(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 χ^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

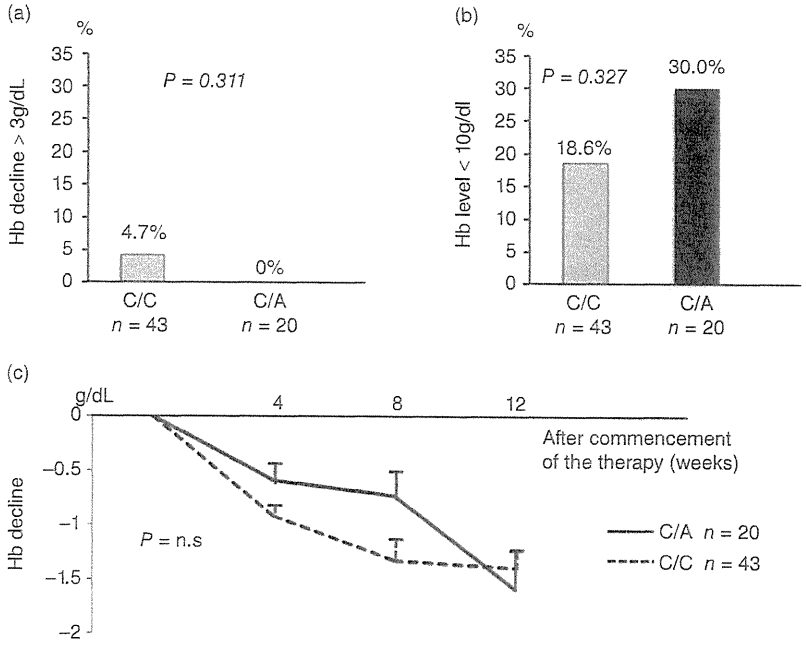

Figure 1 Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and pegylated-interferon/ribavirin (PEG-IFN/RBV)-related anemia after liver transplantation (LT). (a) Hemoglobin (Hb) decline greater than 3 g/dL at 4 weeks after the commencement of therapy was found in 4.7% of CC allele carriers 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

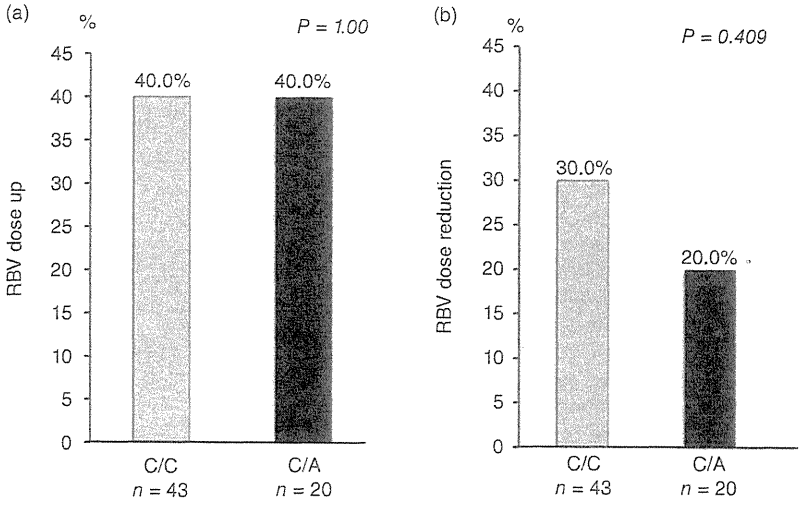

Figure 2 Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and ribavirin (RBV) dosage. (a) The dosage of RBV was increased in 40% of each genotype group. (b) RBV dose reduction due to anemia was found in 30% of those carrying the CC allele and 20% of those carrying the CA allele.

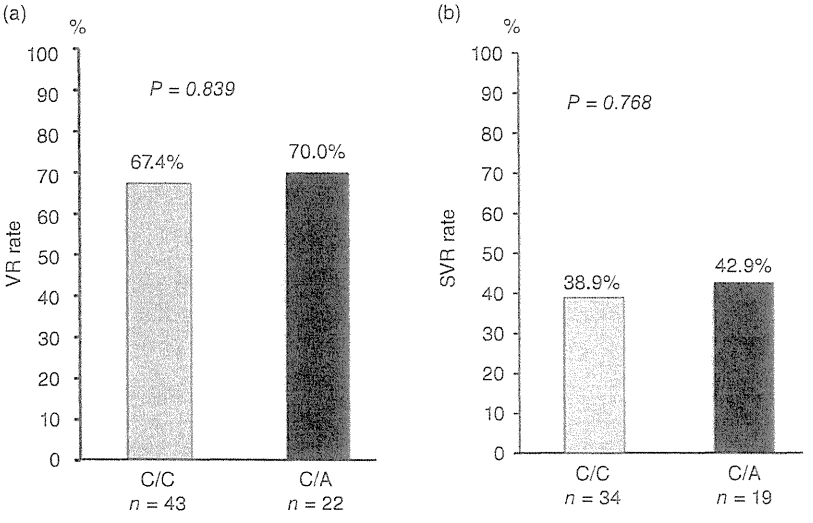

Figure 3 Inosine triphosphate pyrophosphatase (ITPA) genetic polymorphism and virological response. (a) Cirological response (VR) between the two genotypes was 68.9% and 72.7%. (b) The incidence of the sustained virological response (SVR) was 38.9% and 42.9%.

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·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·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.

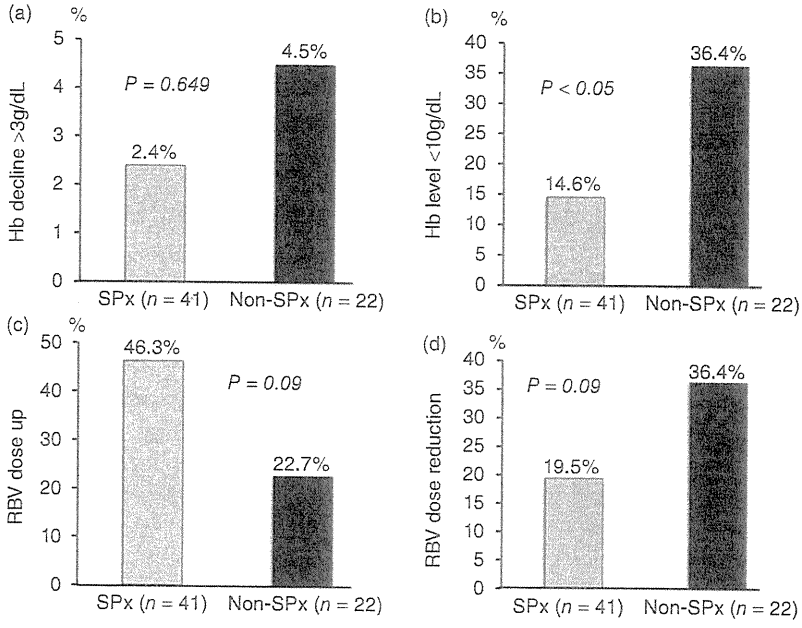

Figure 4 The efficacy of splenectomy for anaemia and ribavirin (RBV) tolerance. (a) The incidence of a hemoglobin (Hb) decline greater than 3 g/dL at 4 weeks was evident in 2.4% of recipients who had simultaneous splenectomy at liver transplantation (LT) (Spx group) and 4.5% of recipients were not subjected 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

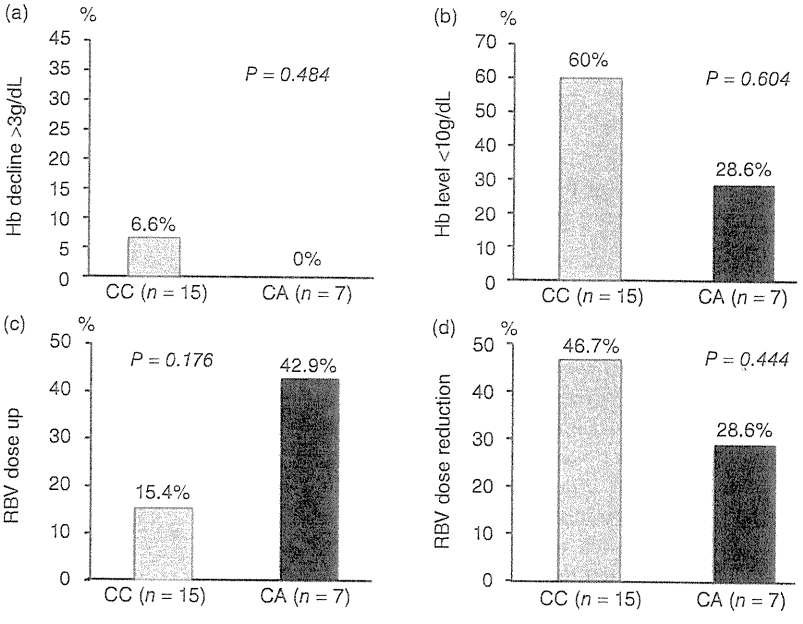

Figure 5 Inosine triphosphate pyrophosphatase (ITPA) genotypes and anemia among non-Spx group. (a) The incidence of hemoglobin (Hb) decline at 4 weeks was higher in CC allele carriers compared with CA allele carriers (6.6 *vs.* 0%). (b) The incidence of Hb levels less than 10 g/dL at 4 weeks was higher 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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