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IL28B polymorphism determines treatment response of patients with hepatitis C genotypes 2 or 3 who do not achieve a rapid virologic response

Alessandra Mangia[1], Alexander J Thompson[2], Rosanna Santoro[1], Valeria Piazzolla[1], Hans L. Tillmann[2], Keyur Patel[2], Kevin V. Shianna[3], Leonardo Mottola[1], Daniela Petruzzellis[1], Donato Bacca[4], Vito Carretta[5], Nicola Minerva[6], David B. Goldstein[3], John G. McHutchison[2]

1- Liver Unit, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy

2- Duke Clinical Research Institute and Duke University Medical Center, Durham, NC, USA

3- Institute for Genome Sciences & Policy, Center for Human Genome Variation, Duke University, Durham, NC, USA

4- Department of Internal Medicine, Hospital Castrano, Italy

5- Department of Internal Medicine Hospital Venosa, Italy

6- Department of Internal Medicine, Hospital Canosa, Italy

Short title: *IL28B* polymorphism predicts SVR in genotype 2/3 HCV

Address correspondence and reprint requests to: Alessandra Mangia, MD, Gastroenterology Unit, IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo, Italy

Fax: +39 0882 455161; E-mail: a.mangia@tin.it

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ABSTRACT

Background & Aims: Polymorphisms in the region of the interleukin (IL)28B gene on chromosome 19 have been associated with peginterferon-α (pegIFN)-induced clearance of genotype 1 hepatitis C virus (HCV); there are no data for patients with genotypes 2 or 3 (G2/3) HCV. We evaluated the effects of IL28B polymorphisms on response to treatment with pegIFN and ribavirin in a well-characterized cohort of G2/3 patients.

Methods: DNA was analyzed from 268 patients (Caucasian, G2=213, G3=55). Patients were randomly assigned to groups that received standard (24 weeks, SD24, n=68) or variable durations of therapy. Patients that received variable durations and had a rapid virological response (RVR) were treated for 12 weeks (VD12, n = 122); those without a RVR were treated for 24 weeks (VD24, n=78). IL28B genotypes (rs12979860) were analyzed for association with treatment response.

Results: The frequencies of the IL28B genotypes were: CC=37%, CT=48%, TT=15% ; 82% of patients with the CC genotype achieved a SVR, compared to 75% with the CT and 58% with the TT genotypes (P = 0.0046). Differences between IL28B genotypes were greatest among patients who failed to attain RVR (VD24 SVR rates: CC=87%, CT=67%, and TT=29%, P=0.0002). Among patients with RVRs (61%), the IL28B genotype was not associated with SVR (>70% for all IL28B genotypes). In a multi-variable logistic regression model, IL28B genotype predicted SVR (odds ratio=1.76; 95% confidence interval=1.16 – 2.7).

Conclusions: An IL28B polymorphism was associated with a SVR in patients infected with genotypes 2 or 3 HCV who did not achieve a RVR. Analysis of IL28B genotype might be used to guide treatment for these patients.

Key words: hepatitis C, IL28B, SNP, personalized medicine, short course therapy

INTRODUCTION

Several viral and host-related factors determine the outcome of peginterferon-α (PegIFN) and ribavirin (RBV) treatment in patients with chronic hepatitis C virus (HCV) infection. In addition to viral genotype, serum HCV RNA level and stage of hepatic fibrosis, host genetic factors have recently been identified to influence treatment responsiveness in patients with chronic hepatitis C. Single nucleotide polymorphisms (SNPs) on a linkage disequilibrium block encompassing two interferon lambda genes on chromosome 19 have been strongly associated with response to pegIFN plus RBV combination treatment in three genome wide association studies of HCV genotype 1 infected patients [1-3]. The strongest association signal to date has been for the SNP rs12979860 [1]. Adherent patients with the CC genotype of rs12979860 were more than twice as likely to respond to 48 weeks of treatment as compared to the non-CC genotypes[1]. Although the mechanism by which this genetic variant acts remains unclear, it is likely to influence innate antiviral immune responses. The influence of this genetic variation on response to shorter treatment courses in patients chronically infected with genotype 2 or 3 HCV is not known.

The current standard therapy for HCV genotype 2 and 3 infection is 24 weeks of pegIFN plus RBV, which cures approximately 80% of patients [4]. In recent years, refinements to this regimen have been developed to minimize side effects and costs. In patients with low viral load at baseline who attain week 4 rapid virological response (RVR), 12 – 16 weeks of therapy is associated with rates of SVR that are comparable to those attained after a standard 24 weeks of treatment [5-7] . Although relapse rates may be

somewhat higher with shorter therapy [8], the cost-effectiveness of this approach has been demonstrated [6, 9]. Individualized therapy for patients with genotype 2/3 HCV infection according to RVR has recently been included in a number of European treatment guidelines [10, 11].

We have now investigated the relevance of *IL28B* genetic variation to treatment response in a well characterized cohort of European patients with genotype 2 or 3 HCV infection who were treated with pegIFN-alpha-2b and weight-based RBV for 12 or 24 weeks duration according to week 4 virological response, as previously published [5].

METHODS

Patient and control population

The primary study was a multi-centre randomized controlled trial that recruited patients from 13 clinical sites in Italy [5]. In the original study, 70 patients were randomized to standard treatment duration (24 weeks, SD24) and 213 to variable duration according to week 4 response: patients achieving RVR were treated for 12 weeks [VD12]; while those not achieving RVR were treated for 24 weeks [VD24]). Patients received PegIFN-alpha-2b (PEG-Intron, Schering Plough, Kenilworth, NJ) 1.0 mcg/kg/week, combined with RBV (Rebetol, Schering Plough, Kenilworth, NJ) 1000mg daily if body weight $\leq$ 75 kg, or 1200 mg if body weight >75 kg. PegIFN and RBV dose modification followed standard criteria and procedures [5]. RVR was defined by undetectable serum/plasma HCV RNA using a sensitive qualitative assay (lower limit of

detection <50 IU/mL). Inclusion and exclusion criteria have been reported previously [5]. 270 of the 283 patients provided informed consent for the collection and storage of peripheral blood mononuclear cells (PBMCs), for testing of their host DNA for research purposes consistent with the current study. The database for this analysis includes clinical and demographical data extracted from the original clinical trial database. In addition, one hundred seventy three healthy volunteers with normal liver enzymes and no serological markers of HCV, HBV, HIV or other hepatic infection were also evaluated as a control population from the same geographical region, to estimate the population frequency of the *IL28B* genotypes in this population. These subjects were all Caucasian and recruited from the Puglia region of Italy. The study was approved by a central ethics committee was conducted in accordance with the provisions of the Declaration of Helsinki, and Good Clinical Practice guidelines.

HCV viral load testing, HCV genotyping

At baseline, all patients had a quantitative measure of serum/plasma HCV RNA performed using the Amplicor Monitor HCV 2.0, Roche Diagnostics, Basel, Switzerland, with a lower limit of detection of 600 IU/mLml. A qualitative measurement of serum/plasma HCV RNA was performed at week 4 and week 8 on-treatment, at the end-of treatment (week 12 in the VD12 arm/ week 24 in the SD24 / VD24 arms), and at 24 weeks post-treatment. HCV RNA was qualitatively analysed by a PCR assay (Amplicor HCV, Roche Molecular Systems, Branchburg) lower limit of detection= 50 IU/mL during and off therapy. HCV genotyping was performed by reverse hybridization (InnoLIPA

HCV, Innogenetics Gent, Belgium) in all patients. Histologic results were classified by local pathologists following standard criteria according to Scheuer's scoring system [12].

IL28B Genotyping

The genomic region associated with HCV treatment response lies on chromosome 19 and contains multiple SNPs in linkage disequilibrium around the *IL28B* gene [1-3]. We selected the most strongly associated SNP, rs12979860, located 3kB upstream of the *IL28B* gene, for genotyping in the cohorts using the allele specific discrimination kit (ABI TaqMan) and the ABI7900HT sequence Detection System (Applied Biosystems) [13]. This SNP has been associated with treatment response in both Caucasians and African Americans [1]. Genotyping was performed at the Duke Institute for Genome Sciences and Policy Genotyping Core and was conducted in a blinded fashion relative to HCV treatment status and other patient or treatment response characteristics. Genotyping calls were manually inspected and verified prior to release. Hardy-Weinberg Equilibrium was assessed in the study population. Two subjects were excluded due to poor quality DNA preventing the accurate determination of the *IL28B* genotype.

Statistical analysis

Differences in baseline characteristics between patients in the SD24 and VD12/24 treatment arms were compared by 2-tailed Chi-square or Fisher's exact test for dichotomous variables. Binary parameters of viral response in the treatment subgroups were compared using the Chi-square or Fisher's exact test. To test the association of

IL28B-genotype with virologic response, a single-marker genotype trend test was used. Logistic regression analyses were performed to identify independent predictors of rapid and sustained virological response. First, candidate predictive factors were examined by univariable analysis. A two-sided p-value <0.05 was considered statistically significant. Multivariable logistic regression with backward selection was then used to identify baseline factors independently associated with SVR. A significance level of 0.05 was used for removal from the model. A second model was built to consider the effect of *IL28B* polymorphism for predicting SVR after adjusting for RVR. A commercially available software program (SAS version 9.2 [SAS institute, Cary, NC, USA]) was used for statistical analyses.

RESULTS

Patients

268 patients were included in this analysis. All patients were Caucasian, 213 patients were infected with genotype 2 HCV and 55 with genotype 3 HCV. Baseline demographic, biochemical and virologic characteristics of the patients are reported in Table 1. Sixty-eight were randomized to 24 weeks of standard therapy and 200 to the variable duration therapy (Supplementary Figure 1). There were no significant differences in baseline characteristics between patients in the standard / variable treatment arms. One hundred and sixty-five (61%) patients achieved RVR overall, 43 (63%) in the standard treatment group and 122 (61%) in the variable treatment group (P=0.74). Overall, 201 patients (75%) attained a SVR, 51/68 (75%) were in the standard treatment and 150/200 (75%) were in the variable treatment arms. Within the variable

treatment arm, 98/122 (80%) RVR patients in the 12 week arm, and 52/78 (67%) non-RVR patients in the 24 week arm attained SVR (P=0.1).

The frequency of *IL28B*-types in the treatment study cohort was evaluated and is summarized in Table 2. The *IL28B*-genotyping was also performed in a second cohort of ethnically matched healthy non-HCV infected control subjects (Table 2). The genotype results were in Hardy-Weinberg Equilibrium (χ^2 = 0.008, P=0.93 for HCV patients, χ^2 = 3.53, P=0.06 for non-HCV controls). The frequency of the *IL28B* CC genotype was numerically lower in the HCV cohort compared to the healthy control group (Table 2). *IL28B* genotype has previously been associated with genotype 1 HCV viral load set point, with the good response CC variant paradoxically associated with a higher viral load [1]. In the current genotype 2/3 cohort, there was a non-significant trend in the same direction (median baseline HCVRNA level, CC patients: = 5.81 (5.45 – 6.03) vs CT = 5.70 (5.11 – 6.0) vs TT = 5.65 (5.36 – 5.93) $\log_{10}$ IU/mL, P=0.2165; baseline HCVRNA level > 800,000 IU/mL, CC patients = 42% vs CT = 37% vs TT = 28%, P = 0.2746).

Sustained virological response

The study protocol randomized patients in the variable treatment arm to 12 or 24 weeks of therapy according to whether patients had attained a RVR. No difference in SVR rate was observed between the standard and variable duration treatment arms (75% in both). In the overall cohort, the C allele was significantly associated with rate of SVR (CC: 82% vs CT 75% vs TT 58%, OR 1.8 [95% CI 1.2 – 2.7], P= 0.0046). This effect was largely driven by the non-RVR patients in the VD24 treatment arm. A comparison of

SVR rates by *IL28B*-type for each treatment arm is presented in Figure 1/Table 3. The rates of SVR were high in the SD arm, and the VD12 arm (RVR patients treated for 12 weeks), independent of *IL28B*-type. In contrast, *IL28B*-type was strongly associated with treatment outcome in the third treatment arm (VD24) that included non-RVR patients exclusively (SVR = 87% vs 67% vs 29% for CC vs. CT vs. TT, OR 4.0 [95% CI 1.9 – 8.5], P=0.0002). In contrast to previous observations in North American genotype 1 HCV patients where CC patients had superior response rates and there was little clinical difference between response rates in CT and TT patients [14], in the genotype 2/3 non-RVR patients the SVR rate for CT patients was intermediate between CC and TT patients.

There was limited power to analyze the role of *IL28B*-type and SVR comparing 12 and 24 week treatment regimens in RVR patients. In CC patients, the SVR rates were similar for 12 or 24 week treatment duration (37/44 (84%) and 13/15 (87%) respectively, P=1.0) (Table 3). There was a trend for SVR rates to be lower in the CT and TT RVR patients treated for 12 weeks (Table 3).

On-treatment, end-of treatment virological responses and relapse

Patients were pooled for analysis of week 4 and week 8 responses as the treatment regimens were identical up to these timepoints in all patients. In contrast to genotype 1 HCV [14], IL28B-type was not associated with an increased rate of RVR. 165 patients (61%) attained an RVR, and the rate of RVR was high for all *IL28B*-types: 53%, 66% and 59% for CC, CT and TT patients, respectively (P=0.8463) (Figure 2A). By week 8,

there was a difference in the rate of viral clearance according to *IL28B*-type, 90% and 89% in CC and CT patients compared to 69% in TT patients (P=0.003). End-of treatment (EOT) virological responses were analyzed for each treatment arm separately. In the VD12 (RVR) treatment arm and the SD24 arm (63% RVR), most patients attained an EOT response at week 12, or week 24, respectively, and there was no difference by *IL28B*-type (Figure 2A). However, in the VD24 (non-RVR) treatment arm, *IL28B*-type was strongly associated with 24 week EOT response (84% vs 70% vs 29% for CC vs CT vs TT patients, P=0.0002, Figure 2A).

Relapse rates were generally low (Figure 2B). Although the sample size limited detailed analysis, there was a non-significant trend for higher relapse rates in the RVR pts treated for 12 weeks compared to RVR patients who were treated for 24 weeks (VD12: 15/116 [12.9%] vs SD24+RVR: 1/41 [2.4%], P=0.06). However, among RVR patients with the CC *IL28B*-type, relapse rates were comparable between the two treatment durations (VD12: 4/42 [9.5%] vs SD24, RVR: 1/14 [7.1%], P=0.7867).

HCV Genotype

No significant differences in the rate of overall on-treatment virological responses or SVR rate were noted according to HCV genotype 2 or 3 (data not shown). Patients who attained an RVR had a high rate of SVR regardless of HCV genotype or *IL28B*-type. However, in patients not attaining an RVR *IL28B*-type was associated with SVR in both genotype 2 and genotype 3 (HCV genotype 2, n = 85, OR = 2.3 [95%CI 1.2 – 4.5], P=0.0143; HCV genotype 3, n = 18, OR = 5.5 [95%CI 1.2 - 25.0], P=0.0273).

Logistic regression modeling for SVR

We analyzed pre-treatment clinical variables that were associated with SVR. *IL28B*-type, BMI ≥ 27 and Scheuer stage F3-4 were the only baseline variables that were associated with SVR on univariable analysis (Table 4A). Multivariable logistic regression then confirmed independent roles for each (*IL28B*-type: OR for SVR = 1.76 [95% CI 1.16 – 2.66], P=0.0077, Table 4A).

We then considered the informativeness of *IL28B*-type in the context of week 4 response, which was strongly associated with SVR in univariable analysis of the overall cohort (OR = 3.25 [95%CI =1.83 - 5.75], $P=5.49x10^{-5}$). For those subjects attaining RVR, *IL28B* genotype was not associated with SVR (P=0.6966). However, *IL28B*-type had strong predictive value in non-RVR patients (OR=2.75 [95%CI = 1.51 - 5.00], P=0.0009). A direct comparison showed that the predictive value of the *IL28B* polymorphism was significantly different between RVR and non-RVR patients (*P*-value for interaction = 0.044). We then tested for independent effects of the *IL28B* polymorphism and RVR for predicting SVR. Unfortunately, regression models that included both RVR and IL28B were difficult to interpret because of this interaction. We therefore created a dummy variable that included week four response and *IL28B* polymorphism as a four-level ordinal variable: week four responders (RVR); week four non-responders, CC genotype; week four non-responders, CT genotype; and week four non-responders, TT genotype. The combined RVR/IL28B-type variable was associated with SVR (OR 2.02 [95% CI

1.52 - 2.69], P=1.13x10^{-6}) independent of the effects of other baseline predictors (Table 4B).

Test characteristics for IL28B SNP genotype compared with RVR

The performance of the *IL28B* SNP genotype as a binary predictor for SVR was evaluated and compared to RVR. For this analysis we considered both CC vs non-CC patients, and non-TT vs TT (poor responder) patients (Table 5). Given the high response rates to treatment observed in genotype 2/3 patients, likelihood ratios were low for both *IL28B* classifiers and RVR.

DISCUSSION

To our knowledge this is the first report of the role and relevance of the recently described *IL28B* genetic polymorphism to PegIFN and RBV treatment outcome in the setting of chronic infection with genotype 2 or 3 HCV. This unique cohort, from a prospectively performed landmark clinical trial [5], has allowed us to carefully evaluate the interaction between this genetic determinant and treatment response in patients who received a variable duration of therapy based upon week 4 virologic response.

The data indicate that the IL28B polymorphism is relevant to treatment outcome, although the effect size was attenuated in these genotype 2/ 3 infected European patients, compared to the original observation in North American patients with genotype 1 HCV infection (genotype 2/3: 1.76 [95% CI 1.16 – 2.66], Table 4A, vs genotype 1: OR 5.2 [95%CI 4.1 – 6.7] [14]). The attenuation of overall effect size was largely due to the much higher rates of RVR that occurred in the genotype 2/3 patients; *IL28B*-type did not influence outcome in these rapid responders. The major effect of this genetic variant was in patients who did not achieve a rapid response at week 4, where *IL28B*-type had a strong influence on the rate of SVR. A second point of contrast between this cohort and the genotype 1 North American cohort previously reported [14] was that the presence of a single C allele was noted to confer significant clinical benefit. In contrast, in genotype 1 HCV infection, the clinical benefit was more restricted to *IL28B* CC homozygotes [14].

Despite the association between *IL28B*-type and overall SVR, there was no significant association between the *IL28B* polymorphism and week 4 viral clearance,

although RVR was numerically higher in CC and CT patients compared to TT patients. However, by week 8, viral clearance was clearly more common in CC and CT patients. We interpret this to mean that the *IL28B* polymorphism is associated with differential viral kinetics, similar to that observed in genotype 1, but that the different rate of decline between *IL28B*-types is attenuated, such that rates of viral clearance were not demonstrably different by week 4, but were significantly different at week 8. It is likely that quantitative measures of viral load may have been more sensitive for differentiating kinetics between patients with different *IL28B*-types, but unfortunately such measures were not available. We therefore acknowledge that the lack of HCV RNA decline evaluation represents a possible limitation of this study and that HCV RNA viral decline could have shown a better correlation with the *IL28B* genetic variant.

The study was not powered to formally evaluate the utility of *IL28B*-type for making decisions about selecting RVR patients for 12 weeks of therapy. Current guidelines recommend this approach for RVR patients with genotype 2/3 HCV infection and low viral load [10, 11]. It is tempting to speculate that such treatment would be most suitable for CC patients. Although our data would be consistent with this being a subgroup of RVR patients most likely to achieve equivalent response rates, no significant difference was noted between 12 and 24 weeks of therapy in this cohort and we cannot draw firm conclusions. What appears more impressive is the potential role for *IL28B* genotyping in selecting non-RVR patients for standard 24 week therapy or more prolonged therapy. Prospective studies randomizing patients according to *IL28B*-type will be necessary to address both of these issues.

Of interest and as previously reported by Ge et al. in a genotype 1 HCV cohort [1], we also noted that the prevalence of the favourable C allele was lower in genotype 2/3 HCV-infected patients than in the non-HCV control population. This suggests that the T allele is associated with persistence, and is consistent with the recent observation that the *IL28B* CC-type is associated with spontaneous clearance following acute HCV infection [13, 15]. The data therefore suggest that the role of *IL28B* variants in spontaneous clearance may be independent of HCV genotype.

Finally, the identification that genetic variation in the IL28B gene region is associated with pegIFN treatment outcome raises the possibility that IL28B might have therapeutic potential. IL28B (or IFN-lambda (λ)-3) is one of three members of the IFN-λ family, first identified in 2003 [16, 17]. All 3 members signal via a common IFN- λ receptor, to trigger an IFN-stimulated gene response that overlaps considerably with the downstream signaling pathway of type 1 IFNs. Antiviral activity against both HCV and HBV has been demonstrated *in vitro* [18]. Phase 1 studies investigating IL29 (IFN-λ-1) have recently confirmed an anti-HCV effect in genotype 1 non-responders [19]. Phase II development in a genotype 1-naïve population is planned. The efficacy of IL29 in the setting of genotype 2/3 HCV is yet to be studied.

In conclusion, these findings highlight the importance of the interaction between HCV genotype and host genetic determinants in influencing treatment response. We have demonstrated that genetic variation in *IL28B* is associated with treatment response in

patients infected with genotype 2/3 HCV. This effect is attenuated compared to previous reports in genotype 1 HCV, consistent with the recognized IFN-sensitivity of these genotypes, and also suggesting that key differences in the ability to evade innate antiviral immune responses are likely to exist between genotype 1 and genotype 2/3 HCV. *IL28B*-type was particularly important in patients who did not achieve RVR. Further prospective randomized studies are needed to investigate whether patients without RVR carrying the *IL28B* non-CC genotypes benefit from extended treatment duration.

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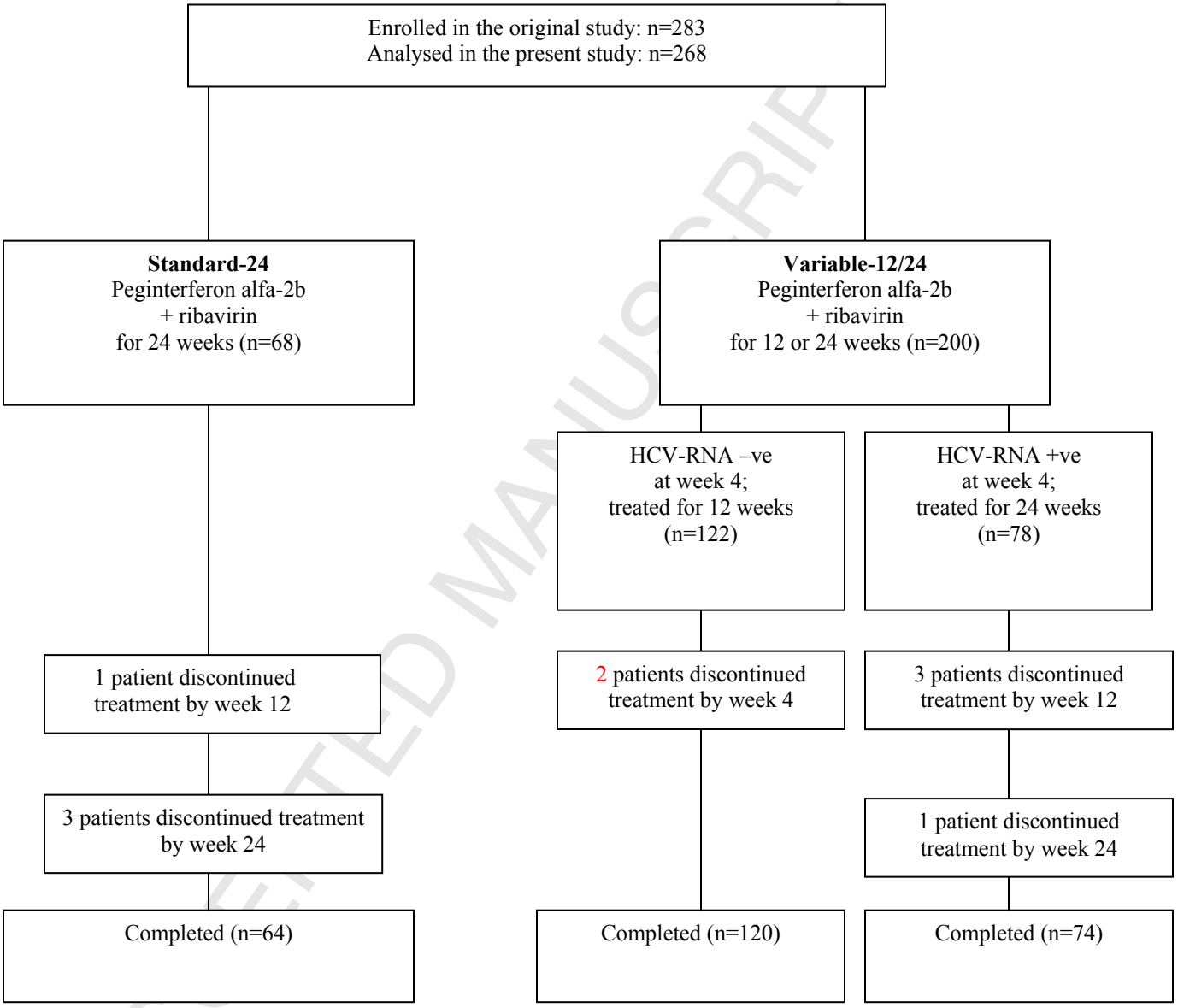
Figure 1. SVR rate by treatment arm and IL28B-type

Figure 2A. On-treatment virological response rate by *IL28B*-type

On-treatment virological response defined as undetectable serum HCV RNA. All patients were treated with identical regimens at week 4 and week 8 of therapy. Treatment duration beyond week 12 differed according to treatment arm. EOT = end-of-treatment; SD24 = standard duration (24 weeks); VD12/24 = variable duration (12/24 weeks).

Figure 2B. Relapse rate by *IL28B*-type

Supplementary Figure 1. Flow chart of the study - Patient Progress Through Trial



Figure
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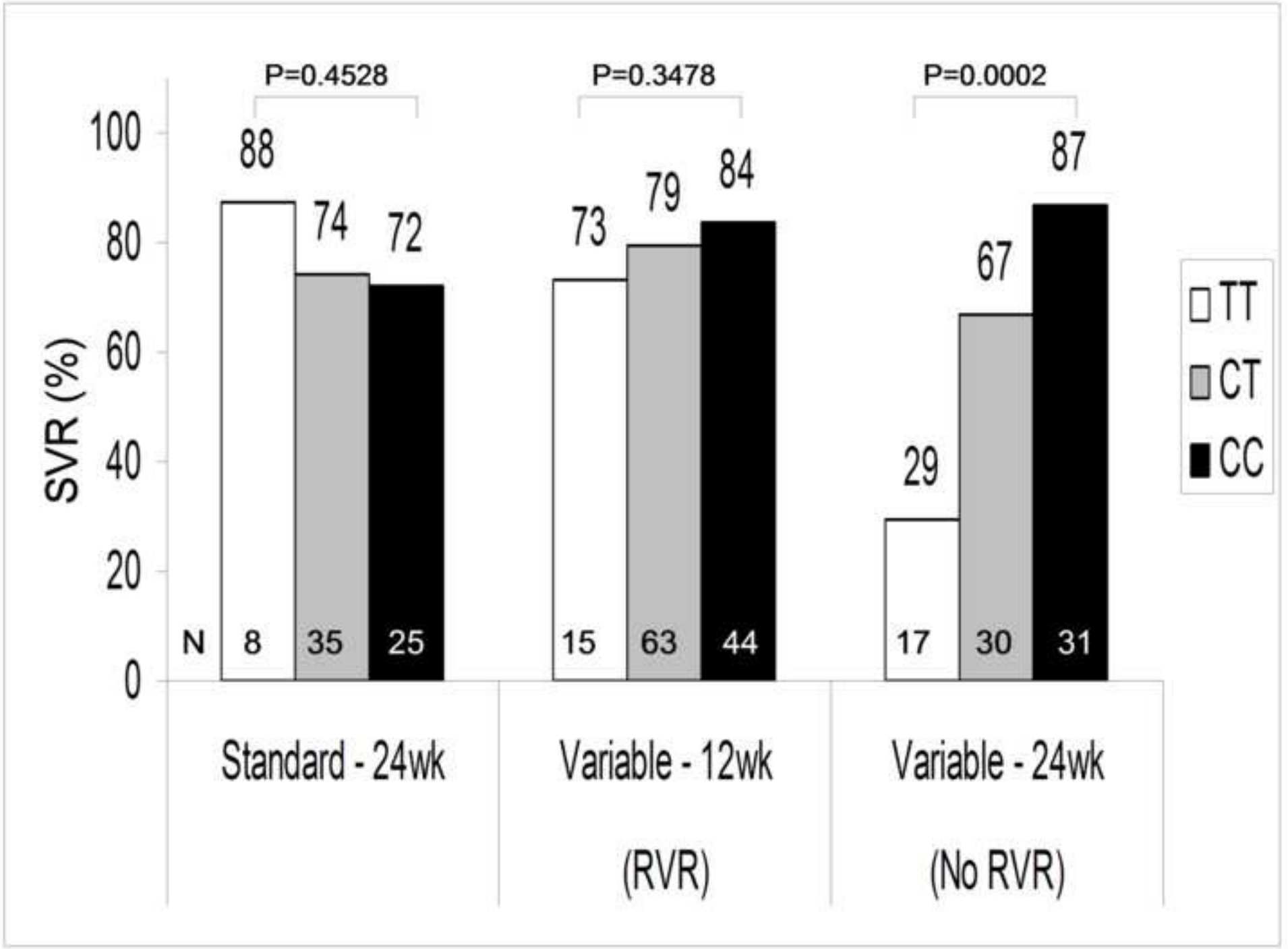

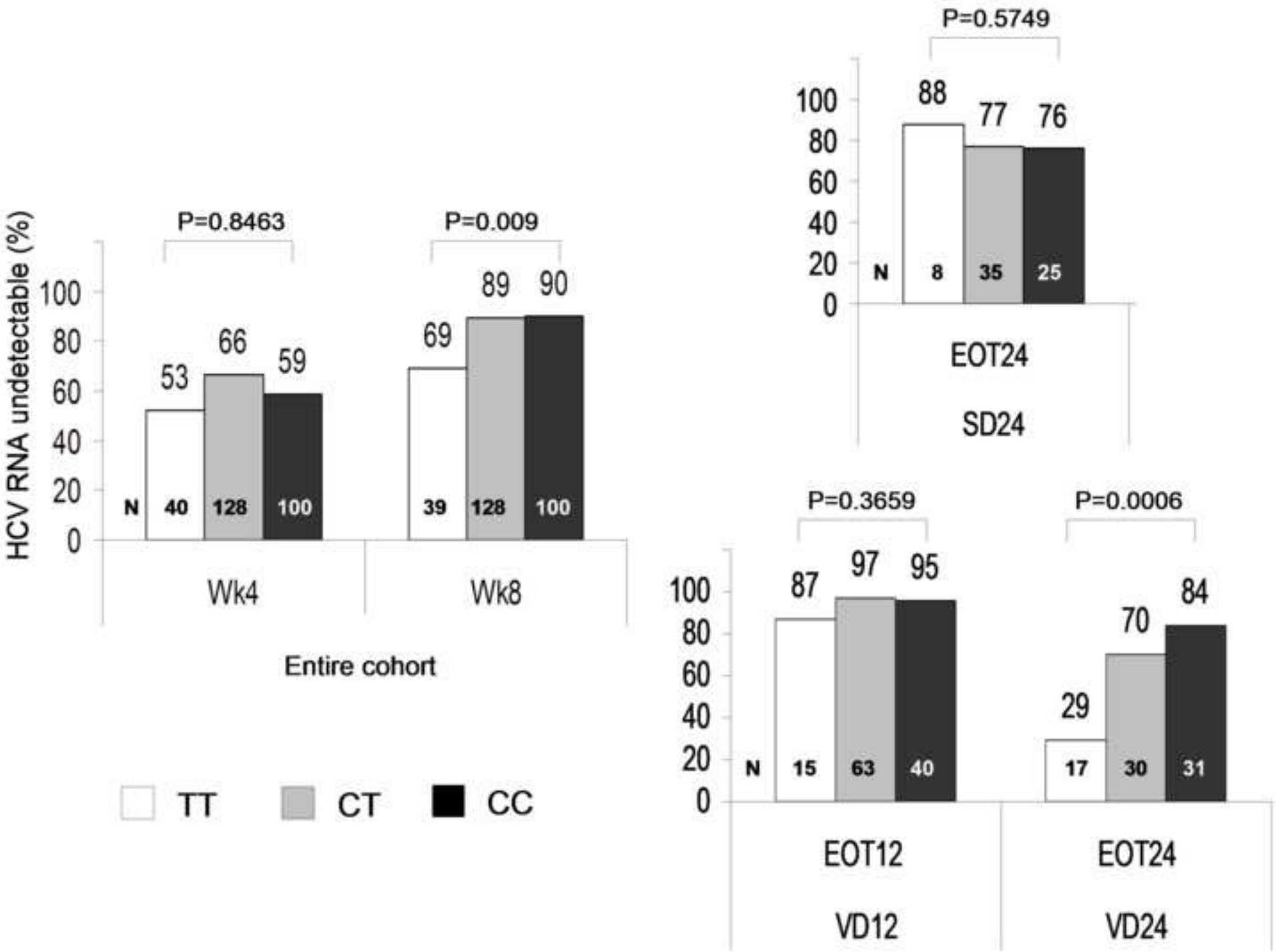

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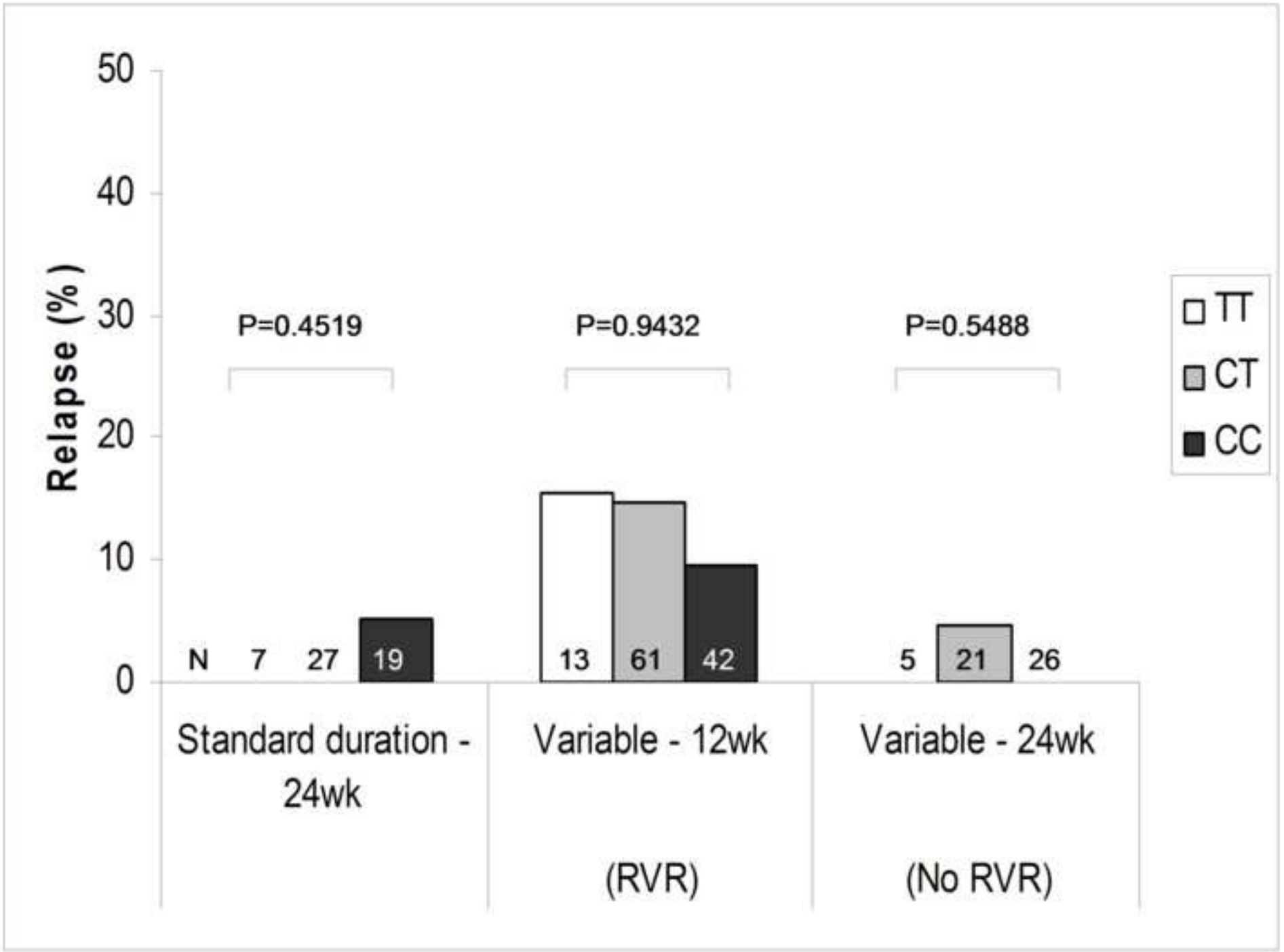

Table 1. Characteristics of the patients

	Overall		Standard duration (SD)		Variable duration (VD)		P SD vs VD
	N	(%)	N	(%)	N	(%)	
N	268		68		200		
Age ≥ 40	208	78.0%	50	73.5%	158	79.0%	0.3498
Male Gender	155	57.8%	39	57.0%	116	58.0%	0.9256
BMI ≥ 27	112	41.8%	28	41.2%	75	37.5%	0.5903
Genotype 2	213	79.5%	53	77.9%	160	80.0%	0.7165
Genotype 3	55	20.5%	15	22.1%	40	20.0%	*
HCV RNA > 800,000 IU/mL	100	37.5%	20	29.4%	80	40.2%	0.1125
ALT > 3 x ULN	61	23.0%	21	30.9%	40	20.3%	0.074
Mod-severe steatosis*	61	25.0%	22	34.9%	44	24.2%	0.0975
Scheuer F3-4	52	19.5%	17	25.0%	35	17.6%	0.1827

* Hepatic steatosis grade was not available in 23 patients

Table 2. Frequency of *IL28B* genotypes

Population frequency of *IL28B*-genotypes, comparing patients with chronic genotype 2/3 HCV with non-HCV healthy controls of the same ethnic background, from the same geographic region (Southern Italy). *P = P-value for comparison of genotype frequency between the two populations

IL28B-type			P*
G2/3 HCV infection	CC (n,%)	100 (37%)	0.0793
N=268	CT (n,%)	128 (48%)	
	TT (n,%)	40 (15%)	
	C allele frequency	0.61	
Healthy control	CC (n,%)	74 (42%)	
N=178	CT (n,%)	90 (51%)	
	TT (n,%)	14 (8%)	
	C allele frequency	0.67	

Table 3. Rates of SVR by treatment arm and *IL28B*-type

SVR	Standard duration (SD)						Variable duration (VD)					
	Overall		RVR		No RVR		Overall		RVR (12 wks)		No RVR (24 wks)	
Entire cohort	51 / 68	75%	40 / 43	93%	11 / 25	44%	150 / 200	75%	98 / 122	80%	52 / 78	67%
TT (n,%)	7	88%*	6 / 6	100%	1 / 2	50%	16 / 32	50%	11 / 15	73%**	5 / 17	29%***
CT (n,%)	26 / 35	74%	21 / 22	95%	5 / 13	38%	70 / 93	75%	50 / 63	79%	20 / 30	67%
CC (n,%)	18 / 25	72%	13 / 15	87%	5 / 10	50%	64 / 75	85%	37 / 44	84%	27 / 31	87%

* P=0.4528, TT vs CT vs CC (SD); ** P=0.3478, TT vs CT vs CC (VD/RVR); *** P=0.0002, TT vs CT vs CC (VD/no RVR)

Table 4. Multivariable logistic regression for SVR

(A) A regression model was built to consider the association between baseline predictors and treatment outcome. **(B)** A regression model was built including both *IL28B*-type and RVR (see text).

Table 4A

Baseline predictors		OR	95% CI	P
Univariable	***IL28B*-type**	1.80	1.20 - 2.71	0.0046
	Age ≥ 40	*		0.3783
	Male gender	*		0.1352
	BMI < 27	2.14	1.22 - 3.75	
	ALT > 3xULN	*		0.3169
	HCV genotype 2 vs 3	*		0.9301
	HCV RNA > 800,000 IU/mL	*		0.1537
	Moderate - severe steatosis	*		0.1859
	Scheuer F0-2	2.56	1.34 - 4.88	0.0042
Multivariable*	***IL28B*-type**	1.76	1.16 - 2.66	0.0077
	BMI < 27	1.88	1.05 - 3.37	0.0334
	Scheuer F0-2	2.35	1.21 - 4.56	0.0118

*Co-variates: *IL28B*-type, BMI ≥ 27, Scheuer F0-2 vs F3-4, ± HCV RNA > 800,000 IU/mL (when baseline HCV RNA level was forced into the model it was excluded by backward selection; the final model did not change)

Table 4B

Baseline predictors + RVR		OR	95% CI	P
Univariable	**RVR**	3.25	1.83 - 5.75	5.49×10^{-5}
	Combined RVR+*IL28B* variable	2.07	1.57 - 2.74	2.60×10^{-7}
	Age ≥ 40	*		0.3783
	Male gender	*		0.1352
	BMI < 27	2.14	1.22 - 3.75	0.0079
	ALT > 3xULN	*		0.3169
	HCV genotype 2 vs 3	*		0.9301
	HCV RNA > 800,000 IU/mL	*		0.1537
	Moderate - severe steatosis	*		0.1859
	Scheuer F0-2	2.56	1.34 - 4.88	0.0042
Multivariable*	**Combined RVR+*IL28B* variable**	2.02	1.52 - 2.69	1.13×10^{-6}
	Scheuer F0-2	2.02	1.01 - 4.04	0.0456

*Co-variates: combined RVR+*IL28B* variable (RVR vs no-RVR+CC vs no-RVR+CT vs no-RVR+TT), BMI ≥ 27, Scheuer F0-2 vs F3-4, ± HCV RNA > 800,000 IU/mL (when baseline HCV RNA level was forced into the model it was excluded by backward selection; the final model did not change)

Table 5

Predictive values of *IL28B*-type (non-TT vs TT or CC vs non-CC) compared to RVR (yes or no) for SVR, in patients with chronic genotype 2/3 HCV infection.

	Sensitivity	Specificity	PPV	NPV	LR +	LR -
IL28B-type (non-TT vs TT)	88.56	25.37	78.07	42.50	1.19	0.45
IL28B-type (CC vs non-CC)	40.80	73.13	82.00	29.17	1.52	0.81
RVR vs no RVR	68.66	59.70	83.64	38.83	1.70	0.53

LR +, positive likelihood ratio; LR -, negative likelihood ratio; NPV, negative predictive value; PPV, positive predictive value; SVR, sustained virological response.