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Genome-wide association study of interferon-related cytopenia in chronic hepatitis C patients

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Conflict of interest: Drs. McHutchison, Goldstein, Muir, Afdhal, Jacobson, Esteban, Poordad, Lawitz, McCone, Shiffman, King, Kwo, Patel and Sulkowski report having received research and grant support from Schering-Plough. Drs. McHutchison, Goldstein, Muir, Afdhal, Jacobson, Esteban, Poordad, Lawitz, Shiffman, Kwo, and Sulkowski have reported receiving consulting fees or acted in an advisory capacity for Schering-Plough. Drs. Noviello, Pedicone, Brass, Pedicone, and Albrecht are employees of Schering-Plough (now Merck & Co., Inc.) and are stock holders in this entity. Drs. Thompson, Goldstein, McHutchison, Ge, Fellay, Shianna, and Urban are co-inventors of a patent application based on the ITPA finding.

Author contributions: A.J.T. performed the primary data analysis and wrote the first draft of the manuscript with assistance and revision from P.J.C., D.G., A.S., J.F., M.F., Q.Z., A.J.M., and J.G.M. D.G., J.F., K.V.S., T.U., and D.B.G. were responsible for genotyping the ITPA variants. J.G.M. and M.S.S. were the principal investigators for the IDEAL study, and together with D.B.G., S.N., L.D.P., C.A.B., and J.K.A., developed the pharmacogenomic study protocol. A.J.M., N.H.A., I.M.J., F.P., E.J.L., J.M., M.L.S., G.W.G., J.W.K., P.Y.K., and K.P. were site investigators for the IDEAL study. All authors had full access to the data in the study and contributed to the interpretation of the results. All authors reviewed the manuscript and provided further contributions and suggestions. All authors read and approved the final manuscript.

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Abstract

Background & Aims—Interferon-alfa (IFN)-related cytopenias are common and may be dose-limiting. We performed a genome wide association study on a well-characterized genotype 1 HCV cohort to identify genetic determinants of peginterferon-α (peg-IFN)-related thrombocytopenia, neutropenia, and leukopenia.

Methods—1604/3070 patients in the IDEAL study consented to genetic testing. Trial inclusion criteria included a platelet (Pl) count ≥80 × 10^9/L and an absolute neutrophil count (ANC) ≥ 1500/mm^3. Samples were genotyped using the Illumina Human610-quad BeadChip. The primary analyses focused on the genetic determinants of quantitative change in cell counts (Pl, ANC, lymphocytes, monocytes, eosinophils, and basophils) at week 4 in patients >80% adherent to therapy (n = 1294).

Results—6 SNPs on chromosome 20 were positively associated with Pl reduction (top SNP rs965469, $p = 10^{-10}$). These tag SNPs are in high linkage disequilibrium with 2 functional variants in the *ITPA* gene, rs1127354 and rs7270101, that cause ITPase deficiency and protect against ribavirin (RBV)-induced hemolytic anemia (HA). rs1127354 and rs7270101 showed strong independent associations with Pl reduction ($p = 10^{-12}$, $p = 10^{-7}$) and entirely explained the genome-wide significant associations. We believe this is an example of an indirect genetic association due to a reactive thrombocytosis to RBV-induced anemia: Hb decline was inversely correlated with Pl reduction (r = −0.28, $p = 10^{-17}$) and Hb change largely attenuated the association between the *ITPA* variants and Pl reduction in regression models. No common genetic variants were associated with pegIFN-induced neutropenia or leucopenia.

Conclusions—Two *ITPA* variants were associated with thrombocytopenia; this was largely explained by a thrombocytotic response to RBV-induced HA attenuating IFN-related thrombocytopenia. No genetic determinants of pegIFN-induced neutropenia were identified.

Keywords

GWAS; ITPA; Thrombocytopenia; Hepatitis C; Neutropenia; IL28B

Introduction

Chronic infection with hepatitis C virus (HCV) affects up to 170 million individuals worldwide [1] and may lead to progressive hepatic fibrosis and cirrhosis with risk of liver failure and hepato-cellular carcinoma. HCV-related liver disease is currently the most common indication for liver transplantation in North America. Antiviral therapy with pegylated-interferon-alfa (pegIFN) plus ribavirin (RBV) may be curative, but is poorly tolerated by many patients.

Bone marrow suppression is an important adverse effect of pegIFN therapy, leading to neutropenia and thrombocytopenia, with risk of secondary sepsis and bleeding, respectively [2,3]. Dose reduction may be required potentially compromising treatment outcome, as rates of viral clearance are significantly reduced in patients who cannot be maintained on at least 80% of their pegIFN and ribavirin dosage for the duration of treatment [4]. Identifying patients at greatest risk for such complications would be clinically useful for selecting patients for therapy, as well as planning the frequency of monitoring and likely need for growth factor support on treatment. Patients with advanced hepatic fibrosis are at highest risk [5], but bone marrow suppression remains prevalent in patients with early stage fibrosis and there is a need for more accurate biomarkers. A genetic biomarker for predicting risk of IFN-related bone marrow suppression would be particularly useful as a pre-treatment test.

A number of lines of evidence suggest that genetic variants may be associated with IFN-induced cytopenia. Firstly, persistently low neutrophil counts are more commonly observed in persons of African American ancestry compared to Caucasians ('benign ethnic neutropenia') [6], and this has recently been linked to a regulatory variant in the Duffy Antigen Receptor for Chemokines gene (*DARC*) [7]. The relevance of this variant to drug-induced neutropenia is not known. Secondly, polymorphism in the region of the interleukin 28B gene (*IL28B*), coding for IFN-lambda(λ)-3, has recently been identified to be strongly associated with viral clearance following pegIFN plus RBV therapy [8–11]. Although the mechanism remains unclear, the polymorphism is believed to regulate sensitivity to the antiviral effects of IFN. Whether *IL28B* polymorphism is relevant to other IFN-mediated effects has not been evaluated. Finally, functional variants in the inosine triphosphatase gene (*ITPA*) causing inosine triphosphatase (ITPase) deficiency, previously recognized as a benign red cell enzymopathy, have recently been identified to protect against RBV-induced hemolytic anemia [12,13]. RBV depletes red cell GTP levels, leading in turn to ATP depletion, oxidative stress, and hemolysis. The protective *ITPA* variants are associated with red cell inosine triphosphate (ITP) accumulation, and it has been shown that ITP is able to substitute for GTP in the biosynthesis of ATP, thereby protecting against RBV-hemolysis [14].

In this study we have performed genome-wide analyses for determinants of treatment-related bone marrow suppression in a large, well characterized cohort of genotype 1 HCV patients treated with pegIFN plus RBV in the IDEAL study. We have focused primarily on treatment-induced neutropenia and thrombocytopenia.

Materials and methods

Patient and control population

1604/3070 patients in the IDEAL study [15] consented to collection of DNA samples for genetic testing (ClinicalTrials.gov number, NCT00081770). Clinical and laboratory data were collected as described previously [15,16]. All patients included in this study were treatment-naïve and infected with genotype 1 HCV [15]. Patients were treated with either pegIFN-alfa-2b (1.0 or 1.5 μg/kg/week) or pegIFN-alfa-2a (180 μg/week) plus weight-based RBV (800–1400 mg for peg-IFN-alfa-2b, and 1000–1200 mg for pegIFN-alfa-2a) [15]. For all patients, the protocol-specified treatment duration was 48 weeks, with an additional 24 weeks follow-up. All patients had a full blood count performed at baseline, weeks 2, 4, 8, 12, 18, 24, 30, 36, 42, and 48 of therapy and at weeks 4, 12, and 24 post-treatment. Inclusion criteria for the parent study required an absolute neutrophil count (ANC) ≥ 1500/mm^3 and platelet count (Pl) ≥80 × 10^9/L. All patients had compensated liver disease. Protocol specified dose reduction of pegIFN was indicated for ANC <750/mm^3 or Pl <50 × 10^9/L, and discontinuation of both pegIFN and RBV was required for ANC <500/mm^3 or Pl <25 × 10^9/L. The use of growth factor support for neutropenia or thrombocytopenia was not permitted. Detailed records of drug compliance were kept for all patients on-treatment. Only patients who were more than 80% adherent to pegIFN to week 4 of treatment were included in the primary analyses (26 patients were excluded from analysis).

Genotyping

A total of 1604 DNA samples were genotyped in the context of a previously reported study of anti-HCV treatment response, using the Illumina Human610-quad BeadChip (Illumina, San Diego, CA, USA) as previously described [8]. Quality control steps are described in Supplementary Material I. Genotyping of the two *ITPA* variants, rs1127354 and rs7270101,

was performed using the ABI TaqMan allelic discrimination kit (Applied Biosystems, Carlsbad, CA, USA) in a previous study of RBV-induced hemolytic anemia [12,17].

Definition of clinical endpoints

The primary analyses focused on the genetic determinants of quantitative change in (i) platelet, and (ii) leukocyte counts, at week 4 of treatment in adherent patients. The following leukocyte sub-populations were separately analyzed: absolute neutrophil count (ANC), lymphocytes, monocytes, basophils, and eosinophils. Week 4 was chosen as a time point to minimize confounding by dose modification of pegIFN and RBV, or confounding by the use of erythropoietin supplementation.

Statistical analysis

The primary association tests involved single-marker genotype trend tests performed in three independent groups (European-Americans, African-Americans, Hispanics), using a linear regression model. Association tests were implemented in the PLINK software [18], correcting for the relevant clinical covariates baseline cell count (Pl, leukocyte cell lines), age, gender, body mass index, liver fibrosis stage (METAVIR F0–2 *vs.* F3–4), pegIFN dose (binary variable: pegIFN-α2b 1.0 μg/kg/week *vs.* pegIFN- α2b 1.5 μg/kg/week and RBV dose (mg/kg). The association signals (*p* values) were then combined using Stouffer's weighted Z-method [19], adjusting for sample sizes, effect sizes and effect directions in each population. This combined *p* value was then reported as the main result, along with the *p* values in each ethnic group. A series of quality control steps resulted in 565,759 polymorphisms being included in the association tests. Methods to assess copy number variants were applied and the relation between copy number variants and reduction of Pl/ leukocyte cell lines was tested. To control for the possibility of spurious associations resulting from population stratification, we used a modified EIGENSTRAT method [20] and corrected for population ancestry within each group. We assessed significance with a Bonferroni correction ($P_{cutoff} = 4.4 \times 10^{-8}$).

Results

Interferon-alfa-mediated thrombocytopenia

We performed a genome-wide association study (GWAS) of genetic determinants of IFN-related thrombocytopenia at week 4 in compliant genotype 1 HCV patients from the IDEAL study. Following quality control steps, 1284 individuals (984 European-Americans, 201 African-Americans, 99 Hispanics) were included in the analysis (patient characteristics are summarized in Table 1). Baseline Pl counts were not significantly different between the 3 populations ($p = 0.8977$, Table 1). We tested each of 565,759 single nucleotide polymorphisms (SNPs) passing quality control measures in a linear regression model incorporating the relevant clinical covariates: age, gender, body mass index (BMI), hepatic fibrosis stage, pegIFN dose (binary: pegIFN-alfa-2b 1.0 μg/kg/week vs. pegIFN-alfa-2b 1.5 μg/kg/week or pegIFN-alfa-2a 180 μg/week), RBV dose (mg/kg) and baseline Pl count.

6 SNPs on chromosome 20 were significantly associated with Pl reduction at week 4 (top SNP rs965469, $p = 9.02 \times 10^{-10}$ in European Americans, Fig. 1 and Table 2). These SNPs have previously been shown to co-segregate with 2 functional variants in the *ITPA* gene on chromosome 20, rs1127354 and rs7270101 (Supplementary Material III), that are each independently associated with reduced ITPase activity and protect against RBV-induced hemolytic anemia (HA) [12]. rs1127354 is a mis-sense variant in exon 2 of the *ITPA* gene (P32T), and rs7270101 is splicing-altering variant located in the second intron (IVS2). Neither variant was included on the genome-wide array but they had been genotyped in the context of a previous GWAS [12]. These 2 functional variants showed strong independent

associations with week 4 Pl reduction (rs1127354, overall $p = 10^{-12}$ and rs7270101 $p = 10^{-7}$, respectively, Table 2). The level of ITPase activity may be predicted according to an individual's *ITPA* genotype, based on previous functional studies (Supplementary Material III), and a combined low activity allele made up of either functional variant may be used to define an ITPase deficiency variable [21–25]. This ITPase deficiency variable was more strongly associated with Pl reduction ($p = 10^{-20}$). Furthermore, when the two functional *ITPA* variants were incorporated into a regression model, they were found to entirely explain the genome-wide significant association between rs965469 and Pl reduction (European American patients: p value fell from $p = 10^{-10}$ to $p = 0.9204$ after adjustment for the 2 functional variants, Table 2). The functional *ITPA* variants remained strongly associated with Pl reduction in this model.

Whereas the *ITPA* variants associated with ITPase deficiency have previously been shown to protect against RBV-induced hemolytic anemia [12], in this study they were associated with more pronounced reduction of Pl counts. The decline in platelet counts that occurs during antiviral therapy is known to be less pronounced when IFN is combined with RBV than in the setting of IFN monotherapy [26,27]. This has been attributed to a relative thrombocytosis occurring in response to RBV-induced hemolysis. In the current cohort, a negative correlation was noted between week 4 hemoglobin (Hb) reduction and Pl reduction (European Americans, r = −0.28, p value = 10^{-17}, Fig. 2). Inclusion of week 4 Hb reduction in the same model with the ITPase deficiency variable largely attenuated the strength of the association with Pl reduction (European Americans, from $p = 10^{-16}$ to $p = 10^{-6}$, Supplementary Table 5).

In order to evaluate the clinical relevance of this observation we considered the relationship between the ITPase deficiency variable and reductions of Pl count over the course of therapy. The ITPase deficiency variable was significantly associated with more profound reductions in Pl count at week 4, 12, and 24 (Fig. 3). Beyond week 24, there were non-significant trends in the same direction. Despite this, the number of patients in whom Pl levels fell to below 50×10^9/L, the level at which dose reduction is indicated, was low (<1.5% at any time point) and there were no significant differences in the frequency of Pl $<50 \times 10^9$/ L according to predicted ITPase deficiency (data not shown). This was true both for the overall cohort, as well as an analysis limited just to those patients treated with pegIFN-alfa-2a 180 μg/week or pegIFN-alfa-2b 1.5 μg/kg/week.

Finally, genetic variation in the region of the *IL28B* gene on chromosome 19 is strongly associated with the pegIFN and RBV response rate [8,10,11,28]. No relationship between *IL28B* genotype and week 4 thrombocytopenia was noted in the 3 ethnic populations.

Interferon-alfa-mediated neutropenia

We performed a second genome-wide analysis focused on the genetic determinants of week 4 reductions in ANC as a continuous variable. The final analysis included 1292 patients (European Americans = 991, African Americans = 203, Hispanics = 98). At baseline, median ANC were lower in the African American population (European Americans = 3.65 (2.96–4.68), African Americans = 3.04 (2.14–4.04), Hispanics = 3.36 (2.77–4.24), $p = 10^{-12}$). Median ANC reduction at week 4 was then less prominent in the AA population (European Americans = 2.0 (1.34–2.68), African Americans = 1.22 (0.61–1.97), Hispanics = 1.72 (1.0–2.38), $p = 10^{-18}$). We tested for genetic determinants of week 4 ANC reduction using linear regression models including the covariates age, gender, BMI, hepatic fibrosis stage (F0–2 *vs.* F3–4), pegIFN dose (binary: alfa-2b 1.0 μg/kg/week vs. 1.5 μg/kg/week or alfa-2a 180 μg/week) and baseline neutrophil level. No common genetic variants were associated with treatment-related reduction in ANC at week 4 at the level of genome-wide

significance. In particular, *IL28B* polymorphism was not associated with IFN-related neutropenia.

A genome-wide analysis of baseline ANC was also negative. In the AA population, we noted associations between baseline ANC and *DARC* gene polymorphism but these did not meet genome-wide significance criteria (top SNp rs3027041, $p = 10^{-6}$, Supplementary Material VI).

Genome-wide analysis for variants associated with other leucopenia

We were also interested in identifying common genetic variants associated with baseline and pegIFN-related week 4 reductions in other white cell counts. Lymphocyte, monocyte, basophil, and eosinophil count were all considered separately. No significant associations were observed in any of these analyses (data not shown).

Discussion

To our knowledge this is the first study to consider genetic determinants of treatment-related cytopenia using a genome-wide approach in chronic hepatitis C patients. We have identified an association between *ITPA* variants causing ITPase deficiency and treatment related thrombocytopenia. We did not detect any common genetic variants that influenced IFN-related neutropenia or leukopenia, an important negative finding. Of note, *IL28B* polymorphisms, recently identified to be strongly associated with pegIFN plus RBV treatment outcome, were not associated with IFN-related cytopenia.

Two functional variants in the *ITPA* gene that cause ITPase deficiency, red cell ITp accumulation and protection against RBV-induced HA [12,14] were associated with more profound pegIFN-induced thrombocytopenia. This association was largely explained by a relative, reactive thrombocytosis in response to RBV-induced HA in those patients with wildtype ITPase activity. Thus the RBV-induced anemia attenuated the pegIFN effect to reduce Pl counts. Thrombocytosis is well-described as a consequence of hemolytic anemia [29], which is in keeping with the original observation in the late 1990s that on-treatment reductions of Pl counts were less marked following the addition of RBV to standard-of-care HCV therapy [26,27]. This therefore represents an indirect genetic association, where wildtype ITPase activity is associated with more profound RBV-related anemia, which in turn stimulates Pl production, manifesting as less pronounced pegIFN-induced thrombocytopenia. The *ITPA* variants, which protect against RBV-hemolysis, are therefore associated with greater IFN-induced thrombocytopenia. The biological mechanism underlying this relationship between Hb levels and Pl counts is not clearly understood, but may involve stimulation of the bipotent erythroid/megakaryocyte progenitor cell by erythropoietin [30,31]. Although adjustment for Hb reduction in the linear regression model largely attenuated the association between the *ITPA* variants and Pl counts, a residual association with the combined ‘low activity’ allele persisted (European Americans, $p = 10^{-16}$ reduced to $p = 10^{-6}$). Although this association was not genome-wide significant, we cannot exclude the possibility of two separate phenomena, with a weaker secondary effect due to a biological relationship between *ITPA* variants, exogenous IFN and Pl levels. This will require further mechanistic studies.

Despite the strong statistical association between *ITPA* variants, Hb reduction and Pl counts, the clinical relevance of this finding remains uncertain. Relatively few patients decreased their Pl counts to levels requiring dose reduction. It is likely that *ITPA* genotyping may find a role in predicting RBV-induced anemia in high risk individuals [12,13], but on the basis of the current data, there does not appear to be great clinical utility for predicting severe thrombocytopenia. We note that the current dataset did not include significant numbers of

patients with advanced stage fibrosis, and it will be important to assess whether *ITPA* variants may predict treatment-limiting Pl reductions in this population.

No common genetic variants were associated with pegIFN-induced neutropenia or leucopenia. It was interesting that the hematological complications of IFN therapy were not associated with *IL28B* variants. Although a negative result, this has important implications for our understanding of the biology of the *IL28B*–pegIFN interaction. The data suggest that the biology of the *IL28B*–pegIFN treatment response association in HCV is not directly relevant to pegIFN-induced bone marrow suppression. *IL28B* polymorphism is strongly associated with on-treatment viral kinetics and pegIFN plus RBV treatment outcome [9]. Although the mechanism by which *IL28B* variation effects pegIFN sensitivity remains unclear, there is evidence that levels of intrahepatic ISG expression are important [32,33] and the effect is believed to primarily reflect sensitivity to exogenous IFN. The current data suggest that this is a liver-specific phenomenon. IFN-λ is induced by similar stimuli to type 1 IFN, and shares a common downstream signaling pathway, however the expression of the IFN-λ-receptor (IFNLR) is more restricted than that of the ubiquitous IFN-α-receptor (IFNABR). Although the IFNLR has been shown to be expressed by hepatocytes, IFNLR gene expression is not expressed in hematopoietic cells, with the exception of B lymphocytes [34,35]. Consistent with this, minimal bone marrow suppression was observed in a recent early phase clinical trial using IFN-λ-1 for the treatment of HCV, despite good antiviral potency [36]. The IL28B polymorphism may therefore act to regulate IFN-α signaling, which is dependent on co-expression of the IFNLR and the IFNABR within the same tissue.

In conclusion, two functional variants in the *ITPA* gene that are strongly associated with protection from RBV-induced HA are also associated with greater thrombocytopenia in chronic hepatitis C patients. This association is largely explained by a relative reactive thrombocytosis in response to RBV-induced HA, which attenuates IFN-related thrombocytopenia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

HCV	hepatitis C virus
pegIFN	pegylated-interferon-alfa
RBV	ribavirin

DARC	Duffy Antigen Receptor for Chemokines
IL28B	Iinterleukin 28B
ITPA	inosine triphosphatase gene
ITPase	inosine triphosphatase
ANC	absolute neutrophil count
Pl	platelet
Hb	hemoglobin
SNP	single nucleotide polymorphism
GWAS	genome-wide association study
BMI	body mass index
HA	he-molytic anemia
IFNLR	IFN-λ-receptor
IFNABR	IFN-α-receptor

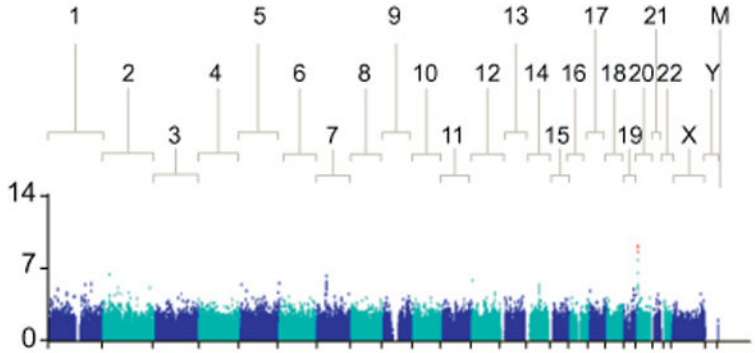

Fig. 1. The Manhattan plot shows a genome-wide view of the *p* values [$2\log_{10}(P)$] for association between SNPs tested and week 4 platelet reduction in patients of European American ancestry

The SNPs that show genome-wide significant association with quantitative reduction in Pl levels are marked in red. [This figure appears in color on the web.]

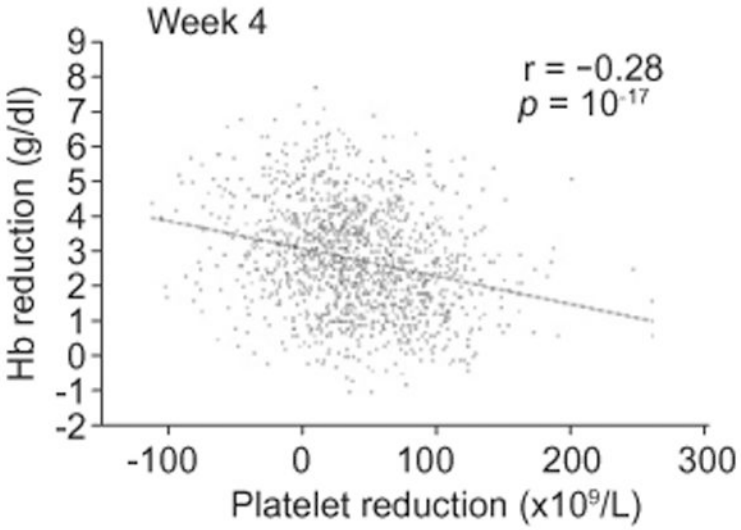

Fig. 2.

Correlation of Pl reduction at week 4 (× 10^9/L) with hemoglobin reduction at week 4 (g/dl), limited to the patients of European American ancestry.

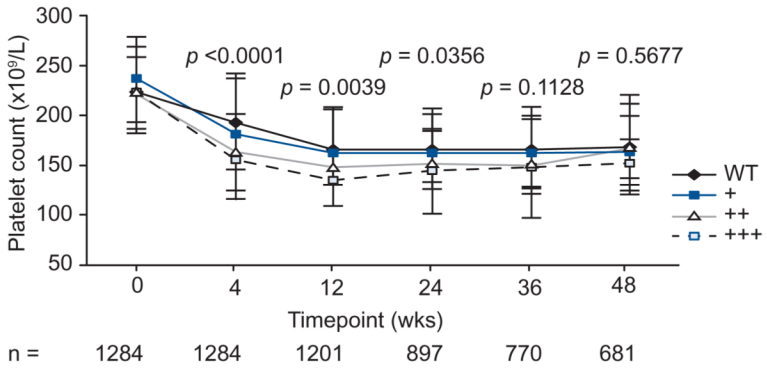

Fig. 3. Median platelet count over time (× 10^9/L) according to predicted ITPase deficiency in the overall population

All patients included in the analysis were >80% adherent to week 4 (n = 1284); for time points beyond week 4, patients were included if they remained on treatment, and a platelet count was available. WT = wildtype (normal ITPase activity); + = mild ITPase deficiency; + + = moderate ITPase deficiency; +++ = severe ITPase deficiency.

Table 1

Patient characteristics

	European Americans	African Americans	Hispanics	*p* value
No (platelet analysis)	984	201	99	
Gender (n, %)	608 (62%)	121 (60%)	63 (64%)	0.8387
Age, yrs *	48 (44-52)	50 (47-54)	46 (39-51)	<0.0001
BMI, kg/m^2	27.4 (24.8-30.4)	29.3 (26.6-32.6)	28.6 (25.1-32.8)	<0.0001
METAVIR fibrosis stage (n, %)	873 (89%)	183 (91%)	85 (86%)	0.3886
Minimal (F0-2)	111 (11%)	18 (9%)	14 (14%)	
Advanced (F3-4)				
RBV starting dose, mg/kg	13.2 (12.4-14.1)	12.8 (12.1-13.7)	13.6 (12.5-14.7)	0.0004
RBV starting dose (n, %)				0.0065
800 mg	86 (9%)	4 (2%)	6 (6%)	
1000 mg	373 (38%)	65 (32%)	41 (41%)	
1200 mg	463 (47%)	118 (59%)	44 (44%)	
1400 mg	62 (6%)	14 (7%)	8 (8%)	
PegIFN starting dose (n, %)				
PegIFN-α-2b 1.0	332 (34%)	71 (35%)	31 (31%)	0.8532
PegIFN-α-2b 1.5	321 (33%)	62 (31%)	37 (37%)	
PegIFN-α-2a	331 (34%)	68 (34%)	31 (31%)	
Baseline Pl count (×10^9/L)	225 (184-269)	228 (184-273)	230 (186-275)	0.8977
Baseline Pl count <100×10^9/L	17 (1.7%)	2 (1%)	1 (1%)	0.6724
Wk 4 Pl reduction (×10^9/L)	37 (11-72)	28 (0-61)	26 (2-65)	0.0052
Wk 4 Pl count (n, %)				
<75×10^9/L	24 (2%)	4 (2%)	0 (0%)	0.2796
<50×10^9/L	2 (<1%)	0 (0%)	0 (0%)	0.7369
<25×10^9/L	0 (0%)	0 (0%)	0 (0%)	1.0000
No (ANC analysis)	991	203	98	
Baseline ANC count (/mm^3)	3.65 (2.96-4.68)	3.04 (2.14-4.04)	3.36 (2.77-4.24)	<0.0001
Week 4 ANC reduction (/mm^3)	2.0 (1.34-2.68)	1.22 (0.61-1.97)	1.72 (1.0-2.38)	<0.0001
<1.0/mm^3 (n, %)	124 (13%)	26 (13%)	12 (12%)	0.9892
<0.75/mm^3 (n, %)	30 (3%)	8 (4%)	1 (1%)	0.3816
<0.5/mm^3 (n, %)	2 (<1%)	2 (1%)	0 (0%)	0.1588

* Continuous data are presented as median (25th – 75th centile).

Table 2

(A) Six variants in the 20p13 were associated with Pl reduction at the genome-wide significant level. These tag SNPs have previously been shown to be in linkage disequilibrium with 2 functional variants in the *ITPA* gene, which cause ITPase deficiency. (B) The two functional *ITPA* variants rs1127354 and rs7270101 entirely explained the GWAS association signals detected in the region. The adjusted *p* value (*) was obtained for each SNP in a linear regression model in which the two *ITPA* functional variants are incorporated

A

Wk 4 Pl reduction	European Americans	African Americans	Hispanics	Combined *p* value
Top discovery SNPs (Illumina 610 chip)				
rs965469	9.02×10^{-10}	0.1818	0.0792	1.29×10^{-9}
rs3310	1.30×10^{-9}	0.4035	0.0816	3.91×10^{-9}
rs6051702	1.30×10^{-9}	0.4621	0.0812	4.41×10^{-9}
rs6051762	2.76×10^{-9}	0.5050	0.1118	1.28×10^{-8}
rs6051841	2.16×10^{-8}	0.0858	0.1424	2.09×10^{-8}
rs6051693	2.21×10^{-8}	0.3207	0.0953	4.96×10^{-8}
ITPA variants				
rs1127354 (P32T)	1.70×10^{-10}	0.0005	0.0600	1.38×10^{-12}
rs7270101 (IVS2)	9.95×10^{-6}	0.0038	0.0231	3.39×10^{-7}
ITPase deficiency variable	2.05×10^{-16}	0.00002	0.0021	8.42×10^{-20}

B

GWAS hit	Population	GWAS *p* value	Adjusted *p* value*
rs965469	European Americans	9.02×10^{-10}	0.9204
rs3310	European Americans	1.30×10^{-9}	0.7914
rs6051702	European Americans	1.30×10^{-9}	0.7914
rs6051762	European Americans	2.76×10^{-9}	0.8065
rs6051841	European Americans	2.16×10^{-8}	0.9204
rs6051693	European Americans	2.21×10^{-8}	0.8876