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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 $\times$ 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 χ^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

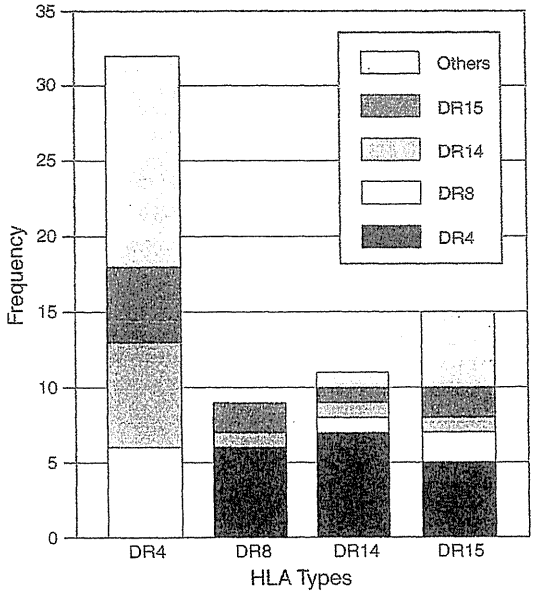

Fig. 1 HLA-DR alleles in the 48 patients with AIH. The allele in the other chromosome is shown in patients with DR4, DR8, DR14, and DR15

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 Response	Number ($n = 48$)	Histological response		
		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 ± 3.5 vs. 7.9 ± 3.3, $P = 0.092$), it improved to comparable extents in both of them after treatment (4.5 ± 0.9 vs. 4.7 ± 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

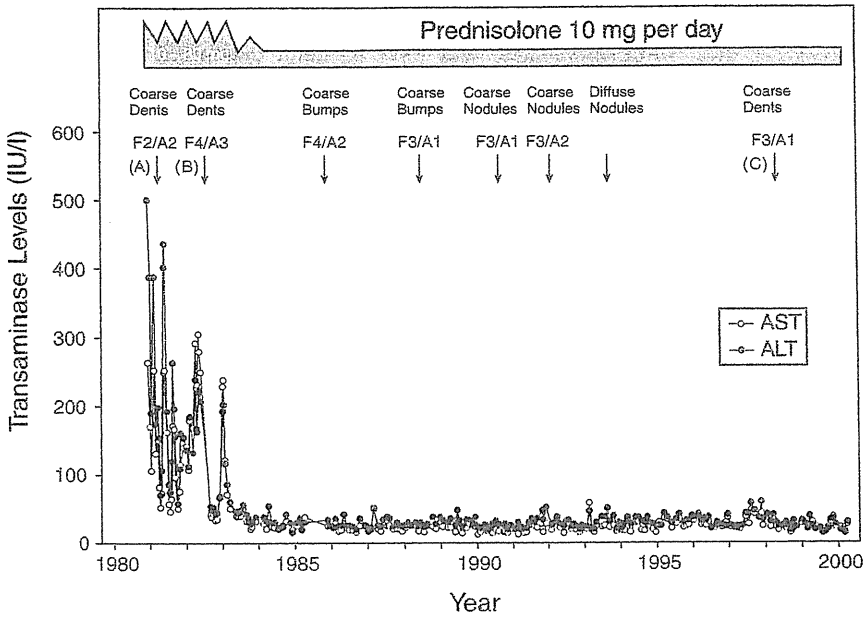

Fig. 2 Clinical course of a patient with AIH (female, 45 years old with HLA-DR4/DR14) who had been followed for 20 years. Doses of prednisolone are indicated at the top, and appearances of the liver surface on laparoscopies, as well as fibrosis stage and inflammation grade on liver biopsies, are shown 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 ≤ 10 mg

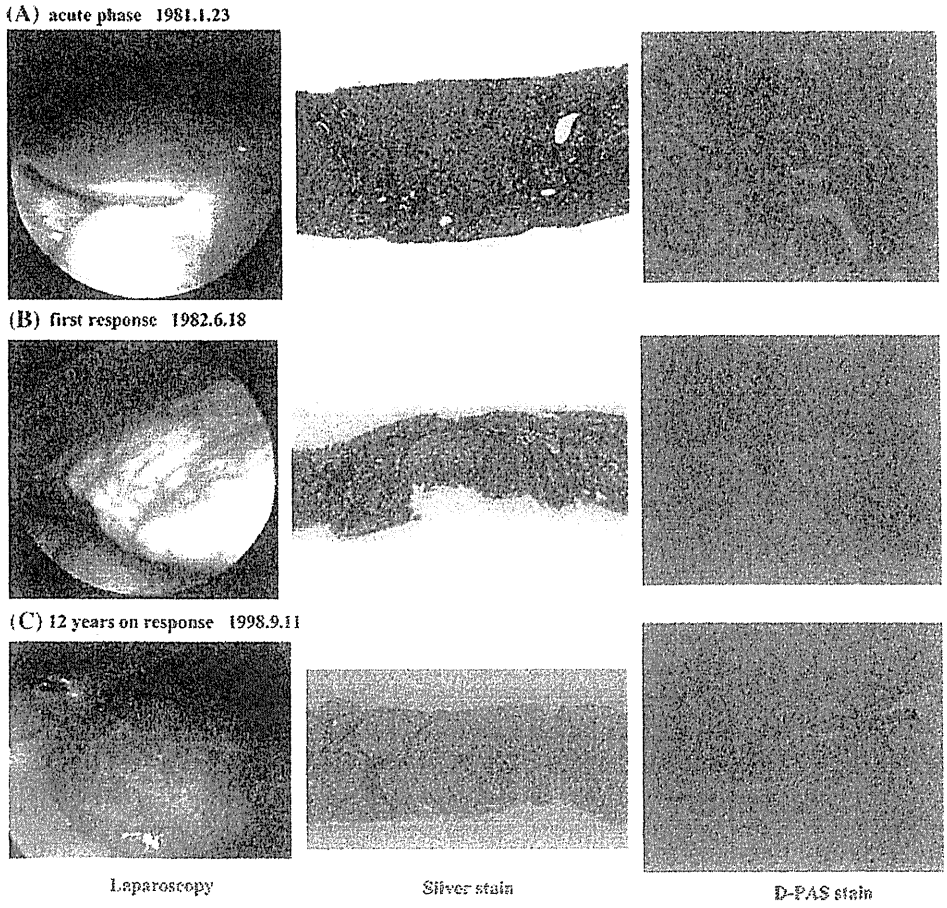

Fig. 3 Laparoscopic findings and histological changes in the patients with AIH. The patient presented in Fig. 2 was examined at three time points (*a*, *b*, and *c* in Fig. 2). Laparoscopic findings were improved since she responded to prednisolone since June, 1982. Histologically, typical submassive 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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HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy

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ABSTRACT

Background and aims A number of recent studies have shown that human polymorphisms near the *IL28B* type III interferon (IFNλ) gene influence the response to peg-interferon plus ribavirin combination therapy for infection with chronic hepatitis C virus (HCV). Viral polymorphisms, including substitutions within the HCV core and NS5A proteins, have also been shown to influence treatment outcome, but it is not known whether these factors act independently of the IL28B polymorphism or if they reflect the same or a different underlying mechanism. Multiple logistic regression was used to determine whether host and viral polymorphisms independently predict sustained virological response (SVR).

Methods Two single nucleotide polymorphisms were genotyped in the IL28B locus (rs12979860 and rs8099917) from 817 patients with chronic HCV infection, and substitutions at amino acids 70 and 91 of the HCV core protein and within the NS5A interferon sensitivity-determining region (ISDR) were analysed.

Results It was found that independent predictors of an SVR included IL28B rs12979860 CC genotype (OR=4.98; p=4.00E-08), core amino acid 70 substitutions (OR=0.53; p=0.016), age and baseline viral load. For non-virological response, the IL28B rs12979860 CT/TT genotype (OR=0.23; p=1.96E-8) and age were independent predictors. IL28B rs12979860 genotype (p=1.4E-8), core amino acid 70 substitutions (p=0.0013), ISDR substitutions (p=0.0019), baseline viral load, γ-glutamyltranspeptidase, alanine aminotransferase and platelet count were independent predictors for change in viral load by week 4 of treatment.

Conclusions IL28B polymorphisms and HCV core amino acid 70 substitutions contribute independently to an SVR to peg-interferon plus ribavirin combination therapy.

Significance of this study

What is already known about this subject?
- Clinical and viral factors influence the outcome of peg-interferon plus ribavirin combination therapy for chronic hepatitis C virus infection.
- Polymorphisms within the human IL28B locus strongly influence treatment outcome.
- Substitutions at amino acids 70 and 91 of the HCV core protein as well as within the interferon sensitivity-determining region (ISDR) also affect response to treatment.

What are the new findings?
- IL28B polymorphisms as well as substitutions at amino acid 70 both independently predict sustained virological response, suggesting that they influence treatment outcome through different mechanisms.
- IL28B polymorphisms, substitutions at core protein amino acid 70 and ISDR substitutions are each independent predictors for change in viral load after 4 weeks of treatment.

How might it impact on clinical practice in the forseeable future?
- The combination of IL28B genotyping and detection of core protein substitutions may yield more accurate pretreatment predictions of treatment efficacy.

INTRODUCTION

Hepatitis C virus (HCV) is a primary cause of chronic hepatitis and often progresses to liver cirrhosis and hepatocellular carcinoma.[1 2] Peg-interferon plus ribavirin combination therapy (PEG-RBV) is the current standard of care, but it is only effective in 50% of patients and has severe side effects often requiring discontinuation or dose modification.[3] Consequently, reliable predictors are needed to identify unsuitable candidates as early as possible.

Genome-wide association studies have reported common single nucleotide polymorphisms (SNPs) predictive of response to interferon treatment. While polymorphisms in MxA,[4 5] interferon α-receptor 1,[6] osteopontin[7] and MAPKAPK3[8] have been reported to be associated with interferon response, several linked SNPs within the IL28B locus on chromosome 19 have recently been shown to be the strongest predictors of early viral kinetics, response to treatment and spontaneous viral clearance.[9–15]

Viral polymorphisms have also been shown to be associated with treatment response. HCV genotypes 1 and 4 in particular are considered more difficult to treat than genotypes 2 and 3,[16 17] and genotype 3 is associated with steatosis.[18] Within genotype 1b, amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity-determining region (ISDR) of the NS5A protein[19 20] have also been shown to be associated with treatment outcome, especially among Japanese patients.

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Consequently, a number of human and viral factors are now known to affect response to treatment, but in order to identify the most important independent predictors and to identify which, if any, may be useful in guiding clinical practice, it is necessary to analyse them simultaneously in a multivariate model. In this study we therefore attempted to identify host and viral factors that independently predict treatment outcome.

MATERIALS AND METHODS

Patients

Data from 817 patients who were treated with PEG-RBV combination therapy for chronic hepatitis C genotype 1b infection between 2002 and 2008 were collected from Toranomon Hospital (Tokyo) and hospitals that belong to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Study subjects tested positive for HCV RNA over a span of >6 months, were negative for hepatitis B and HIV, and showed no evidence of other liver diseases. Patients received weekly injections of peg-interferon-α2b at 1.5 g/kg body weight for 48 weeks and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients with low baseline viral load (<5 log IU/ml) were excluded, as were patients who received <0.89 g/kg of peg-interferon or <8.3 mg/kg of ribavirin. Treatment success was evaluated based on a sustained virological response (SVR), defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. Some patients showed a transient response (TR or relapser), in which HCV RNA dropped to undetectable levels during treatment but then later rebounded. In those with a non-viral response (NVR), HCV RNA levels failed to decline by 2 log10 IU/ml by week 12 of treatment and never dropped below detectable levels. Histopathological diagnosis was made according to the criteria of Desmet *et al.*[21] All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA levels

HCV RNA levels were monitored throughout the course of treatment at 1 or 2 month intervals for a total of at least six time points via reverse transcription–PCR (RT–PCR) using the original Amplicor method, the high range method or the TaqMan RT–PCR test. The measurement ranges of these assays were 0.5–850 kIU/ml, 5–5000 kIU/ml and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with phosphate-buffered saline (PBS) and reanalysed. All values were reported as log IU/ml.

ISDR and core amino acid substitutions

Amino acid substitutions in the HCV core and ISDRs were determined by direct sequencing of PCR products following extraction and reverse transcription of serum HCV RNA. Core amino acid substitutions at positions 70 and 91 (core70 and core91) were determined according to Akuta *et al*,[22 23] and the number of ISDR substitutions was established as in Enomoto *et al.*[19 21 24] Of the 817 patients in the study, substitutions for both ISDR and core70 could be determined for 379 patients.

SNP genotyping

We genotyped each patient for two IL28B SNPs previously reported to be associated with treatment outcome, rs12979860 and rs8099917.[9–11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader assay, as described previously.[25 26] The two SNPs are in strong linkage disequilibrium, with a correlation coefficient of 0.99. SNP genotypes for both rs12979860 and rs8099917 were determined for 815 patients (99.7%).

Statistical analysis

All analyses were performed using the R statistical package (http://www.r-project.org). Non-parametric tests (χ^2 and Mann–Whitney U tests) were used to detect significant associations. All statistical analyses were two sided, and $p<0.05$ was considered significant. Simple and multiple logistic regression analyses were used to examine the association between viral substitutions and clinical factors using $p<0.05$ as the criterion for inclusion in the initial multivariate model. Multivariate logistic regression analysis was performed using forward/backward stepwise selection based on Akaike Information Criterion (AIC) score and validated using the rms package in R. ORs and 95% CIs were calculated for each factor.

RESULTS

Patient characteristics

Patient profiles are shown in table 1. Forty-five per cent of patients achieved an SVR, 22% were transient responders and 33% failed to respond to treatment (NVR). Males were significantly more likely to achieve an SVR than females (50% and 38%, respectively; p=0.0011), and younger patients were more likely to achieve an SVR than older patients (59.2% and 40.9% above and below median age 58, respectively; p=1.57E-6). Patients who achieved an SVR also had lower γ-glutamyltranspeptidase (γGTP) levels (36 IU/l vs 45 IU/l; p= 0.008) and higher platelet counts (17.1 vs 15.3×10^{10}/L; p=3.649E-05) than those who did not.

IL28B SNP genotypes

The genotypes of two IL28B SNPs were measured for each patient. Because of linkage disequilibrium, SNP results are nearly interchangeable. However, six patients showed an intermediate haplotype consisting of the favourable genotype for rs8099917 (TT) but an unfavourable genotype for rs12979860 (CT), whereas only one of the six patients achieved an SVR, suggesting that rs12979860 is a better predictor of SVR in this data set.

The frequency of the risk allele (T) for rs12979860 was 0.15 among all patients and 0.08 in SVR patients, 0.14 in TR patients and 0.27 in NVR patients. Patients homozygous for the rs12979860 favourable allele (CC) were significantly more likely to achieve an SVR compared with those with TC or TT genotypes (53% vs 24%, OR=3.55, p=3.95E-13). Conversely, patients with the risk allele (TC or TT) were significantly more likely to show an NVR (55% vs 25%; OR=0.265; p=4.4E-16). Patients with the rs12979860 CC genotype had a marginally lower baseline viral load (6.6 vs 6.4 log IU/ml; p=0.093), but showed significantly greater reduction in viral load by week 4 of treatment (−3.2 vs −0.8 log IU/ml; p<2.2E-16). The rs12979860 CC genotype was also associated with wild type core70 (78% vs 54%; p=1.6E-6) and non-wild type ISDR (67% vs 83%; p=0.007).

The frequency of the rs8099917 risk allele (G) was 0.15 among all patients, 0.08 in SVR patients, 0.13 in TR patients and 0.26 in NVR patients. Patients with the rs8099917 TT genotype were significantly more likely to achieve an SVR than patients with GT or GG genotypes (53% vs 24%, OR=3.43, p=2.18E-12), and GT/GG patients were significantly more likely to show an NVR

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Table 1 Patient profiles by response to treatment

	All (813)	SVR (366)	TR (176)	NVR (271)
Sex (M/F)	459/354	231/135	84/92	144/127
Age	58 (51–65)	56 (47–63)	60.5 (56–65.25)	59 (52.5–66)
Body weight (kg)	59 (52–67)	60 (52–68.25)	58 (51–66)	60 (52–66.4)
BMI (kg/m^2)	22.61 (20.81–24.65)	22.44 (20.46–24.58)	22.85 (20.85–24.89)	22.76 (21.12–24.63)
Hypertension (yes/no)	141/672	61/305	29/147	51/220
Diabetes (yes/no)	97/716	31/335	25/151	41/230
Fibrosis (0–2/3–4)	138/421	52/227	34/81	52/113
Activity (0–1/2–3)	274/272	136/138	53/56	85/78
ISDR (0, 1/≥2)	78/298	43/128	15/71	20/99
Amino acid 70 (wild-type/mutant)	256/139	137/45	54/35	65/59
Amino acid 91 (wild-type/mutant)	221/178	112/72	51/40	58/66
WBC (/L)	4.71×10^8 (3.9×10^9–5.7×10^9)	4.9×10^9 (4.0×10^9–6.0×10^9)	4.6×10^9 (3.8×10^9–5.4×10^9)	4.6×10^9 (3.7×10^9–5.5×10^9)
Haemoglobin (g/dl)	14.1 (13.2–15)	14.2 (13.3–15.22)	13.9 (13.1–14.8)	14.1 (13.05–14.9)
Platelets ($\times10^4$L)	16.1×10^6 (12.5×10^6–19.9×10^6)	17.1×10^6 (13.7×10^6–20.7×10^6)	15.5×10^6 (11.3×10^6–18.8×10^6)	15.1×10^6 (12×10^6–19.2×10^6)
AST (IU/l)	45 (34–65.5)	43 (32.25–64)	43.5 (33.25–66)	48 (37–66.5)
ALT (IU/l)	55 (37–87)	57 (37–92)	50 (33–78)	53 (39–82.5)
γGTP (IU/l)	40 (25–72)	36 (23–65.75)	36 (23–69)	52 (32–86)a
Albumin (g/dl)	3.9 (3.7–4.1)	3.9 (3.7–4.1)	3.8 (3.7–4)	3.8 (3.7–4.1)
Total cholesterol (mg/dl)	171 (150–192)	169 (149.2–192)	175 (158–191)	170 (148.5–192.5)
Viral load (log IU/ml)	6.5 (6.1–6.9)	6.4 (5.9–6.825)	6.6 (6.3–7)	6.6 (6.2–7)
PEG-IFN-α2b (μg)	80 (80–100)	80 (80–100)	80 (75–100)	80 (60–100)
PEG-IFN-α2b/kg (μg/kg)	1.19 (1.19–1.48)	1.36 (1.19–1.48)	1.19 (1.19–1.48)	1.19 (1.02–1.48)
Ribavirin (mg)	600 (600–800)	600 (600–800)	600 (600–800)	600 (400–800)
Ribavirin/kg (mg/kg)	8.9 (8.9–11.87)	10.29 (8.9–11.87)	8.9 (8.9–11.87)	8.9 (7.8–11.86)
rs12979860 (CC/CT/TT)	582/203/27	311/51/4	128/43/4	143/109/19
rs8099917 (TT/TG/GG)	588/199/25	311/51/3	132/40/4	145/108/18

For categorical data, the number of patients in each category is shown. For continuous data, the median and range are displayed.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; M, male; NVR, non-virological response; PEG-IFN, pegylated interferon; SVR, sustained virological response; TR, transient response; WBC, white blood cells.

(56% vs 25%; OR=0.26; p=3.33E-16). Patients with the rs8099917 TT genotype had marginally higher baseline viral load (6.6 vs 6.4 log IU/ml; p=0.077) but showed a significantly greater drop in viral load by week 4 of treatment (−3.1 vs −0.8 log IU/ml; p<2.2E-16). The rs8099917 TT genotype was also associated with wild-type core70 (79% vs 56%; p= 3.1E-6) and non-wild-type ISDR (68% vs 83%; p=0.015).

Viral substitutions

Patients who achieved an SVR had significantly lower initial HCV RNA levels than those who did not (6.4 vs 6.6 log IU/ml; p=2.1E-6). The 140 patients (17%) with a substitution at position 70 of the HCV core protein (core70) were significantly less likely to achieve an SVR than patients with wild type core70 (33% vs 53%; p=0.00019) and were significantly more likely to show an NVR (42% vs 25%; p=0.0013). The 179 (22%) of patients with a substitution at position 91 (core91) were marginally less likely to achieve an SVR (41% vs 50%; p=0.08) but were significantly more likely to show an NVR (37% vs 27%; p=0.039). The 78 (10%) of patients who had two or more substitutions in the ISDR of NS5A were only marginally less likely to achieve an SVR than those with wild-type ISDR (43% vs 55%; p=0.066) and were not more likely to show an NVR (33% vs 26%; p=0.24).

Predictive factors for an SVR

Significant univariate predictors for an SVR included patient clinical factors (age, sex, diabetes, platelet count, white blood cell count, haemoglobin level, γGTP level); SNP genotype (rs12979860 and rs8099917); and viral factors (baseline viral load and core70, core91 and ISDR substitutions) (table 2). Following multivariate analysis, only age, rs12979860 genotype, core70 substitution and baseline viral load were significant independent predictors (figure 1A). The joint effects of rs12979860 and core70 on response to treatments are illustrated in figure 2.

Predictive factors for an NVR

Significant univariate predictors for an NVR included age, rs12979860 and rs8099917 genotypes, core70 and core91 substitutions, diabetes, aspartate aminotransferase (AST), baseline viral load, platelet count, white blood cell count and γGTP levels (table 3). Following multivariate analysis only age and rs12979860 genotype remained as independent predictors (figure 1B).

Predictive factors for change in viral load by week 4 of treatment

Factors influencing virological response were assessed by examining change in viral load between the start of treatment and week 4. Using linear regression, sex, rs12979860, rs8099917, core70, core91, ISDR, baseline viral load, alanine aminotransferase (ALT), platelet count, white blood cell count, haemoglobin level and γGTP were found to be significant univariate predictors of change in viral load by week 4 (table 4). Independent factors included rs12979860, core70, ISDR, ALT, platelet count and γGTP. We also found a significant positive linear relationship between the total number of ISDR substitutions and change in viral load between week 0 and week 4 (slope=0.2; p=0.0047).

In patients with the favourable rs12979860 CC genotype, core70 wild type was a significant predictor of viral decline (p=0.007; figures 3A,B), but in patients with the CT or TT genotypes, viral decline did not vary with respect to core70 substitutions (p=0.18; figures 3C,D). Conversely, ISDR was not

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Table 2 Predictors for a sustained virological response

	Simple			Multiple			
Variable	**n**	**OR**	**p Value**	**n**	**OR**	**95% CI**	**p Value**
Age	813	0.58	1.22E-08***	362	0.432	0.31 to 0.60	6.61E-07***
Sex (male vs female)	813	1.28	0.0006***	362	1.2	0.95 to 1.54	0.133
BMI (kg/m^2)	800	0.87	0.1286				
rs12979860 (CC vs TC/TT)	812	3.65	2.67E-14***	362	4.98	2.81 to 8.82	4.00E-08***
rs8099917 (TT vs GT/GG)	812	3.53	1.77E-13***				
Hypertension	813	0.92	0.6452				
Diabetes	813	0.53	0.005907**				
Core amino acid 70 (wild type vs mutant)	395	0.42	5.82E-05***	362	0.527	0.31 to 0.89	0.01575*
Core amino acid 91 (wild type vs mutant)	399	0.66	0.0419*				
ISDR	376	1.12	0.1627				
Viral load (log IU/ml)	695	0.68	2.09E-06***	362	0.77	0.62 to 0.96	0.02249*
Fibrosis (F0–1 vs F2–4)	559	0.74	0.0817				
Activity (A0–1 vs A2–4)	546	0.96	0.7975				
Total cholesterol (mg/dl)	663	0.86	0.2151				
AST (IU/l)	687	1.03	0.1069				
ALT (IU/l)	692	1.26	0.0920				
Platelets (×10^4/L)	694	1.49	3.57E-05***	362	1.39	0.97 to 1.99	0.073
WBC (/L)	693	1.31	0.0014**				
Haemoglobin (g/dl)	693	1.28	0.0043**				
γGTP (IU/l)	646	0.96	0.0052**				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

a significant predictor of viral decline in patients with the rs12979860 CC genotype (p=0.078; figures 4A,B), but patients with the CT or TT genotypes and two or more substitutions in the ISDR showed significantly greater viral decline by week 4 than patients with zero or one ISDR substitution (p=0.007; figures 4C,D).

DISCUSSION

In this study we showed that host factors (younger age, male sex, favourable IL28B SNP genotypes) as well as viral factors (baseline viral load, wild-type core70 and two or more substitutions in the ISDR) contribute to the successful outcome of PEG-RBV combination therapy. Although some of these factors independently predict an SVR or NVR in multivariate analysis, collectively they reflect a complex genotype-by-environment interaction involving common polymorphisms in both the virus and the human host.

Genetic variation within the human IL28 locus has been reported as the strongest pretreatment predictor of an SVR,[15] and the results of this study support this finding. Several tightly linked SNPs in the non-coding region of *IL28A* and *IL28B* have been shown to be associated with spontaneous viral clearance, rapid and early virological response and/or SVR following treatment with interferon and ribavirin for HCV genotype 1b.[9–15] *IL28A*, *IL28B* and *IL29* code for type III (λ) interferons, which are similar to type I interferons but use a different receptor and show high tissue specificity.[27 28] It has not been determined which, if any, of the reported SNPs directly affects function, but the functional SNP probably affects gene expression. IRF3- and IRF7-binding sites near the transcription start

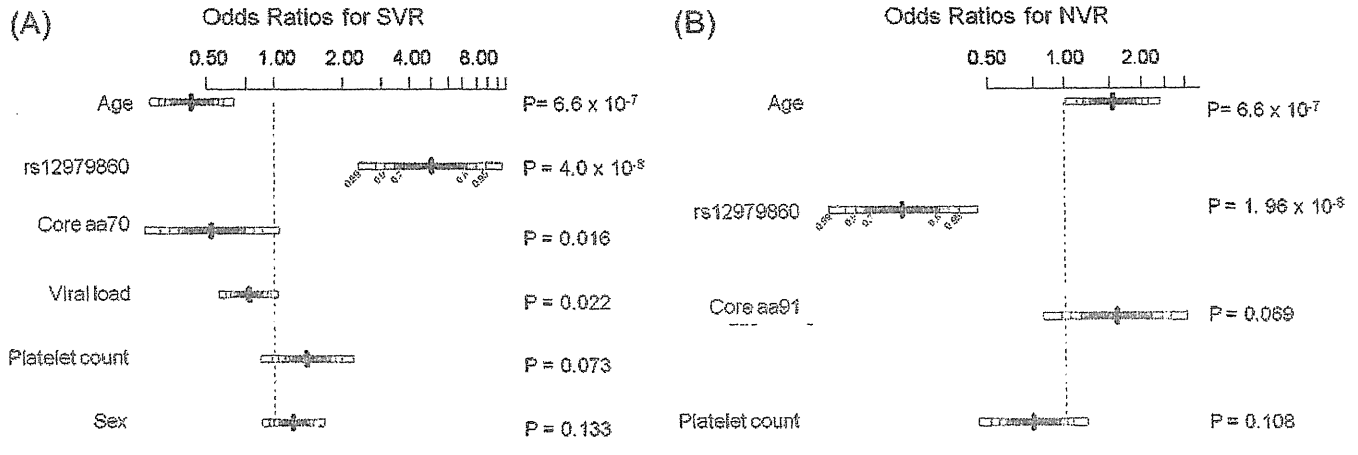

Figure 1 ORs for predictive factors response to treatment. ORs and 95% CIs are shown for predictive factors for (A) sustained virological response (SVR) and (B) non-virological response (NVR) based on multiple logistic regression with stepwise selection.

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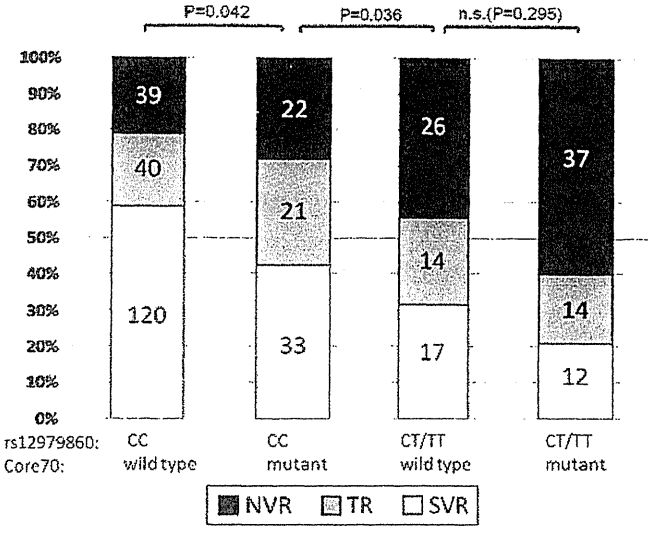

Figure 2 Cumulative effects of rs12979860 genotype and core protein amino acid 70 substitutions. The relative effects of rs12979860 genotype (favourable CC vs non-favourable CT/CC) and core amino acid 70 substitutions (favourable wild type vs unfavourable substitutions) on response to treatment are shown. NVR, non-virological response; TR, transient response/relapser; SVR, sustained virological response.

site of *IL28B* are essential for gene expression, but distal clusters of nuclear factor-κB (NF-κB)-binding sites are necessary for maximal expression,[29 30] suggesting that upstream polymorphisms may potentially disrupt transcription factor-binding sites within a distal promoter or enhancer. Unintuitively, interferon-stimulated genes are downregulated in patients with the favourable rs8099917 TT genotype,[31] implying that responders have a lower baseline expression of immune response genes.[32] This might serve to prevent desensitisation and promote maximal induction of interferon-stimulated genes, but detailed gene regulation studies are needed to resolve the role of IL28B polymorphisms in antiviral defence.

In addition to effects of human genetic polymorphisms, a number of studies have reported significant association between HCV core70/core91 substitutions and treatment outcome.[20 33 34] We found significant independent associations between core70 substitutions and an SVR, as well as change in viral load by week 4, but the association was not significant for an NVR under multivariate analysis despite being highly significant in univariate analysis. Although the role of core70 substitutions is unclear, the core protein interacts with a number of viral and host proteins and disrupts the interferon signalling pathway.[35–37] The proportion of core70 substitutions in the host viral population has been reported to increase during treatment with PEG-RBV therapy, which may indicate positive selection at this position in response to treatment.[38] Substitutions at these positions appear to affect the antiviral response during the early stages of treatment, as wild-type core70 and core91 are associated with a rapid decrease in HCV RNA levels during the first 4 weeks of treatment.[39 40] Because a rapid virological response is also a strong predictor of SVR and NVR, core70 and core91 substitutions may affect treatment outcome either directly or indirectly.[40 41]

Unlike HCV core70 substitutions, we found only a marginal association between ISDR substitutions and SVR, and no association with NVR. However, ISDR substitution was a significant independent predictor of change in viral load by week 4. The presence of two or more mutations in this 40 amino acid stretch of the NS5A protein is associated with an SVR.[24 42] Other studies have found no significant association between ISDR and SVR but have found a higher overall mutation rate in the NS5A protein among SVR patients,[43 44] and one study suggests that the association with ISDR varies by strain and is more pronounced in Japan than in Europe.[45] It is not clear whether mutations in ISDR directly affect function or whether they reflect the genetic distance from an interferon-resistant

Table 3 Predictors for a non-virological response

	Simple			Multiple			
Variable	**n**	**OR**	**p Value**	**n**	**OR**	**95% CI**	**p Value**
Age	813	1.30	0.01306*	370	1.55	1.12 to 2.15	0.008367**
Sex (male vs female)	813	0.90	0.178				
BMI (kg/m^2)	800	1.07	0.3899				
rs12979860 (CC vs TC/TT)	812	0.26	2.73E-17***	370	0.231	0.14 to 0.39	1.96E-08***
rs8099917 (TT vs GT/GG)	812	0.26	1.51E-17***				
Hypertension	813	1.16	0.4323				
Diabetes	813	1.55	0.04685*				
Core amino acid 70 (wild type vs mutant)	395	2.17	0.000496***				
Core amino acid 91 (wild type vs mutant)	399	1.66	0.02029*	370	1.58	0.96 to 2.60	0.06943
ISDR	376	0.92	0.06197				
Viral load (log IU/ml)	695	1.32	0.01716*				
Fibrosis (F0–1 vs F2–4)	559	1.24	0.2608				
Activity (A0–1 vs A2–4)	546	1.12	0.5499				
Total cholesterol (mg/dl)	663	0.98	0.5824				
AST (IU/l)	687	1.02	0.03148*				
ALT (IU/l)	692	0.91	0.8772				
Platelets (×10^4/L)	694	0.76	0.008222**	370	0.739	0.51 to 1.07	0.1077
WBC (/L)	693	0.83	0.04617*				
Haemoglobin (g/dl)	693	0.84	0.1201				
γGTP (IU/l)	646	1.15	1.23E-05***				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

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Table 4 Predictors for change in viral load by week 4 of treatment

	Simple			Multiple		
Variable	**n**	**Coefficient**	**p Value**	**n**	**Coefficient**	**p Value**
Age	500	−0.01	0.138			
Sex (male vs female)	500	−0.23	0.005**			
BMI (kg/m^2)	494	0.00	0.958			
rs12979860 (CC vs TC/TT)	500	2.11	5.18E-38***	221	1.37	1.35E-08***
rs8099917 (TT vs GT/GG)	499	2.10	1.40E-36***			
Hypertension	500	−0.25	0.249			
Diabetes	500	−0.31	0.19			
Core amino acid 70 (wild type vs mutant)	259	−1.01	1.38E-05***	221	−0.665	0.001328**
Core amino acid 91 (wild type vs mutant)	262	−0.77	0.000***			
ISDR	247	0.20	0.006**	221	0.186	0.001878**
Viral load (log IU/ml)	500	0.37	0.000***	221	0.414	0.00012***
Fibrosis (F0−1 vs F2−4)	397	−0.22	0.217			
Activity (A0−1 vs A2−4)	389	−0.10	0.578			
Total cholesterol (mg/dl)	472	0.00	0.064			
AST (IU/l)	490	0.00	0.442			
ALT (IU/l)	493	0.00	0.005**	221	0.00606	0.008895**
Platelets (×10^4/L)	495	0.03	0.048*	221	0.0701	7.24E-05***
WBC (/L)	495	0.00	0.027*			
Haemoglobin (g/dl)	495	0.13	0.013*			
γGTP (IU/l)	460	0.00	0.001***	221	−0.00634	0.002095**

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

strain. Nonetheless, the NS5A protein has been shown to be under purifying selection[44] and plays a critical role in both viral replication[46 47] and modulation of the immune response.[48] Therefore, the number of substitutions in one or more variable regions of the NS5A may be a useful predictor of early viral dynamics and an indirect predictor of SVR, although in this study we found a significant effect only for change in viral load by week 4 of treatment.

A number of factors have now been reported to influence outcome of PEG-RBV therapy, and it is important to determine which of these factors represent independent, clinically useful predictors. Because of the expense and occasionally severe side effects of the current standard of care, reliable pretreatment indicators, especially of poor response, will help guide treatment decisions and steer difficult-to-treat patients towards more

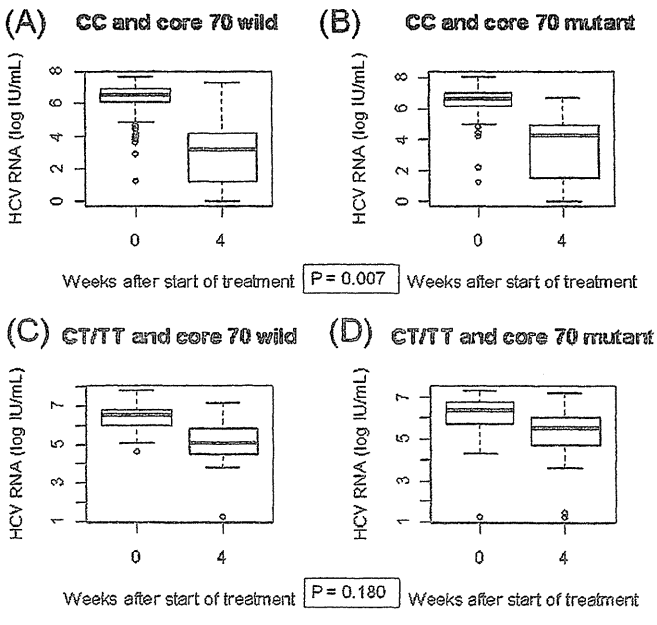

Figure 3 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and hepapitis C virus (HCV) core protein substitutions. The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and wild/mutant amino acid at core70 is shown.

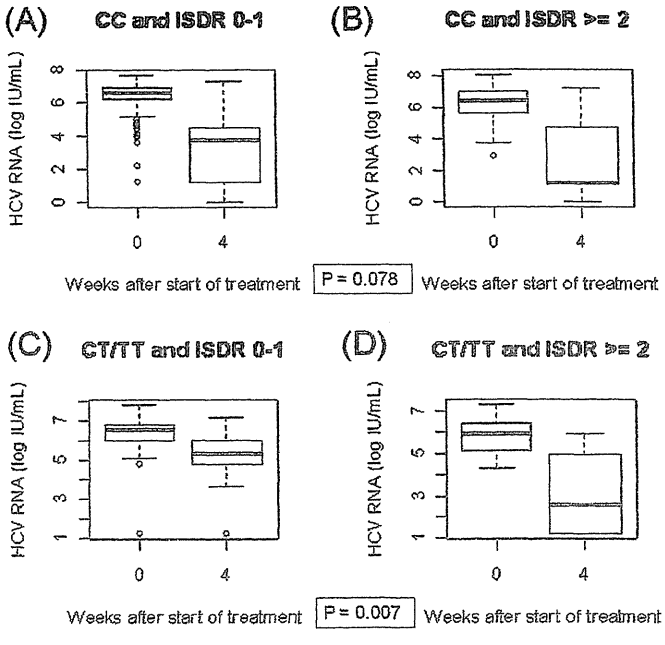

Figure 4 Change in viral load by IL28B single nucleotide polymorphism (SNP) genotype and substitutions in the interferon sensitivity-determining region (ISDR). The change in viral load between the start of treatment and after 4 weeks plotted by rs12979860 genotype and the number of substitutions in the ISDR is shown.

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effective treatments or enrolment in clinical trials. In order to identify the most important independent predictors, it will be necessary to disentangle the intriguing interactions between human and viral polymorphisms as well as gain better understanding of the role of type III interferon in the immune response against HCV.

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Special Report

Management of hepatitis C; Report of the Consensus Meeting at the 45th Annual Meeting of the Japan Society of Hepatology (2009)

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The consensus meeting for the diagnosis, management and treatment for hepatitis C was held in 45th annual meeting for the Japan Society of Hepatology (JSH) in June 2009 where the recommendations and informative statements were discussed including organizers and presenters. The Several important informative statements and recommendations have been shown. This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. Thus, this is the first report of JSH consensus of hepatitis C. The rate of development of hepatocellular carcinoma (HCC) in HCV-infected patients in Japan is higher than in the USA, because the average age of the HCV-infected patients is greater and there are more patients with severe fibrosis of the liver than in the USA. In Japan, more than 60% of HCV-infected patients are genotype 1b infection, and they show lower response to perinterferon and ribavirin combination treatment. To improve the response rate is also an important issue in our country. To establish the original recommendations and informative statements to prevent the development of HCC is a very important issue in Japan.

Key words: chronic hepatitis C, peginterferon, ribavirin, fibrosis of the liver, hepatocellular carcinoma, HCV mutation

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a major public health problem and a leading course of death from liver disease in Japan. Two million people are infected, and more than 30 000 patients die from hepatocellular carcinoma (HCC) and/or liver cirrhosis every year. HCC is the fourth leading cause of death from malignant neoplastic disease, and prevention of the development of HCC is an urgent issue in Japan. The purpose of this consensus is to provide clinicians with consensus-based approaches to diagnosis and treatment of HCV infection.

The consensus meeting for the diagnosis, management and treatment for hepatitis C was held during the 45th annual meeting of the Japan Society of Hepatology (JSH) in June 2009 (Congress President: M. Kudo), where the recommendations and informative statements were discussed and compared with AASLD practice guidelines which has been published in *Hepatology*.[1] This was the fourth JSH consensus meeting of hepatitis C, however, the recommendations have not been published in English previously. This is the first report of the JSH consensus of hepatitis C. Established information regarding the pathogenesis and contributing factors for disease

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Table 1 Grading system for recommendations

	Description
Classification	
Class I	Conditions for which there is evidence and/or general agreement that a given diagnostic evaluation procedure or treatment is beneficial, useful and effective
Class II	Conditions for which there is conflicting evidence and/or a divergence of opinion about the usefulness/efficacy of a diagnostic evaluation, procedure or treatment
Class IIa	Weight of evidence/opinion is in favor of usefulness/efficacy
Class IIb	Usefulness/efficacy is less well established by evidence/opinion
Class III	Conditions for which there is no evidence and/or general agreement that a diagnostic evaluation, procedure/ treatment is not useful/effective and in some cases may be harmful
Level of evidence	
Level A	Data derived from multiple randomized clinical trials or meta-analysis
Level B	Data derived from a single randomized trial, or non-randomized studies
Level C	Only consensus opinion of experts, case studies or standard of care

progression which were agreed by the organizers and presenters are shown as informative statements, and clinically useful consensuses are shown as "Recommendations". The rate of development of HCC in HCV-infected patients in Japan is higher than that in the USA, because the average age of the patient is greater and there are more patients with severe fibrosis of the liver than in the USA. To establish original recommendations and informative statements to prevent the development of HCC is a very important issue in our country. The quality of recommendations or informative statements is required to show a "class" (reflecting benefit vs risk) and "level" (assessing strength or certainty) of evidence according to AASLD practice guidelines (Table 1).[1,2]

PATHOGENESIS OF HEPATITIS C

HEPATITIS C VIRUS infection causes acute and chronic hepatitis (CH), cirrhosis and HCC. The severity and rate of progression of the disease are highly variable and may reflect both host and viral factors, but the mechanisms of pathogenesis are incompletely understood. Thus, understanding the mechanisms of HCV pathogenesis is an important goal of HCV research.

Entry pathway of HCV

For the virus, the first step in propagation is enter into hepatocytes. A decade ago, the HCV envelop protein E2 was shown to bind human CD81, a tetraspanin expressed on various cell types including hepatocytes and B lymphocytes.[3] Next, two other essential proteins, scavenger receptor class B type I (SR-B1)[4] and claudin-1 (CLDN1),[5] and potentially additional accessory factors such as glycosaminoglycans and low-density protein receptors[6] were identified as receptors involved in HCV entry. Finally, the crucial factor was identified as the tight junction protein occludin (OCLN).[7] Interestingly, both CLDN1 and OCLN are components of tight junctions which are structures forming firm seals between adjacent cells. The initial adhesion of HCV to hepatocytes may be mediated by accessory factors and/or direct interaction with SR-B1 and CD81 proteins. On transfer to a tight junction complex, HCV may interact directly with CLDN1 and/or OCLN, allowing viral uptake into the cell.

Hepatitis C virus infects only humans and chimpanzees. Once these HCV entry factors were identified, the next concern was to determine which factors dictate species-specific tropism. CD81 proteins from other mammals, such as the mouse, are used inefficiently by HCV.[8] Although HCV does not discriminate between human and mouse SR-B1 and CLDN1, mouse OCLN like CD81 cannot substitute for the related human protein in aiding viral entry. These findings indicate that CD81 and OCLN represent minimal human-specific entry factors.

Informative statement: CLDN1 and OCLN in addition to CD81 and SR-B1 are required for entering of HCV into hepatocytes, and especially CD81 and OCLN represent minimal human-specific entry factors. (Grade A.)

Evasion of intracellular host defense by HCV

One of the mechanisms by which HCV infection is likely to lead to be persistent is evasion of intracellular host defense through a complex combination of processes that include interference of interferon (IFN) signaling, modulation of its effectors and continual viral genetic variation. The HCV genome contains pathogen-associated molecular pattern (PAMP) signatures which

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are recognized by the retinoic-inducible gene I (*RIG-I*) and specific Toll-like receptors when introduced into naïve cells.[9–11] Viral signaling through *RIG-I* and its adaptor protein, IFN promoter-stimulator 1 (IPS-1), activates IFN regulatory factor-3 (IRF-3) and the host IFN-α/β response that limits virus infection.[12,13] HCV NS3/4A protease cleaves IPS-1, releasing IPS-1 from the mitochondrial membrane.[14] Cleavage results in subcellular redistribution of IPS-1 and loss of interaction with *RIG-I*, thereby preventing downstream activation of IRF-3 and induction of IFNβ.[15]

Secreted IFNβ engages the local tissue through the autocrine and paracrine processes of binding the IFN-α/β receptors. This results in activation of the Jak-signal transducer and activator of transcription (STAT) pathway, in which the receptor-associated Jak and Tyk1 protein kinases catalyze the phosphorylation of STAT proteins. The resulting IFN-stimulated gene factor-3 (ISGF3) transcription factor complex localizes in the cell nucleus, where it binds to the IFN-stimulated response element (ISRE) within the promoter/enhancer region of IFN-stimulated genes (ISG). Jak-STAT signaling leads to a second wave of transcriptional activity stimulating ISG expression in the infected cells. Expression of the HCV core protein has been associated with increased expression levels of suppressor of cytokine signaling (SOCS)-3.[16] The SOCS proteins are known for their role as negative regulators and inhibitors of Jak-STAT signaling, where they mediate a classic negative feedback loop on IFN-α/β receptor signaling events.[17] The HCV NS5A protein has been shown to induce interleukin (IL)-8 production leading to partial inhibition of the IFN-induced antiviral response, probably through the alteration of ISG expression.[18] The HCV NS5A and E2 proteins also bind double-strand RNA-activated protein kinase (PKR) and inhibit its catalytic activity,[19,20] which allows HCV to evade in part the translational-suppressive actions of IFN. Thus, HCV evasion of the host response includes various strategies directed by viral proteins to control IFN signaling, ISG expression or function.

Informative statement: HCV evades intracellular host defenses through a complex combination of processes that include IFN signaling, modulation of its effectors and continual viral genetic variation. These mechanisms include cleavage of IPS-1 by the NS3/4A protease, inhibition of Jak-STAT signaling by HCV-induced SOCS3, inhibition of the IFN-induced antiviral response by NS5A-induced IL-8, and/or inhibition of catalytic activity of PKR by the NS5A and E2 proteins. (Grade A.)

Oxidative stress induced by HCV

Oxidative stress is well known to be present in CH-C to a greater degree than in other inflammatory liver diseases. Although the mechanisms underlying oxidative stress induced by HCV have not been elucidated fully, there are several lines of evidence suggesting that HCV directly generates reactive oxygen species (ROS) *in vitro* and *in vivo*. Hepatic ROS production is significantly higher in HCV core transgenic mice than in normal control mice in the absence of hepatic inflammation.[21] HCV core protein also produces ROS in human hepatoma Huh-7 cells and HeLa cells.[22] Analysis of the interaction of HCV core protein with mitochondria in transgenic mice and direct interaction of recombinant core protein and isolated mitochondria indicated oxidation of the mitochondrial glutathione pool and an increase in ROS production by the mitochondrial electron transport complex I, suggesting that direct interaction of core protein with mitochondria is an important cause of the oxidative stress seen in CH-C.[23]

Informative statement: Mitochondrial dysfunction induced by HCV leads to ROS generation that causes the oxidative stress seen in CH-C. (Grade A.)

Metabolic disorders caused by HCV

Epidemiological studies have suggested a link between type 2 diabetes and chronic HCV infection, which implies HCV-induced insulin resistance. A high level of tumor necrosis factor (TNF)-α and disturbance of tyrosine phosphorylation of the insulin receptor substrate (IRS)1 by TNF-α has been demonstrated in HCV core transgenic mice.[24] Another possible mechanism is that HCV core-induced SOCS3 promotes proteosomal degradation of IRS1 and IRS2 through ubiquitination.[25] Hepatic steatosis is one of the histopathological features in CH-C. HCV core protein inhibits microsomal triglyceride transfer protein activity and secretion of very low density lipoprotein.[26] HCV core protein also upregulates the sterol regulatory element binding protein (SREBP)1c promoter activity through the enhanced binding of the LXRα/RXRα to LXR-response element,[27] which leads to an increase in transcription of genes involved in hepatic fatty acid synthesis. Hepatic iron accumulation is also a histopathological feature of CH-C, even though its levels are not extremely high. HCV-induced ROS down-regulates the transcriptional activity of hepcidin, a negative regulator in iron homeostasis, in transgenic mice expressing the HCV polyprotein[28] and in HCV replicon cells[29] (Fig. 1).

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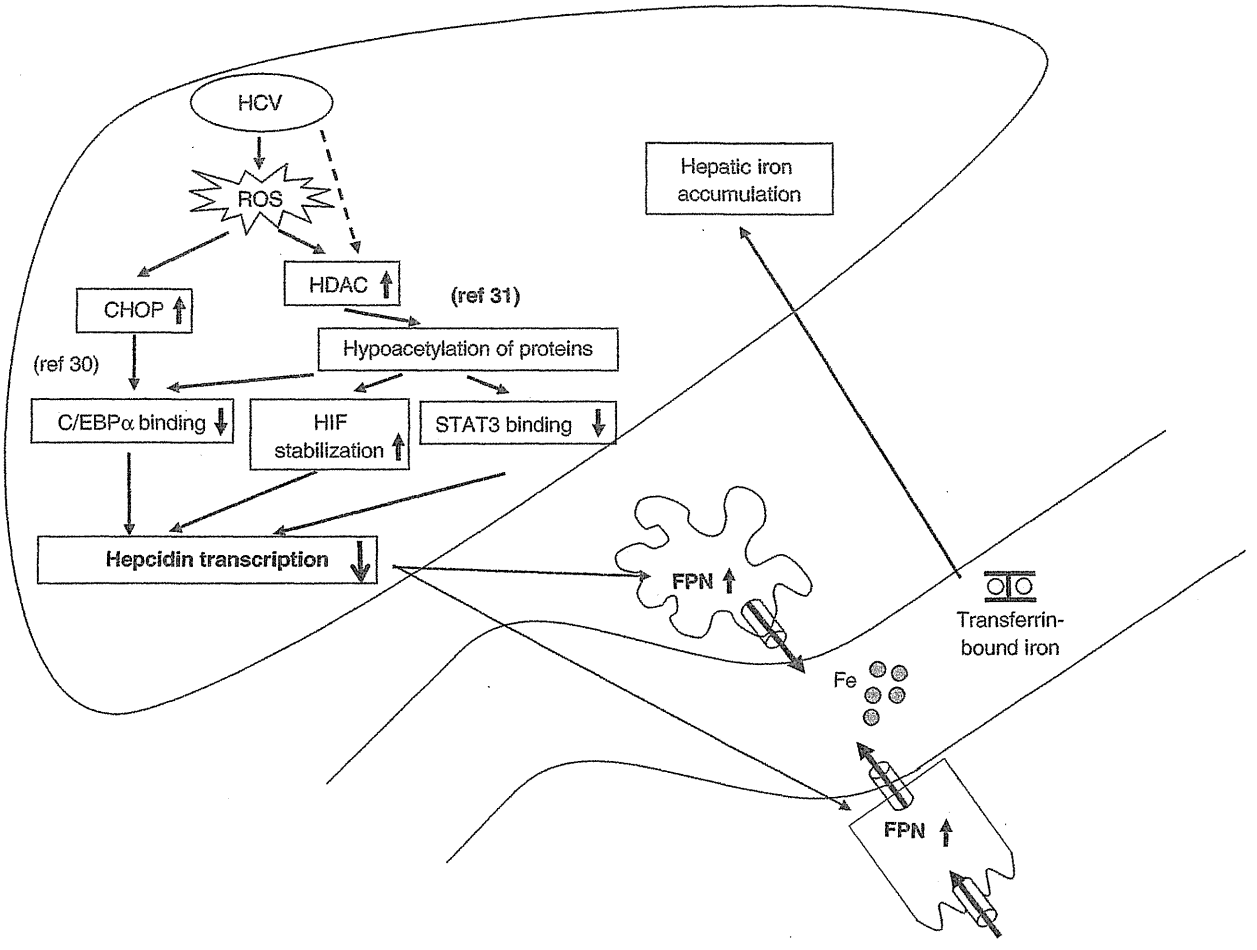

Figure 1 Schematic diagram depicting the mechanisms underlying the hepatic iron accumulation induced by HCV. HCV-induced ROS reduces hepcidin transcription through the inhibited binding of CHOP and/or STAt3 to the hepcidin promoter, and/or stabilization of HIF that is negative hepcidin regulator. C/EBP, CCAAT/enhancer-binding protein; CHOP, C/EBP homology protein; FPN, ferroportin; HCV, hepatitis C virus; HDAC, histone deacetylase; HIF, hypoxia inducible factor; ROS, reactive oxygen species; STAT, signal transducer and activation of transcription.

Metabolic disorders caused by HCV such as insulin resistance, hepatic steatosis and iron accumulation are clinically important in terms of amplification of oxidative stress and involvement in hepatocarcinogenesis in CH-C.[30–33] In addition, these metabolic disorders are related to the response to antiviral therapy. Insulin resistance[34] and hepatic steatosis[35] seem to be negatively correlated with response to antiviral therapy in CH-C.

Informative statement: HCV induces insulin resistance, hepatic steatosis, and/or hepatic iron accumulation, which is associated with hepatocarcinogenesis in CH-C. (Grade A.)

Recommendation 1: Insulin resistance and hepatic steatosis seem to be negatively correlated with response to antiviral therapy in CH-C, whereas it remains controversial whether hepatic iron accumulation is related to a poor response to therapy. (Level 2a, Grade C.)

Liver biopsy for evaluating pathogenesis of hepatitis C

Assessment of the extent of liver fibrosis is still of great importance in terms of predicting the response to antiviral therapy and hepatocarcinogenesis in CH-C. It is also apparent that as many as a quarter of CH-C patients with persistently normal aminotransferase values have significant fibrosis.[36] The recently developed transient elastography that uses ultrasound and low-frequency elastic waves to measure liver elasticity has

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Table 2 Definitions of virological responses to interferon therapy

RVR (rapid virological response)	Undetectable HCV RNA at week 4
cEVR (complete early virological response)	Undetectable HCV RNA at week 12
pEVR (partial early virological response)	Two log drop of HCV RNA without undetectable level at week 12
LVR (late virological response)	Undetectable HCV RNA between week 13 and 24 week
NVR (null virological response)	Positive HCV RNA during treatment
Relapse	Undetectable HCV RNA at end of treatment followed by detectable level after treatment
SVR (sustained virological response)	Undetectable HCV RNA at 24 weeks after treatment

improved the ability to define the extent of fibrosis without a liver biopsy, particularly when combined with other non-invasive markers,[37] but it is not yet ready to replace liver biopsy. Among the pathological features, steatosis and excess hepatocellular iron that affect disease progression and possibly impede treatment response are difficult to diagnose without liver biopsy. Thus, a liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment.

Recommendation 2: A liver biopsy should be considered if it is desirable to determine the stage of fibrosis or presence of steatosis or excess hepatocellular iron for prognostic purposes or making a decision regarding treatment. (Level 1, Grade C.)

VIRAL LOAD, GENOTYPE, VIRAL MUTATIONS

DEFINITIONS OF VIROLOGICAL responses to IFN therapy are summarized in Table 2.

HCV RNA assay and genotype

In clinical practice, the usual approach is to test initially for antibodies to HCV (anti-HCV), then to use HCV RNA to document viremia. The quantity of HCV RNA is useful to know before providing and monitoring HCV treatment. For HCV RNA determination, quantitative tests based on target amplification (reverse transcriptase polymerase chain reaction [RT–PCR]) and signal amplification (branched DNA [bDNA]) techniques with differing sensitivity and linear measuring ranges are commercially available. The COBAS Amplicor HCV Monitor Test v2.0 (Roche Molecular Systems, Branchburg, NJ, USA), however, requires sample dilutions for accurate quantification of high-titer specimens. In addition, the assay displays relatively low sensitivities of approximately 600 IU/mL. Recently, the COBAS AmpliPrep/COBAS TaqMan HCV test (Roche Molecular Systems) and AccuGene m-HCV (Abbott Molecular, Des Plaines, IL, USA) have become available. These meet the requirements for highly sensitive detection and reliable quantification of HCV in clinical samples.

There are six major HCV genotypes. Genotype specificity predicts the likelihood of treatment response and determines the duration of treatment. Therefore, HCV genotype should be determined in all HCV-infected persons prior to treatment in order to determine the duration of therapy and likelihood of response.[38]

Many reports showed that sustained virological response (SVR) rates in IFN monotherapy and IFN plus ribavirin (RBV) combination therapy were higher in patients who had lower pretreatment RNA levels and genotype 2 infections.[39–41]

Recommendation 3: HCV RNA level and genotype should be determined in all HCV-infected persons prior to treatment in order to predict the efficacy of response of therapy. SVR rate in IFN therapy are higher in patients who had lower pretreatment RNA levels and genotype 2 HCV infections in IFN therapy. (Level 1, Grade A.)

HCV mutation

IFN sensitivity determining region (ISDR)

Enomoto *et al.* were able to demonstrate a strong correlation between the number of mutations within the carboxy terminal region of the NS5A gene, the ISDR spanning codons 2209–2248, and response to IFN therapy.[42] Thus, no patient infected with HCV with a wild-type ISDR sequence (identical to the prototype Japanese HCV strain [HCV-J]) responded to IFN therapy whereas all patients infected with the "mutant type", defined by four or more amino acid substitutions in this region, showed an SVR.[43] These initial findings have been confirmed by other Japanese studies but controversial data were reported from other parts of the world, particularly from Europe and the

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USA. This may indicate that geographical factors account for different sensitivities of HCV genotype 1b infection to antiviral therapy. Pascu *et al.* reported that the distribution of wild-, intermediate- and mutant-type ISDR sequences differed significantly between Japanese ($n = 655$) (44.1%, 37.6% and 18.3%, respectively) and European patients ($n = 525$) (24.8%, 63.4% and 11.8%, respectively; $P = 0.001$). However, there was a significant positive correlation between the number of ISDR mutations and SVR rate, irrespective of geographical region.[44]

Moreover, Shirakawa *et al.* reported that a logistic regression model that includes the sequence of ISDR of HCV, and other factors (T-helper cell [Th]1/Th2 ratio, bodyweight and neutrophil count) can be useful for accurately predicting accurately the SVR rate before pegylated (PEG)-IFN and RBV combination therapy.[45]

Recommendation 4: The ISDR should be evaluated before IFN treatment in order to predict the response to treatment. (Level 2b, Grade B.)

IFN/RBV resistance-determining region (IRRDR)

El-Shamy *et al.* have reported recently that a high degree of sequence variation in the V3 and the pre-V3 regions (amino acid [aa]2334-2355) of NS5A, which they refer to collectively as the IRRDR (aa2334-2379), was closely correlated with virological response in HCV-1b-infected patients treated with PEG-IFN and RBV.[46] A high degree (>6 aa substitutions) of sequence variation in the IRRDR should be a useful marker for predicting SVR, whereas a less diverse (<5) IRRDR sequence predicts non-SVR.

Amino acid substitutions in the HCV core region

Akuta *et al.* identified pretreatment substitutions of aa70 and aa91 in the core region as independent and significant pretreatment factors associated with virological non-response, based on 48-week combination therapy of IFN plus RBV.[47] Moreover, they identified aa70 and aa91 substitutions in the core region as predictors of response to PEG-IFN-RBV therapy in Japanese patients infected with HCV genotype 1b[48] (Table 3). Donlin *et al.* reported sequencing the complete pretreatment genotype 1 HCV open reading frame using samples from 94 participants in the Virahep-C study to assess the effects of viral diversity on response to therapy.[49] Genotype 1b sequences from patients with more than 3.5 log declines in viral RNA levels by day 28 (marked responders) were more variable than those from patients with declines of less than 1.4 log (poor responders) in core and NS3. Moreover, arginine (R) at aa70 in the core region was related to a marked response.

Recently evaluations were made of the impact of aa substitutions in HCV core region on hepatocarcinogenesis. Akuta *et al.* reported that cumulative hepatocarcinogenesis rates in double wild-type (arginine at aa70/leucine at aa91) of the HCV core region were significantly lower than those in the non-double wild type in CH-C patients.[50] Moreover, another report showed that a logistic regression model developed

Table 3 Factors associated with sustained virological response to 48-week pegylated interferon plus ribavirin combination therapy in patients infected with hepatitis C virus genotype 1b, identified by multivariate analysis ($n = 114$) 52)

Factor	Category	Risk ratio (95% confidence interval)	*P*
Amino acid substitution in core region	1: double wild	1	
	2: non-double wild	0.102 (0.022–0.474)	0.004
Low-density lipoprotein cholesterol (mg/dL)	1: <86	1	
	2: ≥86	12.87 (2.177–76.09)	0.005
Sex	1: male	1	
	2: female	0.091 (0.017–0.486)	0.005
ICG R15 (%)	1: <10	1	
	2: ≥10	0.107 (0.017–0.678)	0.018
γ-Glutamyltransferase	1: <109	1	
	2: ≥109	0.096 (0.0011–0.819)	0.032
Ribavirin dose (mg/kg)	1: <11.0	1	
	2: ≥11.0	5.173 (1.152–23.22)	0.032

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