (KIU/mL)	593 ± 540
Staging (non-LC/LC)	2,649/193
Blood glucose level (normal/prediabetes)	2,601/241
Fasting plasma glucose (mg/dL)	87 ± 24
Triglyceride (mg/dL)	166 ± 31
Total bilirubin (g/dL)	102 ± 56
AST (IU/L)	74 ± 63
ALT (IU/L)	116 ± 102
IFN monotherapy*/combination therapy†	2,417/425
Efficacy of treatment (SVR/non-SVR)	1,175/1,667
Follow-up period (years)	6.4 ± 5.0

Data are expressed as the number of patients or mean ± standard deviation.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LC, liver cirrhosis; SVR, sustained virological response.

*Outbreak of IFN monotherapy: recombinant IFN-α2a, 304 cases; recombinant IFN-α2b, 235 cases; natural IFN-bα, 1,355 cases; natural IFN-β, 522 cases; total dose of IFN = 598 ± 170 MU.

†Outbreak of combination therapy: recombinant IFN-α2b + ribavirin, 175 cases; total dose of IFN = 537 ± 196 MU; total dose of ribavirin = 182 ± 69 g; pegylated IFN-α2b + ribavirin, 250 cases; total dose of pegylated IFN = 4.28 ± 1.17 mg; total dose of ribavirin = 232 ± 60 g.

IFN monotherapy or combination therapy with IFN and ribavirin. The sustained virological response (SVR) rate was 36.7% (886/2417) in IFN monotherapy and 68% (289/425) in IFN + ribavirin therapy. Thus, the number of patients with SVR was 1,175. The mean period after the termination of antiviral drugs was 6.4 years.

Incidence of T2DM in Patients with HCV. A total of 143 patients (102 men and 41 women) developed T2DM during a mean observation period of 6.4 years. Of these, 26 were SVR and 117 were non-SVR. The cumulative development rate of T2DM was determined to be 3.6% at 5 years, 8.0% at 10 years, and 17.0% at 15 years using the Kaplan-Meier method (Fig. 1). The factors associated with the incidence of T2DM in all 2,842 patients treated with IFN therapy are shown in Table 2.

Multivariate Cox proportional hazard analysis revealed that type 2 diabetes development after the termination of IFN therapy occurred when histological staging was advanced (hazard ratio 3.30; 95% confidence interval [CI] 2.06-5.28; $P < 0.001$), sustained virological response was not achieved (hazard ratio 2.73; 95% CI 1.77-4.20; $P < 0.001$), patient had pre-diabetes (hazard ratio 2.19; 95% CI 1.43-3.37; $P < 0.001$), and age was >50 years (hazard ratio 2.10; 95% CI 1.38-3.18; $P < 0.001$). SVR causes a two-thirds reduction of development of T2DM in patients treated with IFN. In addition to SVR, age ≥50 years, liver cirrhosis, and pre-diabetes contribute to a high risk of developing diabetes. The cumulative development rates of T2DM based on difference of age, efficacy of the IFN therapy, histological diagnosis, and glucose level at the starting time of follow-up are shown in Fig. 2.

Fig. 3 shows the impact of reduction due to SVR on the incidence of T2DM in patients with ≥50 years, liver cirrhosis, or pre-diabetes. When patients with age ≥50 years, liver cirrhosis, and pre-diabetes have SVR after IFN therapy, SVR could statistically reduce the onset of T2DM compared with those without SVR.

Discussion

We have described the development incidence of diabetes after the termination of antiviral therapy in HCV-positive patients treated with IFN therapy in the present study. Diabetes has been reported in less than 0.08% of patients treated with IFN[19,20]; thus, to exclude diabetes originating from IFN-related side effects, patients without diabetes for 3 months after the termination of IFN were enrolled in the present study. The present study indicates that the annual incidence of T2DM for a prolonged follow-up after the termination of IFN therapy among HCV patients is 0.8% to 1.0%. The present study was limited by a retrospective cohort trial. We started the present study in 1991 based on the diabetes mellitus criteria published by Fajans.[21] However, after that, diabetes mellitus criteria were revised. We thus rechecked the diagnosis of T2DM based on the diabetes mellitus criteria of 2003 in patients seen prior to 2003.[16] Because of rechecking the diagnosis of T2DM on the basis of diabetes mellitus criteria in 2003, the present study was regarded as a retrospective cohort study. However, the patients were

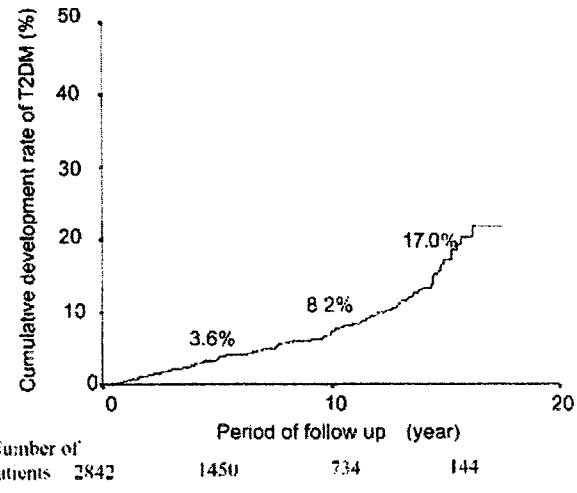

Fig. 1. Cumulative development rate of T2DM in patients treated with IFN.

Table 2. Predictive Factors for T2DM Development

Variables	Univariate Analysis		Cox Regression	
	HR (95% CI)	P Value	HR (95% CI)	P Value
Age, years (≥50/<50)	2.55 (1.74-3.73)	<0.001	2.10 (1.38-3.18)	<0.001
Sex (female/male)	0.84 (0.59-1.19)	0.318		
Body mass index (≥25/<25)	1.44 (0.98-2.08)	0.057		
HCV load (KIU/mL, ≥1,000/<1,000)	0.67 (0.43-1.03)	0.069		
Genotype (1/2)	0.73 (0.50-1.06)	0.098		
ALT (IU/L, ≥50/<50)	1.83 (1.14-2.94)	0.012		
Glucose level (prediabetes/normal)	2.25 (1.53-3.33)	<0.0001	2.19 (1.43-3.37)	<0.001
Triglyceride (mg/dL, ≥150/<150)	1.66 (0.93-2.98)	0.088		
Cholesterol (mg/dL, ≥220/<220)	1.56 (0.62-3.95)	0.346		
Histological diagnosis (LC/non-LC)	4.03 (2.55-6.36)	<0.0001	3.30 (2.06-5.28)	<0.001
Combination of ribavirin (−/+)	1.53 (0.99-2.38)	0.058		
Type of IFN (α/β)	0.88 (0.57-1.35)	0.882		
Total dose of IFN (MU, ≥500/<500)	0.91 (0.59-1.40)	0.672		
Efficacy (non-SVR/SVR)	2.73 (1.77-4.20)	<0.0001	2.78 (1.75-4.41)	<0.001

Data are expressed as the median (range).
Abbreviations: ALT, alanine aminotransferase; HR, hazard ratio; LC, liver cirrhosis.

prospectively followed. Another limitation of the study was that patients were treated with different types of antiviral therapy (IFN monotherapy or combination IFN + ribavirin therapy) for different duration (4 to 52 weeks). This heterogeneity makes it difficult to interpret the results of the study. On the other hand, the strength of the present study is the long-term follow-up in the large numbers of patients included.

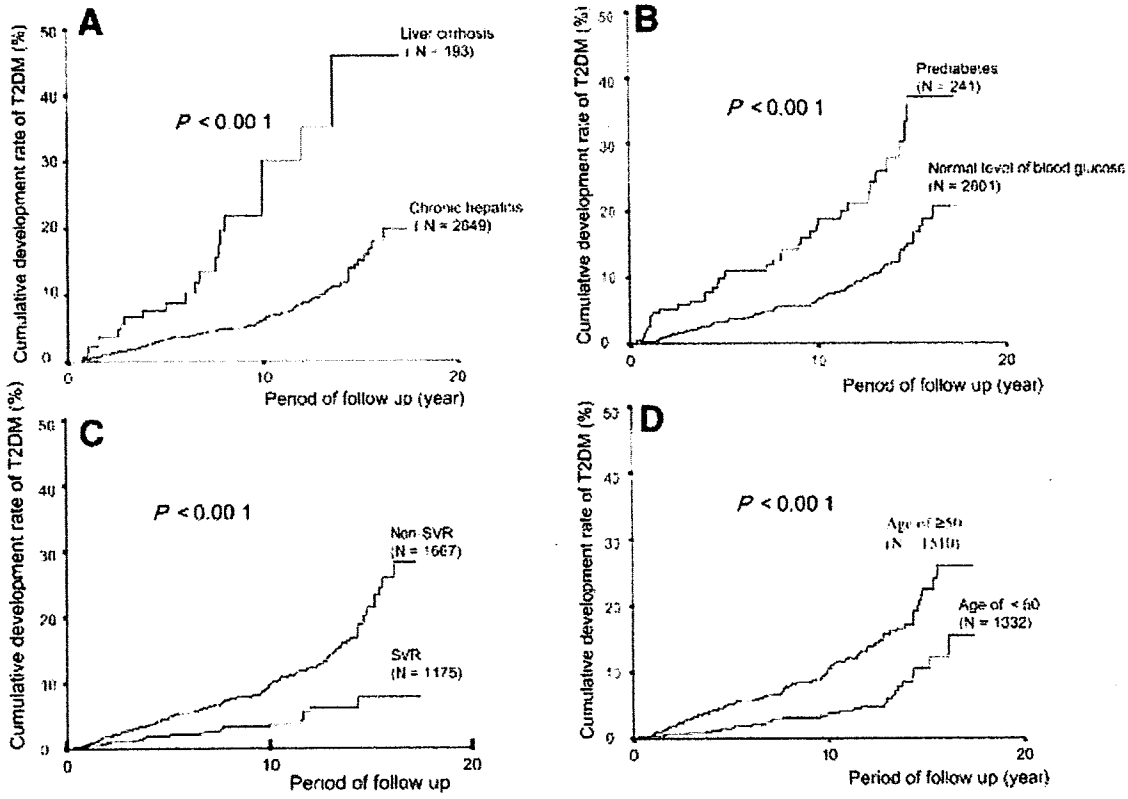

Fig. 2. Cumulative development rate of T2DM in patients treated with IFN. (A) Cumulative development rate of T2DM based on difference of hepatic fibrosis. (B) Cumulative development rate of T2DM based on the difference of glucose level. (C) Cumulative development rate of T2DM based on the difference of efficacy. (D) Cumulative development rate of T2DM based on the difference of age.

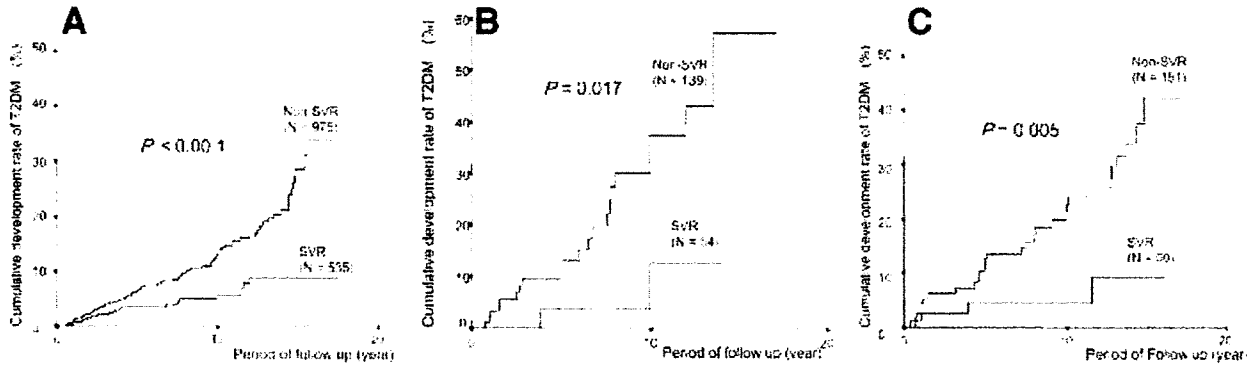

Fig. 3. Cumulative development rate of T2DM in patients with SVR or without SVR after IFN therapy. (A) Cumulative development rate of T2DM based on SVR or non-SVR in patients with age ≥50 years. (B) Cumulative development rate of T2DM based on SVR or non-SVR in patients with liver cirrhosis. (C) Cumulative development rate of T2DM based on the difference of SVR or non-SVR in patients with pre-diabetes.

The present study shows several findings with regard to development of T2DM after the termination of antivirus agents for HCV positive patients. First, the T2DM development rate in the non-SVR group was higher than that in the SVR group. The SVR caused a two-thirds reduction in the onset of T2DM in the course of posttreatment follow-up. That SVR reduced the onset of diabetes mellitus in HCV patients is in accordance with the data reported by Simó et al.[22] and Romero-Gómez et al.[23] Though the role of HCV in the pathogenesis of diabetes mellitus remains speculative, the following possible mechanisms have been reported: (1) patients with HCV have a tendency to attain insulin resistance[24]; (2) in transgenic mice, the expression of HCV core protein is associated with insulin resistance and T2DM development[25]; and (3) SVR in HCV patients reduces insulin resistance and onset of the incidence of abnormal glucose value.[26] Thus, it is accepted that clearance of HCV reduces the onset of T2DM.

Second, in addition to persistence of HCV, the present study suggests that aging, histological progression, and pre-diabetes enhanced the onset of T2DM in patients with HCV infection. However, when HCV was eradicated even in patients with age ≥50 years, pre-diabetes, or liver cirrhosis, the cumulative development rate of T2DM decreased.

T2DM is increasing dramatically in many Asian nations, including Japan, over the past decades.[27] It is widely accepted that 7 to 8 million people are affected by diabetes mellitus in Japan. Approximately 8% to 10% of adults in Japan have T2DM. In general, T2DM is associated with a genetic predisposition, but it is also strongly influenced by lifestyle-related factors, such as eating habits and/or physical activity.[28-33] The risk factors associated with T2DM include family history, age, sex, obesity, smoking, and physical activity. T2DM occurred in elderly patients compared to young patients. Life expectancies are long in Japan; thus, in the near future, a large number of patients with HCV will be >60 years of age. Therefore, it is apparent that the incidence of T2DM will increase in HCV-positive patients.

T2DM is a serious, costly disease. Treatment for T2DM may prevent some of its devastating complications, but does not usually restore normoglycemia or eliminate all the adverse consequences.[28,29] Moreover, HCV patients with T2DM are at major risk for HCC.[34] On the efficacy of IFN therapy, it has been reported that T2DM reduces HCV eradication via combination IFN + ribavirin therapy.[26] Thus, it should be considered whether HCV-positive patients should be treated with antiviral drugs in the histological nonprogression stage and at a non-elderly age for prevention of T2DM onset. If SVR obtained via antiviral therapy for HCV cannot only prevent progression to liver cirrhosis or HCC but also prevent the development of diabetes, the potential impact of IFN therapy is quite significant.

In conclusion, this retrospective study suggests that the annual incidence of T2DM among patients with HCV is 0.8% to 1.0%. Our results indicate that SVR causes a two-thirds reduction of T2DM development in HCV-positive patients treated with antiviral drugs.

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RESEARCH ARTICLES

ERK5 is a Target for Gene Amplification at 17p11 and Promotes Cell Growth in Hepatocellular Carcinoma by Regulating Mitotic Entry

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Using high-density oligonucleotide microarrays, we investigated DNA copy-number aberrations in cell lines derived from hepatocellular carcinomas (HCCs) and detected a novel amplification at 17p11. To identify the target of amplification at 17p11, we defined the extent of the amplicon and examined HCC cell lines for expression of all seven genes in the 750-kb commonly amplified region. Mitogen-activated protein kinase (MAPK) 7, which encodes extracellular-regulated protein kinase (ERK) 5, was overexpressed in cell lines in which the gene was amplified. An increase in *MAPK7* copy number was detected in 35 of 66 primary HCC tumors. Downregulation of *MAPK7* by small interfering RNA suppressed the growth of SNU449 cells, the HCC cell line with the greatest amplification and overexpression of *MAPK7*. ERK5, phosphorylated during the G2/M phases of the cell cycle, regulated entry into mitosis in SNU449 cells. In conclusion, our results suggest that *MAPK7* is likely the target of 17p11 amplification and that the ERK5 protein product of *MAPK7* promotes the growth of HCC cells by regulating mitotic entry.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy in the world and is estimated to cause approximately half a million deaths annually (El-Serag, 2002). Several risk factors for HCC have been reported, including infection with hepatitis B and C viruses, dietary intake of afratoxin, alcohol consumption, and diabetes.

The mitogen-activated protein kinase (MAPK) cascades transmit extracellular signals from cell surface receptors to specific intracellular targets and regulate a wide variety of cellular functions, including cell proliferation, differentiation, and the stress response (Nishimoto and Nishida, 2006). Extracellular stimuli induce sequential activation of MAPK kinase kinase, MAPK kinase, and MAPK. At least four MAPK subfamilies have been identified: extracellular-regulated protein kinase (ERK) 1 and 2, c-Jun-N-terminal kinases, p38, and ERK5 (also know as BMK1). ERK5, which was recently characterized, can be activated by a wide range of growth factors and cellular stresses, including serum, epithelial growth factor, oxidative stress, and hyperosmotic shock (Hayashi and Lee, 2004; Nishimoto and Nishida, 2006; Wang and Tournier, 2006). When stimulated, MAP/ERK kinase kinase 2 and 3 activate MAP/ERK kinase (MEK) 5, a specific kinase for ERK5. Subsequently, MEK5 phosphorylates ERK5, and the activated ERK5 promotes cell proliferation, differentiation, and survival (Hayashi and Lee, 2004; Garaude et al., 2006; Nishimoto and Nishida, 2006; Wang and Tournier, 2006). Some investigators have described the possible involvement of ERK5 in cancers (Esparis-Ogando et al., 2002; Weldon et al., 2002; Mulloy et al., 2003; Carvajal-Vergara et al., 2005; Linnerth et al., 2005).

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