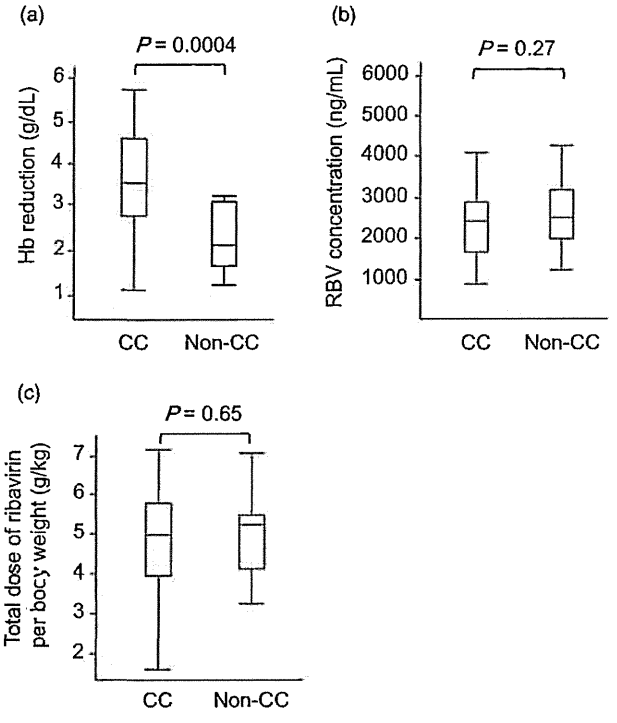

Figure 4 Association between *ITPA* polymorphism and clinical factors: hemoglobin reduction at week 12 (a), ribavirin concentration at week 44 (b), and total dose of administered ribavirin (mg/kg of body weight (c). Hb reduction in wild-type (CC) was significantly higher than those with heterozygous (CA) or homozygous (AA) rs1127354 (3.56 *vs* 2.16 g/dL, respectively, $P = 0.0004$). There were no significant associations between *ITPA* SNP rs1127354 and ribavirin concentration and total ribavirin dose administered ($P = 0.27$ and 0.65, respectively). *ITPA*, inosine triphosphatase gene; SNP, single nucleotide polymorphism.

RNA was undetectable, RBV concentration, and total dose of RBV administered. These factors were categorized below: (i) younger or older than 60 years, (ii) CC or non-CC genotype of *ITPA* SNP rs1127354, (iii) HCV RNA undetectable at < 24 weeks or ≥ 24 weeks, (iv) RBV concentration < 2500 ng/mL or ≥ 2500 ng/mL, and (v) total RBV dose of < 4.9 g/kg or ≥ 4.9 g/kg. Multiple regression analysis indicated that age, *ITPA* rs1127354, and RBV concentration were significant independent predictive factors for SVR ($P = 0.002$, 0.006, and 0.045, respectively Table 3).

Discussion

Previous studies have shown that extended 72-week combination therapy with PEG-IFN/RBV improves SVR rate,[14,15] while extended treatment is recommended only for HCV genotype 1 infection with LVR but not for general HCV patients.[13] However, Buti *et al.* showed that SVR rates were similar among LVR patients who received a standard dose of PEG-IFN alpha-2b and weight-based RBV for 48 or 72 weeks.[17] Although the overall SVR rate has been shown to improve in patients with LVR, it is necessary to determine which group of patients can benefit from extended therapy. The present study showed that age, timing of HCV RNA disappearance, serum RBV concentration, and *ITPA* SNP rs1127354 were related to the outcome of 72-week PEG-IFN/RBV therapy for patients with LVR.

Table 3 Multiple regression analysis

	Odds ratio (95% CI)	*P*-value
Age (≥ 60 years/< 60 years)	9.7 (1.8–82.6)	0.005*
ITPA rs1127354 (CA/AA *vs* CC)	15.8 (1.7–415)	0.012*
At week of undetectable HCV RNA (> 24 weeks/≤ 24 weeks)	1.1 (0.2–6.4)	0.897
Ribavirin concentration on week 44 (≥ 2500 ng/mL/< 2500 ng/mL)	12 (2.2–105.4)	0.003*
Total dose of ribavirin administered (≥ 4.9 g/kg/< 4.9 g/kg)	2 (0.4–9.7)	0.361

*$P < 0.05$.

HCV, hepatitis C virus; *ITPA*, inosine triphosphatase gene.

However, *IL28B* SNPs were not associated with the outcome of 72-week treatment in patients with LVR. *IL28B* SNP was originally reported as a host marker to predict null responders to 48-week treatment.[28] The patients enrolled in our study were late viral responders, but not null responders. Including only patients with a specific on-treatment viral response may reduce the influence of *IL28B* SNP on the outcome. Our results are consistent with those of Mangia *et al.*,[29] showing that *IL28B* genotyping had limited clinical utility in the arrangement of response-guided therapy for patients with genotype 1.

In contrast, 11 (92%) of 12 patients with CA or AA at *ITPA* SNP rs1127354 achieved SVR among 66 patients with LVR. Polymorphic variation in the *ITPA* gene causing ITPase deficiency leads to an elevated concentration of inosine triphosphate (ITP) in erythrocytes. Similarly, RBV-induced anemia is triggered by the accumulation of RBV active forms of triphosphate (RBV-TP) in erythrocytes. ITP competes with RBV-TP, thus protecting cells from the lytic effects of RBV-TP. Patients with the rs1127354 CA/AA genotype have a lower risk for a hemoglobin decline of > 3 g/dL.[22,30] In fact, we found that hemoglobin was significantly lower in patients with the CC genotype than in those with the CA/AA genotype during the initial 12 weeks of treatment (Fig. 4a). It has been reported that a cumulative reduction in RBV is more frequent in patients with the CC genotype than in patients who are non-CC. Additionally, *ITPA* SNP rs1127354 is one of the predictive factors for SVR.[31] However, other studies have shown that *ITPA* SNP is associated with RBV-induced anemia but not with treatment outcome in patients who undergo standard therapy.[22–25] In the 165 patients who underwent 48 weeks of therapy in our hospital, *ITPA* genotype was not related to outcomes of patients who underwent standard therapy (data not shown). In the present study, LVR patients were the subjects. We speculate that LVR patients have different clinical backgrounds, including genotype, related to outcome of PEG-IFN and RBV combination therapy. In a subset of patients with the favorable TT genotype of *IL28B* SNP rs8099917, rs1127354 SNP of *ITPA* seemed to be associated with the outcome of combination therapy.[32] This is the first study to demonstrate an association between *ITPA* SNP and SVR rate in LVR patients who underwent extended treatment.

It is unclear why the *ITPA* genotype was associated with outcomes of LVR patients who underwent extended treatment. It has been reported that expression of several genes before combination therapy is related to *ITPA* genotype.[30] One of these might play an important role in the response to elongated therapy. In the present study, seven of 10 patients with *ITPA* non-CC type showed > 2500 ng/mL RBV at week 44. In contrast, 19 of 41 patients with the *ITPA* CC type had > 2500 ng/mL RBV at week 44. Many patients with *ITPA* non-CC type had > 2500 ng/mL RBV at week 44.

We did not detect associations between *ITPA* variants and RBV concentrations at week 44 (Fig. 4b). RBV concentration is affected by both the dose administered and its clearance; the latter is regulated by renal function.[33] Serum creatinine level was within the normal range in the patients included in the present study, indicating that their renal function is sufficient to receive RBV adjusted by body weight. The RBV dose administered is dependent on body weight and is correlated with RBV-related adverse events, particularly anemia. Recently, it was reported that both *SLC28A2* rs11854484 genotype and *ITPA* genotype were related to RBV-related anemia. However, the factor associated with RBV concentration at weeks 4 and 8 was the *SLC28A2* rs11854484 genotype, but not the *ITPA* genotype.[34] In patients with LVR, RBV concentration and *ITPA* genotype were independently associated with the outcome of extended treatment (Table 3).

Our data suggest that serum RBV concentration at week 44 was significantly higher in patients with SVR than in those with relapse ($P = 0.002$). On the other hand, total dosage of RBV was not related to the outcome of extended therapy. In previously published data regarding 48-week therapy, both the RBV dose administered and the RBV concentration in peripheral blood were associated with the outcome of combination therapy with PEG-IFN and RBV.[35,36] Furusyo *et al.* reported that in both groups with < 60% and ≥ 60% of RBV assigned total dosage, the mean RBV concentration at 48 weeks in patients with SVR was > 1500 ng/mL and was significantly higher than in those with relapse, suggesting that RBV concentration was unaffected by the assigned total dosage.[37] In the present study, no association between RBV concentration on week 44 and the total dose of RBV administered was identified (data not shown).

Many novel interferon-free antiviral regimens for HCV are now under clinical investigation. Some of these include RBV in combination with one or two direct-acting antiviral agents.[38,39] RBV will remain a key drug for treatment of chronic HCV infection in the forthcoming era of oral combination antiviral therapy. Further studies are required to evaluate the significance of *ITPA* SNP as predictors of not only RBV-induced anemia but also of treatment outcome.

In conclusion, age, RBV concentration, timing of HCV RNA disappearance, and *ITPA* SNP rs1127354 were associated with a higher SVR rate in LVR patients given 72-week treatment. These predictive factors may allow more efficient extended treatment with PEG-IFN and RBV for patients with LVR.

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