Role of IL28B Gene Polymorphisms in Response to the Standard of Care Treatment in Egyptian Patients with Chronic HCV Genotype Four

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Abstract: Egypt has the highest prevalence of HCV (predominantly genotype 4) all over the world with 9% countrywide and up to 50% in certain rural areas. Combined PEG-IFN and ribavirin is still the only standard of care treatment in spite of its side effects, high costs and low sustained virological response rates. Hence, this provides a compelling reason for the identification of biomarker predictors of disease response to treatment. Genetic variation in the interleukin 28B (IL28B) genes has been associated with the response to interferon-alfa/ribavirin therapy in hepatitis C virus (HCV) genotype 1-infected patients, however its importance for HCV genotype 4-infected patients is still unevident. This study aimed at assessing whether specific IL28B gene polymorphisms (SNPs), known as rs8099917 and rs12979860 could predict treatment outcomes among chronic HCV genotype4 patients treated with the standard of care treatment. Methods: One hundred of naïve chronic HCV patients were selected and submitted to combined interferon/ ribavirin therapy, 48% of them have sustained viral response and (SVR) while the remaining 52% failed to respond (non responders). SNPs for rs12979860 and rs8099917 were done by PCR-RFLP technique for all patients before therapy. The CC genotype of rs12979860 was identified in 39 patients, 34 of them (87.2%) achieved SVR, while the CT heterozygous was detected in 51 (51%) patients, 13 of them achieved SVR (25.5%) and the TT was found in 10 patients and only one of them (10%) was responder. The SVR was significantly associated with CC genotypes as compared to other two genotypes ($p<0.001$), but TT genotype was associated with failed response to therapy. The TT homozygous of rs8099917 genotype was detected in 46 (46%) of overall HCV patients, 37 of them (80.4%) achieved SVR. The GT heterozygous was detected in 42 (42%) of HCV patients, SVR was achieved in 9 (21.4%) of them. While, the GG genotype was found in 12 patients and two of them only (16.7%) were responders. Conclusion: These data suggest that host genetics may be useful for the prediction of treatment outcomes and that IL28B SNP genotype is an important predictive biomarker for SVR in patients with HCV genotypes 4. Further studies based on a larger number of patients are necessary to investigate the present results.
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Introduction

Egypt has the highest prevalence of HCV worldwide with 9% countrywide and up to 50% in certain rural areas (**Kamal and Nasser, 2008**) and the highest prevalence of HCV-4, (previously called the Egyptian genotype) which is responsible for almost 90% of infections and is considered a major cause of chronic hepatitis, liver cirrhosis, hepatocellular carcinoma, and liver transplantation in the country (**Abdel-Hamid *et al.*, 2007**).

The published studies estimate the overall rates of spontaneous resolution in acute HCV-4 infections to range between 20% and 50%, which is not historically different than other genotypes (**Kamal *et al.*, 2006**). The fibrosis progression rate in patients with chronic HCV-4 was 0.1_0.06 fibrosis units per year, with significantly higher grading and staging scores in Egyptian patients infected with HCV-4a (**Roulot *et al.*, 2007**).

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 and 40–50% in genotype 1 patients can be cured, the response rate of genotype 4 in many clinical repots is showing SVR rates exceeding 60% (**Kamal and Nasser, 2008**). There is no dought that the high treatment cost presents a high economic burden in developing country like Egypt, necessitating a more meticulous research on predictors of (SOC) treatment response. Such as viral factors as viral load,genotype and host factors as steatosis,gender, liver cirrhosis and genetics as IL28 polymorphisms.

Viral 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 virological response (**Kau *et al.*, 2008**).

Cytokines are among the predominant mechanisms of host defence against infection; they induce inflammatory response that often leads to tissue injury, but also act as antiviral effectors as well. Cytokine synthesis capacity has a significant genetic component, which explains why there are differences between individuals in their ability to produce cytokines; it may also be due to single-nucleotide polymorphisms (SNPs) within the coding regions of cytokine genes. Cytokine genes are polymorphic and some of these variants modify the production of the specific cytokines and affect the host immune response. In HCV infection, secretion of inappropriate amounts of cytokines may be associated with chronicity or resistance to interferon (IFN) treatment (**Thio, 2008**).

Recently, three independent research groups have reported the results of separate genome-wide association studies (GWAS), supporting a strong association of two single nucleotide polymorphisms (change at a particular position in the gene sequence) of the IL28B gene on chromosome 9, which encodes type III interferon lambda (IFN-λ-3), rs8099917 and rs12979860, with treatment outcomes of Peg-IFN α-2a plus RBV therapy (**Ge *et al.*, 2009; Suppiah *et al.*, 2009; Tanaka *et al.*, 2009**). These variations in the IL28B gene correlated well with natural clearance of HCV and with SVR . In the first study, performed with European-American, African-American, and Hispanic individuals, the rs12979860 SNP was most strongly associated with SVR, which is located 3 kilo bases upstream of the *IL28B* gene. The minor allele (T) was associated with a lower rate of SVR (26% in those with genotype TT and 79% in those with genotype CC) (**Ge *et al.*, 2009**). In the second study, carried out with 293 Australian patients, a significant association between the SNP rs8099917 and SVR was found. This was further validated by an independent cohort of 555 European individuals. From 392 patients who achieved SVR, 247 (63%) were homozygotes for the allele T, which was significantly higher than genotype GG (SVR of 3.8%) (**Suppiah *et al.*, 2009**). Similar findings were also reported in a Japanese study. Results of a GWAS showed a significant association between treatment response with two SNPs (rs12980275 and rs8099917), both located in the IL28B gene region, with the latter being the same SNP found by Australian researchers. In this case, for the SNP rs8099917, the G allele was associated with a significantly lower SVR (0% for genotype GG and 78% for genotype TT) (**Tanaka *et al.*, 2009**).

Although, several groups have reported an association between several SNPs in the IL28 locus and the effect of PEG-RBV combination therapy for genotype 1 (**Thomas *et al.*, 2009; Rauch *et al.*, 2010**), but only a few studies have examined the role of these SNPs in the treatment of other genotypes especially genotype 4.

Aim:

In this study, we investigated whether the IL28B polymorphisms are associated with response to the therapy of PEG-IFN and ribavirin in Egyptian chronic HCV infection patients mostly with genotype four.

2. Patients and Methods:

The current study was conducted on 100 naïve (not treated before,first time to receive trearment) chronic HCV patients from interferon clinic of National Liver Institute-Menoufiya University in the period from February 2010 to July 2011. Selected patients were planned to receive combined interferon and ribavirin therapy. They had persistent (>6 months) elevation of alanine aminotransferase 1.5 times above the upper normal limit, with detectable HCV antibodies and HCV RNA with negative both HBs and HBe antigens with histological evidence of HCV. The stage of fibrosis was determined according to Ishak scoring system (**Ishak *et al.*, 1995**), fibrosis score from 0-6, 0=no fibrosis, 1-2=portal fibrosis (mild fibrosis), 3-4=bridging fibrosis (moderate fibrosis) and 5-6= cirrhosis (advanced fibrosis). All patients gave their written informed consent before the study and this study was approved by the ethical committee of National Liver Institute-Menoufiya University.

All chronic HCV patients were genotype 4, and were all submitted to the (SOC) therapy. of pegylated interferon-alfa 2a 180 mcg per week or pegylated interferon-alfa 2b 1.5 mcg per kg body weight in combination with ribavirin 600–1400 mg per day according to body weight for 24–48 weeks. Patients will be stratified according to response to (SOC) therapy into two groups: The first group is responders to treatment, the chronic HCV patients who had received the (SOC) therapy and have shown negative HCV RNA 6 months (24 weeks) following completion of a 48 weeks treatment course. The second group is non responders to the (SOC) therapy (no disappearance of HCV RNA at the end of the 12 week). Epidemiological, biochemical, and virological characteristics of these patients are shown in Table 1.

The following investigations were done for the patients:

- Liver function tests were done on Integra-400 (Roche-Germany), Complete blood counts were done on Sysmix KX-21 automatic cell counter (Japan). HCV antibodies were done by EIA (COBAS-Amplicore, Germany). HCV-RNA levels were analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) at 0, 4, 12, 24, 48, 72 weeks of starting treatment, using a commercial kit (Roche Diagnostic, Branchburg, NJ) according to the manufacturer's instructions.
- HCV genotyping was done using INNO-LiPA III (line immunoprobe assay) provided by Innogenetics, Ghent, Belgium.
- Liver biopsies were done for all patients included before therapy to assess fibrosis stage.

Single nucleotide polymorphisms (SNPs) of the IL28B genotype:

Blood was collected into EDTA tubes following standard procedures. Genomic DNA was prepared from peripheral blood lymphocytes by QIAGEN EZ1 DSP DNA Blood System **.** In brief, 2 ml of whole blood was mixed with 8 ml of triton lysis buffer 1 (0.32M Sucrose, 5mM MgCl2.6H2O, 12mM Tris-HCl, pH 7.5, 1%V/V Triton X-100). Leukocytes and nuclei were spun down (3500g, 5min), the pellet was washed with dH_2O and then resuspended in 0.9 ml of lysis buffer 2 (0.375M NaCl, 0.12M EDTA, pH 8.0), 25 µl SDS 10%, and 0.22 ml $NaClO_4$ (4M) and was shaken vigorously, spun down (13000g, 5 min) and subsequently salted out using a saturated NaCl solution. DNA in the supernatant was precipitated with 99.5% ethanol. Finally, DNA pellet was dissolved in 100 µl of ddH_2O. After quantification of DNA by UV spectrophotometer, 100ng of genomic DNA was used for each 20 µl PCR reaction (**Newton *et al.*, 1989**).

The rs12979860 and rs8099917 SNPs genotyping was carried out by polymerase chain reaction (PCR), and restriction fragment length polymorphism (RFLP). For rs12979860, oligonucleotide primers were: 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). For rs8099917, oligonucleotide primers were: 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).

PCR reaction conditions (30 µL) were: 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, and extension at 72 °C for 1 min. The PCR product for rs12979860 and rs8099917 was of 403 and 401 base pairs, respectively.

Restriction fragment length polymorphism (RFLP) analyses for IL28B alleles:

In order to perform RFLP assay for the rs12979860 genotype, 20 µL of amplicons were digested with 5U of *Bst*U I restriction endonuclease (New England Biolabs, MA, United States) 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. For the RFLP assay for the rs8099917 genotype, 20 µL of amplicons were digested with 1U of *Mae* III restriction endonuclease (Roche Molecular Systems, Branchburg, NJ, United States) 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. Restriction digestion products for each were separated on agarose gels stained with ethidium bromide for visualization on a UV trans-illuminator (**Venegas *et al.*, 2011**).

Statistical analysis:

Statistical package for SPSS (statistical package for social science) program version 13 for windows and Epi info computer program was used for data analysis. Quantitative variables were summarized using Mean±SD. Student's t-test was done to compare two normally distributed variables Mann Whitney non parametric variables. Fisher's exact test and the Chi square (X^2) test for categorical variables. Correlation coefficients (r) were calculated using the Pearson's correlation analysis. *p* value was significant at <0.05 level.

3. Results

The characteristics of the total 100 patients with chronic HCV infection (before therapy) are shown in Table (1). The study includes, 27% of stage 1 fibrosis, 38% of stage 2, 20% of stage 3 and 15% of stage 4, all HCV patients were genotype 4. The frequency of SNPs of IL28B showed that: for genotype rs12979860, CC was detected in 39%, CT in 51% and TT in 10% of overall HCV patients, but for rs8099917, the frequency was 46%, 42% and 12% for TT, GT, and GG respectively.

About 48(48%) among overall 100 HCV patients achieved SVR (responders), and the remaining 52(52%) of patients failed to respond (non responders). The CC genotype of rs12979860 was identified in 39 patients, 34 of them (87.2%) were achieved SVR. The unfavourable SNP genotype (TT)

was found in 10 patients and only one of them (10%) was responder. The CT heterozygous was detected in 51 (51%) patients, SVR was achieved in 13 (25.5%) of them, the frequency of CC genotypes was associated with SVR as compared to other genotypes ($p<0.001$). In contrast, the frequency of TT genotypes was associated with non responder patients ($p<0.01$) (Table 2).

As regard the TT homozygous of rs8099917 genotype, it was detected in 46 (46%) of overall HCV patients, and 37 of them (80.4%) were achieved SVR. The GT heterozygous was detected in 42 (42%) of HCV patients, SVR was achieved in 9 (21.4%) of them. While, the GG genotype was found in 12 patients and two of them only (16.7%) were responders ($p<0.001$; <0.05 for SVR and non responders) respectively (Table 3).

The comparison between rs12979860 genotypes revealed a significant increase in ALT, AST ($p<0.05$) and viral load ($p<0.01$) in CC genotype as compared to CT+TT genotypes. As regard rs8099917 genotypes, the ALT, AST levels were significantly higher in TT genotype as compared to GT+GG ($p<0.05$). But no significant difference was detected as regard other parameters (Table 4).

Table (1) Epidemiological, biochemical, and virological characteristics of patients:

	Chronic HCV patients (n=100)
Age (Years)	38.5 ± 10.4 (29-52)
Gender (Male/female)	68/32
BMI (kg/m^2)	23.2±4.6 (18-29)
ALT (U/L)	104.6±21.3 (78-122)
AST (U/L)	83.9 ± 25.7 (61-103)
GGT (U/L)	39.4±10.3 (27-41)
S. albumin (gm/dl)	3.9 ± 0.33 (3.5-4.2)
Prothrombin conc. %	77.2±3.6 (74-82)
Total leukocytic count (x10^3/µl)	5.7±1.5 (4.3-7.6)
Hemoglobin (gm/dl)	12.6 ±1.4 (11-13.9)
Platelet count (x10^3/µl)	230±90 (129-310)
AFP (mg/ml)	22.7±16.2 (6-39)
Viral load (log IU/mL)	7.5± 2.4 (5.1-9.7)
Fibrosis stage: Stage 1 Stage 2 Stage 3 Stage4	 27% 38% 20% 15%
IL28B genotypes frequency: rs12979860 rs8099917	 CC=39%, CT=51%, TT=10% TT=46%, GT=42%, GG=12%

Table (2) rate of IL28B (rs12979860) genotypes among HCV patient groups

Variables	**rs12979860 genotypes**			**p-value**
	CC (n=39)	**CT (n=51)**	**TT (n=10)**	
Responders (SVR) (n=48)	34/39 (87.2%)	13/51(25.5%)	1/10 (10%)	<0.001
Non responders (n=52)	5/39 (12.8%)	38/51(74.5%)	9/10 (90%)	<0.01

Table (3) rate of IL28B (rs8099917) genotypes among HCV patient groups

Variables	**rs8099917 genotypes**			**p-value**
	TT (n=46)	**GT (n=42)**	**GG (n=12)**	
Responders (SVR) (n=48)	37/46 (80.4%)	9/42 (21.4%)	2/12 (16.7%)	<0.001
Non responders (n=52)	9/46 (19.6%)	33/42 (78.6%)	10/12(83.3%)	<0.05

Table (4) Comparison between IL28B genotypes

Variables	rs12979860 genotypes			rs8099917 genotypes		
	CC	CT+ TT	p-value	TT	GT+GG	p-value
Age	43.8 ± 7.3	37.4 ± 4.9	>0.05	48.1 ± 6.5	43.2 ± 4.3	>0.05
Gender	28/11	40/21	>0.05	26/20	42/12	>0.05
ALT	121.6± 32.4	81.2±10.6	<0.05	134.1± 28.5	98.1±16.2	<0.05
AST	113.2 ± 17.4	73.2±12.5	<0.05	102.4 ± 15.2	82.1±9.8	<0.05
Albumin	3.5 ± 0.23	4.0 ± 0.51	>0.05	3.8 ± 0.41	4.1 ± 0.45	>0.05
P. C	75.4±2.3	72.8±4.2	<0.05	77.2±6.2	70.6±2.2	>0.05
Hb	11.9 ± 1.6	12.5 ± 2.1	>0.05	12.1 ± 1.4	11.5 ± 1.1	>0.05
TLC	5.9 ±2.3	4.9±1.8	>0.05	6.2 ±1.9	5.4±1.2	>0.05
Platelets	189±50	210±90	>0.05	197±23	176±65	>0.05
Viral load	7.3±1.9	5.8± 1.2	<0.01	4.9±1.3	5.6±1.8	>0.05

4. Discussion:

IL-28B is a Th1 type cytokine, a class II cytokine receptor ligand, a 200 amino acid long protein, a member of type III IFN-s, that distantly structurally relates to the members of IL-10 superfamily of cytokines, but shares also limited sequence similarity and functional characteristics with the type I IFN-s (α, β). IL-28B has a role in the regulation of intracellular IFN stimulated gene (ISG) expression (**Sheppard *et al.*, 2003**). It is expressed by peripheral blood mononuclear cells; dendritic cells upon infection with viruses. IL-28B exhibits antiviral activity, having an impact on natural clearance of HCV (**Par *et al.*, 2011**). The current study was designed to clarify, the effect of rs12979860 and rs8099917, SNP located nearest to interleukin 28B (*IL28B)*, the gene that encodes for IFN lambda-3 on HCV-4 outcome after combined IFN/RVN therapy.

In the current study, CC genotype of rs12979860 was identified in 39 % of overall HCV patients; the CT heterozygous was detected in 51% and the TT 10%. Mangia et al. (2010) recorded that, the frequencies of the IL-28B genotypes were as follows: CC, 37%; CT, 48%; and TT, 15%

After therapy, 48 (48%) among overall 100 HCV patients were achieved SVR (responders), and the remaining 52 (52%) of patients failed to respond (non responders). The CC genotype of rs12979860 who achieved SVR was 87.2%, which is significantly higher compared to CT (25.5%) and TT (10%) genotypes. This finding was supported by previous studies (**Suppiah *et al.*, 2009; Tanaka *et al.*, 2009**).

Mangia *et al.*(2010) reported that, 82% of patients with the CC genotype achieved a SVR, compared with 75% with the CT and 58% with the TT genotypes (*P*=0.0046). **De Nicola *et al.* (2011)**, found that, of 112 treated patients (98 males, 75 of Egyptian descent, 26 with cirrhosis), 23% were genotype CC, 63% CT and 14% TT. An SVR was achieved in 49%, and 88% of them were CC patients vs 37% of CT/TT (p<0.0001). **Kurbanov *et al.* (2011)** showed that the protective C allele was more common in those with spontaneous viral clearance (76.3% vs 57.9%; *P*=0.0006). Individuals with clearance were 3.4 times more likely to have C/C genotype. Thus, *IL28B* plays a role in spontaneous clearance of HCV genotype 4 in North Africa. **Asselah *et al.* (2011)** showed a better treatment response rate of the C Allele of the IL28B Gene SNP rs12979860. The response rates were 81.8%, 46.5% and 29.4% for genotype CC, CT and TT respectively. **Lin *et al.* (2011)** reported that in patients with genotype 1, SVR was achieved in 68.6% of the patients and it was higher in CC genotype of rs12979860 (p<0.001) but no other SNPs were independent predictors for SVR.

Par *et al.*(2011) reported that, the high IL-28B cytokine producing C/C genotype of this polymorphism was associated with a two- to threefold greater rate of SVR to anti-HCV therapy compared with the low cytokine producing T/T genotype. They added that the frequency of IL-28B C/C genotype was lower in HCV patients than in controls, it may be regarded as being protective against the chronic HCV infection, and that is, it may confer protection against the disease. **Thomas *et al.* (2009)**, reported that the IL-28B C/C genotype enhanced the spontaneous resolution of HCV infection and patients who harbour the *C* allele at *rs12979860* are more prone to respond to treatment and clear HCV than patients who do not possess this genetic polymorphism, which suggests a primary role for IL-28B in the resolution of HCV infection.

Ge *et al.* (2009) suggested that their IL-28B polymorphism was associated with decreased IL-28B (and IL-28A) expression-- a biologically plausible suggestion because IL-28B encodes interferon-lambda3, which may be involved in interfering with viral replication. **Chevaliez *et al.* (2010),** added that, most patients who fail to respond to pegylated IFN and ribavirin carry either TT or CT rs12979860 genotypes. CT patients are significantly more likely

to respond to higher doses of IFN. This indicates that the IL28B genotype is a marker of host cell responsiveness to IFN.

Furthermore, our results revealed that, SVR was detected in 80.4% of patients who harbour homozygous TT of rs8099917, in 21.4% of heterozygous GT and in 16.7% of the GG genotype. Similarly, **Rauch and colleagues (2010)** suggested that, the strongest association with spontaneous recovery was detected for rs8099917, a SNP located nearest to interleukin 28B (*IL28B)*, the gene that encodes for IFN lambda-3. Patients who are homozygous for C at rs12979860 (C/C) have a >2.5-fold increased likelihood of spontaneous resolution of HCV compared with patients who have persistent HCV infection. The association of the *IL28B* locus with natural and treatment-associated control of HCV suggests the importance of innate immunity and IFN lambda-3 in the pathogenesis of HCV infection.

Suppiah *et al.* (2009) reported an association to SVR within the gene region encoding interleukin 28B (rs8099917. *IL28B* contributes to viral resistance and is known to be upregulated by interferons and by RNA virus infection. These data suggest that host genetics may be useful for the prediction of drug response, and they also support the investigation of the role of *IL28B* in the treatment of HCV and in other diseases treated with IFN-α

In a study by **Aparicio *et al.* (2010)**, the rs12979860 SNP genotype was also highly associated with treatment success in HCV genotype 4-infected patients (P<0.0001), and patients carrying rs8099917 G alleles had high rates of treatment failure. The rate of treatment failure in patients infected with HCV genotype 3 was not affected by rs8099917 genotype. Similar results reported by **Pineda *et al.2010* and Rallon *et al.* (2010)**, which have demonstrated a significant influence of the rs12979860 SNP, that is in linkage disequilibrium with rs8099917 (**Rauch *et al.,* 2010**), on the treatment response of HIV-1 patients coinfected with HCV genotypes 1 and genotype 4. **Akuta *et al.* (2010)**, showed that genetic variation near the IL28B gene (rs8099917, rs12979860) are pre-treatment predictors of virological response to PEG-IFN plus ribavirin combination therapy in individuals infected with HCV.

The high prevalence of the rs8099917 G allele in HCV genotype 1- or 4-infected patients shows that the rs8099917 TT genotype may have a protective effect in terms of preventing the persistence of these two HCV genotypes. Since the rs8099917 G allele has been correlated with lower expression levels of IL28 genes (**Tanaka *et al.,* 2009**), the different frequencies of the rs8099917 G allele in patients infected with different HCV genotypes may indicate that the innate immune system interacts differently with the different HCV genotypes. However, the viral factors involved in this interaction remain unknown. The IL28B, IL28A, and IL29 genes are closely related cytokine genes in chromosomal region 19q13 that encode proteins known as type III IFNs (IFN-ls) (**Kotenko *et al.,* 2003**). IFN-l has been proposed as a possible treatment for hepatitis C (**Dodds *et al.,* 2009**; **Muir *et al.,* 2009).**

An interesting finding in our study was the significant higher levels of ALT, AST and viral load were detected in CC genotype. **Pablo *et al.* (2011)** reported that, HIV/HCV-co-infected patients with the C allele at rs12979860 show significantly higher plasma HCV-RNA load than TT carriers. Notably, plasma HCV-RNA levels associated with poorer response to IFN-α based therapy are significantly more frequent in CC/CT than TT carriers. Hypothetically, patients harbouring the rs12979860 allele C could display a lower activity of endogenous IFN-α, allowing higher HCV replication while keeping an enhanced susceptibility to exogenous IFN-α therapy.

Kawaoka *et al.* (2011) reported that, HCV RNA viral load, treatment regimen, and rs8099917 genotypes independently contributed to the effect of the therapy. For patients treated with PEG-RBV, rs8099917 and viral load were independent predictive factors for SVR in genotype 2b but not in genotype 2a. Conversely, in patients treated with interferon monotherapy, viral load and rs8099917 were independent predictive factors for SVR in genotype 2a but not in genotype 2b. The favourable rs8099917 genotype is also associated with a steep decline in viral load by the second week of treatment. On the contrary, **Lin *et al.* (2011**), suggested that, none of the ten SNPs examined were associated with baseline viral load and stages of liver fibrosis, but CC genotype of rs12979860 was the only treatment predictor.

What functional mechanism underlies the *IL28B* response? HCV RNA triggers production of type 1 interferons by hepatocytes; these molecules stimulate transcription of interferon-stimulated genes (ISGs). Exogenous (therapeutic) interferon alpha signals similarly. Given that the polymorphism 3-kb upstream of *IL28B* appears to be associated with natural clearance as well as treatment response, it seems likely that the gene product is involved in the innate control of HCV. The specific *IL28B* polymorphism (*C/C*) is strongly associated with reduced expression of intrahepatic ISGs and the response rate to PEG-IFN and ribavirin (**Tanaka *et al.,* 2009**). Genetic variation in*IL28B* regulates the innate immune response to HCV in the liver, priming

patients for a stronger response to exogenous IFN alpha therapy.

The question may arise whether HCV patients homozygous *for the IL-28 T/T* genotype (and *low IFNλ-3 production*) will need more intensive PEG-IFN/RBV treatment or more complex (e.g. triple) anti-HCV therapy in the future? Alternatively, for patients with IL-28B C homozygosity would a shorter treatment be appropriate? According to the IL-28 deficiency hypothesis, it would also be of importance to find compounds which can increase the expression of IL-28B on leukocytes, or to identify compounds which upregulate IL-28B transcription.

In conclusion: This study identified a polymorphism 3 kb upstream of IL28B (namely; rs12979860 and rs8099917) that is significantly associated with response to PEG-IFN and RBV for patients with chronic genotype 4 HCV infection. The polymorphism explains much of the difference in response between other HCV genotypes. Given that the polymorphism appears to associate with natural clearance as well as treatment response, it seems likely that the gene product is involved in the innate control of HCV. These findings, and further study of the functional mechanism underlying the IL28B response association, may help to identify patients for whom therapy is likely to be successful.

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