end-stage liver disease is the leading cause of orthotopic liver transplantation [23]. This background demands that immediate measures should be taken to prevent fibrosis developing in the elderly with chronic hepatitis C by initiating the appropriate treatment; pegylated IFN combined with ribavirin can eliminate HCV efficiently [24,25].

Management of antiviral treatment in the elderly, however, is not without difficulties. Discontinuation of therapy or dose reduction was required frequently in the Japanese patients older than 60 years with chronic hepatitis C [21]. It is obvious that antiviral treatment needs to be administered with caution in aged patients with chronic hepatitis C, with the indication restricted to those who are likely to derive benefit from it. Early virological response at 12 weeks of treatment is predictive of sustained virological response [26]. The influence of HCV genotypes on the response to combined therapy, which increases with age [27], would have to be taken into consideration, also. In the Japanese patients infected with HCV genotype 1b, substitutions of amino acids at positions 70 and 91 are associated with a better response to combined treatment [28]. In view of the more frequent and serious side effects in elderly patients, these predictors would need to be taken into account when deciding whether to continue or discontinue combined treatment with IFN and ribavirin in elderly patients with chronic hepatitis C.

In order to plan the treatment of elderly patients, the natural history of HCV infection in these patients needs to be elucidated, which has not been done as yet. In the present study, we have followed-up treatment-naive patients aged $\geq 65$ years without antiviral treatment for more than 3 years. None of them had cirrhosis at baseline. They were stratified by baseline transaminase levels $\leq 40$ IU/l (group A ($n=120$)) and $\geq 41$ IU/l (group B ($n=212$)) and classified further into the three age groups, 65–69, 70–74, and 75–85 years. Cirrhosis and HCC developed more frequently in the patients in group B than those in group A ($p<0.001$ for both). Of the patients aged 65–69 years at entry, in particular, cirrhosis and HCC developed more frequently in group B than in group A ($p<0.001$ and $p=0.001$, respectively). Liver-related causes of death were more common in group B than in group A (20/34 (59%) versus 1/9 (11%), $p<0.05$), and HCC developed more frequently in men than in women ($p=0.021$).

Despite the progression of fibrosis that is accelerated with age [6], liver-related deaths were infrequent in patients with normal baseline transaminase levels and much less often than in those with elevated baseline transaminase levels (1/120 (0.8%) versus 20/212 (9.4%), $p=0.002$). Development of cirrhosis or HCC was no different between patients in groups A and B who were aged 70 years or older at entry. Taken altogether, elderly patients with elevated transaminase levels who are younger than 70 years would be the best candidates for antiviral treatment. They would need to be treated, even when side effects appear, by modifying the doses of IFN and ribavirin. In contrast, antiviral treatment may not be necessary for elderly patients with normal ALAT levels, or can be discontinued in these patients when side effects emerge.

There has been some controversy over antiviral treatment for elderly patients with chronic hepatitis C, and no specific guidelines have been drawn up so far [29]. The sustained virological response to antiviral treatment in aged patients is reported to be either poorer than [30–32] or comparable with that in younger patients [19,33]. The difference is most likely ascribed to careful selection of the aged patients who would benefit from treatment [13]. Based on the natural history of elderly patients with chronic hepatitis C described herein, those with elevated transaminase levels would need treatment to prevent progression to cirrhosis and HCC, while others with normal levels may not require treatment. It is to be hoped that the results in this study might be of help in planning a reasonable treatment strategy towards the longevity, without development of cirrhosis or HCC, in elderly patients with chronic hepatitis C, whose numbers are expected to increase progressively in the foreseeable future.

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## References

[1] Cohen J. The scientific challenge of hepatitis C. Science 1999;285:26–30.

[2] Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, et al. Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.

[3] Wong JB, McQuillan GM, McHutchison JG, Poynard T. Estimating future hepatitis C morbidity, mortality, and costs in the United States. Am J Public Health 2000;90:1562–9.

[4] Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006;144:705–14.

[5] Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 1999;340:1228–33.

[6] Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997;349:825–32.
[7] Vogt M, Lang T, Frosner G, Klingler C, Sendl AF, Zeller A, et al. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 1999;341:866–70.
[8] Feray C, Gigou M, Samuel D, Paradis V, Mishiro S, Maertens G, et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. Gastroenterology 1995;108:1088–96.
[9] Garcia-Samaniego J, Soriano V, Castilla J, Bravo R, Moreno A, Carbo J, et al. Influence of hepatitis C virus genotypes and HIV infection on histological severity of chronic hepatitis C. Am J Gastroenterol 1997;92:1130–4.
[10] Pageaux GP, Ducos J, Mondain AM, Costes V, Picot MC, Perrigault PF, et al. Hepatitis C virus genotypes and quantitation of serum hepatitis C virus RNA in liver transplant recipients: relationship with severity of histological recurrence and implications in the pathogenesis of HCV infection. Liver Transpl Surg 1997;3:501–5.
[11] Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248–52.
[12] Kumada T, Toyoda H, Honda T, Kuzuya T, Katano Y, Nakano I, et al. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. Intervirology 2006;49:112–8.
[13] Marcus EL, Tur-Kaspa R. Chronic hepatitis C virus infection in older adults. Clin Infect Dis 2005;41:1606–12.
[14] Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. Intervirology 2006;49:7–17.
[15] El-Serag HB, Davila JA, Petersen NJ, McGlynn KA. The continuing increase in the incidence of hepatocellular carcinoma in the United States: an update. Ann Intern Med 2003;139:817–23.
[16] Arase Y, Ikeda K, Suzuki F, Suzuki Y, Kobayashi M, Akuta N, et al. Interferon-induced prolonged biochemical response reduces hepatocarcinogenesis in hepatitis C virus infection. J Med Virol 2007;79:1485–90.
[17] Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. Hepatology 1999;29:1124–30.
[18] Koyama R, Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. Intervirology 2006;49:121–6.
[19] Honda T, Katano Y, Urano F, Murayama M, Hayashi K, Ishigami M, et al. Efficacy of ribavirin plus interferon-alpha in patients aged (60 years with chronic hepatitis C. J Gastroenterol Hepatol 2007;22:989–95.
[20] Davis GL, Esteban-Mur R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. N Engl J Med 1998;339:1493–9.
[21] Iwasaki Y, Ikeda H, Araki Y, Osawa T, Kita K, Ando M et al. Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. Hepatology 2006;43:54–63.
[22] Poynard T, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet 1998;352:1426–32.
[23] Verna EC, Brown RS Jr. Hepatitis C and liver transplantation: enhancing outcomes and should patients be retransplanted? Clin Liver Dis 2008;12:637–59.
[24] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347: 975–82.
[25] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–65.
[26] Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38:645–52.
[27] Antonucci G, Longo MA, Angeletti C, Vairo F, Oliva A, Comandini UV, et al. The effect of age on response to therapy with peginterferon alpha plus ribavirin in a cohort of patients with chronic HCV hepatitis including subjects older than 65 years. Am J Gastroenterol 2007;102:1383–91.
[28] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 2007;79:1686–95.
[29] Wright TL. Treatment of patients with hepatitis C and cirrhosis. Hepatology 2002;36:S185–94.
[30] Floreani A, Minola E, Carderi I, Ferrara F, Rizzotto ER, Baldo V. Are elderly patients poor candidates for pegylated interferon plus ribavirin in the treatment of chronic hepatitis C? J Am Geriatr Soc 2006;54:549–50.
[31] Hayashi J, Kishihara Y, Ueno K, Yamaji K, Kawakami Y, Furusyo N, et al. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 1998;158:177–81.
[32] Hiramatsu N, Oze T, Tsuda N, Kurashige N, Koga K, Toyama T, et al. Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? Hepatol Res 2006;35:185–9.
[33] Tsui JI, Currie S, Shen H, Bini EJ, Brau N, Wright TL. Treatment eligibility and outcomes in elderly patients with chronic hepatitis C: results from the VA HCV-001 Study. Dig Dis Sci 2008;53:809–14.

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ORIGINAL ARTICLE

# Association of HLA-DR14 with the Treatment Response in Japanese Patients with Autoimmune Hepatitis

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**Abstract**
*Background* Influence of human lymphocyte antigen (HLA) on the therapeutic response in autoimmune hepatitis (AIH) is not known.
*Aims* To evaluate if HLA-DR types influence biological and histological responses to corticosteroids in patients with AIH.
*Methods* During 28 years from 1979 through 2007, 48 patients with definite diagnosis of AIH received long-term corticosteroid therapy (median 9 years [range: 5–28 years]) in a single Japanese center. They were followed for transaminase levels and received liver biopsy before and after the treatment.
*Results* DR4 was detected in 32 and DR14 in 11 patients; seven possessed both DR4 and DR14. DR4 was more frequent in AIH patients than in the general population (67% vs. 22%), while DR14 was comparably frequent between them (23% vs. 17%). Overall, biochemical response was achieved in 43 (90%) of the 48 patients. The sustained biochemical response to a maintenance prednisolone dose $< 10$ mg was gained more frequently in the patients with than without DR14 (10/11 [91%] vs. 10/37 [27%], $P < 0.001$). Marked histological improvement with a decrease in histology activity index (HAI) score by $> 2$ points was achieved in 31 of the 32 (97%) biochemical responders. Histological aggravation with an increase in HAI score occurred in 4 of the 16 (25%) patients without biochemical response (non-responders and relapsers combined), but in none of the 32 responders.
*Conclusion* Long-term immunosuppressive treatment can improve the outcome of Japanese patients with AIH, and DR14 is associated with excellent biochemical response.

**Keywords** Hepatitis · Autoimmune-HLA-DR-corticosteroids-biopsy · Needle

## Introduction

Autoimmune hepatitis (AIH) is the inflammation of hepatocytes of unknown etiology and characterized by histological hallmark of interface hepatitis with infiltration of lymphocytes in the portal area [1–3]. Female preponderance, various auto-antibodies and hyper-γ-globulinemia, as well as excellent response to immunosuppressive therapies, are prominent clinical features. AIH is sub-grouped into types 1–3 by the age of onset, severity of disease, and autoantibody profiles [3]. Loss of immunotolerance to self-antigens expressed on hepatocytes is implicated in the pathogenesis of AIH, in the background of major histocompatibility complex (MHC) genes represented by HLA-DR alleles [4].

The disease entity of AIH is not uniform and influenced by geography and ethnicity, in which HLA-DR types play a major role. For the purpose of dealing with a broad clinical spectrum of AIH, diagnostic criteria were proposed by the International Autoimmune Hepatitis Group (IAIHG) in 1993 [5], and they were modified in 1999 [6]. In Japan, an

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indigenous scoring system for defining AIH was established in 1996 [7]. It has allowed to distinguish AIH from other autoimmune liver disease, such as primary biliary cirrhosis and primary sclerosing cholangitis [8]. Although the treatment response differs in AIH patients with distinct DR profiles, aggressive immunosuppressive treatments with precaution to avoid side-effects can prevent histological deterioration toward favorable long-term outcomes [9, 10].

Since by far the most patients with AIH can merit from immunosuppressive treatment, an effective therapy for an appropriate duration is the primary goal of physicians. AIH can run a rapid course accompanied by cirrhosis in some cases, particularly in young male patients [11], when they fail to receive a therapeutic intervention [12]. Some patients relapse after treatment, often accompanied by rapid deterioration in the liver histology [13]; they need utmost care for timely and effective treatment.

In order to examine a long-term prognosis of AIH, 48 patients with the definite diagnosis of AIH were treated with long-term corticosteroid for up to 28 years, and followed for biochemical and histological responses to treatment, with a special reference to their HLA-DR profiles.

## Methods

### Patients

During 28 years from 1979 to 2007, 118 patients with AIH type-1 visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. Of these patients, 78 (66%) fulfilled the definite diagnostic criteria defined by IAIHG [6], while the remaining 40 (34%) did those of probable AIH. All of the patients were negative for antibodies to liver kidney micosome-1 (anti-LKM-1), and they were classified into AIH type-1. They had a median age of 52 years (range: 19–64 years), and included 45 (67%) women. There were four patients who underwent transient and moderate increases in the serum level of alanine aminotransferase (ALT), and they were followed without treatment. The remaining 60 patients received corticosteroid therapy and were followed for biochemical response during the median of 9 years (range: 5–28 years). Of these patients, 48 (70%) were included in this study, and received serial liver biopsies under laparoscopy for the evaluation of histological improvement. None of them had ongoing infection with hepatitis B or C virus, or possessed antibody to human immunodeficiency virus type-1. The study protocol conformed to the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of the Toranomon Hospital. Every patient or his/her next of kin gave an informed consent on the purpose of this study.

### Serological Tests

Autoantibodies as well as immunoglobulins of IgG and IgM classes were determined by enzyme immunoassay (EIA). Antinuclear antibodies (ANA) were determined by indirect immuno-fluorescence with Hep-G2 cells, and anti-smooth muscle antibodies as well as anti-LKM-1 by indirect fluorescence on cryostat sections of rat organs by the standard procedure. Hepatitis B surface antigen (HBsAg) was determined by radioimmunoassay, antibody to hepatitis C virus (anti-HCV) by EIA of the third generation, and HCV RNA by reversed-transcription polymerase chain reaction (RT-PCR).

### HLA Typing

HLA typing was performed by serological methods, and confirmed by PCR-MPH (microplate hybridization) for patients with inconclusive results [14].

### Prednisolone Treatment and Biochemical Response

As soon as the diagnosis of AIH was established, patients received 30–60 mg prednisolone daily and were followed for transaminase levels during a mean follow-up period of 5 years (range: 5–28 years). Aminotransferase levels were monitored monthly, and the dose of prednisolone was reduced by 10–15% for the patients in whom ALT levels were normalized to below 40 U/l for 3 months or longer. The response was judged 6 months after the normalization of ALT. Complete response was defined by the normalization of transaminase levels with a maintenance dose of $\leq$10 mg prednisolone daily; partial response by that with >10 mg prednisolone (up to 20 mg); and no response by the failure in normalizing transaminase levels with a maintenance dose of prednisolone (10–20 mg). Relapse was an exacerbation with increase in ALT levels exceeding 80 U/Ll (2 × upper limit of normal) after they had been normalized by a maintenance dose.

### Laparoscopic and Histological Examinations

Patients received liver biopsy under laparoscopy before and after the treatment with an interval of 5 years with a minor patient-to-patient variation. Biopsied liver specimens were stained for silver for evaluating fibrosis and with D-periodic acid Schiff (PAS) for examining inflammatory changes.

Statistical Analysis

Categorial variables were compared between groups by the $\chi^2$ test and Fisher's exact test, and non-categorial variables by the Mann–Whitney's $U$ test.

## Results

Baseline Characteristics of AIH Patients

Table 1 lists the baseline characteristics of the 48 patients with AIH for whom HLA typing was performed and who had received a long-term immunosuppressive therapy (median 9 years [range: 5–28 years]) while they were monitored for biochemical and histological responses. Frequencies of HLA-DR are shown in Fig. 1. DR4 predisposing Japanese patients to AIH [15, 16] was detected in 32 of the 48 (67%) patients, DR8 in nine (19%), DR14 in 11 (23%) and DR15 in 16 (33%) of the 48 AIH patients.

Biochemical Responses of AIH Patients with Reference to HLA Types

Biochemical response with the normalization of aspartate aminotransferase (AST) and ALT levels was achieved in 43 of the 48 (90%) patients after the initial aggressive treatment with corticosteroids (30–60 mg/day of prednisolone) followed by a small maintenance dose (10 mg/day or less). However, 16 of the 43 (37%) responders required occasional increased doses (20 mg/day or more) for the treatment of hepatitis flares. Response differed in patients with distinct HLA-DR types (Table 2). Complete biochemical response was more frequent in the patients with than without DR14 (10/11 [91%] vs. 10/37 [27%], $P < 0.001$).

**Table 1** Baseline characteristics of the 48 patients with AIH

| Features | Normal range | |
|---|---|---|
| Age (years) | Not applicable | 52 (22–71) |
| Men | Not applicable | 10 (21%) |
| AST (IU/l) | 11–38 | 93 (16–1,550) |
| ALT (IU/l) | 6–50 | 110 (16–2,640) |
| ALP (IU/l) | 117–350 | 282 (128–949) |
| γ-GTP (IU/l) | 9–109 | 84 (15–651) |
| γ-Globulin (g/dl) | 0.76–1.76 | 2.27 (1.36–4.59) |
| IgG (mg/dl) | 870–1,700 | 2,632 (1,340–2,632) |
| ANA (x) | <80 | 640 (0–10,240) |
| Fibrosis stage | Not applicable | |
| $F_0$ | | 0 |
| $F_1$ | | 19 (40%) |
| $F_2$ | | 17 (35%) |
| $F_3$ | | 10 (21%) |
| $F_4$ | | 2 (4%) |

Data are expressed by the median with the range in parentheses or the number with percentage in parentheses

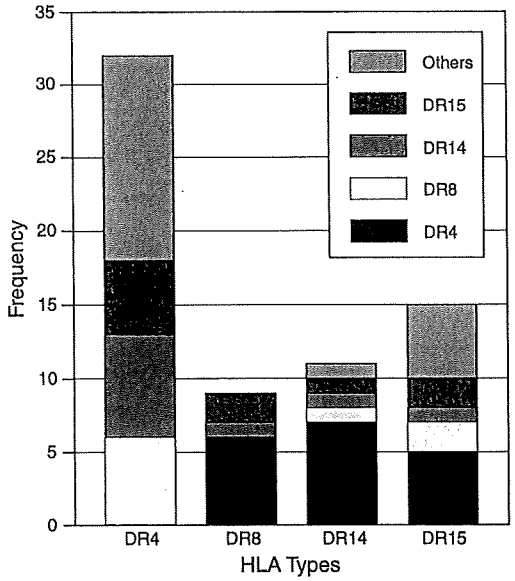


**Fig. 1** HLA-DR alleles in the 48 patients with AIH. The allele in the other chromosome is shown in patients with DR4, DR8, DR14, and DR15

**Table 2** Biochemical response in AIH patients with or without the DR14 allele

| HLA-DR | Number | Biochemical response | | | Relapse |
|---|---|---|---|---|---|
| | ($n = 48$) | Complete | Partial | None | |
| DR14 | 11 (23%) | 10 (91%)* | 1 (9%) | 0 | 0 |
| Non-DR14 | 37 (77%) | 10 (27%) | 11 (29%) | 5 (14%) | 11 (28%) |
| DR4 | 32 (67%) | 11 (34%) | 7 (22%) | 3 (9%) | 11 (34%) |
| DR8 | 9 (19%) | 3 (33%) | 2 (22%) | 1 (11%) | 3 (33%) |
| DR15 | 15 (31%) | 6 (40%) | 7 (47%) | 1 (7%) | 1 (7%) |
| Others | 3 (6%) | | 1 (33%) | 2 (67%) | |

Transaminase levels were normalized with a maintenance dose of ≤10 mg prednisolone in complete responders and with that of >10 mg in partial responders. Relapse was an exacerbation of transaminase levels after they had been normalized by a maintenance dose

* $P < 0.001$ vs. non-DR14

Relationship Between Biochemical and Histological Responses to Prednisolone Therapy in the 48 Patients with AIH

Histological follow-ups were performed in the 48 patients, and the HAI score markedly improved in 42 (88%), moderately improved in two patients (4%) and worsened in the remaining four (8%) (Table 3). Marked histological

**Table 3** Relationship between biochemical and histological responses to prednisolone therapy in the 48 patients with AIH

| Biochemical | Number | Histological response | | |
|---|---|---|---|---|
| Response | ($n = 48$) | Marked | Moderate | Worsened |
| Response | 32 | 31 (97%) | 1 (3%) | 0 |
| Complete | 20 | 19 (95%) | 1 (5%) | 0 |
| Partial | 12 | 12 (100%) | 0 | 0 |
| No Response | 5 | 2 (40%) | 1 (20%) | 2 (40%) |
| Relapse | 11 | 9 (82%) | 0 | 2 (18%) |

Histology activity index (HAI) score decreased by ≥2 points in marked response and by 1 point in moderate response

improvement was accomplished in 31 of the 32 (97%) responders, while it was achieved in two of the four (50%) non-responders and nine of the 11 (82%) relapsers. Histology worsened in four of the 16 (25%) patients without biochemical response (non-responders and relapsers combined), but in none of the 32 responders. Changes in the total HAI score as well as respective scores for specific histological parameters (periportal with/without bridging necrosis; intralobular degeneration with focal necrosis; portal inflammation; and fibrosis) are shown in Table 4. The gain in total HAI score was due to an increase in inflammation and not attributed to aggravation of fibrosis in each of them.

### Histological Responses of AIH Patients with Reference to HLA

Table 5 compares histological responses between the patients with and without DR4. Although the pretreatment HAI score was somewhat higher in the patients with than without DR14 ($9.8 \pm 3.5$ vs. $7.9 \pm 3.3$, $P = 0.092$), it improved to comparable extents in both of them after treatment ($4.5 \pm 0.9$ vs. $4.7 \pm 2.5$). Thus, the marked histological response with a decrease in HAI score ≥2 was no different between the patients with and without DR14

**Table 4** Changes in the total HAI score and those in respective parameters in the four patients in whom histology worsened after prednisolone treatment

| | Total HAI score (scores for each parameter[a]) | |
|---|---|---|
| | Before treatment | After treatment |
| Patient 1 | 6 (1, 1, 1, 3) | 8 (1, 3, 1, 3) |
| Patient 2 | 3 (0, 1, 1, 1) | 6 (1, 3, 1, 1) |
| Patient 3 | 6 (1, 1, 1, 3) | 8 (1, 3, 1, 3) |
| Patient 4 | 13 (3, 3, 3, 4) | 15 (4, 4, 3, 4) |

[a] Four histological parameters were graded, including periportal with/without bridging necrosis; intralobular degeneration with focal necrosis; portal inflammation; and fibrosis

**Table 5** Histological response in AIH patients with or without DR14

| HLA-DR | Number | Histological improvement | | |
|---|---|---|---|---|
| | | Marked | Moderate | Worsened |
| DR14 | 11 (23%) | 10 (91%) | 1 (9%) | 0 |
| Non-DR14 | 37 (77%) | 32 (86%) | 1 (3%) | 4 (11%) |
| DR4 | 32 (67%) | 27 (84%) | 1 (3%) | 4 (13%) |
| DR8 | 9 (19%) | 8 (89%) | 1 (11%) | 0 |
| DR15 | 15 (31%) | 3 (93%) | 0 | 1 (7%) |
| Others | 3 (6%) | 2 (67%) | 0 | 1 (33%) |

(10/11 [91%] vs. 32/37 [86%], $P = 0.697$). Improvement in the histology was mostly due to changes in the necroinflammatory grade; there were few changes in the fibrosis grade from the baseline values.

Figure 2 illustrates clinical and histological courses of a representative patient (female, 50 years old, HLA-DR4/DR14) who received eight laparoscopes and seven liver biopsies during the follow-up for 20 years. Before she received corticosteroid therapy, liver histology had already progressed to cirrhosis, and she had to undertake sclerotherapies for the treatment of esophageal varices. She had to receive 10–30 mg prednisolone during initial few years for the treatment of several hepatitis flares. Thereafter, her liver function improved remarkably and had remained within normal limits by a maintenance dose of ≤ 10 mg prednisolone through 17 years until the last follow-up. Remarkably, she gained improvement not only in the inflammation grade but also in the fibrosis stage. Serial laparoscopic and histological findings of her liver are demonstrated in Fig. 3. In other AIH patients, also, aggressive immnosuppressive therapy prevented histological progression and gained improvement in their long-term outcomes, even though their responses to prednisolone differed.

## Discussion

In the present study, HLA typing was performed in 48 of the 78 (62%) patients with the definite diagnosis of AIH type-1. They had been followed-up during a long-term corticosteroid treatment, with liver biopsies performed as frequently as possible, and histological and biochemical responses were correlated with HLA types. DR14, which has not gained attention in AIH, was detected in 11 of the 48 (23%) patients. Remarkably, the sustained biochemical response was achieved more frequently in the AIH patients with than without DR14 (10/11 [91%] vs. 10/37 [27%], $P < 0.001$).

The association of HLA types and AIH are under regional influence. DR3 and DR4 are the main HLA

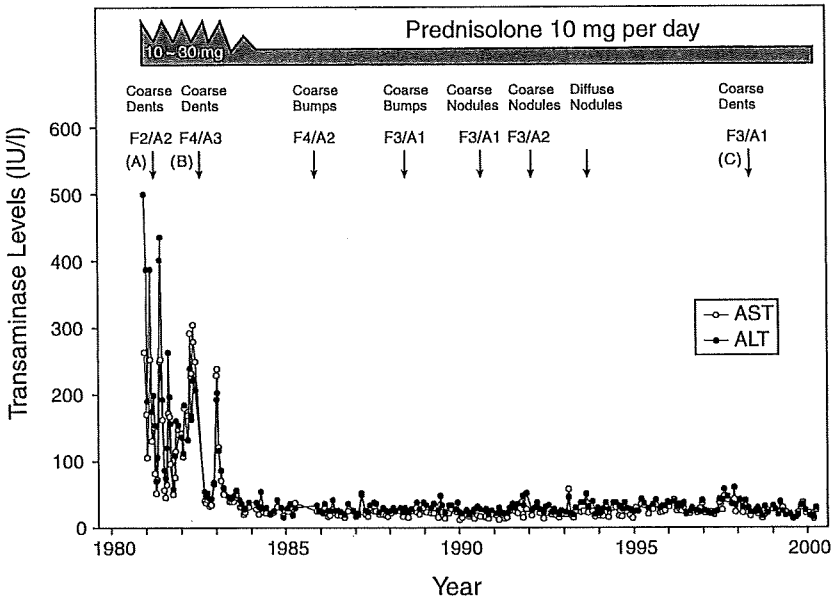


**Fig. 2** Clinical course of a patient with AIH (female, 45 years old with HLA-DR4/DR14) who had been followed for 20 years. Doses of prednisolone are indicated at the top, and appearances of the liver surface on laparoscopies, as well as fibrosis stage and inflammation grade on liver biopsies, are shown in the middle. During the initial few years, she received up to 30 mg prednisolone per day for treatment of several hepatitis flares. Thereafter, her liver function improved remarkably and had stayed within normal limits through 17 years with a maintenance prednisolone dose $\leq$ 10 mg

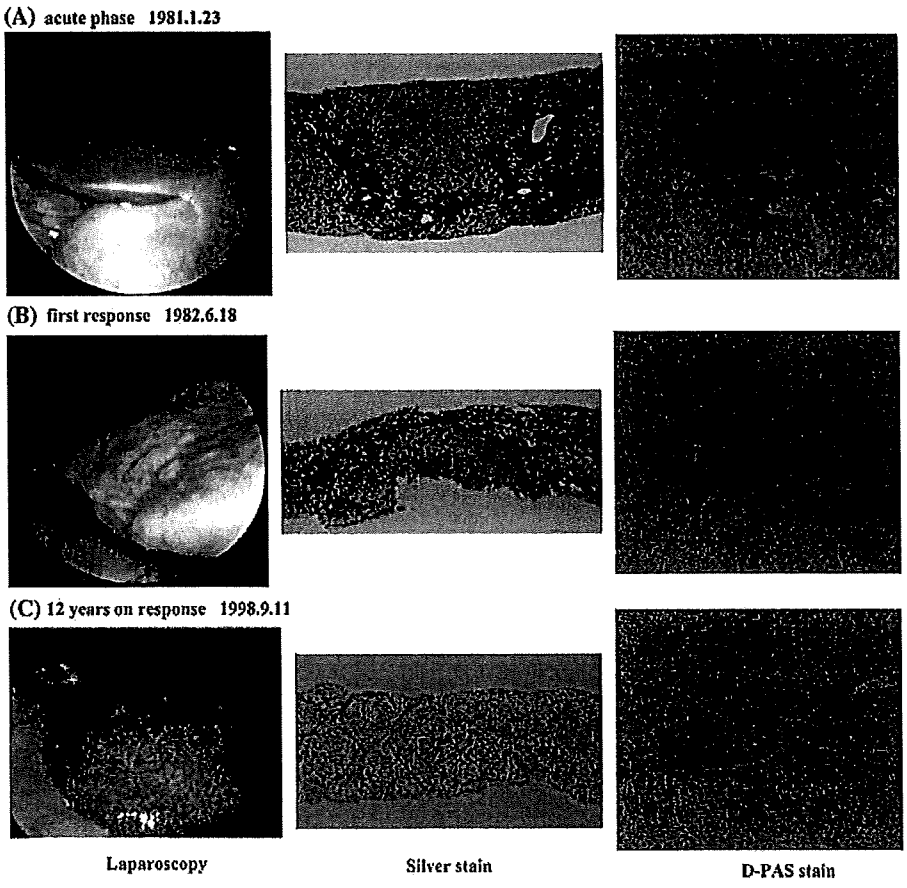


**Fig. 3** Laparoscopic findings and histological changes in the patients with AIH. The patient presented in Fig. 2 was examined at three time points (*a*, *b*, and *c* in Fig. 2). Laparoscopic findings were improved since she responded to prednisolone since June, 1982. Histologically, typical submassive necrosis and interface-hepatitis were found in the first biopsy (a). Since she responded, necroinflammatory changes improved, however (b and c). Laparoscopic findings are shown in right row, low-power fields (×20) by silver staining in the middle row; and high-power fields (×200) by D-PAS staining

susceptibility alleles among Caucasoid Northern Europeans and North Americans, and 84% of adult patients have either or both of these alleles [17, 18]. In contrast, the principal susceptibility allele for AIH in Japan is DR4 [15, 16]; DR3 is detected in none of them, however. DR4 is also frequent in adult patients in Argentina and Mexico [19, 20], while DRB13 prevails in Argentine and Brazilian children with AIH [21, 22]. DR4 is associated with better response and fewer relapse than DR3 in AIH patients from Western countries [1]. However, there have been no reports on the association of HLA types with treatment response in patients with AIH in Japan. In the present study, DR4 was detected in 32 of the 48 (67%) Japanese patients with AIH, with a frequency comparable to those in previous reports [15, 16]; DR4 was more common in AIH patients than in the general Japanese population (67 vs. 22%) [23]. In contrast, DR14 was comparably frequent in AIH patients and the general population of Japan (23 vs. 17%) [23]. Thus, DR4 would predispose the Japanese population to the development of AIH, while DR14 would not, albeit DR14 would increase the response to corticosteroids in AIH patients.

On the basis of DR4 that is more frequent in the individuals with than without DR3, these alleles have been regarded to behave independently and reciprocally toward the susceptibility for AIH. Such a possibility has been evaluated in peripheral blood mononuclear cells and lymphocytes infiltrating in the liver [24]. Liver lymphocytes are sensitized with hepatocytes or hepatic autoantigens. Even among inflammatory cells infiltrating the portal area, CD4+ lymphocytes predominate in the patients with than without AIH. These lines of evidence implicate the class-II MHC in the pathogenesis of AIH, of which DR4 and DR15 would play major roles in Japan. In the patients with AIH who are positive for LKM-1 antibodies, Th1 cells dominate in the cytokine production assay with a T-cell line specific for LKM-1 [25]. Combined, CD4+ lymphocytes would be crucially required in the manifestation of AIH by interacting with class-II MHC antigens.

In conclusion, the association of MHC class-II antigens with biochemical and histological responses to immunosuppressive treatment was evaluated in Japanese patients with AIH, for predicting their long-term outcomes. On the basis of the results obtained, DR14 would be associated with favorable treatment response in Japanese patients with AIH, which needs to be confirmed in an extended series of patients. The validity of such an assumption will be evaluated by in vitro studies, which are underway.

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## References

1. Czaja AJ, Freese DK. Diagnosis and treatment of autoimmune hepatitis. *Hepatology*. 2002;36:479–497.
2. Krawitt EL. Autoimmune hepatitis. *N Engl J Med*. 2006;354: 54–66.
3. Manns MP, Strassburg CP. Autoimmune hepatitis: Clinical challenges. *Gastroenterology*. 2001;120:1502–1517.
4. Czaja AJ, Doherty DG, Donaldson PT. Genetic bases of autoimmune hepatitis. *Dig Dis Sci*. 2002;47:2139–2150.
5. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. *Hepatology*. 1993;18:998–1005.
6. Alvarez F, Berg PA, Bianchi FB, et al. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatol*. 1999;31:929–938.
7. Toda G, Zeniya M, Watanabe F, et al. Present status of autoimmune hepatitis in Japan–correlating the characteristics with international criteria in an area with a high rate of HCV infection. *J Hepatol*. 1997;26:1207–1212.
8. Vergani D, Mieli-Vergani G. Autoimmune hepatitis. *Autoimmun Rev*. 2003;2:241–247.
9. Czaja AJ, Carpenter HA. Decreased fibrosis during corticosteroid therapy of autoimmune hepatitis. *J Hepatol*. 2004;40:646–652.
10. Czaja AJ, Carpenter HA. Progressive fibrosis during corticosteroid therapy of autoimmune hepatitis. *Hepatology*. 2004;39: 1631–1638.
11. Al-Chalabi T, Underhill JA, Portmann BC, McFarlane IG, Heneghan MA. Impact of gender on the long-term outcome and survival of patients with autoimmune hepatitis. *J Hepatol*. 2008; 48:140–147.
12. Czaja AJ, Bianchi FB, Carpenter HA, et al. Treatment challenges and investigational opportunities in autoimmune hepatitis. *Hepatology*. 2005;41:207–215.
13. Czaja AJ, Menon KV, Carpenter HA. Sustained remission after corticosteroid therapy for type 1 autoimmune hepatitis: a retrospective analysis. *Hepatology*. 2002;35:890–897.
14. Ota M, Seki T, Fukushima H, Tsuji K, Inoko H. HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. *Tissue Antigens*. 1992;39:187–202.
15. Miyake Y, Iwasaki Y, Takaki A, et al. Human leukocyte antigen DR status and clinical features in Japanese patients with type 1 autoimmune hepatitis. *Hepatol Res*. 2008;38:96–102.
16. Yoshizawa K, Ota M, Katsuyama Y, et al. Genetic analysis of the HLA region of Japanese patients with type 1 autoimmune hepatitis. *J Hepatol*. 2005;42:578–584.
17. Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR4 and A1–B8-DR3 are independent risk factors. *Hepatology*. 1991;13:701–706.
18. Strettell MD, Donaldson PT, Thomson LJ, et al. Allelic basis for HLA-encoded susceptibility to type 1 autoimmune hepatitis. *Gastroenterology*. 1997;112:2028–2035.
19. Pando M, Larriba J, Fernandez GC, et al. Pediatric and adult forms of type I autoimmune hepatitis in Argentina: evidence for differential genetic predisposition. *Hepatology*. 1999;30:1374–1380.
20. Vazquez-Garcia MN, Alaez C, Olivo A, et al. MHC class II sequences of susceptibility and protection in Mexicans with autoimmune hepatitis. *J Hepatol*. 1998;28:985–990.
21. Fainboim L, Marcos Y, Pando M, et al. Chronic active autoimmune hepatitis in children. Strong association with a particular HLA-DR6 (DRB1*1301) haplotype. *Hum Immunol*. 1994;41:146–150.
22. Czaja AJ, Souto EO, Bittencourt PL, et al. Clinical distinctions and pathogenic implications of type 1 autoimmune hepatitis in Brazil and the United States. *J Hepatol*. 2002;37:302–308.

23. Maeda H, Hirata R, Tokunaga K. HLA-DNA typing and HLA in the Japanese: a review (*in Japanese*). *Nihon Ishokugakkai Zasshi.* 1999;34:55–64.
24. Ichiki Y, Aoki CA, Bowlus CL, Shimoda S, Ishibashi H, Gershwin ME. T cell immunity in autoimmune hepatitis. *Autoimmun Rev.* 2005;4:315–321.
25. Schlaak JF, Lohr H, Gallati H, Meyer zum Buschenfelde KH, Fleischer B. Analysis of the in vitro cytokine production by liver-infiltrating T cells of patients with autoimmune hepatitis. *Clin Exp Immunol.* 1993;94:168–173.

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Case report

# Sustained virological response in a patient with chronic hepatitis C treated by monotherapy with the NS3-4A protease inhibitor telaprevir

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ABSTRACT

Here, we describe for the first time a case of sustained virological response (SVR) achieved in a patient with chronic hepatitis C (CH-C) by monotherapy with a NS3-4A protease inhibitor, telaprevir, without interferon therapy. A 59-year-old treatment-naïve Japanese man was enrolled in a phase II trial of telaprevir by repeat oral administration at a dose of 750 mg every 8 h for 24 weeks. At the start of treatment, he exhibited a low-level viremia with genotype 1b of the hepatitis C virus (HCV). After the first week of treatment with telaprevir, serum HCV RNA was undetectable, and negativity remained until the end of treatment. Moreover, he was evaluated as having a SVR after the post-treatment 24-week follow-up program. Two characteristics may explain the strong antiviral activity of telaprevir in the present case. First, although pre-treatment PCR-direct sequencing and cloning for the N-terminal in the NS3 region showed a protease inhibitor-resistant variant (T54A) in 1 of 32 independent clones, the T54A substitution has only a low-level resistance to protease inhibitors and his viral load was low. Second, when compared to a consequence sequence of 35 treatment-naïve patients with HCV genotype 1b, R130K and Q195K substitutions were unique to the present case. Although it is presently unknown whether the R130K and Q195K substitutions are related to SVR, this case suggests that long-term telaprevir monotherapy may be effective in CH-C patients with genotype 1 and a low viral load.



## 1. Introduction

The goals of antiviral treatment in patients with chronic hepatitis C (CH-C) are long-lasting eradication of the virus and a decrease in disease-related hepatic mortality. Standard treatment uses a combination of pegylated interferon and ribavirin (PEG-IFN-RBV), which provides a sustained virological response (SVR) rate of over 50%.[1,2] In Japan, approximately 70% of patients with CH-C are infected with genotype 1b, and those with a high titer of genotype 1b (≥100 KIU/mL [Amplicor; Roche Diagnostics K.K. Tokyo, Japan]) have lower rates of SVR (<50%), even on 48 weeks of PEG-IFN-RBV combination therapy.[3] Further, although treatment for CH-C is currently based on interferon (IFN), use of this agent is associated with serious adverse effects in some patients, such as mental disorders, apathy, and laboratory abnormalities.[1,2,4] Moreover, most CH-C patients in Japan over 70 years of age cannot receive IFN therapy due to either or both co-morbidities and the risk of adverse effects. For these reasons, a new treatment strategy is needed for patients with CH-C that displays high SVR rates and a favorable side-effect profile.

One recently introduced treatment strategy for CH-C is inhibition of the NS3-4A protease in the HCV polyprotein. Potential inhibitors include telaprevir (VX-950; MP-424; Mitsubishi Tanabe Pharma Co., Osaka, Japan), which has been selected as a clinical therapy candidate for the treatment of CH-C.[5] In some patients with genotype 1 and a high viral load, however, the efficacy of telaprevir monotherapy was limited, and combination therapy of telaprevir plus PEG-IFN-RBV is now standard.[6–10] On this background, we therefore report here for the first time a patient with CH-C who achieved a SVR following monotherapy with telaprevir.

## 2. Case report

A 59-year-old Japanese man was admitted to Toranomon Hospital, Tokyo in July 2007 following a positive result for HCV RNA at general check-up. Laboratory tests before treatment showed mild

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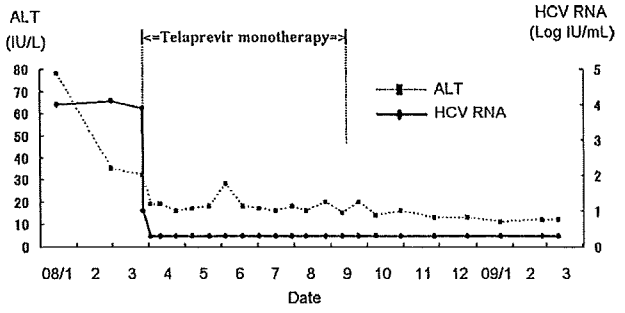


**Fig. 1.** Clinical course during and after 24 weeks of telaprevir monotherapy.

elevation of ALT (46 IU/L), and persistent HCV infection with genotype 1b and low-level viremia (<5 Log IU/mL [COBAS TaqMan HCV test, Roche Diagnostics K.K. Tokyo]) that continued to remain low until the start of treatment. He was diagnosed with CH-C by peritoneoscopy and liver biopsy (mild hepatitis [A1] and moderate fibrosis [F2]) at our hospital in February 2008. He had not received IFN therapy or any other antiviral drugs, and was enrolled in a phase II trial of telaprevir. Written informed consent was obtained, and the study was conducted in compliance with Good Clinical Practice and the Declaration of Helsinki. Treatment with telaprevir was started in March 2008, at which time serum HCV RNA was 3.9 Log IU/mL. Treatment was by repeat oral administration at a dose of 750 mg every 8 h for 24 weeks. Serum HCV RNA was undetectable after the first week and remained negative until the end of treatment (September 2008), and moreover remains undetectable as of March 2009. He was evaluated as having a SVR after the post-treatment 24-week follow-up program (Fig. 1).

The genome sequence for the N-terminal 609 nucleotides (203 amino acids) in the NS3 region of HCV isolates from the patient was analyzed before treatment with telaprevir. HCV RNA was extracted from 100 μL of serum and the nucleotide sequences were determined by direct sequencing and cloning. The primers used to amplify the NS3 region were NS3-F1 (5′-ACACCGCGGCGTGTGGGGACAT-3′; nucleotides 3295–3316) and NS3-AS2 (5′-GCTCTTGCCGCTGCCAGTGGGA-3′; nucleotides 4040–4019) as the first (outer) primer pair and NS3-F3 (5′-CAGGGGTGGCGGCTCCTT -3′; nucleotides 3390–3407) and NS3-AS2 as the second (inner) primer pair.[11] Thirty-five cycles of first and second amplifications were performed as follows: denaturation for 30 s at 95 °C, annealing of primers for 1 min at 63 °C, extension for 1 min at 72 °C, and final extension was performed at 72 °C for 7 min. PCR-amplified DNA was purified after agarose gel electrophoresis and amplification products of the second-round PCR were ligated with plasmid and transformed in *Escherichia coli* in a cloning kit (TA Cloning; Invitrogen, Carlsbad, CA). Dideoxynucleotide termination sequencing was performed with the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems Japan, Tokyo). Sequences of 32 independent clones from the sample were determined and analyzed. The pre-treatment analyses by PCR-cloning showed a variant (T54A) resistant to protease inhibitors in 1 of the 32 clones.

We also made a consensus sequence of the NS3 region from the PCR-direct sequences of 35 treatment-naïve Japanese patients with HCV genotype 1b in our hospital (Fig. 2). Compared to the consensus sequence, there were a total of 5 identical substitution variants (V48I, P89S, S122G, R130K, Q195K) within the 32 independent clones from this patient, among which R130K and Q195K were unique to this patient.

## 3. Discussion

Previous studies showed that telaprevir monotherapy for HCV patients with genotype 1 and a high viral load demonstrated substantial antiviral activity, and the median maximum change was −4.77 Log IU/mL with administration at 750 mg every 8 h for 2 weeks.[6,7] In Reesink et al., HCV RNA decreased below the limit of

```
                1       10        20        30        40        50
CONSENSUS       APITAYSQQT RGLLGCIITS LTGRDKNQVE GEVQVVSTAT QSFLATCVNG
Case clone1     ---------- ---------- ---------- ---------- -------I--
Case clone2     ---------- ---------- ---------- ---------- -------I--
Case clone3     -----H---- ---------- ---------- ---------- -------I--
Case clone4     ---------- ---------- ---------- ---------- -------I--
Case clone5     ---------- ---------- ---------- ---------- -------I--
                51                                                100
CONSENSUS       VCWTVYHGAG SKTLAGPKGP ITQMYTNVDQ DLVGWQAPPG ARSLTPCTCG
Case clone1     ---------- ---------- ---------- --------S- ----------
Case clone2     -----F---- ---------- ---------- --------S- ----------
Case clone3     ---A------ ---------- ---------- --------S- ----------
Case clone4     ---------- ---------- ---------- --------S- -----L----
Case clone5     -----F---- ---------- ---------- --------S- ----------
                101                 130                   150
CONSENSUS       SSDLYLVTRH ADVIPVRRRG DSRGSLLSPR PVSYLKGSSG GPLLCPSGHA
Case clone1     ---------- ---------- -G-------K ---------- ----------
Case clone2     ---------- ---------- -G-------K ---------- ----------
Case clone3     ---------- ---------- -G-------K ---------- ----------
Case clone4     ---------- ---------- -G-------K ---------- ----------
Case clone5     ---------- ---------- -G-------K ---------- ----------
                151                                 195  200
CONSENSUS       VGIFRAAVCT RGVAKAVDFI PVESMETTMR SPVFTDNSSP PAVPQTFQVA
Case clone1     ---------- ---------- ---------- ---------- ----K-----  15
Case clone2     ---------- ---------- ---------- ---------- ----K-----  14
Case clone3     ---------- ---------- ---------- ---------- ----K-----   1
Case clone4     ---------- ---------- ---------- ---------- ----K-----   1
Case clone5     ---------- ---------- ---------- ---------- ----K----V   1
```

**Fig. 2.** Evolution of the HCV NS3 gene sequence at the start of telaprevir monotherapy. Consensus sequence was made from the HCV RNA of 35 treatment-naïve Japanese patients with genotype 1b in our hospital. The number of clones within each sample of identical amino acid sequences is given on the right at the end of each sequence. Dashes indicate identical amino acid sequences.

detection (10 IU/mL) for 2 patients in the group receiving 750 mg every 8 h.[6] In some patients, however, HCV RNA levels increased between days 7 and 14, and mutations that confer resistance to telaprevir were detected. This trial of telaprevir monotherapy was therefore terminated after 2 weeks, and combination therapy of telaprevir plus PEG-IFN-RBV is now used in the USA and Europe.[8–10] Our case may therefore represent an unusual and possibly serendipitous response to long-term telaprevir monotherapy, and the efficacy of monotherapy remains unclear.

To our knowledge, this is the first report of a patient with CH-C achieving SVR by telaprevir monotherapy, without the use of IFN. Three treatment-naïve Japanese patients were enrolled in our hospital for this phase II trial of telaprevir monotherapy over 24 weeks. Before treatment, the 2 non-SVR patients had a high HCV RNA viral load (>5 Log IU/mL), while the viral load in the SVR patient remained low. Further, while HCV RNA decreased below the limit of detection (10 IU/mL) and negativity of HCV RNA remained until the end of treatment in 2 patients, HCV RNA in the other non-SVR patient reappeared after treatment cessation.

The development of drug resistance has been a challenge for treatment strategies in many viral infections. The high replication rate and the error-prone nature of viral RNA polymerases generate a viral quasi-species from which variants resistant to viral inhibitors can be selected. Recently, Kuntzen et al. reported that viral loads were high in the majority of treatment-naïve patients carrying mutations of protease and polymerase inhibitors.[12] Low viral load may therefore be an important factor for achieving SVR by telaprevir monotherapy.

It has recently been reported that CH-C patients never treated with an NS3-4A protease inhibitor may nevertheless possess variants resistant to protease inhibitors involving the HCV RNA NS3 region.[12–14] While there was a resistant variant (T54A) in this case, this mutation exhibits only low-level resistance,[7] and the number of mutant variants may have been few along with substantial suppression of HCV replication by telaprevir. This may also help to explain the effectiveness of telaprevir in this case.

Moreover, two amino acid substitutions (R130K and Q195K) were unique to this patient. We therefore checked the nucleotide sequence data in the DDBJ/EMBL/GenBank databases and found a previous report by Franco et al. on the R130K substitution (EF013801, EF013863, EF013867, EF013869).[15] Interestingly, although only a minor clone (4% of total) in that study, the viral load of the patient with the R130K substitution was also low (2364 IU/mL). To date, however, the Q195K substitution has not been reported. Their presence in this case may indicate that telaprevir has a stronger antiviral activity against HCV with these substitutions.

The NS3-4A protease targeted by protease inhibitors is required for viral polyprotein processing, an essential step in viral replication, but is also responsible for disrupting IFN responses to the infection.[16] Previous studies have shown that high concentrations of protease inhibitors may restore retinoic acid-inducible gene I (RIG-I) signaling in HCV replicon cells, [16–18] and Liang et al. also recently reported that protease inhibitors could restore interferon regulatory factor 3 (IRF-3) signaling in HCV-infected cells.[19] In our patient, telaprevir may have therefore rescued the NS3-4A-mediated blockade of IRF-3 signaling *in vivo*.

Further studies are required, such as sequencing analyses of the HCV NS3 region, and research into the rescue of IFN-β signaling through the RIG-I pathway. It is foreseeable in the future for CH-C patients to be treated by one or a combination of two or more oral drugs with high efficacy and genetic barriers to resistance and low side-effect profiles. Telaprevir may hold promise for being one of these drugs, even if only within a subset of patients, and further studies into telaprevir monotherapy or combination therapy with other oral drugs is therefore warranted. Although still an isolated response, based on our current molecular understanding of HCV infection and pharmacotherapy, this case suggests that long-term telaprevir monotherapy may be effective in other CH-C patients with genotype 1 and a low viral load.

## Conflict of interest

The authors have no commercial or other associations that may pose a conflict of interest.

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## References

1. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001;**358**:958–65.
2. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonçales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;**347**:975–82.
3. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007;**46**:403–10.
4. Hadziyannis SJ, Sette Jr H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004;**140**:346–55.
5. Lin C, Kwong AD, Perni RB. Discovery and development of VX-950, a novel, covalent, and reversible inhibitor of hepatitis C virus NS3.4A serine protease. *Infect Disord Drug Targets* 2006;**6**:3–16.
6. Reesink HW, Zeuzem S, Weegink CJ, Forestier N, Vliet AV, Rooij JVDWD, et al. Rapid decline of viral RNA in hepatitis C patients treated with VX-950: a phase 1b, placebo-controlled, randomized study. *Gastroentrology* 2006;**131**:997–1002.
7. Sarrazin C, Kieffer TL, Bartels D, Hanzelka B, Muh U, Welker M, et al. Dynamic hepatitis C virus genotypic and phenotypic changes in patients treated with the protease inhibitor telaprevir. *Gastroentrology* 2007;**132**:1767–77.
8. Lawitz E, Rodriguez-Torres M, Muir AJ, Kieffer TL, McNair L, Khunvichai A, et al. Antiviral effects and safety of telaprevir, peginterferon alfa-2a, and ribavirin for 28 days in hepatitis C patients. *J Hepatol* 2008;**49**:163–9.
9. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009;**360**:1827–38.
10. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009;**360**:1839–50.
11. Ogata S, Florese RH, Nagano-Fujii M, Hidajat R, Deng L, Ku Y, et al. Identification of hepatitis C virus (HCV) subtype 1b strains that are highly, or only weakly, associated with hepatocellular carcinoma on the basis of the secondary structure of an amino-terminal portion of the HCV NS3 protein. *J Clin Microbiol* 2003;**41**:2835–41.
12. Kuntzen T, Timm J, Berical A, Lennon N, Berlin AM, Young SK, et al. Naturally occurring dominant resistance mutations to hepatitis C virus protease and polymerase inhibitors in treatment-naïve patients. *Hepatology* 2008;**48**:1769–78.
13. Bartels DJ, Zhou Y, Zhang EZ, Marcial M, Byrn RA, Pfeiffer T, et al. Natural prevalence of hepatitis C virus variants with decreased sensitivity to NS3-4A protease inhibitors in treatment-naïve subjects. *JID* 2008;**198**:800–7.
14. Cubero M, Esteban JI, Otero T, Sauleda S, Bes M, Esteban R, et al. Naturally occurring NS3-protease-inhibitor resistant mutant A156T in the liver of an untreated chronic hepatitis C patient. *Virology* 2008;**370**:237–45.
15. Franco S, Parera M, Aparicio E, Clotet B, Martinez MA. Genetic and catalytic efficiency structure of an HCV protease quasispecies. *Hepatology* 2007;**45**:899–910.
16. Foy E, Li K, Wang C, Surnpter Jr R, Ikeda M, Lemon SM, et al. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. *Science* 2003;**300**:1145–8.
17. Johnson CL, Owen DM, Gale Jr M. Functional and therapeutic analysis of hepatitis C virus NS3-4A protease control of antiviral immune defense. *J Biol Chem* 2007;**282**:10792–803.
18. Loo YM, Owen DM, Li K, Erickson AK, Johnson CL, Fish PM, et al. Viral and therapeutic control of IFN-β promoter stimulator 1 during hepatitis C virus infection. *Proc Natl Acad Sci USA* 2006;**103**:6001–6.
19. Liang Y, Ishida H, Lenz O, Lin TI, Nyanguile O, Simmen K, et al. Antiviral suppression vs restoration of RIG-I signaling by hepatitis C protease and polymerase inhibitors. *Gastroentrology* 2008;**135**:1710–8.

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# Influence of Amino-Acid Polymorphism in the Core Protein on Progression of Liver Disease in Patients Infected With Hepatitis C Virus Genotype 1b

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The substitution of amino acid (aa) 70 of arginine for glutamine and/or that of aa91 of leucine for methionine in the core protein in patients infected with hepatitis C virus (HCV) genotype 1b is associated with a poor response to pegylated interferon and ribavirin. Factors influencing these substitutions were sought in 1,097 patients infected with HCV-1b who had not received antiviral treatment. HCV variants with Arg70 and Leu91 (wild-type) decreased, while those with Gln70 and/or Met91 (mutant types) increased with age ($P < 0.001$). Of the 1,097 patients, 464 (42.3%) were infected with the Gln70 variant and the remaining 633 patients with the Arg70 variant. The proportion of patients with the Gln70 variant increased with the severity of liver disease ($P < 0.001$), elevated γ-glutamyl transpeptidase (γ-GTP) levels ($P < 0.001$) and a decrease in platelet count ($P = 0.008$). In univariate analysis patients with hepatocellular carcinoma, elevated aspartate aminotransferase (AST ≥ 58 IU/L) and γ-GTP (≥61 IU/L), and decreased albumin levels (<3.9 g/dl) were more frequent in the patients with the Gln70 variant than the Arg70 variant ($P = 0.003$, 0.005, <0.001, and 0.031, respectively). In multivariate analysis HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and γ-GTP ≥61 IU/L (1.647 [1.268–2.139]) increased the risk for the Gln70 variant. In conclusion, the substitution of amino aa70 of Arg for Gln in patients infected with HCV-1b increases with age, and it is associated with severe liver disease accompanied by elevated AST and γ-GTP levels, as well as the development of hepatocellular carinoma. ***J. Med. Virol. 82:41–48, 2010.*** © 2009 Wiley-Liss, Inc.

**KEY WORDS:** cirrhosis; core protein; hepatitis C; hepatocellular carcinoma; interferon; ribavirin



## INTRODUCTION

Worldwide, an estimated 170 million people are infected with hepatitis C virus (HCV) persistently [Cohen, 1999]. Decompensated cirrhosis and hepatocellular carcinoma (HCC) can develop in about 30% of patients infected with HCV [Alberti et al., 1999; Seeff, 2002]. HCV has six major genotypes and dozens of subgenotypes, and they have distinct geographic distributions and are associated with the progression of liver disease [Simmonds, 1995]. Host and virological factors can influence the severity of liver disease and the response to antiviral treatment. HCV infection in the childhood and women runs a milder course than that in adulthood and men, and the intake of alcohol accelerates the progression of liver disease [Poynard et al., 1997; Kenny-Walsh, 1999; Vogt et al., 1999; Wiese et al., 2000]. Genotypes 1 and 4 aggravate liver disease and decrease the response to antiviral treatment, in comparison with genotypes 2, 3, and 6 [Tsubota et al., 1994; Hui et al., 2003; Hadziyannis et al., 2004; Legrand-Abravanel et al., 2005; Yuen and Lai, 2006]. High levels of HCV RNA in the serum can induce severe liver disease and decrease treatment response [Tsubota et al., 1994].

In Japan, genotype 1b in a high viral load (>100 KIU/ml) accounts for >70% of HCV infection, and decreases the treatment response in patients with chronic hepatitis C [Kumada et al., 2006]. Even with pegylated interferon (PEG-IFN) combined with ribavirin, the sustained virological response for longer than 24 weeks after the withdrawal of treatment is achieved merely in

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50% of the patients with HCV-1b in high levels [Manns et al., 2001; Fried et al., 2002]. It is necessary to predict the response to PEG-IFN/ribavirin before the start of antiviral therapy, to avoid severe side-effects in the patients who will barely gain sustained virological response.

The core protein of HCV is coded for by the C gene, and consists of 191 amino acids (aa) [Rosenberg, 2001]. Although the core protein is conserved better than the other structural and non-structural proteins of HCV, polymorphisms of core protein are known, and they influence the response to antiviral treatment. In patients infected with HCV-1b, for example, the substitution of arginine at position 70 (Arg70) for glutamine (Gln70) and that of leucine at position 91 (Leu91) for methionine (Met70) decrease sustained virological response in the patients with chronic hepatitis C who are treated with PEG-IFN/ribavirin and increase the development of HCC [Akuta et al., 2007a,b,d, 2008].

In the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo, the amino-acid sequence of the core-protein was determined in 1,079 patients infected with HCV-1b who had not received antiviral treatment. The substitution of Arg70 for Gln70 and that of Leu91 or Met 91 were correlated with the age at presentation, liver function tests and the severity of liver disease. Based on the results obtained, Gln70 would contribute to the progression of chronic hepatitis C.

## MATERIALS AND METHODS

### Patients

During 1966–2008, 1,097 patients infected with HCV-1b visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. They were: (1) negative for hepatitis B surface antigen by radioimmunoassay (Dainabot, Tokyo, Japan) or antibody to human immunodeficiency virus type-1; (2) positive for anti-HCV by a third-generation enzyme immunoassay (Chiron Corp., Emeryville, CA) and HCV RNA by the polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor ver.2.0, Roche Diagnostics, Tokyo, Japan); (3) infected with HCV genotype 1b but not with other genotypes; (4) without previous antiviral treatment; (5) without other forms of hepatitis, including hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease and autoimmune liver disease; and (6) had serum samples stored at −80°C. Of the 1,097 patients, 778 (70.9%) had chronic hepatitis, 221 (20.1%) cirrhosis, and 98 (8.9%) HCC. Amino acids in the core protein at positions 70 and 91 were determined, and were correlated with liver disease and biochemical and virological markers. Informed consent was obtained from each patient in this study, and the protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval by Ethic Committee of the institution.

### Histopathological Diagnoses of Liver Disease

Liver biopsy was performed under laparoscopy by a modified Vim Silverman needle (Tohoku University style, Kakinuma Factory, Tokyo). The sample was fixed in 10% formalin, and was stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff. It contained at least six portal areas. The pathological diagnosis was made by one of the authors (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994]. Cirrhosis was diagnosed by imaging on ultrasonography (US), computed tomography (CT), or magnetic resonance imaging (MRI). HCC was diagnosed by US and/or CT. Angiography was performed when HCC was strongly suspected by US, CT, MRI, or liver biopsy. An increasing trend of tumor markers was taken into consideration for the diagnosis of HCC.

### Determination of Amino-Acid Substitutions in the Core Protein

Amino acid (aa) at position 70 of Arg or Gln and aa91 of Leu or Met were determined by PCR with primers specific for each of them [Okamoto et al., 2007]. It is highly reproducible, and has a sensitivity of 94.4% in the determination of aa70 or aa91 in samples with HCV RNA titers >10 KIU/ml. The concordance of the results of this method with those of direct sequencing reached 97.1%. Amino acids at positions 70 and 91 were confirmed by direct sequencing of most samples [Akuta et al., 2005].

### Statistical Analysis

Changes of Arg70/Leu91 (wild-type) and Gln70 and/or Met91 (mutant types) with age were analyzed by the Cochran–Armitage trend test (SAS version 9.1.3; SAS Institute, Inc., Cary, NC). Frequencies were compared between groups by the Kruskal–Wallis test and Fisher's exact test. Univariate and multivariate logistic regression analyses were used for the evaluation of factors independently associated with the substitution of aa70. They included the following ten variables: age, sex, liver disease, platelet count, hemoglobin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), and substitution of aa at position 91 in the core protein. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance on univariate analysis were tested by the multivariate Cox proportional hazard model to identify independent factors. Statistical comparisons were performed using SPSS ver.11.0 (SPSS, Inc., Chicago, IL). A $P$-value $<0.05$ by the two-tailed test was considered significant.

## RESULTS

### Clinical and Virological Characteristics of the 1,097 Patients Who Were Infected With HCV-1b

Table I lists the baseline characteristics of the 1,097 patients who were infected with HCV-1b and had not received antiviral treatment. They had the median age of 60 years and included 590 (53.8%) men. The median transaminase levels were elevated, and alpha-fetoprotein was within the normal limit (<10 μg/L). The majority of the patients (70.9%) had chronic hepatitis, while HCC had developed in 8.9% of the patients. Amino acids at positions 70 and 91 in the core protein were both the wild-type (Arg70 and Leu91) in 37.6% of them, and both mutant types (Gln70 and Met91) in 16.4%. The Gln70 variant was detected in 464 of the 1,097 (42.3%) patients.

### The Prevalence of Amino-Acid Substitutions Stratified by Age and Sex

The 1,097 patients infected with HCV-1b were classified into three age groups, and the prevalence of Arg70/Leu91 (wild-type) and that of Gln70 and/or Met91 (mutant types) were compared (Fig. 1). Ag70/Leu91 decreased with age by trend analysis, from 63.6% in the patients aged ≤30 years to 36.6% in those ≥41 years ($P < 0.001$ by the Cochran–Armitage trend test). Table II lists the prevalence of the Gln70 variant in men and women stratified by the age. There were no sex differences in the prevalence of the Gln70 variant.

### The Prevalence of the Gln70 Variant in Patients With Different Liver Diseases

Figure 2 compares the prevalence of the Gln70 variant among patients infected with HCV-1b who presented with different liver diseases at the baseline. The prevalence of the Gln70 variant increased with the progression of liver disease from chronic hepatitis (32.6%) to cirrhosis (43.0%) and HCC (53.1%) ($P < 0.001$ by the Kruskal–Wallis test). In patients with cirrhosis, the 126 patients with the Arg70 variant were aged with the mean of 62 years (range: 32–78 years) in comparison with the 95 patients with the Gln70 variant who were aged 59 years (25–80). In patients with HCC, the 47 patients with the Arg70 variant were aged with the mean of 66 years (range: 37–81 years) in comparison with the 51 patients with the Gln70 variant who were aged 66 years (46–78).

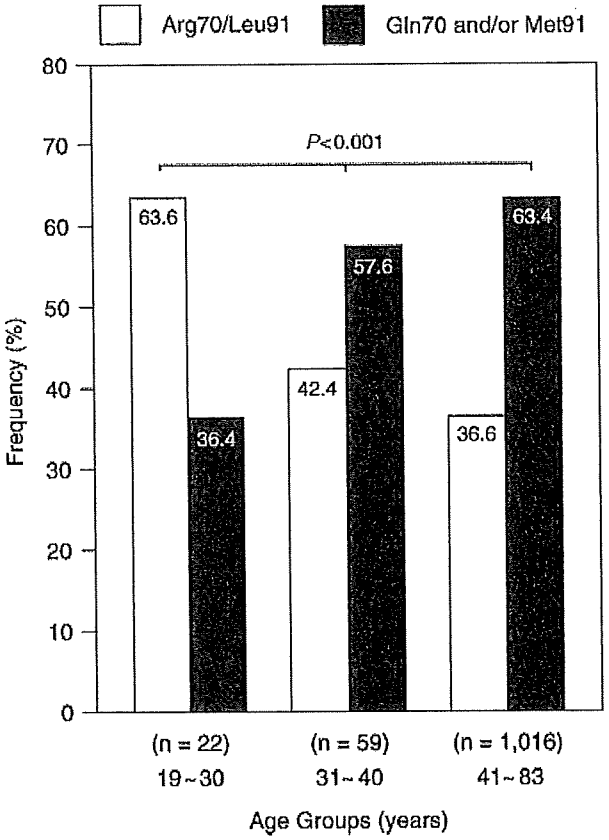


Fig. 1. The age-specific prevalence of Gln70 in treatment-naive patients infected with HCV-1b.

TABLE I. Clinical and Virological Characteristics of the 1,097 Patients Who Were Infected With Hepatitis C Virus of Genotype 1b

| | |
|---|---|
| Age (years) | 60 (19–83) |
| Men | 590 (53.8%) |
| Follow-up period (years) | 8 (3–28) |
| Hemoglobin (g/dl) | 14.0 (4.5–26.8) |
| Platelets ($\times 10^3/mm^3$) | 15.4 (2.0–34.1) |
| Aspartate aminotransferase (IU/L) | 58 (8–617) |
| Alanine aminotransferase (IU/L) | 69 (6–776) |
| Alpha-fetoprotein (μg/L) | 6 (2–65,700) |
| Liver disease | |
| Chronic hepatitis | 778 (70.9%) |
| Cirrhosis | 221 (20.1%) |
| Hepatocellular carcinoma | 98 (8.9%) |
| Amino acids in the core protein | |
| Arg70/Leu91 (double wild-type) | 412 (37.6%) |
| Gln70/Leu91 (mutant type) | 284 (25.9%) |
| Arg70/Met91 (mutant type) | 221 (20.1%) |
| Gln70/Met91 (double mutant type) | 180 (16.4%) |

Values are the median with range in parentheses or the number with percentage in parentheses.

TABLE II. Frequency of Gln70 in the Core Protein in Patients Infected With HCV-1b Stratified by Age and Sex

| Age (years) | Men | Women | Differences |
|---|---|---|---|
| 19–30 | 23.5% (4/17) | 20% (1/5) | 1.0 |
| 31–40 | 34.1% (14/41) | 38.9% (7/18) | 0.773 |
| 41–50 | 37.2% (45/121) | 40% (14/35) | 0.763 |
| 51–60 | 39.1% (72/184) | 40.1% (63/157) | 0.912 |
| 61–70 | 36.0% (62/172) | 30.1% (74/246) | 0.205 |
| 70–83 | 45.5% (25/55) | 43.5% (20/46) | 0.842 |
| Total | 37.6% (222/590) | 35.3% (179/507) | 0.451 |

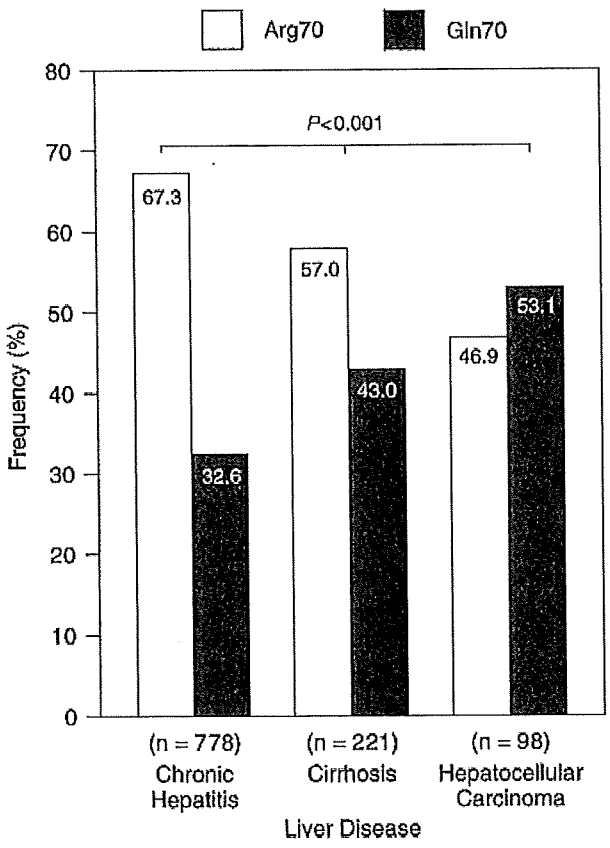


Fig. 2. The prevalence of the Gln70 variant among patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma.

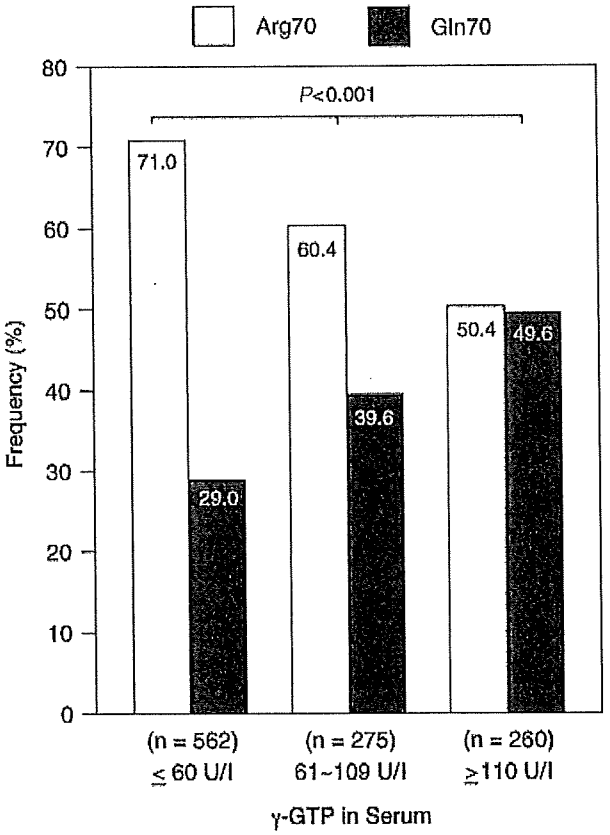


Fig. 3. The prevalence of the Gln70 variant among patients with different γ-GTP levels.

### The Influence of γ-GTP Levels on the Prevalence of the Gln70 Variant

The prevalence of Gln70 was compared among patients with different γ-GTP levels at the baseline (Fig. 3). The prevalence of the Gln70 variant increased in parallel with the γ-GTP levels from 29.0% to 49.6% ($P < 0.001$ by the Kruskal–Wallis test).

### The Influence of Platelet Count on the Prevalence of the Gln70 Variant

The prevalence of the Gln70 variant was compared among three groups of patients with various platelet counts at the baseline (Fig. 4). The prevalence of the Gln70 variant increased as the platelet count decreased ($P = 0.008$ by the Kruskal–Wallis test).

### Factors Associated With the Gln70 Variant in Patients Infected with HCV-1b

Since the Gln70 variant, in comparison with the Arg70 variant, aggravated liver disease in patients infected with HCV-1b (Figs. 2–4), ten factors were evaluated for the association with the Gln70 variant by the univariate analysis (Table III); the cut-off value was set at the median of studied patients. Among them, HCC, elevated levels of AST (≥58 IU/L) and γ-GTP (>61 U/L), as well as decreased albumin concentration (<3.9 g/dl), were associated with the Gln70 variant ($P = 0.003$, 0.005, <0.001, and 0.031, respectively). A similar analysis was performed for the substitution of Leu91 for Met91 (Table IV). Except for the association with the substitution of Arg70 for Gln70, the Met91 variant had no influence on any variable examined.

Two factors associated independently with the Gln70 variant were identified by the multivariate analysis (Table V). The risk for the Gln70 variant was increased by HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917], $P = 0.011$) and γ-GTP ≥61 IU/L (1.647 [1.268–2.139], $P < 0.001$).

## DISCUSSION

The response to PEG-IFN and ribavirin is influenced by genotypes and viral load, and is poorest in patients with HCV-1b in high HCV RNA levels [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. The prediction of sustained virological response would circumvent side-effects and costs in non-responders. Amino-acid substitutions in the core protein are useful

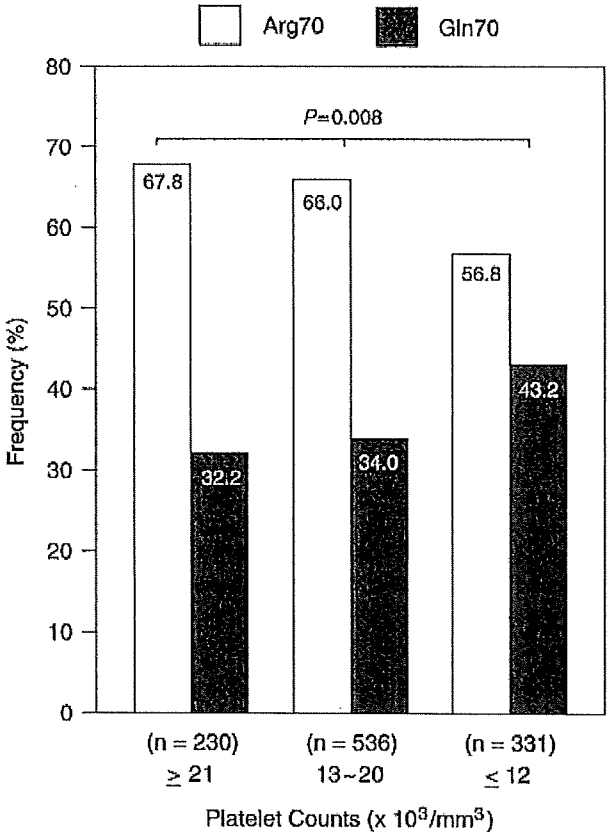


Fig. 4. The prevalence of the Gln70 among three groups of patients with different platelet counts.

for predicting the non-response in patients infected with HCV-1b. The substitution of Arg70 for Gln70 in the prototype sequence of HCV-1b [Kato et al., 1990] and/or that of Leu91 for Met91 can predict the non-response to IFN-based treatment [Akuta et al., 2005, 2006, 2007c,d]. It has been beyond the scope of previous studies, however, whether or not these amino-acid substitutions influence the progression of hepatitis C in the patients who have not received antiviral treatment. The availability of pre-treatment sera from many patients with chronic hepatitis C permitted the evaluation of the influence of aa substitutions in the core protein on the progression of liver disease without therapeutic intervention.

First, the prevalence of the Gln70 variant increased with the age of patients until they had reached 50 years (Fig. 1). It is not certain if HCV-1b with Arg70 underwent a point mutation for Gln70 (G-to-A at nucleotide 209), or these amino-acid residues were present in HCV-1b strains prevalent at the time of infection. Follow-up of patients for aa substitutions will resolve this issue. Another possibility for this difference would be a selection bias. If the patients with the Arg90 variant fare better than those with the Gln70 variant, they would not develop liver disease severe enough to visit hospital.

Secondly, the patients infected with HCV-1b with Gln70 increased in parallel with γ-GTP levels and the severity of liver disease from chronic hepatitis to cirrhosis and HCC, as well as with a decrease in platelet count (Figs. 2–4). Since the Met91 variant did not make such difference, the aggravation of liver disease would have been due to the Gln70 variant, but not to the Met91 variant. Increases in the γ-GTP level may have been related to the development of HCC; γ-GTP has been proposed as a sensitive marker of cirrhosis and HCC [Penn and Worthington, 1983]. Decreased platelet counts have been associated with HCC [Ikeda et al., 2001; Lu et al., 2006; Kumada et al., 2009]. Although the proportion of the Gln70 variant increases with the severity of liver disease (Fig. 2), the median age of patients with cirrhosis or HCC did not differ between the patients with the Arg70 variant and Gln70 variant who

TABLE III. Factors Associated With the Substitution of aa70 of Arginine for Glutamine in the Core Protein in 1,097 Patients Infected With HCV Genotype1b by Univariate Analysis

| Factor | Category | Gln70 | *P*-value |
|---|---|---|---|
| Sex | 1: Male | 38.6% (228/590) | 0.663 |
| | 2: Female | 37.3% (189/507) | |
| Age (years) | 1: <60 | 40.6% (219/540) | 0.093 |
| | 2: ≥60 | 35.5% (198/557) | |
| AST (IU/L) | 1: <58 | 33.9% (184/543) | 0.005 |
| | 2: ≥58 | 42.2% (234/554) | |
| ALT (IU/L) | 1: <75 | 36.9% (213/578) | 0.376 |
| | 2: ≥75 | 39.3% (204/519) | |
| Albumin (g/dl) | 1: <3.9 | 42.5% (194/457) | 0.031 |
| | 2: ≥3.9 | 35.8% (229/640) | |
| γ-GTP (IU/L) | 1: <61 | 29.0% (163/562) | <0.001 |
| | 2: ≥ 61 | 44.4% (238/535) | |
| Hemoglobin (g/dl) | 1: <14 | 35.1% (176/501) | 0.083 |
| | 2: ≥14 | 40.4% (241/596) | |
| Platelet count (×10³/mm³) | 1: <150 | 39.9% (207/519) | 0.253 |
| | 2: ≥150 | 36.3% (210/578) | |
| Hepatocellular carcinoma | 1: No | 36.6% (366/999) | 0.003 |
| | 2: Yes | 53.1% (52/ 98) | |
| Substitutions of core aa91 | 1: Leucine | 35.6% (227/638) | 0.051 |
| | 2: Methionine | 41.4% (190/459) | |

TABLE IV. Factors Associated With the Substitution of aa91 of Leucine for Methionine in the Core Protein in 1,097 Patients Infected With HCV Genotype1b by Univariate Analysis

| Factor | Category | Met91 | *P*-value |
|---|---|---|---|
| Sex | 1: Male | 40.8% (241/590) | 0.500 |
| | 2: Female | 43.0% (218/507) | |
| Age (years) | 1: <60 | 43.5% (235/540) | 0.271 |
| | 2: ≥60 | 40.2% (220/517) | |
| AST (IU/L) | 1: <58 | 43.6% (234/537) | 0.196 |
| | 2: ≥58 | 39.7% (217/547) | |
| ALT (IU/L) | 1: <75 | 42.4% (238/561) | 0.618 |
| | 2: ≥75 | 40.8% (205/502) | |
| Albumin (g/dl) | 1: <3.9 | 42.0% (177/421) | 0.797 |
| | 2: ≥3.9 | 41.2% (249/604) | |
| γ-GTP (IU/L) | 1: <61 | 40.4% (237/586) | 0.327 |
| | 2: ≥61 | 43.4% (222/511) | |
| Hemoglobin (g/dl) | 1: <14 | 40.8% (193/473) | 0.658 |
| | 2: ≥14 | 42.3% (240/567) | |
| Platelet count ($\times 10^3/mm^3$) | 1: <150 | 40.5% (202/499) | 0.454 |
| | 2: ≥150 | 42.9% (240/559) | |
| Hepatocellular carcinoma | 1: No | 42.3% (423/999) | 0.334 |
| | 2: Yes | 36.7% (36/ 98) | |
| Substitutions of core aa71 | 1: Arginine | 49.0% (269/680) | 0.051 |
| | 2: Glutamine | 45.6% (190/417) | |

had cirrhosis (62 years vs. 59 years) of HCC (66 years vs. 66 years). This would indicate a possibility that the Gln70 variant would be a factor for the aggravation of liver disease that might be independent of age.

The distinct capacity of Gln70 and Met91 for decreasing the response to combined treatment in patients infected with HCV-1b was proposed in a recent study [Okanoue et al., 2008]. The Gln70 variant decreased sustained virological response, while the Met91 variant did not, although the Met91 variant reduced the rate of rapid virological response within 4 weeks after the start of therapy. The role of the Gln70 variant greater than that of the Met91 variant in the progression of liver disease has been confirmed in this study (Tables III and IV). In the multivariate analysis, the risk for Gln70 was increased by HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and γ-GTP ≥61 U/L (1.647 [1.268–2.139]). The Gln70 variant would aggravate liver disease toward the development of HCC in patients infected with HCV-1b who have not received antiviral treatment.

It would be a matter of conjecture how the Gln70 variant influences the severity of liver disease. Previous suggestions for a reduced response of patients with the Gln70 variant were confined to interaction of the core protein with IFN receptors and IFN-signaling pathways [Alexander, 2002; Blindenbacher et al., 2003; Bode et al., 2003]; these studies were restricted to patients receiving IFN-based treatments [Akuta et al., 2007a,b,d, 2008]. The ability of the Gln70 variant for accelerating the progression of liver disease, in the absence of exogenous IFN, has changed this issue into a wider perspective. There still remains a possibility, however, that the Gln70 variant would interact with the endogenous IFN induced by HCV infection, and aggravate liver disease.

Another possibility may be the cytotoxic T-cell (CTL) response, as has been demonstrated for the pathogenesis of chronic hepatitis B [Chisari and Ferrari, 1995]. Since both hepatitis B virus (HBV) and HCV do not have a cytopathic capacity, hepatitis B and C would be mediated by immune responses directed at viral proteins. Amino-acid sequences bearing a CTL epitope restricted by the MHC class-I are demonstrated in the HBV core protein [Bertoletti et al., 1993; Bertoletti and Gehring, 2006], and are implicated in liver disease in the patients with the HLA-2 phenotype [Penna et al., 1991; Bertoletti et al., 1994]. It is tempting to speculate that the substitution of Arg70 for Gln70 might generate a CTL epitope and stimulate cytotoxic lymphocytes toward inflammation of the liver [Kita et al., 1993; Jackson et al., 1999].

In conclusion, amino-acid substitutions in the core protein influence the progression of liver disease, and the Gln70 variant aggravates hepatic inflammation and increases the risk for HCC in the patients who have not received antiviral treatment. The ability of the Gln70

TABLE V. Factors Associated with the Substitution of aa70 of Arginine for Glutamine in the Core Protein in 1,097 Patients Infected with HCV Genotype1b by Multivariate Analysis

| Factor | Category | Odds ratio (95%CI) | *P*-value |
|---|---|---|---|
| Hepatocellular carcinoma | 1: No | 1 | 0.011 |
| | 2: Yes | 1.829 (1.147–2.917) | |
| γ-GTP (IU/L) | 1: <61 | 1 | <0.001 |
| | 2: ≥61 | 1.647 (1.268–2.139) | |

variant to aggravate liver disease, in the absence of exogenous IFN, would lend further support on its capacity of predicting sustained virological response before the start of therapy. It is possible that mechanisms other than the resistance to IFN, such as cytotoxic T-cell responses, might be involved in an increased pathogenetic potential of HCV-1b with Gln70.

## REFERENCES


Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Matsuda M, Arase Y, Ikeda K, Kumada H. 2005. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 48:372–380.

Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Arase Y, Ikeda K, Kumada H. 2006. Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. J Med Virol 78:83–90.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007a. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. Hepatology 46:1357–1364.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007b. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: Amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 46:403–410.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2007c. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. J Med Virol 79:1686–1695.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Miyakawa Y, Kumada H. 2007d. Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphisms in the viral core protein and very early dynamics of viremia. Intervirology 50:361–368.

Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Arase Y, Ikeda K, Kumada H. 2008. Substitution of amino acid 70 in the hepatitis C virus core region of genotype 1b is an important predictor of elevated alpha-fetoprotein in patients without hepatocellular carcinoma. J Med Virol 80:1354–1362.

Alberti A, Chemello L, Benvegnu L. 1999. Natural history of hepatitis C. J Hepatol 31:S17–S24.

2002. Suppressors of cytokine signalling (SOCS) in the immune system. Nat Rev Immunol 2:410–416.

Bertoletti A, Gehring AJ. 2006. The immune response during hepatitis B virus infection. J Gen Virol 87:1439–1449.

Bertoletti A, Chisari FV, Penna A, Guilhot S, Galati L, Missale G, Fowler P, Schlicht HJ, Vitiello A, Chesnut RC, Fiaccardori F, Ferrari C. 1993. Definition of a minimal optimal cytotoxic T-cell epitope within the hepatitis B virus nucleocapsid protein. J Virol 67:2376–2380.

Bertoletti A, Costanzo A, Chisari FV, Levrero M, Artini M, Sette A, Penna A, Giuberti T, Fiaccadori F, Ferrari C. 1994. Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. J Exp Med 180:933–943.

Blindenbacher A, Duong FH, Hunziker L, Stutvoet ST, Wang X, Terracciano L, Moradpour D, Blum HE, Alonzi T, Tripodi M, La Monica N, Heim MH. 2003. Expression of hepatitis c virus proteins inhibits interferon alpha signaling in the liver of transgenic mice. Gastroenterology 124:1465–1475.

Bode JG, Ludwig S, Ehrhardt C, Albrecht U, Erhardt A, Schaper F, Heinrich PC, Haussinger D. 2003. IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J 17:488–490.

Chisari FV, Ferrari C. 1995. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 13:29–60.

Cohen J. 1999. The scientific challenge of hepatitis C. Science 285:26–30.

Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. 1994. Classification of chronic hepatitis: Diagnosis, grading and staging. Hepatology 19:1513–1520.

Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL, Jr., Haussinger D, Diago M, Carosi G, Dhumeaux D, Craxi A, Lin A, Hoffman J, Yu J. 2002. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 347:975–982.

Hadziyannis SJ, Sette H, Jr., Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Jr., Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. 2004. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: A randomized study of treatment duration and ribavirin dose. Ann Intern Med 140:346–355.

Hui CK, Yuen MF, Sablon E, Chan AO, Wong BC, Lai CL. 2003. Interferon and ribavirin therapy for chronic hepatitis C virus genotype 6: A comparison with genotype 1. J Infect Dis 187:1071–1074.

Ikeda K, Saitoh S, Kobayashi M, Suzuki Y, Suzuki F, Tsubota A, Arase Y, Murashima N, Chayama K, Kumada H. 2001. Long-term interferon therapy for 1 year or longer reduces the hepatocellular carcinogenesis rate in patients with liver cirrhosis caused by hepatitis C virus: A pilot study. J Gastroenterol Hepatol 16:406–415.

Jackson M, Smith B, Bevitt DJ, Steward M, Toms GL, Bassendine MF, Diamond AG. 1999. Comparison of cytotoxic T-lymphocyte responses to hepatitis C virus core protein in uninfected and infected individuals. J Med Virol 58:239–246.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 87:9524–9528.

Kenny-Walsh E. 1999. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. N Engl J Med 340:1228–1233.

Kita H, Moriyama T, Kaneko T, Harase I, Nomura M, Miura H, Nakamura I, Yazaki Y, Imawari M. 1993. HLA B44-restricted cytotoxic T lymphocytes recognizing an epitope on hepatitis C virus nucleocapsid protein. Hepatology 18:1039–1044.

Kumada T, Toyoda H, Honda T, Kuzuya T, Katano Y, Nakano I, Goto H. 2006. Treatment of chronic hepatitis C with interferon alone or combined with ribavirin in Japan. Intervirology 49:112–118.

Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kanamori A, Atsumi H, Takagi M, Nakano S, Arakawa T, Fujimori M. 2009. Incidence of hepatocellular carcinoma in hepatitis C carriers with normal alanine aminotransferase levels. J Hepatol 50:729–735.

Legrand-Abravanel F, Nicot F, Boulestin A, Sandres-Saune K, Vinel JP, Alric L, Izopet J. 2005. Pegylated interferon and ribavirin therapy for chronic hepatitis C virus genotype 4 infection. J Med Virol 77:66–69.

Lu SN, Wang JH, Liu SL, Hung CH, Chen CH, Tung HD, Chen TM, Huang WS, Lee CM, Chen CC, Changchien CS. 2006. Thrombocytopenia as a surrogate for cirrhosis and a marker for the identification of patients at high-risk for hepatocellular carcinoma. Cancer 107:2212–2222.

Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. 2001. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: A randomised trial. Lancet 358:958–965.

Okamoto K, Akuta N, Kumada H, Kobayashi M, Matsuo Y, Tazawa H. 2007. A nucleotide sequence variation detection system for the core region of hepatitis C virus-1b. J Virol Methods 141:1–6.

Okanoue T, Itoh Y, Yotsuyanagi H, Tanaka E, Yoshioka K, Izumi N, Kumada H. 2008. Substitution of core amino acid 91 lowers rapid virological response and substitution of amino acid 70 lowers sustained virological response to peginterferon alfa-2b plus ribabirin in chrnoic hepatitis C patients with genotype 1b—Nationwide Study (Abstract). Hepatology 48:868A.

Penn R, Worthington DJ. 1983. Is serum gamma-glutamyltransferase a misleading test? Br Med J 286:531–535.

Penna A, Chisari FV, Bertoletti A, Missale G, Fowler P, Giuberti T, Fiaccadori F, Ferrari C. 1991. Cytotoxic T lymphocytes recognize an

HLA-A2-restricted epitope within the hepatitis B virus nucleocapsid antigen. J Exp Med 174:1565–1570.

Poynard T, Bedossa P, Opolon P. 1997. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 349:825–832.

Rosenberg S. 2001. Recent advances in the molecular biology of hepatitis C virus. J Mol Biol 313:451–464.

Seeff LB. 2002. Natural history of chronic hepatitis C. Hepatology 36:S35–S46.

Simmonds P. 1995. Variability of hepatitis C virus. Hepatology 21:570–583.

Tsubota A, Chayama K, Ikeda K, Yasuji A, Koida I, Saitoh S, Hashimoto M, Iwasaki S, Kobayashi M, Hiromitsu K. 1994. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. Hepatology 19:1088–1094.

Vogt M, Lang T, Frosner G, Klingler C, Sendl AF, Zeller A, Wiebecke B, Langer B, Meisner H, Hess J. 1999. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. N Engl J Med 341:866–870.

Wiese M, Berr F, Lafrenz M, Porst H, Oesen U. 2000. Low frequency of cirrhosis in a hepatitis C (genotype 1b) single-source outbreak in Germany: A 20-year multicenter study. Hepatology 32:91–96.

Yuen MF, Lai CL. 2006. Response to combined interferon and ribavirin is better in patients infected with hepatitis C virus genotype 6 than genotype 1 in Hong Kong. Intervirology 49:96–98.