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Role of Interleukin 28B rs12979860 C/T Polymorphism on the Histological Outcome of Chronic Hepatitis C: Relationship with Gender and Viral Genotype

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Abstract
Background This study aimed to determine whether the single-nucleotide polymorphism (rs12979860 C/T) of the interleukin 28B (IL-28B) gene, which is associated with hepatitis C virus (HCV) clearance, is also associated with fibrosis in chronic HCV infection.
Methods An RFLP-PCR technique was used to genotype 629 HCV-positive patients (200 with cirrhosis) and 428 healthy control subjects.
Results The genotype frequencies in the controls and chronic hepatitis C patients were as follows: C/C 47.0% vs. 32.6%, C/T 41.8% vs. 52.8% and T/T 11.2% vs. 14.6% ($p<0.0001$). The C allele frequency was higher in HCV-2- (0.635) and 3- (0.692) infected patients in comparison to those infected with HCV-1 (0.550) or 4–5 (0.600) ($p<0.001$). Infected T/T homozygotes had a mean staging score higher than other patients (3.50 vs. 3.04, $p<0.05$).
Conclusions IL-28B rs12979860 C/T polymorphism is associated with a greater likelihood of HCV persistence, particularly in HCV genotypes 1 and 4. The T allele affects the severity of liver fibrosis.

Keywords Interleukin 28B polymorphism · hepatitis C virus · viral genotypes · liver fibrosis · gender

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Introduction

The natural history of hepatitis C virus (HCV) infection varies greatly. Spontaneous HCV clearance occurs in a minority of cases, with the majority of patients progressing to chronic disease [1]. During chronic hepatitis C, progressive fibrosis deposition occurs; however, this deposition ends in cirrhosis in only 20–30% of chronic HCV carriers. In fact, most HCV-infected patients never progress beyond a mild disease stage [2].

To date, the reasons for the high variability in the outcomes of chronic HCV infection are not fully explained. However, it is interesting to note that several of the factors thought to be responsible for the progression of fibrosis [2–4] are also related to failure to respond to antiviral treatment, for example, male gender [5], ethnicity [6], insulin resistance [7] and advanced age [8]. Fibrosis itself is a strong predictor of non-responsiveness to antivirals [9]. Therefore, antiviral treatment is least effective in those for whom it is most needed.

Recently, the rs12979860 C/T polymorphism, located 3-kb upstream of the interleukin 28B (IL-28B) gene that encodes the type III interferon λ3 (IFN-λ3), was shown to

be strongly associated with more than a twofold difference in response to HCV drug treatment both in patients of European ancestry and in African–Americans [10]. Moreover, this IL-28B rs12979860 C/T polymorphism appeared to predict the rate of spontaneous clearance of HCV, which could be observed in the 53.0% of patients with the C/C genotype but only 23.4% of patients with the T/T genotype [11]. Furthermore, the IL-28B rs12979860 C/T polymorphism has been shown to influence the severity of recurrent hepatitis C infections following liver transplantation [12]. In immune-competent patients, the IL-28B rs12979860 T allele is more prevalent in patients with viral cirrhosis due to HCV infection in comparison to other aetiologies and in patients with mild, chronic hepatitis C [13]. To date, it has not been shown whether the IL-28B rs12979860 C/T may affect the natural history of chronic hepatitis C. HCV is not cytopathic itself; rather, it is the inflammatory process evoked by the inability to clear HCV that damages the liver parenchyma and results in fibrotic repair. Allelic variants of the IL-28B polymorphism may be linked to the efficiency of the inflammatory process during HCV infection and to the mechanisms that HCV adopts to escape elimination by innate and adaptive immunity [14]. These mechanisms may favour viral clearance during the acute phases of infection and modulate the infection course when chronic infection is established.

The aims of the present study were as follows: (a) to assess the allelic and genotypic frequencies of the IL-28B rs12979860 C/T polymorphism in patients with chronic HCV infection at various stages of the disease in comparison to healthy control subjects; (b) to verify whether this polymorphism is an independent predictor of the degree of fibrosis in chronic hepatitis C; and (c) to investigate the interaction between the IL-28B rs12979860 C/T polymorphism and other factors known to influence the evolution of chronic hepatitis C.

Materials and Methods

Patients This retrospective study included 629 Italian patients of Caucasian ethnicity affected by chronic hepatitis C. The majority of these patients (N=523, 83.1%) were referred to our regional liver and transplant unit for diagnosis and treatment of chronic hepatitis C, while the remainder (N=106, 16.9%) had undergone transplantation for HCV-related end-stage liver disease. When patients were categorised according to the severity of their HCV-related liver disease, 429 were classified as being affected by chronic hepatitis naive to antiviral treatment, 301 had a mild disease (Ishak staging score ≤2) and 128 had an Ishak staging score of 3–4. The diagnosis was confirmed in all cases by histological evaluation of a liver biopsy specimen. Two hundred patients had liver cirrhosis, and 51 of these cases were complicated by HCC. In 106 patients in this group who underwent liver transplantation, the diagnosis of liver cirrhosis and of HCC (N=35) were confirmed by histological evaluation of the explanted liver. In the remaining 94 non-transplanted patients, liver cirrhosis was diagnosed clinically on the basis of the presence of signs of portal hypertension, pertinent imaging features and laboratory findings such as hypoalbuminemia, INR increase and low platelet count. In 52 cases (55.3%), the diagnosis was indicated by liver histology performed by percutaneous liver biopsy. The diagnosis of HCC (N=16) was largely based on the results of dynamic imaging studies in accordance with the AASLD practice guidelines [15]. A liver biopsy was performed in two cases in which imaging studies were not conclusive and confirmed the presence of HCC. The main demographic and clinical characteristics of the patients are reported in Table I. Four hundred twenty-eight healthy Italian blood donors of Caucasian ethnicity served as controls. They were 314 males (73.4%) and 114 females (26.6%); the median age was 49 years with a range of 18–77 years. Control subjects did not have any clinical or laboratory evidence of liver disease or other major pathological conditions such as diabetes mellitus. Informed consent to participate in the study was obtained from each subject in accordance with the Declaration of Helsinki and following the local ethical committee indications. All study participants approved the storage of their frozen DNA specimens, for research purposes, in our laboratory.

Histology Evaluation Liver biopsy specimens were formalin-fixed, paraffin-embedded and stained with haematoxylin–eosin; they included a median number of 7 portal triads (range 4–12). Histological liver staging was assessed using the Ishak staging score [16] corresponding to the following: 0=no fibrosis, 1=fibrous expansion of some portal areas (Fig. 1a), 2=fibrous expansion of most portal areas, 3=fibrous expansion of most portal areas with occasional portal to portal bridging, 4=fibrous expansion of portal areas with marked bridging, 5=marked bridging with occasional nodules (incomplete cirrhosis–Fig. 1b) and 6=probably or definite cirrhosis.

Molecular Biology

Genotyping for the IL-28B rs12979860 C/T polymorphism was performed by a PCR-based restriction fragment length polymorphism assay as previously described [13]. Genomic DNA was extracted from whole blood samples using the QIAamp DNA Blood Mini Kit (Qiagen, Milan, Italy)

Table I Main demographic and clinical characteristics of the study population (N=629)

Male gender, *N*	357 (56.8)
Age (years)	53 (17–86)
Body mass index (kg/m^2)	24.1 (15.1–42.6)
Presence of diabetes mellitus, *N*	75 (11.9)
History of alcohol intake, *N*	
≤20 g/day	67 (10.7)
>20 g/day	22 (3.5)
HCV genotype, *N*	
1	377 (59.9)
2	144 (22.9)
3	78 (12.4)
4–5	30 (4.8)
Patient presentation, *N*	
HCV-related chronic liver disease	523 (83.1)
Follow-up after liver transplantation for HCV-related liver disease	106 (16.9)
Status of liver disease, *N*	
Mild (Ishak staging score 0–2)	301 (47.9)
Moderate (Ishak staging score 3–4)	128 (20.3)
Severe (Ishak staging score 5–6)	200 (31.8)
Presence of hepatocellular carcinoma, *N*	51 (8.1)
Child-Pugh score (only in patients with cirrhosis)	7 (5–13)

Continuous variables are reported as medians (range) and categorical variables as frequencies (%)

HCV hepatitis C virus

according to the manufacturer's instruction. A 242-bp product was obtained using the forward primer 5′-GCTTATCGCATACGGCTAGG-3′ and the reverse primer 5′-AGGCTCAGGGTCAATCACAG-3′, designed with the aid of NCBI Primer-Blast Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). PCR amplification was performed in a total volume of 10 μL containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, Tween-20 0.01%, 0.2 mM deoxyribonucleotides, 24 pmol of each primer, 2.0 mM $MgCl_2$, and 0.5 U Hot-Start Taq DNA polymerase (RighTaq, Euroclone, Milan, Italy). Samples containing 10 ng of genomic DNA were subjected to 40 cycles of denaturation (at 95°C for 30 s), annealing (at 62°C for 30 s) and elongation (at 72°C for 30 s) using a Techne TC-412 thermal cycler. In a total volume of 20 μL, 10 μL of the amplicons was digested with 1 U of the BstU-I restriction endonuclease (New England Biolabs, Hitchin, UK) at 60°C overnight. The digested fragments were 135+82+25 bp for the C allele and 160+82 bp for the T allele variant. The fragments were resolved by electrophoresis in a 3.5% agarose gel after staining with ethidium bromide. In 18 patients, the genomic region encompassing the IL-28B rs12979860 C/T polymorphism was sequenced, with results confirming those obtained by the RFLP assay.

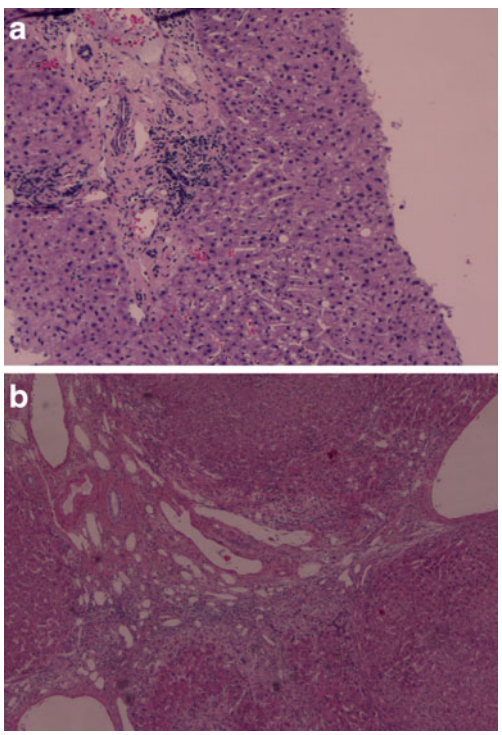

Fig. 1 **a** Ishak staging score 1: presence of portal fibrosis without septa and lymphoid infiltrate within the portal tracts (H&E, ×100). **b** Ishak staging score 5: presence of marked fibrosis with portal to central bridging and moderate lymphoid infiltration. Nodules of hepatic regeneration are present (H&E, ×100)

Statistical Analysis Statistical analysis of data was performed using the BMDP Dynamic Statistical Software Package 7.0 (Statistical Solutions, Cork, Ireland). Continuous variables are presented as median (range) or mean± standard error, while categorical variables are expressed as frequencies (%). The chi-square "Goodness of Fit" G test was employed to verify whether the proportions with the

Table II Allele and genotype frequencies of IL-28B rs12979860 C/T polymorphism in control subjects and in patients with chronic HCV infection

IL-28	Control subjects (*N*=428)	Chronic HCV infection (*N*=629)	O.R.	95% C.I.	*p* value
rs12979860	C=0.679	C=0.590	1	Reference	
	T=0.321	T=0.410	1.469	1.225–1.763	<0.001
rs12979860	C/C=201 (47.0%)	C/C=205 (32.6%)	1	Reference	
	C/T=179 (41.8%)	C/T=332 (52.8%)	1.819	1.394–2.373	<0.001
	T/T=48 (11.2%)	T/T=92 (14.6%)	1.879	1.262–2.798	0.002

The odds ratios were constructed considering the wild type as the reference for each polymorphism. The odds ratios for the minor allele dominant and recessive models are also presented here. The statistical analysis was performed using the Pearson's chi-square test

IL-28B interleukin 28B, *O.R.* odds ratio, *C.I.* confidence interval, *N.S.* not significant, *HCV* hepatitis C virus

T/T+C/T vs. C/C—O.R.=1.831, 95% C.I.=1.423–2.357, $p<0.001$

T/T vs. C/T+C/C—O.R.=1.356, 95% C.I.=0.935–1.966, p=N.S.

polymorphism are distributed in both controls and patients in accordance with the Hardy–Weinberg equation. The existence of differences in allelic and genotypic frequencies between groups was assessed using the Pearson's chi-square test (chi-square test for linear trend when appropriate) and calculating the odds ratio with 95% confidence intervals. Stepwise logistic regression analysis with a forward approach was used to verify whether the IL-28B rs12979860 C/T polymorphism was associated with chronic HCV infection independently of other confounding covariates. Furthermore, analysis was employed to assess whether this polymorphism or its interaction with the patient's gender could be considered as independent predictors of liver fibrosis in patients with chronic hepatitis C. Covariance analysis was performed to assess whether the mean values of the dependent variables significantly differed after adjustment for the results of the independent variables.

Results

IL-28B rs12979860 C/T Polymorphism in Controls and Patients with Chronic Hepatitis C The IL-28B rs12979860 C/T polymorphism genotype frequencies in controls and patients with chronic hepatitis C differed with a significant linear trend: C/C 47.0% vs. 32.6%, C/T 41.8% vs. 52.8% and T/T 11.2% vs. 14.6% ($p<0.0001$). In control subjects, the genotype frequencies did not depart from those expected on the basis of Hardy–Weinberg equilibrium ($p>0.2$); however, in patients with chronic HCV infection, the observed and expected frequencies were significantly different ($p<0.05$). After comparing patients and controls, it was evident that the presence of the T allele was strongly associated with chronic HCV infection (Table II). Using a stepwise logistic regression analysis with a forward approach, the IL-28 polymorphism was observed to be significantly associated with chronic HCV infection (C/T

Table III Allele and genotype frequencies of the IL-28B rs12979860 C/T polymorphism in patients with chronic HCV infection stratified according to HCV genotype

IL-28	HCV genotype						
rs12979860	1 *N*=377	4–5 *N*=30	2 *N*=144	3 *N*=78	O.R.	95% C.I.	*p* value
C	0.550	0.600	0.635	0.692	1	Reference	
T	0.450	0.400	0.365	0.308	1.531	1.205–1.945	<0.001
C/C	106 (28.1%)	11 (36.6%)	55 (38.2%)	33 (42.3%)	1	Reference	
C/T	203 (53.8%)	14 (46.7%)	73 (50.7%)	42 (53.9%)	1.419	0.994–2.027	N.S.
T/T	68 (18.1%)	5 (16.7%)	16 (11.1%)	3 (3.8%)	2.890	1.632–5.112	<0.001

The odds ratios were constructed comparing HCV unfavourable (1–4–5) vs. favourable (2–3) genotypes. The wild type was considered to be the reference for each polymorphism. The odds ratios for the minor allele dominant and recessive models are also presented here. The statistical analysis was performed using the Pearson's chi-square test

IL-28B interleukin 28B, *O.R.* odds ratio, *C.I.* confidence interval, *N.S.* not significant, *HCV* hepatitis C virus

T/T+C/T vs. C/C—O.R.=1.628, 95% C.I.=1.155–2.295, p=0.005

T/T vs. C/T+C/C—O.R.=2.335, 95% C.I.=1.375–3.963, p=0.001

odds ratio 1.87, 95% confidence interval 1.42–2.46; T/T odds ratio 1.72, 95% confidence interval 1.14–2.59, improvement chi-square $p<0.001$) independently of gender and age.

IL-28B rs12979860 C/T Polymorphism and HCV Genotypes The IL-28B genotypic frequencies among patients with chronic HCV infection differed greatly according to viral genotype (Table III). The frequency of the C allele was higher in patients infected with "favourable" HCV genotypes 2 (0.635) and 3 (0.692), while it was lower in those infected with HCV genotype 1 (0.550) and 4–5 (0.600). Conversely, presence of the T allele was found to be significantly associated with HCV infection with genotypes 1–4–5 as compared to genotypes 2–3. Increasing frequencies of the T allele were detected ($p<0.0001$ for linear trend) as being lowest in controls, higher in patients infected with HCV 2–3 and highest in patients infected with HCV 1–4–5 (Fig. 2a). Accordingly, in chronic hepatitis C, the frequency of HCV infection due to unfavourable HCV genotypes 1–4–5 increased ($p=0.0002$) from IL-28B rs12979860 C/C genotype to the C/T and T/T genotypes (Fig. 2b).

IL-28B rs12979860 C/T Polymorphism and Fibrosis in Patients with Chronic HCV Infection Allelic and genotypic frequencies in patients with chronic HCV infection stratified according to the severity of liver disease are presented in Table IV. Patients with Ishak staging scores ≥3 were likely to have the T/T genotype in comparison to the C/T+C/C genotypes more frequently than patients with mild disease (Ishak staging score ≤2, Table IV footnote). Stepwise logistical regression analysis was performed to assess whether the IL-28B rs12979860 C/T polymorphism (C/* vs. T/T) could be considered a predictor of an Ishak staging score ≥3 independently of age (≤/>55 years), gender (female/male), BMI (≤/>25 kg/m^2), alcohol intake (≤/>20 g/day), diabetes mellitus (absent/present) and HCV genotype (2–3/1–4–5). Based on this analysis, the presence of the T/T genotype of IL-28B rs12979860 was shown to be an independent predictor of a higher staging score (odds ratio 1.68, 95% confidence interval 1.03–2.75; improvement of chi-square $p<0.05$). Analysis of covariance with staging score was performed using age (≤/>55 years), gender (female/male), BMI (≤/>25 kg/m^2), alcohol intake (≤/>20 g/day), diabetes mellitus (absent/present) and HCV genotype (2–3/1–4–5) as independent variables. Patients were classified according to IL-28B rs12979860 C/T polymorphism (C/* vs. T/T). Patients with the T/T genotype had an adjusted mean±standard error Ishak staging score that was significantly higher (3.50±0.20) than the patients carrying the C/T+C/C genotypes (3.04±0.08, $p<0.05$).

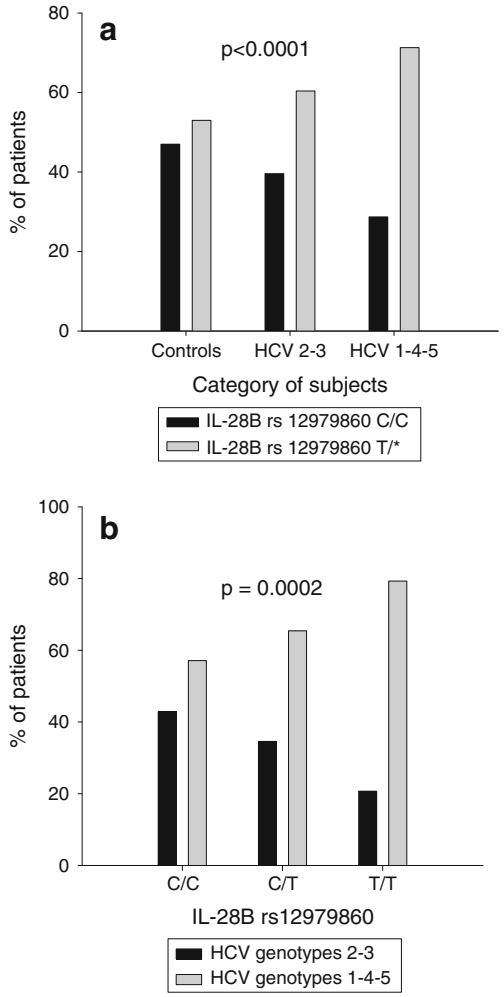

Fig. 2 **a** Percentage of subjects carrying the IL-28B rs12979860 C/C and T/* genotypes in control subjects, patients with chronic infection with HCV genotypes 2–3 and patients with chronic infection with HCV genotypes 1–4–5. The statistical analysis was performed using the chi-square test for linear trend. **b** Percentage of patients with chronic infection with favourable (HCV 2–3) and unfavourable (HCV 1–4–5) genotypes stratified according to the *IL-28B rs12979860 C/C*, *C/T* and *T/T* genotypes. The statistical analysis was performed by means of a chi-square test for linear trend

IL-28B rs12979860 C/T Polymorphism and Gender in Patients with Chronic HCV Infection In females, but not in males, a significant linear trend was observed for increasing frequencies of the IL-28B rs12979860 T/T genotype, ranging from patients with mild infection to those with severe HCV chronic infection (Table V). Therefore, a possible interaction between the IL-28B rs12979860 C/T polymorphism and gender was examined. Moderate/severe disease was detected at a lower frequency among females carrying the C/* genotype (95/224, 42.4%); the frequency of patients with moderate/severe disease increased in C/* males (176/313, 56.2%) and was maximal in patients with the T/T genotype irrespective of

Table IV Allele and genotype frequencies of the IL-28B rs12979860 C/T polymorphism in patients with chronic HCV infection (N=629) stratified according to the severity of liver disease

	IL-28	Mild	Moderate	Severe	Mild vs. others		
		N=301	N=128	N=200	O.R.	95% C.I.	p value
rs12979860	C	0.601	0.594	0.570	1	Reference	
Alleles	T	0.399	0.406	0.430	1.096	0.875–1.372	N.S.
	C/C	96 (31.9%)	46 (35.9%)	63 (31.5%)	1	Reference	
rs12979860	C/T	170 (56.5%)	60 (46.9%)	102 (51.0%)	0.839	0.593–1.189	N.S.
Genotypes	T/T	35 (11.6%)	22 (17.2%)	35 (17.5%)	1.434	0.870–2.365	N.S.

Allele and genotype frequencies of the IL-28B rs12979860 C/T polymorphism stratified according to mild, Ishak staging score=0–2; moderate, Ishak staging score=3–4; severe, Ishak staging score=5–6. The odds ratios were constructed considering the wild type as the reference for each polymorphism. The odds ratios for the minor allele dominant and recessive models are also presented here. The statistical analysis was performed using the Pearson's chi-square test; patients with mild disease were compared to those with moderate and severe disease grouped together

IL-28B interleukin 28B, *O.R.* odds ratio, *C.I.* confidence interval, *N.S.* not significant

T/T+C/T vs. C/C—O.R.=0.941, 95% C.I.—0.674–1.313, p=N.S.

T/T vs. C/T+C/C—O.R.=1.599, 95% C.I.=1.018–2.509, $p<0.05$

gender (57/92, 62.0%; chi-square test for linear trend $p<0.0005$). This interaction was associated with an Ishak staging score >2 independently of other well-known predictors of fibrosis progression in chronic hepatitis C (Table VI). Compared to females carrying the IL-28B rs12979860 C/* genotypes, males with the same genotypes had an adjusted odds ratio of 2.06 (95% confidence interval 1.39–3.05) of being affected by moderate/severe chronic hepatitis C, while the adjusted value was 2.39 (1.40–4.08) in patients carrying the IL-28B rs12979860 T/T genotype, irrespective of gender. Finally, a significant linear trend was detected in the adjusted mean±standard error staging scores (covariates, age ≤/>55 years; BMI ≤/>25 kg/m^2; alcohol intake ≤/>20 g/day; diabetes mellitus absent/present, HCV genotype 2–3/1–4–5) in the following order: (a) females carrying the IL-28B rs12979860 C/* genotypes (2.69±0.13), (b) males carrying the IL-28B rs12979860 C/* genotypes (3.30±0.11) and (c) carriers of the IL-28B rs12979860 T/T genotype irrespective of gender (3.46±0.20, $p<0.002$ for linear trend).

Discussion

In the present study, a clear difference was observed in IL-28B rs12979860 polymorphism allele and genotype frequencies between patients with chronic HCV infection and healthy controls. Patients harboured the T allele much more frequently than controls (0.410 vs. 0.321); moreover, the C/C genotype was detected in 47.0% of controls and in 32.6% of HCV carriers. While the frequency of the C allele in our control subjects largely overlapped the frequencies previously reported in other studies of European populations [10], our chronically HCV-infected patients had the C/C genotype and the C allele less frequently than previously reported in American and European HCV-infected patients [10, 11, 17, 18]. In fact, the frequency of this genotype in HCV-infected patients of European ancestry has been reported to vary from 38% [10] to 45% [18]. In agreement with the data from Montes-Cano et al. [18], our results suggest that the presence of the T allele appears to favour the persistence of the virus and the development of chronic HCV infection.

Table V Genotype frequencies of the IL-28B rs12979860 C/T polymorphism (C/C+C/T vs. T/T) in patients with chronic HCV infection

	IL-28B rs12979860	Mild N=301	Moderate N=128	Severe N=200	p value
Males (N=357)	C/*	137 (88.4)	63 (87.5)	113 (86.9)	N.S.
	T/T	18 (11.6%)	9 (12.5%)	17 (13.1%)	
Females (N=272)	C/*	129 (88.4%)	43 (76.8%)	52 (74.3%)	<0.01
	T/T	17 (11.6%)	13 (23.2%)	18 (25.7%)	

Genotype frequencies of the IL-28B rs12979860 C/T polymorphism stratified according to mild, Ishak staging score=0–2; moderate/severe, Ishak staging score=3–6 and gender. The statistical analysis was performed using the Pearson's chi-square test for linear trend

IL-28B interleukin 28B, *N.S.* not significant

Table VI Stepwise logistical regression analysis with a forward approach performed to identify independent predictors of an Ishak staging score >2 in patients with chronic HCV infection

	Coefficient	S.E.	Coefficient/S.E.	O.R.	95% C.I.
Age >55 years	1.165	0.184	6.34	3.21	2.23–4.60
Alcohol intake >20 g/day	1.632	0.657	2.48	5.12	1.41–18.6
Diabetes mellitus present	1.625	0.350	4.64	5.08	2.55–10.10
HCV genotype 1–4–5	0.472	0.185	2.56	1.60	1.12–2.30
IL-28B rs12979860 C/* males	0.723	0.199	3.63	2.06	1.39–3.05
IL-28B rs12979860 T/T	0.870	0.273	3.18	2.39	1.40–4.08

The following variables were included as covariates: age (≤/>55 years), gender (female/male), BMI (≤/>25 kg/m^2), alcohol intake (≤/>20 g/day), diabetes mellitus (absent/present) and HCV genotype (2–3/1–4–5), IL-28B rs12979860 C/T polymorphism (C/* vs. T/T) and the interaction between gender and IL-28B rs12979860 C/T polymorphism (IL-28B rs12979860 C/* females vs. IL-28B rs12979860 C/* males vs. IL-28B rs12979860 T/T irrespective of gender)

HCV hepatitis C virus, *BMI* body mass index, *IL-28B* interleukin 28B, *S.E.* standard error, *O.R.* odds ratio, *C.I.* confidence interval

Until now, two studies have reported data regarding the IL-28B rs12979860 C/T polymorphism in relation to HCV genotypes [17, 18]. In both studies, a higher frequency of the IL-28B rs12979860 C/C genotype was detected in patients infected with "favourable" HCV genotypes 2–3. In agreement with McCarthy et al. and Montes-Cano et al., a clear relationship was detected between the IL-28B rs12979860 polymorphism and HCV genotypes. Patients with HCV genotypes 1, 2 and 3 had the IL-28B rs12979860 C/C genotype in 28%, 38% and 42% of cases. The corresponding frequencies in the study by McCarthy et al. were 33%, 46% and 55% for HCV genotypes 1, 2 and 3, respectively, while those of Montes-Cano et al. were 39% and 66% for HCV genotypes 1 and 2–3, respectively. In our study, in addition to what has been previously reported, genotypes 4–5, which behave clinically similarly to HCV genotype 1, showed an IL-28B rs12979860 polymorphism distribution very close to that presented by HCV genotype 1. Consequently, the T allele and the IL-28B rs12979860 T/T genotype were detected at increased frequencies in patients infected with "unfavourable" HCV genotypes (1–4–5) in comparison to favourable genotypes (2–3). Thus, the observed interaction between HCV genotypes and the IL-28B rs12979860 polymorphism may not be random. An ethnic association has been described in relation to the frequencies of infection with HCV genotype 1 and of the presence of IL-28B rs12979860 non-C/C genotypes: patients of African ancestry have the highest prevalence of HCV genotype 1 and of the T allele, while the opposite is observed in East Asian populations where non-1 HCV and the IL-28B rs12979860 C/C genotype are generally more represented [11, 19]. It is well known that African–American patients have lower rates of response to antiviral therapy than Caucasians and are more likely to have progressive disease [6], while the opposite is observed in East Asian populations. It has been reported that IL-28B rs12979860 C allele is associated with a higher probability of clearing the virus during the natural history of chronic HCV infection [11, 18], and inversely, the presence of the less-frequent T allele might be associated with HCV persistence. Therefore, it could be hypothesised that the same could occur in the natural history of HCV infection: the presence of the IL-28B rs12979860 T allele could be associated with a lesser degree of HCV clearance in HCV 1–4 than in HCV 2–3 genotypes.

Another important finding of this study is the demonstration that the IL-28B rs12979860 C/T polymorphism appears to be linked to disease progression in chronically infected HCV patients. In this context, a recessive model for the minor allele rather than a dominant one, i.e. the presence of the T/T genotype, exerts a major role. The T/T genotype was found to be more frequent in patients with higher rates of liver fibrosis (Ishak staging score ≥3) than in patients with mild, chronic hepatitis C (Ishak staging score ≤2). The association between the IL-28B rs12979860 C/T polymorphism and liver fibrosis persisted even when the principal confounding factors responsible for a worse course of chronic hepatitis C were taken into account. These results expand our preliminary observations [13] and suggest a possible role for the IL-28B rs12979860 C/T polymorphism in modulating liver fibrosis progression in chronic hepatitis C. This is not unexpected; in fact, one could hypothesise that factors shown to be able to clear the hepatitis C virus might also blunt the pathologic activity of this infectious agent.

The relationship between the IL-28B rs12979860 C/T polymorphism and HCV infection was found to be related to gender, at least in part. In particular, while the presence of the T/T genotype was independently associated with higher degrees of liver fibrosis, even after adjusting for the other covariates responsible for fibrosis progression in chronically infected HCV patients, an interaction was

detected between the C allele and gender. In the presence of the C/C or C/T IL-28B rs12979860 genotypes, female gender was found be protective in comparison to male gender. The possible influence of gender in the context of immune response, in particular on the action of pro-inflammatory cytokines, has been reported. The diethylnitrosamine mouse model of HCC, in which HCC develops predominantly in male mice, has demonstrated that oestrogens, via the oestrogen receptor-α, interfere with the MyD88-dependent activation of NF-κB [20]. Moreover, the effect of 17β-estradiol on cytokine production has been tested in human whole blood cultures. It was shown that E2 inhibits the spontaneous secretion of cytokines, namely interleukin-1β (IL-1β) and IL-6 [21]. Finally, gender and interleukin 6 polymorphisms were found to interact in predicting the occurrence of HCC in patients with end-stage liver disease: a low producer phenotype independent of gender was seldom associated with HCC; the frequency of this cancer rose in IL-6 high producers of female gender and peaked in high-producer males [22]. Similar considerations may apply to the IL-28B rs12979860 polymorphism, given that the inflammatory response shares, at least in part, common final effectors. In this regard, it should be pointed out that IL-28B plays a central role in the immune response to viral infections [23]; in a differentiated murine hepatocyte cell line, IFN-λ inhibits HBV replication with kinetics and efficiency similar to those of type I IFNs [24]. Moreover, IFN-λ blocks the replication of a sub-genomic and a full-length genomic HCV replicon in the human hepatoma cell line Huh7 [24]. Furthermore, IFN-λ, through both extracellular and intracellular antiviral mechanisms, inhibits HIV-1 replication in macrophages [25]. Finally, type III IFN has been demonstrated to possess potent antiviral and immuno-stimulatory activities in response to poxvirus infection [26].

In conclusion, the IL-28B rs12979860 C/T polymorphism appears to be associated with the persistence of HCV infection in relation to the HCV genotype. HCV patients with genotype 1 more frequently have the T/T genotype, which is associated with higher scores of liver fibrosis. Female gender in association with the presence of the C allele predicts the occurrence of lower degrees of liver fibrosis.

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References

1. Thomas DL, Seeff LB. Natural history of hepatitis C. Clin Liver Dis. 2005;9:383–98. vi.
2. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet. 1997;349:825–32.
3. Poynard T, Ratziu V, Charlotte F, et al. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. J Hepatol. 2001;34:730–9.
4. Moucari R, Asselah T, Cazals-Hatem D, et al. Insulin resistance in chronic hepatitis C: association with genotypes 1 and 4, serum HCV RNA level, and liver fibrosis. Gastroenterology. 2008;134:416–23.
5. Poynard T, Marcellin P, Lee SS, et al. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT). Lancet. 1998;352:1426–32.
6. Pearlman BL, Hepatitis C. Virus infection in African Americans. Clin Infect Dis. 2006;42:82–91.
7. Grasso A, Malfatti F, De Leo P, et al. Insulin resistance predicts rapid virological response in non-diabetic, non-cirrhotic genotype 1 HCV patients treated with peginterferon alpha-2b plus ribavirin. J Hepatol. 2009;51:984–90.
8. Horsmans Y. Treatment of chronic hepatitis C in elderly patients. Expert Opin Pharmacother. 2010;11:571–7.
9. Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol. 2008;49:634–51.
10. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461:399–401.
11. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461:798–801.
12. Charlton MR, Thompson A, Veldt BJ, et al. Interleukin-28B polymorphisms are associated with histological recurrence and treatment response following liver transplantation in patients with hepatitis C virus infection. Hepatology. 2010;53:317–24.
13. Fabris C, Falleti E, Cussigh A, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. J Hepatol. 2011;54:716–22.
14. Balagopal A, Thomas DL, Thio CL. IL28B and the control of hepatitis C virus infection. Gastroenterology. 2010;139:1865–76.
15. Bruix J, Sherman M. Management of hepatocellular carcinoma. Hepatology. 2005;42:1208–36.
16. Ishak K, Baptista A, Bianchi L, et al. Histological grading and staging of chronic hepatitis. J Hepatol. 1995;22:696–9.
17. McCarthy JJ, Li JH, Thompson A, et al. Replicated association between an IL28B gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology. 2010;138:2307–14.
18. Montes-Cano MA, Garcia-Lozano JR, Abad-Molina C, et al. Interleukin-28B genetic variants and hepatitis virus infection by different viral genotypes. Hepatology. 2010;52:33–7.
19. Yu ML, Chuang WL. Treatment of chronic hepatitis C in Asia: when East meets West. J Gastroenterol Hepatol. 2009;24:336–45.
20. Naugler WE, Sakurai T, Kim S, et al. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science. 2007;317:121–4.
21. Rogers A, Eastell R. The effect of 17beta-estradiol on production of cytokines in cultures of peripheral blood. Bone. 2001;29:30–4.

22. Falleti E, Fabris C, Toniutto P, et al. Interleukin-6 polymorphisms and gender: relationship with the occurrence of hepatocellular carcinoma in patients with end-stage liver disease. Oncology. 2009;77:304–13.
23. Ank N, West H, Bartholdy C, et al. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. J Virol. 2006;80:4501–9.
24. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol. 2005;79:3851–4.
25. Hou W, Wang X, Ye L, et al. Lambda interferon inhibits human immunodeficiency virus type 1 infection of macrophages. J Virol. 2009;83:3834–42.
26. Bartlett NW, Buttigieg K, Kotenko SV, et al. Murine interferon lambdas (type III interferons) exhibit potent antiviral activity in vivo in a poxvirus infection model. J Gen Virol. 2005;86:1589–96.