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The relationship between *ITPA* rs1127354 polymorphisms and efficacy of antiviral treatment in Northeast Chinese CHC patients

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Abstract

This prospective study investigated the relationship between 2 inosine triphosphatase (*ITPA*) polymorphisms (rs7270101 and rs1127354) and the efficacy of ribavirin-based antiviral therapy in hepatitis C virus (HCV)-infected Chinese patients.

A total of 906 patients diagnosed with chronic hepatitis C receiving pegylated interferon (PEG-IFN) plus ribavirin combination therapy between January 2011 and January 2014 from 5 hepatitis centers in Northeast China were enrolled. The patients were divided into genotype 1 and non-genotype 1 groups according to the genotype of infected HCV. ITPA single nucleotide polymorphism (SNP) genotyping was performed for all patients. Ribavirin-induced hemolytic anemia and virological response (VR) were monitored during treatment and follow-up. Multivariate regression analysis was used to analyze the predictors for sustained virological response (SVR).

IPTA rs7270101 variants were not detected. *IPTA* rs1127354 variants were detected and showed no difference between the genotype 1 and non-genotype 1 groups. *IPTA* rs1127354 genotype CC was related to a higher incidence of ribavirin-induced hemolytic anemia. For patients who received >80% of the planned ribavirin dose, rs1127354 variants and related ITPase were related to better SVR. Multivariate analysis showed that *IPTA* rs1127354 non-genotype CC, HCV genotype, a baseline HCV RNA level $<4 \times 10^5$ IU/mL, IL-28B rs12979860 genotype CC, and low liver fibrosis were independent predictors for SVR during the combination therapy.

IPTA rs1127354 variants and related ITPase were not only related with ribavirin-induced hemolytic anemia but also directly affected the SVR to PEG-IFN plus ribavirin combination therapy in Chinese HCV-infected patients.

Abbreviations: ADSS = adenylosuccinate synthase, DAAs = direct-acting antiviral agents, ETR = end-of-treatment response, EVR = early virological response, GTP = guanosine-5′-triphosphate, HCV = hepatitis C virus, IMP = inosine monophosphate, ITP = inosine triphosphate, *ITPA* = inosine triphosphatase, ITPase = inosine triphosphate pyrophosphohydrolase, PEG-IFN = pegylated interferon, RBV = ribavirin, SNP = single nucleotide polymorphism, SVR = sustained virological response, VR = virological response, XTP = xanthosine triphosphate.

Keywords: chronic hepatitis C, hemolytic anemia, *ITPA* polymorphisms, ITPase activity, ribavirin, sustained virological response

1. Introduction

The inosine triphosphatase (*ITPA*) gene encodes an inosine triphosphate pyrophosphohydrolase (ITPase), which specifically recognizes and catalyzes the pyrophosphohydrolysis of inosine triphosphate (ITP)/dITP and xanthosine triphosphate (XTP)/dXTP to maintain the concentration of nucleoside triphosphate in cells.

Sumi et al[1–3] first found that *ITPA* mutation resulted ITPase deficiency, thus affecting the metabolism of some purine drugs such as 6-dithiopurine and azathioprine. The metabolic products of purine drugs are involved the synthesis of 6-thio-ITP which is toxic, while 6-thio-ITP can be degraded to inosine monophosphate (IMP) and 6-thio-derivatives by ITPase. When *ITPA* mutates, the hydrolysis of 6-thio-ITP is affected, leading to the accumulation of 6-thio-ITP and increasing the toxicity of purine drugs, thus increasing the side-effects of purine drugs.[4–7] However, this phenomenon is neglected because *ITPA* polymorphisms do not affect the efficacy of purine drugs. Until recent years, researchers found that *ITPA* polymorphisms affect the metabolism of ribavirin (RBV)[8] and RBV-based therapy in hepatitis C virus (HCV)-infected patients, the relationship between *ITPA* polymorphisms and metabolism of some drugs attracts the attention of the researchers. Before the development

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of direct-acting antiviral agents (DAAs), pegylate interferon (PEG-IFN) plus RBV combination therapy has been the only standard therapy for HCV infection for many years.[8] Although DAAs have been wildly applied in the treatment of chronic hepatitis C (CHC), PEG-IFN plus RBV combination therapy continues to be the recommended standard therapy in many developing countries. Moreover, some DAAs are recommended in combination with RBV due to its synergistic effect on DAAs.[9]

RBV is orally absorbed into blood circulation and intaken by red cells, where RBV is phosphorylated to be activated by phosphotransferase and released to function against virus. However, during the phosphorylation of RBV in red cells, guanosine-5′-triphosphate (GTP) is consumed, which affects the synthesis of ATP in red cells, thereby inducing hemolytic anemia.[10,11] ITPase deficiency due to *ITPA* mutation results in the accumulation of ITP in red cells.[1–3,12–15] ITP can be substituted for GTP to be used for synthesis of ATP by adenylosuccinate synthase (ADSS), which supplements the effect of consumption of GTP by RBV on the ATP synthesis,[16] thus decreasing the incidence of hemolytic anemia. In 2010, using a genome-wide association study, Fellay et al[17] from the USA found that 2 *ITPA* mutants were related to the incidence of RBV-induced hemolytic anemia in patients with CHC receiving RBV-based antiviral therapy. One single nucleotide polymorphism was located at Chromosome 2 (ITPA rs1127354, P32T); the other single nucleotide polymorphism was also located at Chromosome 2 (ITPA rs7270101, IVS2). However, studies from Japan and Korea only found ITPA rs1127354 to be related with RBV-induced hemolytic anemia. These studies did not detect the ITPA rs7270101 mutant.[18,19] No Chinese reports regarding the relationship between *ITPA* polymorphisms and RBV-induced hemolytic anemia are available.

More evidence is needed to determine whether *ITPA* polymorphisms are directly associated with the anti-HCV efficacy of RBV. Moreover, whether *ITPA* polymorphisms directly affect the efficacy of RBV-based therapy in HCV-infected patients and whether the effect of *ITPA* polymorphisms on RBV-induced hemolytic anemia affect the dose of RBV in treatment (thereby affecting efficacy) remain unclear. Thompson et al[20] found in genotype 1 HCV-infected patients and Eskesen et al[21] found in genotype 2/3 HCV-infected patients that the *ITPA* polymorphisms rs7270101 and rs1127354 are related with the occurrence of RBV-induced hemolytic anemia but not efficacy. Sakamoto et al[18] also found that *ITPA* polymorphisms affected the RBV dose during treatment, thereby affecting the sustained virological response (SVR) in genotype 2/3 and low viral load of genotype 1 HCV-infected patients but not in the high viral load of genotype 1 HCV-infected patients. Rembeck et al[22] found that the *ITPA* polymorphisms rs7270101 and rs1127354 were directly related with the efficacy of RBV-based therapy independently of the RBV dose in chronic genotype 2/3 HCV-infected patients.

HCV infection was the fourth most common infectious disease in China and the HCV prevalence in China ranges from 1.5% to 28.9%.[23,24] Our previous epidemic study also showed that the HCV prevalence in 50 cities of Northeastern China was 3%.[25] HCV infection in China shows unique distributions of HCV genotypes and host IL-28 polymorphisms and the concept of anti-viral therapy is needed to be improved. Investigating the relationship between *ITPA* polymorphisms and RBV-induced hemolytic anemia as well as anti-viral efficacy of RBV-based therapy in China is very important because RBV plus PEG-IFN therapy continues to be recommended for anti-HCV treatment in China and worldwide. The present study investigated the distribution of 2 *ITPA* polymorphisms (rs7270101 and rs1127354) in genotype 1 as well as non-genotype 1 HCV-infected Chinese patients and sought to determine whether the *ITPA* polymorphisms rs7270101 and rs1127354 affect the incidence of RBV-induced hemolytic anemia and the efficacy of RBV-based therapy.

2. Patients and methods

2.1. Patients

This prospective study included patients diagnosed with CHC between January 2011 and January 2014 from 5 regional representative hepatitis centers (China-Japan Union Hospital of Jilin University and the People's Hospital of Jilin City in the middle region, the Fourth Hospital of Harbin Medical University in the north, the Yanbian People's Hospital of Hunchun City in the east, and the Second Hospital of Daqing in the west) in the Heilongjiang and Jilin provinces, the most northeastern in China. The patients were diagnosed according to the EASL Clinical Practice Guidelines for the management of hepatitis C virus infection.[26] The inclusion criteria were as follows: the patients were anti-HCV core and HCV RNA-positive (HCV RNA was quantified using the Roche COBAS AmpliPrep/TaqMan 48 [Roche Diagnostics, Branchburg, NJ] with a lower limit of 15 IU/mL) before the treatment and were appropriate for the treatment according to the EASL Guidelines [26]. The exclusion criteria were as follows: the patients were receiving or had been receiving a combination of IFN and RBV therapy for HCV infection; they also had comorbidities including decompensated liver cirrhosis, chronic hepatitis B or co-infection with human immunodeficiency virus, alcoholic liver disease, drug-induced liver disease, or autoimmune liver disease. A total of 943 patients were enrolled in the present study. Of them, 37 quit because they could not tolerate the treatment or were lost of follow-up. Finally, 906 patients were included in the present study.

This study was approved by the Institutional Review Board of China-Japan Union Hospital affiliated with Jilin University and has been registered in the Chinese Clinical Trial Registry (No. ChiCTR-ONRC-12002207). Written informed consent was obtained from each participant before therapy.

2.2. Treatment and outcomes

All patients received a combination regimen of PEG-IFN-2α (subcutaneous injection, 180 μg/w) and RBV (1000 mg/d for genotype 1 and 800 mg/d for non-genotype 1) according to the EASL HCV Guidelines.[26] The regimen was adjusted according to the HCV genotype and the virological response during treatment. If patients infected with HCV genotype 1 achieved a rapid virological response (RVR, HCV RNA is undetectable at Week 4 post therapy) and had a low viral load at baseline (HCV RNA < 400,000 IU/mL), the treatment course was 24 weeks; if the patient achieved a complete early virological response (cEVR, HCV RNA is undetectable in the Week 12 post therapy), the treatment course was 48 weeks; if no cEVR was achieved, the treatment course was 72 weeks. In patients not infected with HCV genotype 1 who achieved RVR without low response risk factors (insulin resistance, metabolic syndrome, liver cirrhosis, and advanced age), the treatment course was 12 weeks; otherwise, the treatment course was 24 weeks. If the patient

achieved cEVR, the treatment course was 48 weeks. The treatment was stopped when the patient only achieved a partial response (PR, the decrease in HCV RNA at Week 12 post therapy compared with baseline is >2Log and HCV RNA is detectable at Week 12 and 24 post therapy) or null response (NR, the decrease in HCV RNA at Week 12 post therapy compared with the baseline is <2Log).

During treatment, half the routine dose of PEG-IFN is applied when the number of neutrophil granulocytes is $<0.75 \times 10^9$/L or the number of platelets is $<50 \times 10^9$/L. The treatment was stopped when the number of neutrophil granulocytes was $<0.5 \times 10^9$/L or the number of platelets was $<25 \times 10^9$/L. The dose of RBV was adjusted according to the hemoglobin level after 12 weeks of treatment. When the hemoglobin level decreased to 10 g/dL, the dose of RBV was decreased to 600 mg/d; when the hemoglobin level decreased to 8 g/dL, the dose of RBV was further decreased to a minimum of 200 mg/d. No erythrogenin or other therapy for anemia was provided during treatment.

The outcomes were as follows: early virological response (EVR) was indicated by a decrease in HCV RNA at Week 12 post therapy compared with the baseline of >2Log; for end-of-treatment response (ETR), HCV RNA was undetectable at the end of treatment; for SVR, HCV RNA was undetectable at Week 24 after the end of treatment.

2.3. Biochemical analysis

Before treatment, the patients received examinations for routine blood, routine urine, serum biochemical indexes (automatic biochemical analyzing equipment, Beckmann, San Diego, CA), thyroid function, quantification of HCV RNA (Roche COBAS AmpliPrep/TaqMan 48 with a lower limit of 15 IU/mL), and liver fibrosis staging (FibroScan, Echosens, France).

Before 12 weeks post therapy, routine blood examination, serum biochemical indexes, and HCV RNA level were detected every 4 weeks. After 12 weeks post therapy, these parameters were detected every 12 weeks until 24 weeks after the end of therapy.

Hemolytic anemia was defined as a decrease in hemoglobin of >3 g/dL or hemoglobin levels <10 g/dL.

2.4. SNP genotyping

Peripheral blood was collected from all patients before starting treatment. Human genomic DNA was extracted from the peripheral blood using a commercial Puregene DNA extraction kit SK8224 (Sangon Biotech, Shanghai, China) according to the manufacturer's protocol. The quantity of the extracted genomic DNA was determined using the absorbance ratio of 260 nm/280 nm on ultraviolet spectroscopy (NanoDrop model ND-1000 Peqlab Erlangen, Erlangen, Germany). Genetic polymorphisms were detected by real-time polymerase chain reaction (PCR) using the ABI7900HT Fast Real-Time PCR System.

ITPA rs1127354:
Forward: 5′-AGGAGATGGGCAGCAGAGT-3′
Backward: 5′-GCTCAACAAACTGTGTCTACTCTATT-3′
ITPA rs7270101:
Forward: 5′-TTGGTGGCACAGAAAATTGAC-3′
Backward: 5′-GGGAAACAGACACACAGAAAGTCA-3′

The PCR reaction solution was composed of 50 μL containing 1 μL template, 1 μL primers, 1 μL dNTPs, 5 μL Taq buffer, 5 μL $MgCl_2$ (25 mmol/L), 0.5 μL Taq DNA polymerase, and 35.5 μL ddH_2O. The PCR procedure for IPTA was as follows: pre-denaturation at 95 °C for 3 minutes, then 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 35 seconds and elongation at 72 °C for 40 seconds, and finally elongation at 72 °C for 5 minutes. The PCR procedure for IPTA was as follows: predenaturation at 95 °C for 3 minutes, then 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 62 °C for 30 seconds and elongation at 72 °C for 30 seconds, and finally elongation at 72 °C for 5 minutes.

IL-28B rs12979860:
Forward: 5′-CCTCTGCACAGTCTGGGATTC-3′
Backward: 5′-GCTCAGGGTCAATCACAGAAG-3′

2.5. HCV genotyping

HCV genotyping was performed using a commercial kit based on PCR and direct sequencing according to the manufacturer's protocol (SinoMDgene, Beijing, China).

2.6. Grading of ITPase activity

ITPase activity was graded according to previous reports.[1–3] The details are shown in Table 1.

2.7. Statistical analysis

Statistical analysis was performed using SPSS for Windows, Version 19.0 (SPSS, Chicago, IL). Measurement data were presented as the means ± standard deviation (SD). Measurement data were used to perform a normality and homogeneity of variance test. Normally distributed data were compared using Student *t* test, and non-normally distributed data were compared using the Wilcoxon Rank Sum Test. The enumeration data were presented as a frequency and carried out using Fisher exact test. The relative risk associated was estimated as an odds ratio (OR) with a 95% confidence interval (CI). Logistic regression analysis was performed to identify predictors for the factors affecting SVR during treatment. A two-tailed value of $P < .05$ was considered statistically significant.

3. Results

3.1. Baseline characteristics of the included patients

The included 906 patients were divided into genotype 1 and non-genotype 1 according to HCV genotyping. The baseline characteristics between these 2 groups were compared. As shown in Table 2, no significant differences were found between the 2 groups regarding sex, age, body mass index, homeostasis model assessment of insulin resistance, hemoglobin, platelet, aspartame aminotransferase, alanine aminotransferase, creatinine clearance rate, HCV RNA, liver fibrosis staging or IL-28B rs12979860.

Table 1

Grading of ITPase activity.

ITPA rs1127354	ITPA rs7270101	ITPase activity (%)
CC	AA	100
CC	AC	60
CC	CC	30
CA	AA	25
CA	AC	10
AA	CC	<5

ITPA = inosine triphosphatase, ITPase = inosine triphosphate pyrophosphohydrolase.

Table 2

Baseline characteristics of the included 906 patients.

	All chronic HCV-infected patients		
	Genotype 1 (n = 612)	Non-genotype 1 (n = 294)	*P*
Gender (male/female)	323/289	152/142	.761
Age (y)	49.76 ± 10.68	50.69 ± 12.05	.238
BMI, kg/m^2	22.89 ± 4.16	22.98 ± 4.10	.783
HOMA-IR	1.98 ± 0.59	2.01 ± 0.56	.681
Hemoglobin, g/dL	13.42 ± 1.42	13.38 ± 1.44	.682
Platelet, 109/L	134.21 ± 14.18	133.79 ± 14.44	.682
AST, IU/L	53.34 ± 11.52	53.26 ± 11.24	.986
ALT, IU/L	48.25 ± 12.31	48.14 ± 11.95	.976
CCR, mL/min	97.65 ± 13.89	99.46 ± 13.87	.076
HCV RNA, log10 IU/mL	4.65 ± 1.46	4.65 ± 1.43	.972
Fatty liver			
Yes	140 (22.9%)	73 (24.8%)	
No	472 (77.1%)	221 (75.2%)	.516
Fibrosis (FibroScan):			
F0–2 (n, %)	506 (82.7%)	234 (79.6%)	
F3–4 (n, %)	106 (17.3%)	60 (20.4%)	.261
RBV >80% of the planned dose			
Yes	420 (68.6%)	212 (72.1%)	
No	192 (31.4%)	82 (27.9%)	.283
IL-28B rs12979860			
CC	508 (83.0%)	250 (85.0%)	
CT	104 (17.0%)	44 (15.0%)	.437

ALT = alanine aminotransferase, AST = aspartame aminotransferase, BMI = body mass index, CCR = creatinine clearance rate, HCV = hepatitis C virus, HOMA-IR = homeostasis model assessment of insulin resistance, RBV = ribavirin, .

3.2. The distribution of the ITPA polymorphisms rs7270101 and rs1127354 and ITPase activity

An analysis of the *ITPA* polymorphisms rs7270101 and rs1127354 showed no significant differences in the distribution of rs1127354 genotype frequencies between genotype 1 and non-genotype 1 groups, and rs7270101 showed no variants (Table 3). ITPase activity data showed that in the genotype 1 group, 76.3% of the patients showed 100% ITPase activity, 22.7% showed 25% ITPase activity, and 1.0% showed ITPase activity <5%; however, in the non-genotype 1 group, 77.9% of the patients showed 100% ITPase activity, 21.4% showed 25% ITPase activity, and 0.7% showed ITPase activity <5% (Table 3).

3.3. Relationship between ITPA rs1127354 genotypes and RBV-induced hemolytic anemia

The incidence rate of hemolytic anemia (defined as a decrease in hemoglobin of >3 g/dL or hemoglobin levels <10 g/dL) during the first 4 weeks of treatment in all patients was analyzed. As shown in Fig. 1, 29.0% of the patients showed a decrease in hemoglobin of >3 g/dL, and 7.2% of the patients had a hemoglobin level <10 g/dL. Patients with rs1127354 genotype CC showed a higher incidence rate of hemolytic anemia than those with rs1127354 non-genotype CC regardless of HCV genotype. The baseline hemoglobin level in the HCV genotype 1 group showed no difference between rs1127354 genotype CC and rs1127354 non-genotype CC, whereas the hemoglobin in patients with rs1127354 genotype CC decreased more rapidly than those with rs1127354 non-genotype CC and was lowest at Week 12 (Fig. 2A). Similar changes were observed in the HCV non-genotype 1 group (Fig. 2B).

3.4. Relationship between ITPA rs1127354 genotypes and the total dose of RBV during treatment

ITPA rs1127354 variants affect the incidence of hemolytic anemia; we questioned whether this could affect RBV use during the treatment. As shown in Table 4, the percentage of patients who achieved 80% of the total planned RBV dose was significantly lower in patients with the rs1127354 genotype CC than those with rs1127354 non-genotype CC (65.3% vs 79.3% in HCV genotype 1 group, 69.0% vs 83.1% in HCV non-genotype 1 group).

3.5. Relationship between ITPA rs1127354 genotypes as well as between ITPase and the virological response

For 632 patients who achieved 80% of the total planned RBV dose, the RVR, EVR, ETR, and SVR rates were compared between the genotype 1 and non-genotype 1 groups and further analyzed between the rs1127354 genotype CC (with 100% ITPase) and rs1127354 non-genotype CC (with ITPase <25%) groups. As shown in Table 5, for patients infected with HCV genotype 1, ETR and SVR (but not RVR or EVR rates) were significantly lower in the rs1127354 genotype CC group than in the rs1127354 non-genotype CC group ($P < .001$ for ETR and 0.018 for SVR, respectively). For patients infected with HCV

Table 3

Distribution of *ITPA* polymorphisms rs7270101 and rs1127354 and ITPase activity between 906 chronic HCV-infected patients.

	Genotype 1 (n = 612)	Non-genotype 1 (n%) (n = 294) (%)	OR (95% CI)	*P*
ITPA rs1127354				
CC	467 (76.3%)	229 (77.9%)	1	
CA	139 (22.7%)	63 (21.4%)	1.082 (0.772–1.516)	.647
AA	6 (1.0%)	2 (0.70%)	1.471 (0.295–7.346)	.636
C	1073 (87.7%)	521 (88.6%)	1	
A	151 (12.3%)	65 (11.4%)	1.128 (0.828–1.536)	.445
ITPA rs7270101				
AA	612 (100.0%)	294 (100.0%)		
Non-AA	0	0	–	–
ITPase activity (%)				
100	467 (76.3%)	229 (77.9%)	1	
25	139 (22.7%)	63 (21.4%)	1.082 (0.447–1.516)	.647
<5	6 (1.0%)	2 (0.7%)	1.471 (0.295–7.346)	.636

CI = confidence interval, HCV = hepatitis C virus, *ITPA* = inosine triphosphatase, ITPase = inosine triphosphate pyrophosphohydrolase; OR = odds ratio.

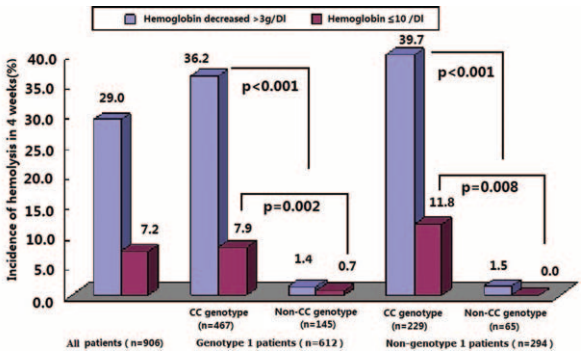

Figure 1. Relationship between *ITPA* rs1127354 genotypes and ribavirin-induced hemolytic anemia at Week 4 posttreatment.

non-genotype 1, EVR, ETR, and SVR (but not RVR) rates were significantly lower in the rs1127354 genotype CC group than in the rs1127354 non-genotype CC group ($P = .034$ for EVR, 0.035 for ETR, and 0.005 for SVR, respectively).

3.6. Univariate and multivariate analysis of the factors affecting SVR during treatment

Next, the factors affecting SVR during the PEF-IFN combination with RBV treatment were analyzed. A univariate analysis showed that age <40 y, low liver fibrosis (FibroScan 0–2), HCV non-genotype 1, a baseline HCV RNA level $<4 \times 10^5$ IU/mL, IL-28B rs12979860 genotype CC, and IPTA rs1127354 non-genotype CC were the factors affecting SVR rates. A multivariate analysis showed that low liver fibrosis (FibroScan 0–2), HCV non-genotype 1, a baseline HCV RNA level $<4 \times 10^5$ IU/mL, IL-28B rs12979860 genotype CC, and IPTA rs1127354 non-genotype CC were independent predictors for SVR (Table 6).

4. Discussion

In the present study, IPTA rs1127354 variants but not rs7270101 were found in Chinese patients infected with HCV. IPTA rs1127354 genotype CC was related with a higher incidence of RBV-induced hemolytic anemia, a lower total RBV dose, and lower ETR and SVR rates regardless of the HCV genotype. IPTA rs1127354 non-genotype CC was an independent predictor for SVR during PEG-IFN combination with RBV treatment.

RBV plus PEG-IFN combination treatment is always the standard recommended therapy for CHC. Although DAAs have been widely applied in CHC therapy, RBV plus PEG-IFN combination treatment is currently recommended for CHC therapy.[8,9] Moreover, RBV is recommended for combination with many DAAs during CHC treatment. Thus, it is important to investigate the relationship between *ITPA* polymorphisms and RBV-induced hemolytic anemia as well as the anti-viral efficacy of RBV-based therapy in various regions and races.

Sumi et al[1–3] first found that an *ITPA* mutation resulted in ITPase deficiency, thereby affecting the metabolism of some purine drugs, such as 6-dithiopurine and azathioprine, and resulting in adverse events in patients. However, this phenomenon is neglected because *ITPA* polymorphisms do not affect the efficacy of purine drugs. Researchers recently found that *ITPA* polymorphisms affect the metabolism of RBV[8] and RBV-based therapy in HCV-infected patients; the relationship between *ITPA* polymorphisms and metabolism of some drugs has attracted the attention of the researchers.

A genome-wide association study of ITPA found that the 2 *ITPA* polymorphisms rs7270101 and rs1127354 were related with the occurrence of RBV-induced hemolytic anemia during RBV-based therapy for CHC. *ITPA* rs1127354 genotype CA/AA and rs7270101 genotype AC/CC were related with a lower incidence of hemolytic anemia.[27] Different from this report, 2 other reports from Japan and Korea did not find rs7270101 polymorphisms.[18,19] The present study found that the rate of rs1127354 non-genotype CC was 23.7% (genotype CA 22.7% and AA 1.0%) in Chinese patients infected with HCV genotype 1 and 22.1% (genotype CA 21.4% and AA 0.7%) in Chinese patients infected with HCV non-genotype 1, which is similar to those reported in Japan and Korea (27.2% and 19.0%, respectively)[18,19] but higher than those reported from Europe and the USA (15.0% and 13.2%, respectively).[20,21] No rs7270101 polymorphisms were found in the present study. ITPase activity in Chinese patients with a different rs1127354 genotype showed greater difference than those observed in European and American studies.

Reports regarding the relationship between *ITPA* polymorphisms and the incidence of RBV-induced hemolytic anemia are currently controversial due to regional differences.[17–19] Our data showed that the incidence of hemolytic anemia was higher in

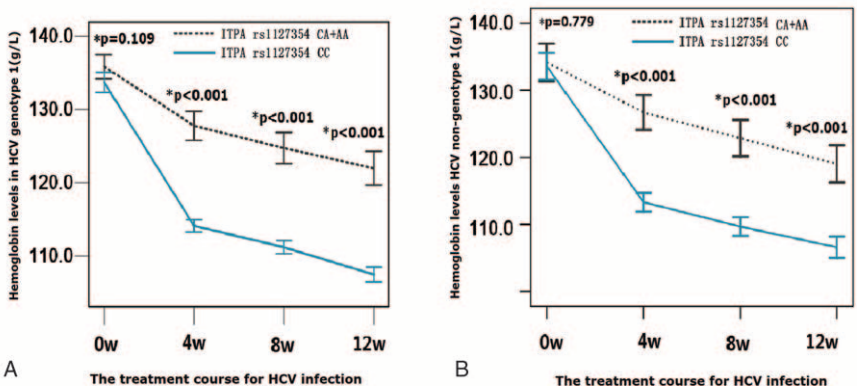

Figure 2. Dynamic changes in ribavirin-induced hemolytic anemia during the first 12 weeks of treatment. (A) HCV genotype 1 group; (B) HCV non-genotype 1 group. *Compared with ITPA rs1127354 CC.

Table 4

Relationship between *ITPA* rs1127354 genotypes and the total dose of RBV during treatment.

	RBV planned dose			
	>80% (%)	<80%	OR (95% CI)	*P*
Genotype 1 (n = 612)				
ITPA rs1127354				
CC	305 (65.3%)	162 (34.7%)	1	–
CA/AA	115 (79.3%)	30 (20.7%)	2.036 (1.305–3.176)	.002
Non-genotype 1 (n = 294)				
ITPA rs1127354				
CC	158 (69.0%)	71 (31.0%)	1	–
CA/AA	54 (83.1%)	11 (16.9%)	2.206 (1.089–4.470)	.025

CI = confidence interval, *ITPA* = inosine triphosphatase, OR = odds ratio, RBV = ribavirin.

Table 5

Relationship between *ITPA* rs1127354 genotypes as well as between ITPase and virological response.

	100% ITPase activity (rs1127354CC)	ITPase activity ≤25% (rs1127354 CA/AA)	OR (95% CI)	*P*
HCV genotype 1 (n = 420)				
RVR				
No	204 (66.9%)	72 (62.6%)	1	
Yes	101 (33.1%)	43 (37.4%)	0.829 (0.530–1.296)	.410
EVR				
No	174 (57.0%)	60 (52.2%)	1	
Yes	131 (43.0%)	55 (47.8%)	0.821 (0.534–1.263)	.370
ETR				
No	73 (23.9%)	9 (7.8%)	1	
Yes	232 (76.1%)	106 (92.2%)	0.270 (0.130–0.560)	<.001
SVR				
No	123 (40.3%)	32 (27.8%)	1	
Yes	182 (59.7%)	83 (72.2%)	0.570 (0.357–0.911)	.018
HCV non-genotype 1 (n = 212)				
RVR				
No	63 (39.9%)	21 (38.9%)	1	
Yes	95 (60.1%)	33 (61.1%)	0.960 (0.510–1.807)	.898
EVR				
No	50 (31.6%)	9 (16.7%)	1	
Yes	108 (68.4%)	45 (83.3%)	0.432 (0.192–0.952)	.034
ETR				
No	18 (11.4%)	1 (1.9%)	1	
Yes	140 (88.6%)	53 (98.1%)	0.147 (0.019–1.127)	.035
SVR				
No	40 (25.3%)	4 (7.4%)	1	
Yes	118 (74.7%)	50 (92.6%)	0.236 (0.080–0.695)	.005

CI = confidence interval, ETR = end-of-treatment response, EVR = early virological response , HCV = hepatitis C virus, *ITPA* = inosine triphosphatase, ITPase = inosine triphosphate pyrophosphohydrolase, OR = odds ratio, RVR = rapid virological response, SVR = sustained virological response.

Table 6

Univariate and multivariate analysis of the factors affecting SVR during treatment.

Baseline characteristics	Univariate analysis		Multivariate analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Female vs male	1.792 (1.342–2.338)	.001	1.279 (0.959–1.705)	.094
Age <40 vs age ≥40	1.030 (0.726–1.459)	.870	1.113 (0.768–1.072)	.528
BMI <27 vs ≥27, kg/m^2	1.161 (0.845–1.593)	.357	1.178 (0.883–1.666)	.353
ALT > 2ULN vs ALT ≤ 2ULN, IU/L	1.145 (0.820–1.600)	.427	1.074 (0.718–1.608)	.728
AST > 2ULN vs AST ≤ 2ULN, IU/L	1.326 (0.837–2.100)	.228	1.420 (0.814–2.478)	.271
HCV-RNA ≤ 4×10^5 IU/mL vs > 4×10^5 IU/mL	2.221 (1.690–2.891)	<.001	2.236 (1.676–2.983)	<.001
Non-genotype 1 vs HCV genotype 1	1.790 (1.342–2.388)	<.001	2.034 (1.488–2.779)	.001
IL-28B rs12979860 CC vs CT	3.660 (2.505–5.347)	<.001	7.31 (3.67–16.74)	<.001
ITPA rs1127354 CC vs CA+AA	2.228 (1.634–3.180)	<.001	2.184 (1.520–3.138)	.001
4W HGB ≤10 g/dL No vs Yes	2.327 (1.381–3.922)	.002	2.954 (1.698–5.140)	<.001
Fatty liver Yes vs No	0.807 (0.593–1.098)	.171	1.355 (0.969–1.854)	.075
Liver fibrosis (F0–2) vs F3–4	0.485 (0.345–0.683)	<.001	1.553 (1.062–2.269)	.023

ALT = alanine aminotransferase, AST = aspartame aminotransferase, BMI = body mass index, CI = confidence interval, HCV = hepatitis C virus, OR = odds ratio, ULN = upper limits of normal.

patients with rs1127354 genotype CC than those with rs1127354 non-genotype CC during the first 4 weeks during treatment. As treatment was prolonged, hemoglobin decreased more rapidly in patients with rs1127354 genotype CC than those with rs1127354 non-genotype CC. The changes showed no difference between patients infected with HCV genotype 1 and non-genotype 1. The incidence of hemolytic anemia in the present study is 29.0%, which is similar to previous studies from Japan and Korea (35.4% and 16.5%, respectively) and is lower than that in the USA.[17–19] This may be attributed to the high percentage of patients with rs1127354 non-genotype CC in an Asian population.

The present study showed that the percentage of patients who achieved 80% of the planned RBV dose was significantly higher in those with rs1127354 non-genotype CC than in those with rs1127354 genotype CC, which is in accordance with the results of the effects on hemolytic anemia and hemoglobin. Kurosaki et al[28] found that patients with rs1127354 genotype AA/CA can receive RBV at >80% of the planned dose and achieved a higher SVR and lower relapse rate. A decreased incidence of hemolytic anemia avoids reducing the RBV dose or stopping RBV, thereby improving the virological response. Thus, we speculated that the high virological response in Chinese patients infected with HCV genotype 1 (compared with those from Europe and USA) is not only related with IL-28B polymorphisms but is also related with the high percentage of patients with rs1127354 non-genotype CC, which is related with a higher RBV dose.

The direct effect of *ITPA* polymorphisms on the efficacy of PEG-IFN combined with RBV therapy was recently studied with no consistent conclusions.[18,22]*ITPA* polymorphisms were first thought to affect SVR indirectly by affecting the incidence of RBV-induced hemolytic anemia and thus the RBV dose used during therapy. However, Rembeck et al[22] found that in patients who received RBV at >80% of the planned dose, those with lower ITPase activity because of *ITPA* polymorphisms achieved better SVR. They speculated that the *ITPA* polymorphisms rs7270101 and rs1127354 could affect the SVR independently of the RBV dose. However, they only included patients infected with HCV genotype 2/3 but not genotype 1. The present study showed no effect for the HCV genotype. Patients with a higher ITPase activity achieved a lower SVR rate that was not related with the RBV dose or treatment course. Notably, in 2010, Sakamoto et al[18] found that patients with rs1127354 non-genotype CC achieved a higher SVR rate than those with rs1127354 genotype CC in patients with low viral load and HCV genotype 1. However, their study did not apply individual therapy for the patient. Based on the theoretical hypothesis, if they analyzed the data from patients who finished receiving >80% of the planned RBV dose, they should conclude that patients with rs1127354 non-genotype CC achieved a higher SVR rate than those with rs1127354 genotype CC independent of the serum HCV RNA level and genotype, as well as RBV-induced hemolytic anemia; they should have also found that rs1127354 non-genotype CC directly affects the response to Peg-INF combined with RBV therapy.

In conclusion, the present study found *ITPA* rs1127354 variants in Chinese patients infected with HCV. The rate of rs1127354 genotype CC was similar to that in other Asian populations but higher than that in European and American populations. During PEG-IFN combined with RBV treatment, patients with rs1127354 genotype CC showed a higher incidence of hemolytic anemia, a slower reduction in hemoglobin, a smaller percentage of patients who received RBV at >80% of the planned dose, and lower EVR, ETR and SVR rates. We also found that *IPTA* rs1127354 non-genotype CC was an independent predictor for SVR. Our data suggested *IPTA* polymorphism rs1127354 should be screened before RBV-based therapy to avoid hemolytic anemia and obtain a better SVR.

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