that STING could associate with Cardif by MAM interaction. Castanier et al.[41] reported that Cardif-STING interaction was enhanced in cells with elongated mitochondria. In addition, Horner et al.[42,43] observed NS3/4A targeting of MAM-anchored synapse and cleavage of Cardif at MAM but not in mitochondria. These results led us to speculate that interaction between STING and Cardif was enhanced by altering their subcellular localization during viral infection and that NS4B inhibits Cardif activation by interfering with the association between STING and Cardif on MAM-like NS3/4A behavior against host innate immunity.

HCV-NS4B is an ER-localized 27-kDa protein with several functions in the HCV life cycle. Cellular expression of NS4B induces convolution of the ER membrane and formation of a membranous web that harbors HCV replicase complex.[44,45] NS4B also has RNA-binding capacity.[46] In addition, several point mutations of NS4B were found to alter viral replication activity.[33,46,47] The studies above indicate that NS4B provides an important protein-protein or protein-RNA interaction platform within the HCV replication complex and is essential for viral RNA replication. However, there are few reports on the involvement of NS4B with antiviral immune responses. Consistent with our previous study, Moriyama et al.[48] reported that NS4B partially inhibited dsRNA-induced but not TRIF-induced activation of IFN-β. In NS4B-expressing cells, IFN-α induced activation of STAT1 was suppressed.[49] The present study has demonstrated that NS4B functions against the host IFN response, such that NS4B directly interacts with STING and suppresses downstream signaling, resulting in the induction of IFN production.

STING contains a domain homologous to the N terminus of NS4B derived from several flaviviruses, including HCV. In our previous NS4B truncation assay, the NS4B N-terminal domain (amino acids 1-110) was important for suppression of RIG-I–induced IFN-β expression.[19] Consistent with these results, N-terminally truncated NS4B (NS4Bt1-84) significantly suppressed STING and Cardif-induced IFN-β promoter activation, whereas the C terminus of NS4B (NS4Bt85-261) did not (Fig. 7). These results reinforce our hypothesis that NS4B binds STING at its homology domain and blocks the ability of STING to induce IFN-β production.

A small molecule inhibitor of NS4B has been developed and is under preliminary clinical trials.[50] Einav et al.[51] identified clemizole hydrochloride, an H1 histamine receptor antagonist, as an inhibitor of the RNA-binding function of NS4B and HCV RNA replication. A phase 1B clinical trial of clemizole in hepatitis C patients has been completed.[52] Other two NS4B inhibitors which are a compound of amiloride analog and anguizole are under preclinical development.[53,54] The possibility remains that such NS4B inhibitors may suppress HCV replication partly through inhibiting the ability of NS4B to suppress IFN-β production and restore cellular antiviral responses.

In conclusion, IFN production signaling induced by HCV infection and mediated by RIG-I is suppressed by NS4B through a direct interaction with STING. These virus-host interactions help to elucidate the mechanisms of persistent HCV infection and constitute a potential target to block HCV infection.

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Association of ITPA *gene variation and serum ribavirin concentration with a decline in blood cell concentrations during pegylated interferon-alpha plus ribavirin therapy for chronic hepatitis C*

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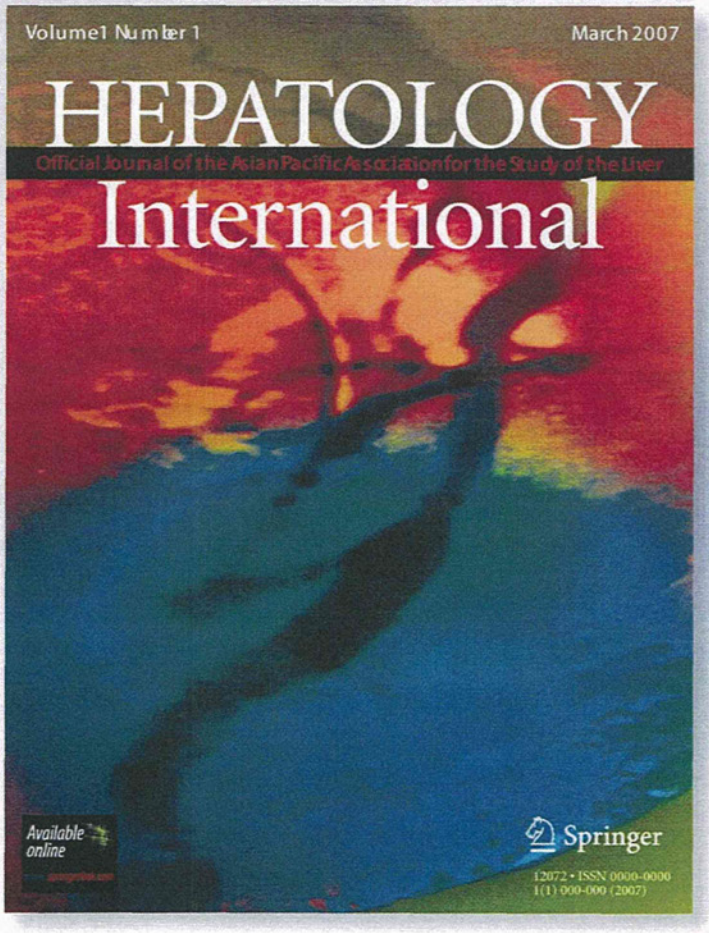

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ORIGINAL ARTICLE

Association of *ITPA* gene variation and serum ribavirin concentration with a decline in blood cell concentrations during pegylated interferon-alpha plus ribavirin therapy for chronic hepatitis C

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Abstract
Background Genetic variation leading to inosine triphosphatase (*ITPA*) deficiency protects chronic hepatitis C patients receiving ribavirin against hemolytic anemia. The relationship between *ITPA* gene variation and serum ribavirin concentration was analyzed in association with a reduction in blood cells and dose reduction of pegylated interferon (PEG-IFN) or ribavirin.

Patients and methods A total of 300 hepatitis C patients treated with PEG-IFN plus ribavirin were analyzed. Genetic polymorphisms were determined in *ITPA* and the quantitative reduction in blood cells from the baseline was analyzed every 4 weeks for the duration of treatment and after the end of therapy. The decline in hemoglobin (Hb) or platelet (PLT) level at week 4 compared to baseline was also assessed according to ribavirin concentrations.
Results Patients with the *ITPA-CA/AA* genotypes showed a lower degree of Hb reduction throughout therapy than those with the *ITPA-CC* genotype and a marked difference in mean Hb reduction was found at week 4 (*CA/AA* −1.0 vs. *CC* −2.8, $p < 0.001$). The *ITPA-CC* genotype had significantly less reduction in the mean platelet count than the *ITPA-CA/AA* genotypes early during treatment ($p < 0.001$ for weeks 4 and 8). Patients with the *ITPA-CA/AA* genotypes were less likely to develop anemia, regardless of the concentration of ribavirin. Patients with baseline PLT counts below $130 \times 10^3/\mu l$ had a significantly lower tendency to achieve sustained virological response (SVR), especially those with the *ITPA-CA/AA* genotypes. ITPA gene variation was not extracted by multivariable analysis as an important predictor of SVR.
Conclusions Despite the fact that ITPA variants were less likely to develop anemia, patients with low baseline PLT counts were difficult to treat, especially those with the *ITPA-CA/AA* genotype. These results may give a valuable pharmacogenetic diagnostic tool for the tailoring of dosing to minimize drug-induced adverse events.

Keywords Hepatitis C virus (HCV) · Pegylated interferon plus ribavirin therapy · *ITPA* (inosine triphosphatase)

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Introduction

Hepatitis C virus (HCV) is a major causative agent of chronic liver disease, and persistent HCV infection may result in liver cirrhosis and hepatocellular carcinoma over the course of 20–30 years [1–3]. Antiviral treatment has been shown to improve liver histology and decrease the incidence of hepatocellular carcinoma in chronic hepatitis C (CHC) patients [4, 5]. A combination of ribavirin plus pegylated interferon (PEG-IFN)-alpha [6, 7] is effective, but less than 50 % of patients infected with HCV genotype 1 treated in this way achieved a sustained virological response (SVR) or a cure of the infection [6, 8]. In particular, failure of treatment is due to either a lack of virological response or relapse after the completion of therapy, despite an initial virological response. Hematologic abnormalities and ribavirin-induced hemolytic anemia necessitate dose reduction and premature withdrawal from therapy in 10–14 % of patients [6, 9–12]. Although new drugs and therapeutic approaches for CHC are being developed actively and several candidates are in early phase trials [13, 14], ribavirin remains mandatory for improving clinical anti-HCV chemotherapeutic responses [15–17].

Given this background, several recent studies have demonstrated that genetic variation leading to inosine triphosphatase (*ITPA*) deficiency, a condition not thought to be clinically important, protects CHC patients receiving ribavirin against hemolytic anemia [18]. However, factors other than *ITPA* gene polymorphism also contribute to the risk of severe anemia and consequent ribavirin dose reduction, and the impact of the ITPA genotype on treatment outcome has been studied with conflicting results [19–23]. The aims of this study were to analyze the relationship between *ITPA* gene variation and serum ribavirin concentration associated with reduction in blood cell concentrations.

Patients and methods

Patients

In this retrospective, cross-sectional case–control study, 300 patients with chronic HCV infection who were treated at Tokyo Medical and Dental University Hospital and associated hospitals, part of the Ochanomizu-Liver Conference Study Group, were enrolled from December 2004 to November 2010. Each patient was treated with combination therapy comprising PEG-IFN (Peg-Intron; Schering-Plough Nordic Biotech, Stockholm, Sweden) 1.2–1.5 μg/kg subcutaneously and ribavirin (Rebetol; Schering-Plough Nordic Biotech) (b.w. < 60 kg: 600 mg po daily; b.w.: 60–80 kg: 800 mg po daily; b.w. > 80 kg: 1,000 mg po daily; in two divided doses). The treatment duration was set at a standard 48 weeks for patients infected with genotype 1b with high viral loads (≥5 log copies/ml) and 24 weeks for patients with genotype 1 with low viral loads (≤5 log copies/ml) or with genotype 2. On-treatment dose reduction and discontinuation of PEG-IFN or ribavirin were decided based on the recommendations of package inserts or the clinical situations of individual patients to avoid possible side effects. The amounts of PEG-IFN and ribavirin administered were expressed as percentages of the target standard total dose over 48 or 24 weeks, according to body weight before therapy. All patients had histologically or clinically proven chronic active hepatitis and were positive for anti-HCV antibodies and serum HCV RNA by RT-PCR. Patients with a positive test for serum hepatitis B surface antigen, coinfection with other HCV genotypes, coinfection with human immunodeficiency virus, other causes of hepatocellular injury (such as alcoholism, autoimmune hepatitis, primary biliary cirrhosis, or a history of treatment with hepatotoxic drugs), and a need for hemodialysis were excluded. During treatment, patients were assessed as outpatients at weeks 2, 4, 6, and 8, and then every 4 weeks for the duration of treatment and at every 4 weeks after the end of therapy. Biochemical and hematological testing was carried out by a central laboratory.

Informed consent was obtained from each patient who participated in the study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the ethics committee of this hospital and of all the participating hospitals.

Patient evaluation

The following factors were analyzed to determine whether they were related to the efficacy of combination therapy: age, gender, body mass index (BMI), grade of inflammation and stage of fibrosis on liver biopsy, pretreatment biochemical parameters, such as white blood cells (WBC), hemoglobin (Hb), platelet (PLT) count, alanine transaminase (ALT) level, gamma-glutamyl transpeptidase (γ-GTP) level, serum creatinine, and serum HCV RNA level (log IU/ml). Liver biopsy specimens were evaluated blindly, to determine the grade of inflammation and stage of fibrosis, by an independent interpreter who was not aware of the clinical data. The activity of inflammation was graded on a scale of 0–3: A0 shows no activity, A1 shows mild activity, A2 shows moderate activity, and A3 shows severe activity. Fibrosis was staged on a scale of 0–4: F0 shows no fibrosis, F1 shows mild fibrosis, F2 shows moderate fibrosis with few septa, F3 shows severe fibrosis with numerous septa without cirrhosis, and F4 shows cirrhosis.

Single nucleotide polymorphism genotyping

Human genomic DNA was extracted from whole blood from each patient. Genetic polymorphisms, rs1127354 in *ITPA* and rs8099917 around the *IL28B* gene, were determined by real-time detection PCR with the TaqMan probe or DigiTag2 assay, typing one tag SNP located within each locus [24]. Another functional SNP, rs727010 within the *ITPA* gene, was excluded because it does not vary in the Asian population, as reported in the International HapMap Project database. Preliminary genotyping of 100 patients from the study population did not reveal variation in that SNP.

Measurement of the ribavirin concentration

The ribavirin concentration was measured using high-performance liquid chromatography (HPLC; SRL, Tokyo, Japan); the detection limit was 50 ng/ml.

Outcomes

The primary end point showed a decline in the blood cell concentration and dose reduction in PEG-IFN or ribavirin at week 4; the secondary end point was an SVR. SVR was defined as undetectable serum HCV RNA at 24 weeks after the end of treatment. Adverse events and drug adherence were recorded.

Statistical analyses

The association between the individual *ITPA* SNP and the occurrence of a significant decline in the Hb concentration was evaluated by a basic allelic test and calculated using the Chi-square test. Multivariate logistic regression analysis with stepwise forward selection was performed with p values of less than 0.05 as the criteria for model inclusion. These statistical analyses were conducted using the SPSS software package (SPSS 18 J; SPSS, Chicago, IL, USA). Discrete variables were evaluated by Fisher's exact probability test. The p values were calculated by two-tailed Student's t-test for continouous data and the Chi-square test for categorical data; values less than 0.05 were considered as statistically significant.

Results

Association between *ITPA* rs1127354 genotypes and decline in blood cells

The clinical characteristics of the 300 patients are summarized in Table 1. On an intention-to-treat (ITT) analysis, 79 (41 %) of the 195 patients infected with HCV genotype 1 achieved SVRs and 85 (81 %) of the 105 patients infected with HCV genotype 2 achieved SVR.

The quantitative reduction in blood cells from the baseline according to the *ITPA* rs1127354 genotypes is shown in Fig. 1. Patients with the *ITPA-CA/AA* genotypes showed a lower degree of Hb reduction throughout the therapy than those with the *ITPA-CC* genotype (Fig. 1a), and a marked difference in the mean Hb reduction was found at week 4 (*AA/CA* −1.0 vs. *CC* −2.8, $p < 0.001$). These results show that the *ITPA-CA/AA* genotypes are

Table 1 Baseline characteristics of participating patients

Total number	300
HCV genotype (1/2)	195/105
ITPA gene (rs1127354); *AA/CA/CC*	2/80/218
IL28B gene (rs8099917); TT/non-TT	225/75
Age (years)[a]	57 (20–78)
Gender (male/female)	153/147
Body mass index (kg/m^2)[a]	23.5 (15.3–33.7)
Histology at biopsy	
Grade of inflammation; A0–1/A2–3/ND	96/109/95
Stage of fibrosis; F0–2/F3–4/ND	165/40/95
Baseline white blood cells (/μl)[a]	5270 (2000–10,300)
Baseline hemoglobin (g/dl)[a]	14.2 (9.7–17.5)
Baseline platelet count (×10^3/μl)[a]	170 (61–458)
Baseline ALT (IU/l)[a]	85 (9–541)
Baseline γ-GTP (IU/l)[a]	67 (10–731)
Baseline serum creatinine (mg/dl)[a]	0.72 (0.4–1.4)
Serum HCV RNA level ($\log_{10}$ IU/ml)[a, b]	6.1 (2.9–7.6)
Initial RBV dose (mg/kg)[a]	11.2 (6.0–15.7)
Serum RBV concentration at week 4 (μg/ml)[a]	2.3 (0.4–5.2)

HCV hepatitis C virus, *ALT* alanine transaminase

[a] Data are shown as median (range) values

[b] Data are shown as log (IU/ml)

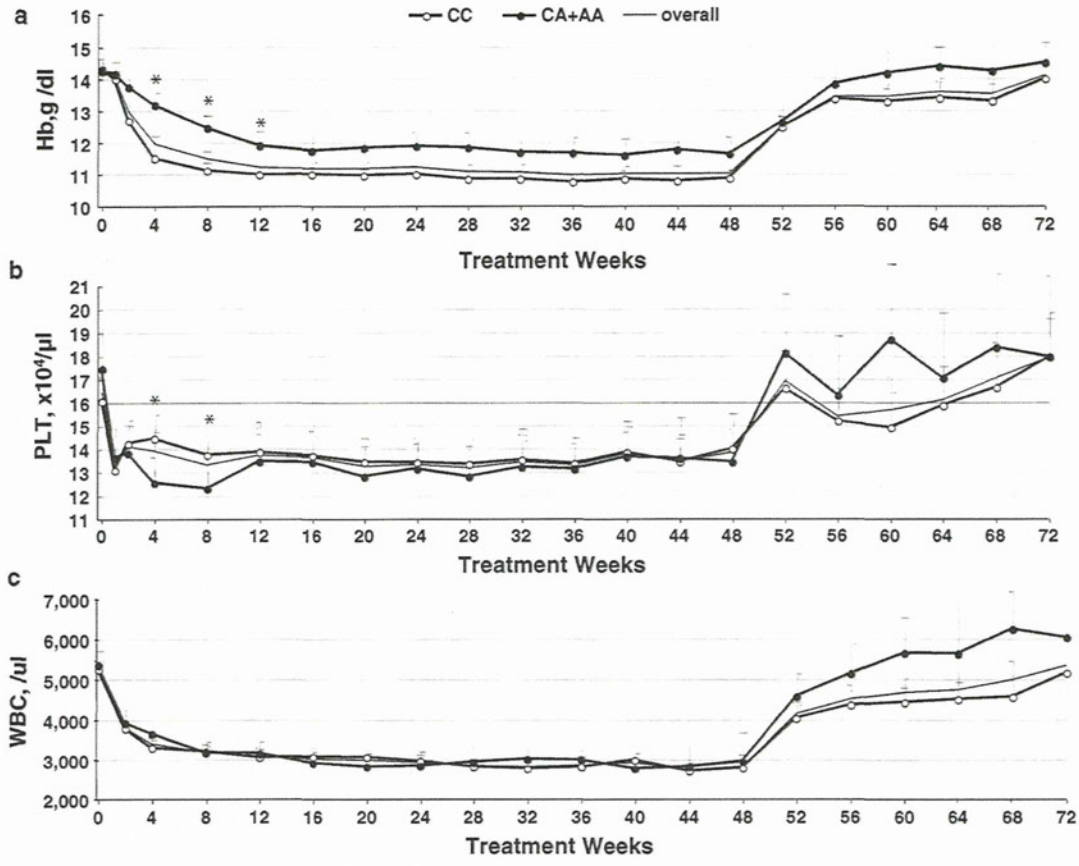

Fig. 1 The quantitative reduction of blood cells from the baseline according to the *ITPA* rs1127354 major and minor variants. Blood cell counts were determined every 4 weeks for the duration of treatment and at every 4 weeks after the end of therapy for Hb (**a**), PLT (**b**), and WBC (**c**). Patients with *ITPA-CC* are indicated by open circles and those with *ITPA-CA/AA* by closed circles. *Error bars* indicate mean ± SE. *Asterisks* indicate statistical significance. *WBC* white blood cells, *Hb* hemoglobin, *PLT* platelet

associated significantly with a lower reduction in Hb levels throughout the therapy and protect against the development of severe hemolytic anemia. Figure 1b shows that the *ITPA-CC* genotype is associated with a significantly lower reduction in the mean PLT count than the *ITPA-CA/AA* genotypes at the early stages of treatment ($p < 0.001$ for weeks 4 and 8), possibly due to a reactive increase in the PLT count. That is to say that a severe decline in the Hb concentration, which was associated particularly with the *ITPA-CC* genotype, was inversely correlated with platelet reduction. There were no differences in the WBC count between the *ITPA-CC* and *ITPA-CA/AA* variants (Fig. 1c).

To evaluate the clinical relevance of the *ITPA* genotype and decline in the Hb concentration or PLT counts, the decline in Hb and PLT levels was analyzed at week 4 and compared with baseline according to ribavirin concentrations in patients with *ITPA-CC* and *ITPA-CA/AA* (Fig. 2). Patients with the *ITPA-CA/AA* genotype were less likely to develop anemia regardless of the concentration of ribavirin. These results show that the *ITPA* minor variant *A* has a protective phenotype against treatment-induced anemia, and the quantitative reduction of Hb in patients with *ITPA-CC* was greater, especially with a high concentration of ribavirin. In contrast, the patients with the *ITPA-CC* genotype had a lower reduction in the PLT count than the patients with the *ITPA-CA/AA* genotypes, as reported previously [25].

Relationship between *ITPA* rs1127354 variants and treatment outcome due to dose reduction of PEG-IFN or ribavirin

The percentages of patients requiring PEG-IFN or ribavirin dose reduction at week 4 among those with the *ITPA* rs1127354 major and minor variants in accordance with the incidence of anemia or thrombocytopenia were investigated. There was a significant difference between the *ITPA-CC* and *ITPA-CA/AA* variants in terms of the need for ribavirin dose reduction. At week 4 of treatment, ribavirin

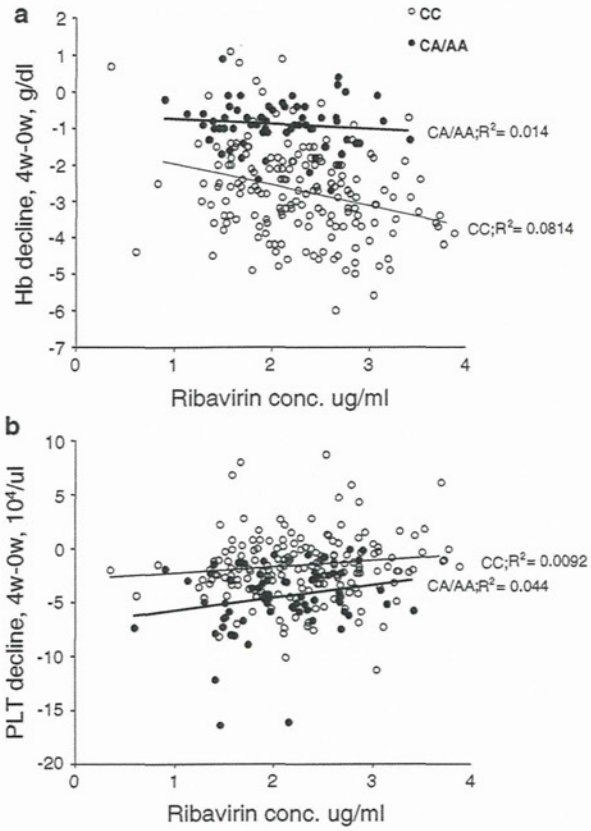

Fig. 2 ITPA genotype and decline in Hb and platelets at week 4 compared with baseline according to ribavirin concentrations in patients with *ITPA-CC* and *ITPA-CA/AA*. The decline in Hb (**a**) and PLT (**b**) levels was analyzed at week 4 and compared with baseline according to ribavirin concentrations in patients with *ITPA-CC* and *ITPA-CA/AA*, to evaluate the clinical relevance of ITPA genotypes and decline in Hb or PLT. Patients with *ITPA-CC* are indicated by open circles and those with *ITPA-CA/AA* by *closed circles*. The *Y* axis indicates Hb or PLT concentrations (g/dl or 10,000/μl) and the *X* axis indicates ribavirin concentration (μg/ml)

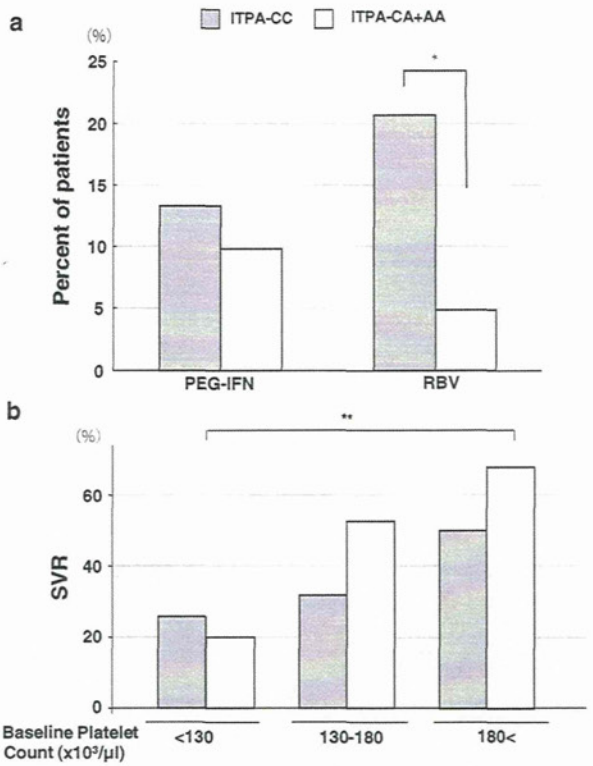

Fig. 3 Relationship between *ITPA* rs1127354 major and minor variants and treatment outcome due to dose reduction of PEG-IFN or ribavirin. **a** The percentages of patients requiring PEG-IFN or ribavirin dose reduction at week 4 among patients with *ITPA* rs1127354 major and minor variants. The *Y* axis indicates the percentage of patients who required dose reduction. *Asterisk* indicates statistical significance, $p = 0.001$. **b** Percentages of SVR in patients infected with genotype 1 according to the baseline platelet count. *Asterisks* indicate statistical significance, $p = 0.024$

doses were reduced in 20.6 % of patients with *ITPA-CC*, but in only 4.9 % of patients with *ITPA-CA/AA* ($p = 0.001$; Fig. 3a). Similar to ribavirin, PEG-IFN dose reduction was apparently more common in patients with *ITPA-CC*, although this did not reach statistical significance.

The treatment outcome in patients with *ITPA-CC* and *ITPA-CA/AA* was analyzed according to baseline PLT counts because an inverse correlation was observed in the Hb and PLT decline between the *ITPA-CC* and *ITPA-CA/AA* variants. Figure 3b shows the percentages of SVR in the patients infected with genotype 1 according to the baseline PLT count. Patients with baseline PLT counts below $130 \times 10^3/\mu l$ had a significantly lower tendency to achieve SVR than patients with baseline PLT counts above $180 \times 10^3/\mu l$ ($p = 0.024$) and the difference was more pronounced in patients with the *ITPA-CA/AA* genotypes. No difference was observed between the *ITPA-CC* and *ITPA-CA/AA* variants according to the baseline PLT count in patients infected with genotype 2.

Knowing that significantly less frequent ribavirin dose reduction was necessary in patients with the *ITPA-CA/AA* genotypes, it was determined whether the *ITPA* gene variation affected final treatment outcomes. The treatment outcomes were available for 195 patients infected with genotype 1 and 105 patients infected with genotype 2. On multivariable analysis of patients infected with genotype 1 (Table 2), the IL28B genotype was the most important predictor of SVR at baseline [adjusted odds ratio 16.949 (95 % confidence interval 0.014–0.248), $p < 0.0001$], along with baseline serum HCV RNA level [7.813 (0.046–0.359), $p = 0.003$]. *ITPA* gene variation was not significant in patients infected with genotype 1 (Table 2) or in those infected with genotype 2 (Table 3).

Table 2 Comparison of clinical and laboratory characteristics of patients infected with genotype 1 based on the therapeutic response

All patients	SVR ($n = 79$)	Non-SVR ($n = 116$)	Univariate analysis p value	Multivariate analysis OR	95 % CI	p value
Age (years)[a]	57 (24–8)	61 (21–78)	0.027	1.024	0.972–1.080	0.373
Gender (male/female)	42/37	56/60	0.560	–		
BMI (kg/m^2)[a]	23.4 (16.9–3.7)	23.3 (15.3–30.8)	0.730	–		
Grade of inflammation (A0–1/2–3/ND)	27/36/16	28/44/44	0.043	3.658	0.533–25.086	0.187
Stage of fibrosis (F0–2/3–4/ND)	55/8/16	51/21/44	0.002	2.847	0.788–10.285	0.110
Baseline white blood cells (/μl)[a]	5,230 (2,600–9,650)	4,900 (2,000–9,700)	0.038	1.000	1.000–1.000	0.505
Baseline hemoglobin (g/dl)[a]	14.5 (11.9–7.5)	14.0 (9.7–17.0)	0.027	1.423	0.949–2.134	0.088
Baseline platelet count ($\times 10^3$/μl)[a]	174 (73–458)	145 (61–309)	0.001	1.075	0.984–1.173	0.108
Baseline γGTP (IU/l)[a]	35 (10–731)	50 (10–618)	0.012	1.003	0.998–1.008	0.284
Serum HCV RNA level [log (IU/ml)][a, b]	6.1 (2.9–7.3)	6.3 (5.1–7.4)	0.003	7.813	0.046–0.359	0.000
RBV concentration at week 4 (μg/ml)[a]	2.4 (1.4–5.2)	2.3 (0.4–4.8)	0.154	–		
Substitutions in the ISDR (≤ 1/ ≥ 2/ND)	52/18/9	91/7/18	0.004	2.469	0.105–1.555	0.188
Substitutions of core amino acid 70 (wild type/mutant/ND)	44/23/12	60/39/17	0.799	–		
IL28B SNPs (rs8099917; TT/non-TT)	71/8	68/48	0.000	16.949	0.014–0.248	0.000
ITPA gene (rs1127354; *CC*/non-*CC*)	51/28	89/27	0.064	–		

Medians (ranges) are shown

OR odds ratio, *CI* confidence interval, *RBV* ribavirin, *SVR* sustained virological response

[a] Data are shown as median (range) values

[b] Data are shown as log (IU/ml)

Table 3 Comparison of clinical and laboratory characteristics of patients infected with genotype 2 based on the therapeutic response

All patients	SVR ($n = 85$)	Non-SVR ($n = 20$)	Univariate analysis p value	Multivariate analysis OR	95 % CI	p value
Age (years)[a]	56 (20–72)	61 (48–69)	0.019	1.091	0.838–1.004	0.061
Gender (male/female)	42/43	13/7	0.226	–		
BMI (kg/m^2)[a]	23.0 (16.9–33.5)	24.3 (19.4–27.7)	0.071	–		
Grade of inflammation (A0–1/2–3/ND)	36/22/27	5/7/8	0.356	–		
Stage of fibrosis (F0–2/3–4/ND)	50/8/27	9/3/8	0.506	–		
Baseline white blood cells (/μl)[a]	5,200 (2,600–10,300)	4,310 (2,380–7,900)	0.124	–		
Baseline hemoglobin (g/dl)[a]	14.2 (11.0–17.3)	14.0 (10.3–17.4)	0.967	–		
Baseline platelet count ($\times 10^3$/μl)[a]	186 (68–340)	175 (80–284)	0.172	–		
Baseline γ-GTP (IU/l)[a]	31 (11–209)	74 (14–292)	0.017	1.014	0.972–1.000	0.050
Serum HCV RNA level [log (IU/ml)][a, b]	6.1 (3.6–7.4)	6.2 (4.0–7.6)	0.601	–		
RBV concentration at week 4 (μg/ml)[a]	2.1 (0.6–4.1)	2.1 (1.3–3.5)	0.877	–		
Substitutions in the ISDR (≤ 1/≥ 2/ND)	46/26/13	11/4/5	0.466	–		
IL28B SNPs (rs8099917; TT/non-TT)	70/15	16/4	0.115	–		
ITPA gene (rs1127354; *CC*/non-*CC*)	60/25	18/2	0.092	–		

Medians (ranges) are shown

OR odds ratio, *CI* confidence interval, *RBV* ribavirin, *SVR* sustained virological response

[a] Data are shown as median (range) values

[b] Data are shown as log (IU/ml)

Discussion

This study confirmed the most recent report that the ITPA rs1127354 genotype is a useful marker for predicting hematological side effects of treatment with ribavirin [25, 26]. The results show that the severe Hb decline, which is found predominantly in patients with the *ITPA-CC* genotypes, was inversely correlated with platelet reduction and that the opposite correlation is observed in Hb and PLT decline, but not in WBC concentration, as reported previously [25]. While patients with the *ITPA-CA/AA* genotype were less likely to develop anemia, regardless of the concentration of ribavirin (Fig. 2), patients with baseline PLT counts below $130 \times 10^{-3}/\mu l$ had a significantly lower tendency to achieve SVR, especially those with the *ITPA-CA/AA* genotype, possibly due to PEG-IFN dose reduction in response to the PLT decline. As a result, ITPA gene variation was not extracted as an important predictor of SVR in CHC patients with either genotype 1 or 2, which is consistent with a very recent report [27].

Ribavirin is directly toxic to erythrocytes and is associated with hemolysis, which is usually reversible and dose related [28, 29]. Ribavirin is incorporated into erythrocytes where it undergoes phosphorylation by adenosine kinase to its pharmacologically active forms. The ribavirin–phosphate conjugates are unable to cross the erythrocyte cell membrane and are, therefore, accumulated intracellularly and cleared slowly from red cells, with a half-life of ~40 days [30]. The possible mechanism of protection against ribavirin-induced hemolysis is that ITP deficiency or low-activity variants (*ITPA-CA/AA* groups) are associated with the accumulation of ITP in red blood cells [31, 32] and ITP confers protection against ribavirin-induced ATP reduction by substituting for erythrocyte GTP, which is depleted by ribavirin, in the biosynthesis of ATP [33]. In addition, the sequence homology of thrombopoietin (TPO) and erythropoietin (EPO) may explain the synergy of the physiological role of TPO and EPO in platelet production [25]. Ochi et al. [23] analyzed the genomes of Japanese patients, including the *ITPA* and *DDRGK1* loci, which are located together on chromosome 20. Their report indicates that the *ITPA* SNP, rs1127354, which was genotyped in this study, represents the dominant variant of *ITPA* deficiency that protects against ribavirin-induced anemia in Japanese/Asian populations. Ribavirin is a synthetic guanosine analog and has in vitro activity against a wide range of RNA and DNA viruses [34]. Possible antiviral mechanisms of ribavirin include immune modulation by switching the T-cell phenotype from type 2 to type 1 [35], antiproliferative effects by the inhibition of cellular GTP synthesis [34], and direct inhibition of virus replication [36]. Although monotherapy with ribavirin showed a minimal effect on the viral load and almost no effect on viral clearance [37–40], the combinatory use of ribavirin with IFN elicits strong synergistic effects against HCV in vitro [41] and in vivo [28, 29]. Interestingly, Snoeck et al. [42] reported that the probability of SVR was not influenced by the ribavirin dose in patients with HCV genotype 2 or 3 infection, but increased as a function of ribavirin dose in patients with HCV genotype 1 infection (40–50 % increase in the probability of SVR for a ribavirin dose increase of 12–6 mg/kg^1). Indeed, while there are several directly acting antiviral (DAA) agents being tested for clinical efficacy against hepatitis C [13, 14], most experts believe that ribavirin remains mandatory for improving clinical anti-HCV chemotherapeutic effects when new drugs are approved to treat hepatitis C.

There are a number of ongoing trials registerd with ClinicalTrials.gov. Although the limited results have been presented thus far, the addition of ribavirin without IFN was shown to accelerate the HCV RNA level decline and reduce the incidence of virological breakthroughs, at least in the short term [43]. New therapeutic approaches using combinations of DAA agents in the IFN-spared regiments with or without ribavirin are currently under study, but ribavirin appears to exert its own effect independently of IFN in some studies. While *ITPA* gene variation was not extracted as an important predictor of SVR in combination therapy with PEG-IFN as reported, including our data, the *ITPA* gene may play an important role as a significant and independent pretreatment marker to predict SVR in IFN-free regiments.

In conclusion, the results presented here show that an inverse correlation is observed in the reduction in Hb and PLT count in patients with the *ITPA-CC* and *ITPA-CA/AA* genotypes. Despite the fact that *ITPA* variants were less likely to develop anemia, regardless of a high concentration of ribavirin, patients with baseline PLT counts below $130 \times 10^3/\mu l$ had a significantly lower tendency to achieve SVR, especially those with the *ITPA-CA/AA* genotype. These results may give a valuable pharmacogenetic diagnostic tool for the tailoring of ribavirin dosing to minimize drug-induced adverse events and for further optimization of the clinical anti-HCV treatment outcomes.

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Research Article

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Data mining model using simple and readily available factors could identify patients at high risk for hepatocellular carcinoma in chronic hepatitis C

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Background & Aims: Assessment of the risk of hepatocellular carcinoma (HCC) development is essential for formulating personalized surveillance or antiviral treatment plan for chronic hepatitis C. We aimed to build a simple model for the identification of patients at high risk of developing HCC.

Methods: Chronic hepatitis C patients followed for at least 5 years (n = 1003) were analyzed by data mining to build a predictive model for HCC development. The model was externally validated using a cohort of 1072 patients (472 with sustained virological response (SVR) and 600 with nonSVR to PEG-interferon plus ribavirin therapy).

Results: On the basis of factors such as age, platelet, albumin, and aspartate aminotransferase, the HCC risk prediction model identified subgroups with high-, intermediate-, and low-risk of HCC with a 5-year HCC development rate of 20.9%, 6.3–7.3%, and 0–1.5%, respectively. The reproducibility of the model was confirmed through external validation ($r^2 = 0.981$). The 10-year HCC development rate was also significantly higher in the high- and intermediate-risk group than in the low-risk group (24.5% *vs.* 4.8%; $p < 0.0001$). In the high-and intermediate-risk group, the incidence of HCC development was significantly reduced in patients with SVR compared to those with nonSVR (5-year rate, 9.5% *vs.* 4.5%; $p = 0.040$).

Conclusions: The HCC risk prediction model uses simple and readily available factors and identifies patients at a high risk of HCC development. The model allows physicians to identify patients requiring HCC surveillance and those who benefit from IFN therapy to prevent HCC.

Keywords: Decision tree; Prediction; Pegylated interferon; Ribavirin; Risk.
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Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide [1] and its incidence is increasing in many countries [2]. Chronic viral hepatitis is responsible for 80% of all HCC cases [2]. The need to conduct HCC surveillance should be determined according to the risk of HCC development because this surveillance is cost-effective only in populations with an annualized cancer development rate of ⩾1.5% [3]. The annualized rate of developing HCC from type C liver cirrhosis is 2–8% [4–6], indicating that this population with type C liver cirrhosis needs surveillance. However, the annualized rate of HCC development is <1.5% in patients with chronic hepatitis C but without cirrhosis and the benefit of surveillance for all patients with chronic hepatitis has not yet been established [3]. HCC surveillance may be needed for patients with advanced fibrosis because the risk of HCC development increases in parallel with the progression of liver fibrosis [7,8]. Liver biopsy is the most accurate means of diagnosing fibrosis, but a single liver biopsy cannot indicate long-term prognosis because liver fibrosis progresses over time. Serial liver biopsies are not feasible because of the procedure's invasiveness. Moreover, factors other than fibrosis, such as advanced age, obesity, sex, lower albumin, and low platelet counts, also contribute to the development of HCC from chronic hepatitis C [8–11]. Therefore, these factors must be considered while assessing the risk of HCC development.

A meta-analysis of controlled trials [12] has shown that interferon (IFN) therapy reduced the rate of HCC development in patients with type C liver cirrhosis. However, there was a marked heterogeneity in the magnitude of the prevention effect

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of IFN on HCC development among the studies, probably due to the large differences in the baseline rate of HCC development among the different trials [12]. Whether the incidence of HCC development could be reduced in all patients with chronic hepatitis C, especially in those without liver cirrhosis, remains to be elucidated.

Data mining analysis, unlike conventional statistical analysis, is performed in an exploratory manner without considering a predefined hypothesis. Decision tree analysis, the major component of data mining analysis, is used to extract relevant factors from among various factors. These relevant factors are then combined in an orderly sequence to identify rules for predicting the incidence of the target outcome [13]. Data mining analysis has been used to define prognostic factors in various diseases [14–20]. In the field of hepatic diseases, data mining analysis has proven to be a useful tool for predicting early response [21], sustained virological response (SVR) [22–25], relapse [26], and adverse events [27] in patients with chronic hepatitis C treated with pegylated interferon (PEG-IFN) plus ribavirin (RBV). The findings of data mining analysis are expressed as flowcharts and are therefore easily understood [28] and readily available for clinical use, even by physicians without a detailed understanding of statistics.

In the present study, data mining analysis was used to identify risk factors for HCC development in a cohort of patients with chronic hepatitis C who had been followed for at least 5 years. An HCC risk prediction model was constructed on the basis of simple and generally available tests because the goal was to make the model easy to use in the clinic. The suitability, reproducibility, and generalizability of the results were validated using the data of an external cohort that was independent of the model derivation cohort.

Materials and methods

Patients

The model derivation cohort consisted of 1003 chronic hepatitis C patients without cirrhosis who had a non-sustained virological response (nonSVR) to previous IFN administered at the Musashino Red Cross Hospital and were followed for at least 5 years. Patients who had SVR or those who were followed for less than 5 years were not included. An analytical database on age, body mass index, albumin, aspartate aminotransferase (AST) levels, alanine aminotransferase (ALT) levels, γ-glutamyltransferase (GGT) levels, total bilirubin levels, total cholesterol levels, hemoglobin levels, and platelet count at the start of the observation was created. Histological data such as fibrosis stage, activity grade, or degree of steatosis was not included in the database because the goal of the present study was to make the model on the basis of simple and generally available tests. The patients who developed HCC more than 5 years after the start of the observation were considered not to have developed HCC by the 5-year point because the model was intended to predict HCC development within 5 years. The 1072 chronic hepatitis C patients included in the external validation cohort were treated with PEG-IFN and RBV at the University of Yamanashi, Tokyo Medical and Dental University, Osaka University, Osaka City University, Nagoya City University, or Toranomon Hospital and followed for at least 5 years. Among them, 600 had nonSVR and 472 had SVR. Data from nonSVR patients in this external cohort were used for external validation of the HCC prediction model. To assess the preventive effect of PEG-IFN plus RBV therapy on HCC development, the cumulative HCC development rate was compared between SVR and nonSVR patients in the external validation cohort after stratification by the risk of HCC development as determined by data mining analysis. Informed consent was obtained from each patient. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional review committees of all concerned hospitals.

HCC surveillance and diagnosis

HCC surveillance was conducted by performing abdominal ultrasonography every 4–6 months. Contrast-enhanced computer tomography, magnetic resonance imaging, or angiography were performed when abdominal ultrasonography suggested a new lesion suspicious for HCC. Classical HCC was diagnosed for tumors showing vascular enhancement with washout on at least two types of diagnostic imaging. Tumor biopsy was used to diagnose tumors with non-classical imaging findings.

Statistical analysis

The IBM-SPSS Modeler 13 (IBM SPSS Inc., Chicago, IL, USA) was used for decision tree analysis. The statistical methods used have been described previously [21,22,24–27]. In brief, the software searched the analytical database for the factor that most effectively predicted HCC development and for its cutoff value. The patients were divided into two groups according to that predictor. Each divided group was repeatedly assessed and divided according to this 2-choice branching method. Branching was stopped when the number of patients decreased to ⩽20 to avoid over fitting. Finally, an HCC risk prediction model was created through this analysis. The model classified patients into subgroups with different HCC development rates in a flowchart form. For model validation, nonSVR patients from an external cohort were individually fitted into the model and classified into the subgroups and the HCC development rates of those subgroups were then calculated. The suitability and reproducibility of the model were validated by comparing the subgroup HCC development rates of the model derivation group to those of the validation group.

On univariate analysis, Student's *t*-test was used for continuous variables and Fisher's exact test was used for categorical data. Logistic regression was used for multivariate analysis. A log-rank test for Kaplan–Meier analysis was used to statistically test HCC development rates over time. *p*-Values of <0.05 were considered significant. SPSS Statistics 18 (IBM SPSS Inc.) was used for these analyses.

Results

Univariate and multivariate analysis of factors associated with HCC development

The baseline characteristics of patients are shown in Table 1. The 5-year HCC development rate in the model derivation group was 6.2%, which did not differ significantly from the rate of 6.0% in the nonSVR group of the external cohort, but the rate of 2.0% in the SVR group of the external cohort was significantly lower than that in the model derivation group ($p = 0.0003$) and the nonSVR group of the external cohort ($p = 0.0012$). On univariate analysis, the factors found to be associated with HCC development in the model derivation cohort were age, AST levels, albumin levels, total cholesterol levels, and platelet count. On multivariate analysis, age (odds ratio 1.086), albumin levels (odds ratio 0.248), and platelet count (odds ratio 0.842) were significant predictors of HCC development (Table 2).

HCC risk prediction model by data mining analysis

The results of decision tree analysis are presented in Fig. 1. Age was selected as the first predictor. The 5-year HCC development rate was 3.4% in younger patients (<60 years) and 8.6% in older patients (⩾60 years). The second predictor for younger patients (<60 years) was platelet count. The HCC development rate was 6.9% in patients with a lower platelet count ($<150 \times 10^9$/L) and 0.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The second predictor for older patients (⩾60 years) was also platelet count. The HCC development rate was 13.1% in patients with a lower platelet count ($<150 \times 10^9$/L) and 1.8% in patients with a higher count ($\geqslant 150 \times 10^9$/L). The third predictor was albumin levels,

Table 1. Baseline characteristics of patients for model deviation and external validation.

	Model derivation (n = 1003)	External cohort, non-SVR (n = 600)	External cohort, SVR (n = 472)
Sex: Male/Female*	463 (46%)/540 (54%)	306 (51%)/294 (49%)	299 (63%)/173 (37%)
Age (yr)	57.3 (11.1)	55.9 (9.6)	51.4 (10.6)
Body mass index (kg/m^2)	23.5 (3.2)	23.4 (3.3)	23.3 (3.1)
Albumin (g/dl)	4.1 (0.3)	4.0 (0.4)	4.0 (0.3)
AST (IU/L)	64.2 (36.5)	67.3 (43.8)	62.5 (48.3)
ALT (IU/L)	80.6 (55.1)	81.2 (62.3)	88.6 (82.1)
GGT (IU/L)	59.3 (50.5)	67.6 (65.1)	55.7 (71.2)
Total cholesterol (mg/dl)	172.1 (31.5)	168.2 (31.0)	174.3 (33.7)
Platelet (10^9/L)	154.0 (53.0)	153.7 (53.2)	176.6 (49.7)
Hemoglobin (g/dl)	13.3 (1.5)	14.2 (1.5)	14.4 (1.4)
HCC development within 5 years: n (%)*	62 (6.2%)	36 (6.0%)	10 (2.0%)

Data expressed as mean (standard deviation) unless otherwise indicated.
AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; HCC, hepatocellular carcinoma; SVR, sustained virological response.
*Data expressed as number of patients (percentage).

whose cutoff value was 3.75 g/dl in patients with a higher platelet count ($\geq 150 \times 10^9$/L). The HCC development rate was 6.3% when albumin levels were lower (<3.75 g/dl) and 1.5% when levels were higher ($\geq$3.75 g/dl). The cutoff value for albumin levels was 4.0 g/dl in patients with a lower platelet count ($<150 \times 10^9$/L). The HCC development rate was 20.9% when albumin levels were lower (<4.0 g/dl) and 6.4% when levels were higher ($\geq$4.0 g/dl). The fourth and final predictor was AST levels. The HCC development rate was 7.3% when AST levels were at least 40 IU/L and 0% when the levels were <40 IU/L. On the basis of this analysis, seven subgroups with a 5-year HCC development rate of 0–20.9% were identified. The area under the receiver operating characteristic curve according to the HCC risk prediction model was 0.817.

External validation of the HCC risk prediction model with an independent external cohort

Six hundred nonSVR patients from an external cohort were fitted into the HCC risk prediction model and classified into the seven subgroups. The 5-year HCC development rate of these subgroups was 0–17.9%. The HCC development rate in the individual subgroups of the model derivation group was closely correlated to that in the corresponding subgroups of the external validation group (Fig. 2; correlation coefficient $r^2 = 0.981$). The HCC development rate in the subgroup of patients with the highest risk of HCC development (high-risk group) according to the model older age ($\geq$60 years) with a lower platelet count ($<150 \times 10^9$/L) and lower albumin levels (<4.0 g/dl) was 20.9% in the model derivation group and 17.9% in the external validation group. The intermediate-risk group or the patients with an HCC development rate of at least 5% consisted of the following three subgroups: (1) older age ($\geq$60 years), lower platelet count ($<150 \times 10^9$/L), higher albumin levels ($\geq$4.0 g/dl), and higher AST levels ($\geq$40 IU/L); (2) older age ($\geq$60 years), higher platelet count ($\geq 150 \times 10^9$/L), and lower albumin levels (<3.75 g/dl); and (3) younger age (<60 years) and lower platelet count ($<150 \times 10^9$/L). In these intermediate-risk groups, the 5-year HCC development rate was 6.3–7.3% in the model derivation group and 5.3–7.9% in the external validation group. The low-risk group consisted of the following three subgroups: (1) younger age (<60 years) and higher platelet count ($\geq 150 \times 10^9$/L); (2) older age ($\geq$60 years), lower platelet count ($<150 \times 10^9$/L), higher albumin levels ($\geq$4.0 g/dl), and lower AST levels (<40 IU/L); and (3) older age ($\geq$60 years), higher platelet count ($\geq 150 \times 10^9$/L), and higher albumin levels ($\geq$3.75 g/dl). In these low-risk groups, the 5-year HCC development rate was 0–1.5% in the model derivation group and 0–2.9% in the external validation group.

Table 2. Multivariable analysis of factors associated with subsequent development of HCC within 5 years.

	Odds ratio	95% CI	*p* value
Age	1.086	1.029-1.146	0.003
Albumin	0.248	0.100-0.613	0.003
Platelet	0.842	0.769-0.921	<0.0001

CI, confidence interval.

Predictability of the HCC risk prediction model on HCC development rate beyond 5 years

Cumulative HCC development rates in the high-, intermediate-, and low-risk groups were compared over time using the Kaplan–Meier method. The 10-year rates were 28.9% in the high-risk group, 22.9% in the intermediate-risk group, and 4.8% in the low-risk group (Fig. 3A). The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups had a significantly higher cumulative HCC development rate than the low-risk group beyond 5 years (Fig. 3B; 5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; p <0.0001).

Effect of response to PEG-IFN plus RBV therapy in the reduction of HCC development: analysis stratified by the HCC risk prediction model

The 600 nonSVR patients and 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and

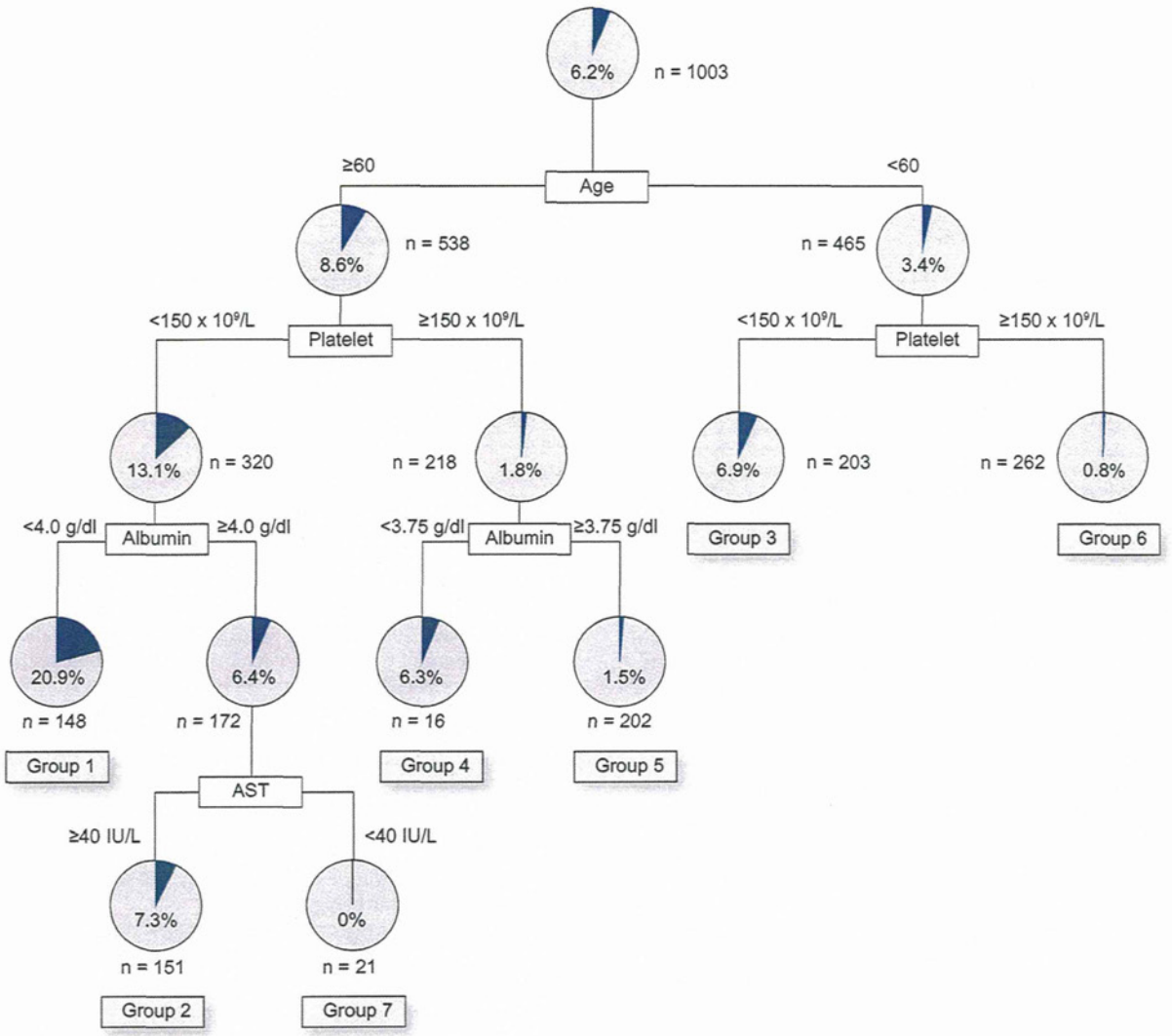

Fig. 1. The decision tree model of HCC development within 5 years. Boxes indicate the factors used to differentiate patients and the cutoff values for those different groups. Pie charts indicate the HCC development rate within 5 years for each group of patients after differentiation. Terminal groups of patients differentiated by analysis are numbered from 1 to 7.

classified into the high-and intermediate-risk group or the low-risk group, as defined above. The HCC development rate was significantly lower in SVR patients than in nonSVR patients in the high-and intermediate-risk group (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$, log-rank test). In the low-risk group, the 5-year rate was 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates were low and not significantly different ($p = 0.331$, log-rank test) (Fig. 4).

Discussion

An awareness of the risk of HCC development in the context of routine care for chronic hepatitis C is essential for formulating an HCC surveillance plan personalized for individual patients. The risk of developing HCC from chronic hepatitis is lower than that from cirrhosis [7]; therefore, across-the-board surveillance for chronic hepatitis C is not recommended [3]. A method to easily determine this risk, without performing serial liver biopsies, would be extremely significant clinically. In the present study, an HCC risk prediction model that included the factors such as age, platelet count, albumin levels, and AST levels was constructed. The model was found to have excellent reproducibility when validated with an external cohort. This model could identify subgroups of chronic hepatitis C patients at high risk of HCC development; the 5-year HCC development rate for the high- and intermediate-risk groups was 11.6%, yielding an annual incidence of 2.3%. This HCC risk prediction model requires only

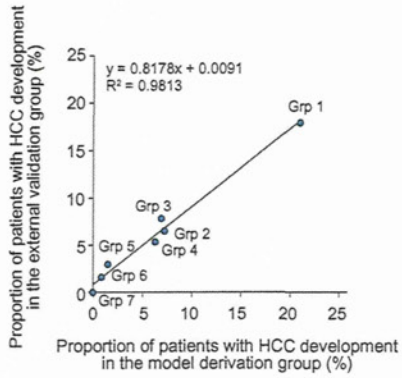

Fig. 2. External validation of the decision tree model with an independent cohort. Each patient in the external validation group was allocated to groups 1–7 following the flowchart of the decision tree. The HCC development rates were then calculated for each group and the graph plotted. The *x*-axis represents the HCC development rate in the model derivation group, and the *y*-axis represents the HCC development rate in the external validation group. The HCC development rates in each subgroup of patients are closely correlated between the model derivation group and the external validation group (correlation coefficient: $R^2 = 0.981$).

simple test values that are readily obtained in routine care and can therefore be easily used at the patient bedside. The model can be used to identify patients with a high risk of HCC development and therefore requiring surveillance, thereby allowing the formulation of surveillance plans personalized for individual patients.

Advanced fibrosis has been reported as independent risk factors for HCC development [7,8]. Platelet counts and albumin levels, which were factors selected for discrimination of the risk of HCC development, are closely related to the stage of fibrosis. Their correlation with the HCC risk has been repeatedly demonstrated [9–11,29–31]. The present study confirmed the impact of old age and advanced fibrosis, as reflected by low platelet counts and albumin levels. These results are consistent with our previous report [32]. What is unique to the present study was the study design to build a simple and reliable model for

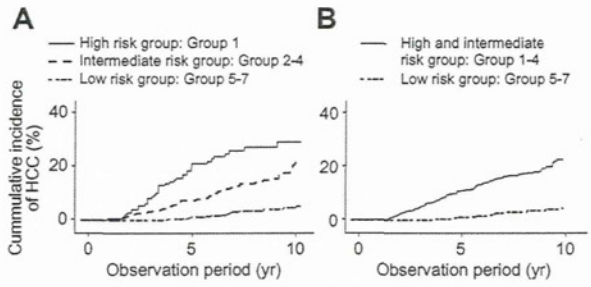

Fig. 3. Cumulative incidence of HCC development beyond 5 years in subgroups of patients defined by the decision tree model. Cumulative incidences of HCC in the groups classified by the decision tree model are compared. (A) The cumulative HCC development rate beyond 5 years is higher in the high- (group 1) and intermediate-risk (groups 2–4) groups compared to the low-risk group (groups 5–7). (B) The high and intermediate-risk group created by pooling data from the high- and intermediate-risk groups has a significantly higher cumulative HCC development rate than the low-risk group (5-year rate, 11.6% *vs.* 1.0%; 10-year rate, 24.5% *vs.* 4.8%; $p < 0.0001$).

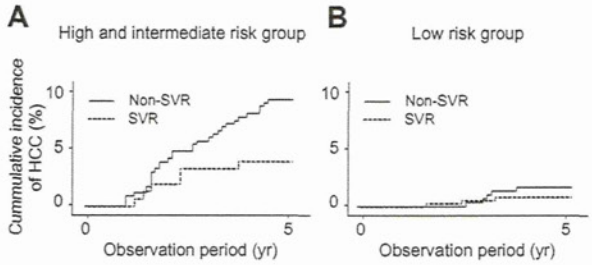

Fig. 4. Sustained virological response to PEG-IFN plus RBV therapy reduces the incidence of HCC development after stratification by the HCC risk. The 600 nonSVR patients and the 472 SVR patients in the external cohort were fitted into the HCC risk prediction model and classified into the high and intermediate-risk group or the low-risk group. The HCC development rate is significantly lower in SVR patients than in nonSVR patients in the high and intermediate-risk group (groups 1–4) (5-year HCC rate, 9.5% *vs.* 4.5%; $p = 0.040$). In the low-risk group (groups 5–7), the 5-year rate is 1.8% in nonSVR patients and 0.9% in SVR patients. Both rates are low and not significantly different ($p = 0.331$).

the prediction of HCC development that could be easily used in the clinic. For this purpose, a novel statistical method was used, histological factors were excluded in the analysis, the model derivation cohort was restricted to those who had nonSVR and had a long follow-up period duration (5 years), and the reproducibility of the model was independently validated by an external cohort. These are the major differences of the present study compared to our previous report. Many researchers have put a lot of efforts to formulate regression models for HCC prediction [9,10,33]. These prediction models are useful for identifying high-risk patients but are somewhat complicated to use at the bedside because they require calculations to be performed. Our prediction model is used simply by incorporating patients' data obtained through simple tests into the decision tree and following the flowchart. These prediction models based on factors easily accessible in routine clinical settings help physicians identify high-risk patients out of chronic hepatitis.

Viral eradication is the short-term goal of IFN therapy, but the ultimate goal is the prevention of HCC occurrence. Previous reports have shown that SVR to IFN therapy suppresses HCC occurrence in patients with type C liver cirrhosis and chronic hepatitis [7,12,30,34,35]. However, there is a marked heterogeneity in the magnitude of the treatment effect on the risk of HCC among studies, probably due to differences in the baseline risk of HCC among different trials [12]. Thus, the question remains whether the preventive effect of IFN therapy on HCC development could apply to all patients with chronic hepatitis C, especially those without liver cirrhosis. The result of the present study indicated that among high- and intermediate-risk patients, as assessed with our HCC risk prediction model, the cumulative HCC development rate was significantly reduced in SVR patients compared with nonSVR patients. This finding suggests that patients with chronic hepatitis, in whom disease has not yet progressed to hepatic cirrhosis but who are at a high risk of HCC development, benefit from antiviral treatment. The preventive effect of IFN on HCC development was not evident in low-risk patients within 5 years of observation. A longer observation term may be required to analyze the possible effect of antiviral therapy in these patients. Application of the present model on treatment decision may have limitations in that effect to prevent HCC development may differ in newer therapeutic agents such as protease

inhibitors [36,37], and that low-risk patients may also benefit from therapy after a longer term observation period such as 15–20 years.

Patients with chronic hepatitis often have no subjective symptoms accompanying their disease and therefore have a low consciousness of the disease. The broad array of adverse reactions and the high cost of IFN therapy are frequent hurdles in motivating patients to undergo therapy. However, patients may be convinced to undergo therapy or remain motivated for continued therapy if they are made aware of their risk of HCC development and the preventive effect of IFN on HCC development.

In conclusion, a reproducible HCC risk prediction model, which includes the factors such as age, platelet count, albumin levels, and AST levels, was constructed to predict the 5-year HCC development rate in patients with chronic hepatitis C. The model requires only a combination of readily available test values and can therefore be easily used at the bedside. The information provided by the model allows the physician to identify patients requiring IFN therapy for the prevention of HCC and formulate plans for imaging HCC surveillance.

Conflict of interest

The authors who have taken part in this study declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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