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VIRAL HEPATITIS

Variation in genes encoding for interferon λ-3 and λ-4 in the prediction of HCV-1 treatment-induced viral clearance

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Keywords
chronic hepatitis – HCV – IL28B/interferon lambda-3 gene – interferon lambda-4 gene – peg-interferon/ribavirin

Abbreviations
CI, confidence interval; DAAs, direct-acting antivirals; EOT, undetectable serum HCV RNA at the end of treatment; HCV, hepatitis C virus; HWE, Hardy–Weinberg equilibrium; IFN λ-3, interferon Lambda 3; IFN λ-4, interferon Lambda 4; IL28B, interleukin-28B; LD, linkage disequilibrium; non-SVR, non sustained virological response; OR, odds ratio; PCR, polymerase chain reaction; Peg-IFN, pegylated interferon; RBV, ribavirin; RVR, rapid virological response; SNP, single-nucleotide polymorphism; SVR, sustained virological response.

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Abstract

Background & Aims: In patients with chronic HCV-1 infection, recent evidences indicate that determination of a dinucleotide polymorphism (ss469415590, ΔG/TT) of a new gene, designated IFN λ-4, might be more accurate than the 12979860CC type of the IL28B locus in predicting sustained virological response (SVR) following peg-interferon and ribavirin. In addition, combined genotyping of different SNPs of the IL28B locus was shown to help dissect patients most prone to SVR among those with rs12979860CT. We examined whether single or combined genotyping of two IL28B SNPs, rs12979860 and rs8099917, and ss469415590 variation might improve the prediction of SVR. *Results:* In the study cohort of 539 patients, 38% had SVR. The SNPs 12979860CC, rs8099917TT, and rs469415590TT/TT correlated significantly with SVR (68%, 50%, and 67%). Carriers of either the triplotype rs12979860CC_ss469415590TT/TT_rs8099917TT or the diplotype rs12979860CC_ss469415590TT/TT had the highest SVR rate (72%). In carriers of the rs12979860 T allele, neither the rs8099917 nor the ss469415590 improved the response prediction. After pooling this finding with data from previous studies, in rs12979860 T heterozygous individuals the co-presence of the rs8099917TT SNP was associated with improved response prediction. *Conclusion:* In HCV-1 patients, the rs12979860 polymorphism appeared as the hit SNP better predicting response following peg-interferon and ribavirin treatment. Additional ss469415590 or rs8099917 genotyping had no added benefit for response prediction. In the subset of carriers of the rs12979860 T allele, genotyping of the rs8099917 SNP was unhelpful in the present investigation, but may inform clinical prediction of treatment response when our data were pooled with previous investigations.

Evidence has accumulated suggesting that the individual ability to clear HCV infection in the context of therapy with peg-interferon (Peg-IFN) and ribavirin (RBV) partly reflects differences in the genetic make-up of the human host. Since the landmark discovery that variants on chromosome 19 located within a

genomic block encompassing the interleukin 28B (IL28B) gene, which encodes for interferon lambda-3 (IFN λ-3), were associated with either spontaneous or treatment-induced viral clearance (1–6), assessment of the host IL28B genotype is increasingly used among HCV genotype-1 patients to inform clinical prediction of treatment outcome (7). Many single nucleotide polymorphisms (SNPs) were found to play a fundamental role in the response to interferon-based therapy (3–7). More specifically, the rs12979860 and rs8099917 SNPs were the two most examined: they were found in linkage disequilibrium each other, and to be similarly informative as host predictors of sustained viral clearance (SVR). In a recent meta-analysis of results from nine studies regarding the correlation of IL28B genotype with SVR in 3110 Caucasian HCV-1 patients, the favourable IL28B genotype CC was present in 41% of individuals, and 67% of them achieved SVR compared to 37% of those with the unfavourable genotypes (8). In addition, rs8099917 was also indicated as the best tagger of the common haplotype associated with SVR (6).

To better elucidate the mechanisms underpinning the host genetic contribution to the Peg-IFN and RBV treatment, two different strategies have been pursued. In the first approach, combined genotyping of different IL28B SNPs were correlated with treatment outcome: while patients carrying rs12979860CC or rs8099917TT variants may reach high SVR rates (9), in rs12979860 T heterozygous individuals the co-presence of the rs8099917TT SNP has been reported to improve response prediction (10). This finding implies that diplotype analysis could help identify more individuals prone to achieve SVR. However, this claim was not validated in a cohort of patients from Italy (11)

A different approach was taken by Prokunina-Olsson *et al.* who discovered a dinucleotide polymorphism (ss469415590, ΔG/TT) between interferon IFN λ-3 and -λ2 in a new gene, designated IFN λ-4, located 3 kb upstream of and in the same orientation as IFN λ-3 (12). This dinucleotide variant was in high linkage disequilibrium with rs12979860 and, compared with the latter SNP, strongly associated with HCV clearance in individuals of African ancestry, but yielded comparable information in Europeans and Asians (12) and in Hispanic (13). At variance, Bibert *et al.* were able to detect the strong impact of this genetic variation on SVR prediction even in a cohort of European (Swiss) patients (14). Despite the strong linkage disequilibrium in the locus and the biological role released to new IFN λ-4 gene, it remains to be independently determined the lead marker across the IFN λ region as the key determinant for the differences in HCV-1 clearance following standard therapy. All together, previous findings would indicate that either the combination of different IFN λ-3 variants and/or the use of IFN λ-4 genotyping would improve patient management better than that afforded by rs12979860 SNP evaluation.

To externally validate the lead predictive SNP(s), triplo- or diplotype, able to better picture the SVR, we investigated the rs12979860, ss469415590 and rs8099917 polymorphisms across the IFNL region in an Italian cohort of 539 naïve HCV-1 patients who received Peg-IFN plus RBV. In an attempt to reconcile discrepancies among previous studies on the contribution of genes encoding for IFNL -3 and -4 proteins to SVR, we run a meta-analysis of available data.

Material and methods

Patients

Current analysis refers to 539 HCV-1 patients extracted from a larger cohort of 1249 patients who were treated at 16 Italian centers from 2005 to 2010. The full characteristics of the entire cohort have been presented elsewhere (7). Briefly, all patients included were treatment-naïve Caucasians with a diagnosis of HCV-1 chronic hepatitis or compensated cirrhosis. Patients received either Peg-IFN α-2b or α-2a plus RBV outside of any clinical trial, and the therapeutic regimen administered was according to recommended guidelines (15). All HCV-1 infected patients enrolled in this investigation agreed to donate a blood sample for genotyping, after having signed an informed consent. At the recall visit, information about the latest HCV RNA testing, liver chemistry, and abdominal ultrasound was recorded. This study was approved by the Ethical Committee of 'Casa Sollievo della Sofferenza' Hospital, San Giovanni Rotondo, Italy, and conducted according to provisions of the Declaration of Helsinki and Good Clinical Practice Guidelines.

Methods

Genomic DNA was extracted from whole blood samples by a standard non-enzymatic method, using the QIAamp DNA Blood Maxi Kit (Qiagen GmbH, Hilden, Germany). Samples were genotyped for the SNPs ss469415590 and rs8099917 using 5′exonuclease TaqMan genotyping assays on an ABI Prism 7900 Real-Time polymerase chain reaction (PCR) system, according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). Assays for the rs12979860 genotyping were performed at the Department of Clinical and Experimental Medicine, University of Foggia, Italy, whereas ss469415590 and rs8099917 polymorphisms and data management were under the responsibility of the Research Laboratory of the Division of Gastroenterology, at the IRCCS 'Casa Sollievo della Sofferenza' Hospital, San Giovanni Rotondo, Italy. Briefly, genotyping was performed using made-to-order TaqMan assays for rs8099917, whereas using custom-designed primers for ss469415590, as previously reported (12),

according to manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The rs12979860 variant was analysed by direct DNA sequencing with an automated procedure using the 3100 Genetic Analyzer (Foster City, CA, USA) and previously described primers (1).

Viral load, and HCV genotyping

HCV genotyping was performed using the Inno-LiPA assay (Innogenetics, Zwijndrecht, Belgium). Serum HCV RNA was quantified at baseline by reverse transcription-PCR, using the Cobas Amplicor HCV Monitor Test, v 2.0 (Roche, Basel, Switzerland). Qualitative HCV RNA assessment was made at weeks 4, 24, 48 during treatment, and at week 24 after stopping therapy. We then defined successful treatment response or no response according to standard definitions (15), concentrating on SVR, which is the absence of detectable virus at the end of follow-up evaluation.

Statistical analysis

All data were analysed on the intention to treat basis, using SPSS Statistical Package (v17 Chicago, IL, USA). Genotypic frequencies for all investigated polymorphisms were tested for consistency with the Hardy-Weinberg equilibrium (HWE) by means of the Haploview 4.2 (Broad Institute, Cambridge, MA, USA). Linkage disequilibrium (LD) between markers, haplotype structures and haplotype associations analyses were also performed. Strong linkage disequilibrium was defined as an $r^2 > 0.8$. The association between genetic and clinical data was calculated by means of univariate and subsequently logistic regression analysis. *P*-values of less than 0.05 were considered as significant.

Meta-analysis

A meta-analysis was undertaken to establish whether the combination of different variants at the IFN λ-3 locus or at the IFN λ-4 locus might extend the prediction power for SVR. A PUBMED computer database search of manuscripts published between September 2009 and July 2013 was performed using the keywords: IL28B AND response AND 'therapy' OR 'therapeutics' AND 'HCV'. After reviewing titles and abstracts of pertinent citations reporting treatment outcome of antiviral therapy in relation to the combined evaluation of different IFN λ-3 or λ-4 variants were deemed eligible, and full papers read by two investigators (OP, AL) who independently extracted data. Results from the selected studies were pooled and analysed using the Comprehensive Meta-analyses (version 1.0.25; Biostat, Englewood, NJ, USA). The random-effects method was used to estimate Odds Ratio (OR) with 95% confidence intervals (95% CI).

Results

Patients

The study cohort included 539 Caucasian patients with chronic HCV hepatitis; main patients' characteristics are shown in Table 1. All patients were infected with genotype 1, received Peg-IFN α-2a (n = 292, 54%) or α-2b (n = 247, 46%) plus RBV at a mean dosage of 14 ± 2 mg/kg of body weight, and had virological response determined 6 months after completion of therapy. Standard therapy applied to these patients led to SVR in 204 (38%), to rapid virological response (RVR) in 120 (22%), and undetectable serum HCV RNA at the end of treatment (EOT) in 338 (63%) of them. All responder and non-responder patients received therapy for 48 weeks, except when HCV RNA levels were low (400,000 IU) at baseline, and undetectable at treatment week 4. In this case, treatment duration was 24 weeks.

IFN λ-3 and λ-4 genotype distribution

The distribution of the three SNPs encompassing the IFN λ loci in the total cohort of 539 Caucasian patients was as follows: for the rs12979860 SNP, prevalence rates of CC, CT, and TT genotypes were 22%, 61%, and 17%; for the rs8099917 SNP, the values for TT, TG, and GG genotypes were 44%, 49%, and 7%; for the ss469415590 SNP, the rates were 18%, 62%, and 20% for the TT/TT, ΔG/TT, and ΔG/ΔG dinucleotide genotypes respectively. The allele frequencies of the three SNPs were in

Table 1. Demographic and Baseline Characteristics of 539 HCV-1 patients.

	N	%
Gender (*N*, %)		
Male	310	58
Female	229	42
Age (mean ± SD)	55 ± 12	
BMI (mean ± SD)	26 ± 4	
ALT (U.N.L.) (mean ± SD)	2.14 ± 1.4	
Platelet count (mean ± SD)	190 ± 66	
Liver fibrosis (*N*, %)		
Stage F0–F2	314	58
Stage F3–F4	225	42
Type 2 diabetes (*N*, %)		
Yes	52	10
No	487	90
HCV genotypes (*N*, %)		
1a	82	15
1b	456	85
Serum HCV RNA levels (*N*, %)		
<400.000 IU/ml	159	29
≥400.000 IU/ml	380	71
Type of Peg-IFN (*N*, %)		
alpha 2a	292	54
alpha 2b	247	46

accordance with the predicted Hardy-Weinberg's equilibrium ($P > 0.05$) only in patients who attained SVR. The linkage disequilibrium was moderate either between rs12979860 and rs8099917 ($r^2 = 0.38$), or between ss469415590 and rs8099917 ($r^2 = 0.47$) (Fig. 1). The rs12979860 SNP was in strong linkage disequilibrium with ss469415590 ($r^2 = 0.81$). The D prime (D') for the rs12979860, ss469415590, and rs8099917 SNPS was >0.87.

Predictive value of candidate SNPs for treatment-induced viral clearance

Two hundred and four patients (38%) of the study cohort attained SVR. The genotypic distribution of the three SNPs between responders and non-responders is shown in Table 2. For the rs12979860 SNP, SVR rates were 68%, 31%, and 24%, respectively for CC, CT, and TT genotypes. For the ss469415590 variation, the values were 67% for patients with TT/TT, 31% for ΔG/TT, and 29% for ΔG/ΔG. For the rs8099917 polymorphism, SVR rates were 50% in patients with TT, 30% for those carrying the TG variant, and 19% for GG individuals. At univariate analysis of individual SNPs, genotypes CC for rs12979860, TT/TT for ss469415590, and TT for rs8099917 were all significantly associated with SVR. The prediction power for SVR differed among the three SNPs: the CC homozygous genotype of rs12979860 SNP was more strongly associated (OR = 5.15, 95% CI 3.32–7.98 for CC vs. CT/TT) than the TT/TT genotype of ss469415590 (OR = 4.73, 95% CI 3.02–7.40 for TT/TT vs. ΔG/TT + ΔG/ΔG), or the TT genotype of rs8099917 (OR = 2.46, 95% CI 1.72–3.51 TT vs. TG/GG).

We next developed a logistic regression model that related clinical and genetic predictors to response rates. In the model including the three favourable genotypes (CC vs. CT/TT, TT vs. GT/ GG, and TT/TT vs. TT/ ΔG + ΔG/ΔG), the rs12979860 CC type retained a significant association with SVR (OR = 3.39; 95% CI 1.61–7.13), however significance was lost either for the ss469415590 TT/TT (OR = 1.40, 95% CI 0.61–3.21), or for the rs8099917 TT (OR = 1.34, 95% CI 0.86–2.09). In addition, adjusting for covariates of age, gender, HCV RNA levels, and fibrosis stage, the rs12979860 variant remained the strongest independent predictor of SVR (OR = 4.72; 95% CI 2.82–7.90).

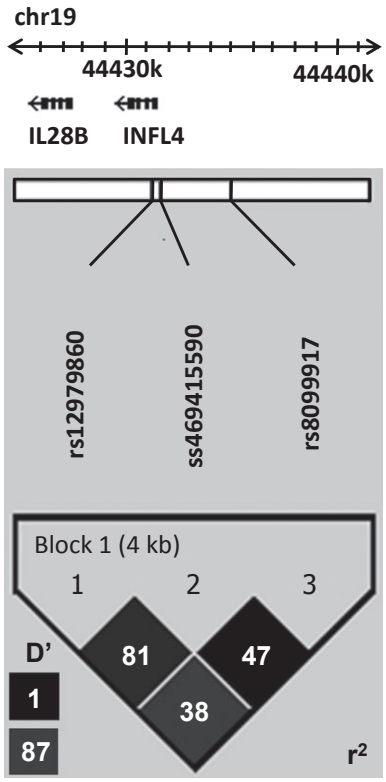

Fig. 1. Linkage disequilibrium (LD) plot for the cohort. D′ = proportion of possible LD present between the SNPs. D′ varies from 0 (complete equilibrium) to 1 (complete disequilibrium) r^2 = correlation between SNPs. When $r^2 = 1$, two SNPs are in perfect LD and allelic frequencies are identical for both SNPs. The diagram shows the D′ value in red, whereas the number represent the r^2.

Combination of IFN λ-3 and -4 variants and treatment-induced viral clearance

The impact of the combined evaluation of the two IL28B SNPs, rs12979860 and rs8099917, with the ss469415590 variation on SVR prediction is shown in Table 3. In a triplotype analysis, haplotypes characterized by carriage of the three favourable alleles rs12979860CC_ss469415590TT/TT_rs8099917TT had the strongest association with SVR: 72% of 99 carriers of the triplotypes attained SVR, as compared with the 30% SVR rate registered in those with different triplotypes (OR = 5.85, 95% CI 3.61–9.48). The strength of the association remained unchanged when the analysis was restricted to diplotypes. In 99 carriers of the rs12979860CC_ss469415590TT diplotype, the SVR rate amounted to 72%, as compared to a value of 32% in carriers of different diplotypes (OR = 5.85, 95% CI 3.61–9.48). In a similar fashion, the 104 carriers of the rs12979860CC_rs8099917TT diplotype attained a SVR rate of 71%, as compared with the 30% value registered in those with different diplotypes (OR = 5.79, 95% CI 3.61–9.27). In addition, SVR rates were 67% in 110 carriers of the ss469415590TT/TT_rs8099917TT diplotype, and 30% in those with different diplotypes (OR = 4.73, 95% CI 3.02–7.40).

Predictive value of single or combined SNPs for treatment-induced Rapid Virological Response

One hundred and twenty patients (22%) of the study cohort attained RVR. The genotypic distribution of the three SNPs is shown in Table 2. For the rs12979860 SNP, RVR rates were 46%, 17%, and 9%, respectively

Table 2. Association between IFN λ-3 SNPs (rs12979860 and rs8099917) and IFN λ-4 variation (ss469415590) with Sustained Virological Response (SVR) and Rapid Virological Response (SVR).

	Study cohort		SVR		Non-SVR					RVR		Non-RVR				
	n = 539		*n* = 204 (38%)		*n* = 335 (62%)					*n* = 120 (22%)		*n* = 419 (78%)				
	N	%	*N*	%	*N*	%	*P*-value	OR	IC 95%	*N*	%	*N*	%	*P*-value	OR	IC 95%
rs12979860																
CC	119	22	81	68	38	32	1.36e-14	5.15	3.32–7.98	55	46	64	54	1.11e-12	4.69	3.00–7.34
CT	329	61	101	31	228	69				57	17	272	83			
TT	91	17	22	24	69	76				8	9	83	91			
ss469415590																
ΔG/ΔG	98	18	28	29	70	71				11	11	87	89			
ΔG/TT	331	61	102	31	229	69				54	16	277	84			
TT/TT	110	20	74	67	36	33	9.87e-13	4.73	3.02–7.40	55	50	55	50	4.58e-15	5.60	3.54–8.85
rs8099917																
GG	36	7	7	19	29	81				3	8	33	92			
TG	265	49	79	30	186	70				39	15	226	85			
TT	238	44	118	50	120	50	5.92e-07	2.46	1.72–3.51	78	33	160	67	1.84e-07	3.01	1.97–4.59

CI, confidence interval; Non-SVR, non sustained virological response; Non-RVR, non rapid virological response; OR, odds ratio; SVR, sustained virological response.

for CC, CT, and TT genotypes. For the ss469415590 variation, the values were 50% for patients with TT/TT, 16% for ΔG/TT, and 11% for ΔG/ΔG. For the rs8099917 polymorphism, RVR rates were 33% in patients with TT, 15% for those carrying the TG variant, and 8% for GG individuals. At univariate analysis the studied SNPs were all significantly associated with RVR ($P \leq$ 1.84e-07; OR $\geq$ 3.01).

By stratifying carriers of the rs12979860CC, ss469415590TT/TT or rs8099917TT, (119, 110 and 208 patients respectively) that achieved RVR, 48 (87%) for rs12979860CC, 47 (85%), and 78 (85%) had an SVR. In particular, the achievement of an RVR was related to SVR rates for all the variations, as the SVR rates were 87% in those with an RVR and 52% in those without an RVR for rs12979860CC carriers (P = 3.1e-05), 87% vs. 49% in those ss469415590TT/TT (P = 4.82e-05), and 85% vs. 32% in rs8099917TT (P = 4.42e-14).

The impact of the combined evaluation of the three studied SNPs on RVR prediction is shown in Table 3. In a triplotype analysis, haplotypes rs12979860CC _ss469415590TT/TT_rs8099917TT had the strongest association with RVR, although only 53% of 99 carriers of the triplotypes attained RVR, as compared with the 15% RVR rate registered in those with different triplotypes (OR = 6.05, 95% CI 3.78–9.70).

In a diplotypes analysis, in 99 carriers of the rs12979860CC_ss469415590TT diplotype, the RVR rate amounted to 53% (OR = 6.05, 95% CI 3.78–9.70). In a similar fashion, the 104 carriers of the rs12979860CC_rs8099917TT diplotype attained a RVR rate of 52%, as compared with the 12% value registered in those with different diplotypes (OR = 6.04, 95% CI 3.79–9.62). In addition, RVR rates were 50% in 110 carriers of the ss469415590TT/TT_rs8099917TT diplotype, and 15% in those with different diplotypes (OR = 5.60, 95% CI 3.54–8.85).

SVR rates in HCV-1 patients with the rs12979860 T allele

In 329 patients with the heterozygous variant of the rs12979860 SNP, genotyping for the rs8099917 SNP did not improve the prediction of SVR: genotyping for the rs8099917 SNP yielded 33% and 30% SVR rates in TT or TG carriers. In a similar fashion, genotyping for the ss469415590 variant in the same subset of patients yielded 30% and 30% SVR rates in TT/TT or ΔG/TT carriers. For the 91 carriers of the homozygous rs12979860 TT non-responder genotype, SVR rates remained unaffected whether the rs8099917 SNP or the ss469415590 variant was evaluated (data not shown).

Meta-analysis of available studies on IFN λ-3

Of the 314 published studies on IFN λ-3 and therapeutic outcome following Peg-IFN and RBV, two studies (10,11) were identified that reported the combined evaluation of the IFN λ-3 rs12979860 and rs8099917 SNPs and its impact on SVR. Summary data for genotypic distribution of the two IFN λ-3 SNPs from these studies are shown in supplementary Table 1 and in Table 4: the distribution of either the rs12979860 or rs8099917 SNPs differed among the studies. By pooling SVR data from the studies, genotyping for the latter SNP improved the prediction of treatment-induced viral clearance in the subset of patients with the heterologous T allele of the rs12979860 SNP: OR = 1.57 (95% CI 1.15–2.17).

Table 3. Association of IFN λ-3 and λ-4 triplotypes or diplotypes with Sustained Virological Response (SVR) and Rapid Virological Response (SVR).

			SVR		Non-SVR					RVR		Non-RVR				
			n = 204 (38%)		*n* = 335 (62%)					*n* = 120 (22%)		*n* = 419 (78%)				
rs12979860	ss469415590	rs8099917	*N*	%	*N*	%	*P*-value	OR	IC 95%	*N*	%	*N*	%	*P*-value	OR	IC 95%
CC	TT/TT	TT	71	72	28	28	1.47e-14	5.85	3.61–9.48	52	53	47	47	1.14e-15	6.05	3.78–9.70
ALL TRIPLOTYPES			133	30	307	70				68	15	372	85			
CC	TT/TT		71	72	28	28	1.47e-14	5.85	3.61–9.48	52	53	47	47	1.14e-15	6.05	3.78–9.70
ALL DIPLOTYPES			133	30	307	70				68	15	372	85			
CC		TT	74	71	30	29	6.42e-15	5.79	3.61–9.27	54	52	50	48	5.81e-16	6.04	3.79–9.62
ALL DIPLOTYPES			130	30	305	70				66	15	369	85			
	TT/TT	TT	74	67	36	33	9.87e-13	4.73	3.02–7.40	55	50	55	50	4.58e-15	5.60	3.54–8.85
ALL DIPLOTYPES			130	30	299	70				65	15	364	85			
CT		TT	39	33	79	67	0.543	1.16	0.72–1.89	23	19	95	81	0.472	1.24	0.69–2.22
CT		TG	62	30	146	70				34	16	174	84			
CT	TT/TT		3	30	7	70	1	1.02	0.26–4.01	3	30	7	70	0.220	2.23	0.56–8.91
CT	ΔG/TT		92	30	218	70				50	16	260	84			

CI, confidence interval; Non-SVR, non sustained virological response; Non-RVR, non rapid virological response; OR, odds ratio; SVR, sustained virological response.

Discussion

With the approval of the first two direct-acting antivirals (DAAs) protease inhibitors in 2011, several other molecules have been approved for HCV-1 infections. These compounds used in different combinations, till to quadruple therapy, have increased SVR rates to about 90% (16). In this scenario, IFNL3 and INFL4 genes typing might become appropriate to target patients who could more likely respond to conventional dual therapy with Peg-interferon and ribavirin.

The aim of this study was to establish whether there was an interaction between different IFN λ-3 and -4 polymorphisms with respect to predicting treatment-induced clearance in chronic HCV-1 infection. The SNPs evaluated were two variants of the IFN λ-3 locus, rs12979860 and rs8099917, in moderate linkage disequilibrium and likely to be tagging the same haplotype block, and a dinucleotide variation present in the IFN λ-4, ss469415590TT/TT, which is also in strong linkage disequilibrium with rs12979860 (12, 13). These linkage data were confirmed in our cohort of patients.

In the initial analysis of the total cohort of 539 patients, we ascertained the prediction power of each of the three investigated polymorphisms on viral clearance following Peg-IFN plus RBV. The homozygous variants of each genotype were associated with treatment response. At univariate analysis, by genotyping rs12979860CC, ss469415590 TT/TT, and rs8099917TT SVR could be predicted, respectively, in 68% (81 of 119), 67% (74 of 110), and 50% (118 of 238) of the study cohort. Therefore, in our Italian cohort the clinical information provided by ss469415590 genotyping appeared comparable to that of the rs12979860 SNP, a finding which is in accordance with the original study by Prokunina-Olsson *et al.* (12), but at odds with the Bibert *et al.* (14) who found the TT/-G genotype to predict response to treatment better than the rs12979860. Finally, in our series of patients genotyping the rs8099917 polymorphism was less informative. On the whole, our results would suggest that either the rs12979860 SNP or the ss469415590TT/TT variation might be indifferently genotyped in HCV-1 infected patients before starting antiviral therapy. To establish which of the investigated polymorphisms encompassing the IL28B/IFN λ-4 locus could more likely be causal, we entered the three polymorphisms into a multiple logistic model: the hit SNP appeared to be the rs12979860CC genotype as it was the only one to retain an independent prediction power (OR = 3.39; CI 1.61–7.13) even when other predictive factors were taken into account.

Our next step was to observe the effect of triplotype/diplotype in predicting treatment response. In fact, carriers of the triplotype rs12979860CC_ss469415590TT/TT_rs8099917TT had SVR rate of 72%, a marginally higher value than the 67–68% rates afforded by the single evaluation of either the rs12979860 SNP or the dinucleotide ss469415590TT/TT variation. However, it

Table 4. Genotype distribution of single IL28B rs12979860 and rs8099917 SNPs in the cohorts studied in the meta-analysis.

	Present			Fischer J.*			Fischer J.§			Galmozzi E.		
	All *n* = 539	SVR *n* = 204	Non-SVR *n* = 335	All *n* = 942	SVR *n* = 495	Non-SVR *n* = 447	All *n* = 377	SVR *n* = 137	Non-SVR *n* = 240	All *n* = 187	SVR *n* = 91	Non-SVR *n* = 96
rs12979860												
CC	22	68	32	34	68	32	26	67	33	30	72	28
CT	61	31	69	52	46	54	55	26	74	57	40	60
TT	17	24	76	14	41	59	19	31	69	12	30	70
P-value	reference			9e-6			0.24			0.044		
rs8099917												
GG	7	19	81	5	35	65	9	31	69	5	30	70
TG	49	30	70	40	42	58	46	21	79	42	37	63
TT	44	50	50	56	62	38	45	54	46	53	60	40
P-value	reference			9e-5			0.31			0.11		

Values are given as percentage.

*Discovery cohort.

§Confirmation cohort.

CI, confidence interval; Non-SVR, non sustained virological response; OR, odds ratio; SVR, sustained virological response.

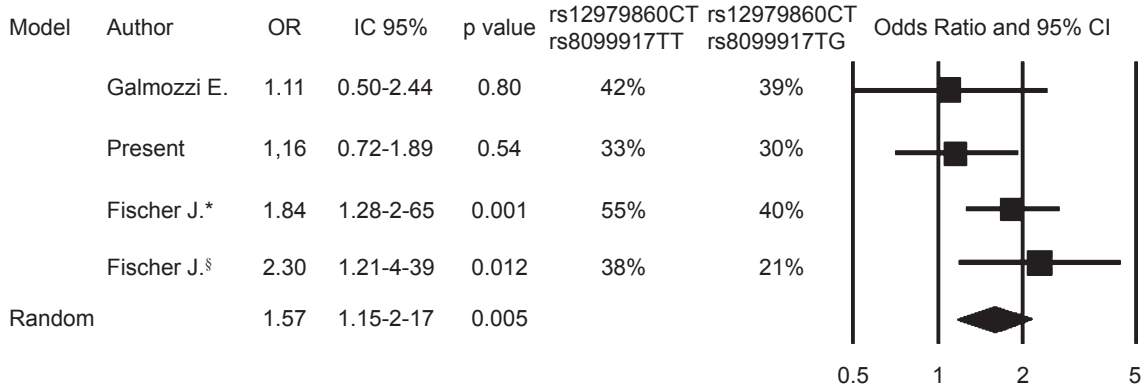

Fig. 2. Meta-analyses of studies that have analysed the rs12979860CT/rs8099917TT diplotype. Fischer *et al.* *discovery and §confirmation cohorts.

seems most likely that the gain in information provided by the triplotype evaluation was essentially carried over by diplotype analysis. Indeed, equally high rates of SVR (72–71%) were registered in carriers of either the rs12979860CC_ss469415590TTTT diplotype and in those with ss469415590TTTT/rs809917TT. Being the modest gain in SVR prediction afforded by either the triplotype or the diplotype analysis, our results would suggest that in homozygous carriers of the rs12979860 responder C allele, additional genotyping of the rs8099917 SNP or the ss469415590 variation had no effect on response prediction.

When the week 4 treatment response was taken into account, at univariate analysis rs12979860CC, ss469415590 TT/TT, and rs8099917TT variations were associated with RVR in 46%, 50%, and 30% patients of the study cohort respectively. In addition, the triplotype rs12979860CC_ss469415590TT/TT_rs8099917TT and the diplotype rs12979860CC_ss469415590TT/TT were associated with RVR in 53% and 53% of subjects, and comparable predictive value were found also for the other diplotypes combination. It follows that a low utility of genotyping different polymorphisms at the interferon lambda locus, at least for the prediction of an RVR.

A more cogent clinical need is to explore the added clinical value of evaluating different polymorphisms of the IFN λ-3 and -4 genes in the subset of HCV-1 infected patients carrying the heterozygous rs12979860CT genotype. Although the CC type of this SNP has been consistently shown to best predict SVR (1–5), it has a limited distribution in HCV-1 infected patients (22% in the total cohort of the present investigation). The majority of patients carried a non-CC type and exhibited low values of SVR. Consequently, it could be of major clinical interest to verify whether the combined evaluation of more than a single genetic variant could help identify heterozygous rs12979860CT

patients most prone to achieve a favourable outcome. Of interest, Fischer *et al.* provided a positive answer to this question by showing that in carriers of the rs12979860 T allele, SVR rates were 55% in the presence of the rs8099917TT genotype and 40% in carriers of the rs8099917TG (10). At variance, Galmozzi *et al.* (11) were unable to replicate the findings in a limited series of patients heterozygous for the rs12979860T allele. In this study, carriers of the rs8099917TT genotype attained SVR rates similar to those seen in rs8099917TG carriers (42% vs. 39%). The analysis of our large cohort of 539 HCV-1 patients produced data in accordance with the Galmozzi' study (11), as heterozygous rs12979860CT patients attained equal rates of SVR whether they were genotyped TT or TG/GG at the rs8099917 SNP (33% vs. 30%; $P = 0.5$). We can offer no explanation for the discrepant data from these studies, however would recall the higher SVR rates registered in the Fischer' study in comparison with the rate observed in the present investigations: 53% vs. 38%.

To solve discrepancies among results from the three studies, we performed a meta-analysis after pooling data from the two previous investigations (10, 11) and the present study. As shown in Fig. 2, in more than 1100 heterozygous rs12979860CT patients, the evaluation of the rs8099917 SNP was of clinical utility, as SVR rates were higher (45%) in those genotyped TT as compared with a figure of 33% registered in those genotyped TG (OR = 1.57, 95% CI 1.15–2.17). However, the meta-analytical results appeared to be driven essentially by the positive data from the Fischer *et al.*' study (10). We noted a few dissimilarities in patients' features between the present investigation and the German one, in particular in the proportion of those with advanced fibrosis or cirrhosis, which may have impacted on the registered SVR rates and in the overall meaning of our results. We agree with Fischer *et al.* (17) that more studies on more patients are warranted on this topic.

In conclusion, in the overall population of HCV-1 patients evaluated in the present investigation, the rs12979860 polymorphism at the IL28B locus appeared as the hit SNP better predicting response following Peg-IFN and RBV treatment: carriers of the homozygous CC genotype attained a 68% rate of SVR. When evaluated individually, additional ss469415590 or rs8099917 genotyping had no added benefit for response prediction. However, the diplotype rs12979860CC/ss469415590TTTT was associated with marginally higher rate of SVR (72%), a gain of seemingly modest clinical utility. Even in the subset of our patients carrying the rs12979860 T allele, there appears no clinical benefit of evaluating variants at the rs8099917 SNP. However, after pooling this latter results with similar data from previous studies (10, 11), we note that in rs12979860 T heterozygous individuals the co-presence of the rs8099917TT SNP was associated with improved response prediction.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline Characteristics of HCV Type 1–Infected patients according to treatment outcome in present and Fischer *et al.*