J Clin Immunol (2009) 29:620–628
DOI 10.1007/s10875-009-9302-z

Interleukin 18 Promoter Variants (−137G>C and −607C>A) in Patients with Chronic Hepatitis C: Association with Treatment Response

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Received: 29 March 2009 / Accepted: 28 April 2009 / Published online: 20 May 2009

Abstract
Background Recently, two functional *IL18* promoter variants, −607C>A (rs1946518) and −137G>C (rs187238), were associated with viral clearance in patients with hepatitis C. The present study focused on their relevance for treatment response.
Methods Seven hundred fifty-seven chronically infected European patients and 791 controls were enrolled in the study. *IL18* genotyping was performed by allele-specific PCR. Liver histology was available in 67.9%.
Results Genotype and allele frequencies were equally distributed in patients and controls. No significant association with various disease characteristics was observed. However, when comparing patients with sustained virological response (SR) and non-SR, statistically significant associations were found for both variants (p=0.0416 and p=0.0274, respectively). In viral genotype 1, the *−607A* allele was positively associated with treatment response (p=0.0190; OR 1.537; 95% CI, 1.072–2.205) and the *−137G* allele with a higher rate of nonresponse (p=0.0302; OR 1.524; 95% CI, 1.040–2.233).
Conclusions The association of *IL18* variants with treatment response in genotype 1 hepatitis C patients implies a predictive and modifying role of these genetic variants.

Keywords Hepatitis C · antiviral therapy · treatment outcome · genetic alterations · interleukin 18

Introduction

Hepatitis C virus (HCV) infection is the leading cause of chronic liver disease worldwide with an estimated number of 170 million chronically infected individuals [1]. The clinical course is highly variable, but up to 30% of the patients eventually present with liver cirrhosis carrying an annual risk of 1–6% for the development of hepatocellular carcinoma [2]. Numerous viral and host-related factors have been shown to accelerate progression of liver disease such as older age at infection, male sex, alcohol consumption, overweight, hepatic iron status, and co-infection with the hepatitis B or human immunodeficiency virus [3, 4]. Antiviral treatment with polyethylene-glycol-conjugated IFN-α (Peg-IFN-α) in combination with ribavirin aims to prevent progression of the disease by eradication of the virus; it is currently considered the gold standard of care [5].

There is increasing evidence that host immunologic and genetic factors play an important role for disease suscep-

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tibility, hepatic inflammation, progression to fibrosis and cirrhosis, risk of hepatocellular carcinoma, and response to therapy [6–10]. Recently, numerous investigators focused on polymorphisms in cytokine, chemokine, and receptor genes which were previously shown to be implicated in HCV pathogenesis. IL-18 is a pleiotropic cytokine with a wide range of proinflammatory biological effects [11–13]. It is a unique member of the IL-1 cytokine family and synthesized as a 24-kDa proform which is activated to the bioactive 18-kDa mature IL-18, initially described as an interferon (IFN)-γ inducing factor [14]. IFN-γ production is synergistically enhanced in conjunction with IL-12. The main sources of IL-18 are monocytes, macrophages, Kupffer cells, and intestinal epithelial cells. Chief immunomodulatory functions of IL-18 comprise stimulation of the differentiation of naïve T-cells to T-helper 1 cells, enhancement of Fas ligand, and perforin-mediated T-cell and NK-cell cytotoxicity as well as modulation of immunoglobulin secretion by B cells. Proinflammatory activity is mediated by the production of nitric oxide (NO), prostaglandins, and inflammatory cytokines (e.g., TNFα, IL-1β, IL-13), as well as the recruitment of monocytes and macrophages by upregulation of chemokines (IL-8, MIP-1α, MIP-1β, MCP-1) [11–13, 15]. In animals, inhibition of IL-18 by antibodies or an IL-18bp-Fc fusion product abrogated liver damage [14, 16, 17]. In patients with chronic hepatitis C, IL-18 serum levels are increased as compared to healthy controls and positively correlated with the serum activity of alanin-aminotransferase (ALAT) [18, 19].

Giadraitis and colleagues described three genetic alterations in the promoter and two variants in the 5′-untranslated region of *IL18* [20]. Evidence for a functional relevance was given in their study for the −137 and the −607 *IL18* variants. The −137G>C transversion changes the H4TF1 nuclear factor binding site to a binding site for an unknown factor found in the GM-CSF promoter. The −607C>A promoter variant leads to a disruption of a cAMP-responsive element protein binding site. In the meantime, associations of *IL18* variants with different chronic inflammatory conditions were reported comprising hepatitis B [21], atopic asthma bronchiale [22], cardiovascular diseases [23], sarcoidosis in a Japanese population [24], Alzheimer's disease [25, 26], Graves' disease [27], and juvenile idiopathic arthritis [28].

We investigated the frequencies of the two abovementioned *IL18* promoter alterations in HCV patients and control subjects to determine whether these variants might represent susceptibility factors for hepatitis C in Europeans. Further, we investigated whether −137G>C and −607C>A are correlated with HCV patients' characteristics and, in particular, with treatment response.

Methods

Patients

In the present study, we genotyped 1,548 European individuals, comprising 757 patients with chronic hepatitis C infection and 791 healthy controls. Patients were recruited from the Department of Hepatology and Gastroenterology, Campus Virchow-Klinikum, Charité in Berlin. The study was approved by the local ethics committee, and all patients gave their written consent. Controls consisted of 791 healthy individuals from the same geographical origin.

Patients' diagnosis was based on elevation of liver enzymes for at least 6 months with corresponding detection of serum HCV-RNA. All patients proved to be negative for hepatitis B surface antigen and antibodies to human immunodeficiency virus.

Prior to treatment, a liver biopsy was performed in 514/757 (67.9%) of the patients. Hepatic fibrosis (stage) and inflammation (grade) was classified according to the scoring system established by Scheuer [29].

Patients were treated with a combination of standard interferon-α-2a or standard interferon-α-2b plus ribavirin (800–1,200 mg/day) or pegylated interferon-α and ribavirin for 24 weeks in HCV genotype 2/3 infection and 48 weeks in HCV genotype 1 infection, respectively. Data regarding the treatment schedule were unavailable in 282 patients. For the remaining 475 patients, outcome was classified as sustained virological response (HCV-RNA negative at 6 months after end of treatment; $n=176$, 37.1%; SR), relapse (HCV-RNA negative at the end of treatment but recurrence of HCV-RNA thereafter; $n=103$, 21.7%), viral breakthrough (HCV-RNA recurrence during treatment after initial clearance, $n=23$, 4.8%), or nonresponse (failure to clear virus during treatment at week 24 $n=173$, 36.4%). For further analysis, patients with relapse, viral breakthrough, and nonresponse were comprised in one group (non-SR).

HCV-RNA Measurements and Genotyping

Virological response was determined by qualitative HCV-RNA assays with a lower detection limit of 30–50 IU/ml (HCV Amplicore 2.0, Roche Diagnostics, Mannheim, Germany or Superquante NGI, Los Angeles, CA, USA). Quantification of HCV-RNA was performed by different standardized, quantitative HCV-RNA assays (Amplicore HCV Monitor 2.0, Roche Diagnostics, Mannheim, Germany, Versant Quantitative HCV, Bayer, Emeryville, CA, USA, or Superquante NGI, Los Angeles, CA, USA). All results for HCV-RNA levels were reported or transformed in IU/ml. HCV genotypes were assessed by a reverse hybridization assay (Ino LiPA HCV II, Innogenetics, Gent, Belgium).

IL18 Gene Promoter Variants

Genetic alterations at position −607C>A (rs1946518) and −137G>C (rs187238) in the *IL18* promoter were determined by allele-specific PCR (Table 1) [20]. For each variant, four amplifications were carried out in two reactions. For characterization of the −607C>A variant, PCR reaction was performed by applying a sequence-specific sense primer which yielded a 196-bp product with the common antisense primer. A second sense primer was included to amplify a 301-bp fragment representing the positive control. Accordingly, the −137G>C alteration was determined by amplification of a 261-bp fragment with an allele-specific sense primer and a common reverse primer. A 446-bp fragment—using a common sense and common antisense primer—served as the positive control.

Statistical Analysis

Quantitative variables are presented as mean ± standard deviations or (for skewed data) as median together with minimum and maximum. Hardy–Weinberg equilibrium was assessed by using a Chi^2 test with 1 *df*. Categorical variables including treatment responses were compared by χ^2 statistics. When appropriate, odds ratios (ORs) together with 95% confidence intervals were estimated. Two-sample *t* tests or 1-way ANOVAs were applied in order to compare continuous variables if the data were approximately normally distributed. For skewed data, the nonparametric Kruskal–Wallis test was used. Furthermore, odds ratios together with 95 % confidence intervals have been calculated in order to determine the association of IL-18 genotypes with treatment response.

SAS software, release 9.01 (SAS Institute Inc., Cary, NC, USA) was used for all statistical calculations. Test results were considered statistically significant when $p<0.05$.

Results

Patients' characteristics are listed in Table 2. Altogether, 757 HCV patients comprising 382 (50.6%) males and 373 (49.4%) females were enrolled in the study. Additionally, 791 healthy subjects from the same geographical area served as controls. No statistically significant deviation from the Hardy–Weinberg equilibrium was observed.

In 84.4% of the patients, viral genotype was known. More than 70% of patients with known genotype presented with a viral genotype 1. In 67.9% (514/757) of the patients, a diagnostic liver biopsy was performed, whereas data regarding inflammatory activity were available in 64.1% (485/757) of the patients. In 59.4% (450/757) of the HCV-infected patients, complete information regarding genotype and treatment response was available for further analysis.

The association of genotypes and alleles with treatment response was tested in 475 HCV-infected patients. The −607C>A variant was found in similar frequency in patients and controls ($p=0.4742$, Table 3). When evaluating the −137G>C alteration, the CC genotype was relatively rare. Again, patients and controls did not differ significantly in their genotype frequencies ($p=0.2061$). Moreover, allele frequencies for both variants were similar in patients and controls ($p=0.2871$ or $p=0.1024$, respectively; Table 3).

Lack of significant differences in genotype frequencies was stated for the −607C>A variant as far as sex ($p=0.7254$), route of transmission ($p=0.8800$), viral genotype ($p=0.8530$), and other parameters were concerned (Table 4). Further analyses for the −137G>C SNP substantiated that genotype frequencies did not differ according to sex ($p=0.6785$), transmission route ($p=0.1960$), viral genotype ($p=0.8918$), and other parameters (Table 5).

Analysis of −137 and −607 *IL18* allele frequencies in relation to treatment response revealed significant differences (Tables 6, 7) for both variants. Patients with −607A had a higher chance for sustained virological response than those with the −607C allele ($p=0.0416$ OR 1.331; [CI 95%, 1.011–1.1752]). In parallel, −137C was associated with a higher probability for viral clearance ($p=0.0274$; CI 95%, 1.037–1.872). Since viral genotype has been identified as one of the strongest predictors for virological response, we performed subgroup analyses. Surprisingly, positive correlations were observed in genotype 1 HCV patients for −607A ($p=0.019$; OR=1.537 [CI 95%, 1.072–2.205]) and

Table 1 Sequences of Oligonucleotides Used for Characterization of *IL18* SNPs by Allele-Specific PCR

Primer	Sequence (5′–3′)
IL18 607control	F: 5′-CTTTGCTATCATTCCAGGAA-3′
IL18 607A	F: 5′-GTTGCAGAAAGTGTAAAAATTATTA **A**-3′
IL18 607C	F: 5′-GTTGCAGAAAGTGTAAAAATTATTA **C**-3′
IL18 607R	R: 5′-TAACCTCATTCAGGACTTCC-3′
IL18 137control	F: 5′-CCAATAGGACTGATTATTCCGCA-3′
IL18 137C	F: 5′-CCCCAACTTTTACGGAAGAAAA **C**-3′
IL18 137G	F: 5′-CCCCAACTTTTACGGAAGAAAA **G**-3′
IL18 137R	R: 5′-AGGAGGGCAAAATGCACTGG-3′

F forward primer, *R* reverse primer

Table 2 Characteristics of 757 Patients with Chronic Hepatitis C Infection

Variable		
Sex	Male	382 (50.6 %)
	Female	373 (49.4 %)
	Unknown	2
Age (years)		Mean ± SD (range)
		49.41 ± 12.21 (18-82)
Transaminases		
ALAT (U/L)		Median (range) 45 (1–2,036)
ASAT (U/L)		Median (range) 30 (5–971)
Viremia x 1000 IE/ml		Mean ± SD, 3,096 ± 11,698
		Median (range) 887 (0–247,000)
BMI (kg/m^2)		Mean ± SD (range)
		25.13 ± 4.82 (13.46–70.50)
BMI class	Normal	370 (54.3%)
	Pre-obese	226 (33.2%)
	Obese	85 (12.5%)
	Unknown	76
Transmission route	Transfusion	169 (48.1%)
	IV-drug abuse	121 (34.5%)
	Other	61 (17.4%)
	Unknown	406
Viral genotype	1	457 (71.5%)
	Non-1	182 (28.5%)
	Unknown	118
Fibrosis	Stage 1	201 (39.1%)
	Stage 2	158 (30.7%)
	Stage 3	155 (30.2%)
	Unknown	243
Inflammatory activity	Grade 1	189 (39.0%)
	Grade 2	251 (51.8%)
	Grade 3	45 (9.3%)
	Unknown	272
Treatment response	SR	176 (37.1%)
	Relapse or breakthrough	126 (26.5%)
	Nonresponse	173 (36.4%)
	Non-SR	299 (62.9%)
	Unknown	282

−607C (p=0.0302; OR=1.524; [CI 95%, 1.037–1.872]) with treatment response, whereas in patients with non-1 genotypes no association was demonstrated (p=0.8562 and 0.3770, respectively).

Similar results were observed when analyzing −607C>A and −137G>C genotype frequencies after stratification for treatment response. There was a trend towards a higher frequency of the AA genotype among patients with a treatment success (p=0.0869; Table 6) for the −607C>A variant. Concurrently, CC genotypes of the −137G>C variant were more prevalent among patients with a treatment success by tendency (p=0.0620, Table 7).

Again, in the subgroup of patients with viral genotype 1, –607 and −137 *IL18* genotypes turned out to be stronger predicting factors than in the non-1 viral genotype subgroup (Tables 6, 7). Additionally, odds ratios were calculated to further explore the role of genotypes for treatment response. When using a binary outcome (nonresponse or relapsed response versus sustained response), the odds ratio of genotypes CA versus CC was 1.558 [95% CI, 1.011–2.401] for −607 indicating that CA carriers have a higher probability of a sustained response. For −137 genotypes (GC versus GG), the corresponding odds ratio was 1.563 [95 % CI, 1.046–2.337].

Table 3 −607C>A and −137G>C *IL18* Allele and Genotype Frequencies in Patients with Chronic Hepatitis C Infection and Controls

		Patients (n=757)	Controls (n=791)	p Value
Genotype frequencies				
Locus −607	CC	276 (36.5%)	300 (37.9%)	0.4742
	CA	347 (45.8%)	369 (46.7%)	
	AA	134 (17.7%)	122 (15.4%)	
Locus −137	GG	386 (51%)	439 (55.5%)	0.2061
	GC	315 (41.6%)	299 (37.8%)	
	CC	56 (7.4%)	53 (6.7%)	
Allele frequencies				
Locus −607	C	899 (59.4%)	969 (61.3%)	0.2871
	A	615 (40.6%)	613 (38.7%)	
Locus −137	G	1087 (71.8%)	1177 (74.4%)	0.1024
	C	427 (28.2%)	405 (25.6%)	

Table 4 Clinical and Demographic Characteristics of Patients with Chronic Hepatitis C Infection According to the −607 *IL18* Genotype, Respectively

Parameters	Group size	CC (n=276)	AC (n=347)	AA (n=134)	p Value	Test
Age (years) mean ± SD		50.2±12.0	48.6±12.2	49.8±12.4	0.2661	ANOVA
BMI (kg/m^2) mean ± SD		25.2±4.3	25.1±4.9	25.1±5.5	0.9821	ANOVA
Sex (n (%))						
Male	382	138(36.1%)	172 (45.0%)	72 (19.4%)	0.7254	Chi^2
Female	373	138(37.0%)	173 (46.4%)	62 (16.6%)		
Transaminases median (range)						
ALAT (U/L)		48 (1–2,036)	44 (4–367)	44 (8–1,828)	0.9529	KW
ASAT (U/L)		30 (7–971)	30 (5–669)	30 (6–849)	0.9218	KW
Viremia/1,000 (IU/mL)		2349±3873	3083±7536	4662±24314	0.9363	KW
Median		859	985	784		
HCV genotype (n (%))						
1	457	164 (35.9%)	211 (46.2%)	82 (17.9%)	0.8530	Chi^2
non-1	182	65 (35.7%)	81 (44.5%)	36 (19.8%)		
Route of infection (n (%))						
Transfusion	169	63 (37.3%)	72 (42.6%)	34 (20.1%)	0.8800	Chi^2
IV-drug abuse	121	42 (34.7%)	58 (47.9%)	21 (17.4%)		
Other	61	19 (31.1%)	30 (49.2%)	12 (19.7%)		
Unknown	406	152 (37.4%)	187 (46.1%)	67 (16.5%)		
Liver histology						
Inflammation (n (%))						
0–1	189	75 (39.7%)	82 (43.4%)	32 (16.9%)	0.5107	CMH
2	251	88 (35.1%)	114 (45.4%)	49 (19.5%)		
3	45	15 (33.3%)	24 (53.3%)	6 (13.3%)		
Fibrosis (n (%))						
0–1	201	69 (34.3%)	91 (45.3%)	41 (20.4%)	0.6832	CMH
2	158	68 (43.0%)	63 (39.9%)	41 (20.4%)		
3–4	155	49 (31.6%)	78 (50.3%)	28 (18.1%)		

Symmetrically distributed data are presented as mean value ± standard deviation; skewed data as median and range; qualitative data are given in absolute and relative frequencies

ANOVA one-way ANOVA, *KW* Kruskal–Wallis test; *Chi^2* Chi^2 test, *CMH* Cochran–Mantel–Haenszel test

Table 5 Clinical and Demographic Characteristics of Patients with Chronic Hepatitis C Infection According to the −137 *IL18* Genotype, Respectively

Parameters	Group size	GG (n=386)	GC (n=315)	CC (n=56)	p Value	Test
Age (years) mean±SD		50.2±12.0	48.5±12.3	49.4±12.7	0.1849	ANOVA
BMI (kg/m^2) mean±SD		25.1±4.4	25.0±4.7	26.0±7.6	0.3527	ANOVA
Sex (n (%))						
Male	382	200 (52.3%)	156 (40.8%)	26 (6.8%)	0.6785	Chi^2
Female	373	185 (49.5%)	158 (42.4%)	30 (8.0%)		
Transaminases median (range)						
ALAT (U/L)		48 (1–2,036)	42 (4–422)	42.5 (8–346)	0.4401	KW
ASAT (U/L)		31 (5–971)	29 (7–669)	26.5 (6–333)	0.5902	KW
Viremia/1,000 (IU/mL)		2,558±6,830 median: 848	2,890±4,930 median: 912	7,591±36,192 median: 1107	0.4475	KW
HCV genotype (n (%))						
1	457	228 (49.9%)	194 (42.5%)	35 (7.6%)	0.8918	Chi^2
non-1	182	90 (49.5%)	76 (41.8%)	16 (8.8%)		
Route of infection (n (%))						
Transfusion	169	80 (47.3%)	70 (41.4%)	19 (11.4%)	0.1960	Chi^2
IV-drug abuse	121	60 (49.6%)	53 (43.8%)	8 (6.6%)		
Other	61	26 (42.6%)	29 (47.5%)	6 (9.8%)		
Unknown	406	220 (54.2%)	163 (40.1%)	23 (5.7%)		
Liver histology						
Inflammation (n (%))						
0–1	189	98 (51.8%)	75 (39.7%)	16 (8.5%)	0.9812	CMH
2	251	117(46.6%)	111 (44.2%)	23 (9.2%)		
3	45	26 (57.7%)	15 (33.3%)	4 (8.9%)		
Fibrosis (n (%))						
0–1	201	93 (46.3%)	90 (44.7%)	18 (9.0%)	0.7498	CMH
2	158	85 (53.8%)	61 (38.6%)	12 (7.6%)		
3–4	155	77 (49.6%)	64 (41.3%)	14 (9.0%)		

Symmetrically distributed data are presented as mean value ± standard deviation; skewed data as median and range; qualitative data are given in absolute and relative frequencies

ANOVA one-way ANOVA, *KW* Kruskal–Wallis test, *Chi^2* Chi^2 test, *CMH* Cochran–Mantel–Haenszel test

Discussion

This is the first study characterizing the impact of functional *IL18* promoter variants in a large cohort of patients with hepatitis C on treatment response and various additional clinical and biochemical features. Both the −137 and the −607 IL-18 promoter variants were significantly associated with treatment response in patients infected with HCV genotype 1.

There is a growing body of evidence that genetic host factors have a significant impact on outcome and resolution of HCV infection—both treatment-related and spontaneously occurring. A strong Th 1 antiviral immune response is considered a prerequisite for HCV elimination [30]. Furthermore, functional variants of involved cytokines were recently shown to be associated with HCV disease pathogenesis. Huang and coworkers demonstrated an association of the −764 IFN-γ alteration with spontaneous recovery of HCV infection and treatment response [16]. In a recent multicenter study, the functional 174C>G *IL6* variant was significantly associated with treatment response in acutely and chronically HCV-infected patients with HIV co-infection [14]. It was suggested that higher IL6 expression may favor Jak-STAT3-signaling pathways in the liver of affected subjects, thus, stimulating a strong antiviral response. Genetic alteration in *IL10*-encoding the main anti-inflammatory cytokine—were found to be associated with HCV clearance in African Americans in contrast to Americans with European ancestry from the same geographical area [31]. Others did not find a significant impact of *IL10* variants on severity and clearance of HCV infection [32, 33]. A European study reported a positive association of *IL10* receptor (*IL10R*) and *IL22* variants with outcome in HCV-infected patients [34].

The biological role of the proinflammatory IL-18 in hepatic pathology is complex and still not fully character-

Table 6 Treatment Response According to Allele Frequencies in Patients with Chronic Hepatitis C Infection (n=450)

	SR (*n* (%))	non-SR (*n* (%))	*p* Value	OR	95% CI
Locus −607					
All viral genotypes					
A	148 (45%)	214 (38%)	0.0416	1.331	1.011–1.752
C	184 (55%)	354 (62%)			
Genotype 1					
A	77 (48%)	175 (37%)	0.0190	1.537	1.072–2.205
C	85 (52%)	297 (63%)			
Non-1 genotype					
A	71 (42%)	39 (41%)	0.8562	1.048	0.630–1.743
C	99 (58%)	57 (59%)			
Locus −137					
All viral genotypes					
C	110 (33%)	149 (26%)	0.0274	1.369	1.037–1.872
G	222 (67%)	419 (74%)			
Genotype 1					
C	57 (35%)	124 (26%)	0.0302	1.524	1.040–2.233
G	105 (65%)	348 (74%)			
Genotype non-1					
C	53 (31%)	25 (26%)	0.3770	1.287	0.735–2.251
G	117 (69%)	71 (74%)			

SR patients with sustained response, *non-SR* patients with nonresponse/breakthrough, *OR* odds ratio, *95% CI* 95% confidence interval

Table 7 Treatment Response According to Genotype Frequencies in Patients with Chronic Hepatitis C infection (n=450)

	SR (*n* (%))	non-SR (*n* (%))	*p* Value	OR	95% CI
Locus −607					
All viral genotypes					
CC	50 (30 %)	115 (40 %)	0.0869	1.000	Reference
CA	84 (51 %)	124 (44 %)		1.558	1.011–2.401
AA	32 (19 %)	45 (16 %)		1.636	0.933–2.868
Genotype 1					
CC	21 (26 %)	96 (41 %)	0.0523	1.000	Reference
CA	43 (53 %)	105 (44 %)		1.872	1.037–3.379
AA	17 (21 %)	35 (15 %)		2.220	1.0521–4.689
Genotype non-1					
CC	29 (34%)	19 (40%)	0.6286	1.000	Reference
CA	41 (48%)	19 (40%)		1.414	0.639–3.128
AA	15 (18%)	10 (21%)		0.983	0.366–2.638
Locus −137					
All viral genotypes					
GG	71 (43%)	154 (54%)	0.0620	1.000	Reference
GC	80 (48%)	111 (39%)		1.563	1.046–2.337
CC	15 (9 %)	19 (7 %)		1.712	0.823–3.564
Genotype 1					
GG	31 (38%)	127 (54%)	0.0542	1.000	Reference
GC	43 (53%)	94 (40%)		1.874	1.099–3.194
CC	7 (9%)	15 (6%)		1.912	0.718–5.089
Genotype non-1					
GG	40 (47%)	27 (56%)	0.5912	1.000	Reference
GC	37 (44%)	17 (35%)		1.469	0.691–3.122
CC	8 (9%)	4 (8%)		1.350	0.370–4.932

SR patients with sustained response, *non-SR* patients with nonresponse/breakthrough, *OR* odds ratio, *95% CI* 95% confidence interval

ized. Activated macrophages, Kupffer cells, natural killer (NK) cells, and NK T cells are believed to be the main sources of IL-18 in the liver [35]. IL-18 potentiates liver injury due to the induction of IFN-γ in conjunction with IL-12. Okamura and coworkers demonstrated that anti-IL-18 antibodies prevented liver damage in mice primed with *Propionibacterium acnes* and subsequently challenged with LPS [14]. In a similar mouse model, anti-IL-18 antibodies diminished T-cell-mediated liver injury in leptin deficient (ob/ob) mice [16]. In *IL18* transgenic mice, upregulated IL-18 expression was associated with severe hepatic injury and spontaneous apoptosis of hepatocytes [35]. In contrast, IL-18 displayed features of a protective factor in viral infection, for instance, by inhibition of hepatitis B viral replication. In the livers of HBV transgenic mice, IL-18 stimulated natural killer cells and NK T cells to secrete IFN-γ. This coincided with a rapid and reversible inhibition of viral replication [36]. Others have demonstrated antiviral effects in models of herpes simplex virus and vaccinia virus infection [37, 38]. On the contrary, HIV replication was stimulated by IL-18 in a monocytic cell line [39].

Regarding the role of *IL18* variants in viral hepatitis, there is only limited data available. Zhang and coworkers performed analyses on −137 and −607 *IL18* variants in 231 Chinese patients with HBV infection and in 300 healthy controls. The frequency of the −137GG genotype was found to be significantly higher in the patient group leading to the assumption of a protective role of this allele in chronic HBV infection. In addition, the −607AA genotype was linked to a lower viral load [40]. Studying a population of 140 Thai HCV patients and 140 matched controls, the −607AA genotype was more frequently observed in HCV patients when compared with matched controls [21]. In a recent publication by An and coworkers, both the −137C and the −607A alleles were associated with spontaneous HCV clearance in African American intravenous drug users, when comparing 91 drug abusers who cleared the virus with 182 patients with persistent viral infection [41]. Bouzgarrou studied the outcome of HCV patients and IL18 serum levels in relation to the −137 and −607 variants [42]. When comparing HCV patients with different stages of disease (no cirrhosis, cirrhosis, and hepatocellular carcinoma (HCC), IL-18 serum levels increased with disease progression. Furthermore, the −607C allele was associated with cirrhosis and HCC.

In our study, no association of *IL18* promoter variants with severity of hepatic inflammation and fibrosis was apparent. In parallel, no association of *IL18* alterations with transaminase serum levels could be shown. Of note, alleles with lower transcriptional IL18 promoter activity (*−607A* and *−137C*) were associated with treatment response indicating a more complex role of IL-18 than previously assumed. IL-18 is not a mere proinflammatory Th1 cytokine but additionally modulates Th2 functions. Following this concept, downregulated IL18 expression enables an optimal Th1 and Th2 balance for viral eradication. The relevance of our finding is corroborated by the comparison of spontaneous hepatitis C viral eradication in IV drug abusers in association with *IL18* alterations [41]. In analogy to our study, alleles with low *IL18* promoter activity were associated with a higher eradication rate. In hepatitis B patients, the "low IL18" −137C allele was attributed a protective role when comparing 231 HBV patients and 300 normal controls from China [40].

Here, associations of *IL18* promotor alleles with treatment response were only observed in patients infected with HCV genotype 1. This patient subgroup represents a particular challenge in clinical practice since the rate of rapid and sustained virological response is lower than in genotype 2/3 HCV patients. Identifying predictive genetic markers bears the potential of a more individualized treatment regimen.

Acknowledgement The authors would like to thank Sigrid Hugues for helping with the preparation of the manuscript.

This work was supported by the German Competence Network for Viral Hepatitis (Hep-Net), funded by the German Ministry of Education and Research (BMBF, Grant No. 01 KI 0437, Project No. 10.1.3 Core Project No. 10.1 Genetic host factors in viral hepatitis and Genetic Epidemiology Group in viral hepatitis) and by the EU-Vigilanz network of excellence combating viral resistance (VIRGIL, Projekt No. LSHM-CT-2004-503359) and by the BMBF Project: Host and viral determinants for susceptibility and resistance to hepatitis C virus infection.

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