21 Pagliaro L, Amico G, Sorenson TI *et al.* Prevention of first bleeding in cirrhosis. A meta-analysis of randomized clinical trials of non-surgical treatment. *Ann Intern Med* 1992; **117**: 59–70.
22 Kovacs TOG, Jensen DM. Initial management of UGI hemorrhage in patients with portal hypertension. In: Rutherford RB, ed. *Vascular Surgery*, 5th edn. Philadelphia, PA: Saunders, 1999; 1554–66.
23 Satin SK, Lamba GS, Kumar M, Misra A, Murthy NS. Comparison of endoscopic ligation and propranolol for the primary prevention of variceal bleeding. *N Engl J Med* 1999; **340**: 988–93.
24 Satin SK, Guptan RK, Jain AK, Sundaram KR. A randomized controlled trial of endoscopic variceal band ligation for primary prophylaxis of variceal bleeding. *Eur J Gastroenterol Hepatol* 1996; **8**: 337–42.
25 The Japan Society for Portal Hypertension. *The General Rules for Study of Portal Hypertension*, 2nd edn. Tokyo: Kanehara, 2004; 37–8. (in Japanese).
26 Liver Cancer Study Group of Japan. *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, 5th edn. Tokyo: Kanehara, 2008; 20–4. (in Japanese).
27 Therasse P, Arbuck SG, Eisenhauer EA *et al.* New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* 2000; **92**: 205–16.
28 Kim DY, Park W, Lim DH *et al.* Three-dimensional conformal radiotherapy for portal vein thrombosis of hepatocellular carcinoma. *Cancer* 2005; **103**: 2419–26.
29 Toya R, Murakami R, Baba Y *et al.* Conformal radiation therapy for portal vein tumor thrombosis of hepatocellular carcinoma. *Radiother Oncol* 2007; **84**: 266–71.
30 Wu SS, Yen HH, Chung CY. Oesophageal variceal bleeding in hepatocellular carcinoma with portal vein thrombosis: improved outcome in response to molecular target therapy. *Clin Oncol* 2008; **20**: 566–7.
31 Katamura Y, Aikata H, Takaki S *et al.* Intra-arterial 5-fluorouracil/interferon combination therapy for advanced hepatocellular carcinoma with or without three-dimensional conformal radiotherapy for portal vein tumor thrombosis. *J Gastroenterol* 2009; **44**: 492–502.

JOURNAL OF VIRAL HEPATITIS JVH

Journal of Viral Hepatitis, 2011, **18**, e550–e560 doi:10.1111/j.1365-2893.2011.01468.x

IL28B polymorphism may guide pegylated interferon plus ribavirin therapy even after curative treatment for hepatitis C virus-related hepatocellular carcinoma

T. Kawaoka,[1,2] H. Aikata,[1] S. Takaki,[1] A. Hiramatsu,[1] K. Waki,[1] N. Hiraga,[1] D. Miki,[1,2] M. Tsuge,[1] M. Imamura,[1] Y. Kawakami,[1] S. Takahashi,[1] H. Ochi,[1,2] H. Tashiro,[3] H. Ohdan[3] and K. Chayama[1,2]

[1]*Department of Medicine and Molecular Science, Division of Frontier Medical Science, Minami-ku, Hiroshima University, Hiroshima, Japan;* [2]*Laboratory for Digestive Diseases, Centre for Genomic Medicine, RIKEN (The Institute of Physical and Chemical Research), Minami-ku, Hiroshima, Japan; and* [3]*Department of Surgery, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan*

Received January 2011; accepted for publication February 2011

SUMMARY. The present study was designed to determine the predictive factors for the viral response to pegylated interferon-alpha plus ribavirin combination therapy (PEGIFN/RBV) administered after curative treatment for hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC). The study group was 78 patients treated between January 2005 and January 2009. The sustained viral response (SVR) rate was 25.8% (15/58) in patients infected with HCV-genotype 1 and 55.0% (11/20) in those with genotype 2. Among the 78 patients, 32 (41.0%) could not complete the treatment protocol, and this was because of HCC recurrence in 17 (53%) of them. Multivariate analysis identified partial early viral response (pEVR) as the only independent determinant of SVR [odds ratio (OR) 14.73, $P = 0.013$] for patients with genotype 1. Multivariate analysis identified male gender (OR 8.72, $P = 0.001$) and interleukin-28B (IL-28B) genotype (rs8099917) TT (OR 7.93, $P = 0.007$) as independent predictors of pEVR. Multivariate analysis also identified IL-28B genotype GG+TG (OR 14.1, $P = 0.021$) and α-fetoprotein >30 (OR 5.4, $P = 0.031$) as independent predictors of null response. Patients with SVR showed a better survival rate than those without SVR ($P = 0.034$). The second HCC recurrence rate tended to be lower in patients with SVR than in those without SVR ($P = 0.054$). With regard to the prognosis of patients with SVR, it is desirable to achieve SVR with interferon therapy even when administered after HCC treatment. IL-28B genotype is a potentially useful marker for the response to PEGIFN/RBV therapy administered after curative treatment of HCV-related HCC.

Keywords: curative treatment, hepatitis C virus, hepatocellular carcinoma, interleukin-28B, pegylated interferon-alpha plus ribavirin combination therapy.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Chronic infection with hepatitis C virus (HCV) has been associated with hepatocarcinogenesis [1–3]. Recent advances in imaging and treatment modalities have brought about some improvement in the prognosis of patients with HCV-related HCC, but the overall outcome remains unsatisfactory; the 5-year survival rate is only 50–70%, even after curative treatment such as hepatic resection or local ablation [4]. The reasons for this unfavourable prognosis are considered to include high intrahepatic tumour recurrence rates and sustained hepatic damage, both resulting from HCV infection [5]. Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumour recurrence within 1 year is 20–40%, rising to about 80% by 5 years [4,6,7].

Intrahepatic recurrence of HCC may result from intrahepatic metastasis originating from the primary HCC or from ongoing multicentric carcinogenesis related to chronic HCV infection. The background HCV-related hepatic damage may

Abbreviations: AFP, α-fetoprotein; cEVR, complete early viral response; HCV, hepatitis C virus; IFN, interferon; IL-28B, interleukin-28B; NR, null response; PEGIFN, pegylated interferon; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response; RBV, ribavirin; SNP, single-nucleotide polymorphism; SVR, sustained viral response; TR, transient viral response.

Correspondence: Hiroshi Aikata, MD, Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3, Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
E-mail: aikata@hiroshima-u.ac.jp

also compromise hepatic functional reserve and worsen clinical outcome. Thus, the prevention of HCC recurrence as well as preservation of liver function constitutes high priorities for the improvement of prognosis of patients with HCV-related HCC.

Interferon (IFN) therapy for patients with HCV infection is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV [8,9] and thus IFN could be of value in minimizing hepatic necrosis, inflammation and fibrosis, as well as reducing the incidence of HCC. Several recent studies have reported that IFN therapy, applied even after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival [10–21].

The recent introduction of pegylated interferon-alpha plus ribavirin combination therapy (PEGIFN/RBV) has improved the treatment efficacy [22,23]. Recent studies have highlighted the relationship between various single-nucleotide polymorphisms (SNPs) in the IL-28 locus and the effect of PEGIFN/RBV in patients infected with HCV [24–29]. Further, the results of few recent studies suggest that PEGIFN/RBV could prevent HCC recurrence and improve survival even when used after curative treatment of HCV-related HCC [30,31]. To our knowledge, however, there are no studies on the factors that could predict a sustained viral response (SVR) to PEGIFN/RBV after treatment of HCC (e.g. IL-28B as a host factor). The present study was designed to determine the predictive factors of viral response to PEGIFN/RBV in patients with HCV treated for HCC.

MATERIAL AND METHODS

Patients

The study subjects were 78 patients treated with PEGIFN/RBV after curative intent treatment (hepatic resection or radiofrequency ablation) for HCV-related HCC between January 2005 and January 2009 in this retrospective cohort study. Tumour staging was defined based on the Liver Cancer Study Group of Japan/Tumour-Node-Metastasis staging system of the Liver Cancer Study Group of Japan (LCSGJ): stage I [fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion, n = 28 (36%)], stage II [two of the three intrahepatic conditions, n = 27 (35%)], stage III [one of the three intrahepatic conditions, n = 23 (29%)], stage IVa (none of the three intrahepatic conditions, with no distant metastases or any intrahepatic conditions with lymph node metastases) and stage IVb (any intrahepatic condition with distant metastases) [stage IV, n = 0 (0%)] [32]. The median duration was 7 months (range, 1–60) from curative intent treatment for HCC to the start of PEGIFN/RBV therapy.

Antiviral treatment protocol

Each patient received 1.5 μg/kg body weight (BW) pegylated interferon-alpha (PEGIFN) (Peg-Intron; Schering-Plough, Segrate, Italy) subcutaneously (s.c.) once weekly, together with ribavirin (RBV) (Rebetol; Schering-Plough). The RBV dose was adjusted according to BW to 600 mg for patients <60 kg BW, 800 mg for >60 but ≤80 kg BW and 1000 mg for >80 kg BW, based on the drug information for RBV supplied by the manufacturer. The above durations and dosages are those approved by the Japanese Ministry of Health, Labour and Welfare.

The daily dose of RBV was reduced by 200 mg when haemoglobin (Hb) fell below 10 g/dL, acute fall in Hb concentration followed by stabilization at more than 3 g/dL from baseline, or appearance of clinical symptoms of anaemia (e.g. fatigue, pallor, palpitation, dyspnoea on efforts and fatigue) associated with a decrease in Hb of >2 g/dL from baseline. Once the RBV dose was reduced, it was maintained at that level throughout the rest of study. The protocol also included withdrawal of RBV when Hb fell below 8.5 g/dL or when patients manifested more severe anaemia including orthostatic hypotension. After the end of the treatment, the patients were followed up for 24 more weeks without treatment. The treatment term was 48 weeks for patients infected with HCV genotype 1 and 24 weeks for those with genotype 2.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committees of all participating centres. Written informed consent was obtained from each patient. At each visit, information on possible side effects was obtained by questioning the patients in a structured manner about specific, commonly observed and expected side effects of the study medication, such as flu-like symptoms, fatigue, nausea, vomiting, diarrhoea, dizziness, depression and hair loss.

Single-nucleotide polymorphism genotyping and quality control

Because the two reported significant IL-28 SNPs (rs8099917 and rs12979860) are in strong linkage disequilibrium, we analysed only rs8099917 in this study. Some samples obtained from patients with HCV were determined using the Illumina HumanHap610-Quad Genotyping BeadChip, whereas the remaining samples were genotyped using the Invader assay, as described previously [33,34].

Analysis of nucleotide sequence of the core and NS5A region

The amino acid (aa) substitutions at aa 70 and aa 91 of the HCV core region and mutation at the interferon sensitivity-determining region (ISDR) in the nonstructural 5A (NS5A) region of HCV were analysed by the direct sequencing method as described previously by our group [35–37].

Assessment of viral response

Serum HCV RNA was determined at baseline, after 4, 8, 12, 16 and 20 weeks of treatment, at the end of treatment and

at the end of the 24-week drug-free follow-up period. HCV RNA was assessed by qualitative reverse transcription polymerase chain reaction (TaqMan RT-PCR). SVR represented a negative HCV RNA at 24-week follow-up without treatment after the end of active treatment. Transient viral response (TR) was defined as positive HCV RNA at 24-week follow-up after a negative HCV RNA at the end of active treatment. Complete early viral response (cEVR) was defined as negative HCV RNA at week 12 of active treatment. Partial early viral response (pEVR) was defined as HCV RNA ≥ 2 log10 drop from baseline at week 12 of active treatment. Null response (NR) was defined as HCV RNA that never dropped by ≥2 log10 from baseline at week 12 of active treatment.

Histopathological stage was assessed before treatment and determined based on the histological scoring system of Desmet *et al.* [38].

Assessment of hepatocellular carcinoma recurrence

The concentrations of serum tumour markers α-fetoprotein (AFP) and des-γ-carboxy prothrombin were measured once a month after hepatic resection or radiofrequency ablation. Follow-up US was performed every 3 months; and CT or MR imaging was performed every 6 months. IFN therapy was discontinued upon suspicion of HCC recurrence.

Statistical analysis

Nonparametric tests (chi-square test and Fisher's exact probability test) were used to compare the clinical and laboratory parameters of the two groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to early viral dynamics. The odds ratio and 95% confidence intervals (95% CI) were also calculated. All P values <0.05 using two-tailed tests were considered significant. Variables that achieved statistical significance ($P < 0.05$) or marginal significance ($P < 0.10$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors.

Cumulative survival and recurrence rates were calculated from the initial date of hepatic resection or radiofrequency ablation and assessed by the Kaplan–Meier life-table method, with differences evaluated by the log rank test. All statistical analyses were performed using PASW 18 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

Patient characteristics

Table 1 shows the baseline characteristics of the patients treated with PEGIFN/RBV after hepatic resection or radiofrequency ablation for HCC. The median age of the patients (55 men and 23 women) was 66 years. The median body mass index was 22.4 kg/m^2. The median pretreatment serum HCV RNA viral load was 6.0 log IU/mL. Most patients were infected with HCV genotype 1 ($n = 58$) followed by genotype 2 ($n = 20$). IL-28B genotype (rs8099917) was TT ($n = 51$), GG+TG ($n = 25$) and no date ($n = 2$).

Table 1 The baseline characteristics of the all 78 patients treated with PEGIFN/RBV

	$n = 78$
Gender (male/female)	55/23
Age (years)*	66 (48–83)
Body mass index (kg/m^2)*	22.4 (15.6–40.1)
IL28B genotype (TT/GG+TG/ND)	51/25/2
White blood Cell (×10^3/μL)*	4.2 (2.4–7.5)
Haemoglobin (g/dL)*	13.3 (8.7–18.1)
Platelet count (×10^4/mm^3)*	11.1 (3.9–20.5)
T-bilirubin (mg/dL)*	0.7 (0.2–2.8)
Alanine aminotransferase (IU/L)*	44 (8–189)
Prothrombin time activity (%)*	87 (58–121)
Albumin (g/dL)*	4.0 (2.7–5.2)
γ-glutamyl transpeptidase (IU/L)*	45 (12–371)
HbA1c (%)*	5.3 (3.9–10.8)
Indocyanine green retention rate (%)*	15.4 (3.5–45.4)
Fibrosis stage (F1-3/F4/ND)	20/19/39
Genotype (1/2)	58/20
HCV viral load (Log IU/mL)*	6.0 (2.1–7.2)
Tumour stage (I/II/III/IV)†	28/27/23/0
α-Fetoprotein (ng/mL)*	11 (0.5–286)
Des-γ-carboxy prothrombin (mAU/mL)*	29 (10–4550)
Tumour size (mm)*	21 (7–110)
Number of tumour*	1 (1–4)
Hepatic resection/radiofrequency ablation	28/50

ND, not done; HCV, hepatitis C virus; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy. *Data are median and (range). †Tumour staging was defined based on the Liver Cancer Study Group of Japan/Tumor-Node-Metastasis staging system of the Liver Cancer Study Group of Japan.

Efficacy and tolerance of therapy and adverse events

Figure 1 shows the effects of PEGIFN/RBV treatment according to genotype. The SVR rate was 33.3% (26/78) for all patients. The PRGIFN/RBV treatment protocol could not be completed by 32 (41%) patients; 17 (53%) of the 32 developed HCC recurrence. In 58 patients with genotype 1, PEGIFN and RBV were discontinued in 29% (17/58) patients because of HCC recurrence and because of other reasons in another 9 (15.5%) [general fatigue ($n = 3$), cancer of the

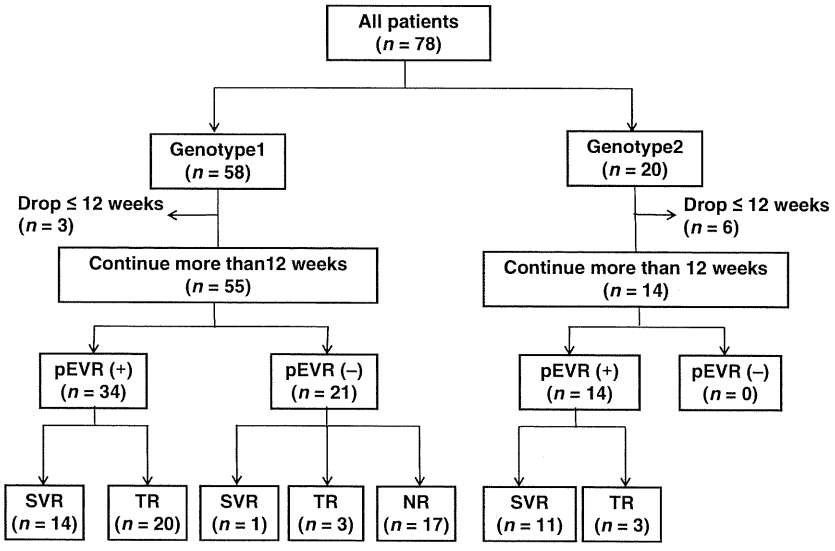

Fig. 1 Flow diagram showing the course of Peg-related interferon plus ribavirin therapy after curative treatment for hepatitis C virus (HCV)-related hepatocellular carcinoma. According to HCV genotype, 78 patients treated with pegylated interferon-alpha plus ribavirin combination therapy were divided into three groups, namely the sustained virological response, transient response and null response. *n*, number of patients.

throat (n = 1), vomiting (n = 1), itching (n = 1), pulmonary haemorrhage (n = 1), jumpiness (n = 1), sarcoidosis (n = 1)] within 48 weeks. Thus, PEGIFN and RBV treatment could be achieved in 55% (32/58) of the patients. Furthermore, 95% (55/58) of the patients continued the treatment for more than 12 weeks. Among 55 patients, 34 achieved pEVR, including 21 patients achieved cEVR, 14 achieved SVR and 20 showed TR. In the other 21 patients who did not achieve pEVR, one patient achieved SVR and three patients showed TR while 17 patients showed NR. Thus, the SVR rate was 25.8% (15/58) for patients infected with HCV genotype 1.

Among the 20 patients infected with genotype 2, 6 discontinued treatment because of side effects [general fatigue (n = 3), thrombocytopenia (n = 1), diabetes mellitus (n = 1), bleeding from oesophageal varices (n = 1)] within 12 weeks. The remaining 14 (70%) patients completed the treatment protocol. All 14 patients achieved pEVR, including 11 who showed SVR and three achieved TR. Thus, the SVR rate was 55.0% (11/20) for patients infected with genotype 2 (Fig. 1).

Relationship between IL-28B and viral response in patients infected with hepatitis C virus genotype 1

In patients infected with HCV genotype 1, number of patients with TT genotype of IL-28B was 44 (TT group) and GG+TG was 14 (GG+TG group). The SVR rate of the TT group [34.3% (n = 14/41)] was higher than that of the TG+GG group [7% (n = 1/14), P = 0.08, Fig. 2A]. The pEVR rate of TT group [73.1% (n = 30/41)] was also significantly higher than that of the TG+GG group [28.5% (n = 4/14), P = 0.009, Fig. 2B]. The NR rate of the TT group [19.5% (n = 8/41)] was significantly lower than that of the TG+GG group [64.2% (n = 9/14), P = 0.005, Fig. 2C].

Determinants of sustained viral response in patients infected with hepatitis C virus genotype 1

Next, we analysed the factors that determine SVR using data of 55 patients infected with HCV genotype 1 who continued PEGIFN/RBV therapy for more than 12 weeks (Table 2). Univariate analysis identified five parameters that correlated with SVR: pEVR (P = 0.004), viral load (<6.0 g/dL; P = 0.008), completion of therapy (P = 0.06), IL-28B genotype (TT genotype; P = 0.08) and gender (man; P = 0.043). Multivariate analysis identified pEVR as the only significant and independent factor that influenced the SVR: (odds ratio, 14.73, 95%CI 1.7–123.2, P = 0.013).

Determinants of partial early viral response in patients infected with hepatitis C virus genotype 1

Next, we analysed the factors that determine pEVR using data of 55 patients infected with HCV genotype 1 who continued PEGIFN/RBV treatment for >12 weeks. Univariate analysis identified three parameters that correlated with pEVR: IL-28B genotype (TT genotype; P = 0.009), gender (man; P = 0.005) and viral load (<6.0 g/dL; P = 0.068) (Table 3). Multivariate analysis identified two parameters that independently influenced the pEVR: gender (male; odds ratio 8.72, 95%CI 2.1–41.6, P = 0.001) and IL-28B genotype (TT genotype; odds ratio 7.93, 95%CI 1.7–36.0, P = 0.007, Table 4). Mutations of aa 70 and aa 91 in the core region of the HCV protein and fewer mutations in its ISDR region were not significantly different between the pEVR and non-pEVR groups among patients infected with HCV genotype 1b in our study.

Determinants of null response in patients infected with hepatitis C virus genotype 1

Next, we analysed the factors that determine the NR in patients infected with HCV genotype 1 (n = 55). Univariate

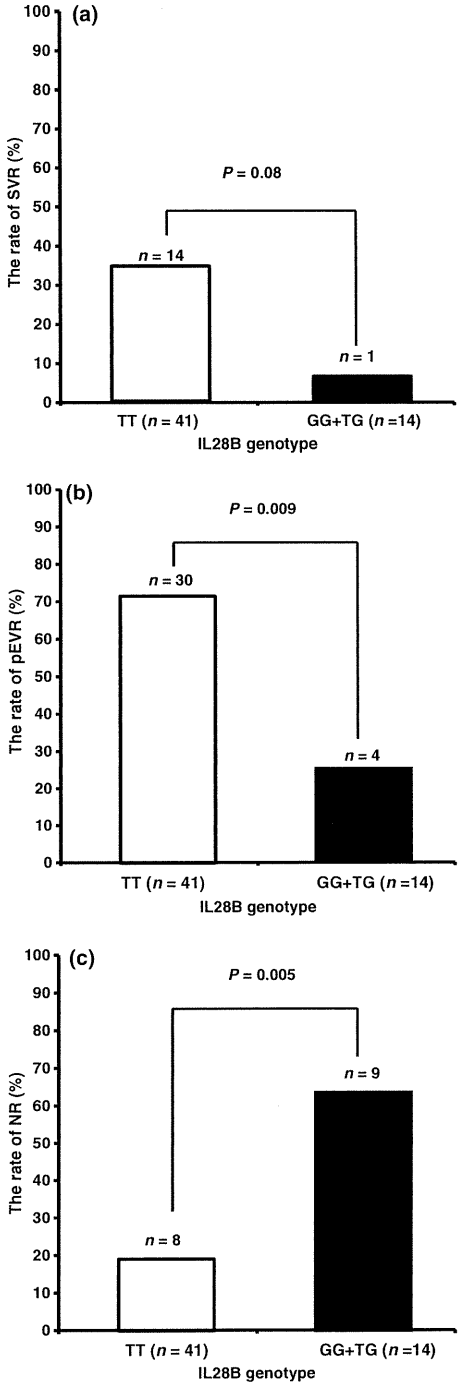

Fig. 2 Relationship between IL-28B and viral response in patients infected with hepatitis C virus genotype 1. (a) Sustained viral response rate, (b) Partial early viral response rate, (c) null response rate.

analysis identified three parameters that influenced NR: IL-28B genotype (genotype; GG+TG, $P = 0.005$), AFP (>30 ng/dL; $P = 0.054$) and gender (male; $P = 0.022$) (Table 5). Multivariate analysis identified two parameters that independently influenced the NR: IL-28B genotype (genotype GG+TG; odds ratio 7.8, 95%CI 1.81–34.4, $P = 0.006$) and AFP (>30; odds ratio 5.6, 95%CI1.40–22.8, $P = 0.015$) (Table 6). Mutations of aa 70 and aa 91 in the core region of the HCV protein and fewer mutations in its ISDR region were not significantly different between the NR and SVR+TR groups among patients infected with HCV genotype 1b.

Survival rates

The overall survival rate was significant different between patients of the SVR and non-SVR groups ($P = 0.034$). The survival rate of the SVR groups was 100% at 1 year, 100% at 3 years and 100% at 5 years. In contrast, the rates of the non-SVR group were 100%, 96% and 74%, respectively (Fig. 3).

Comparison of the first and second recurrence rates of hepatocellular carcinoma

Finally, we compared the overall cumulative rates of the first and second recurrence of HCC between the SVR and non-SVR groups (Fig. 4). The 1-, 3- and 5-year rates of the first recurrence of HCC in the SVR and non-SVR group were not different (0% *vs* 6.7%, 38.1% *vs* 37% and 48% *vs* 68%, respectively, Fig. 4A, $P = 0.41$). The 1-, 3- and 5-year rates of the second recurrence in the SVR and non-SVR groups were 0% *vs* 0%, 41% *vs* 64% and 48% *vs* 78%, respectively (Fig. 4(B), $P = 0.054$). These results demonstrated that patients of the SVR group tended to have a better chance of escaping a second HCC recurrence compared with those of the non-SVR group.

DISCUSSION

Several recent studies have reported that IFN therapy can prevent HCC recurrence and improve survival, especially in patients with SVR, even when administered after curative treatment for HCV-related HCC [10–21,31,39]. While there are a few reports of the use of PEGIFN/RBV after curative treatment for HCV-related HCC [30,31], none have discussed the SVR rate and the factors that determine the viral response to PEGIFN/RBV in such patients. In the present study, we reported the viral response and determinants (specially SNPs) of viral response with PEGIFN/RBV after treatment of HCC.

In our study, the SVR rate was 33.3% (26/78) for all patients, while that for patients with genotype 1 was 25.8% (15/58) and genotype 2 was 55.0% (11/20). These SVR rates are lower than that of patients with chronic hepatitis. The lower rate in the present study was probably because of the low number of patients who completed the therapy. The reason for the latter was the relatively high rate of HCC recurrence [53% (17/32)].

One of the major reasons of the low SVR rate was probably of discontinuation of therapy because of HCC recurrence.

Table 2 Univariate analysis of factors associated with SVR in 55 patients with genotype 1 continued PEGIFN/RBV >12 weeks

	SVR (n = 15)	TR+NR (n = 40)	P
Gender (male/female)	1/14	25/15	0.043
Age (years)*	65 (54–74)	65 (53–83)	0.94
Body mass index (kg/m^2)*	21.2 (18.4–28.5)	23.0 (18.7–40.1)	0.174
White blood Cell (×10^3/μL)*	5050 (4390–6130)	4280 (2470–6660)	0.8
Haemoglobin (g/dL)*	13.7 (11.2–14.8)	13.4 (9.3–18.1)	0.96
Platelet count (×10^4/mm^3)*	12.5 (3.9–19.6)	10.0 (4.7–20.8)	0.138
T-bilirubin (mg/dL)*	0.7 (0.4–1.8)	0.7 (0.2–1.7)	0.58
Alanine aminotransferase (IU/L)*	33 (12–189)	45 (17–166)	0.25
Prothrombin time activity (%)*	88 (80–106)	86 (64–121)	0.49
Albumin (g/dL)*	4.1 (3.7–4.9)	4.0 (2.7–4.9)	0.52
Fibrosis stage (F1-3/F4/ND)	2/2/11	10/15/15	1.0
γ-glutamyl transpeptidase (IU/L)	43 (12–87)	46 (15–294)	1.2
HbA1c (%)	5.1 (4.2–10.2)	5.4 (3.9–10.8)	0.41
Indocyanine green retention rate (%)	17.7 (7.5–37.8)	17.4 (3.5–45.4)	0.92
HCV viral load (Log IU/mL)	5.59 (4.3–7.1)	6.23 (1.2–7.2)	0.08
HCV Core70(mutant/wild)	8/7	23/17	1.0
HCV Core91 (mutant/wild)	5/10	21/19	0.23
HCV ISDR (0–1/>2)	9/6	26/14	0.75
α-Fetoprotein (ng/mL)*	6.9 (5–286.8)	19.7 (5–63240)	0.11
IL28B genotype (TT/GG+TG)	14/1	27/13	0.08
pEVR (yes/no)	14/1	20/20	0.004
Dose of PEGIFN at administration (μg/kg)*	80 (40–100)	80 (50–120)	0.74
Dose of RBV at administration(mg)*	600 (200–800)	600 (200–1000)	0.26
Therapy were completed (yes/no)	12/3	20/20	0.06

ND, not done; HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NR, null response; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response; SVR, sustained viral response; TR, Transient viral response. *Data are median and (range).

Alternatively, the low rate could be because of a high proportion of patients with advanced liver fibrosis. In fact, 19 (11.5%) patients were classified as F4 stage, and the median of platelet count was 11.1 × 10^4/mm^3. These reasons may explain the low IFN therapy continuation rate (55.2%) and the low SVR rate.

We analysed the factors that affect SVR in 55 patients infected with HCV genotype 1 who were able to continue therapy for more than 12 weeks. Multivariate analysis identified a single parameter that independently influenced the SVR: pEVR. Among the 55 patients, 34 (61.8%) achieved pEVR. Among the pEVR group, 14 (41.1%) patients achieved SVR. Recent studies reported the importance of the response guide-based therapy in the treatment of chronic hepatitis; i.e. 70–80% of patients of the cEVR group achieved SVR [40–43].

On the other hand, gender (male) and IL-28B genotype (TT) were identified as significant and independent predictors of pEVR. These factors are probably also significant and independent predictors of SVR in patients with chronic hepatitis C.

Thus, male patients with IL-28B genotype TT were more likely to achieve pEVR even when PEGIFN/RBV treatment was introduced after curative treatment for HCV-related HCC.

Evidence suggests that the SVR rate could be improved by IFN therapy (long-term low-dose IFN of 72 weeks instead of 48 weeks). In fact, Pearlman *et al.* [43] reported that the SVR rate was superior in patients treated for 72 *vs* 48 weeks (38% *vs* 18%, respectively; P = 0.026) in the pEVR groups. Furthermore, the SVR rate could be improved by combination therapy for HCC and HCV. For example, to achieve SVR, it might be better to restart PEGIFN/RBV therapy immediately after curative treatment of HCC.

On the other hand, multivariate analysis identified IL-28B genotype (GG+TG) as an independent parameter that influenced the NR. In this group, it is better to select low-dose intermittent IFN therapy than PEGIFN/RBV based on the SVR. In fact, it is reported that low-dose intermittent IFN therapy after hepatectomy for HCC improved liver function of patients with HCV-related HCC, and the preservation of hepatic function increased the chance of successful treatment against recurrence [10]. In contrast, mutations of aa 70 and aa 91 in the core region of the HCV protein and fewer mutations in its ISDR region were not significant and independent predictors of pEVR and NR.

Table 3 Univariate analysis of factors associated with pEVR in 55 patients with genotype 1 continued PEGIFN/RBV >12 weeks

	pEVR (n = 34)	non-pEVR (n = 21)	P
Gender (male/female)	29/5	10/11	0.005
Age (years)*	67 (54–83)	63 (53–72)	0.977
Body mass index (kg/m^2)*	23.6 (18.7–40.1)	22.2 (18.4–30.0)	0.151
White blood Cell (×10^3/μL)*	5150 (4390–6660)	3610 (2470–4930)	0.8
Haemoglobin (g/dL)*	13.8 (10.2–18.1)	12.3 (9.3–17.4)	0.745
Platelet count (×10^4/mm^3)*	11.1 (3.9–20.8)	10.0 (4.7–18.2)	0.126
T-bilirubin (mg/dL)*	0.7 (0.2–1.8)	0.7 (0.5–1.7)	0.53
Alanine aminotransferase (IU/L)*	44 (17–189)	37 (12–134)	0.319
Prothrombin time activity (%)	88 (68–114)	85 (64–121)	0.41
Albumin (g/dL)*	4.0 (3.4–4.9)	4.0 (2.7–4.9)	0.405
Fibrosis stage (F1-3/F4/ND)	8/8/18	9/4/8	0.43
γ-glutamyl transpeptidase (IU/L)	52 (12–219)	26 (15–294)	0.172
HbA1c (%)	5.5 (4.2–8.8)	5.0 (3.9–10.8)	0.49
Indocyanine green retention rate (%)	17.4 (3.5–37.8)	18.7 (7.6–45.4)	0.92
HCV viral load (Log IU/mL)	6.04 (4.3–7.2)	6.23 (1.2–6.7)	0.068
HCV Core70(mutant/wild)	19/15	13/8	0.78
HCV Core91 (mutant/wild)	13/21	13/8	0.17
HCV ISDR (0–1/>2)	19/15	14/7	0.24
α-Fetoprotein (ng/mL)*	9.1 (5.0–909.2)	42.0 (5.0–63240)	0.116
IL28B genotype (TT/GG+TG)	30/4	11/10	0.009
Dose of PEGIFN at administration (μg/kg)*	80 (40–120)	80 (50–100)	0.689
Dose of RBV at administration (mg)*	600 (200–1000)	600 (200–800)	0.20
Therapy were completed (yes/no)	21/13	11/10	0.4

HCV, hepatitis C virus; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response. *Data are median and (range).

Table 4 Multivariate analysis of factors associated with pEVR

Factor	Category	Odds ratio (95%CI)	P
Gender	Female	1	0.001
	Male	8.72 (2.1–41.6)	
IL28B genotype	GG+TG	1	0.007
	TT	7.93 (1.7–36.0)	

pEVR, partial early viral response.

Achieving SVR by PEGIFN/RBV treatment, even when administered after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival. Although achieving SVR had no impact on the occurrence of HCC at the initial site, patients of the SVR group tended to show a lower rate of second HCC recurrence in this and another study [31]. It was reported that IFN therapy had no impact on the occurrence of HCC shortly after IFN therapy was started. It was speculated that IFN therapy does not suppress latent HCC. In our study, although the first recurrence rate of HCC was similar between patients with and without SVR, the second HCC recurrence rate tended to be lower in patients with SVR than in those without SVR (P = 0.054). Therefore, efforts should be directed to achieve SVR by PEGIFN/RBV therapy after curative treatment of HCV-related HCC, whenever possible. Importantly, the SVR rate for PEGIFN/RBV combination therapy was better than that for IFN monotherapy. On the other hand, the high rate of incomplete PEGIFN/RBV therapy (44.8%) was one of the causes of the high HCC recurrence rate and the advanced liver fibrosis. Our study identified factors that affect the viral response to PEGIFN/RBV therapy, and the identification of these factors should help in the selection of patients who will best benefit from such therapy.

On the other hand, the SVR rate was 55.0% (11/20) in patients infected with HCV genotype 2. Although the sample size was small, 78.5% (11/14) patients who showed pEVR achieved SVR. Therefore, continuation of treatment is likely to result in achievement of SVR even when PEGIFN/RBV treatment is started after curative treatment for HCV-related HCC. Efforts should be made to achieve SVR by PEGIFN/RBV therapy in patients infected with HCV genotype 2 after curative treatment for HCV-related HCC. Recently, the relationship between IL-28B and the effect of PEGIFN/RBV

Table 5 Univariate analysis of factors associated with NR in 55 patients with genotype 1 continued PEGIFN/RBV >12 weeks

	NR (n = 17)	TR+SVR (n = 38)	P value
Gender (male/female)	8/9	31/7	0.022
Age (years)*	66 (53–83)	67 (48–80)	0.75
Body mass index (kg/m^2)*	22.2 (19.3–30.0)	21.2 (15.6–28.5)	0.86
White blood Cell (×10^3/μL)*	5050 (4390–6130)	4280 (2470–6660)	0.6
Haemoglobin (g/dL)*	12.6 (9.3–17.4)	13.7 (8.7–15)	0.66
Platelet count (×10^4/mm^3)*	10.1 (4.7–18.2)	12.1 (3.9–19.6)	0.43
T-bilirubin (mg/dL)*	0.7 (0.4–1.7)	0.8 (0.4–2.3)	0.45
Alanine aminotransferase (IU/L)*	45 (19–134)	45 (12–189)	0.75
Prothrombin time activity (%)*	86 (64–121)	88 (69–112)	0.79
Albumin (g/dL)*	3.8 (2.7–4.9)	4 (3.4–5.2)	0.106
Fibrosing stage(F1-3/F4/ND)	3/8/6	9/9/20	0.21
γ-glutamyl transpeptidase (IU/L)*	52 (12–219)	26 (15–294)	0.113
HbA1c (%)*	5.3 (4–10.8)	5.2 (4.2–8.8)	0.99
Indocyanine green retention rate (%)	18.7 (7.6–45.4)	15.4 (8–29.2)	0.21
HCV viral load (Log IU/mL)*	6.28 (2.1–6.7)	6.18 (1.2–6.7)	0.25
HCV Core70 (mutant/wild)	11/6	20/18	0.55
HCV Core91 (mutant/wild)	10/7	16/22	0.38
HCV ISDR (0–1/>2)	12/5	21/17	0.23
α-Fetoprotein (ng/mL)*	45.3 (5–63240)	10 (0.5–909.2)	0.054
IL28B genotype (TT/GG+TG)	8/9	33/5	0.005
Dose of PEGIFN at administration (μg/kg)*	80 (40–120)	80 (50–100)	0.34
Dose of RBV at administration (mg)*	600 (200–1000)	600 (200–800)	0.77
Therapy were completed (yes/no)	9/8	23/15	0.76

HCV, hepatitis C virus; ISDR, interferon sensitivity-determining region; NR, null response; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; SVR, sustained viral response; TR, Transient viral response. *Data are median and (range).

Table 6 Multivariate analysis of factors associated with NR

Factor	Category	Odds rate (95%CI)	P value
IL28B genotype	TT	1	0.006
	GG+TG	7.8 (1.81–34.4)	
AFP	<30	1	0.015
	>30	5.6 (1.40–22.8)	

AFP, α-fetoprotein; NR, null response.

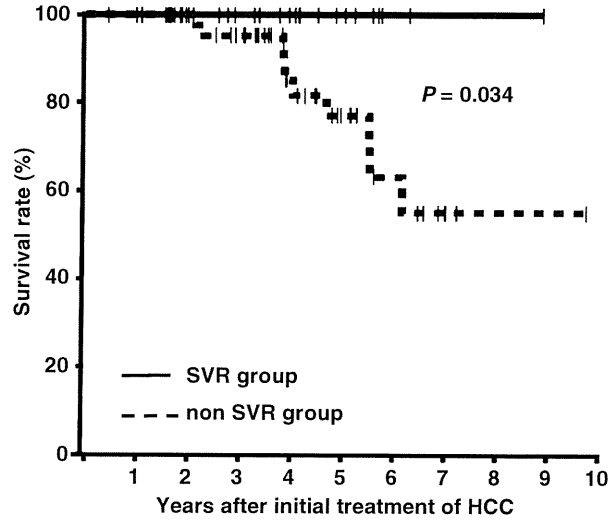

Fig. 3 Comparison of cumulative survival rates in the sustained viral response (SVR) and non-SVR groups. The cumulative survival rate was significantly higher in the SVR group than in the non-SVR group (P = 0.034).

therapy in patients with HCV genotype 2 was reported in two independent studies [28,29]. Further studies of larger sample size are needed to confirm the relationship between IL-28B genotype and the viral response to PEGIFN/RBV after treatment of HCV-related HCC in patients infected with HCV genotype 2.

Our results suggest that IL-28B genotype could be potentially used as a marker for the viral response to PEGIFN/RBV therapy. Furthermore, PEGIFN/RBV therapy should be recommended after curative treatment for HCV-related HCC for patients who are likely to achieve pEVR [those with IL-28B genotype (TT)]. In addition, the SVR rate might improve by IFN therapy and combination therapy HCC and HCV. On the other hand, it might be better to

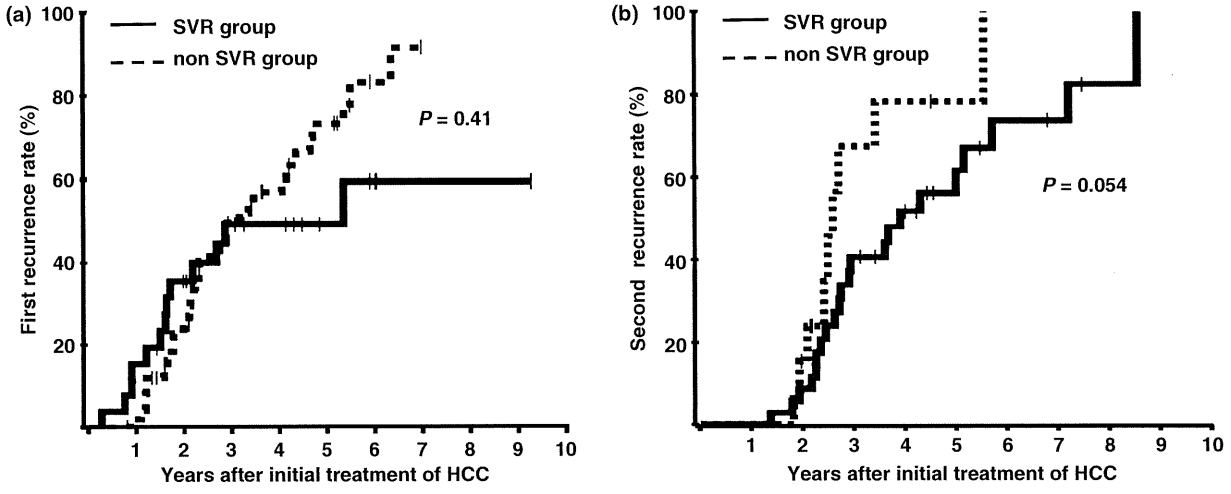

Fig. 4 Cumulative recurrence rates after curative treatment of hepatocellular carcinoma. (a) Rates of first recurrence for the sustained viral response (SVR) and non-SVR groups ($P = 0.41$). (b) Rates of second recurrence for the SVR and non-SVR groups. The second recurrence rate for the SVR group tended to be lower than that for the non-SVR group ($P = 0.054$).

administer low-dose intermittent IFN therapy for patients considered to show NR [those with IL-28B genotype (GG+TG)]. This therapy might result in the improvement of liver function and prevention of HCC recurrence, even if not to obtain SVR.

In conclusion, with regard to the prognosis of patients who undergo curative treatment for HCC, it is desirable to achieve SVR with interferon therapy even after treatment of HCC. IL-28B genotype could potentially be a suitable marker for the response to PEGIFN/RBV combination therapy after treatment of HCV-related HCC.

DISCLOSURES

The authors declare no conflict of interest.

REFERENCES

1 Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. *Hepatology* 1995; 21: 650–655.
2 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; 328: 1797–1801.
3 Shiratori Y, Shiina S, Imamura M *et al.* Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. *Hepatology* 1995; 22: 1027–1033.
4 Ikeda K, Saitoh S, Tsubota A *et al.* Risk factors for tumor recurrence and prognosis after curative resection of hepatocellular carcinoma. *Cancer* 1993; 71: 19–25.
5 Kubo S, Nishiguchi S, Shuto T *et al.* Effects of continuous hepatitis with persistent hepatitis C viremia on outcome after resection of hepatocellular carcinoma. *Jpn J Cancer Res* 1999; 90: 162–170.
6 Kumada T, Nakano S, Takeda I *et al.* Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology* 1997; 25: 87–92.
7 Nagasue N, Uchida M, Makino Y *et al.* Incidence and factors associated with intrahepatic recurrence following resection of hepatocellular carcinoma. *Gastroenterology* 1993; 105: 488–494.
8 Davis GL, Balart LA, Schiff ER *et al.* Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. *N Engl J Med* 1989; 321: 1501–1506.
9 Di Bisceglie AM, Martin P, Kassianides C *et al.* Recombinant interferon alfa therapy for chronic hepatitis C. A randomized, double-blind, placebo-controlled trial. *N Engl J Med* 1989; 321: 1506–1510.
10 Jeong S, Aikata H, Katamura Y *et al.* Low-dose intermittent interferon-alpha therapy for HCV-related liver cirrhosis after curative treatment of hepatocellular carcinoma. *World J Gastroenterol* 2007; 13: 5188–5195.
11 Mazzaferro V, Romito R, Schiavo M *et al.* Prevention of hepatocellular carcinoma recurrence with alpha-interferon after liver resection in HCV cirrhosis. *Hepatology* 2006; 44: 1543–1554.
12 Sakaguchi Y, Kudo M, Fukunaga T, Minami Y, Chung H, Kawasaki T. Low-dose, long-term, intermittent interferon-alpha-2b therapy after radical treatment by radiofrequency ablation delays clinical recurrence in patients with hepatitis C virus-related hepatocellular carcinoma. *Intervirology* 2005; 48: 64–70.
13 Kubo S, Nishiguchi S, Hirohashi K, Tanaka H, Shuto T, Kinoshita H.

Randomized clinical trial of long-term outcome after resection of hepatitis C virus-related hepatocellular carcinoma by postoperative interferon therapy. *Br J Surg* 2002; 89: 418–422.
14 Ikeda K, Arase Y, Saitoh S *et al.* Interferon beta prevents recurrence of hepatocellular carcinoma after complete resection or ablation of the primary tumor-A prospective randomized study of hepatitis C virus-related liver cancer. *Hepatology* 2000; 32: 228–232.
15 Kubo S, Nishiguchi S, Hirohashi K *et al.* Effects of long-term postoperative interferon-alpha therapy on intrahepatic recurrence after resection of hepatitis C virus-related hepatocellular carcinoma. A randomized, controlled trial. *Ann Intern Med* 2001; 134: 963–967.
16 Nishiguchi S, Tamori A, Kubo S. Effect of long-term postoperative interferon therapy on intrahepatic recurrence and survival rate after resection of hepatitis C virus-related hepatocellular carcinoma. *Intervirology* 2005; 48: 71–75.
17 Suou T, Mitsuda A, Koda M *et al.* Interferon alpha inhibits intrahepatic recurrence in hepatocellular carcinoma with chronic hepatitis C: a pilot study. *Hepatol Res* 2001; 20: 301–311.
18 Shiratori Y, Shiina S, Teratani T *et al.* Interferon therapy after tumor ablation improves prognosis in patients with hepatocellular carcinoma associated with hepatitis C virus. *Ann Intern Med* 2003; 138: 299–306.
19 Lin SM, Lin CJ, Hsu CW *et al.* Prospective randomized controlled study of interferon-alpha in preventing hepatocellular carcinoma recurrence after medical ablation therapy for primary tumors. *Cancer* 2004; 100: 376–382.
20 Hung CH, Lee CM, Wang JH, Tung HD, Chen CH, Lu SN. Antiviral therapy after non-surgical tumor ablation in patients with hepatocellular carcinoma associated with hepatitis C virus. *J Gastroenterol Hepatol* 2005; 20: 1553–1559.
21 Jeong SC, Aikata H, Katamura Y *et al.* Effects of a 24-week course of interferon-alpha therapy after curative treatment of hepatitis C virus-associated hepatocellular carcinoma. *World J Gastroenterol* 2007; 13: 5343–5350.
22 Manns MP, McHutchison JG, Gordon SC *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: 958–965.
23 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; 347: 975–982.
24 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; 41: 1100–1104.
25 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; 461: 399–401.
26 Tanaka Y, Nishida N, Sugiyama M *et al.* Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; 41: 1105–1109.
27 Thomas DL, Thio CL, Martin MP *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461: 798–801.
28 Kawaoka T, Hayes CN, Ohishi W *et al.* Predictive value of the IL28B polymorphism on the effect of interferon therapy in chronic hepatitis C patients with genotypes 2a and 2b. *J Hepatol* 2011; 54: 408–414.
29 Mangia A, Thompson AJ, Santoro R *et al.* An IL28B polymorphism determines treatment response of hepatitis C virus genotype 2 or 3 patients who do not achieve a rapid virologic response. *Gastroenterology* 2010; 139: 821–827.
30 Huang JF, Yu ML, Huang CF *et al.* The efficacy and safety of pegylated interferon plus ribavirin combination therapy in chronic hepatitis c patients with hepatocellular carcinoma post curative therapies – a multicenter prospective trial. *J Hepatol* 2011; 54: 219–226.
31 Hagihara H, Nouso K, Kobayashi Y *et al.* Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *Int J Clin Oncol* 2010; doi: 10.1007/s10147-010-0150-x.
32 Liver Cancer Study Group of Japan. The general rules for the clinical and pathological study of primary liver cancer. *Jpn J Surg* 1989; 19: 98–129.
33 Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. *J Hum Genet* 2001; 46: 471–477.
34 Suzuki A, Yamada R, Chang X *et al.* Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. *Nat Genet* 2003; 34: 395–402.
35 Kitamura S, Tsuge M, Hatakeyama T *et al.* Amino acid substitutions in core and NS5A regions of the HCV genome can predict virological decrease with pegylated interferon plus ribavirin therapy. *Antivir Ther* 2010; 15: 1087–1097.
36 Mori N, Imamura M, Kawakami Y *et al.* Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: Correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. *J Med Virol* 2009; 81: 640–649.
37 Kawaoka T, Hiraga N, Takahashi S *et al.* Prolongation of interferon therapy for recurrent hepatitis C after living donor liver transplantation: analysis of predictive factors of sustained virological response, including amino acid sequence of the core and NS5A regions of hepatitis C virus. *Scand J Gastroenterol* 2010; 45: 1488–1496.
38 Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer PJ. Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; 19: 1513–1520.
39 Tomimaru Y, Nagano H, Eguchi H *et al.* Effects of preceding interferon therapy on outcome after surgery for hepatitis C virus-related hepatocellular carcinoma. *J Surg Oncol* 2010; 102: 308–314.

40 Elefsiniotis IS, Vezali E, Mihas C, Saroglou G. Predictive value of complete and partial early virological response on sustained virological response rates of genotype-4 chronic hepatitis C patients treated with PEG-interferon plus ribavirin. *Intervirology* 2009; 52: 247–251.

41 Lagging M, Wejstal R, Uhnoo I *et al.* Treatment of hepatitis C virus infection: updated Swedish Consensus recommendations. *Scand J Infect Dis* 2009; 41: 389–402.

42 Reau N, Satoskar R, Te H *et al.* Evaluation of early null response to pegylated interferon and ribavirin as a predictor of therapeutic nonresponse in patients undergoing treatment for chronic hepatitis C. *Am J Gastroenterol* 2011; 106: 452–458.

43 Pearlman BL, Ehleben C, Saifee S. Treatment extension to 72 weeks of peginterferon and ribavirin in hepatitis c genotype 1-infected slow responders. *Hepatology* 2007; 46: 1688–1694.

nature genetics

Variation in the *DEPDC5* locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers

Daiki Miki[1,2,10], Hidenori Ochi[1,2,10], C Nelson Hayes[1,2], Hiromi Abe[1,2], Tadahiko Yoshima[1,3], Hiroshi Aikata[2], Kenji Ikeda[4], Hiromitsu Kumada[4], Joji Toyota[5], Takashi Morizono[6], Tatsuhiko Tsunoda[6], Michiaki Kubo[7], Yusuke Nakamura[8], Naoyuki Kamatani[9] & Kazuaki Chayama[1,2]

Chronic viral hepatitis is the most important risk factor for progression to hepatocellular carcinoma (HCC). To identify genetic risk factors for progression to HCC in individuals with chronic hepatitis C virus (HCV), we analyzed 467,538 SNPs in 212 Japanese individuals with chronic HCV with HCC and 765 individuals with chronic HCV without HCC. We identified one intronic SNP in the *DEPDC5* locus on chromosome 22 associated with HCC risk and confirmed the association using an independent case-control population (710 cases and 1,625 controls). The association was highly significant when we analyzed the stages separately as well as together (rs1012068, $P_{combined} = 1.27 \times 10^{-13}$, odds ratio = 1.75). The significance level of the association further increased after adjustment for gender, age and platelet count ($P = 1.35 \times 10^{-14}$, odds ratio = 1.96). Our findings suggest that common variants within the *DEPDC5* locus affect susceptibility to HCC in Japanese individuals with chronic HCV infection.

HCC is the third leading cancer-related cause of death and the seventh most common form of cancer worldwide[1]. In many western countries and Japan, HCV infection is the most common risk factor for HCC[2,3]. Chronic hepatitis caused by HCV often leads to fibrosis and cirrhosis (stage F4 fibrosis), which markedly increase the risk of developing HCC. The annual incidence of HCC correlates with the severity of liver fibrosis, from 0.5% among individuals with stage F0 or F1 fibrosis to 7.9% among individuals with stage F4 fibrosis[4]. Recently, age at initial diagnosis of HCV-related HCC has been increasing in Japan, and most affected individuals are diagnosed at age 55 or older[5–8]. To date, many studies have examined individuals with HCV and identified several predictive factors for HCC in addition to fibrosis and age, including male gender, alcohol consumption, diabetes mellitus, obesity, ethnicity and co-infection with hepatitis B virus (HBV)[1,5,7,9]. In spite of recent progress in anti-HCV therapy, it remains difficult to achieve complete eradication of the virus[10]. Particularly among individuals with HCV who are unable to clear the virus, screening of any SNPs associated with susceptibility to HCC may help improve prognosis and target surveillance efforts more efficiently to high-risk individuals. Researchers from another study[11] recently identified a SNP within the *KIF1B* locus associated with progression to HCC among chronic HBV carriers; however, the virological effects of HBV and HCV are entirely different[12], and so far, SNPs associated with risk of HCC among individuals with chronic HCV have not been fully investigated. To identify genetic markers associated with risk of HCV-related HCC development in the Japanese population, we conducted a two-phase case-control study consisting of a genome-wide association study (GWAS) and a replication study using a total of 3,312 Japanese individuals over the age of 55 with chronic HCV infection. In the GWAS phase, we performed SNP genotyping using the Illumina HumanHap610-Quad BeadChip. We analyzed 467,538 SNPs that passed quality control filters using an additive model for genotype-phenotype association in 212 chronic HCV carriers with HCC (cases) and 765 chronic HCV carriers without HCC (controls). Principal component analysis revealed no population substructure in our population. In addition, a quantile-quantile plot using the results of the Cochran-Armitage trend test showed that the inflation factor, λ, was 1.00, indicating a low probability of false-positive associations resulting from population stratification (**Supplementary Fig. 1a**). Using the additive model, one intronic SNP, rs1012068, within isoform 1 of the *DEPDC5* locus on chromosome 22, showed strong association with HCC ($P = 8.05 \times 10^{-8}$) with odds ratio (OR) = 2.20 (95% confidence interval (CI) 1.64–2.97) (**Table 1**). We also found that rs1012068 showed a statistically significant association

[1]Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN, Hiroshima, Japan. [2]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan. [3]Pharmacology Research Laboratories, Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan. [4]Department of Hepatology, Toranomon Hospital, Tokyo, Japan. [5]Department of Gastroenterology, Sapporo Kosei General Hospital, Hokkaido, Japan. [6]Laboratory for Medical Informatics, RIKEN Center for Genomic Medicine, Yokohama, Japan. [7]Laboratory for Genotyping Development, RIKEN Center for Genomic Medicine, Yokohama, Japan. [8]Laboratory of Molecular Medicine, Human Genome Center, The Institute of Medical Science, University of Tokyo, Tokyo, Japan. [9]Laboratory for Statistics, RIKEN Center for Genomic Medicine, Yokohama, Japan. [10]These authors contributed equally to this work. Correspondence should be addressed to K.C. (chayama@hiroshima-u.ac.jp).

Received 4 January; accepted 7 June; published online 3 July 2011; doi:10.1038/ng.876

Table 1 Summary of GWAS and replication study

SNP	Gene	Study	Allele (1/2)	Case 11	Case 12	Case 22	Control 11	Control 12	Control 22	MAF Case	MAF Control	OR	95% CI[a]	P^b	P^c_{het}
rs1012068	*DEPDC5*	GWAS	T/G	138	68	6	624	136	5	0.189	0.095	2.20	1.64–2.97	8.05×10^{-8}	
		Replication		470	221	19	1262	334	29	0.182	0.121	1.63	1.37–1.93	2.41×10^{-8}	
		Combined studies[d]										1.75	1.51–2.03	1.27×10^{-13}	0.082

MAF, minor allele frequency; OR, odds ratio; CI, confidence interval.
[a]Odds ratios of risk allele from two-by-two allele frequency table. [b]*P* value of Cochran-Armitage trend test. [c]Result of Breslow-Day test. [d]Combined meta-analysis was performed using the Mantel-Haenszel method.

with HCV-related HCC after Bonferroni correction for multiple testing (calculated as $P < 0.05/467{,}538 = 1.07 \times 10^{-7}$)[13,14]. No other SNPs reached genome-wide significance (**Supplementary Table 1** and **Supplementary Fig. 1b**). As shown in **Table 1**, we next performed a replication study using 710 cases and 1,625 controls and again found that rs1012068 was strongly associated with HCC ($P = 2.41 \times 10^{-8}$, OR = 1.63). The association between rs1012068 and HCC remained highly significant when we combined results of the GWAS and replication sets using the Mantel-Haenszel method (combined $P = 1.27 \times 10^{-13}$, OR = 1.75). We observed no heterogeneity across the two studies (heterogeneity test $P = 0.082$).

On the other hand, platelet count is known to correlate significantly with the stage of liver fibrosis in individuals with HCV, and a platelet count of $<10 \times 10^4/\mu l$ has also been used as a marker for cirrhosis[4,15–19]. After adjusting for age, gender and platelet count using multiple logistic regression analysis, the significance level of rs1012068 increased ($P = 1.35 \times 10^{-14}$, OR = 1.96) (**Supplementary Table 2**). Other predictive factors for HCV-related HCC have been reported, including alcohol consumption, diabetes mellitus, obesity, ethnicity and co-infection with HBV[1,5,7,9]. As all subjects enrolled were of Japanese ethnicity, and there were no HBV co-infected subjects, the effect of the SNP was reevaluated using only 994 subjects (480 cases and 514 controls) for whom data was fully available for other factors (**Supplementary Table 3**). After adjusting for each of these six factors using multiple logistic regression analysis, rs1012068 remained highly significant with an OR = 1.87 (95% CI 1.39–2.52) (**Supplementary Table 4**). However, we cannot rule out the possibility that other confounding factors influenced the results. In addition to examining the effect of rs1012068 on HCC independently of other predictive factors, we performed stratified analysis using gender, age and platelet count (**Supplementary Table 5**). Notably, this SNP tended to show a greater effect in males (OR = 1.99 (95% CI 1.63–2.42)) than females (OR = 1.51 (95% CI 1.18–1.93)), as well as in elderly subjets (age ≥65 years: OR = 1.84 (95% CI 1.52–2.24) compared to age <65: OR = 1.73 (95% CI 1.36–2.19)) and in subjects with low platelet counts ($<10 \times 10^4/\mu l$: OR = 2.35 (95% CI 1.67–3.31) compared to $\geq 10 \times 10^4/\mu l$: OR = 1.71 (95% CI 1.42–2.05)). Each of these factors (male gender, older age and lower platelet count) are well known risk factors for HCV-related HCC. rs1012068 seems to more strongly affect individuals with multiple risk factors for HCC, but we detected no heterogeneity among subgroups stratified by gender, age and platelet count (heterogeneity test $P = 0.086$, $P = 0.675$ and $P = 0.103$, respectively, for each factor).

To examine whether rs1012068 genotypes are associated with any specific HCC phenotypes, we analyzed clinical phenotypes of cases with HCC with regard to rs1012068 genotype. We observed no differences between individuals with TT and TG+GG genotypes (**Supplementary Table 6**), but when we evaluated the case to control ratio of each 5-year age group with respect to rs1012068 genotype (TT compared to TG+GG), we found that the ratio was higher among subjects with the TG+GG genotype over all 5-year age ranges, and the slope of the ratio with increasing age was steeper among these individuals (**Supplementary Fig. 2**).

In order to explore the region around the landmark SNP rs1012068 in more detail, we performed fine mapping in the GWAS-stage subjects of a 350-kb genomic region between 22q12.2 and 22q12.3 upstream and downstream of the *DEPDC5* locus, including the neighboring genes *C22orf30* and *YWHAH* (**Fig. 1** and **Supplementary Fig. 3**). We successfully genotyped 43 tagging SNPs and identified another intronic SNP, rs5998152, located about 2.7 kb upstream of the landmark SNP (rs1012068) that is in strong linkage disequilibrium (LD) with rs1012068 ($r^2 = 0.99$). However, no SNPs showed stronger association than rs1012068 (**Supplementary Fig. 3** and **Supplementary Table 7**). To investigate the existence of any functional coding SNPs linked to rs1012068, we resequenced all 42 exons of *DEPDC5* using genomic DNA from 48 individuals

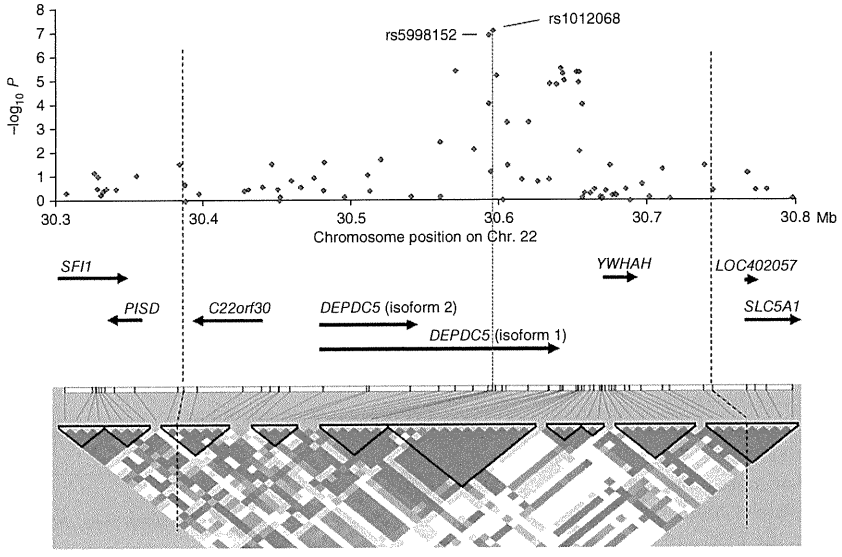

Figure 1 Case-control association plots and linkage disequilibrium (LD) map and genomic structure of the *DEPDC5* region in chromosome 22q12.2–3. The candidate region is indicated by two black dashed lines. We performed fine mapping in the region from 30.39–30.74 Mb. Blue diamonds represent $-\log_{10} P$ obtained from the GWAS and fine mapping using GWAS samples. We drew the LD map based on *D'* values using the genotype data of the cases and controls in the GWAS samples. The landmark SNP (rs1012068) is indicated by the red dotted line.

with HCV. We identified two new SNPs that were not registered in the dbSNP database (**Supplementary Table 8**). However, both SNPs had low minor allele frequencies (MAF) of 0.010 and were not linked to rs1012068 ($r^2 = 0.00$) nor were they significantly associated with HCC. We also performed haplotype analysis to investigate the effect of combinations of SNPs that were strongly associated with HCC susceptibility; however, no haplotype showed stronger association than the single-marker association of rs1012068 (**Supplementary Fig. 4**). Finally, rs1012068 had the strongest independent association with HCV-related HCC in our study.

Then we investigated the association between rs1012068 genotype and *DEPDC5* mRNA expression using paired tumor (HCC) and adjacent non-tumor liver tissues from 43 individuals with HCV. As shown in **Supplementary Figure 5**, real-time quantitative PCR assays revealed a significantly higher level of *DEPDC5* mRNA expression in tumor tissues than non-tumor tissues ($P = 0.025$), but we observed no significant difference with regard to rs1012068 genotype in tumor tissues as well as in non-tumor tissues ($P = 0.610$ and $P = 0.400$, respectively). On the other hand, we also evaluated *DEPDC5* expression using paired tumor and adjacent non-tumor tissues and calculated the tumor to non-tumor ratio as the *DEPDC5* expression level in tumor tissue divided by the expression level in paired non-tumor tissue from the same subject. As shown in **Supplementary Table 9**, we found that the frequency of the risk allele (G) was significantly higher in subjects with a tumor to non-tumor ratio ≥5 as well as a ratio of ≥1 in males ($P = 0.014$ and $P = 0.036$, respectively) but not in females (a ratio of ≥5: $P = 0.500$ and a ratio of ≥1: $P = 0.226$). This finding may suggest a differential effect of the SNP on *DEPDC5* expression due to gender. Although there is insufficient data to show a direct functional effect of rs1012068 on *DEPDC5* expression and HCV-related hepatocarcinogenesis, the data suggest a possible genetic association between a polymorphism within the *DEPDC5* locus and HCV-related HCC that requires further functional analysis.

In this study, we identified a common SNP associated with HCV-related HCC, and the effect of the SNP remained highly significant even after adjusting for other predictive factors. We observed no significant heterogeneity between the GWAS and replication studies, but the odds ratios for each study differed somewhat, and the 95% CIs for one phase of the study did not include the odds ratio for the other. In addition, the MAFs for the controls differed between the GWAS and replication phases (**Table 1**). We speculate that the differences between the two phases partially explain the different observed effects of the SNP. The female to male ratio was significantly higher in the replication phase than the GWAS phase for both cases and controls (**Supplementary Table 10**). The ages of the cases were significantly lower and the platelet counts of the controls were significantly higher in the GWAS phase than in the replication phase. As shown in **Supplementary Table 5**, rs1012068 showed a weaker risk in females than in males, and the frequency of the risk allele among older cases (≥65 years old) was lower than among younger cases (0.183 compared to 0.186, respectively). The risk allele frequency was also higher among controls with platelet count ≥10 ($\times 10^4/\mu l$) than in controls with platelet count <10 ($\times 10^4/\mu l$) (0.116 compared to 0.088, respectively). These unexpected differences between subjects in the two phases seem to contribute jointly to the observed differences in the effect of the SNP between the two phases. It is important to note that the controls used in this study were not healthy controls (MAF = 0.116 based on HapMap JPT data) but are chronic HCV carriers who still have the potential of developing HCC in the future, especially those who have one or more other strong predictive factors (for example, gender, age or platelet count). When we stratified samples by each predictive factor, the MAFs in the controls were varied (**Supplementary Table 5**). We speculate that after HCV infection becomes chronic, individuals with risk alleles may more easily develop HCC, and conversely, those without risk alleles are relatively less likely to progress to HCC (**Supplementary Fig. 2**), but these other risk factors influence the ultimate course of the disease. Consideration of the genetic background of subjects will likely play a role in personalized medicine, and understanding the mechanism underlying the association may suggest new therapeutic targets.

On the other hand, given the relatively small number of cases in the GWAS phase, we calculated the statistical power to detect an effect caused by rs1012068 to be only 50%, compared to the 80% recommended to detect an association of the expected effect size (**Supplementary Fig. 6**). It remains to be determined whether other SNPs influence susceptibility to HCV-related HCC in the Japanese population. The question also remains whether this susceptibility locus within *DEPDC5* is associated with HCV-related HCC in other ethnic groups, as allele frequencies of rs1012068 vary among ethnic groups, even among those with Asian ethnicities (**Supplementary Table 11**).

DEPDC5 has not been previously reported in association with HCC, but deletion of the region containing *DEPDC* has been reported in malignant brain glioblastomas[20]. Although the function of this gene is still unknown[21], it is noteworthy that DEPDC1, which contains a DEP domain similar to DEPDC5, has been reported to affect bladder carcinogenesis[22,23].

In summary, we conducted a GWAS followed by an independent replication study and fine mapping to detect polymorphisms associated with HCC in Japanese individuals with HCV. We report a common SNP within the *DEPDC5* locus associated with a twofold increased risk of HCC. Further research is required to determine the role of this gene in development of HCV-related HCC.

URLs. PLINK1.03, http://pngu.mgh.harvard.edu/~purcell/plink/; R statistical environment, http://www.cran.r-project.org/; EIGENSOFT, http://genepath.med.harvard.edu/~reich/Software.htm.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturegenetics/.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

The authors thank the subjects who agreed to participate in this study. We also thank the team members at Toranomon Hospital, Sapporo Kosei General Hospital, Hiroshima University Hospital and Hiroshima Liver Study Group for clinical sample collection. We thank T. Yokogi, Y. Hayashida and K. Izumoto for technical assistance, J. Sakamiya for clerical assistance and other members of the RIKEN Center for Genomic Medicine and Hiroshima University for assistance with various aspects of this study.

AUTHOR CONTRIBUTIONS

K.C. conceived the study. D.M., H.O. and K.C. designed the study. D.M. and H.O. performed genotyping. D.M., H.O., C.N.H. and K.C. wrote the manuscript. T.M., T.T., M.K. and N.K. performed data analysis at the genome-wide phase. H. Abe and T.Y. performed functional analyses. H. Aikata, K.I., H.K., J.T. and K.C. managed DNA samples. D.M., H.O. and K.C. summarized the whole results. Y.N., N.K. and K.C. obtained funding for the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

Published online at http://www.nature.com/naturegenetics/.
Reprints and permissions information is available online at http://www.nature.com/reprints/index.html.

1. Yang, J.D. & Roberts, L.R. Hepatocellular carcinoma: a global view. *Nat. Rev. Gastroenterol. Hepatol.* **7**, 448–458 (2010).

2. Barrera, J.M. *et al.* Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. *Hepatology* **21**, 639–644 (1995).
3. Welzel, T.M. *et al.* Variants in interferon-alpha pathway genes and response to pegylated interferon-Alpha2a plus ribavirin for treatment of chronic hepatitis C virus infection in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Hepatology* **49**, 1847–1858 (2009).
4. Yoshida, H. *et al.* Interferon therapy reduces the risk for hepatocellular carcinoma. *Ann. Intern. Med.* **131**, 174–181 (1999).
5. Kiyosawa, K. *et al.* Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* **127**, S17–S26 (2004).
6. Taura, N. *et al.* Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. *Oncol. Rep.* **16**, 837–843 (2006).
7. Miki, D. *et al.* Clinicopathological features of elderly patients with hepatitis C virus-related hepatocellular carcinoma. *J. Gastroenterol.* **43**, 550–557 (2008).
8. Takata, A. *et al.* HCC develops even in the early stage of chronic liver disease in elderly patients with HCV infection. *Int. J. Mol. Med.* **26**, 249–256 (2010).
9. Yuen, M.F., Hou, J.L. & Chutaputti, A. Hepatocellular carcinoma in the Asia pacific region. *J. Gastroenterol. Hepatol.* **24**, 346–353 (2009).
10. Manns, M.P. *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* **358**, 958–965 (2001).
11. Zhang, H. *et al.* Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. *Nat. Genet.* **42**, 755–758 (2010).
12. Ura, S. *et al.* Differential microRNA expression between hepatitis B and hepatitis C leading disease progression to hepatocellular carcinoma. *Hepatology* **49**, 1098–1112 (2009).
13. Pe'er, I., Yelensky, R., Altshuler, D. & Daly, M.J. Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. *Genet. Epidemiol.* **32**, 381–385 (2008).
14. Yamauchi, T. *et al.* A genome-wide association study in the Japanese population identifies susceptibility loci for type 2 diabetes at UBE2E2 and C2CD4A–C2CD4B. *Nat. Genet.* **42**, 864–868 (2010).
15. Ono, E. *et al.* Platelet count reflects stage of chronic hepatitis C. *Hepatol. Res.* **15**, 192–200 (1999).
16. Poynard, T. & Bedossa, P. Age and platelet count: a simple index for predicting the presence of histological lesions in patients with antibodies to hepatitis C virus. *J. Viral Hepat.* **4**, 199–208 (1997).
17. Forns, X. *et al.* Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* **36**, 986–992 (2002).
18. Wai, C.T. *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* **38**, 518–526 (2003).
19. Pohl, A. *et al.* Serum aminotransferase levels and platelet counts as predictors of degree of fibrosis in chronic hepatitis C virus infection. *Am. J. Gastroenterol.* **96**, 3142–3146 (2001).
20. Seng, T.J. *et al.* Complex chromosome 22 rearrangements in astrocytic tumors identified using microsatellite and chromosome 22 tile path array analysis. *Genes Chromosom. Cancer* **43**, 181–193 (2005).
21. Kharrat, A. *et al.* Conformational stability studies of the pleckstrin DEP domain: definition of the domain boundaries. *Biochim. Biophys. Acta* **1385**, 157–164 (1998).
22. Harada, Y. *et al.* Cell-permeable peptide DEPDC1–ZNF224 interferes with transcriptional repression and oncogenicity in bladder cancer cells. *Cancer Res.* **70**, 5829–5839 (2010).
23. Kanehira, M. *et al.* Involvement of upregulation of DEPDC1 (DEP domain containing 1) in bladder carcinogenesis. *Oncogene* **26**, 6448–6455 (2007).

ONLINE METHODS

Samples. We conducted a two-phase case control study consisting of GWAS and replication phases using 3,312 Japanese subjects over the age of 55 with chronic HCV infection diagnosed at Toranomon Hospital Department of Hepatology (n = 727), Sapporo Kosei Hospital (n = 153) and Hiroshima University–affiliated hospitals (n = 2,432) between 2002 and 2010. Individuals with chronic HCV with HCC were enrolled as cases, and those without were enrolled as controls. Cases and controls were then each randomly divided into two sets, totaling 212 cases and 765 controls in the GWAS phase and 710 cases and 1,625 controls in the replication phase. All subjects had abnormal levels of serum alanine transaminase for more than 6 months and were positive for both HCV antibody and serum HCV RNA. All subjects were negative for hepatitis B surface antigen, had no evidence of other liver diseases and had not received immunosuppressive therapy before enrollment in the study. Clinical information such as age, gender and platelet count were available in all subjects. For cases, age and platelet count at initial diagnosis of HCC were used. The subject characteristics are shown in **Supplementary Table 10**. The diagnosis of HCC was based on hypervascularity confirmed by dynamic computed tomography, magnetic resonance imaging, angiography or computed tomography angiography when the serum levels of HCC-related tumor markers, such as alpha fetoprotein or protein induced in the absence of vitamin K or antagonist II (PIVKA-II), were increased or a mass lesion was detected by ultrasonography. When a nodule was not proven to be hypervascular, percutaneous biopsy under ultrasonography was performed for confirmation of the diagnosis of HCC. Staging of HCC adopted in this study was the revised version of Liver Cancer Study Group of Japan[24]. The average quantity of alcohol consumed per day was evaluated regardless of alcohol beverage. Habitual alcohol intake was defined as ≥80 g/day for more than 5 years. All subjects in the present study received a detailed explanation and all signed a written informed consent form. The study was approved by the ethical committee of each participating medical center and by the Ethical Committee at the SNP Research Center, the Institute of Physical and Chemical Research (RIKEN), Yokohama, Japan.

SNP genotyping. Genomic DNA was extracted from peripheral blood leukocytes using a standard method. For the GWAS stage, we genotyped 981 Japanese subjects with chronic HCV infection using the Illumina HumanHap610-Quad BeadChip. We excluded two samples with call rate <0.98, and two other samples suggesting kinship or sample duplication were excluded from the analysis based on PI_HAT value (>0.4). We assessed population stratification using the smartpca program in the EIGENSOFT package using SNPs informative for the Japanese population according to a previously described method[25]. Analysis was performed based on the GWAS data and the Japanese (JPT), Han-Chinese (CHB), European (CEU) and African (YRI) individuals from the HapMap project. Principal component analysis identified no outliers from the JPT/CHB clusters. In total, 467,538 autosomal SNPs passed the quality control filters (call rate ≥ 0.99 in both cases and controls, MAF ≥ 0.01 and a Hardy-Weinberg equilibrium $P \geq 1.0 \times 10^{-6}$ in controls). We used multiplex-PCR–based Invader assays (Third Wave Technologies) for the replication study (710 cases and 1,625 controls) and fine mapping[26]. Samples for both cases and controls were distributed randomly on genotyping plates in both phases of the study, and all persons performing genotyping and interpretation of results were blind to case or control status.

Fine mapping and resequencing. We performed fine mapping using all GWAS-stage case and control samples. Haploview was used to select tag SNPs with a pairwise $r^2 > 0.80$ and MAF ≥ 0.05 on the basis of Phase II HapMap JPT data. Resequencing of candidate regions was performed by direct sequencing of DNA from 48 unrelated Japanese individuals with HCV from among the enrolled subjects.

Quantitative analysis of mRNA of *DEPDC5*. A total of 43 paired primary hepatocellular carcinomas and adjacent non-tumor tissues, derived from 43 unrelated HCC cases enrolled, were examined. Total RNA was extracted from liver tissues using the RNeasy Mini Kit (QIAGEN). One microgram of each RNA sample was reverse transcribed with ReverseTra Ace (TOYOBO Co. Ltd.) and Random Primer (Takara Bio). We quantified the mRNA for *DEPDC5* with the SsoFast EvaGreen Supermix (Bio-Rad Laboratories). Primers were designed for the conserved region between isoforms 1 and 2 (**Supplementary Table 12**). Amplification and detection were performed using a CFX Real-Time PCR Detection System (Bio-Rad Laboratories). Results were normalized to the transcript levels of beta-actin (*ACTB*).

Statistical analysis. Genotype-based associations were tested using a Cochran-Armitage trend test[27,28]. The OR and 95% CI were calculated from a two-by-two allele frequency table. In the GWAS stage, significance levels after Bonferroni correction for multiple testing were $P = 1.07 \times 10^{-7}$ (calculated as 0.05/467,538)[13,14]. Combined analysis was performed following the Mantel-Haenszel method. Heterogeneity among studies was examined using the Breslow-Day test. We used Haploview software to analyze the association of haplotypes and LD values between *DEPDC5* and SNPs. Power analysis was performed using Power for Genetic Association Analyses software[29]. The Mann-Whitney U test was used to analyze continuous variables, and χ^2 or Fisher exact tests were used to analyze categorical data, as appropriate.

Software. For general statistical analysis, we used the R statistical environment version 2.12.0 or PLINK 1.03 (ref. 30). To draw the LD map and analyze the association of haplotypes, we used Haploview software[31].

24. The general rules for the clinical and pathological study of primary liver cancer. *Liver Cancer Study Group of Japan* 3rd English edn. (Kanehara & Co., Ltd., Tokyo, Japan, 2010).
25. Yamaguchi-Kabata, Y. *et al.* Japanese population structure, based on SNP genotypes from 7003 individuals compared to other ethnic groups: effects on population-based association studies. *Am. J. Hum. Genet.* **83**, 445–456 (2008).
26. Ohnishi, Y. *et al.* A high throughput SNP typing system for genome-wide association studies. *J. Hum. Genet.* **46**, 471–477 (2001).
27. Nam, J.M. A simple approximation for calculating sample sizes for detecting linear trend in proportions. *Biometrics* **43**, 701–705 (1987).
28. Margolin, B.H. Test for trend in proportions. in *Encyclopedia of statistical sciences.* (eds. Klotz, S. & Johnson, N.L.) 334–336 (John Wiley & Sons, Inc., New York, New York, USA, 1988).
29. Menashe, I., Rosenberg, P.S. & Chen, B.E. PGA: Power calculator for case-control genetic association analyses. *BMC Genet.* **9**, 36 (2008).
30. Purcell, S. *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* **81**, 559–575 (2007).
31. Barrett, J.C., Fry, B., Maller, J. & Daly, M.J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265 (2005).

Ann Surg Oncol (2012) 19:418–425
DOI 10.1245/s10434-011-1866-1

Annals of
SURGICAL ONCOLOGY
OFFICIAL JOURNAL OF THE SOCIETY OF SURGICAL ONCOLOGY

ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Impact of Pegylated Interferon Therapy on Outcomes of Patients with Hepatitis C Virus-Related Hepatocellular Carcinoma After Curative Hepatic Resection

Yoshisato Tanimoto, MD[1], Hirotaka Tashiro, MD[1], Hiroshi Aikata, MD[2], Hironobu Amano, MD[1], Akihiko Oshita, MD[1], Tsuyoshi Kobayashi, MD[1], Shintaro Kuroda, MD[1], Hirofumi Tazawa, MD[1], Shoichi Takahashi, MD[2], Toshiyuki Itamoto, MD[3], Kazuaki Chayama, MD[2], and Hideki Ohdan, MD[1]

[1]Department of Gastroenterological Surgery, Hiroshima University Hospital, Hiroshima, Japan; [2]Department of Gastroenterology, Hiroshima University Hospital, Hiroshima, Japan; [3]Department of Surgery, Prefectural Hiroshima Hospital, Hiroshima, Japan

ABSTRACT

Background. Several published reports investigating the effects of interferon (IFN) therapy on survival and tumor recurrence after curative resection of hepatocellular carcinoma (HCC) have been inconclusive. The aim of this study is to investigate the efficacy of pegylated-IFN (peg-IFN) therapy after curative hepatic resection for HCC in patients infected with hepatitis C virus (HCV).

Methods. Data from 175 patients who underwent curative hepatic resection for HCC associated with HCV were retrospectively collected and analyzed; 75 patients received peg-IFN therapy after surgery, whereas 100 patients did not receive IFN therapy. To overcome biases resulting from the different distribution of covariates in the two groups, a one-to-one match was created using propensity score analysis. After matching, patient outcomes were analyzed.

Results. After one-to-one matching, patients ($n = 38$) who received peg-IFN therapy after surgery and patients ($n = 38$) who did not receive IFN therapy had the same preoperative and operative characteristics. The 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.00135$). The 3- and 5-year overall survival rates were 100 and 91.7% and 76.6 and 50.6% in the peg-IFN group and non-IFN group, respectively. There was no significant difference in disease-free survival between the two matched groups ($P = 0.886$).

Conclusion. Peg-IFN therapy may be effective as an adjuvant chemopreventive agent after hepatic resection in patients with HCV-related HCC.

First Received: 14 February 2011;
Published Online: 28 June 2011

H. Tashiro, MD
e-mail: htashiro@hiroshima-u.ac.jp

Hepatic resection is a well-accepted therapy for hepatocellular carcinoma (HCC), but many patients show cancer recurrence and the cumulative 5-year HCC recurrence rate exceeds 70%.[1–3] This high incidence of tumor recurrence after hepatic resection remains a major drawback. Some benefits of interferon (IFN) therapy on tumor recurrence and survival have been reported.[4–10] IFN suppresses replication of hepatitis C virus (HCV) and exerts a tumoricidal effect on a number of tumors, including HCC.[10,11] However, several randomized controlled trials (RCTs) have revealed inconclusive results regarding the effects of IFN on survival and tumor recurrence after curative resection or ablation of HCC, either because the effects were not statistically significant or because they were considered only with respect to defined subpopulations.[12–15]

Recently, combination therapy consisting of pegylated interferon (peg-IFN) plus ribavirin (RBV) has been developed, and the effect of this combination has been reported to be higher than that of conventional IFN therapy.[16,17] Peg-IFN has an extended serum half-life that provides viral suppression for 7 days, thus allowing weekly administration and enhanced clinical efficacy.[17] Most Japanese patients infected with HCV are infected with HCV genotype Ib and have high viral load. Moreover, treatment with conventional IFN is complicated by a low sustained viral response (SVR) rate of 20–30%.[18–20]

However, peg-IFN plus RBV combination therapy has good tolerability in Japanese patients with HCV and resulted in an SVR rate of approximately 40–50%.[21–23] The impact of adjuvant immunotherapy with IFN after curative resection of HCC is debatable, and few studies have investigated the effects of peg-IFN plus RBV combination therapy on survival and recurrence after curative resection of HCC.

In the present study, we aim to investigate the impact of peg-IFN plus RBV combination therapy on survival and HCC recurrence after curative resection in patients infected with HCV.

PATIENTS AND METHODS

Patients and HCV Diagnosis

From June 2003 to June 2009, 370 HCC patients underwent hepatectomy as initial treatment at the Department of Gastroenterological Surgery, Hiroshima University Hospital, Japan. Of the 370 patients, 175 patients who were HCV RNA-positive/hepatitis B surface antigen-negative underwent curative hepatectomy. Of the 175 patients, 75 patients received IFN therapy after hepatectomy, and 100 patients did not receive any IFN therapy. Of the 75 patients who received IFN, 20 patients who received IFNs such as IFN-α or IFN-β were excluded. Of the 55 patients who received peg-IFN therapy, 43 patients who started peg-IFN within 9 months after curative resection were enrolled in this analysis. Twenty-four patients who had early recurrence of HCC within 9 months after surgery were excluded from the 100 patients who did not receive any IFN therapy, because these patients could lose the opportunity to receive IFN therapy for HCC recurrence if these patients were assigned to the peg-IFN therapy. Consequently, 119 patients were eventually enrolled in this study. Of these 119 patients, 43 received peg-IFN therapy within 9 months after hepatectomy, and 76 did not receive any IFN therapy.

Curative hepatectomy was defined as removal of all recognizable tumors. HCV RNA levels were measured by quantitative reverse-transcription polymerase chain reaction (RT-PCR; Amplicor, Roche Diagnostic Systems, CA, USA). HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region. HCV negativity was evaluated by quantitative RT-PCR. The lower limit of the assay was 5 kIU/ml (equivalent to 5,000 copies/ml) in the quantitative method and 50 IU/ml (equivalent to 50 copies/ml) in the qualitative method. SVR was defined as undetectable HCV RNA at 24 weeks after completion of IFN therapy. The study was approved by the concerned institutional review boards. Written informed consent was obtained from all patients.

Preoperative Diagnosis and Evaluation of HCC

Hepatocellular carcinoma was diagnosed on the basis of routine imaging modalities such as Doppler ultrasonography (US), computed tomography (CT) during hepatic angiography (CTHA) and CT during arterial portography (CTAP), and magnetic resonance imaging. Tumor stage, liver damage classification, and surgical procedures were defined according to the General Rules for Clinical and Pathologic Study of Primary Liver Cancer, fifth edition, by the Liver Cancer Study Group of Japan.[24]

Hepatectomy

The surgical procedure was determined according to tumor extent and hepatic reserve function. Liver function was assessed by liver damage classification, Child–Pugh classification, and indocyanine green retention rate at 15 min (ICGR 15).[25,26] If permitted by liver function, anatomic resection was performed.[27,28] In patients with insufficient hepatic reserve, limited resection was performed. We divided the liver parenchyma by using an ultrasonic dissector.[29] Postoperative complications were graded according to the method described by Clavien et al.[30]

Follow-Up

Follow-up evaluation after the surgery consisted of monthly blood chemistry tests and measurements of levels of tumor markers, including alpha-fetoprotein and des-gamma-carboxy prothrombin. Patients were examined by US every 3 months and by CT every 6 months. When recurrence was indicated by any of these examinations, patients were examined by CTAP and CTHA.

Patient Selection for IFN Therapy

Patients with HCV genotype 1b in the IFN group received peg-IFNα-2b (Pegintron; Schering-Plough, NJ, USA) at weekly dosage of 1.5 μg/kg subcutaneously for 48 weeks. Daily RBV (Rebetrol, Schering-Plough) was administered orally for 48 weeks, and the dosage was adjusted according to weight (600 mg for patients weighing $\leq$60 kg, 800 mg for those weighing 60–80 kg). Patients with HCV genotype 2 received IFN monotherapy for 24 weeks. Blood samples were obtained every 4 weeks and analyzed for HCV RNA levels. All patients were informed about IFN therapy after hepatectomy, and only consenting patients received IFN therapy. The eligibility criteria for IFN therapy were as follows: (1) detectable serum HCV RNA level, (2) Eastern Cooperative Oncology

Group (ECOG) performance score of 0 or 1, (3) platelet count ≥70,000/μl, (4) patients with no uncompensated cirrhosis (Child class C), and (5) hemoglobin concentration ≥10 g/dl. Peg-IFN therapy was commenced within 24 weeks of surgery or after the eligibility criteria were fulfilled.

Safety Assessments and Dose Modification of Peg-IFN Therapy

Adverse events were graded as mild, moderate, severe, or potentially life-threatening according to a modified World Health Organization grading system. The dose of peg-IFN was decreased by 50% and that of RBV was lowered to half in case of severe adverse events or when laboratory results revealed any of the following: hemoglobin concentration <10 g/dl in patients with no cardiac disease, decrease in hemoglobin concentration >2 g/dl in patients with cardiac disease, white blood cell count <3,000/mm^3, or platelet count <50,000/mm^3. Full dosage could be resumed on resolution of the adverse events. Treatment was permanently discontinued in case of life-threatening events or when laboratory results revealed hemoglobin concentration <7.5 g/dl after 4 weeks of dose reduction, white blood cell count <1,500/mm^3, or platelet count <30,000 mm^3.

Treatment for Recurrence

Patients with intrahepatic HCC recurrence were managed with ablative therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection therapy, transarterial chemoembolization, or surgery including living-donor liver transplantation according to the tumor characteristics (number, size, and location of the tumors) and liver function.

Statistical Analyses

Categorical variables were compared using the chi-square test, and continuous variables were compared using the Mann–Whitney *U*-test. Overall survival and disease-free survival analyses were performed using Kaplan–Meier methods; comparisons between different groups were performed using the log-rank test. *P* value of less than 0.05 was considered significant. Calculations were performed using SPSS software (version 16; SPSS Inc., IL, USA).

Propensity analysis was performed using logistic regression to create a propensity score for the IFN and non-IFN therapy groups.[31,32] Variables entered in the propensity model were age, sex, HCV genotype, liver function test, tumor factors, and operative factors. The model was then used to provide a one-to-one match between the two groups by using the nearest-neighbor matching method.[33,34] Survival and disease-free survival analyses were performed in each matched subgroup to assess the impact of peg-IFN therapy on mortality after adjusting for the confounding factors.

RESULTS

Characteristics and Postoperative Course of the Entire Population

Differences in the characteristics of patients who received peg-IFN therapy after hepatic resection and those who did not receive IFN therapy after hepatic resection are presented in Table 1. Patients who received peg-IFN therapy were younger (65 vs. 71 years; $P = 0.0003$). Regarding tumor characteristics, there was no significant difference between the two groups. Operation times tended to be longer in patients who received peg-IFN therapy than in those who did not receive IFN therapy (260 vs. 242 min; $P = 0.05$). There were no hospital-related deaths in this study. Postoperative complications did not differ between the two groups. In the entire population, the 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.0024$) (Fig. 1a). However, there was no significant difference in disease-free survival between the two groups ($P = 0.795$) (Fig. 1b).

Results After Propensity Score Matching

Characteristics of the patients after propensity score analysis are presented in Table 1. Thirty-eight of the 43 patients who received peg-IFN therapy after hepatic resection and an equal number of the 76 patients who did not receive IFN therapy were matched after covariate adjustment. The study group of 76 patients was well matched; in particular, all covariates that significantly affected recurrence and postoperative liver failure in the entire study group were equally distributed between the two matched groups. Matched patients who received peg-IFN therapy after hepatic resection had similar total bilirubin and serum albumin levels and similar platelet counts to matched patients who did not receive IFN therapy. Similarly, the tumor characteristics, the surgical procedure, operation times, and blood loss during the operation in matched patients who received peg-IFN therapy were almost similar to those in patients who did not receive IFN therapy. There were no hospital-related deaths in the matched groups. Postoperative complications also did not differ between the two groups. The median follow-up period for patients who received peg-IFN and those who