IL28B polymorphisms influence stage of fibrosis and spontaneous or interferon-induced viral clearance in thalassemia patients with hepatitis C virus infection

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The online version of this article has a Supplementary Appendix.

ABSTRACT

Background
Polymorphisms in the interleukin-28B are important determinants in the spontaneous and drug-induced control of hepatitis C virus infection.

Design and Methods
We assessed the association of *rs8099917* and *rs12979860* polymorphisms with spontaneous viral clearance, severity of liver fibrosis, and response to interferon-monotherapy in 245 thalassemia major patients with hepatitis C virus infection.

Results
Ninety-eight patients (40%) had a spontaneous viral clearance while 147 patients (60%) developed a chronic infection. Spontaneous viral clearance was more frequent among patients with the T/T genotype of *rs8099917* polymorphism (OR 2.130; *P*=0.008) or C/C genotype of *rs12979860* polymorphism (OR 2.425; *P*=0.001). During observation, 131 patients with chronic infection underwent a liver biopsy; age (OR 1.058; *P*=0.01) G/T or G/G genotypes of *rs8099917* polymorphism (OR 3.962; *P*=0.001), and C/T or T/T genotypes of *rs12979860* polymorphism (OR 3.494; *P*=0.005) were associated with severe liver fibrosis, independent of liver iron concentration. Finally, T/T genotype of *rs8099917* polymorphism (OR 3.014; *P*=0.03) or C/C genotype of *rs12979860* polymorphism (OR 3.285; *P*=0.01), age (OR 0.902; *P*=0.001), female gender (OR 3.418; *P*=0.01) and 2 or 3 virus C genotypes (OR 4.700; *P*=0.007) were independently associated with sustained virological response in 114 patients treated with alpha-interferon .

Conclusions
Polymorphisms in the interleukin-28B are associated with the control of hepatitis C virus infection in thalassemia major patients, and understanding allelic patterns has an important role in determining prognosis and therapeutic management.

Key words: thalassemia major, hepatitis C virus, IL28b polymorphisms, spontaneous viral clearance, liver fibrosis, cirrhosis, interferon, sustained virological response.

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Introduction

Viral and host factors influence the development of liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) in patients with hepatitis C virus (HCV)[1] and affect the rate of sustained virological response (SVR) to interferon- (IFN) based therapies.[2]

Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) located in and near the interleukin 28B (IL28B) locus, which encodes for IFN-λ3. They are associated with spontaneous clearance of HCV,[3] and with a higher rate of SVR in patients with chronic hepatitis treated with PEG-IFN and RBV.[4-6] Further evidence that *rs12979860* and *rs8099917* SNPs of the IL28B locus are associated with spontaneous clearance is provided by cohorts of Swiss, Spanish, Australian and German patients with acute HCV infection followed during the course of hepatitis.[7-10] Few data are available on the association between IL28B SNPs and severity of liver fibrosis or presence of cirrhosis, and, in any case, these remain controversial.[11-13]

We studied the relations of host genetic patterns of the IL28B locus (*rs12979860* and *rs8099917* SNPs), viral factors (positivity of serum HCV-RNA and HCV genotype), and liver iron overload, with spontaneous HCV clearance, severity of liver fibrosis, and SVR to interferon in a large, ethnically homogeneous cohort of thalassemia major (TM) patients with HCV infection.

Design and Methods

Study design

Three hundred and one patients with transfusion-dependent TM born in Sicily before 1990 on regular follow up at five thalassemia centers in western Sicily were included in a cohort study and were followed from 1993 to 2010. All patients had undergone regular blood transfusions since the first two years of life to maintain pre-transfusion hemoglobin values over 9 g/dL, and were treated with iron chelating drugs (deferoxamine, deferiprone or deferasirox) to maintain ferritin values below 2.500 ng/mL. In this cohort, 56 patients (18.6%) were anti-HCV negative at baseline and during the entire follow-up period. These were excluded from the study, while 245 patients (81.4%) who were anti-HCV positive at baseline underwent analysis for the IL28B polymorphisms (Figure 1). The study was carried out in accordance with the principles of Good Clinical Practice and was approved by the Hospital's Ethical Committee. All patients gave their consent to have all clinical data recorded in a database and to have blood samples taken for SNP evaluation.

Virological data

Anti-HCV antibodies were tested with enzyme-immunoassay (EIA-2 and, later, EIA-3 by Ortho Diagnostic Systems, Raritan, NJ, USA) at baseline. The test was repeated every year thereafter. Anti-HCV positive patients were tested for HCV RNA with polymerase chain reaction (PCR) at baseline and every year thereafter. HCV genotype was determined by line probe assay (InnoLipa, Innogenetics, Belgium). Anti-HCV positive patients at baseline with undetectable HCV RNA for the entire follow-up period were classified as having had spontaneous HCV clearance. Anti-HCV positive patients with detectable HCV RNA at baseline and during the initial follow up were considered to have a chronic HCV infection.

Liver biopsy

During the observation, a liver biopsy was proposed to all HCV RNA-positive patients to evaluate the stage of liver damage, the liver iron concentration (LIC) and to determine the indication for antiviral therapy. The grading of necro-inflammation and the staging of liver fibrosis were evaluated according to the Scheuer score[14] by a single pathologist. The LIC was measured on fresh tissue cores that weighed more than 4 mg to reduce the variability of the measurement. Measurements were performed by atomic absorption spectrometry using the Spectra 880 (Varian, Australia). Results were expressed as mg of iron per gram of liver, dry weight, and 1.8 mg/g was considered the normal limit.

Antiviral treatment

No patients had been treated with antivirals before baseline evaluation in 1993. Patients with a diagnosis of chronic HCV hepatitis at liver biopsy were treated with interferon monotherapy (recombinant IFN α-2a or α-2b) at a dose of 3 MU/m^2 for 12 months.[15,16] No patients were treated with PEG-IFN plus ribavirin.

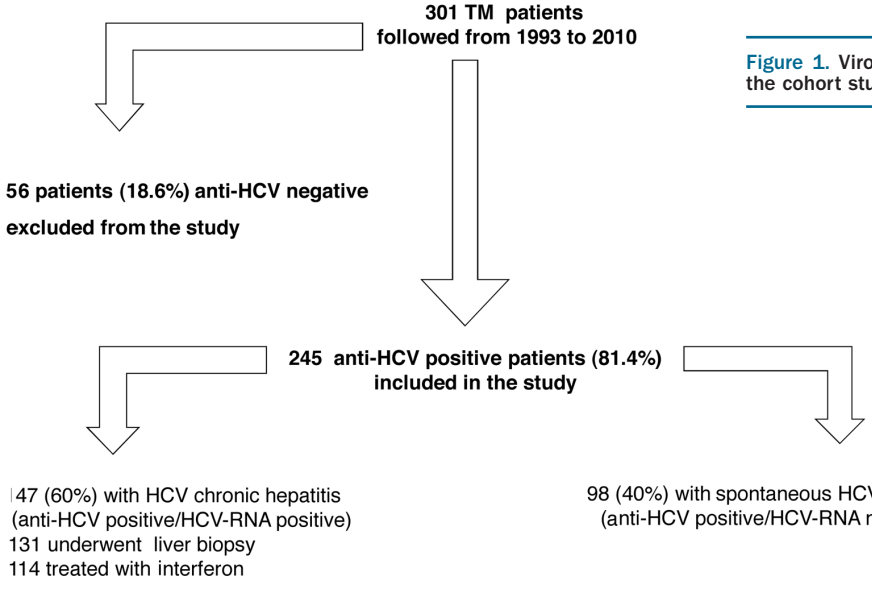

Figure 1. Virological features of 301 patients included in the cohort study.

SVR was considered achieved if HCV RNA was undetectable at 24 weeks after the end of treatment. A negative result was confirmed during follow up.

IL28B genotyping

DNA was purified from whole-blood patient samples using the QIAmp DNA Blood Mini Kit (Qiagen, Mainz, Germany). DNA samples were quantified using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen, Paisley, UK) and normalized to 4 ng/μL with a Tecan Freedom EVO Robot (Tecan, Switzerland). Then 2.5 uL aliquots of each DNA sample were transferred to 384-well plates, dried and stored at 20°C. Genotyping for *rs12979860* and *rs8099917* was carried out using the TaqMan SNP genotyping allelic discrimination method (Applied Biosystems, Foster City, CA, USA). A commercial genotyping assay was available for the *rs8099917* (cat. C_11710096_10) while a custom assay was created by AB for *rs12979860*. Twelve additional SNPs located within the IL28B locus (*rs12980275*, *rs12972991*, *rs8109886*, *rs4803223*, *rs12980602*, *rs8105790*, *rs8103142*, *rs28416813*, *rs4803219*, *rs7248668*, *rs10853727*, *rs10853728*) were genotyped using the FRET-based KASPar SNP genotyping assay method (KBioscience, Herts, UK) on an Applied Biosystems Thermocycler (ABI Prism 9700, Foster City, CA, USA).The *rs12979860* and *rs8099917* were also genotyped by the KASPar method as a control. Custom genotyping assays were designed by submitting the SNP sequences to KBioscience. The genotyping call was performed with 7900 SDS software (ABI Prism 7700, Foster City, CA, USA). Results were confirmed by direct sequencing of PCR fragments amplified in the IL28 gene from random selected samples (8 samples per genotype).

Statistics

Data were analyzed using SPSS 13.0 for Windows software (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean ±standard deviation (SD) or as median with interquartile range (IQR), and categorical variables as absolute and relative frequencies. The differences between continuous data were analyzed by t-test, and corrected χ^2 analysis was used for dichotomous or categorical variables. We analyzed three different classes of events observed during the clinical course of HCV infection: first, the spontaneous clearance of HCV infection; second, the frequency of severe liver fibrosis in patients who underwent liver biopsy; and, finally, the rate of SVR to IFN therapy in treated patients. We defined liver fibrosis as mild-moderate if the Scheuer score was between F0 and F2, and severe if the Scheuer score was F3 or F4. Analysis of the IL28B genotype effect according to a recessive model was pre-planned, based on previous results.[2-10] Multiple logistic regression models were used to assess the relationships among genetic (*rs12979860* and *rs8099917* SNPs), demographics (age and gender), and histological (classes of liver fibrosis and the liver iron concentration) features and spontaneous clearance of HCV, evidence of severe liver fibrosis, and response to antiviral therapy. Variables with a threshold value of P=0.1 at univariate analysis were included in the model, and variables with a threshold value of $P \leq 0.05$ were considered significant in the final model. The results were expressed as odds ratio (OR) and their 95% confidence interval (CI).

Results

Patients' characteristics

Genotyping at the polymorphic sites *rs12979860* and *rs8099917* on chromosome 19 was available in 245 anti-HCV-positive patients. Ninety-eight (40%) had a spontaneous clearance of virus, and 147 (60%) had chronic HCV infection (Figure 1). At the start of the observation period, there were no differences between the two groups in terms of mean age (18.6±8.0 *vs.* 18.7±6.5) and the percentage of male gender (50% *vs.* 51%). Anti-HCV-positive/HCV-RNA-positive patients had significantly higher median ALT values than anti-HCV positive/HCV-RNA negative patients (55 IU/mL *vs.* 22 IU/mL; P<0.001), while there were no differences in the median values of ferritin between the two groups (1,427 ng/mL *vs.* 1,460 ng/mL; P=0.67). Among the 147 patients with chronic HCV infection, 116 (78.9%) were infected with genotype 1, 23 (15.6%) with genotype 2, 6 (4.1%) with genotype 3, and 2 (1.4%) with genotype 4. In the entire cohort, the frequencies of the C/C, C/T and T/T genotypes of *rs12979860* were 46.1%, 42.5% and 10.6%, respectively, reflecting a C allele frequency of 67.3%. The frequencies of the T/T, G/T and G/G genotypes of *rs8099917* were 64.5%, 31.4% and 4.1%, respectively, reflecting a T allele frequency of 80.2%. The calculated distribution of the alleles according to the Hardy-Weinberg equilibrium was 67% for the C allele, 33% for T allele of *rs12979860*, 79% for the T allele, and 21% for the G allele of *rs8099917*.

IL28B genotypes and spontaneous viral clearance

As shown in Figure 2, patients carrying the C/C genotype of rs12979860 had more frequent spontaneous viral clearance than patients who carried the C/T or T/T genotypes (51.3% *vs.* 31.3%, OR 2.425; CI 95% 1.437- 4.093; P=0.001). Similarly, patients who carried the T/T genotype of rs8099917 had more frequent spontaneous HCV clearance than those with G/T or G/G genotypes (46.2% *vs.* 28.7%, OR 2.130; CI 95% 1.217-3.728; P=0.008). During follow up, no spontaneous clearance of HCV RNA was observed at any time during the observation period among the 147 anti-HCV-positive/HCV RNA-positive patients. None of the 98 anti-HCV-positive patients who

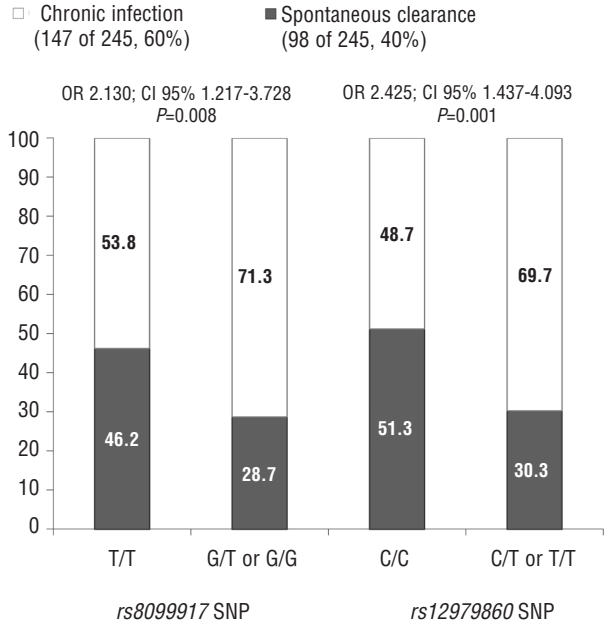

Figure 2. Likelihood of spontaneous HCV clearance in 245 anti-HCV positive thalassemia major patients according to IL28B status.

were HCV RNA-negative at baseline became HCV RNA-positive during follow up. Sixteen (16.3%) of the 98 anti-HCV-positive/HCV RNA negative patients lost anti-HCV antibodies during follow up, but no association between this serological event and *rs12979860* or *rs8099917* genotypes was observed.

IL28B polymorphisms and liver fibrosis

During observation, 131 (89.1%) of the 147 patients with chronic HCV infection underwent a liver biopsy, while 16 patients refused the diagnostic procedure. Their mean age at the time of liver biopsy was 22 ±9 years and none of them had undergone interferon therapy. One hundred and four patients (79.4%) were infected with genotype 1 or 4, and 27 patients (21.6%) with genotype 2 or 3. By the Scheuer score, 3 patients (2.3%) had no fibrosis, 49 (37.4%) had mild fibrosis (F1), 32 (24.4%) moderate fibrosis (F2), 17 (13%) severe fibrosis (F3), and 30 (22.9%) had cirrhosis (F4). The median LIC was 2.5 (IQR 1.4-4.7) mg/gr of dry weight liver tissue.

The frequency of the *rs12979860* C/C genotype was 47.6% among the 84 patients with F0-F2 and 21.3% among the 47 patients with F3-F4 (P=0.005). Similarly, the frequency of the *rs8099917* T/T genotype was 70.4% and 38.3% among the F0-F2 and F3-F4 patients, respectively (P=0.001) (Figure 3). On univariate analysis, the other factors associated with the presence of F3-F4 fibrosis were age (P=0.015) and infection with HCV genotype 1b or 4 (P=0.041), while LIC (P=0.594) and gender (P=0.726) were not correlated with the stage of liver fibrosis. On logistical regression analysis, only age (OR 1.061; 95% CI 1.014-1.109; P=0.010) and *rs12979860* C/T or T/T genotypes (OR 3.494; 95% CI 1.472-8.293; P=0.005) were significantly associated with F3-F4 liver fibrosis. When the *rs8099917* SNP was included in the multivariate analysis, the T/G or G/G genotypes (OR 3.962; 95% CI 1.798-8.730; P=0.001) and age (OR 1.058; 95% CI 1.012-1.106; P=0.013) remained significantly associated with F3-F4 liver fibrosis (Table 1).

IL28B polymorphisms and response to IFN

Among the 147 chronically infected patients, 114 (77.6%) were treated with IFN monotherapy and were evaluable for virological response. SVR was achieved in 46 of 114 (40.3%) patients. SVR was achieved in 30 of the 89 patients with HCV genotype 1 (33.7%), and in 16 of the 25 patients with genotype 2 or 3 (64%). Factors associated with SVR included female gender (P=0.018), age (P<0.001), HCV genotype 2 or 3 (P=0.006), and F3-F4 liver fibrosis (P=0.001), but not LIC (P=0.322). The rate of the

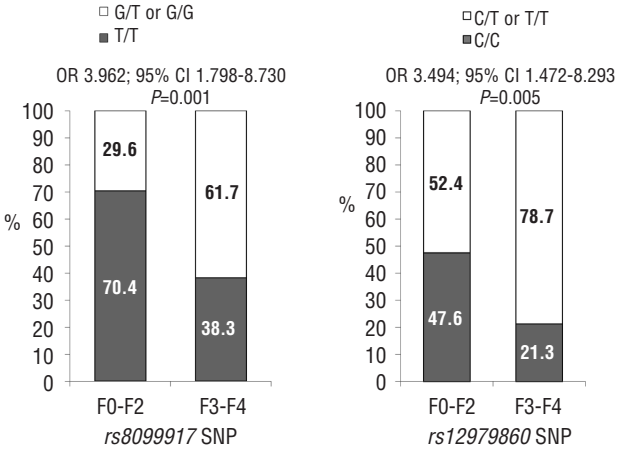

Figure 3. IL28 status and stage of fibrosis in 131 thalassemia major patients with chronic HCV infection.

Table 1. Univariate and multivariate analysis of factors correlated with severe fibrosis at liver biopsy performed during the observation.

	Liver fibrosis in 131 patients with liver biopsy F0-F2	F3-F4	Univariate analysis P	Multivariate analysis, including *rs18099917* SNP OR (95% CI)	P	Multivariate analysis, including *rs1279860* SNP OR (95% CI)	P
N. patients (%)		84 (64.2)	47 (35.8)				
Age (years, mean, SD)	20.9±8.0	24.9±9.8	**0.015**	1.058 (1.012-1.106)	**0.013**	1.061 (1.014-1.109)	**0.010**
Gender (%):							
Male	42 (50)	25 (53.2)					
Female	42 (50)	22 (46.8)	0.726				
LIC (median, IQR)	2.2 (1.3-4.3)	2.8 (1.6-4.6)	0.594				
HCV genotypes (%) :							
1 or 4	62 (73.8)	42 (89.4)					
2 or 3	22 (26.2)	5 (10.6)	**0.041**	2.549 (0.853-7.619)	0.094	1.975 (0.675-9.345)	0.109
rs18099917 SNP alleles (%)							
T/T	59 (70.2)	18 (38.3)					
G/T or G/G	25 (29.8)	29 (61.7)	**< 0.001**	3.962 (1.798-8.730)	**0.001**		
rs1279860 SNP alleles (%)							
C/C							
T/C or T/T	40 (47.6)	10 (21.3)					
	44 (52.4)	37 (78.7)	**0.004**			3.494 (1.472-8.293)	**0.005**

Table 2. Univariate and multivariate analysis of factors correlated with SVR in patients treated with IFN monotherapy.

	114 patients treated with interferon		Univariate analysis	Multivariate analysis, including *rs18099917* SNP		Multivariate analysis, including *rs1279860* SNP	
	No SVR	SVR	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
N. of patients (%)	68 (59.7)	46 (40.3)					
Age (years, mean, SD)	23.8±9.4	17.8±7.0	< 0.001	0.902 (0.847-0.959)	0.001	0.899 (0.844-0.957)	0.001
Gender (%):							
Male	42 (61.2)	18 (40.4)	0.018	3.418 (1.329-8.795)	0.011	3.852 (1.472-10.082)	0.006
Female	26 (38.8)	28 (59.6)					
Fibrosis stage (*)							
F0-F2	34 (53.1)	37 (80.4)	0.001	0.479	0.188	0.453 (0.153-1.342)	0.153
F3-F4	31 (46.9)	8 (19.6)		(0.160-1.431)			
LIC (median, IQR)	2.4 (1.4-3.9)	3.4 (1.7-5.4)	0.322				
HCV genotypes (%)							
1 or 4	59 (86.7)	30 (65.3)	0.006				0.011
2 or 3	9 (13.3)	16 (34.7)		4.700 (1.529-14.448)	0.007	4.279 (1.404-13.036)	
rs18099917 SNP alleles (%)							
T/T	33 (48.5)	35 (76.1)	0.003	3.014 (1.096-8.286)	0.033		
G/T or G/G	35 (51.5)	11 (23.9)					
rs1279860 SNP alleles (%)							
C/C	18 (26.5)	25 (54.3)	0.003			3.285 (1.234-8.743)	0.017
T/C or T/T	50 (73.5)	21 (45.7)					

rs12979860 C/C genotype was 54.3% in patients with SVR, and 26.5% in those without SVR (*P*=0.003). Similarly, the rate of the *rs8099917* T/T genotype was 76.1% in patients with SVR, and 48.5% in those with treatment failure (*P*=0.003). On logistical regression analysis, age (OR 0.902; 95% CI 0.847-0.959; *P*=0.001), female gender (OR 3.418; 95% CI 1.329-8.795; *P*=0.011), HCV genotype 2 or 3 (OR 4.700; 95% IC 1.529-14.448; *P*=0.007), and *rs12979860* C/C genotype (OR 3.285; 95% IC 1.234-8.743; *P*=0.017) were significantly associated with SVR. Similar results were obtained when the *rs8099917* T/T genotype was included in the logistical regression model (OR 3.014; 95% CI 1.096-8.286; *P*=0.033) (Table 2).

Next, we restricted the analysis to HCV genotype 1 patients. Seventeen of 31 (54.8%) HCV genotype 1 patients carrying the *rs12979860* C/C genotype achieved an SVR *versus* 13 of 58 patients (22.4%) with a C/T or T/T genotype (*P*=0.004). Similarly, SVR was achieved in 25 of 52 patients (48.1%) with the *rs8099917* T/T genotype *versus* 5 of 37 patients (13.5%) carrying the G/T or G/G genotype (*P*=0.005). The analysis of HCV genotype 2 or 3 patients showed no significant association with the *rs12979860* or *rs8099917* genotype (Figure 4).

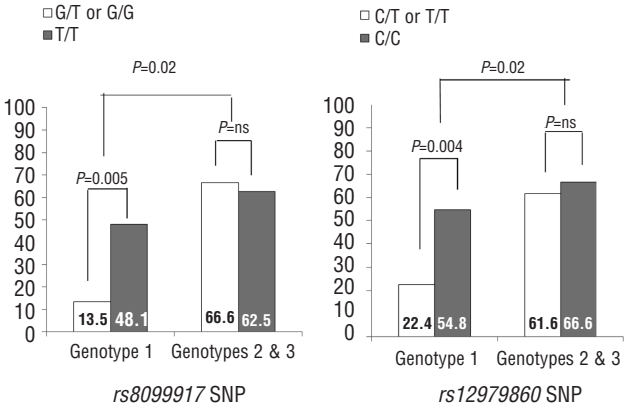

Figure 4. IL28B status and SVR in 114 thalassemia major patients receiving IFN monotherapy.

Analysis of additional polymorphisms at the IL28B locus

We analyzed 12 additional SNPs located within the IL28B locus that have also been shown to link with patient response to pegylated interferon-α and ribavirin therapy in GWAS studies.[3,5-7] A detailed description of their genomic localization and their association with spontaneous and treatment-induced HCV clearance is provided in the *Online Supplementary Appendix*.

Online Supplementary Table S1 shows the association among the prevalence of the major genotypes for these SNPs, as well as *rs1279860* and *rs18099917*, and spontaneous viral clearance, the presence of a mild liver fibrosis, and an SVR to IFN-based therapy. Major genotypes of 10 SNPs (T/T of *rs18099917*, C/C of *rs1279860*, G/G of *rs7248668*, A/A of *rs11881222*, A/A of *rs8113007*, A/A of *rs4803223*, T/T of *rs12980602*, C/C of *rs28416813*, G/G of *rs10853728* and T/T of *rs8105790*) showed a significant association with all the assessed disease events. The strongest association with spontaneous viral clearance in our TM patients was observed for major genotypes of five SNPs (C/C of *rs1279860*, G/G of *rs7248668*, A/A of *rs11881222*, A/A of *rs4803223*, C/C of *rs28416813*); the strongest association with mild fibrosis was observed for major genotypes of five SNPs (T/T of *rs18099917*, C/C of *rs1279860*, G/G of *rs7248668*, *rs12972991* and *rs8105790*); and the strongest association with SRV to IFN was observed for major genotypes of five SNPs (T/T of

rs18099917, C/C of *rs1279860*, G/G of *rs7248668*, A/A of *rs11881222*, and A/A of *rs12980275*). However, only the C/C genotype of *rs1279860* SNP scored among the top five SNPs in all three clinical events studied.

Discussion

To evaluate the influence of single nucleotide polymorphisms of interleukin 28B locus on the natural course of HCV infection we need to analyze large cohorts of patients with a high prevalence of HCV infection who have an identifiable time of infection, have long-term follow up, and have recorded data of virus- and disease-related events.

All TM patients in our cohort were born in Sicily before the implementation of blood-donor screening for hepatitis C and had, therefore, been exposed to a high risk of HCV infection.[17] In a 1992 epidemiological study by Prati *et al.*,[18] 85% of 1,481 TM patients of the Italian Cooleycare program were anti-HCV-positive, and the risk of HCV infection was associated with the total number of blood transfusions administered. Patients in our cohort were infected in the first years of life, and the overall prevalence of anti-HCV antibodies was over 80%. Testing for serum HCV RNA indicated that 40% of HCV infected TM patients achieved a spontaneous clearance. The same high rate of spontaneous viral clearance has been reported in other studies on infected children. Vogts *et al.* reported that 45% of 67 anti-HCV-positive children who underwent cardiac surgery and received blood transfusions in the first years of life (mean age at first operation 2.8 years) were persistently HCV RNA-negative after a mean interval of 19.8 years.[19] Locasciulli *et al.* reported that 28% of children parentally infected with HCV during therapy for leukemia in infancy were HCV RNA-negative 17 years after the last blood transfusion.[20] Finally, approximately 20% of a large cohort of 266 vertically infected children cleared HCV in the first 10-15 years of life.[21]

Virological data recorded during follow up confirmed that spontaneous viral clearance must have been achieved soon after primary infection since no late spontaneous HCV clearance was seen during the observation period. The frequency of the C allele of the *rs12979860* SNP was 67.8% while the frequency of the T allele of *rs8099917* SNP was 80%, as observed in other cohorts of European ancestry.[3,7,10] Patients carrying the T/T genotype of rs8099917 SNP more frequently obtained spontaneous viral clearance than patients who carried the G/T or G/G genotypes. Patients who carried the C/C genotype of the *rs12979860* SNP more frequently obtained spontaneous viral clearance than patients who carried the C/T or T/T genotypes. When we analyzed the other 12 SNPs located within the IL28B locus, we observed a significant association between major alleles of the eight SNPs and the spontaneous HCV clearance. These data confirm that spontaneous clearance of HCV is common in children infected in the first years of life,[19-21] and that the genetic mechanisms related to SNPs of IL28B factors actively participate in this event. Finally, it is very difficult to establish an association between the spontaneous viral clearance of anti-HCV antibodies during follow up and IL28B polymorphisms because of the small number of patients.

The second aim of our study was to evaluate the association between IL28B polymorphisms and the stage of liver fibrosis. In TM patients, hepatic iron overload and HCV infection are the major risk factors for progression of fibrosis. Angelucci *et al.*[22] evaluated the progression of liver fibrosis in a large cohort of TM patients undergoing periodic liver biopsy after bone marrow transplantation. They reported that anti-HCV negative patients with an LIC of less than 16 mg/g dry weight liver tissue showed no progression of liver fibrosis, but patients with HCV infection showed significant liver fibrosis progression regardless of LIC values. The median LIC of patients in our cohort undergoing liver biopsy was less than 3 mg/g of dry weight liver tissue, reflecting a very high compliance with iron chelation therapy. A previous study by our group[23] confirmed that TM patients with a good adherence to chelation therapy, and without HCV infection, did not develop liver fibrosis and, conversely, HCV RNA-positive patients had more severe necroinflammation and more frequent severe fibrosis or cirrhosis. We observed that among 131 TM patients with chronic HCV hepatitis who underwent liver biopsy, age and the presence of C/T or T/T genotype of the *rs12979860* SNP or of T/G or G/G genotype of the *rs8099917* SNP were the factors associated with F3-F4 fibrosis.

The correlation between ILB28 polymorphisms and progression of liver fibrosis is still controversial, and its possible mechanisms are unknown. Romero-Gomez *et al.*[24] reviewed the evidence that polymorphisms of specific genes that encode for inflammatory cytokines may exert a protective or accelerating effect on progression of liver fibrosis. Published data suggest that IL28B genotypes contribute both to the grade of necroinflammation and to the stage of fibrosis. Sarrazin *et al.*[25] observed a trend towards higher stages of fibrosis in European HCV genotype 1 patients who carried the *rs8099917* T/T genotypes, and Abe *et al.*[26] reported that *rs8099917* T/T homozygosity was associated with necroinflammation and progression of chronic hepatitis in a cohort of Asian patients. Conversely, an Italian group reported that in a large cohort (n=629) of patients with HCV chronic liver disease, the presence of the T/T genotype of IL28B rs12979860 was an independent predictor of severe liver fibrosis,[11] and patients with cirrhosis had a higher frequency of T/T or C/T genotype of *rs12979860*. Carriage of the T allele was also found to be an independent predictor of the presence of HCC.[12] Recently, Marabita *et al.*[13] reported interesting data on the role of rs8099917 and rs12979860 polymorphisms, and host and environmental factors on fibrosis progression in a cohort of 247 consecutive patients with chronic HCV who had an accurate estimate of the date of infection. Their paper concluded that age at infection, male gender, HCV genotype 3 and steatosis were associated with liver disease progression, but that IL28B polymorphisms were not associated with the development of severe liver fibrosis.

Unlike other studies, patients in our cohort were infected by HCV in the first years of life; the majority of them were infected with genotype 1 and they had no other risk factors, such as steatosis, obesity or alcohol intake, for the progression of fibrosis. A special risk factor for the development of liver fibrosis in TM was the liver iron overload, but all patients received adequate iron chelation, as proven by the low values of LIC at the time of liver biopsy.

Our data suggest a protective role of homozygous C/C alleles of *rs12979860* and homozygous T/T alleles of *rs8099917*. This observation is confirmed by the analysis of another 12 SNPs of the IL28B locus, suggesting that the association with the stage of liver fibrosis was primarily

driven by one or other of these SNPs.

We suggest that the IL28B polymorphism can influence the spontaneous clearance of HCV, the progression of liver fibrosis, and the response to IFN, especially in genotype 1b patients, through various mechanisms that regulate the replication of the C virus and the immunological response of the host. This hypothesis, and its underlying mechanisms, need to be confirmed by further studies of large cohorts of patients prospectively observed for many years, and in whom there are no co-factors of liver damage.

Finally, we have confirmed the role of IL28B polymorphisms in conditioning the response to IFN. Our study is limited by the fact that study patients were given IFN monotherapy. In the last few years, TM patients with chronic hepatitis C have been treated with IFN alone because of the risk of worsening anemia and transfusion with ribavirin treatment. A systematic review with meta-analysis on data from 429 TM patients with chronic HCV hepatitis treated with conventional or PEG-IFN monotherapy, or combination therapy with ribavirin, reported a pooled SVR of 44.7%, and concluded that genotype 1 TM patients significantly benefit from the addition of ribavirin to their therapeutic regimen.[27] There are no published data on the role of IL28B polymorphisms in genotype 1 TM patients treated with PEG-IFN and ribavirin but, as reported in large cohort studies of patients with chronic hepatitis and no hemoglobinopathies,[5-7] 'favorable' genotypes of *rs12979860* or *rs8099917* SNPs should be associated with a best response to standard of care also in this subset of patients, and the evaluation of their predictive values may become a component of future treatment decision-making algorithms.

The SVR in genotype 1 patients treated with IFN monotherapy was 37-39% *versus* an SVR higher than 70% in patients treated with combination therapy.[27] In our cohort, 114 patients were treated with IFN monotherapy and were assessable for virological response. The overall SVR was 40.3% for treated patients, with a significant difference between genotype 1 (33.7%) and genotype 2 or 3 patients (64%). On logistical regression analysis, age, female gender, viral genotypes 2 or 3, and a T/T genotype of *rs8099917* SNP or C/C genotype *rs12979860* SNP were significantly associated with SVR. But the major genotypes (T/T of *rs8099917* and C/C of *rs12979860*) of two analyzed SNPs were significantly associated with SVR only in genotype 1 patients. The data on other analyzed SNPs showed that at least three different SNPs had a similar performance in association with SVR in genotype 1 patients. As reported in a previous analysis,[16] and in a meta-analysis by Alavian *et al.*,[27] the rate of SVR in genotype 2 or 3 patients was higher than 60%, and there was no significant association between the SNPs analyzed and SVR in this group of patients. Despite the fact that the lack of correlation with IL28B SNPs may be related to very small numbers of genotype 2 or 3 patients in our cohort, this observation confirms data from other studies[25,28-30] indicating that the prognostic value of SNPs for SVR may be limited to patients with difficult-to-treat genotypes even in this subset of patients.

In conclusion, our study shows that 'favorable' genotypes of IL28B SNPs are associated with control of HCV infection in TM patients in terms of spontaneous clearance of HCV infection, progression of liver fibrosis, and response to IFN. As a result, an understanding of the IL28B allelic pattern has an important role in determining prognosis and therapeutic indications in this group of patients.

Authorship and Disclosures

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