Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Importance of *IL28B* gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients

Christoph Sarrazin[1,*], Simone Susser[1], Alexandra Doehring[2], Christian Markus Lange[1], Tobias Müller[3], Christina Schlecker[2], Eva Herrmann[4], Jörn Lötsch[2], Thomas Berg[5]

[1]*Medizinische Klinik 1, J. W. Goethe-University Hospital, Frankfurt am Main, Germany;* [2]*pharmazentrum frankfurt, Institute of Clinical Pharmacology, J. W. Goethe-University, Frankfurt am Main, Germany;* [3]*Medizinische Klinik m. S. Hepatologie und Gastroenterologie, Campus Virchow Klinikum, Charité Universitätsmedizin Berlin, Germany;* [4]*Institute of Biostatistics and Mathematical Modeling, J. W. Goethe-University, Frankfurt am Main, Germany;* [5]*Klinik für Gastroenterologie, Sektion Hepatologie, Universitätsklinikum Leipzig, Germany*

Background & Aims: Genetic variation in the interleukin 28B (*IL28B*) gene has been associated with the response to interferon-alfa/ribavirin therapy in hepatitis C virus (HCV) genotype 1-infected patients. The importance of three *IL28B* single nucleotide polymorphisms (rs8099917, rs12980275 and rs12979860) for HCV genotype 2/3-infected patients is unknown.

Methods: In patients with chronic hepatitis C genotype 2/3 ($n = 267$), *IL28B* host genotypes (rs8099917, rs12980275 and rs12979860) were analyzed for associations with sustained virologic response (SVR) to antiviral therapy with (pegylated) interferon-alfa and ribavirin and with respect to epidemiological, biochemical, and virological parameters. For comparison, hepatitis C genotype 1 patients ($n = 378$) and healthy controls ($n = 200$) were included.

Results: The rs12979860 CC genotype, lower age, and genotype 2 were significantly associated with SVR in HCV genotype 2/3-infected patients ($p = 0.01$, $p = 0.03$ and $p = 0.03$, respectively). No association was observed for rs8099917 and rs12980275. In addition, an SVR in patients with rapid virologic response (RVR) was associated with the rs12979860 CC genotype ($p = 0.05$), while for non-RVR no association was found. Furthermore, a significant association with a higher baseline viral load was observed for all three *IL28B* genotypes in genotype 1/2/3-infected patients. Finally, increasing frequencies of the rs12979860 CC genotypes were observed in genotype 1- (33.9%), genotype 3- (38.9%), and genotype 2-infected (51.9%) patients in comparison with healthy controls (49.0%) ($p < 0.01$).

Conclusions: In genotype 2/3-infected patients, rs12979860 was significantly associated with SVR. The frequency of the rs12979860 CC genotype is lower in HCV genotype 1 vs. genotype 2/3 patients. All major *IL28B* genotypes are associated with HCV-RNA concentration.

Introduction

Chronic hepatitis C virus infection is still a major cause for developing cirrhosis and hepatocellular carcinoma which often results in liver failure and thus in liver transplantation. According to the World Health Organisation, 180 million people are infected worldwide and 3–4 million new infections per year were estimated [1]. Up to now, the standard of care (SOC) treatment consists of (pegylated) interferon-alfa and ribavirin. However, depending on the viral genotype, treatment response rates differ significantly among infected patients. While up to 80% of the genotype 2 and 3 infected patients can be cured, the response rate is only 40–50% in genotype 1 infections [2,3]. Virus-specific characteristics (viral load, genotype, viral variants for example within the interferon sensitivity determining region, ISDR) may be responsible for these differences but also clinical parameters (age, gender, BMI, fibrosis stage, liver enzymes) have been shown to be associated with virologic response [4,5]. The impact of genetic variation near the interleukin 28B (*IL28B*) gene for response in HCV genotype 1 infected patients was shown recently [6–9]. *IL28B* encodes interferon λ-3 (IFN-λ3) a cytokine distantly related to type I interferons and the IL-10 family. Together with interleukin 28A (IFN-λ2) and interleukin 29 (IFN-λ1), *IL28B* forms a cytokine gene cluster on a chromosomal region mapped to 19q13. Expression of the cytokines encoded by these three genes can be induced by RNA virus infection [10].

While the association of the virologic response to (pegylated) interferon-alfa/ribavirin combination therapy with *IL28B* variants

Keywords: *IL28B*; Gene polymorphism; Hepatitis C virus; Genotype 2; Genotype 3.

Received 1 April 2010; received in revised form 9 June 2010; accepted 5 July 2010; available online 22 September 2010

* Corresponding author. Address: Medizinische Klinik 1, J. W. Goethe-Universität, 60590 Frankfurt am Main, Germany. Tel.: +49 69/6301 5122; fax: +49 69/6301 83112.

E-mail address: sarrazin@em.uni-frankfurt.de (C. Sarrazin).

Abbreviations: IL28B, interleukin 28B gene; HCV, hepatitis C virus; SNP, single nucleotide polymorphism; WHO, World Health Organisation; SOC, standard of care; SVR, sustained virologic response; BMI, body mass index; IFN, interferon; RNA, ribonucleic acid; IU, international unit; µg, microgram; kg, kilogram; mg, milligram; mL, milliliter; HBV, hepatitis B virus; HIV, human immunodeficiency virus; EDTA, ethylendiamin-tetraacetat; DNA, desoxyribonucleicacid; OD, optical density; GWAS, genome-wide association studies; ALT, alanine-aminotransferase; PPV, positive predictive value; NPV, negative predictive value; GT, genotype; n.a., not available.

Research Article

in HCV genotype 1 infected patients was shown and confirmed in several independent studies, little is known about the importance of these *IL28B* polymorphisms for genotype 2 or 3 infected patients [11,12].

In the present study, the three main *IL28B* single nucleotide polymorphisms (SNPs), which had so far shown the strongest association with virologic response (rs12979860, rs8099917, and rs12980275) in genotype 1 infected patients, were investigated in HCV genotype 2/3 infected patients (n = 267) in correlation with epidemiological, biochemical, and virological parameters as well as in response to antiviral therapy with (pegylated) interferon-alfa and ribavirin. For comparison, epidemiological *IL28B* genotype frequencies in patients with chronic HCV genotype 1 infection (n = 378) and healthy controls (n = 200) were investigated.

Methods

Patients

Consecutive patients infected with the HCV genotype 2/3 (n = 267; genotype 2, n = 77; genotype 3, n = 190), who presented at tertiary hepatology referral centers at University Hospitals in Frankfurt, Homburg/Saar, and Berlin between 1998 and 2008, were enrolled. Epidemiological, biochemical, and virological characteristics of these patients are shown in Table 1. Antiviral therapy with known virologic outcome was performed in 205/267 patients. The treatment consisted of standard interferon-alfa 2a/b 3 million IU three times per week, pegylated interferon-alfa 2a 180 μg per week or pegylated interferon-alfa 2b 1.5 μg per kg body weight and week in combination with ribavirin 600–1400 mg per day according to body weight for 24–48 weeks. Sustained virologic response was defined as HCV RNA negativity by a sensitive assay (detection limit <50 IU/ml) at least 24 weeks after termination of antiviral therapy. Virologic relapse was defined as HCV RNA undetectable at the end-of-treatment but positive thereafter and virologic non-response as HCV RNA detectability throughout the entire therapy of at least 24 weeks. In addition, patients from an ongoing German multicenter study (INDIV-2) [13] and a random sample of healthy volunteers (n = 200) were enrolled for *IL28B* genotyping, as controls.

Race was obtained from patient charts based on self-definition. All patients and controls in the present study were of Caucasian origin.

Analyses on viral load were limited to patients with available HCV RNA concentration at baseline before initiation of antiviral therapy. HCV RNA viral load was measured by Cobas Amplicor Monitor 2.0, Cobas TaqMan HCV (Roche Diagnostics, Mannheim, Germany), Siemens Versant Quantitative bDNA 3.0 (Siemens Diagnostics, Eschborn, Germany), or National Genetics Institute SuperQuant (NGI, Culver City, CA, USA) assays.

HCV genotyping was performed by a reverse hybridisation assay (InnoLipa, HCV assay vs1, Innogenetics, Zwijnaarde, Belgium).

Co-infection with hepatitis B virus (HBV) and human immunodeficiency virus (HIV) was excluded in all patients by standard serological tests (HBs antigen, HIV-1/2 antibodies).

Histological results of liver biopsies were classified by local pathologists at the different study sites according to internationally standardized criteria. For better comparison between the different local pathologists the individual fibrosis stage was documented as stage 0–1, stage 2 or stage 3–4 (i.e., absence or minimal fibrosis, moderate fibrosis, or advanced fibrosis/presence of cirrhosis).

The clinical studies were approved by local ethics committees. Written informed consent was obtained from all patients and healthy controls and the study was performed in accordance with provisions of the Declaration of Helsinki and Good Clinical Practice guidelines.

DNA collection and extraction

Blood was collected into EDTA tubes. Genomic DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quality was assessed by calculating the absorbance ratio $OD_{260nm/280nm}$ using NanoDrop model ND-1000 (PeqLab, Erlangen).

IL28B genotyping

IL28B variants rs8099917T>G, rs12979860C>T and rs12980275A>G were diagnosed from whole blood samples using validated Pyrosequencing™ assays. Primers for amplification were rs8099917: forward primer: 5′-TCATCCCACTTCTGGAACAAA-3′, reverse primer: 5′-biotin-TGGGAGAATGCAAATGAGAGATAA-3′; rs12979860: forward primer: 5′-ATTCCTGGACGTGGATGGGTACT-3′, reverse primer: 5′-biotin-GGAGCGCGGAGTGCAATT-3′; rs12980275: forward primer: 5′-GTCAGTGAAATAAGCCAGTCTCAA-3′, reverse primer: 5′-biotin-TACATTGTTCGGCAAGCAATCT-3′.Sequencing primers required for the detection of a short DNA sequence around the SNP of interest were rs8099917: 5′-TTTTCCTTTCTGTGAGC-3′; rs12979860: 5′-AGCTCCCCGAAGGCG-3′; and rs12980275: 5′-GAAGTCAAATTCCTAGAAAC-3′). Sequencing analysis took place on a PSQ 96 MA System using Pyrosequencing-specific enzyme mix, substrate mix, and nucleotides (PyroMark™ Gold Q96 reagents set for SNP genotyping and mutation analysis; Qiagen GmbH, Hilden, Germany). The development of *IL28B* genotyping assays is described in detail elsewhere [14]. Two samples of each genotype per SNP were conventionally sequenced by an independent laboratory (AGOWA GmbH, Berlin, Germany) and implemented as positive controls into all local sequencing runs. Genotyping was performed at Frankfurt University Hospital in a blinded fashion relative to the HCV genotype and treatment status.

Table 1. Epidemiological, biochemical, virological, and histological characteristics of patients infected with HCV genotypes 1 and 2/3.

Variable	GT1 (n = 378)	GT2/3 (n = 267)	GT2 (n = 77)	GT3 (n = 190)
Age (years, mean ± SD)	45.8 ± 11.3	45.6 ± 10.7	51.4 ± 11.9	43.3 ± 9.3
Sex (male)	194 (52.1%)	145 (54.1%)	43 (56.6%)	101 (53.4%)
ALT (mean ± SD)	82 ± 60	78 ± 73	63.5 ± 53.1	84.3 ± 78.8
HCV RNA (log IU/ml ± SD)	5.6 ± 0.7	5.9 ± 0.7	6.0 ± 0.7	5.9 ± 0.7
HCV RNA				
<600.000 IU/ml	203 (55.3%)	90 (39.3%)	21 (32.3%)	69 (42.1%)
≥600.000 IU/ml	164 (44.7%)	139 (60.7%)	44 (67.7%)	95 (57.9%)
SVR*	n.a.	160 (78.0%)	51 (87.9%)	109 (74.2%)
Relapse*	n.a.	25 (12.2%)	5 (8.6%)	20 (13.6%)
Non-response*	n.a.	20 (9.8%)	2 (3.5%)	18 (12.2%)
METAVIR fibrosis stage				
F0-1	227 (65%)	72 (41.1%)	22 (46.8%)	50 (39.1%)
F2	74 (21.2%)	51 (29.1%)	10 (21.3%)	41 (32.0%)
F3-4	48 (13.8%)	52 (29.7%)	15 (31.9%)	37 (28.9%)

GT, genotype; SD, standard deviation; n.a., not available.
*Antiviral therapy with virologic response data is available in 205/267 genotype 2/3 patients.

Table 2. Predictors of sustained virologic response (*IL28B* rs12979860 CC genotype, age, sex, ALT, HCV RNA concentration, liver fibrosis) in genotype 2/3 infected patients.

Predictor	Univariate nonparametric analysis			Multivariate logistic regression
	GT2/3	GT2	GT3	
HCV genotype 2	$p = 0.03$			$p = 0.006$ (OR 4.0; 95% CI 1.5, 10.7)
rs12979860 CC vs. CT/TT	$p = 0.01$	$p = 0.16$	$p = 0.05$	$p = 0.009$ (OR 2.8; 95% CI 1.3, 6.0)
rs8099917 TT vs. TG/GG	$p = 0.37$	$p = 0.13$	$p = 0.91$	n.a.
rs12980275 AA vs. AG/GG	$p = 0.12$	$p = 0.13$	$p = 0.50$	n.a.
Lower Age	$p = 0.03$	$p = 0.07$	$p = 0.02$	$p = 0.004$ (OR 1.1; 95% CI 1.02, 1.1)
Sex	$p = 0.94$	$p = 0.88$	$p = 0.91$	n.a.
ALT	$p = 0.10$	$p = 0.92$	$p = 0.11$	n.a.
HCV RNA concentration	$p = 0.11$	$p = 0.20$	$p = 0.33$	n.a.
Fibrosis stage	$p = 0.04$	$p = 0.09$	$p = 0.11$	n.a.

Odds ratios are for age (per year), HCV genotype (3 vs. 2), rs12979860 (CT/TT vs. CC). n.a., not applicable, because only parameters which were significant in GT2/3 patients and additionally in GT2 or in GT3 patients, when analyzed separately, were included in the multivariate analysis.

Statistical analysis

Predictors for sustained virological response were assessed by univariate and multivariate logistic regression analysis. Multivariate analysis included all significant parameters from univariate analysis as well as the parameters which were available from all patients. Differences between groups were assessed by χ^2 test and Wilcoxon–Mann–Whitney *U* test as appropriate. The Yates-Cochran-Test was used to determine the association between *IL28B*, the rs12979860 CC genotype, SVR, and rapid virologic response. The Hardy–Weinberg equilibrium was assessed with an exact conditional test implemented in the hwde package version 0.61 of R (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-sided and *p*-values below 5% were considered significant.

For correlation with virologic response, all 205 HCV genotype 2 or 3 patients were included in the analysis with 160 SVR and 45 non-SVR patients. According to a power analysis, we were able to detect a difference of at least 25% in the prevalence of the CC genotype with a power of at least 87.5%.

Results

Association of IL28B genotypes with virologic response to PEG-IFNα and ribavirin therapy in HCV genotype 2/3 infected patients

Clinical characteristics of HCV genotype 2/3 infected patients are shown in Table 1. In 205/267 patients antiviral combination therapy with (pegylated) interferon and ribavirin was completed and the virologic treatment outcome was known. From the known parameters associated with sustained virologic response, in the present study only age ($p = 0.03$), genotype 2 ($p = 0.03$), and fibrosis stage ($p = 0.04$) were significant, while sex, ALT, and HCV RNA concentration at baseline were not associated with sustained virologic response (Table 2). Fig. 1A–C show the *IL28B* genotyping results of patients with chronic hepatitis C genotype 2/3 infection for the three SNPs related to virologic response.

Statistical analysis identified rs12979860 as being significantly associated with virologic response to (PEG)-IFNα/ribavirin treatment in HCV genotype 2 and 3 infected patients ($p = 0.01$). Rates of sustained virologic response, relapse, and non-response were 78%, 12%, and 10%, respectively in the entire cohort of genotype 2/3 infected patients. For patients with the rs12979860 CC genotype a higher SVR rate (87.4%) was observed in comparison with CT and TT genotypes (70.7% and 73.1%) (Fig. 1A). For virologic relapse, a variable association with the different rs12979860 genotypes was observed (Fig. 1B). The most pronounced difference was observed for virologic non-response. Only 3.4% of patients with the CC genotype vs. 13.0% and 19.2% with the CT and TT genotypes, respectively, were virologic non-responders (Fig. 1C).

Due to the high SVR rates obtained in genotype 2/3 infected patients, the sensitivity for prediction of a sustained virologic response in patients with the rs12979860 CC genotype vs. the CT/TT genotype was only 47.5% (47.5% of all SVR patients had the rs12979860 CC genotype, Fig. 2), while a high specificity for the exclusion of non-response in patients with the rs12979860 CC genotype with 85.0% (85.0% of non-responders had rs12979860 CT/TT genotypes) was observed (Fig. 2).

The rapid virologic response (RVR) rates for the rs12979860 CC, CT, and TT genotypes were 87%, 77%, and 64%, respectively, but this failed to be statistically significant ($p = 0.17$). For SVR in patients who achieved an RVR, a significant correlation with the rs12979860 genotype was observed ($p = 0.05$), while for SVR in non-RVR patients no association was found ($p = 0.48$) (Fig. 4).

Genome-wide association studies also described the rs12980275 and rs8099917 genotypes as predictive for treatment outcome in HCV genotype 1 patients. In the present study, in genotype 2/3 infected patients with the favorable genotypes (rs8099917 TT and rs12980275 AA) high SVR rates of 80.5% and 83.9%, but also in the unfavorable genotypes (rs8099917 GG and rs12980275 GG) high SVR rates of 87.5% and 80.0% in comparison with 78.0% in the overall cohort, were observed (Fig. 1A). The unfavorable genotype (rs8099917 GG and rs12980275 GG) was more rarely associated with virologic relapse. Furthermore, a trend towards lower frequency of the genotypes rs8099917 TT and rs12980275 AA was observed in non-responder patients (Fig. 1B and C). However, in the present study, for genotype 2/3 infected patients, no statistically significant association between the *IL28B* rs8099917 and rs12980275 genotypes with virologic response, was observed.

Multivariate analysis revealed that lower age, HCV genotype 2, and the rs12979860 CC genotype were significantly associated with sustained virologic response (Table 2).

Association of the IL28B genotype with ALT, HCV RNA concentration and fibrosis

In addition to an association with virologic response, the *IL28B* genotypes could also be related to biochemical, virological, and histological parameters. ALT levels, HCV RNA concentration at base-

Research Article

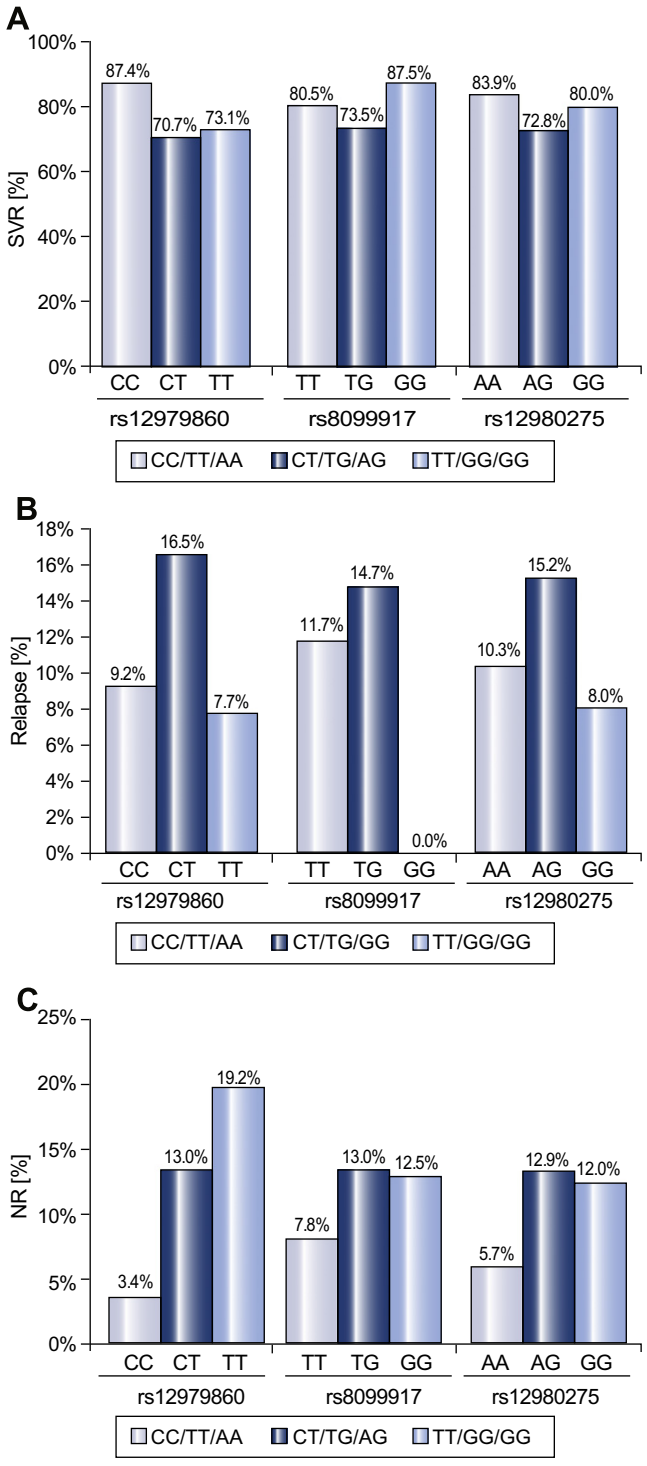

Fig. 1. Association of the *IL28B* genotypes with (A) sustained virologic response (SVR), (B) relapse, (C) non-response (NR) to (pegylated) interferon-alfa and ribavirin in HCV genotype 2/3 infected patients.

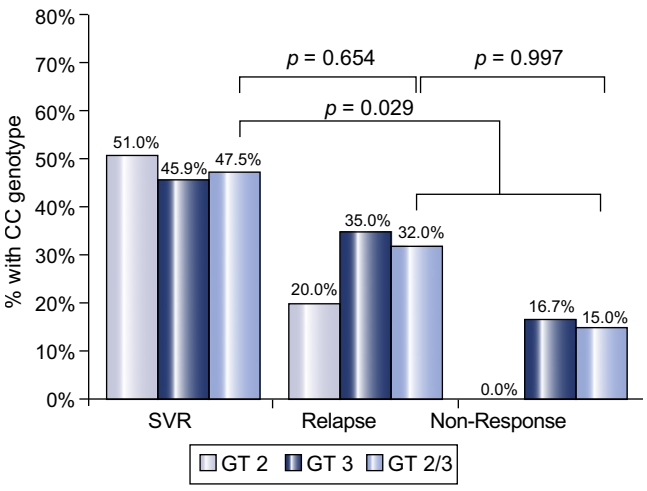

Fig. 2. Frequency of rs12979860 CC genotype in HCV genotype 2, genotype 3, and genotype 2/3 infected patients and association to different treatment outcomes (SVR, Relapse, Non-response).

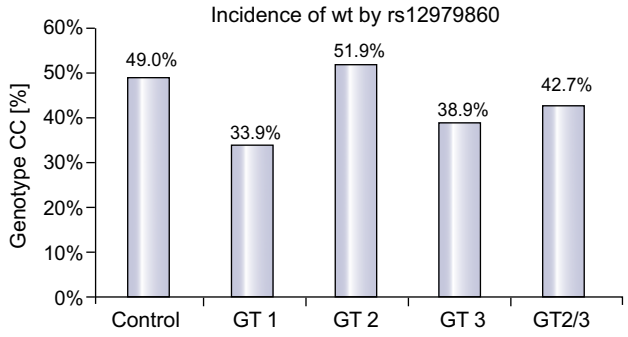

Fig. 3. Frequencies of the *IL28B* rs12979860 CC genotypes in HCV genotype 1, 2, and 3 infected patients compared to healthy controls.

line and the fibrosis stage were correlated with the *IL28B* SNPs rs12979860, rs8099917, and rs12980275. For higher ALT values, a significant association was observed for the rs12979860 CC genotype in HCV genotype 2/3 infected patients and for the rs12980275 AA genotype in HCV genotype 1 infected patients. In addition, for the remaining major *IL28B* genotypes, we observed a trend towards increased association with higher ALT levels (Table 3). A higher HCV RNA concentration was significantly associated with the TT genotype of rs8099917, the CC genotype of rs12979860, and the AA genotype of rs12980275 for HCV genotype 1 and genotype 2/3 infected patients (Table 3). Finally, the rs8099917 TT genotype was significantly associated with a higher fibrosis stage in HCV genotype 1 infected patients (Table 3).

Frequencies of the IL28B genotypes in HCV genotype 2, 3 compared with HCV genotype 1 and healthy controls

Frequencies of the *IL28B* genotypes in HCV genotype 1, 2, and 3 infected patients as well as healthy controls are shown in Table 4. There were no significant deviations from the Hardy–Weinberg equilibrium ($p > 0.10$). In the present study, only the rs12979860 CC genotype was associated with virologic response in genotype 2/3 infected patients. For genotype 1 infected patients, a lower frequency of the rs12979860 CC genotype in comparison with the healthy control was reported previously [6] and this could be confirmed in the present study (genotype 1, 33.9% vs. healthy control, 49%). Interestingly, for the HCV geno-

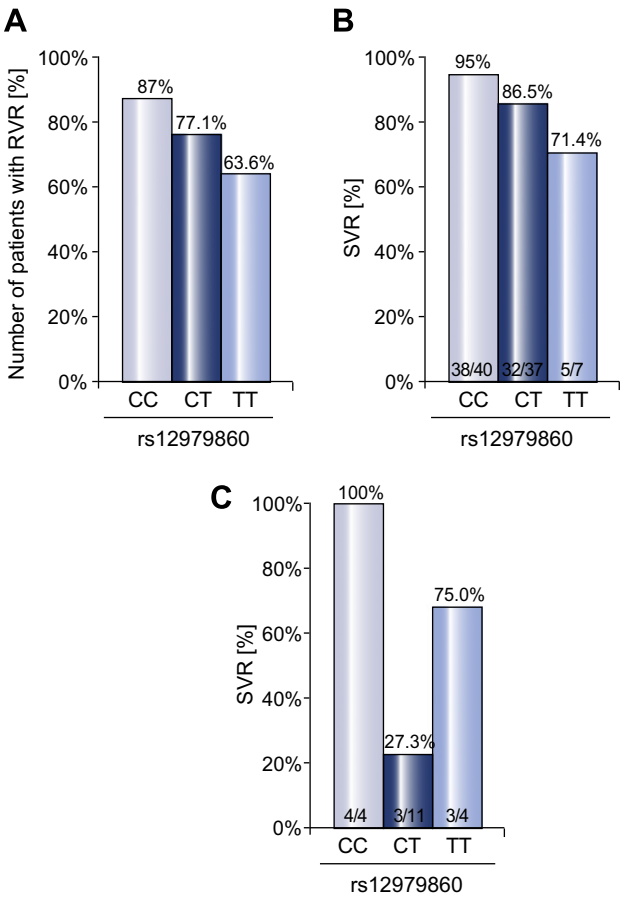

Fig. 4. Correlation of the *IL28B* rs12979860 genotype with (A) RVR, (B) SVR in RVR patients and (C) SVR in non-RVR patients infected with HCV genotype 2/3.

type 2/3 patients, an intermediate frequency of the rs12979860 CC genotype (42.7%) was obtained (Table 4, Fig. 3). The difference between HCV genotype 1 infected patients and healthy controls had a high level of statistical significance (p <0.001), while the difference between control subjects and HCV genotype 2/3 patients was not significant (p = 0.116). Separate analysis of genotype 2 and 3 infected patients showed that a significant difference is present between genotype 1 and 2 (p = 0.045), but not for genotype 1 and 3 (p = 0.43) infected patients, for the frequency of the rs12979860 CC genotype (33.9%, 51.9%, 38.9%) (Fig. 3).

Discussion

Parameters for the prediction of sustained virologic response in patients with chronic hepatitis C before initiation of antiviral therapy are important in order to be able to estimate the potential for treatment success. They can help clinicians in the decision on whether or not to start antiviral therapy and this information can also motivate patients who might have a high chance for virologic response. Different studies have shown that HCV genotype, HCV RNA concentration, age, gender, BMI, fibrosis stage, alanine aminotransferase (ALT), and gamma glutamyltranspeptidase (GGT) levels, insulin resistance as well as host genetic polymorphisms of several genes (HLA, chemokines, interleukins and IFN-stimulated genes) are associated with sustained virologic response [4,5,15,16]. However, in clinical practice guidelines only the HCV genotype and HCV RNA concentration at baseline are currently recommended to be used in order to determine treatment duration in response to guided therapy approaches [17–19]. Most recently, three genome wide association studies reported associations of different SNPs in *IL28B* (interferon lambda gene region) with response to antiviral therapy [6–8]. Here, in HCV genotype 1 infected patients a highly significant correlation of sustained virologic response to interferon-alfa/ribavirin combination treatment was observed with the genotypes rs12979860 CC, rs8099917 TT, and rs12980275 AA. In the present study, the importance of these three major *IL28B* SNPs for European patients with HCV genotype 2/3 infection was investigated.

The main result of this study is that in genotype 2/3 infected patients only a significant association of the rs12979860 CC genotype with SVR was observed. This is in line with a recent study on a relative small cohort of genotype 2/3 infected patients (n = 45) by McCarthy et al., in which only rs12979860 was investigated and also found to be associated with SVR [12]. Because of the high SVR rates of genotype 2/3 infected patients to interferon-alfa/ribavirin combination therapy, differences among patients with and without the rs12979860 CC genotype were much smaller than in genotype 1 patients. Ge et al. reported a twofold greater rate of SVR for the rs12979860 CC genotype in comparison with the TT genotype [6], while in the present study 87.4% of patients with the rs12979860 CC genotype vs. 73.1% of patients with the TT genotype achieved an SVR. Because of the high natural SVR rates of genotype 2/3 infected patients, differences between the rs12979860 CC vs. the non-CC genotype patients became more evident for virologic non-response. Only 47.5% of all patients with SVR had the rs12979860 CC genotype but 85% of patients with virologic non-response had CT or TT genotypes.

Table 3. Correlation of biochemical, virological, and histological parameters of genotype 1 (n = 371) and 2/3 (n = 241) infected patients with different *IL28B* genotypes.

Variable	rs12979860 CC vs. CT/TT		rs8099917 TT vs. TG/GG		rs12980275 AA vs. AG/GG	
	GT1 *P*	GT2/3 *P*	GT1 *P*	GT2/3 *P*	GT1 *P*	GT2/3 *P*
Higher ALT	0.065	0.011	0.095	0.077	0.030	0.066
Higher HCV RNA concentration	<0.001	<0.001	<0.001	0.017	<0.001	<0.001
Higher fibrosis stage	0.173	0.850	0.032	0.802	0.137	0.589

Calculation is based on genotype 2/3 and genotype 1 infected patients with available data on ALT, HCV RNA concentration and fibrosis stage.
A significant association of higher ALT values was observed for rs12980275 AA genotype in HCV genotype 1 infected patients and for rs12979860 CC genotype in HCV genotype 2/3 infected patients. A higher HCV RNA concentration was observed for rs8099917 TT, rs12979860 CC, and rs12980275 AA genotypes in genotype 1 and 2/3 infected patients. A higher fibrosis stage was associated with rs8099917 TT genotype in HCV genotype 1 infected patients only. Data are not corrected for multiple testing.

Research Article

Table 4. Frequencies of *IL28B* genotypes in patients with HCV genotype 1, 2, 3 infection and healthy controls.

	GT1 (n = 378)	GT2/3 (n = 267)	GT2 (n = 77)	GT3 (n = 190)	Healthy controls (n = 200)
rs12979860					
CC	128 (33.9%)	114 (42.7%)	40 (51.9%)	74 (38.9%)	98 (49.0%)
CT	191 (50.5%)	122 (45.7%)	29 (37.7%)	93 (48.9%)	85 (42.5%)
TT	59 (15.6%)	31 (11.6%)	8 (10.4%)	23 (12.1%)	17 (8.5%)
rs8099917					
TT	216 (57.1%)	169 (63.3%)	53 (68.8%)	116 (61.1%)	138 (69.0%)
TG	143 (37.8%)	88 (32.9%)	22 (28.6%)	66 (34.7%)	60 (30.0%)
GG	19 (5.0%)	10 (3.8%)	2 (2.6%)	8 (4.2%)	2 (1.0%)
rs12980275					
AA	140 (37.0%)	116 (43.4%)	44 (57.1%)	72 (37.9%)	105 (52.5%)
AG	185 (48.9%)	122 (45.7%)	27 (35.1%)	95 (50.0%)	82 (41.0%)
GG	53 (14.0%)	29 (10.9%)	6 (7.8%)	23 (12.1%)	13 (6.5%)

In addition, SVR in patients who achieved an RVR was significantly associated with the rs12979860 CC genotype.

Generally, the separate analysis of genotype 2 and 3 infected patients in the present study showed a significant association of the *IL28B* rs12979860 genotype only in genotype 3 infected patients. However, this might be due to the larger number of genotype 3 patients together with the rare event of virologic relapse or non-response in genotype 2 patients. For all single parameters (SVR, RVR, and HCV RNA concentration), and also for genotype 2 infected patients with the rs12979860 CC vs. CT/TT genotype, a trend towards higher SVR and RVR rates as well as higher HCV RNA concentrations at baseline was observed and *p*-values increased in the combined analysis together with HCV genotype 3 infected patients (data not shown).

For the other two *IL28B* SNPs we studied, rs8099917 and rs12980275, no significant association with virologic treatment response was observed. Investigation of a comparable number of genotype 2/3 infected patients in a study by Rauch et al. also showed no correlation between the rs8099917 genotype and virologic response to pegylated interferon/ribavirin combination therapy [11]. In the present study, only a slightly reduced frequency of the genotypes rs8099917 TT and rs12980275 AA in comparison with carriers of the risk alleles (rs8099917 TG/GG and rs12980275 AG/GG) in patients with non-response was detected and it remains unclear whether in a larger patient cohort this association might become significant.

In addition, an association of the major *IL28B* genotypes with higher baseline HCV RNA concentration in genotype 1 infected patients is known from previous studies. Interestingly, in the present study for all three *IL28B* SNPs, a highly significant association with higher baseline viral loads was observed in genotype 2/3 as well as in genotype 1 infected patients carrying the genotypes rs12979860 CC, rs8099917 TT, and rs12980275 AA. Usually a lower baseline viral load is associated with a higher chance of SVR and it remains unclear why here the same *IL28B* genotype is associated with SVR and higher viral load. Furthermore, a trend towards higher ALT levels was observed in carriers of the rs12979860 CC genotype in HCV genotype 2/3 patients and in carriers of the rs12980275 AA genotype in HCV genotype 1 infected patients. In addition, a trend towards higher fibrosis stages in HCV genotype 1 infected patients was seen with the rs8099917 TT genotype. Generally, it is possible that the *IL28B* genotypes are also associated with inflammatory activity and fibrosis stage. However, genotype 2 and 3 patients in the present study were not completely balanced for the frequency of different fibrosis stages and larger patient populations are required to prove this potential association.

An interesting finding from a previous study describes a strong association between the rs12979860 CC genotype with the resolution of acute HCV infection in comparison to those who developed chronic hepatitis C [9]. This implies that an unfavorable *IL28B* rs12979860 genotype may predispose patients to chronic HCV infection [8]. The favorable rs12979860 CC genotype seems to be protective against the development of chronic hepatitis C and thus in patients who did not clear the virus spontaneously, non-favorable rs12979860 genotypes are enriched. However, this may be different according to different HCV genotypes. In the present study, the frequency of the rs12979860 CC genotype in healthy controls was 49.0%. However, in patients with chronic hepatitis C, different rs12979860 CC genotype frequencies according to different HCV genotypes were observed. The lowest frequency of the favorable rs12979860 CC genotype for SVR was detected in genotype 1 infected patients (33.9%), followed by genotype 3 patients (38.9%) and genotype 2 patients (51.9%). For chronic hepatitis C, the lowest SVR rates are observed in genotype 1 infected patients while for genotype 3 patients, higher SVR rates are observed and the highest SVR rates can be seen in genotype 2 infected patients [20–22]. Thus, the different SVR rates seen in the different HCV genotypes might be in part explained by different rs12979860 CC genotype frequencies. In a recent study by McCarthy et al., lower rs12979860 CC genotype frequencies in genotype 1 vs. genotype 2/3 infected patients were also observed. However, in this study, the rs12979860 CC genotype frequencies were higher in genotype 3 vs. genotype 2 infected patients [12]. Thus, additional studies are required to estimate the differences between *IL28B* genotype frequencies in patients with different HCV genotypes. Furthermore, the underlying functional mechanisms for the development of chronic hepatitis C with different HCV geno- and subtypes in the presence of a specific *IL28B* genotype needs to be elucidated.

In conclusion, the rs12979860 CC genotype but not the rs8099917 and rs12980275 genotypes was significantly associ-

ated with sustained virologic response to (pegylated) interferon-alfa/ribavirin combination therapy in genotype 2/3 infected patients. All three major *IL28B* genotypes previously observed to be associated with sustained virologic response and HCV RNA concentration in genotype 1 infected patients are also associated with HCV RNA concentration in genotype 2/3 infected patients. In addition to a correlation with virologic response and viral load, *IL28B* genotypes may also contribute to the grade of inflammation and the stage of liver fibrosis. Finally, a correlation between the frequencies of the rs12979860 CC genotype with SVR rates in genotype 1-, 2- and 3-infected patients was observed which needs further investigation.

Conflict of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Financial support

This study was supported by a BMBF grant titled "Host and viral determinants for susceptibility and resistance to hepatitis C virus infection" for C.S., E.H., S.Z., and T.B., (TP B, TP F) and a DFG grant for C.S., E.H., and S.Z. (Klinische Forschergruppe, KFO 129, TP2, TP1).

References

[1] WHO, World Health Organisation. Hepatitis C. Initiative For Vaccine Research. Available from: <http://www.who.int/vaccine_research/diseases/viral_cancers/en/index2.html> [accessed January 21, 2009] 2009.
[2] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Gonzales FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975–982.
[3] Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958–965.
[4] Wohnsland A, Hofmann WP, Sarrazin C. Viral determinants of resistance to treatment in patients with hepatitis C. Clin Microbiol Rev 2007;20:23–38.
[5] Kau A, Vermehren J, Sarrazin C. Treatment predictors of a sustained virologic response in hepatitis B and C. J Hepatol 2008;49:634–651.
[6] Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in *IL28B* predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399–401.
[7] Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. *IL28B* is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100–1104.
[8] Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of *IL28B* with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105–1109.
[9] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in *IL28B* and spontaneous clearance of hepatitis C virus. Nature 2009;461:798–801.
[10] Asselah T. Genetic polymorphism and response to treatment in chronic hepatitis C: the future of personalized medicine. J Hepatol 2010;52:452–454.
[11] Rauch A, Kutalik Z, Descombes P, Cai T, Di IJ, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138:1338–1345.
[12] McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, et al. Replicated Association between an *IL28B* Gene Variant and a Sustained Response to Pegylated Interferon and Ribavirin. Gastroenterology 2010;138:2307–2314.
[13] Sarrazin C, Schwendy S, Moller B, Dikopoulos N, Buggisch P, Encke J, et al. J Hepatol 2009;50:S236.
[14] Doehring A, Hofmann WP, Schlecker C, Zeuzem S, Susser S, Geisslinger G, et al. Screening for *IL28B* gene variants identifies hepatitis C disease risk. Antivir Ther 2010;15. in press.
[15] Dai CY, Huang JF, Hsieh MY, Hou NJ, Lin ZY, Chen SC, et al. Insulin resistance predicts response to peginterferon-alpha/ribavirin combination therapy in chronic hepatitis C patients. J Hepatol 2009;50:712–718.
[16] Asselah T, Bieche I, Paradis V, Bedossa P, Vidaud M, Marcellin P. Genetics, genomics, and proteomics: implications for the diagnosis and the treatment of chronic hepatitis C. Semin Liver Dis 2007;27:13–27.
[17] Ghany MG, Strader DB, Thomas DL, Seeff LB. Diagnosis, management, and treatment of hepatitis C: an update. Hepatology 2009;49:1335–1374.
[18] McCaughan GW, Omata M, Amarapurkar D, Bowden S, Chow WC, Chutaputti A, et al. Asian Pacific Association for the Study of the Liver consensus statements on the diagnosis, management and treatment of hepatitis C virus infection. J Gastroenterol Hepatol 2007;22:615–633.
[19] Zeuzem S, Berg T, Moeller B, Hinrichsen H, Mauss S, Wedemeyer H, et al. Expert opinion on the treatment of patients with chronic hepatitis C. J Viral Hepat 2009;16:75–90.
[20] Fried MW, Hadziyannis SJ. Treatment of chronic hepatitis C infection with peginterferons plus ribavirin. Semin Liver Dis 2004;24:47–54.
[21] Shiffman ML, Suter F, Bacon BR, Nelson D, Harley H, Sola R, et al. Peginterferon alfa-2a and ribavirin for 16 or 24 weeks in HCV genotype 2 or 3. N Engl J Med 2007;357:124–134.
[22] Zeuzem S, Hultcrantz R, Bourliere M, Goeser T, Marcellin P, Sanchez-Tapias J, et al. Peginterferon alfa-2b plus ribavirin for treatment of chronic hepatitis C in previously untreated patients infected with HCV genotypes 2 or 3. J Hepatol 2004;40:993–999.