# Hepatitis B virus strains of subgenotype A2 with an identical sequence spreading rapidly from the capital region to all over Japan in patients with acute hepatitis B

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# Early dynamics of viremia in patients with genotype 1b chronic hepatitis C: Peg-IFNα2a shows earlier viral decline than peg-IFNα2b in combination therapy with ribavirin

**Authors' Contribution:**
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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## Summary

**Background:** We aimed to assess differences in early viral dynamics following treatment with either peg-IFNα2a or peg-IFNα2b in combination with ribavirin in patients with chronic genotype 1b HCV infection.

**Material/Methods:** Sixty-one patients in the peg-IFNα2a + ribavirin treatment (group α2a) and 88 patients in the peg-IFNα2b + ribavirin treatment (group α2b) were retrospectively analyzed. The early dynamics of HCV RNA over 12 weeks were evaluated. Sustained virological response (SVR) was defined as undetectable HCV RNA at week 24 after end of therapy. First- (day 0–1) and second-phase (day 1–28) viral decline rates were calculated in accordance with theoretical formulae.

**Results:** Baseline HCV RNA concentrations were almost similar between the 2 groups. In group α2a, viral decline was significantly greater than in group α2b at weeks 4, 8, and 12. In group α2a, viral decline was significantly greater in SVR patients than in non-SVR patients at week 2, whereas significantly greater viral decline in SVR patients was found during weeks 1–12 in group α2b. The first-phase viral decline rate was significantly larger in group α2a than in group α2b (1.31±0.84 *vs.* 0.70±0.97 log IU/mL/day; $p<0.0001$). Within SVR patients, first-phase viral decline rate was significantly larger in group α2a compared with group α2b (1.45±0.85 *vs.* 0.78±1.0 log IU/mL/day; $p<0.0001$). Second-phase viral decline rate was comparable between the groups.

**Conclusions:** Peg-IFNα2a showed earlier viral decline than peg-IFNα2b and the difference was obvious, especially in the first-phase viral decline.

**key words:** chronic hepatitis C • HCV • peg-interferon • viral kinetics

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## Background

Approximately 170 million people are infected with hepatitis C virus (HCV) worldwide and natural history studies show that 5–20% of patients develop cirrhosis after approximately 20 years of infection [1]. Currently, the pegylated-interferon (peg-IFN) plus ribavirin combination therapy has become the standard of care for chronic HCV-related liver disease because it achieves the highest rates of sustained virological response (SVR), defined as undetectable HCV RNA in blood 24 weeks after completion of therapy [2]. Moreover, peg-IFN and ribavirin are effective in treating chronic hepatitis C in children [3]. However, in patients infected with genotype 1 or 4 HCV, only about half achieve SVR following combination therapy, and genotype 1b in high viral loads accounts for >70% of patients with HCV infection in Japan [4]. The response to IFN is influenced by viral factors including viral load and genotypes, and host factors such as sex, age, insulin resistance, staging of the disease, and responses to previous antiviral therapies, as well as therapeutic factors such as dose and duration of treatment [5–8].

The stability of HCV RNA levels in individual patients with chronic HCV infection represents a steady state in which viral production is equivalent to viral elimination [9]. Initial viral dynamic studies of HCV showed the standard biphasic decline model after initiation of unmodified IFNα [9–11]. Peg-IFN + ribavirin therapy produced a biphasic viral decline, as was illustrated in initial studies. The first-phase decline in viral loads was rapid, usually occurring within the first 24 h, and was followed by a second, slower phase. The first-phase decline was dose-dependent and the second-phase decline, which was predictive of an SVR, showed considerable variability among individual patients [12,13]. Recently, mathematical modeling approaches have been developed to interpret the complex HCV kinetics observed in patients treated with peg-IFN and ribavirin [14–17]. The studies of viral kinetics in chronic hepatitis C patients during antiviral therapies have been described and early monitoring of viral decline was used to predict treatment outcomes [18–21].

In the IDEAL trial, antiviral efficacy was compared between peg-IFNα2a and peg-IFNα2b in combination therapy with ribavirin for patients with HCV genotype 1 infection, and the SVR rates, as well as the adverse effects, did not differ between the 2 groups in their standard dosing regimens [22–24]. However, there is limited information on the difference of viral kinetics, especially in the early-phase viral decline, between peg-IFNα2a and peg-IFNα2b in combination therapy with ribavirin for chronic hepatitis C. In the present study, the early dynamics of serum HCV RNA and the rate of viral decline were retrospectively analyzed in Japanese patients with genotype 1b chronic hepatitis C with high viral loads who received treatment with peg-IFNα2a + ribavirin or peg-IFNα2b + ribavirin.

## Material and Methods

Patients with chronic hepatitis C who were treated with peg-IFN + ribavirin combination therapy in the National Hospital Organization Group of Japan between 2007 and 2009 were enrolled for this study and retrospectively analyzed. The study protocol was approved by the Ethics Committee of the National Hospital Organization, and written informed consent was obtained from all patients. According to the standard protocols in Japan, patients received subcutaneous injection of peg-IFNα2a (180 μg) or peg-IFNα2b (1.5 μg/kg) once weekly for 48 or 72 weeks. Ribavirin (15 mg/kg/day) was included in both protocols. Doses of peg-IFN and ribavirin were reduced in some patients because of anemia, leukocytopenia, thrombocytopenia, or other adverse events, but not within the first 4 weeks. Serum HCV RNA concentrations were determined by COBAS TaqMan PCR HCV test (Roche Diagnostics, Tokyo, Japan) at baseline and at weeks 1, 2, 4, 8, and 12 after treatment initiation. SVR was defined as undetectable HCV RNA at week 24 after completion of therapy. The first-phase (24-h virological response) and second-phase (day 1–28) viral decline rates by treatment were calculated as shown in Figure 1. The calculation is based on a biphasic viral decline model where the first-phase is the sharp decline observed over the first 24 h of treatment and the dull decline of the second-phase continues for the following 27 days [25–27]. This calculation method was first introduced in the International Liver Congress 2009, 44th Annual Meeting of the European Association for the Study of the Liver [28].

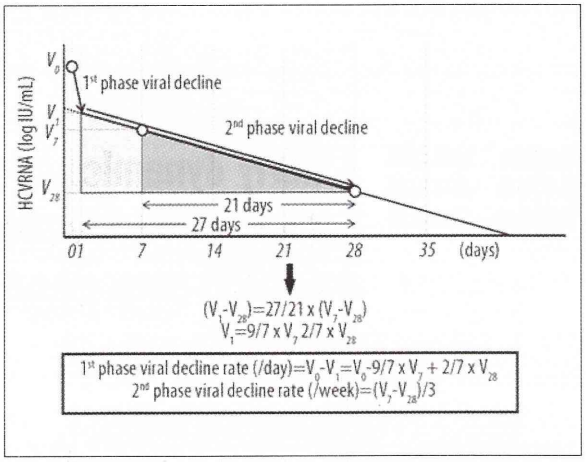


**Figure 1.** Early viral kinetic model during peg-IFN plus ribavirin combination therapy. The first-phase decline slope for 24h should be sharp whereas the subsequent second-phase decline slope should be dull. Expected first-phase and second-phase viral decline rates were calculated from the formulae presented under the graph.

Results are expressed as means ±standard deviation. Differences between categorical variables were analyzed by Fisher's exact test or chi-square test. Mann-Whitney *U* test was used for continuous variables. *P*-values <0.05 were considered statistically significant.

## Results

A total of 149 patients were retrospectively analyzed; their baseline characteristics are shown in Table 1. All patients were infected with genotype 1b HCV with high viral loads; their baseline HCV RNA levels in serum were ≥5.0 log IU/mL. The patients were divided into 2 groups: group α2a included 61 patients with peg-IFNα2a + ribavirin treatment and group α2b included 88 patients with peg-IFNα2b + ribavirin treatment (Table 1). Baseline serum HCV RNA concentrations were similar between the 2 groups (6.1±0.5

**Table 1.** Patient characteristics.

| | Group α2a | Group α2b | P value |
|---|---|---|---|
| Number | 61 | 88 | |
| % of retreatment cases | 41.0% | 25.0% | NS |
| Gender (male/female) | 30/31 | 42/46 | NS |
| Age (years) | 58.3±8.7 | 57.6±10.5 | NS |
| Body mass index (kg/m$^2$) | 22.4±2.1 | 23.5±2.5 | NS |
| HCV RNA (log IU/mL) | 6.1±0.5 | 6.2±0.6 | NS |
| AST (IU/L) | 51.0±26.5 | 55.5±40.3 | NS |
| ALT (IU/L) | 64.8±40.7 | 69.8±66.8 | NS |
| GGT (IU/L) | 54.4±64.2 | 52.2±54.8 | NS |
| Hemoglobin (g/dL) | 14.0±1.4 | 13.8±1.4 | NS |
| White blood cell (/μL) | 5.008±1.320 | 5.002±1.374 | NS |
| Platelet (×10$^4$/μL) | 16.1±4.7 | 19.1±9.0 | NS |

AST – aspartate aminotransferase; ALT – alanine aminotransferase; GGT – γ-glutamyl transpeptidase; NS – not significant.

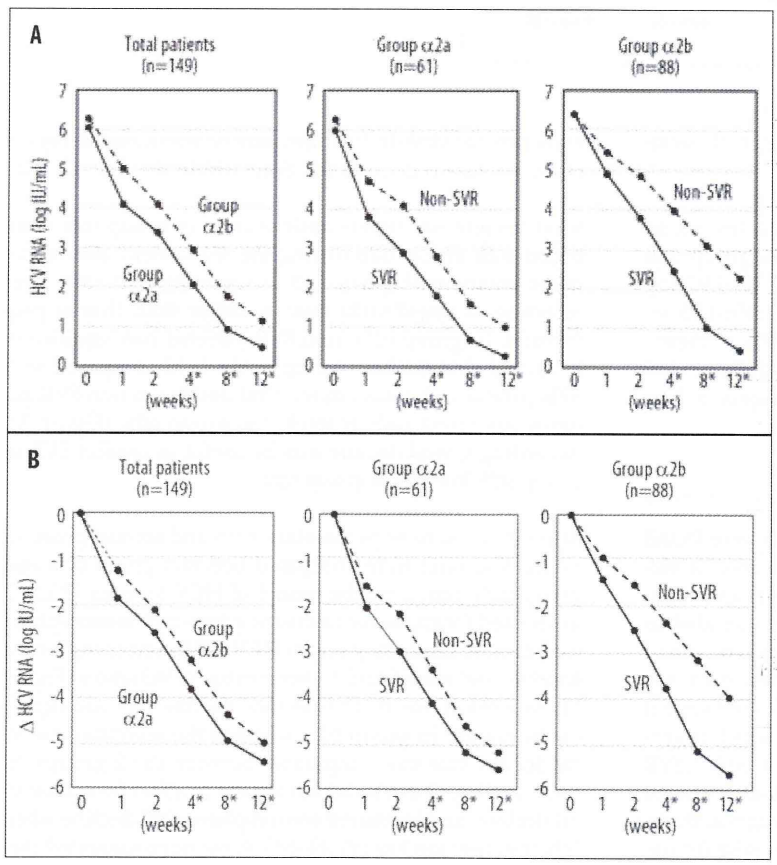


**Figure 2.** (**A**) Serum HCV RNA values in patients with chronic hepatitis C during peg-IFN plus ribavirin combination therapy. The levels were compared between group α2a and group α2b (left panel), between SVR and non-SVR patients in group α2a (center panel), and between SVR and non-SVR patients in group α2b (right panel). * $p<0.01$, ** $p<0.05$. (**B**) Levels of viral decline (HCV RNA) from baseline in patients with chronic hepatitis C during peg-IFN plus ribavirin combination therapy. Levels were compared between group α2a and group α2b (left panel), between SVR and non-SVR patients in group α2a (center panel), and between SVR and non-SVR patients in group α2b (right panel). * $p<0.01$, ** $p<0.05$.

*vs.* 6.2±0.6 log IU/mL, respectively). SVR rate was lower in group α2a (54.1%) than in group α2b (61.4%), although the difference was not statistically significant (data not shown).

HCV RNA concentrations declined earlier in group α2a, and group α2a showed significantly lower concentrations than group α2b at weeks 4, 8, and 12 after starting treatment (Figure 2A). In both groups, HCV RNA levels were significantly lower at weeks 1, 2, 4, 8, and 12 in SVR patients compared with non-SVR patients (Figure 2A). The level of viral decline to baseline levels (net viral decline) was significantly greater at weeks 4, 8, and 12 in group α2a than in group α2b (Figure 2B). The level of viral decline in SVR patients was significantly greater than that in non-SVR patients at

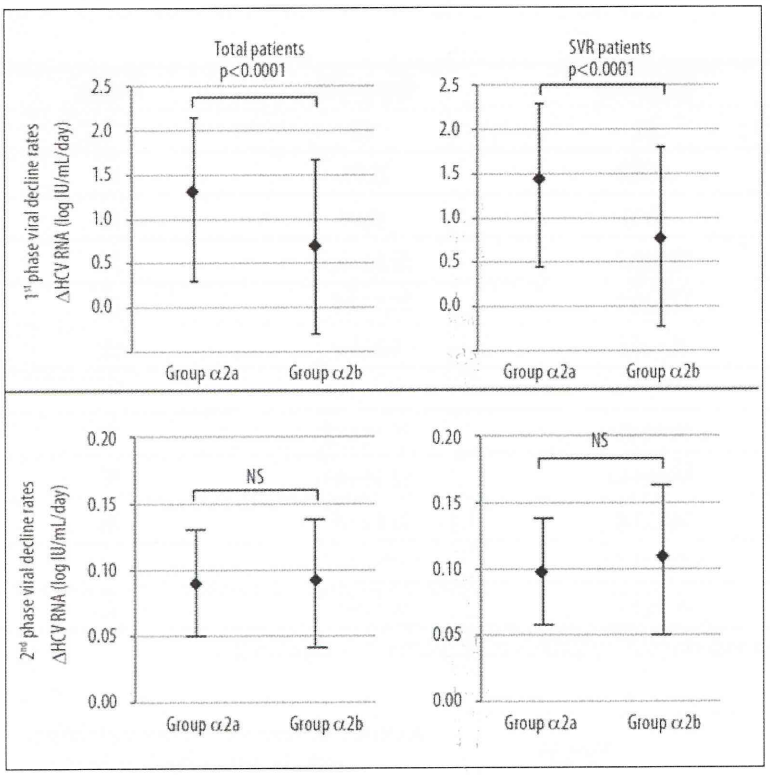


**Figure 3.** Comparison of first- and second-phase decline rates between group α2a and group α2b in total patients (left panels) and in SVR patients (right panels). Diamonds with lines indicate means ± standard deviation.

week 2 in group α2a, whereas this was evident at all assessment time-points in group α2b (Figures 2B).

In both the total population and SVR patients, first-phase viral decline rates were significantly higher in group α2a compared with group α2b (1.31±0.84 *vs.* 0.70±0.97 log IU/mL/day in total population, p<0.0001; 1.45±0.85 *vs.* 0.78±1.0 log IU/mL/day in SVR patients, p<0.0001) (Figure 3, upper panels). On the other hand, second-phase viral decline rates were similar in the 2 groups (Figure 3, lower panels).

## Discussion

As shown in Table 1, no significant differences were found in sex, age, viral load, body weight, platelet counts, or biochemical analysis that would influence the response to antiviral treatments. Retreatment patients were included in this study; their percentage was higher in group α2a than in group α2b (41.0% *vs.* 25.0%), but the difference was not significant. Of note, previous treatments in all retreatment patients were unmodified IFN monotherapy, which is generally ineffective for patients with genotype 1 HCV (SVR rate <5%). Therefore, "retreatment patient" does not mean lower responder to peg-IFN + ribavirin combination therapy and all patients enrolled in this study were naïve for the combination therapy. In patients who had experienced liver biopsies (group α2a, 57 cases; group α2b, 60 cases) there were no significant intergroup differences in histopathological staging and grading (data not shown). Dose reduction of peg-IFNα and/or ribavirin, which weakens the antiviral effect, was not considered in this study, and the duration of treatment was not fixed (48 or 72 weeks). Therefore the final outcome of the treatments, SVR rates, cannot be fairly compared between the groups. However, early viral kinetics, especially the viral decline rate, may be worth evaluating because no dose reduction was done within the first 4 weeks.

Viral decline was significantly greater in group α2a compared with group α2b during the 4-12 weeks after treatment initiation (Figures 2, 3), suggesting that early viral response to peg-IFNα2a may be better than that to peg-IFNα2b. In group α2b, non-SVR patients had significantly limited viral decline during weeks 1–12 compared with SVR patients, whereas limited viral decline in non-SVR patients was found only at week 2 in group α2a (Figure 3). Accordingly, viral decline may be useful to predict SVR in group α2b but not in group α2a.

As pharmacokinetic parameters, first- and second-phase viral decline rates were compared between group α2a and group α2b. Based on the model of HCV kinetics [25,26], we devised formulae for calculating first- and second-phase viral decline rates using serum HCV RNA concentrations at baseline and week 1 and 4 after treatment initiation (Figure 1). As a result, the first-phase viral decline rate was significantly greater in group α2a, whereas the second-phase viral decline rate was comparable between the 2 groups. In some studies, ribavirin did not appear to affect first-phase viral decline, and increased second-phase viral decline when IFN response was low [27,29–32]. It has been suggested that first-phase decline reflects a dose-effect and the pharmacokinetic properties of peg-IFNs, and that the slope of the second-phase decline reflects inter-patient variability [9,33]. Peg-IFNα2a and peg-IFNα2b have different pharmacokinetics; their half-lives in plasma are approximately 77 and 40 h, respectively [34,35]. Therefore, among therapeutic factors, administered dose and half-life may be the main factors affecting the difference in first-phase viral decline rate between treatments with peg-IFNα2a *vs.* peg-IFNα2b.

In practice, it is difficult to fairly evaluate the effect of different antiviral protocols, because virological and host factors that also affect outcomes are complex. For example, novel factors such as substitution of amino acids 70 and 91 in the core region of HCV-1b [36] and genetic variation in IL28B [37–39] are associated with outcomes of antiviral therapy. In future, if these factors can be evaluated more simply and easily, more successful therapeutic protocols may be selected for individual patients as tailor-made therapy.

## Conclusions

In our study population, peg-IFNα2a showed earlier viral decline than peg-IFNα2b, and the difference was particularly obvious in the first-phase viral decline, although no significant difference was shown in SVR rate between the treatments.

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Hepatology Research

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***Original Article***

# Efficacy of splenectomy in preventing anemia in patients with recurrent hepatitis C following liver transplantation is not dependent on inosine triphosphate pyrophosphatase genotype

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*Aim:* A genetic polymorphism of inosine triphosphate pyrophosphatase (ITPA) has been associated with pegylated-interferon/ribavirin (PEG-IFN/RBV)-induced anemia in chronic hepatitis C patients. However, correlation of the genetic variant with anemia following liver transplantation has not been determined.

*Methods:* Sixty-three hepatitis C virus (HCV)-positive patients who underwent liver transplantation and PEG-IFN/RBV therapy were enrolled. The rs1127354 was determined for each individual.

*Results:* There was no relationship with anemia or RBV dosage in patients carrying the CC allele (CC group, $n = 43$) and those carrying the CA allele (CA group, $n = 20$). The incidence of hemoglobin (Hb) decline >3 g/dL (CC: 4.7%, CA: 0%) was relatively low, whereas the incidence of Hb levels <10 g/dL (CC: 18.6%, CA: 30.0%) was high. Univariate analysis revealed that splenectomy inversely correlated with Hb levels <10 g/dL at 4 weeks ($P = 0.04$). Among the 22 patients who did not undergo splenectomy, the incidence of Hb levels <10 g/dL tended to be lower in the seven patients carrying the CA allele (28.6%) than in the 15 patients with the CC allele (60.0%).

*Conclusion:* The ITPA genetic polymorphism does not correlate with post-transplant PEG-IFN/RBV-induced anemia. Splenectomy is useful in preventing anemia regardless of the ITPA genotype.

**Key words:** inosine triphosphate pyrophosphatase genetic polymorphism, liver transplantation, recurrent hepatitis C, splenectomy

## INTRODUCTION

HEPATITIS C VIRUS (HCV) and its related diseases are the leading cause of liver transplantation (LT) worldwide.[1] The incidence of HCV re-infection is increased in almost all cases after LT and the outcome of post-transplant antiviral therapy is very poor.[2] Although the combination of pegylated interferon and ribavirin (PEG-IFN/RBV) is the standard antiviral therapy for HCV, it is expensive and has some side effects such as flu-like symptoms, thrombocytopenia, and anemia. Of these problems, anemia is a serious matter, especially for Japanese patients, as erythropoietin replacement therapies are not covered by public medical insurance and are seldom performed. Furthermore, the incidence of anemia after LT is as high as 50%, even without anti-HCV therapy.[3] PEG-IFN/RBV therapy for recurrent hepatitis C after LT has been reported to cause anemia in no less than 71% of recipients.[4] To prevent these side effects, various techniques have been trialed, including: (i) simultaneous splenectomy at transplantation,[5] and (ii) PEG-IFN-α2 therapy with 200 mg RBV daily followed by an increase in dosage according to the tolerance of the individual.[6]

Recently, two single nucleotide polymorphisms (SNPs) in the inosine triphosphatase pyrophosphatase

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**Conflict of interest:** There are no competing interests regards to this work.

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(ITPA) gene were reported to correlate with treatment-induced anemia in chronic hepatitis C patients. These were identified as rs1127354 and rs7270101.[7] These two SNPs are known to be responsible for ITPA deficiency[7] and inosine triphosphate (ITP) accumulation in erythrocytes, and are thought to confer protective effects in ribavirin-related hemolytic anemia. Of these two SNPs, rs7270101 is not polymorphic in Japanese people,[8] but variants at rs1127354 have been demonstrated to be significantly associated with treatment-induced anemia in Japanese hepatitis C patients.[9,10]

If this genetic polymorphism is a predictor of anemia after post-transplant PEG-IFN/RBV therapy, then that would lead to a new tailored anti-HCV treatment post-LT. In this article, we describe the relationship of the ITPA genetic polymorphism to anemia in LT patients undergoing PEG-IFN/RBV therapy, and demonstrate the usefulness of our strategies against the aforementioned side effects.

## METHODS

### Patients

FROM APRIL 1999 to March 2009, 112 HCV-RNA-positive patients underwent LT at our institute, of which 78 patients were administered PEG-IFN/RBV therapy. Of these 78 patients, five patients who were under treatment, six patients who dropped out from treatment because of its side effect other than anemia such as depression, and three patients whose Hb levels after treatment were unavailable, were excluded from this study. Therefore, 63 patients were retrospectively analyzed. The current study was approved by the ethics committee of Kyushu University.

### Antiviral treatment

The primary doses of PEG-IFN α2b (Pegintron®; Schering-Plough Inc, Kenilworth, NJ, USA) and RBV (Rebetol®; Schering-Plough Inc) were 0.5 μg/kg per week and 200 mg daily, respectively. They were increased to 1.5 μg/kg per week and 800 mg daily in a stepwise manner according to individual tolerance as previously described.[6] Neither granulocyte colony-stimulating factor nor erythropoietin was used in any individual.

### Assessment of the therapeutic effects and anemia

A virological response (VR) was defined as a lack of HCV RNA in response to the treatment regimen regardless of whether a relapse occurred when treatment was terminated. A sustained virological response (SVR) was defined as a lack of HCV RNA at 6 months after completion of the treatment. Treatment-induced anemia was defined as a decline in hemoglobin (Hb) greater than 3 g/dL at 4 weeks, or a Hb level less than 10 g/dL at 4 weeks as previously described.[7]

### DNA extraction and ITPA genotyping

DNA was extracted from the recipient's exenterated liver tissue at transplantation, and direct sequencing was performed using a Big Dye Terminator v1.1 Cycle Sequence Kit (Applied Biosystems Inc., Tokyo, Japan) according to the manufacturer's protocol. The primers used to identify the ITPA genetic polymorphism (rs1127354) were 5′-AGA GTT ATC GAT GAG AAA-3′ (sense) and 5′-GAG AAA TCC AAC CAT CTT-3′ (antisense).

### Statistical analysis

All data was analyzed using JMP® statistical software. A $\chi^2$ test was performed for qualitative variables and a Wilcoxon test was performed for quantitative variables.

## RESULTS

### ITPA genotyping and anemia

THE ITPA MAJOR homozygote allele (rs1127354: CC) was seen in 43 recipients (68.3%) and the heterozygote allele (CA) was seen in 20 recipients (31.7%). No recipient enrolled in the current study carried the minor homozygote allele (AA). The patients' backgrounds between these two genotypes have been outlined in Table 1. None of the pre-transplant, operative, and pre-treatment factors exhibited any differences, except for pre-treatment viral titre.

Among those carrying the CC allele, only two recipients (4.7%) showed a decline in Hb greater than 3 g/dL at 4 weeks after the commencement of PEG-IFN/RBV therapy; whereas none of the recipients carrying the CA allele showed a Hb decline greater than 3 g/dL ($P = 0.311$; Fig. 1a). In contrast, eight recipients whose Hb level was less than 10 g/dL at 4 weeks carried the CC allele and six carried the CA allele ($P = 0.327$; Fig. 1b). In addition, the progression of anemia during the treatment between two groups were compared by each Hb decline at 4, 8, and 12 weeks after commencement of the therapy to reveal that there was no difference (−0.92 g/dL vs. −0.59 g/dL; $P = 0.59$, −1.33 g/dL vs. −0.74 g/dL; $P = 0.27$, −1.39 g/dL vs.

**Table 1** Comparison of the data among patients carrying CC allele and CA allele at rs1127354

| rs1127354 | CC ($n = 43$) | CA ($n = 20$) | *P*-value |
|---|---|---|---|
| *Pretransplantation factor* | | | |
| Recipient's age (years), mean ± SD | 57 ± 1 | 56 ± 2 | n.s |
| Recipient's sex (male / female), *n* | 24 / 19 | 14 / 6 | n.s |
| Recipient's BMI ($kg \cdot m^{-2}$), mean ± SD | 24.9 ± 0.62 | 24.0 ± 0.88 | n.s |
| Donor's age (y), mean ± SD | 33 ± 2 | 34 ± 2 | n.s |
| Donor's sex (male / female), *n* | 31 / 12 | 12 / 8 | n.s |
| Donor's BMI ($kg \cdot m^{-2}$), mean ± SD | 23.3 ± 0.61 | 21.3 ± 0.89 | n.s |
| Pretransplant Hb level (g/dL), mean ± SD | 10.9 ± 0.36 | 11.2 ± 0.48 | n.s |
| MELD score, mean ± SD | 10.3 ± 0.79 | 10.8 ± 1.1 | n.s |
| *Operative factor* | | | |
| Operative time (min), mean ± SD | 793 ± 31 | 839 ± 44 | n.s |
| Simultaneous splenectomy (yes/no), *n* | 28 / 15 | 13 / 7 | n.s |
| Intraoperative bleeding (mL), mean ± SD | 5752 ± 891 | 6105 ± 1260 | n.s |
| GV/SLV (%), mean ± SD | 40.5 ± 1.4 | 42.3 ± 2.0 | n.s |
| *Post-transplantation factor* | | | |
| Bile duct complication (yes / no), *n* | 40 / 3 | 16 / 4 | n.s |
| Pretreatment viral load (logIU/mL), mean ± SD | 6.2 ± 0.1 | 6.6 ± 0.2 | 0.02 |
| Pathological activity score, mean ± SD | 1.3 ± 0.12 | 1.4 ± 0.16 | n.s |
| Pathological fibrosis score, mean ± SD | 1.1 ± 0.20 | 0.88 ± 0.28 | n.s |
| Immunospressive agents (CyA / FK), *n* | 21 / 22 | 15 / 5 | n.s |
| Total dose of RBV during the first 4 weeks (mg), mean ± SD | 8882 ± 703 | 8755 ± 1034 | n.s |
| Pretreatment Hb level (g/dL), mean ± SD | 12.3 ± 0.27 | 11.9 ± 0.40 | n.s |

BMI, body mass index; CyA, cyclosporine; FK, tacrolimus; GV, graft volume; Hb, hemoglobin; MELD, model for end-stage liver disease; n.s, not significant; SLV, standard liver volume.

−1.59 g/dL; $P = 0.81$, respectively, Fig. 1c). The ITPA genetic polymorphism did not correlate with PEG-IFN/RBV-induced anemia after LT.

## ITPA genotype and RBV dosage

The dosage of PEG-IFNα2b and RBV were adjusted for each individual so as not to cause any side effects, including anemia. If the ITPA minor allele was able to protect post-transplant patients from RBV-related hemolytic anemia, the RBV dosage could be increased in recipients carrying the CA allele. As described in Table 1, total dose of RBV administered during the first 4 weeks were similar in each group (8882 mg vs. 8875 mg, $P = 0.787$). It was possible to increase the RBV dosage in 16 recipients (40%) carrying the CC allele and eight recipients (40%) carrying the CA allele ($P = 1.00$; Fig. 2a). Twelve patients carrying the CC allele and four carrying the CA allele had their RBV dosage decreased because of anemia ($P = 0.409$; Fig. 2b).

Ochi *et al.*[9] reported that there was marginal correlation between ITPA genetic polymorphism and the outcome of PEG-IFN/RBV therapy, probably because of the dose reduction of RBV in patients showing severe anemia. In patients enrolled in the current study, the therapeutic effects between recipients carrying the CC and those carrying the CA allele were not significantly different; with a VR of 68.9% and 72.7% ($P = 0.746$; Fig. 3a), respectively. The SVR for these two groups was 38.9% and 42.7% ($P = 0.768$, Fig. 3b), respectively.

## Efficacy of splenectomy

We performed simultaneous splenectomy at transplantation for HCV-related liver diseases to prevent PEG-IFN/RBV therapy-induced blood cytopenia. Univariate analysis showed that splenectomy was significantly related to a Hb level less than 10 g/dL after 4 weeks (Table 2). Therefore, to prove the efficacy of splenectomy against treatment-induced anemia, the incidence of anemia and RBV dose reduction were compared between 41 recipients who had undergone spontaneous splenectomy (Spx group) and 22 recipients who had not undergone splenectomy (Non-Spx group). Although the incidence of Hb decline greater than 3 g/dL was not significantly different between the two groups (2.4 *vs.* 4.5%, $P = 0.649$; Fig. 4a), the Spx group showed a

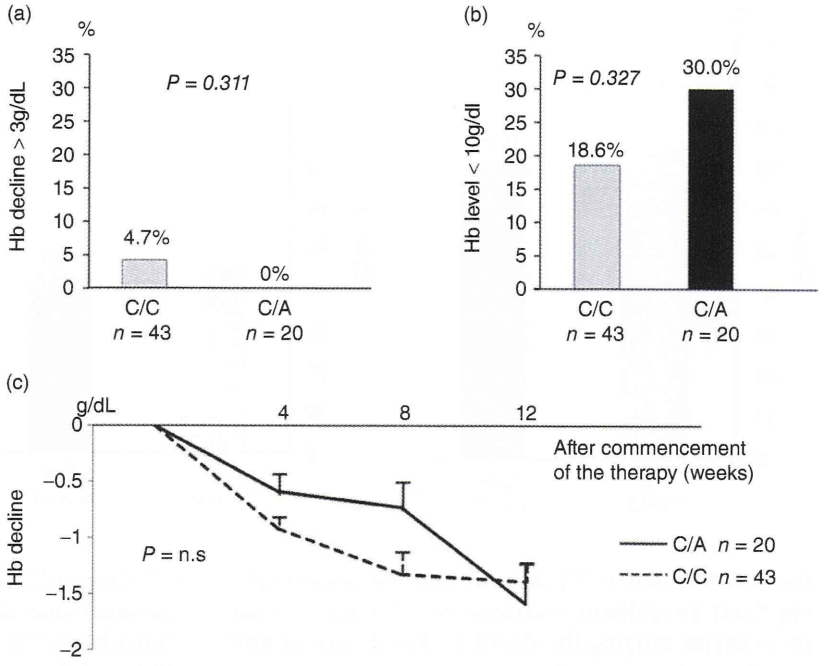


**Figure 1** Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and pegylated-interferon/ribavirin (PEG-IFN/RBV)-related anemia after liver transplantation (LT). (a) Hemoglobin (Hb) decline greater than 3 g/dL at 4 weeks after the commencement of therapy was found in 4.7% of CC allele carriers and in none of the CA allele carriers. (b) hemoglobin (Hb) levels less than 10 g/dL at 4 weeks were found in 18.6% of CC allele carriers and in 30.0% of CA allele carriers. (c) Hb decline at 4, 8, and 12 weeks after commencement of the therapy were compared. There was no statistical difference in the progression of anemia during the treatment between two groups. (—): C/A $n = 20$; (....): C/C $n = 43$.

significantly lower incidence of Hb levels lower than 10 g/dL compared with the Non-Spx group (14.6 *vs.* 36.4%, $P < 0.05$; Fig. 4b). Additionally, the RBV dosage tended to be increased more often in the Spx group than in the Non-Spx group (46.3 *vs.* 22.7%, $P = 0.09$; Fig. 4c); and at the same time was not reduced because of anemia (19.5 *vs.* 36.4%, $P = 0.09$; Fig. 4d), though there was no statistical difference.

The incidence of treatment-induced anemia between those carrying the CC and CA alleles among the non-Spx group was evaluated. Of the 22 recipients in the non-Spx group, 15 carried the CC allele and seven carried the CA allele. Although there was no significant difference because of the small numbers involved, a Hb decline greater than 3 g/dL and Hb levels less than 10 g/dL at 4 weeks were found more often in recipients carrying

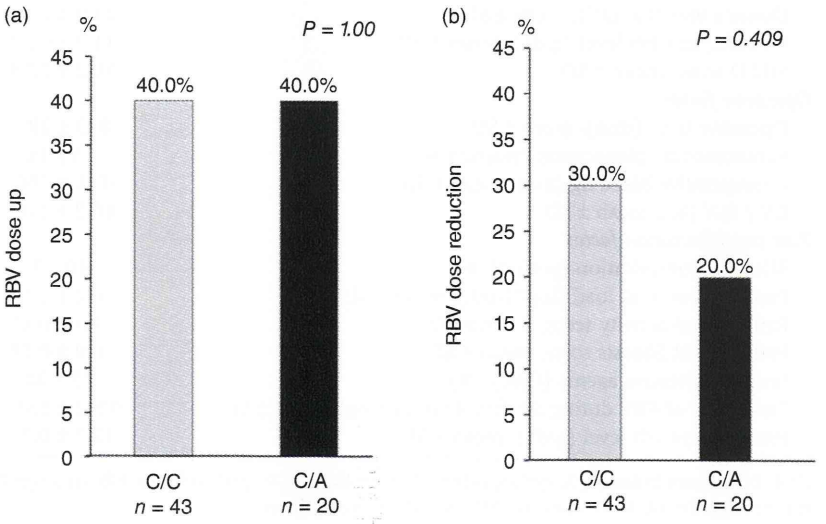


**Figure 2** Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and ribavirin (RBV) dosage. (a) The dosage of RBV was increased in 40% of each genotype group. (b) RBV dose reduction due to anemia was found in 30% of those carrying the CC allele and 20% of those carrying the CA allele.

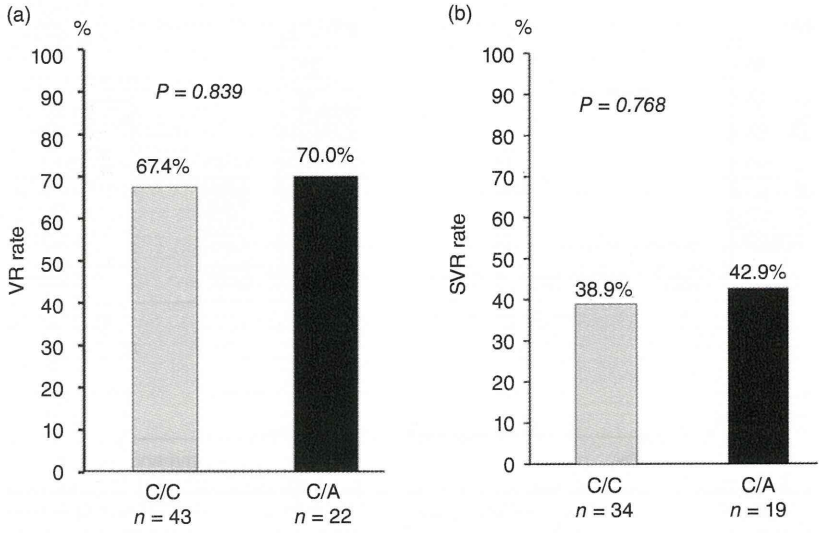


**Figure 3** Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and virological response. (a) Cirological response (VR) between the two genotypes was 68.9% and 72.7%. (b) The incidence of the sustained virological response (SVR) was 38.9% and 42.9%.

the CC allele (6.6 *vs.* 0% and 60 *vs.* 28.6%, respectively; Fig. 5a,b). In addition, tolerance to RBV seemed better in recipients carrying the CA allele. The dosage of RBV was able to be increased in 15.4% of those carrying the CC allele and in 42.9% of those carrying CA (Fig. 5c). At the same time, RBV dose reduction due to anemia was found in 46.7% of those carrying the CC allele and in 28.6% of those with the CA allele (Fig. 5d).

**Table 2** Comparison of the data among patients whose Hb level < 10 g/dL and ≥10 g/dL at 4 weeks

| Hb level at 4 weeks | Hb ≥ 10 g/dL ($n$ = 49) | Hb < 10 g/dL ($n$ = 14) | $P$-value |
|---|---|---|---|
| ***Pretransplantation factor*** | | | |
| Recipient's age (year), mean ± SD | 56 ± 1 | 58 ± 2 | n.s |
| Recipient's sex (male/female), $n$ | 32 / 17 | 6 / 8 | n.s |
| Recipient's BMI ($kg \cdot m^{-2}$), mean ± SD | 24.6 ± 0.6 | 24.5 ± 1.3 | n.s |
| Donor's age (year), mean ± SD | 33 ± 2 | 34 ± 4 | n.s |
| Donor's sex (male/female), $n$ | 33 / 16 | 10 / 4 | n.s |
| Donor's BMI ($kg \cdot m^{-2}$), mean ± SD | 23.0 ± 0.6 | 21.5 ± 1.2 | n.s |
| Pretransplant Hb level (g/dL), mean ± SD | 11.2 ± 0.32 | 9.9 ± 0.68 | n.s |
| MELD score, mean ± SD | 10.2 ± 0.73 | 10.9 ± 1.2 | n.s |
| ***Operative factor*** | | | |
| Operative time (min), mean ± SD | 823 ± 29 | 730 ± 63 | n.s |
| Simultaneous splenectomy (yes/no), $n$ | 35 / 14 | 6 / 8 | 0.04 |
| Intraoperative bleeding (mL), mean ± SD | 5721 ± 786 | 5332 ± 1260 | n.s |
| GV / SLV (%), mean ± SD | 40.2 ± 1.0 | 44.5 ± 2.3 | n.s |
| ***Post-transplantation factor*** | | | |
| Bile duct complication (yes/no), $n$ | 40 / 3 | 16 / 4 | n.s |
| Pretreatment viral load (logIU/mL), mean ± SD | 6.2 ± 0.1 | 6.7 ± 0.2 | 0.03 |
| Pathological activity score, mean ± SD | 1.3 ± 0.11 | 1.2 ± 0.22 | n.s |
| Pathological fibrosis score, mean ± SD | 0.9 ± 0.19 | 1.2 ± 0.38 | n.s |
| Immunospressive agents (CyA / FK) | 25 / 24 | 11 / 3 | n.s |
| Total dose of RBV during the first 4 weeks (mg), mean ± SD | 9282 ± 633 | 7000 ± 1294 | n.s |
| Pretreatment Hb level (g/dL), mean ± SD | 12.7 ± 0.21 | 10.4 ± 0.40 | <0.0001 |

BMI, body mass index; CyA, cyclosporine; FK, tacrolimus; GV, graft volume; Hb, hemoglobin; MELD, model for end-stage liver disease; n.s, not significant; RBV, ribavirin; SLV, standard liver volume.

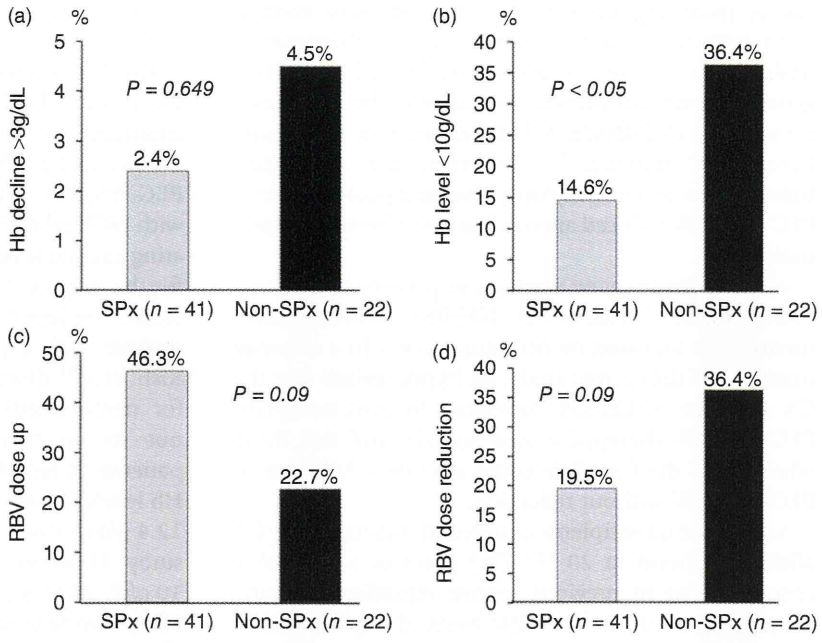


**Figure 4** The efficacy of splenectomy for anaemia and ribavirin (RBV) tolerance. (a) The incidence of a hemoglobin (Hb) decline greater than 3 g/dL at 4 weeks was evident in 2.4% of recipients who had simultaneous splenectomy at liver transplantation (LT) (Spx group) and 4.5% of recipients were not subjected to a splenectomy (non-Spx group). (b) The incidence of Hb level less than 10 g/dL at 4 weeks was significantly lower in the Spx (14.6%) as compared with the non-Spx group (36.4%). (c) The dosage of RBV tended to increase more often in the Spx group (46.3%) than in the non-Spx group (22.7%). (d) RBV dose reduction due to anemia tended to be less frequent in the Spx group (19.5%) than in the non-Spx group (36.4%).

## DISCUSSION

HCV-RELATED LIVER DISEASES are the main reason for liver transplantation worldwide.[1] The post-transplant prognosis for HCV is worse than with other diseases because of the recurrence of hepatitis C[11]. Although PEG-IFN/RBV is the only standardized anti-HCV therapy after LT, the outcome is poor with less than 30% of cases exhibiting a SVR. This is likely because of immunosuppressive agents used and severe side

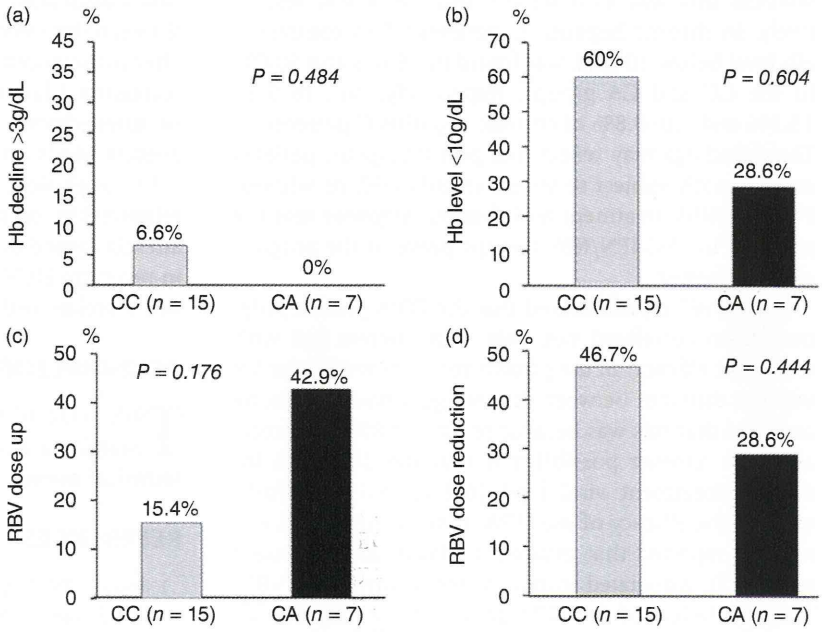


**Figure 5** Inosine triphosphate pyrophosphatase (ITPA) genotypes and anemia among non-Spx group. (a) The incidence of hemoglobin (Hb) decline at 4 weeks was higher in CC allele carriers compared with CA allele carriers (6.6 *vs.* 0%). (b) The incidence of Hb levels less than 10 g/dL at 4 weeks was higher in the CC allele carriers (60 *vs.* 28.6%). (c) The dosage of RBV could be increased more often in CA allele carriers than in CC allele carriers (42.9 *vs.* 15.4%). (d) RBV dose reduction due to anemia was found more often in CC allele carriers than in CA allele carriers (46.7 *vs.* 28.6%).

effects, including anemia.[2] Treatment-induced anemia is an important issue in Japan where erythropoietin-replacement therapy is seldom performed. The ITPA genetic polymorphism was recently reported to be associated with PEG-IFN/RBV-induced anemia in chronic hepatitis C patients.[7,9,10] However, the correlation between this genetic polymorphism and post-transplant PEG-IFN/RBV induced anemia has never been examined until now.

We have made many attempts to prevent side effects, such as minimal dose of PEG-IFN/RBV at therapy commencement followed by dose adjustment in a stepwise manner.[6] In the current study, we hypothesized that the CA allele at rs1127354 correlated to post-transplant PEG-IFN/RBV therapy-induced anemia, and that those who carried the CA allele could tolerate a full dose of PEG-IFN/RBV without reduction.

Among the 63 recipients enrolled in this study, the CA allele was found in 20 (31.7%) patients, a frequency corresponding to previous reports regarding Japanese people.[8] Contrary to the hypothesis, the ITPA genetic polymorphism did not correlate with treatment-induced anemia after LT as shown in Figures 1 and 2. The incidence of Hb decline greater than 3 g/dL was relatively low, whereas the numbers of patients with Hb levels less than 10 g/dL were high at 4 weeks of post-transplant PEG-IFN/RBV therapy compared with those in previous reports for chronic hepatitis C patients.[7,10] In the current study, Hb decline was found in 4.7% of individuals in the CC group and none in the CA group, whereas this was 47.6–48.7% and 0.8–4.5%, respectively, in chronic hepatitis C patients.[7,10] In contrast, a Hb level below 10 g/dL was found in 18.6% and 30.0% in the CC and CA groups, respectively, and in 9.3–15.9% and 0.0–0.8% of chronic hepatitis C patients.[7,10] These findings may reflect that post-transplant patients are originally subject to severe anemia with or without PEG-IFN/RBV treatment and that our stepwise manner protocol in PEG-IFN/RBV therapy prevents the progression of anemia.

Ochi *et al.*[9] demonstrated that the ITPA genetic polymorphism correlated not only with anemia but with treatment efficacy. In the present study, however, the VR was not different between the two genotypes. It can be assumed that this was because of similar RBV tolerance, although another possibility is that the difference for each pretreatment viral load (6.2 *vs.* 6.6 logIU/mL) affected the efficacy of the ITPA minor genotype. It was recently reported that treatment-related anemia would possibly be associated with a greater occurrence of VR[12]. The correlation of the ITPA genetic polymorphism or anemia with the efficacy of PEG-IFN/RBV therapy requires further investigation.

Another strategy against the side effects of post-transplant PEG-IFN/RBV therapy at our institute is simultaneous splenectomy at LT[5]. Splenectomy is known to be effective and safe in combination with PEG-IFN/RBV therapy for thrombocytopenic patients with HCV-related cirrhosis,[13–15] but its efficacy in alleviating anemia is yet to be demonstrated. In the guidelines for the treatment of chronic hepatitis and cirrhosis due to HCV in Japan,[16] a splenectomy is recommended for patients with a platelet count less than 50 000/mm$^3$. Kishi *et al.*[17] described that a splenectomy was effective for treating leukocytopaenia, thrombocytopenia, but not for anemia in post-transplant recurrent HCV patients. In fact, there was no difference in pretreatment Hb levels between the Spx and non-Spx groups (12.0 *vs.* 12.4 g/dL, $P = 0.39$; data not shown) in the present study. However, the incidence of Hb levels less than 10 g/dL after treatment was significantly lower in the Spx group as compared with the non-Spx group, which shows the efficacy of a splenectomy for treatment-induced anemia after LT. At the same time, the ITPA genetic polymorphism tended to be associated with treatment-induced anemia and RBV tolerance in the non-Spx group only, similar to chronic hepatitis C patients. Conversely, it could be said that a splenectomy prevents PEG-IFN/RBV-related anemia regardless of the ITPA genetic polymorphism. However, neither splenectomy nor other factors that were suggested to be significantly associated with anemia by univariate analysis was shown to be associated with Hb level <10g/dl at 4 weeks after commencement of the therapy by multiple logistic regression (data not shown). The proof of the efficacy of splenectomy in preventing PEG-IFN/RBV induced anemia needs further investigation.

In conclusion, this is the first report regarding the relationship of the ITPA genetic polymorphism and anemia caused by post-transplant PEG-IFN/RBV therapy in recurrent HCV. The ITPA genetic polymorphism does not correlate with treatment-induced anemia after LT.

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# グライコプロテオミクスを基軸とした腫瘍マーカー開発技術

久野 敦[*1]・池原 譲[*2]・成松 久[*3]


## abstract

近年，質量分析計による大規模解析技術の発展から，プロテオミクスを基軸としたバイオマーカー探索が盛んに行われ，この進展はグライコプロテオミクスを基軸としたバイオマーカー探索にも及び，糖鎖腫瘍マーカー開発は確実に加速している．しかしながら「同定されたマーカー候補タンパク質群は，本当に疾患特異的な糖鎖変化を有しているのか」という答えを引き出すための検証試験に有効な技術はきわめて乏しく，開発に急ブレーキがかかっている．その結果，大規模解析の成果から発展した分子で疾患特異的マーカーとして認可されたものはないのが現状だ．この問題を解決すべく，疾患特異的糖鎖変化を捉えるのに最適な技術として抗体オーバーレイ・レクチンマイクロアレイが開発された．その検証試験からバリデーション試験への登用により，実効性の高い糖鎖バイオマーカー開発パイプラインが確立された．今後，腫瘍マーカー開発への活用が期待される．


## I はじめに

私たちの血液には，各組織を形成する細胞や血球から分泌されたタンパク質が高濃度で流れており，アルブミンを例外としてその大部分が糖鎖修飾を受けている．疾患の引き金となる組織細胞の形態異常が生じると，その細胞の分泌するタンパク質は量的に変化するだけでなく，質的な変化，すなわちタンパク質上の糖鎖が変化してしまう．というのも，100以上にも及ぶ修飾に関連する遺伝子が細胞の状態に応じ発現調節され，糖鎖は合成されるためである．また，疾患に伴う糖鎖変化が固有なもので，かつ血中の組織特異的糖タンパク質に反映する可能性が高ければ，このような糖タンパク質は糖鎖バイオマーカーとして活用できるであろう．近年，質量分析計による大規模解析技術の発展から，プロテオミクスを基軸としたバイオマーカー探索が盛んに行われ，プロテオミクス専門誌をにぎわせている[1),2)]．この進展は糖タンパク質の大規模解析，すなわちグライコプロテオミクスを基軸としたバイオマーカー探索にも及び，開発は確実に加速している[3)]．本稿では，血中糖鎖バイオマーカー開発の現状と問題点について概説した後に，その問題を解決すべく著者らが確立した，レクチンマイクロアレイ[4)]を用いたマーカー候補分子検証システム，およびマーカー簡易検出キット構築手法について実施例を交えて紹介したい．

## II 血中糖鎖バイオマーカー開発の現状と問題点

疾患バイオマーカーとは，血中に存在する量を測定した際，その量的変化が特定疾患の進行と相関があるものを指し，検出感度が高く，かつ健常者における血中濃度が変動しないものが好まれる．タンパク質の量的変化を網羅的に捉えることから始まるプロテオミクスを基軸とした血清バイオマーカー探索


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のパイプラインは，実験の性質の違いから，

フェーズ1（P1）：大規模解析による候補分子の同定

フェーズ2（P2）：定量的比較解析による候補分子の検証・絞り込み

フェーズ3（P3）：マーカー簡易検出キットの構築

フェーズ4（P4）：バリデーション試験

という段階に分けるのが一般的である[2]．各フェーズの実施に妥当な検体数は，フェーズが進むに従い増加する．例えばP1では10を超えないサンプルで検討するが，P4では数千検体を対象として実施しなければならない．一方，対象となる候補タンパク質分子数はフェーズが進むに従い減少し，P1では数百にも及ぶ候補分子が同定されるが，その後の絞り込みを経て2，3分子のみバリデーション試験で検証される．候補分子の絞り込みには，インフォマティクスを活用するが，より効率的なマーカー開発には，実験的なマーカー候補の絞り込みを併用することが望ましい．この段階での実験は，罹患の有無が明確であり，かつ臨床情報が豊富な検体シリーズを対象にすると，絞り込みの確度が増す．また，P2の結果を基に最適な検出キット（サンドイッチELISAなどの免疫検出キットが主流）が構築できると，さらなる開発期間の短縮にもつながる．大規模解析がより一般的になった昨今では，マーカー開発でほかを圧倒するには，P2でより優れた技術を有していることが鍵となる．プロテオミクスにおいてはmultiple reaction monitoring[5]などの有力な手法が確立しており，100検体レベルの血清を対象とした複数の候補分子の定量比較解析が可能となっている．

では糖鎖マーカーを探索する場合はどうであろうか．基本的には上述のパイプラインと同じ戦略をとる[6]が，①P1で質量分析による大規模解析を行う前段階として，疾患特異的な糖鎖を有する糖タンパク質（ペプチド）を試料溶液から効率よく捕獲する工程，および②100検体レベルでタンパク質上の病気の進展に伴う糖鎖変化を検証する工程，を含む特徴がある．工程①で糖タンパク質（ペプチド）を捕獲する手段としては，化学的な方法[7]と，レクチンを用いた方法[8]があるが，前者は糖鎖全般に有効である反面，選択性には欠ける．これに対し後者は特異的な糖鎖へ結合を示すレクチンを捕獲子として用いるため，選択的な捕獲が可能である．疾患特異的糖鎖を捕獲するにはレクチンを用いるのが好ましく実施例も多い．一方，工程②に最適な技術は現状ではきわめて乏しく，開発推進の大きな問題となっている．

## Ⅲ 糖鎖バイオマーカー検証システムの開発

100検体レベルでタンパク質上の病気の進展に伴う糖鎖変化を検証するためには，「高スループット，高感度，高再現性，迅速性」に優れた比較糖鎖解析技術が必須である．このニーズに最も合致する技術は，著者らが開発した抗体オーバーレイ・レクチンマイクロアレイ[9]であり，その特徴を**図1**に示す．レクチンマイクロアレイとは，40種以上の特異性の異なるレクチンを同一基板上に固相化したものであり，通常ガラス1枚あたり複数のサンプルを同時に分析できる形態をとる．その改法のひとつである抗体オーバーレイ検出法は，分析対象である糖タンパク質の蛍光標識などの処理をせずにそのままレクチンマイクロアレイに添加し反応させ，基板上のレクチンへ結合した糖タンパク質をコアタンパク質認識蛍光標識抗体で検出する．従来の液体クロマトグラフィーや質量分析器を用いた糖鎖解析では，糖鎖をタンパク質から切り離し，蛍光標識しなければならず，多くの工程数と時間を要していたため，それと比較すると圧倒的に簡便な手法である．感度は抗体の質に依存するが，おおむねウェスタンブロットで検出可能な量（ng程度）の標的糖タンパク質があれば分析できる．また，抗体により標的糖タンパク質の結合シグナルのみが特異的に検出されるため，サンプル調製は免疫沈降などの簡易精製程度で問題なく分析が可能である．事実，著者らはこれまでに50種を優に超える糖タンパク質を，血清や細胞培養上清，組織切片中から数10ng程度を効率よくエンリッチし，抗体オーバーレイ・レクチンマイクロアレイで比較糖鎖解析することに成功している．本技術の候補分子検証試験への活用により，実効性の高い糖鎖バイオマーカー開発パイプラインが確立され

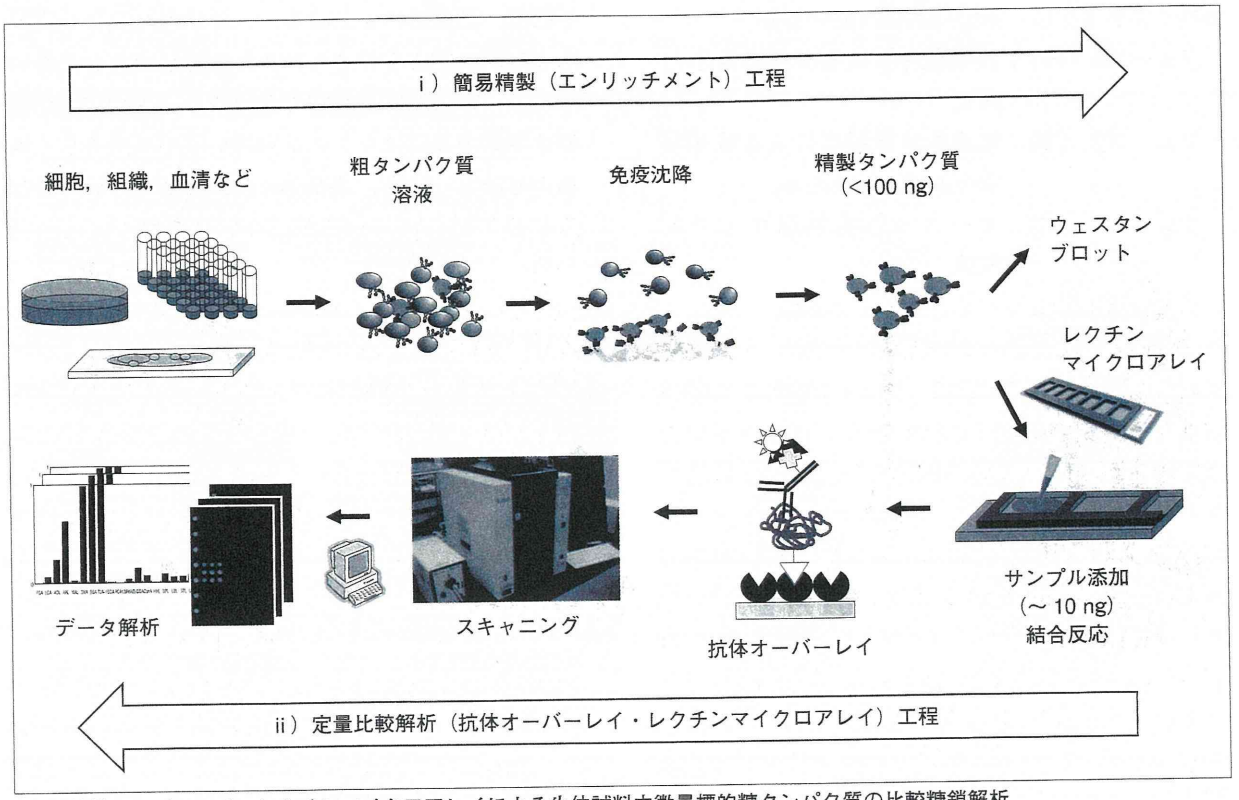


図1　抗体オーバーレイ・レクチンマイクロアレイによる生体試料中微量標的糖タンパク質の比較糖鎖解析

た[10]．この研究戦略については詳解した実験書があるのでそちらを参照いただきたい[11]．

## Ⅳ 実施例）肝線維化の進展度を定量化するための糖タンパク質マーカー開発

レクチンマイクロアレイを用いた検証試験が有効活用された実施例として，肝線維化の進展度を定量化するのに有効な糖タンパク質マーカーの開発について紹介する．

現在日本のC型肝炎ウイルス（hepatitis C virus：HCV）感染者は，およそ150～200万人と推定されている．感染した患者の肝臓では，炎症が慢性化し，20～25年程度の歳月を経て肝硬変に進展後，肝細胞がんへと至る．なお，2009年度には原発性肝がんによって32,725人が死亡したと報じられている（独立行政法人国立がん研究センターがん対策情報センターがん情報サービスの最新がん統計より：http://ganjoho.jp/public/statistics/pub/statistics01.html）が，実にその9割以上は肝炎ウイルス感染の既往をもっており，HCV感染が約70％を占めるとされている．ウイルス性肝炎から肝硬変や肝細胞がんへ至る疾病の進行度は，肝組織の針生検・病理組織診断による線維化の進展度（F0～F4に分類し，F4は肝硬変と診断される）を加味して診断する．この進展度は肝細胞がんの発がんリスクの高さを反映し，肝細胞がん出現頻度はF0～F2では年率1％以下であるのに対し，F3では3～4％，F4では7～8％に達する．一方で，現状gold standardとなっている上述の侵襲的診断法は出血などのリスクもあり，身体的負担が大きいため，非（低）侵襲的な手法が求められている．最近，超音波とせん断波の2種類の波で物体の硬さを計測する原理（エラストグラフィ）に基づいた，非侵襲的な計測機器（FibroScan®）が注目されている．しかしオペレーターの熟練度や患者のBMI値により数値が変化することや，血中ALT値が高値の場合に測定値が見掛け上高くなる，などの問題点が指摘されている[12]．現在，FibroScan®を補完する[13]，より好ましくは凌駕する血清診断法の開発が求められている．

そこで筆者らは，先に述べたシステムを駆使して，

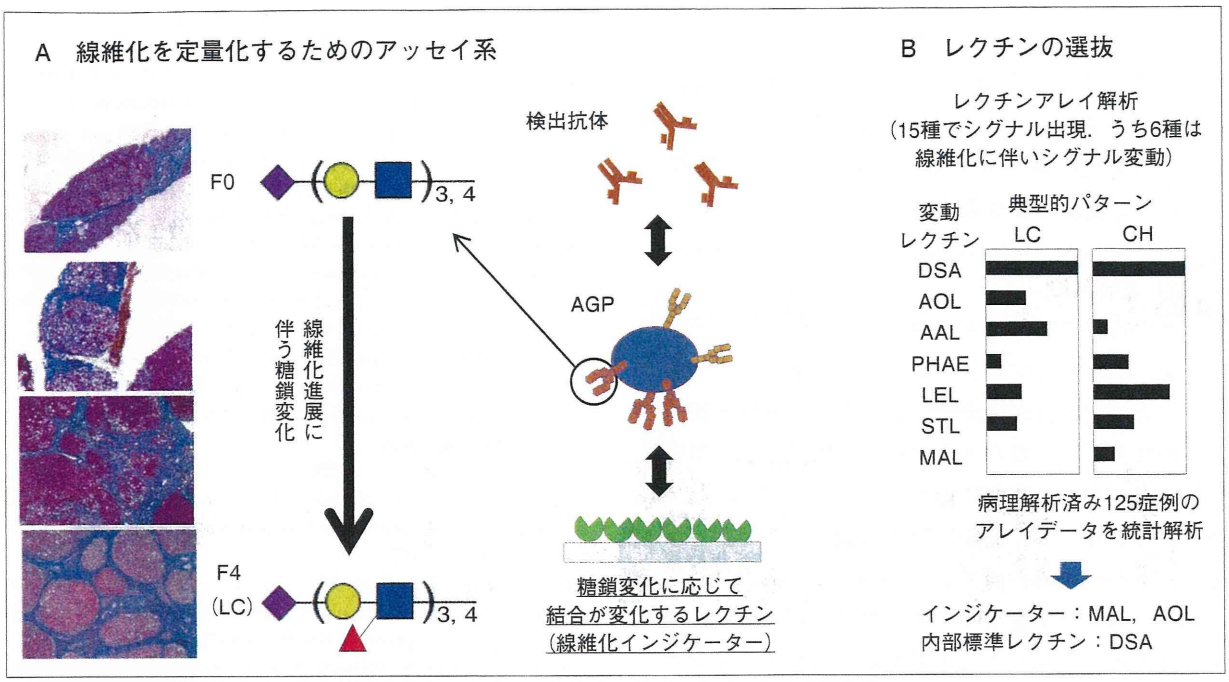


図2　AGP上の肝線維化進展に伴う糖鎖変化をレクチン―抗体サンドイッチアッセイで捉える

〔参考文献14）より引用改変〕

線維化の程度を血清により測定できる新規糖タンパク質マーカーならびに検出システムを開発した．そのひとつがα1酸性糖タンパク質（α1-acid glycoprotein：AGP）である（**図2A**）[14]．レクチンマイクロアレイは高感度な分析方法であるため，AGPの分析に必要な血清量はわずか0.5μLだった．HCVに罹患し，肝生検により線維化の程度（ステージ）が病理診断された患者125症例（F1：33症例，F2：32症例，F3:31症例，F4:29症例）を対象に分析を行った．その結果，AGPはレクチンマイクロアレイ上の15種のレクチンと反応し，うち6種のレクチンがAGP上糖鎖の肝線維化進展に伴う質的変化に応じてシグナル変動した（**図2B**）[14]．さらにこのデータは，線維化進展に最も相関があるレクチンを統計学的に選抜するために用いることができ，結果として2種のレクチン（AOL，MAL）が選出された．また，内部標準レクチン（DSA）を設定することで，線維化進展の定量化に再現性が伴い，さらに2つのシグナルを組み合わせることで，精度が向上した．以上より，線維化進展を定量することを目的とした3種のレクチン（AOL，MAL，DSA）とAGP抗体とのサンドイッチ免疫検出を並列測定するためのキットが構築された．

糖鎖とレクチンの相互作用は一般的に弱く，抗原抗体反応のような迅速自動化が困難であると考えられる．しかし，糖タンパク質がより多くの糖鎖を有し，かつ濃度が十分高ければその限りでない．AGPはN結合型糖鎖を5本もち，かつ血中濃度は0.5～1.0mg/mLと高く，迅速自動化が期待できる．そこで，3種レクチンのサンドイッチアッセイを全自動免疫測定装置（HISCL®）で測定することを試みた．測定値の妥当性を評価するために，HCV患者175症例と健常者100例の275検体を対象にレクチンマイクロアレイおよびHISCL®で測定した結果，各レクチン－抗体サンドイッチの測定値はレクチンマイクロアレイのシグナル値と強い相関を示した[15]．レクチンマイクロアレイで17時間を要していた測定が，血清使用量を10倍にすることで（それでも5μLのため血液1滴以下），1サンプルあたり20分弱（1時間で60サンプル分自動測定）にまで短縮でき，これによりバリデーション試験が一気に加速された．このマーカーの肝硬変の検出力を検討したところ，それぞれ感度94%，特異度86%，AUC 0.95，および感度100%，特異度86%，AUC 0.97であった．既存の線

維化マーカーおよびインデックスは，ヒアルロン酸（感度94%，特異度74%，AUC 0.92），Ⅳ型コラーゲン（感度100%，特異度70%，AUC 0.92），およびFIB-4[16]（感度78%，特異度83%，AUC 0.88）であり，線維化中期から後期の定量化において既存マーカーを凌駕する結果であった．

## V おわりに

近年，グライコプロテオミクスを基軸としたバイオマーカー探索は世界規模で実施され，腫瘍を標的とした糖タンパク質バイオマーカー候補分子の同定に関する報告が飛躍的に増加している．一方，レクチンマイクロアレイ開発に関する論文は2005年に発表されたが，その引用件数は2009年以降で上昇傾向にあり，特に分子腫瘍マーカー開発に関する論文が多いことは注目すべきことである．これは，レクチンマイクロアレイが疾患特異的糖鎖変化を捉えるのに最適な技術であると認知された証といっても過言ではないだろう．本技術が現状において最善であるが，すべてのマーカーを検証できるものとはいえない．著者らはこれまで10程度のがん種のマーカー探索と検証試験を実施してきたが，これらは鑑別診断や予後診断に有効なマーカーが主であり，早期発見につながるマーカーを検証するまでには至っていない．マーカー血中濃度がきわめて低い段階での検出が必要なためである．マーカーを濃縮する技術との組み合わせが突破口になると思われる．


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# Discovery of critical host factor, IL-28B, associated with response to hepatitis C virus treatment

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**Key words**
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The 2011 Okuda lecture.

## Abstract

Chronic hepatitis C affects 2.2–3.0% of the world population (130 million–170 million). Pegylated interferon-α (PEG-IFN-α) in combination with ribavirin (RBV), the approved and standard therapy, leads to viral eradication in about 50% of treated patients. In 2009, genome-wide association studies (GWAS) identified host genetic variation to be critical for predicting treatment response and spontaneous clearance in patients infected with hepatitis C virus (HCV). A correlated set of polymorphisms in the region of the interleukin-28B (IL-28B) gene on chromosome 19, coding for interferon (IFN)-λ3 were associated with clearance of genotype 1 hepatitis C virus (HCV) in patients treated with PEG-IFN-α and RBV. The same polymorphisms were subsequently associated with spontaneous clearance of HCV in untreated patients. In addition, prediction of viral response to PEG-IFN-α and RBV therapy of patients with recurrent HCV infection after orthotopic liver transplantation depends on the IL-28B genotype of both recipient and donor tissues. Diagnosis of a patient's IL-28B genotype is likely to aid in clinical decision making with standard-of-care regimens. Future studies will investigate the possibility of individualizing treatment duration and novel regimens according to IL-28B genotype. As GWAS yield unexpected data, this approach could lead to the development of novel drug therapy, such as already appears promising with IFN-λ. In this Okuda lecture, I present the current understanding in regard to the relationship between host variations and clinical outcome of hepatitis C.

## Introduction

Along with the achievement of high-throughput single nucleotide polymorphism (SNP) genotyping, using whole genome SNP data for linkage or association analysis is now an efficient strategy to reveal heritable factors. The current medical literature is increasing weekly with studies identifying genetic variants and their possible interaction with environmental factors that may have an impact on risk of disease. The growth of such studies has been spurred by the promise of understanding the genetic and environmental basis of complex disorders, and the possibility of identifying therapeutically responsive targets for drug development. Enormous numbers of genetic variants have been associated with diseases and traits, and this number will only grow as it becomes economically feasible to sequence an individual patient's entire genome.[1]

A key challenge of data interpretation lies in how to assess the phenotypic and risk factor heterogeneity within the affected patient population. Even in situations where the association between a risk factor and disease is highly significant, there are individuals with the disease who do not manifest all risk factors and those with risk factors who manifest no disease.[2] It is therefore evident that the presence of a risk factor is not a sufficient determinant of disease. Most researchers would deduce that fatal or drastic diseases based on genetic variation, in addition to those based on lethal mutations, are eliminated by natural selection on the long road of human evolution. Therefore, for the discovery of genetic variations showing a strong association with phenotype, the most effective research objective is directed at patients treated with curative medicines that target that host factor. These drugs can be made with small molecular weight chemicals, human antibodies, or they can be obtained from natural products, modified to bring out the positive effect against disease. Because human beings have not been under selective pressure of these medicines since recorded history, their contemporary pressure will reveal the fine results associated with clinical response to drug treatment.

Based on this theory, we have started to discover genetic variations associated with response to chronic hepatitis C treatment using pegylated interferon and ribavirin. The SNPs obtained from whole genome analysis were reported by a number of research groups simultaneously in 2009 and many related studies have been uploaded to September 2011. The aim of this review is to summarize the relationship between genetic variation and hepatitis C infection.