Polymorphisms of the ITPA gene have been associated with anemia during combination therapy in hepatitis C virus (HCV)-monoinfected patients. Our aim was to confirm this association in HIV/HCV-coinfected patients. In this prospective, observational study, 73 HIV/HCV-coinfected patients treated with pegylated interferon plus ribavirin (RBV) were enrolled. Two single nucleotide polymorphisms within or adjacent to the ITPA gene (rs1127354 and rs7270101) were genotyped. The associations between the ITPA genotype and anemia or treatment outcome were examined. Fifty-nine patients (80.8%) had CC at rs1127354, whereas 14 (19.2%) had a CA/AA ITPA genotype. Percent decreases from baseline hemoglobin level were significantly greater in patients with the CC genotype than in those with the CA/AA genotype at week 4 (P = 0.0003), week 12 (P < 0.0001), and week 36 (P = 0.0102) but not at the end of treatment. RBV dose reduction was more often needed in patients with the CC genotype than in those with the CA/AA genotype (odds ratio [OR] = 11.81; 95% confidence interval [CI] = 1.45 to 256.17; P = 0.0039), as was erythropoietin therapy (OR = 8.28; 95% CI = 1.04 to 371.12; P = 0.0057). Risk factors independently associated with percent hemoglobin nadir decrease were RBV dose reduction (OR = 11.72; 95% CI = 6.82 to 16.63; P < 0.001), baseline hemoglobin (OR = 1.69; 95% CI = 0.23 to 3.15; P = 0.024), and body mass index (OR = -0.7; 95% CI = -1.43 to 0.03; P = 0.061). ITPA polymorphism was not an independent predictor of sustained virological response. Polymorphisms at rs1127354 in the ITPA gene influence hemoglobin levels during combination HCV therapy and the need for RBV dose reduction and erythropoietin use in HIV/HCV-coinfected patients.

Materials and Methods

Study Population. The cohort of patients in the present study is derived from a well-characterized cohort of 389 HIV/HCV-coinfected patients on active follow-up at the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain. All the patients consented to the provision of genetic material as part of their coinfection assessment. To be included in the study, all the patients had to be stable, either treated or untreated, with respect to HIV infection. Nonresponders to previous IFN-based therapies were not included in the study. Patients with chronic renal disease or creatinine clearance of ≤50 ml/min, hemoglobin concentration of ≤11.5 g/dl, neutrophil count of ≤1,500/mm³, or platelet count of ≤70,000/mm³ at baseline were also excluded. Subjects who were hospitalized or had a frank cognitive impairment such as delirium or dementia on enrollment were not eligible.
Patients with opportunistic infections, neoplasms, or fever of undetermined origin were not considered for HCV therapy. The diagnosis of AIDS was based on the 1993 revised case definition of the Centers for Disease Control and Prevention (6). All the patients were negative for hepatitis B surface antigen, did not have evidence of other liver diseases, and had not received other therapies, except for combined antiretroviral therapy. All patients had had abnormal levels of serum alanine aminotransferase (ALT) for more than 6 months and were positive for anti-HCV antibody and serum HCV RNA. The study was approved by the Ethics Committee of the Hospital de la Santa Creu i Sant Pau.

**HCV RNA levels**. Plasma HCV RNA was measured using a real-time PCR assay (Cobas TaqMan; Roche, Barcelona, Spain), which has a detection limit below 15 IU/ml. HCV genotyping was performed using a commercial real-time PCR hybridization assay (Versant HCV Genotype version 2.0 line probe assay; Siemens, Barcelona, Spain).

**Liver fibrosis staging**. The extent of liver fibrosis was measured using transient elastography by a FibroScan apparatus (Echosens, Paris, France). The median value of all tests per patient is expressed in kilopascals. Cirrhosis, corresponding to a METAVIR score of F4, was defined for liver stiffness values of 14 kPa or higher.

**HCV combination therapy**. Treatment regimens included PEG-IFN alpha 2α at standard doses (180 μg per week) plus weight-adjusted RBV (1,000 mg/day for patients weighing <75 kg and 1,200 mg/day for patients weighing ≥75 kg). Patients with HCV genotype 1 or 4 received either 48 or 72 weeks of treatment; patients with HCV genotype 3 received 24 or 48 weeks of treatment, according to the virological response at week 4 (patients with a positive HCV load at week 4 had 6 months more of treatment). Therapy was stopped in patients with a suboptimal virological response at weeks 12 (HCV load decrease under 2 log units with respect to baseline values) and 24 (positive HCV load). Sustained virological response (SVR) was defined as undetectable HCV RNA in serum at the end of follow-up (24 weeks after cessation of treatment). Patients in whom the qualitative serum HCV RNA test result was positive at 24 weeks were considered nonresponders, and therapy was stopped.

PEG-IFN and RBV dose modifications followed the standard criteria and procedures (16). Specifically, the RBV dose was cut by 200 mg in patients receiving 1,000 mg or 1,200 mg when hemoglobin decreased to <12 g/dl and by another 200 mg when it was below 10 g/dl. RBV treatment was stopped when hemoglobin decreased to <8.5 g/dl. Patients with baseline Hb concentrations of <13 g/dl were given reduced doses of RBV (200 mg lower than the standard dose determined by body weight) to prevent early discontinuation of therapy.

Recombinant human erythropoietin (r–huEPO) was administered in the event of (i) a decline in Hb concentration from baseline of more than 4 g/dl, (ii) an Hb concentration of ≤10 g/dl, or (iii) symptomatic anemia, which may occur at any Hb concentration following a rapid fall in Hb concentration. Treatment with r–HuEPO was continued until either of the following: resolution of anemia-related symptoms or recovery of Hb concentrations to the lower of 12 g/dl or 1.0 g/dl below pretreatment values.

**IL-28B and ITPA genotyping**. Genomic DNA was automatically extracted from blood samples using QIAxymphony SP equipment (Qiagen, Hilden, Germany). We analyzed two functional polymorphisms (rs1127354 and rs7270101) in the ITPA gene as well as polymorphism rs12979860 in the interleukin-28 (IL-28B) gene by real-time PCR on an ABI Prism 7000 sequence detection system using TaqMan single nucleotide polymorphism (SNP) genotyping assays (Applied Biosystems, Foster City, CA), according to the manufacturer’s instructions. Homozygous and heterozygous sequenced samples of each SNP were included as internal controls.

**Definition of clinical endpoints**. The primary genetic association analyses considered association of polymorphisms in the ITPA gene with the following: (i) the percent reduction in Hb level from baseline to week 4 (continuous variable); (ii) the absolute Hb reduction from baseline to week 4 (continuous variable); (iii) Hb reduction of >3 g/dl; (iv) Hb reductions over the course of therapy, defined both quantitatively (absolute and percent decreases) and qualitatively (>3-g/dl reduction); (v) the need for RBV dose reduction; (vi) the need for r–huEPO therapy; and (vii) the rate of SVR.

**Statistical analyses**. Data are expressed as mean and standard deviation (SD) or as otherwise specified. Continuous variables were assessed with the Mann-Whitney test for two groups or a nonparametric analysis of variance (ANOVA) by applying a rank transformation on the dependent variable (rank-ANOVA) for more than two groups with the Bonferroni alpha adjustment for post hoc comparisons. Categorical data such as genotype and allele frequencies were compared by use of Fisher’s exact test. The level of significance was established at 0.05, and all reported P values are two-sided. All analyses were performed with SAS software (SAS Institute Inc., Cary, NC). Stepwise logistic regression analysis was used to examine the association of SVR, anemia, and other parameters with polymorphisms in the ITPA gene. The variables selected to enter into stepwise regression were those that correlated significantly with ITPA gene polymorphisms (after Bonferroni correction for multiple testing).

**RESULTS**

**Study population**. Among 389 patients with HIV/HCV coinfection, 73 received therapy with PEG-IFN plus RBV for a median time of 9.6 ± 3.7 months (range, 3 to 18 months). Fifty-six patients (76%) had a complete HCV treatment course. The mean duration of treatment in the 17 patients (24%) who did not complete treatment was 5.8 ± 1.1 months (range, 3 to 8 months). Baseline characteristics of the 73 patients and their genotypes at rs1127354 in the ITPA gene are shown in Table 1. There were 22 cirrhotic patients, without differences between groups (odds ratio [OR] = 1.10; 95% confidence interval [CI] = 0.27 to 5.43; P = 0.8566).

There were no statistically significant differences between treated and not treated HCV–coinfected patients in terms of genotypes at rs1127354 in the ITPA gene (OR = 1.55; 95% CI = 0.80 to 3.17; P = 0.2240). There were also no differences between both groups in terms of genotypes at rs7270101 in the ITPA gene (OR = 1.25; 95% CI = 0.28 to 7.88; P = 0.9736).

Duration of HCV infection and HCV genotypes are shown in Table 1. There were no significant differences between treated patients according to genotype at rs1127354 in the ITPA gene and HCV genotype (Table 1). There were also no differences between groups according to genotype at rs1127354 in the ITPA gene and genotype of the IL-28B gene (44.1% versus 28.6% for CC genotype; OR = 0.51; 95% CI = 0.11 to 2.04; P = 0.4488) and genotype at rs7270101 in the ITPA gene (27.2% versus 14.3% for AC/CC genotype; OR = 0.45; 95% CI = 0.04 to 2.39; P = 0.4938) (Table 1).

Most of the patients were virologically well-controlled in terms of HIV infection (79.4% had undetectable HIV-1 RNA). There were no differences between the two groups of patients in terms of antiretroviral drug exposure (Table 2). There were no differences between HCV-treated patients according to genotype at rs1127354 in the ITPA gene and abacavir-based combination antiretroviral therapy (38.9% versus 37.7%; P = 0.8205). Twelve patients had low hemoglobin levels at baseline and were given an RBV dose decreased by 200 mg. There were 8 patients with the CC genotype and 4 with the CA/AA genotype of the ITPA gene (P = 0.2271) (Table 1).

**Sustained virological response and IL-28B and ITPA polymorphism**. Thirty-nine patients (53.4%) achieved SVR. SVR was associated with the HCV genotype (88.9% for genotype 3 and
TABLE 1 Baseline characteristics of HIV/HCV-coinfected patients treated with pegylated interferon and ribavirin

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Result by ITPA genotype at rs1127354</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>73</td>
<td>59 14</td>
</tr>
<tr>
<td>Sex (no. male/no. female)</td>
<td>43/30</td>
<td>35/24 8/6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.8 ± 5.5</td>
<td>46.7 ± 5.2 47.1 ± 6.6</td>
</tr>
<tr>
<td>Body wt (kg)</td>
<td>68.1 ± 8.5</td>
<td>68.6 ± 8.5 66.3 ± 8.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.2 ± 3.2</td>
<td>23.0 ± 3.0 23.9 ± 3.6</td>
</tr>
</tbody>
</table>

No. (%) of patients acquiring HIV by the following means:
- MsM: 11 (15.1) 8 (13.6) 3 (21.4)  |
- HTSX: 20 (27.4) 18 (30.5) 2 (14.3)  |
- IDU: 42 (57.5) 33 (55.9) 9 (64.3)  |

Duration of HCV infection (yr) | 15.9 ± 6.2 | 15.9 ± 6.5 15.5 ± 4.8  |

No. (%) of patients with cirrhosis | 22 (30.1) | 18 (30.5) 4 (28.6)  |

No. (%) of patients with indicated genotypes of gene:
- IL-28B
  - CC | 30 (41.1) | 26 (44.1) 4 (28.6)  |
  - CT/TT | 43 (58.9) | 33 (55.9) 10 (71.4)  |
- rs270101
  - AA | 55 (75.3) | 43 (72.8) 12 (85.7)  |
  - AC/CC | 18 (24.6) | 16 (27.2) 2 (14.3)  |

Baseline hemoglobin concn (g/dl) | 14.5 ± 1.6 | 14.6 ± 1.6 13.9 ± 1.5  |

No. (%) of patients with baseline hemoglobin concn of <13 g/dl | 12 (16.4) | 8 (13.6) 4 (28.6)  |

Baseline platelet count (10⁹/mm³) | 16.3 ± 4.4 | 16.4 ± 4.7 15.4 ± 3.4  |

Baseline WBC count (10⁹/mm³) | 5.41 ± 1.4 | 5.52 ± 1.44 9.5 ± 1.08  |

Albumin concn (g/liter) | 43.4 | 44.0 41.2  |

AST concn (IU/liter) | 61.9 ± 27.8 | 62.3 ± 29.8 60.3 ± 17.7  |

ALT concn (IU/liter) | 74.6 ± 33.4 | 75.0 ± 35.8 72.6 ± 21.3  |

HCV RNA load (log₁₀ IU/ml) | 6.2 ± 0.7 | 6.2 ± 0.7 6.1 ± 0.5  |

No. (%) of patients with HCV genotype:
- 1 and 4 | 55 (75.3) | 46 (77.9) 9 (64.3)  |
- 3 | 18 (24.7) | 13 (22.1) 5 (35.7)  |

Fibrosis (kPa) | 10.7 ± 11.8 | 10.5 ± 11.5 11.8 ± 13.5  |

*All parameters are means ± standard deviations unless otherwise specified. ITPA, inosine triphosphatase; BMI, body mass index; MsM, contact by men who have sex with men; HTSX, heterosexual contact; IDU, intravenous drug use; WBC, white blood cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus.

41.8% for genotypes 1 and 4; OR = 11.13; 95% CI = 2.20 to 105.90; P = 0.0013), the IL-28B genotype at rs12979860 (70.0% for the CC genotype and 41.9% for the CT/TT genotype; OR = 3.24; 95% CI = 1.09 to 9.29; P = 0.0311), and the genotype at rs1127354 in the ITPA gene (78.6% for the CA/AA genotype and 47.4% for the CC genotype; OR = 4.06; 95% CI = 0.92 to 24.52; P = 0.0371). SVR was not associated with the genotype at rs7270101 in the ITPA gene (OR = 1.52; 95% CI = 0.45 to 5.32; P = 0.6305). A multivariable analysis was performed taking SVR as the dependent variable and age, sex, body mass index (BMI), baseline HCV RNA load, HCV genotype, RBV dose reduction, platelet count, baseline fibrosis, IL-28B genotype, and ITPA genotype as independent variables. Independent predictors of SVR were HCV genotype, age, baseline HCV RNA, and RBV dose reduction (Table 3).

Decrease in hemoglobin levels during PEG-IFN plus RBV therapy. Figure 1 shows the percent decreases in Hb levels between 59 patients with the CC genotype and 14 patients with the CA/AA genotype of the ITPA gene. Hb levels decreased more in patients with the CC genotype than in those with the CA/AA genotype at week 4 (−2.6 ± 1.3 versus −0.90 ± 0.9, P = 0.0002) and week 12 (−3.9 ± 1.8 versus −1.7 ± 0.7, P = 0.0003). The Hb nadir was reached earlier in patients with the CC genotype (13.7 ± 9.9 weeks) than in patients with the CA/AA genotype (25.0 ± 10.5 weeks) (P = 0.0004). The percent decrease in Hb level at week 4 was −17.4% ± 10.3% for patients with the CC genotype, whereas it was −6.1% ± 6.9% for those with the CA/AA genotype (P = 0.0003). At week 12, the percent decrease in Hb was −23.4% ± 10.9% for patients with the CC genotype, whereas it was −11.9 ± 5.2% for those with the CA/AA genotype (P = 0.0003). At week 36, the percent decrease in Hb levels was −18.6% ± 15.3% for patients with the CC genotype, whereas it was −7.0% ± 11.7% for those with the CA/AA genotype (P = 0.0192), whereas at week 48 the respective decreases were −15.4% ± 17.5% and −12.7% ± 10.9% (P = 0.6605). The percentage of patients who presented an Hb decrease of ≥3 g/dl from baseline at each time point is shown in Fig. 2. Genotypes at rs7270101 in the ITPA gene were not associated with an Hb decrease measured in any form. Genotypes at rs1127354 in the ITPA gene were not associated with maximal
white blood cell count decrease (−3.48 × 10³ ± 1.69 × 10³ versus −2.86 × 10³ ± 1.19 × 10³/mm³, respectively, for the CC and CA/AA genotypes; *P = 0.2910*).

**Modification of RBV during PEG-IFN plus RBV therapy.** The RBV dose was reduced ≥200 mg in 15 patients (34.2%) because of an Hb decrease. During the first 12 weeks of therapy, the proportion of patients receiving the full dose of RBV was higher for patients with the CA/AA genotype than for those with the CC genotype (100% versus 54.2%; OR = 11.81; 95% CI = 1.45 to 256.17; *P = 0.0039*). Therefore, none of the CA/AA carriers needed to reduce the RBV dosage. Patients who needed RBV dosage modi-
fication showed a significant decrease in percentages of SVR (32.0% versus 64.6%; OR = 3.88; 95% CI = 1.25 to 12.50; \( P = 0.0163 \)). Among the 48 patients who did not require a reduction of RBV dosage, 64% (31/48) had SVR. Although no significant differences were observed between the CA/AA and CC genotypes (78.6% versus 58.8%; OR = 2.57; 95% CI = 0.57 to 16.64; \( P = 0.3201 \)), a higher percentage of SVR was observed in patients with the CA/AA genotype. Genotypes at rs7270101 in the ITPA gene were not associated with a modification of the RBV dose.

Administration of erythropoietin (r-huEPO) during PEG-IFN plus RBV therapy. Twenty patients (27.4%) needed administration of r-huEPO because of anemia. The Hb level at baseline (15.4 ± 1.5 versus 14.4 ± 1.5 g/dl, \( P = 0.0189 \)) and the percent decrease at week 4 (−20.3% ± 11.3% versus −12.6% ± 9.3%, \( P = 0.0064 \)) and at week 12 (−32.1% ± 10.7% versus −16.1% ± 7.4%, \( P < 0.0001 \)) for patients who did or did not receive r-huEPO were statistically significantly different. Forty-one percent of patients with the CC genotype needed r-huEPO, whereas none of the patients with the CA/AA genotype needed r-huEPO (OR = 8.28; 95% CI = 1.04 to 371.12; \( P = 0.0057 \)).

Factors influencing a decrease in hemoglobin levels during PEG-IFN plus RBV therapy. To determine the factors associated with an Hb decrease during PEG-IFN plus RBV therapy in HIV/HCV-coinfected patients, a logistic regression analysis was performed taking maximum percent Hb decrease as the dependent variable and age, sex, BMI, baseline Hb level, baseline platelet count, RBV dose (reduced versus not reduced), and genotype at rs1127354 in the ITPA gene as independent variables. Independent predictors of Hb decrease were RBV dose reduction, baseline hemoglobin level, and BMI (Table 4).

**DISCUSSION**

Our study shows a strong association of the development of RBV-induced anemia, measured in any form, with polymorphisms in the rs1127354 of the ITPA gene in HIV/HCV-coinfected patients treated with PEG-IFN plus RBV. This finding is similar to the

**TABLE 4** Independent predictors of maximum percent decrease in Hb in 73 HIV/HCV-coinfected patients treated with pegylated interferon plus ribavirin*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBV dose reduction</td>
<td>11.72</td>
<td>6.82-16.63</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Baseline Hb level</td>
<td>1.69</td>
<td>0.23-3.15</td>
<td>0.024</td>
</tr>
<tr>
<td>BMI</td>
<td>0.7</td>
<td>−1.43-0.03</td>
<td>0.061</td>
</tr>
</tbody>
</table>

* HIV, human immunodeficiency virus; HCV, hepatitis C virus; RBV, ribavirin; Hb, hemoglobin; BMI, body mass index.
associations found in clinical trials of treated HCV-monoinfected patients (20, 31, 32). This finding suggests that, whatever the operating protective mechanism is, the toxic effects of RBV triphosphate on the red blood cells may be modulated by functional polymorphisms in the ITPA gene. Moreover, this is in agreement with the dose-dependent mechanism through which RBV causes its toxic effects on the red blood cell membrane (24). Although the effect was most evident early in treatment, it persisted throughout. The protective effect of polymorphisms in ITPA resulted in no need for RBV dose reductions and thus a greater cumulative RBV exposure. Additionally, patients with the protective genotype had no need for r-huEPO.

Adverse effects of combination antiviral therapy for HCV infection are the most common cause of treatment discontinuation and can jeopardize treatment adherence, thus compromising the effectiveness of treatment. The rates of treatment discontinuation in monoinfected patients range from 24.5% to 27%, and discontinuation usually occurs within the first 6 months of treatment, with anemia being the cause of discontinuation in one-third of these patients (11). Therefore, anemia not only is highly incidental but also is of significant magnitude, and in two studies of combination HCV antiviral therapy, Hb decreased by at least 3 g/dl in 54% of the patient population and by more than 25% from baseline in approximately 28% of patients (29). Furthermore, in HIV/HCV-coinfected patients, combination therapy for HCV infection is associated with anemia more profound than that seen in monoinfected patients (29). In fact, the Hb level decreased by at least 3 g/dl in 50.6% of patients in the present study, and a decrease of ≥25% of baseline Hb level was observed in 67.1%, figures similar to those reported by others (12). This is most likely due to a higher prevalence of pretreatment anemia in coinfected patients as well as to the potential need for treatment with antiretrovirals or other medications that may cause anemia. Apart from its effects on therapy discontinuation and adherence to HCV combination therapy, anemia is the main cause of RBV dose reduction. RBV-induced anemia requires RBV dose modification in 9% to 22% of HCV-coinfected patients (10, 17). Dose reduction is usually needed early in treatment (during the first 12 weeks), and this reduction in RBV dose appears to be critical to achieve SVR (10, 28). It is recommended to avoid dose reduction in patients in whom the response rate is lower and in whom a maximal effort is required to increase the individual’s chance of response. This group includes, among others, patients with HIV/HCV coinfection (10, 33). Among coinfected patients, RBV dose reduction because of anemia is needed in about a third of patients (4, 12), a figure similar to the 34.2% found in our study. The association of RBV dose reduction and anemia is so strong that it prevented ITPA from being an independent predictor of anemia in our multivariate model, because of colinearity between both variables.

To avoid both discontinuation and RBV dose reduction, r-huEPO has been successfully used in both monoinfected and coinfected patients to stimulate erythropoiesis, otherwise compromised in HCV-infected patients (1, 21). As r-huEPO therapy, which is needed in about a third of treated coinfected patients (27% among our patients) (21), is a surrogate marker of RBV-induced anemia in treated HIV/HCV-coinfected patients, the difference in its use with respect to polymorphism at rs1127354 in the ITPA gene found in our work was expectable. However, even with these correcting measures, the Hb level between CC and CA/AA patients was significantly different until week 48.

Although anemia complicating combination HCV therapy may be caused by PEG-IFN or RBV, the driving force is hemolytic anemia caused by RBV triphosphate accumulation in erythrocytes, which in turn induces oxidative damage to membranes, eventually leading to extravascular hemolysis (8, 24). The mechanism of protection from hemolysis by increased ITP intraerythrocytic levels is poorly understood, but it has been suggested that an increase in intracellular ITP may in turn cause a decrease in intracellular phosphate concentration, which may prevent the conversion of RBV into RBV triphosphate, or, alternatively, that ITP complexes with RBV triphosphate, thus conferring protection against hemolysis (10, 31). Recent evidence indicates that ITP protects against RBV-induced anemia by substituting for GTP (depleted by RBV) in the biosynthesis of ATP (13). Since ITP intracellular levels are dependent on ITPA activity, which in turn is modulated by functional polymorphisms in the ITPA gene (26), these polymorphisms may in the end determine the degree of protection against RBV-induced hemolytic anemia in HIV/HCV-coinfected patients, as has been recently reported (19, 23) and our work suggests.

Data regarding the association of ITPA polymorphism and combination HCV treatment outcome are quite controversial, with some studies showing such an association (14), whereas others (7, 19, 23, 31, 32) do not see any association between the ITPA gene, anemia, and SVR. This association may only be the reflection of decreased treatment efficacy due to dose reduction of RBV in patients with severe anemia, because the potential of RBV dose reduction to limit treatment efficacy is well-known (28). This is probably the case in our study since ITPA polymorphism was not an independent predictor of SVR.

Treatment with inhibitors of the HCV serine protease together with pegylated-interferon plus ribavirin is associated with anemia beyond that seen with pegylated-interferon plus ribavirin therapy (18, 22). In this setting, two Japanese studies have shown that ITPA polymorphisms are still able to influence hemoglobin levels during triple HCV therapy (7, 30).

However, the present results have inherent limitations. First, this is a cross-sectional study, and, therefore, no causal relationships can or should be drawn. Second, we have not measured ITPA expression, nor have we looked at ITP levels in erythrocytes. Notwithstanding that, several mutations leading to ITPA deficiency, which is a benign cell enzymopathy characterized by accumulation of ITP in erythrocytes, and increased toxicity of purine analogue drugs have been well characterized (27). Among them, mutation at rs1127354 of the ITPA gene causes a substantial reduction in ITPA activity, and homozygosity for the P32T mutation causes nondetectable ITPA activity (9). Altogether these data indicate that there is a good correlation between ITPA gene polymorphisms, ITPA functional activity, and, therefore, ITP accumulation in red blood cells (15, 26). Third, the number of patients included in the present study is relatively small.

In summary, polymorphism at rs1127354 in the ITPA gene is strongly associated with RBV-induced anemia in HIV/HCV-coinfected patients treated with PEG-IFN plus RBV. This finding has the potential to inform clinical decision making, especially in patients who need aggressive dose escalation strategies with RBV or those who are at high risk of anemia or related morbidity, such as older patients, patients with chronic renal dysfunction, or patients with hemoglobinopathies.
ACKNOWLEDGMENTS

We have no conflict of interest to declare.

P.D., J.M.G., and M.B. conceived the research, designed the database, and wrote the article. J.S. performed the genetics for the study. A.F. and J.M. retrieved and processed the blood samples. M.G.M., M.D.M.G., C.P., J.M., and K.L. enrolled the patients and monitored them throughout HCV therapy.

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