Cell Biochem Biophys (2015) 71:307–314
DOI 10.1007/s12013-014-0199-7

ORIGINAL PAPER

# Is rs8099917 Polymorphism of IL-28B Gene a Good Predictor of Response to Therapy of HCV than rs12979860? An Egyptian Study

**Olfat Shaker · Amal Rashad · Ghada Abd El Aziz · Maissa El Raziky**

Published online: 13 August 2014


**Abstract** Hepatitis C virus (HCV) infection is the major etiology of chronic liver disease. Polymorphisms in the IL-28B gene region are important in predicting outcome following therapy for chronic hepatitis C virus infection. The aim of this study was to detect the relationship between IL-28B polymorphism and responses to therapy in patients infected with genotype 4. This study included one hundred chronic hepatitis C patients infected with genotype 4, received PEG-IFNα2b plus ribavirin for 24 weeks, as well as, 20 healthy subjects serving as control. Clinical and laboratory parameters, including genetic variation near the IL-28B gene (rs8099917 and rs12979860), were assessed. The results of this study showed significant difference between responders and non-responders as regard SNPs in the interleukin 28B gene at rs8099917 and rs12979860. In rs8099917, TT genotypes had more frequency in responders than GG genotypes. On the other hand, CC genotype in rs12979860 had more frequency in responders than TT genotype. By multiple regression analysis, rs8099917 (TT), total bilirubin, and prothrombin time were independent factors affecting the response to treatment. This results demonstrate that in HCV genotype 4-infected patients, rs12979860 (CC) and rs8099917 (TT) genotypes may identify patients who are likely to respond to treatment. IL-28B SNPs are good predictors of response to combination therapy of HCV.

**Keywords** Hepatitis C · Interleukin 28B · Polymorphism · Interferon · Response

O. Shaker (✉) · A. Rashad
Medical Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Giza, Egypt
e-mail: olfatshaker@yahoo.com

G. A. E. Aziz
Medical Biochemistry, Faculty of Medicine, Beni Sueif University, Beni Suef, Egypt

M. E. Raziky
Endemic Medicine and Hepatology Department, Faculty of Medicine, Cairo University, Giza, Egypt

## Introduction

Hepatitis C virus (HCV) infection is a problem worldwide affecting approximately 170 million individuals. HCV causes chronic hepatitis [1]. Chronic HCV infection leads to cirrhosis in about 10–20 % of patients, increasing the risk of complications of chronic liver disease, including portal hypertension, ascites, hemorrhage, and Hepatocellular carcinoma [2].

Genotype 4 of HCV is the cause of approximately 20 % of the 170 million global case of CHC. Although rarely encountered in western nations, it is the most common variant of HCV in Africa and Middle East. In Egypt, the nation which has the highest global prevalence of HCV, more than 90 % of cases are caused by genotype 4 [3].

In the past, the best antiviral therapies produced a long-term virological remission (sustained virological response, SVR) in only 5–10 % of treated patients, but significant advances in treatment have increased the SVR rate almost to 54–61 %. These high response rates were obtained by modifying the standard interferon α by attaching a polyethylene glycol (PEG) moiety (pegylation) to produce a longer-active peginterferon [4].

This combination therapy is expensive, effective in only a certain proportion of patients who have HCV, and has many unpleasant adverse effects. Therefore, identifying

predictive factors of therapeutical response in patients with HCV is important [5].

Epidemiological, viral, and host factors have been associated with the differences in HCV clearance or persistence, and studies have demonstrated that a strong host immune response against HCV favors viral clearance. Thus, variation in genes involved in the immune response may contribute to the ability to clear the virus [6].

Interleukin 28B is a member of family of pleiotropic cytokines that exhibit potent antiviral, antiproliferative, apoptotic, and immune regulatory activities. These activities are controlled, in part, by a set of cellular genes that are rapidly induced upon binding of IL-28B to specific membrane-bound receptors [7].

Four genome-wide association studies concurrently provided the overwhelming evidence that single nucleotide polymorphisms (SNPs) of IL-28B on chromosome 19q13 contribute to IFN treatment response and spontaneous HCV clearance in HCV infection [8].

Single nucleotide polymorphism is the most common form of genetic variation that has been employed for the prediction of both disease progression and therapeutic response to interferon therapy [9]. Two of these SNPs, rs12979860 (located ~3 kb upstream of IL-28B) and rs8099917 (located ~8 kb upstream of IL-28B) were identified as the variant most strongly associated with SVR [10].

Therefore, the aim of our study was to detect single nucleotide polymorphisms in IL-28B gene (rs12979860 and rs8099917) on chromosome 19 in Egyptian HCV-infected patients. These patients are under treatment with interferon plus ribavirin (responders versus non-responders) and their results are compared with non-infected subjects (controls).

## Subjects and Methods

The study was conducted on 100 HCV chronically infected Egyptian patients (44 females and 56 males). All of them attended the liver unit of Tropical Medicine Department at Kasr El-Aini Hospital, Cairo University outpatient's Clinic to receive combined treatment of Interferon and Ribavirin during the period from 2002 to 2008. The diagnosis of chronic hepatitis was made by histological findings in liver biopsy specimens and/or by serum biochemical tests and peripheral blood cell counts. A written informed consent was obtained from patients to participate in the study in accordance with the ethical guidelines of the Declaration of Helsinki. As well as, 20 (11 females and 9 males) age- and sex-matched healthy controls were included in the study.

Patients chronically infected with HCV aged between 18 and 60 years had serological, virological, and histological diagnosis of chronic HCV, elevated ALT level above the upper limit of normal range within 6 months, and had not been previously treated with interferon-based therapy. The exclusion criteria were: Decompensated liver disease, hemoglobin <13 g/dl for men and <12 g/dl for women, white blood cell count of <3,000/mm$^3$, neutrophil count of <1500/mm$^3$, or platelet count of <100,000/mm$^3$, patients with hepatitis B surface antigen (HBsAg) seropositivity or infected with the human immunodeficiency virus (HIV), active schistosomiasis, serum creatinine above upper limit of normal, poorly controlled diabetes mellitus, hypertension, or psychiatric diseases, presence of ANA titre (antinuclear antibodies) >1/160, and TSH out of normal range (0.5–5 million units/l).

Each patient received subcutaneous injection of pegylated (Peg) IFN-α2b, at 1.5 μg/kg body weight once a week, combined with daily oral administration of ribavirin at a dosage which was determined based on the patient's body weight (600 mg for <60 kg, 800 mg for 60–80 kg, and 1000 mg for >80 kg). Successful treatment was ascertained based on SVR, defined as HCV RNA-negative 6 months after cessation of therapy.

According to response to treatment, patients were classified into two groups: Responders (60 patients) who had initial response to treatment with normalization of aminotransferases (ALT and AST) levels and clearance of the virus denoted by negative HCV RNA by PCR after 6 months of receiving treatment and those complete treatment for another 6 months and remain negative after completion of the treatment course, and Non-Responders (40 patients) who received treatment for 6 months and failed to clear the virus and give positive HCV RNA by PCR.

Liver biopsies were evaluated by a single expert pathologist and scored using the Ishak system [11] in separate reports for grading and staging. The score for staging ranged from 0 (no fibrosis) to 6 (cirrhosis).

### Methods

Venous blood samples were collected from patients and controls. Serum was separated and used for biochemical characterization of HCV-specific antibody titers by ELISA and enzymatic evaluation of liver functions and complete blood picture. These tests were done at weeks 1, 2, 4 and monthly thereafter during treatment to detect the development of any adverse side effects to the drugs necessitating dose modification, temporary or permanent stoppage of treatment. Markers of Hepatitis virus including HBsAg, Anti-HBc, and Anti-HCV were assessed by routine methods using commercially available assays. Viral RNA was extracted using viral RNA extraction kit (Qiagen) and stored at −80 °C. HCV RNA titer was measured before and after treatment by Real-time PCR. Thyroid function

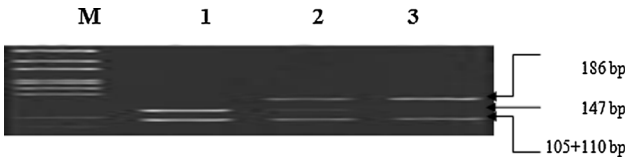


**Fig. 1** Agarose gel electrophoresis 3.5 % stained by ethidium bromide showing gene amplification of IL-28B. *Lanes* 1–3 showing PCR amplification after *Mae* III restriction endonuclease cleavage. *M* is molecular DNA marker (50 base each). *Lane* 1 Homozygous (GG) at (147, 105 + 110 bp), *Lane* 2 Heterozygous (TG) at (186, 147, 105 + 110 bp), *Lane* 3 *Homozyg*ous (TT) at (186, 105 + 110 bp)

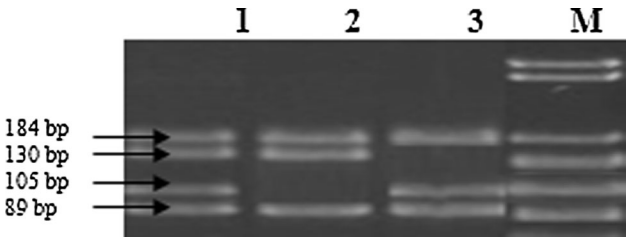


**Fig. 2** Agarose gel electrophoresis 3.5 % stained by ethidium bromide showing gene amplification of IL-28B. *Lanes* 1–3 showing PCR amplification after BstU I restriction endonucleases cleavage, *M* is molecular DNA marker (50 base each). *Lane* 1 Heterozygous (CT) at (184, 130, 105, 89 bp), *Lane* 2 Homozygous (TT) at (184, 130, 89 bp), *Lane* 3 Homozygous (CC) at (184, 105, 89 bp)

tests (T3, T4, and TSH) were done (using Immulite) to all patients before receiving the treatment. Autoantibodies (ANA and Anti-DNA) were detected using Immunofluorescence kits.

### Analysis of SNPs in the Promoter Region of IL-28B

Genomic DNA was extracted from peripheral blood mononuclear cells in the collected EDTA blood using the QIAamp DNA minikit (Qiagen, USA) for genotyping of the two SNPs of IL-28B gene. A polymerase chain reaction (PCR) was performed in a final volume of 50 µl to which 50 pmol of each primer (forward and reverse) and 200 ng genomic DNA template were added to (5 µl of 10× reaction buffer with $MgCl_2$, 5 µl 10× Taq polymerase buffer, 2 mmol dNTPs, and two units Taq DNA polymerase).

For amplification of rs8099917, oligonucleotide primers: 5′-TTC ACC ATC CTC CTC TCA TCC CTC AT-3′ (sense) and 5′-TCC TAA ATT GAC GGG CCA TCT GTT TC-3′ (antisense) were used. For amplification of rs12979860, oligonucleotide primers: 5′-AGG GCC CCT AAC CTC TGC ACA GTC T-3′ (sense), and 5′-GCT GAG GGA CCG CTA CGT AAG TCA CC-3′ (antisense) were used. The cycling condition was initial denaturation at 94 °C for 10 min, followed by 40 cycles of: denaturation at 94 °C for 1 min, annealing at 58 °C for 40 s, extension at 72 °C for 1 min, and final extension cycle of 72 °C for 10 min. The PCR amplification products were 441 base pair (bp) and 403 bp for rs8099917 and rs12979860, respectively. The PCR products were checked by DNA 2 % agarose gel electrophoresis.

For the RFLP assay for the rs8099917 genotype, 20 µl of amplicons were digested with 1 U of *Mae* III restriction endonuclease (Roche Molecular Systems, Branchburg, NJ, USA) at 55 °C for 2 h. *Mae* III digestion of allele TT yields fragments of 105, 110, and 186 base pairs, whereas DNA containing the allele GG polymorphism yields fragments of 105, 110, 39, and 147 base pairs (Fig. 1).

For the RFLP assay for the rs12979860 genotype, 20 µl of amplicons were digested with 5 U of *Bst*U I restriction endonuclease (New England Biolabs, MA, USA) at 60 °C for 2 h. *Bst*U I digestion of allele CC yields fragments of 184, 105, 89, and 25 base pairs, whereas DNA containing the allele TT polymorphism yields fragments of 184, 130, and 89 base pairs. Restriction digestion products for each were separated on 3 % agarose gels stained with ethidium bromide for visualization on a UV trans-illuminator (Fig. 2).

### Statistical Analysis

Statistical Package of social science version 15.0 was used for analysis of data. The difference between two means was statistically analyzed using the student's (*t*) test and Paired *t* test used in comparison between the difference of parameters before and after the treatment, when Coefficient of variance (CV) was less than 30 %. Mann–Whitney *U* test was used when CV is equal to or more than 30 %. Stepwise multiple regression analysis was performed to show factors affecting the response to treatment. Univariate logistic regression analysis was done to show the significant predictors affecting the response to interferon therapy of hepatitis C patients. *P* values $<0.05$ were considered statistically significant.

## Results

One hundred patients were 60 men and 40 women with their mean age 39.18 ± 7.80 years. All were genotype 4a, and were treated with PEG-IFNα 2b 1.5 µg/kg weekly and ribavirin (800–1000 mg/day) for 24 weeks.

As regard the genotype and alleles frequencies of IL-28B gene in patients and control (Table 1), rs8099917 genotype showed no significant difference in SNPs between patients

**Table 1** Prevalence of SNPs and allele frequencies in IL-28B gene in chronic hepatitis C patients and controls

| SNPs | Number (%) | | | *P* value | Allele frequency | | *P* value |
|---|---|---|---|---|---|---|---|
| *rs8099917* | T/T | T/G | G/G | 0.08 | T allele | G allele | 0.03* |
| Patients | 47 (47 %) | 37 (37 %) | 16 (16 %) | | 65.5 % | 34.5 % | |
| Control | 20 (20 %) | 55 (55 %) | 25 (25 %) | | 55 % | 45 % | |
| *rs12979860* | C/C | C/T | T/T | 0.001* | C allele | T allele | 0.0001* |
| Patients | 54 (54 %) | 34 (34 %) | 12 (12 %) | | 71 % | 29 % | |
| Control | 15 (15 %) | 45 (45 %) | 40 (40 %) | | 37.5 % | 62.5 % | |

* A statistically significant difference

**Table 2** Prevalence of SNPs and allele frequencies in IL-28B gene in responders and non-responders to treatment

| SNPs | Number (%) | | | *P* value | Allele frequency | | *P* value |
|---|---|---|---|---|---|---|---|
| *rs8099917* | T/T | T/G | G/G | 0.0001* | T allele | G allele | 0.0001* |
| Responders | 41 (68.3 %) | 16 (26.7 %) | 3 (5 %) | | 81.7 % | 18.3 % | |
| Non-responders | 6 (15 %) | 21 (52.5 %) | 13 (32.5 %) | | 42.2 % | 58.8 % | |
| *rs12979860* | C/C | C/T | T/T | 0.0001* | C allele | T allele | 0.0001* |
| Responders | 44 (73.3 %) | 11 (18.3 %) | 5 (8.3 %) | | 82.5 % | 17.5 % | |
| Non-responders | 10 (25 %) | 23 (57.5 %) | 7 (17.5 %) | | 53.7 % | 46.3 % | |

* A statistically significant difference

**Table 3** Multivariate regression analysis showing factors affecting efficacy of treatment

| Variables | B | 95 % C.I | *P* value |
|---|---|---|---|
| Constant | 2.2 | 1.6–2.8 | 0.0001* |
| rs8099917 (TT) | 0.3 | 0.4–0.2 | 0.0001* |
| Total bilirubin | −0.2 | −0.3–0.08 | 0.002* |
| Prothrombin time | −0.06 | −0.1– −0.01 | 0.01* |

* *P* value is significant

**Table 4** Logistic regression showing factors affecting the response to treatment

| Variables | Odds ratio | 95 % C.I | *P* value |
|---|---|---|---|
| Age (years) | 0.98 | 0.91–1.06 | 0.7 |
| ALT (IU/L) | 1.01 | 0.99–1.03 | 0.2 |
| AST (IU/L) | 1.01 | 1.0–1.03 | 0.1 |
| Total bilirubin (mg/dl) | 0.17 | 0.01–2.31 | 0.18 |
| Direct bilirubin(mg/dl) | 0.25 | 0.001–60.4 | 0.6 |
| Alkaline phosphatase (IU/L) | 0.99 | 0.96–1.0 | 0.2 |
| Albumin | 7.91 | 0.84–74.43 | 0.07 |
| AFP | 0.98 | 0.90–1.06 | 0.6 |
| PT | 0.67 | 0.45–0.99 | 0.04* |
| PCR | 1.0 | 1.0–1.0 | 0.1 |
| Fibrosis (1) | 0.0007 | 0.0–2005 | 0.005* |
| Fibrosis (2) | 0.0001 | 0.0–1865 | 0.8 |
| Fibrosis (3) | 0.0007 | 0.0–2282 | 0.8 |
| Viremia (− ve) | 1.1 | 0.2–7.4 | 0.8 |
| Viremia (+ ve) | 1.2 | 0.3–4.9 | 0.9 |
| rs8099917 (TT vs. GG) | 29.6 | 6.47–135.3 | 0.0001* |
| rs8099917 (T allele vs. G allele) | 3.86 | 2.06–7.027 | 0.001* |
| rs12979860 (CC vs. TT) | 6.16 | 1.617–23.46 | 0.007* |
| rs12979860 (C allele vs. T allele) | 4.056 | 2.13–7.7 | 0.0001* |

* *P* value is significant if <0.05; Odds ratio = $P/(1-P)$. *P* is the probability that the event *y* occurs; *C.I* Confidence interval (it is an interval in which true population parameters fall)

and controls, however, there was a significant difference in the allele frequencies between them. On the other hand, rs12979860 genotype showed a significant difference in SNPs as well as the allele frequencies between patients and controls.

According to response to interferon treatment, patients were classified into two groups: responders included sixty patients and non-responders included forty patients in which the mean values of ALT, AST, alkaline phosphatase, prothrombin time, and HCV RNA were significantly lower in responders than non-responders ($P < 0.0001$ for all of them), however, no significant difference between them in the mean values of direct and total bilirubin, albumin, and α-fetoprotein. As shown in Table 2, there was significant difference in the prevalence of SNPs, as well as, the allele frequencies in IL-28B gene (both rs8099917 and rs12979860) between responders and non-responders to interferon therapy of chronic hepatitis C patients.

Univariate correlation analysis showed that total bilirubin ($r = -0.5$, $P < 0.0001$), direct bilirubin ($r = -0.4$, $P < 0.0001$), alkaline phosphatase ($r = -0.2$, $P = 0.03$), prothrombin time ($r = -0.4$, $P < 0.0001$), α-fetoprotein ($r = -0.2$, $P < 0.05$), fibrosis stage ($r = -0.3$, $P < 0001$), and rs12979860 (CT) ($r = -0.4$, $P < 0.0001$) were significantly negatively associated with response to treatment, while rs8099917 (TT) ($r = 0.5$, $P < 0.0001$) and albumin

($r = 0.3$, $P = 0.002$) were positively associated with SVR. Further multivariate analyses were conducted using significant factors identified by the univariate analysis (Table 3). The multiple regression analysis showed that only rs8099917 (TT), total bilirubin, and prothrombin time were independent risk factors for response to treatment.

Logistic regression analyses were performed to show the factors that could affect and predict the response of chronic hepatitis C patients to interferon treatment. Prothrombin time, stage 1 fibrosis, and polymorphisms at rs8099917 (TT vs. GG genotype and T allele vs. G allele) and at rs12979860 (CC vs.TT and C allele vs. T allele) were the independent predictors affecting the treatment in HCV patients as shown in Table 4.

## Discussion

The genetic variations in the region near the IL-28B gene on chromosome 19, coding for IFN-λ3, recently reported to be associated with treatment response in individuals infected with HCV, they have a potential to better identify patients with HCV infection who are likely to benefit from PEG-IFN/ribavirin therapy, and they may reveal mechanisms associated with viral clearance and immunity [12].

Our study revealed that at the end of treatment, virological response (ETVR) to interferon was 60 % (60 % responders and 40 % non-responders), which is comparable to the result of Kurbanov et al. [13], who studied individuals infected with genotype 4 with about 50 % response to treatment (80 out of 162 patients), and also with Rauch et al. [8], who included individuals infected with HCV genotypes 1, 2, 3, or 4 with the clearance rate.

The results of our study revealed that polymorphisms at (rs12979860) and (rs8099917) of IL-28B were associated with significant difference between HCV patients and controls, also significant difference between responders and non-responders to interferon therapy was found.

As regards the percentage distribution of different genotypes of (rs12979860) in responders, we found that the CC genotype had greater percentage than CT and TT genotypes (73.3, 18.3, and 8.3 %, respectively). Furthermore, CC genotype is associated with sixfold (OR = 6.16, 95 % CI = 1.6–23) greater rate of SVR versus TT genotype.

In particular, numerous studies have shown that individuals with the C/C genotype at the rs12979860 SNP have higher rates of rapid SVR to treatment with PEG-IFN and Ribavirin, compared to those carrying the T allele (C/T and T/T genotypes) [14, 15]. Also, Kurbanov et al. [13] found that in genotype 4, the protective C allele was more common in those with spontaneous clearance (76.3 vs. 57.9 %; $P < 0.0006$). Individuals with clearance were 3.4 (95 %confidence interval, 1.8–6.5) times more likely to have C/C genotype, and also Ghany et al. [16] found that in patients of European ancestry, the CC genotype is associated with twofold (95 % confidence interval 1.8–2.3) greater rate of SVR than the TT genotype with similar ratios in both the African–American threefold (95 % confidence interval 1.9–4.7) and the Hispanic twofold (95 % confidence interval 1.4–3.2) population groups. Furthermore, Ge et al. [10] showed that stratification of patients by ethnicity indicated that the strength of the protective C/C effect was similar in individuals of African and European ancestry (OR 50.32 and 50.38, respectively).

As regards alleles frequencies (C vs. T), we detected significant difference between responders and non-responders and the C allele was associated with higher response rate than T allele (OR = 4.056, 95 % confidence interval = 2.13–7.7).

This is in accordance with previous studies [17, 18] which found that there were significant differences in allele frequencies (C vs. T) between the responders and non-responders, where the C allele showed greater frequencies in the responders than in the non-responders (80.3 and 66.7 %, respectively) of European ancestry and African ancestry (56.2 % and 37 %, respectively).

The underlying mechanism for this finding is not clear, as rs12979860 CC does not seem to negatively influence the replication of HCV at least in untreated patients. This SNP has strong linkage disequilibrium with a non synonymous coding variant in the IL28 gene. Thus, it is possible that changes in rs12979860 genotype are associated with abnormalities in the IFN-λ3 signal transduction pathway, although functional data are lacking [10]. IFN-λ1, another type of interferon III inhibits HCV replication, increases the levels of interferon-stimulated genes, and enhances the antiviral effect of interferon-α [19]. It is conceivable that IFN-λ3, a closely related cytokine with activity against other viruses comparable to that of IFN-λ1, works in a similar way against HCV [20].

As regard genotypes and alleles of single nucleotide polymorphism of the IL-28B gene at nucleotide (rs8099917), we found that TT genotype had higher prevalence (68.3 vs. 15 %) in responders than non-responders. In addition, patients with TT genotype had more than 29-fold to respond to treatment than those with GG genotype (OR = 29.6 and CI = 6.47–135.3). Also, T allele is more than threefold to respond to treatment than G allele (OR = 3.86 and CI = 2.06–7.027). So, TT genotype of rs8099917 is an important predictor for SVR.

This finding was in accordance with Sheppard [21, 22], who found that the TT genotype was significantly associated with better rapid viral response and SVR rates. Also, some authors [23–25] found that TT genotype was an important predictor for SVR. On the other hand, in the

studies of HCV genotype 2-infected Asian patients, the rs8099917 TT genotype was not associated with SVR [26]. Rauch et al. [8] also showed no effect of rs8099917 in HCV genotype 2/3-infected patients in a smaller cohort.

In addition, we found higher prevalence in GG genotype in non-responders (32 %) versus responders (5 %). As regard allele percentage, there is also more prevalence of G allele in non-responders (58.7 %) than in responders (18.3 %). Also, we found that homozygous carriers of the rs8099917 G allele had more prevalence (58.7 %) than T allele (42.2 %). So, GG genotype of (rs8099917) is an important predictor for response failure.

This finding agrees with Rauch et al. [8], who found several SNPs near the IL-28B locus that were associated with chronic HCV infection at a genome-wide significance level. The strongest association with treatment failure was found with rs8099917. Individuals carrying one or two copies of the rs8099917 risk G allele had higher risks of treatment failure compared with individuals carrying the common genotype TT. Also, Suppiah et al. [27], and Tanaka et al. [28] found multiple SNPs that were significantly associated with the response to HCV therapy. Homozygous carriers of the rs8099917 G allele had more than a twofold higher risk to fail HCV therapy compared with TT homozygous.

Ge et al. [10] explained these results that the associated SNP is inherited together with SNPs in or near the IL-28B gene. Therefore, individuals who carry rs8099917 risk alleles are highly likely to carry polymorphism in the promoter or coding region of IL-28B. Several efforts were already performed to identify the functional variant that is critical for the biological effects.

Rauch et al. [8] also explained that the large majority (97 %) of individuals homozygous for the risk allele (G allele) carried haplotypes characterized by multiple SNPs in the IL-28B gene. In contrast, 75 % of the individuals homozygous for the protective T allele carried haplotypes without polymorphisms in IL-28B. Together, these results indicate that rs8099917 tags genetic polymorphisms in IL-28B that might alter expression and/or function of IL-28B.

Both Tanaka et al. [27] and Suppiah et al. [28] explained the consequences of the IL-28B polymorphisms on the expression and function of IL-28B, which are yet to be established. As the causal variant has not yet been identified, it is impossible to delineate whether the polymorphisms influence cytokine levels or function. Carriage of IL-28B risk alleles could result in lower IL-28B expression levels, or could diminish cytokine functions. Both mechanisms would result in lower interferon-stimulated genes (ISG) expression levels. This could diminish the antiviral response to HCV infection and explain the association between carriage of IL-28B risk alleles and poorer control of HCV infection. However, it is important to note that an up-regulation of ISGs before starting HCV treatment has been clearly associated with worse treatment response.

By univariate correlation analysis, showing the factors affecting response to interferon therapy in hepatitis C patients, only total bilirubin, direct bilirubin, alkaline phosphatase, albumin, AFP, PT, fibrosis, SNP at (rs12979860) (CT), and SNP (rs8099917) (TT) were found to be significant predictors for response.

Wang et al. [29] demonstrate that prothrombin time ($P = 0.002$), body mass index ($P = 0.003$), fibrosis score of liver histology ($P = 0.002$), and aspartate aminotransferase ($P = 0.0.17$) were found to be significant prognostic factors of interferon response in HCV.

Also, Hosogaya et al. [30] analyzed the factors that can best estimate the therapeutic efficacy of IFN and revealed that HCV genotype, HCV RNA, and total protein (TP) are shown to be most closely correlated with the therapeutic efficacy, followed by IFN dose, body mass index (BMI), bilirubin, and AST. AST and bilirubin levels represent the level of hepatic damage or impairment of its function, and they should be negatively correlated with the IFN therapeutic efficacy.

Furthermore, Gad et al. [31] reported that among genotype 4 chronic hepatitis C patients, severe fibrosis, severe steatosis, treatment with standard interferon, and a high serum AFP level were all negatively associated with SVR.

However, by multivariate regression analysis showing the significant predictors affecting response to interferon therapy in hepatitis C patients, only Total bilirubin, PT, and SNP at (rs8099917) (TT) were found to be significant predictors for response.

Comparable with results of Ge et al. [10] and Rauch et al. [8], IL-28B variants remained highly associated with response to HCV therapy after adjusting for known predictors of treatment response (HCV RNA levels, fibrosis scores, gender, and HCV genotypes). Remarkably, in multivariate analysis, IL-28B variants were the strongest predictors of response to therapy, with stronger effects compared with the well-established predictors of treatment response (baseline HCV RNA levels, fibrosis scores, gender, and ethnicity). Taken together, these studies suggest that IL-28B genotype is a determinant of treatment-induced clearance in HCV genotype 4 infections.

## Conclusion

In HCV genotype 4-infected patients, rs12979860 (CC) and rs8099917 (TT) genotypes may identify patients who are likely to respond to treatment. This polymorphism explains much of the difference in response between

different genotypes and different alleles. Further studies of the genetic determinants associated with risk of liver disease progression in hepatitis C should represent a high priority of research, with the aim of both allowing a better understanding of disease pathogenesis and guiding an improved patient-selection process for eligibility to antiviral therapy.

**Acknowledgments** This work was supported by the Egyptian Science and Technology Development Fund (Project number 1512 to PI; Olfat Shaker).

**Conflict of interest** The authors have declared that there is no conflict of interest.

## References

1. Shepard, C. W., Finelli, L., & Alter, M. J. (2005). Global epidemiology of hepatitis C virus infection. *The Lancet Infectious Diseases, 5*, 558–567.
2. Jou, J. H., & Muir, A. J. (2008). In the clinic. Hepatitis C. *Annals of Internal Medicine, 148*, 1–16.
3. Kamal, S. M., & Naser, L. A. (2008). Hepatitis C genotype4: what we know what we don't yet know. *Hepatology, 47*, 1371–1383.
4. Fried, M. W., Shiffman, M. L., & Reddy, K. R. (2002). Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *New England Journal of Medicine, 347*(13), 975–982.
5. Strader, D. B., Wright, T., Thomas, D. L., & Seeff, L. B. (2004). American Association for the Study of Liver Diseases. Diagnosis, management, and treatment of hepatitis C. *Hepatology, 39*(4), 1147–1171.
6. Rehermann, B., & Nascimbeni, M. (2005). Immunology of hepatitis B virus and hepatitis C virus infection. *Nature Reviews Immunology, 5*, 215–229.
7. Pestka, S., Krause, C. D., & Walter, M. R. (2004). Interferons, interferon-like cytokines, and their receptors. *Immunological Reviews, 202*, 8–32.
8. Rauch, A., Kutalik, Z., & Descombes, P. (2010). Genetic variation in IL28B isassociated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology, 138*, 1338–1348.
9. Houldsworth, A., Metzner, M., Rossol, S., Shaw, S., Kaminiski, E., Demaine, A. C., et al. (2005). Polymorphism in the IL12B gene and outcome of HCV infection. *Journal of Interferon and Cytokine Research, 25*, 271–276.
10. Ge, D., Fellay, J., & Thompson, A. J. (2009). Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature, 461*, 399–401.
11. Ishak, K., Baptista, A., & Bianchi, L. (1995). Histological grading and staging of chronic hepatitis. *Journal of Hepatology, 22*, 696–699.
12. Afdhal, N. H., McHutchison, J. G., Zeuzem, S., Mangia, A., Pawlotsky, J. M., Murray, J. S., et al. (2011). Hepatitis C pharmacogenetics. *Hepatology, 53*, 336–345.
13. Kurbanov, F., Abdel-Hamid, M., Latanich, R., Astemborski, J., Mohamed, M., Mikhail, N. M., et al. (2011). Genetic polymorphism in IL28B is associated with spontaneous clearance of hepatitis C virus genotype 4 infection in an Egyptian cohort. *Journal of Infectious Diseases, 204*(9), 1391–1394.
14. Shaker, O. G., & Sadik, N. A. (2012). Polymorphisms in interleukin-10 and interleukin-28B genes in Egyptian patients with chronic hepatitis C virus genotype 4 and their effect on the response to pegylated interferon/ribavirin-therapy. *Journal of Gastroenterology and Hepatology, 27*(12), 1842–1849.
15. Khairy, M., Fouad, R., Mabrouk, M., El-Akel, W., Awad, A. B., Salama, R., et al. (2013). The impact of interleukin 28b gene polymorphism on the virological response to combined pegylated interferon and ribavirin therapy in chronic HCV genotype 4 infected egyptian patients using data mining analysis. *Hepatitis Monthly, 13*(7), e10509.
16. Ghany, M. G., Strader, D. B., Thomas, D. L., & Seeff, L. B. (2009). American Association for the Study of Liver Diseases. Diagnosis, management and treatment of hepatitis C. *Hepatology, 49*(4), 1335–1374.
17. McHutchison, J. G., Lawitz, E. J., & Shiffman, M. L. (2009). Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitisC infection. *New England Journal of Medicine, 361*(6), 580–593.
18. Thomas, D. L., Thio, C. L., & Martin, M. P. (2009). Genetic variation in IL28B and to spontaneous clearance of hepatitis C virus. *Nature, 461*, 780–798.
19. Marcello, T., Grakoui, A., Barba-Spaeth, G., Machlin, E. S., Kotenko, S. V., MacDonald, M. R., et al. (2006). Interferons α and λ inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. *Gastroenterology, 131*, 1887–1898.
20. Dellgren, C., Gad, H. H., Hamming, O. J., Melchjorsen, J., & Hartmann, R. (2009). Human interferonlambda3 is a potent member of the type III interferon family. *Genes and Immunity, 10*, 125–131.
21. Sheppard, P., Kindsvogel, W., Xu, W., Henderson, K., Schlutsmeyer, S., Whitmore, T. E., et al. (2003). IL-28, IL-29 and their class II cytokine receptor IL-28R. *Nature Immunology, 4*, 63–68.
22. Fried, M. W., Hadziyannis, S. J., Shiffman, M., Messinger, D., & Zeuzem, S. (2011). Rapid virological response is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection. *Journal of Hepatology, 55*(1), 69–75.
23. Aparicio, E., Parera, M., Franco, S., Prez-Alvarez, N., & Tural, C. (2010). IL28B SNP rs8099917 is strongly Associated with pegylated interferon-a and ribavirin therapy treatment failure in HCV/HIV-1 Coinfected Patients. *PLoS ONE, 5*, e13771.
24. Grebely, J., Petoumenos, K., Hellard, M., Matthews, G. V., & Suppiah, V. (2010). Potential role for Interleukin-28B genotype in treatment decision-making in recent hepatitis C virus infection. *Hepatology, 52*, 216–224.
25. Stättermayer, A. F., Stauber, R., Hofer, H., Rutter, K., & Beinhardt, S. (2011). Impact of IL28B Genotype on the Early and Sustained Virologic Response in Treatment-naive Patients With Chronic Hepatitis C. *Clinical Gastroenterology and Hepatology, 9*(4), 344–350.
26. Yu, M., Huang, C., Huang, J., Chang, N., Yang, J., Lin, Z., et al. (2011). Role of interleukin-28B polymorphisms in the treatment of hepatitis C virus genotype 2 infection in Asian patients. *Hepatology, 53*(1), 7–13.
27. Suppiah, V., Moldovan, M., Ahlenstiel, G., Berg, T., Weltman, M., & Abate, M. L. (2009). IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nature Genetics, 41*, 1100–1104.
28. Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M., Matsuura, K., & Sakamoto, N. (2009). Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic Hepatitis C. *Nature Genetics, 41*, 1105–1109.
29. Wang, T. S., Mo, L. R., Lim, R. T., Kuo, J. J., Chang, K. K., & Wu, J. J. (2008). Artificial neutral net work model is superior to logistic regression model in predicting treatment outcomes of interferon-combination of therapy in patient of chronic hepatitis C. *Intervirology, 51*, 14–20.

30. Hosogaya, S., Ozaki, Y., Enomoto, N., & Akahine, Y. (2006). Analysis of prognostic factors in therapeutic responses to interferon in patients with CHC. *Translational Research, 148*, 79–86.

31. Gad, R. R., Males, S., El Makhzandy, H., Showman, S., Hassan, A., & Atallah, N. (2008). Predictors of sustained virological response in patients infected with hepatitis C virus genotype4. *Liver International, 28*, 1112–1119.