we analyzed the associations between IP-10 concentrations and treatment outcome in subgroups according to *IL28B* genotype. In the rs8099917: TT group, the SVR rate was higher in the patients with IP-10 <300 pg/mL than those with IP-10 ≥300 pg/mL (69% vs. 35%, *P*=0.005), whereas no patient achieved SVR among those with rs8099917: TG/GG and IP-10 ≥300 pg/mL (Fig. 3).

Virological response to PEG-IFN/RBV/telaprevir therapy

The percentages of patients in whom HCV RNA became undetectable in each period of treatment with PEG-IFN/RBV/telaprevir are shown in Fig. 4. The RVR rate was high regardless of *IL28B* genotype: 82% (37/45) of all patients. All the patients with the *IL28B* favorable genotype achieved SVR but, of the eight patients with the unfavorable genotype, two failed to achieve SVR. Therefore, it was difficult to evaluate the association between pretreatment serum IP-10 concentrations and RVR or SVR. Then, we examined the factors associated with vRVR, defined as HCV RNA undetectable at week 2 after the start of therapy. The vRVR rate was 45% (18/45) of all patients. By univariate analysis, low concentrations of IP-10 and HCV RNA at baseline were significantly associated with vRVR (Table 4). There was a weak correlation between pretreatment serum IP-10 concentrations and HCV RNA levels in patients treated with PEG-IFN/RBV/telaprevir, but it was not significant (r=0.256,

P=0.090) (Fig. S3), therefore, they were considered almost independent predictors of vRVR. In addition, in the subgroup with the *IL28B* favorable genotype, IP-10 concentrations were also significantly lower in vRVR (*P*=0.009) (Fig. 5).

DISCUSSION

In this study, we have identified an association between pretreatment serum IP-10 concentrations and treatment efficacy in patients infected with HCV genotype 1 and treated with PEG-IFN/RBV. Considering a previous report,[24] the pretreatment serum IP-10 concentrations tended to be lower in this study than in Caucasian and African American populations. Some previous reports have mentioned differences in serum IP-10 concentrations between in African-American and white patients,[20, 24] and our findings suggest that serum IP-10 concentrations may be lower in Asian patients infected with HCV. Therefore, there is the likelihood that the appropriate cutoff values of pretreatment serum IP-10 concentrations for predicting SVR vary among different races. To assess the potential predictive value of IP-10 measurements, other studies stratified the patients according to a 600 pg/mL cutoff of IP-10 concentrations,[22, 24] while we set the cutoff value as 300 pg/mL on the basis of the ROC analysis. In multivariate analysis, the *IL28B* favorable genotype and pretreatment serum IP-10 <300 pg/mL were independent factors for predicting SVR.

Therefore, the ability to predict treatment efficacy was improved by considering the *IL28B* genotype and pretreatment serum IP-10 concentration together in PEG-IFN/RBV therapy: the positive predictive value of *IL28B* favorable genotype and IP-10 <300 pg/mL for predicting SVR was 69% and the negative predictive value of *IL28B* favorable genotype or IP-10 <300 pg/mL was 100% (Fig. 3 and Table S1).

It has been reported that serum IP-10 concentrations correlated with *IL28B* genotype, activity of hepatitis, progressive liver fibrosis and HCV RNA concentrations.[23, 25, 28] In this study, the serum IP-10 concentrations were not correlated with the *IL28B* genotype but were weakly corrrelated with ALT, γ-GTP and HCV RNA concentrations.

In addition, we identified that pretreatment serum IP-10 concentrations and HCV RNA levels almost independently affected the early viral kinetics of HCV in patients treated with PEG-IFN/RBV/telaprevir. IP-10 concentrations were significantly lower in patients with vRVR, defined as HCV RNA undetectable at week 2 after the start of therapy, than in those with non-vRVR. Moreover, that result was similar in the subgroup of patients with the *IL28B* favorable genotype, suggesting that pretreatment serum IP-10 concentrations are associated with early viral kinetics of HCV, independently of the *IL28B* genotype. We selected patients for treatment with PEG-IFN/RBV/telaprevir according to age, *IL28B* genotype and past IFN-based treatment response. As a result, the virological responses were excellent regardless

of *IL28B* genotype and it was difficult to evaluate the association between pretreatment serum IP-10 concentrations or *IL28B* genotype and RVR or SVR because of the strong antiviral effect of treatment with telaprevir on the selected patients in this study. However, patients with vRVR during triple therapy might benefit from a reduction of the duration of treatment, i.e., 12 weeks to achieve SVR; *IL28B* genotype, pretreatment serum IP-10 concentrations, and HCV RNA levels that predict vRVR might be useful for shortening the triple therapy. Further studies are necessary to reveal the impact of pretreatment IP-10 concentrations on treatment efficacy, especially in refractory patients treated with regimens including DAAs.

IP-10 is a CXC chemokine that lacks chemotactic activity for neutrophils but rather targets T lymphocytes, NK cells and monocytes,[29-31] through its receptor, CXCR3,[32, 33] IP-10 is produced by a variety of cells, including hepatocytes,[34, 35] and IP-10 concentrations in plasma are mirrored by intrahepatic IP-10 mRNA and strongly predict the first phase of HCV RNA decline during PEG-IFN/RBV therapy.[36] On the other hand, it has been reported that high levels of expression of intrahepatic IFN-stimulated genes (ISGs) were associated with a poor response to PEG-IFN/RBV therapy.[37, 38] This is consistent with data, including our results, showing that elevated pretreatment serum IP-10 concentrations correlate with nonresponse to PEG-IFN/RBV therapy,[19-23] In addition, two recent studies have revealed an association

between *IL28B* genotype and the expression levels of intrahepatic ISGs,[39, 40] and others have reported that the serum IP-10 concentration, as well as *IL28B* genetic variants, was a predictive factor for spontaneous clearance of acute HCV infection.[41] However, the mechanisms through which ISGs, IP-10 and *IL28B* genetic variants affect the elimination of HCV RNA are not known yet and further studies are warranted.

In conclusion, pretreatment serum IP-10 concentrations are associated with treatment efficacy in PEG-IFN/RBV and with early viral kinetics in PEG-IFN/RBV/telaprevir therapy.

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Figure legends

Figure 1. Correlations between pretreatment serum IP-10 concentrations and *IL28B* genotype or other variables. Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. The serum IP-10 concentrations were not correlated with the *IL28B* genotype but were weakly corrrelated with ALT, γ-GTP and HCV RNA concentrations. ALT, alanine aminotransaminase; γ-GTP, γ-glutamyl transpeptidase.

Figure 2. Pretreatment serum IP-10 concentrations according to treatment efficacy in patients treated with PEG-IFN/RBV. Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. The serum IP-10 concentrations were lower in SVR than in TVR and NVR (P=0.017 and P=0.005, respectively). There was a significant difference in IP-10 concentrations between SVR and non-SVR (TVR + NVR) (P=0.002). PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response.

Figure 3. Treatment outcome according to *IL28B* genotype and pretreatment serum IP-10 concentrations in patients treated with PEG-IFN/RBV. In the rs8099917: TT group, the SVR rate was higher in the patients with IP-10 <300 pg/mL than in those with IP-10 ≥300 pg/mL (69% vs. 35%, *P*=0.005) whereas no patient with rs8099917: TG/GG and IP-10 ≥ 300 pg/mL achieved SVR. *P* values were obtained by comparing the SVR rates among the groups. PEG-IFN, pegylated interferon; RBV, ribavirin; SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response.

Figure 4. Percentages of patients in whom HCV RNA became undetectable in each period of treatment with PEG-IFN/RBV/telaprevir, according to *IL28B* genotype. The percentages with vRVR, defined as HCV RNA undetectable at week 2 after the start of therapy, and RVR among all patients were 45% (18/45) and 82% (37/45) respectively. All patients with rs8099917: TT (*IL28B* favorable genotype) achieved a SVR but 2 of the 8 patients with rs8099917: TG/GG did not achieve SVR. PEG-IFN, pegylated interferon; RBV, ribavirin , vRVR, very rapid virological response, RVR, rapid virological response; SVR, sustained virological response.

Figure 5. Pretreatment serum IP-10 concentrations in patients with or without a very rapid

virological response to treatment with PEG-IFN/RBV/telaprevir. Boxes represent the interquartile range of the data. The lines across the boxes and the numbers indicate the median values. The hash marks above and below the boxes indicate the 90th and 10th percentiles for each group, respectively. The serum IP-10 concentrations were significantly lower in vRVR than in non-vRVR (P=0.009). PEG-IFN, pegylated interferon; RBV, ribavirin , vRVR, very rapid virological response.

Table 1. Clinical characteristics of 149 patients infected with HCV genotype 1.

	PEG-IFN/RBV (n = 104)	PEG-IFN/RBV/telaprevir (n=45)
Gender, male	46	27
Age, years	59 (16 - 73)	55 (28 – 70)
Body weight, kg	57 (34 - 92)	61 (42 – 102)
Hemoglobin, g/dL	13.7 (9.9 – 17.7)	14.7 (12.0 – 16.7)
Platelet count, $\times 10^4/\mu L$	15.6 (6.3 – 28.1)	14.3 (9.8 – 31.9)
ALT, IU/L	45 (12 – 426)	37 (13 – 212)
γ-GTP, IU/L	32 (10 – 222)	27 (12 – 258)
IP-10, pg/mL	325 (58 – 2053)	261 (57 – 1438)
HCV RNA, log IU/mL	6.5 (5.1 – 7.5)	6.7 (4.8 – 7.6)
rs8099917, TT / TG+GG	72 / 32	37 / 8
Treatment efficacy		
SVR / TVR / NVR	39 / 36 / 29	43 / 1 / 1

PEG-IFN, pegylated interferon; RBV, ribavirin; ALT, alanine aminotransaminase; γ-GTP, γ-glutamyl transpeptidase; IP-10, interferon-gamma-inducible protein-10; SVR, sustained virological response; TVR, transient virological response; NVR, non-virological response. Data are expressed as number for categorical data or the median (range) for continuous data.

Table 2. Univariate analysis of factors associated with sustained virological response in patients treated with PEG-IFN/RBV.

	SVR (n=39)	non-SVR (n=65)	*P* value
Gender, male	20	26	n.s.
Age, years	57 (47 - 61)	60 (53 - 65)	0.035
Body weight, kg	58 (52 - 67)	56 (51 - 61)	n.s.
Hemoglobin, g/dL	13.9 (13.0 – 14.7)	13.6 (12.7 – 14.5)	n.s.
Platelet count, $\times 10^4$/μL	16.9 (12.5 – 20.5)	14.0 (10.9 – 18.1)	0.027
ALT, IU/L	49 (34 - 83)	44 (32 - 70)	n.s.
γ-GTP, IU/L	26 (17 - 45)	33 (23 - 67)	0.024
IP-10, pg/mL	212 (144 - 384)	356 (239 - 509)	0.002
HCV RNA, log IU/mL	6.4 (5.9 – 6.9)	6.5 (6.2 – 6.8)	n.s.
rs8099917, TT / TG+GG	37 / 2	35 / 30	<0.0001

PEG-IFN, pegylated interferon; RBV, ribavirin; ALT, alanine aminotransaminase; γ-GTP, γ-glutamyl transpeptidase; IP-10, interferon-gamma-inducible protein-10.

Data are expressed as number for categorical data or the median (first-third quartiles) for continuous data.

Table 3. Logistic regression analysis of factors associated with sustained virological response in patients treated with PEG-IFN/RBV.

	Odds ratio (95% CI)	*P* value
Age, ≤58 years	1.46 (0.53 – 4.03)	0.467
Platelet count, $\geq 15 \times 10^4/\mu L$	1.84 (0.68 – 4.95)	0.228
γ-GTP, ≤31 IU/L	1.09 (0.40 – 3.00)	0.861
IP-10, <300 pg/mlL	3.86 (1.39 – 10.75)	0.010
rs8099917, TT	17.44 (3.62 – 83.94)	<0.001

PEG-IFN, pegylated interferon; RBV, ribavirin; γ-GTP, γ-glutamyl transpeptidase; IP-10, interferon-gamma-inducible protein-10.

Table 4. Univariate analysis of factors associated with very rapid virological response in patients treated with PEG-IFN/RBV/telaprevir.

	vRVR (n=18)	non-vRVR (n=27)	*P* value
Gender, male	12	14	n.s.
Age, years	57 (41 - 63)	55 (48 - 62)	n.s.
Body weight, kg	65 (58 - 76)	57 (50 - 70)	n.s.
Hemoglobin, g/dL	15.1 (13.6 – 15.6)	14.2 (13.1 – 15.3)	n.s.
Platelet count, $\times 10^4/\mu L$	15.9 (13.6 – 19.4)	13.6 (11.8 – 17.4)	n.s.
ALT, IU/L	34 (25 - 60)	45 (30 - 73)	n.s.
γ-GTP, IU/L	28 (20 - 51)	26 (19 - 58)	n.s.
IP-10, pg/mL	190 (126 - 336)	300 (223 - 616)	0.007
HCV RNA, log IU/mL	6.3 (5.8 – 6.9)	6.8 (6.6 – 7.2)	0.006
rs8099917, TT / TG+GG	14 / 4	23/ 4	n.s.

PEG-IFN, pegylated interferon; RBV, ribavirin; vRVR, very rapid virological response; ALT, alanine aminotransaminase; γ-GTP, γ-glutamyl transpeptidase; IP-10, interferon-gamma-inducible protein-10.

Data are expressed as number for categorical data or the median (first-third quartiles) for continuous data.

Fig. 1

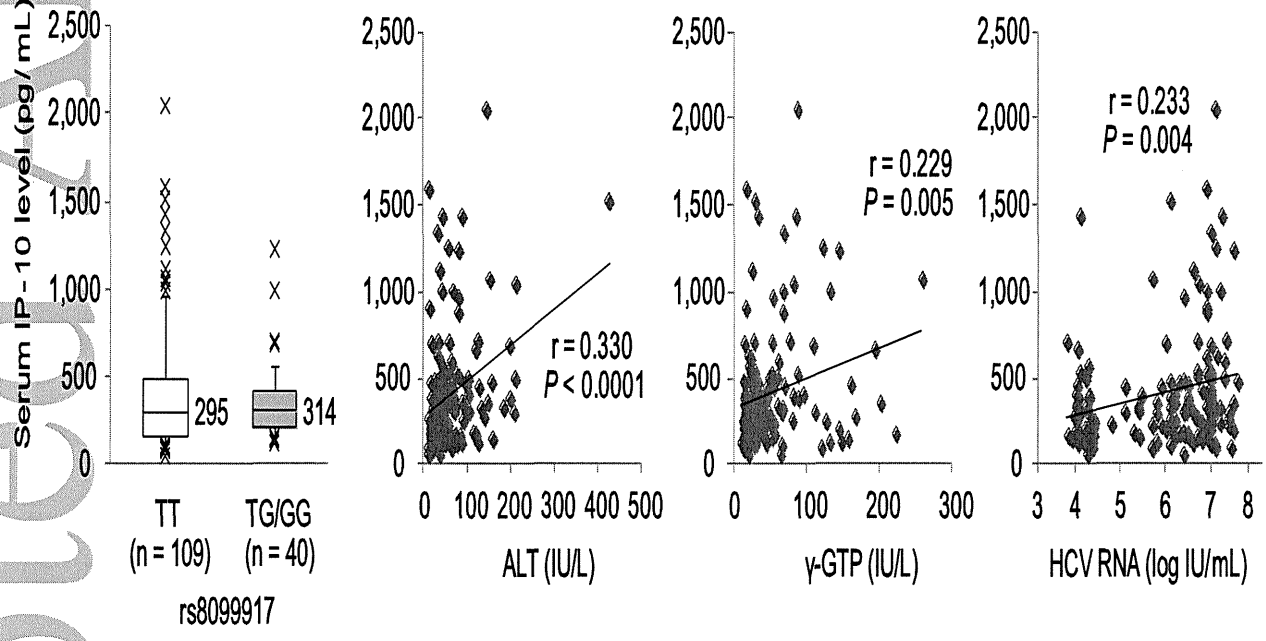

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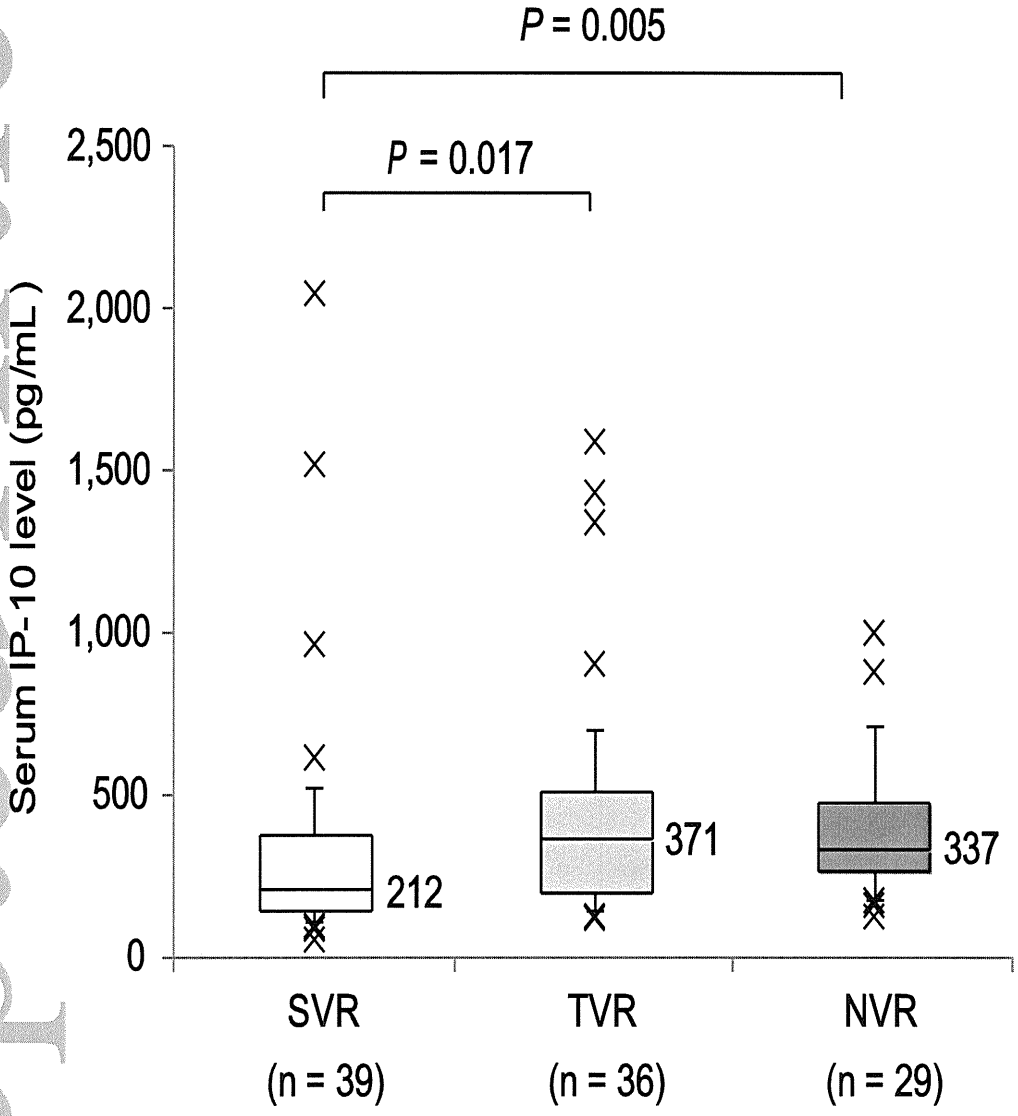
Fig. 2



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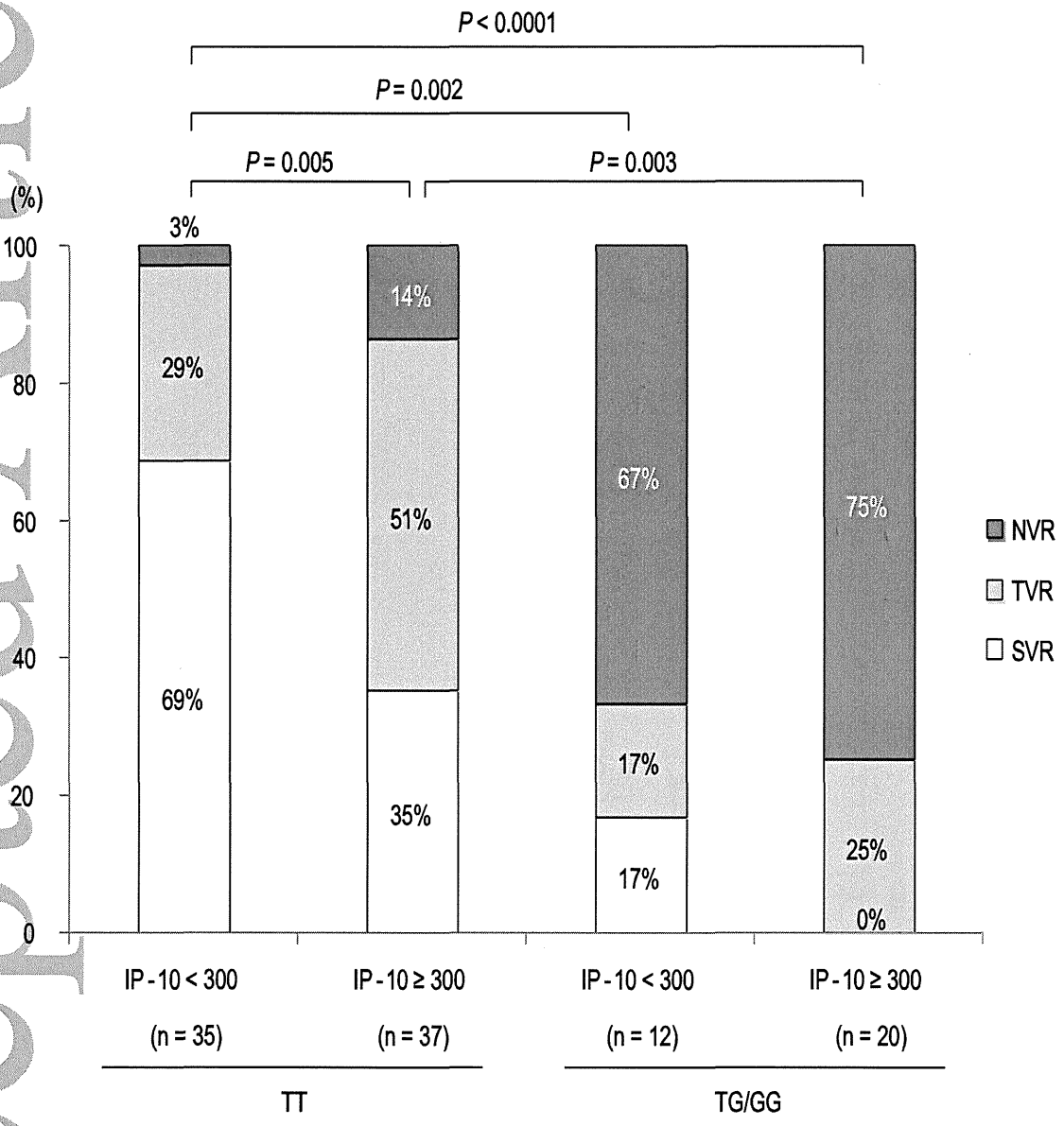

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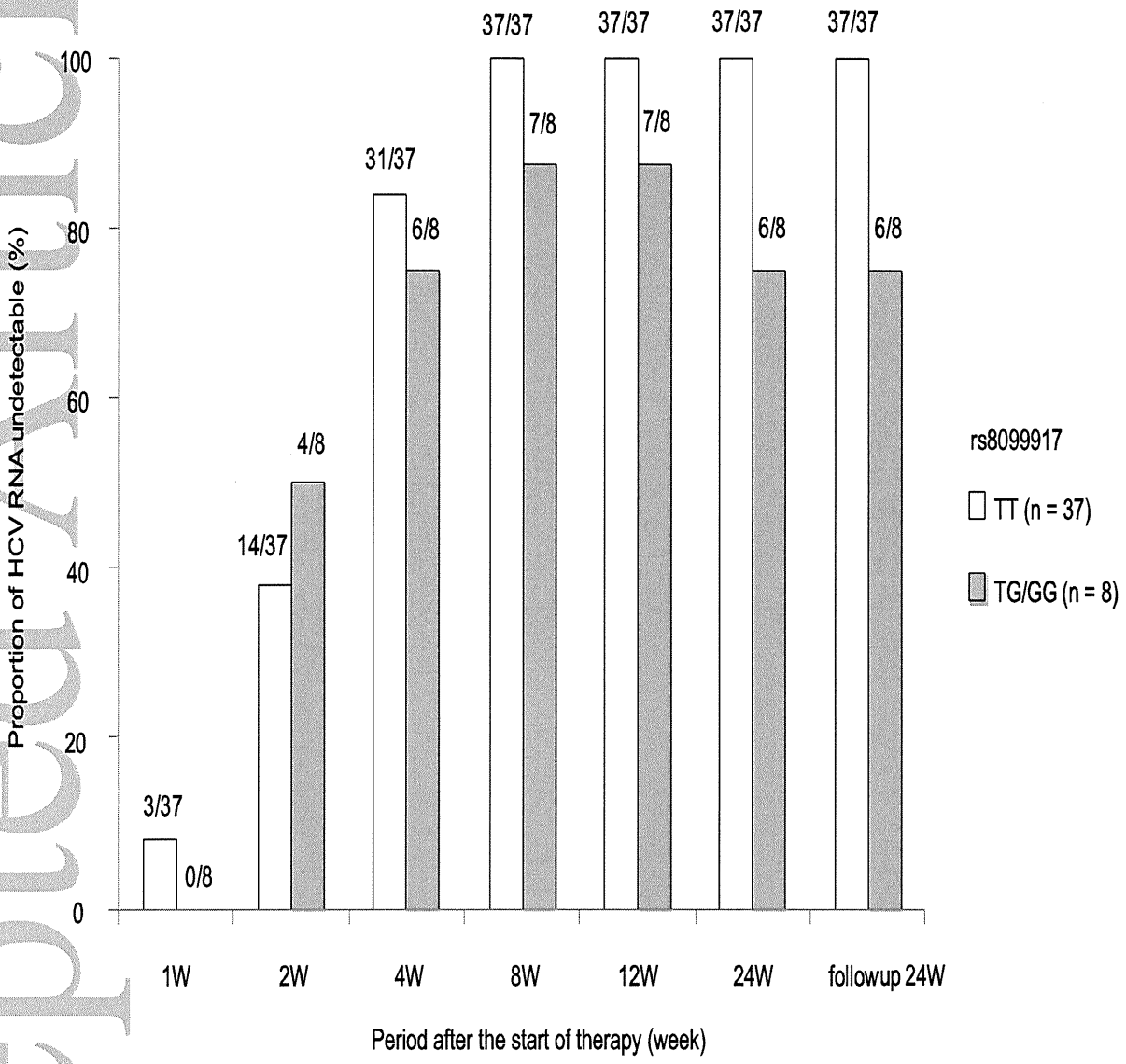

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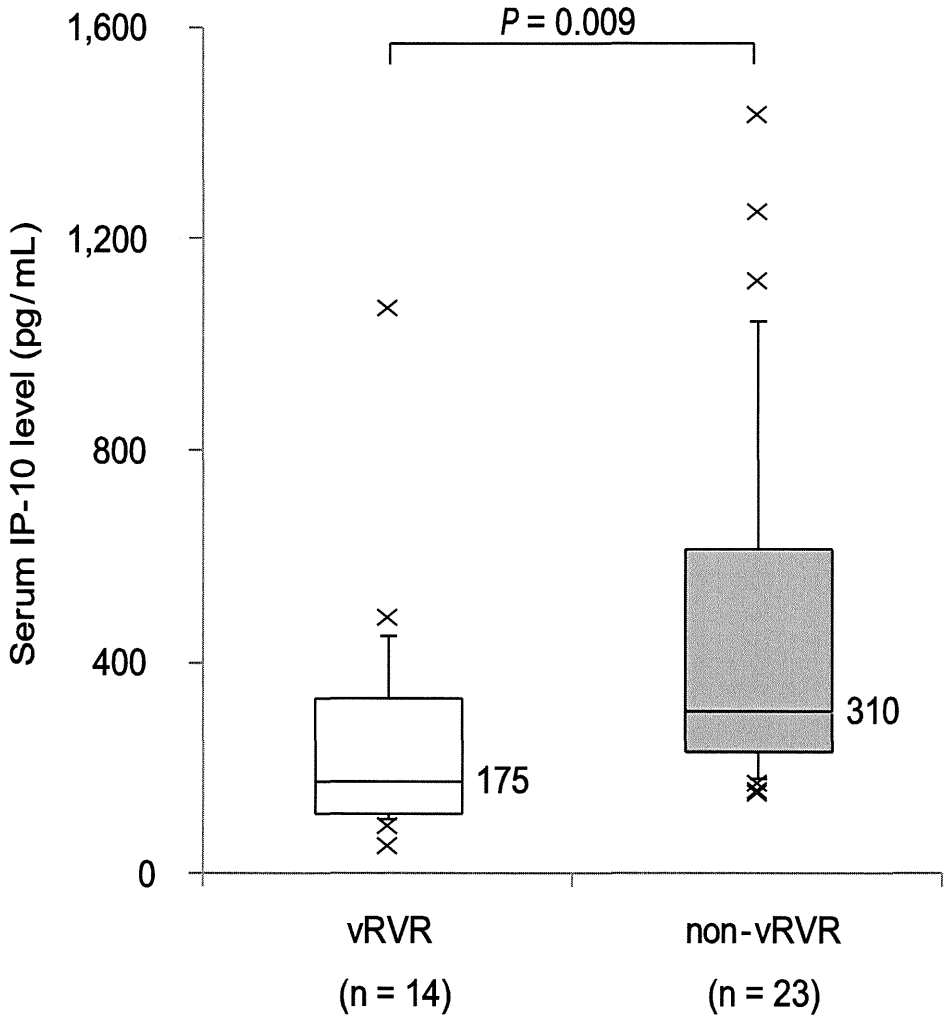
Fig. 5



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