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The predictive value of IL28B rs12979860, rs11881222 and rs8099917 polymorphisms and IP-10 in the therapeutic response of Egyptian genotype 4 patients

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ABSTRACT

Interleukin-28B (IL28B) polymorphisms have previously been reported to be strongly associated with spontaneous and treatment-induced HCV viral clearance.

Aim: To assess the impact of four different IL28B polymorphisms and their haplotype combination and interferon-c inducible protein 10 (IP-10) in response to treatment in Egyptian genotype 4 patients.

Method: 159 HCV-genotype 4 patients were included. All patients were treated with Peginterferon alph2a/Ribavirin for 48 wk. The following polymorphisms rs12979860, rs11881222, rs8103142 and rs8099917 and rs80803142 of Il-28 were known to be associated with the sustained virological response. They were genotyped using the TaqMan assay. IP-10 was assessed by Eliza.

Results: The data indicated that all SNPs are within the Hardy-Weinberg Equilibrium (HWE) except for rs8103142 ($p=6.255^{-9}$), therefore it was excluded from the study since it deviates from HWE-P. The CC, AA and TT genotypes of rs12979860, rs11881222 and rs8099917 were the more frequent genotypes among the responders at RVR, EVR, ETR and SVR, respectively. The frequency of CC, CT, and TT genotype was 46.4%, 38.1% and 15.5% among responders of RVR, and was 46.9%, 45.9% and 7.2 among responders of SVR for rs12979860, respectively. The relapse rate was 18.0% and 16.0 % during EVR and ETR, while the response rate was 52.8%, 58.5%, 59.7% and 61.6% after 4, 12, 48 and 72 weeks of treatment. The transient virological response (TVR) was 6.9% among HCV patients. The results showed that the odds ratio and 95% CI of HCV genotype 4 patients to have a better sustained response to treatment (SVR) was 2.92, (1.83–4.68, $p=2.01^{-5}$), 2.89 (1.79–4.70, $p=2.53^{-5}$), and 2.73 (0.21–0.65, $p=0.0007$) for those with the major allele "C" of rs12979860, the "A" allele of rs11881222, and the "T" allele of rs8099917, respectively. Furthermore, the positive predictive value (PPV) of the major homozygous alleles for SVR with better response to therapy was in the following order: 78.69%, 68.42%, and 32.14% with a positive likelihood ratio of 1.95, 1.25, and 0.86 for rs12979860, rs11881222 and rs8099917, respectively. The haplotype formed between the 3 studied SNPs (rs12979860, rs11881222 and rs8099917) showed that two haplotypes (TGG and TGT) increased the probability of a poor response to therapy, but the CAT haplotype had the opposite effect. Multinomial logistic regression analysis revealed that the viral load and rs12979860 are the only significant actors involved in the efficacy of the treatment response among the cohort study. In addition, patients with SVR had significantly lower values of IP-10 than non-responder patients (NR), with a *P*-value $< =0.001$.

Conclusions: In genotype 4 cases, the IL28B SNPs rs12979860 rs8099917, and rs11881222 are the strongest predictors of a response, while IP-10 is a strong negative biomarker of a response. Accounting for this factor is important in the individualization of treatment and enhances the degree of predictiveness of the IL28 polymorphism in the final treatment outcome. The frequent distribution of C, A and T alleles of IL28 polymorphism are higher among TVR, which may reflect sensitivity to prolonged course.

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Introduction

The hepatitis C virus (HCV) genotype is an important predictor of disease progression and treatment response. Genotype 1b has a worldwide distribution and is often found to be the most common genotype. HCV-genotype 4 causes approximately 20% of the 180 million cases of chronic hepatitis C in the world, is predominant in the Middle East and Northern Africa, and has recently spread to Southern Europe (Fernandez-Arcas et al., 2006). Genotype 4 accounts for more than 90% of the reported cases from Egypt, a country with a massive HCV-related disease burden (Kamal and Nasser, 2008). However, knowledge of factors predicting sensitivity to combined antiviral treatment is still limited and restricted in populations infected with HCV-4.

Many viral and host factors have been investigated as predictors for achieving viral clearance. Higher age, advanced liver fibrosis, and diabetes have been shown to significantly reduce the response rates in patients infected with HCV-4, and Egyptian ethnicity has been claimed to be a positive predictor of a sustained virological response (Papastergiou et al., 2012) that is not related to insulin resistance (Cieśla et al., 2012). Serum adiponectin at baseline levels appears to be an independent predictor in the achievement of SVR in Genotype 4 (Derbala et al., 2009).

Recently, viral genetic polymorphisms, especially within the core and NS5A regions, have been shown to affect the response to treatment in HCV-4 infection (El-Shamy et al., 2012). Differences in host genetics are believed to influence the outcome of hepatitis C virus (HCV) infection. The interleukin 28B (IL28B) variability has a strong impact on viral clearance, either spontaneously or drug-induced, in HCV infected patients. Moreover, several recent findings have demonstrated that some SNPs in the IL28B gene are closely associated with pegylated-interferon (Peg-IFN)/Ribavirin responsiveness, but the true mutation has not been identified yet. Therefore, new SNPs still need to be discovered. In total, 20 polymorphisms of IL28B have been studied in HCV genotype 1; however, very few reports have been published on Genotype 4.

The CC genotype of the IL28B SNP was found to be associated with higher sustained virological response (SVR) rates than the TT or TC genotypes across all population groups (Ge et al., 2009b). The magnitude of this association between the CC genotype and SVR was greater than that of other clinical factors currently used to predict treatment responses in patients infected with genotype 1 HCV, including baseline viral load, baseline fibrosis, and ethnicity (McCarthy et al., 2010). Additionally, the expression of this SNP at the mRNA level was significantly reduced in the chronically infected cohort compared with ethnically matched controls, which reflects the relationship between the C allele and the natural clearance of HCV (Shi et al., 2012).

Among Egyptians, who have the highest prevalence of Genotype 4 worldwide, HCV clearance has been associated with a region of IL28B that is smaller compared to European populations, involving the non-synonymous IL28B SNP rs8103142 (Pedergnana et al., 2012). Inheritance of the IL4 polymorphism may be associated with resistance to combined antiviral therapy in Egyptian HCV patients (Shalaby et al., 2012). In addition, a strong association between the intracellular adhesion molecule-1 gene marker rs281437 and the progression of hepatic fibrosis has been reported, but not a response to treatment (Rizk and Derbala, 2012).

Abnormal cytokine levels may contribute to viral persistence and may affect the response to antiviral therapy. Serum IL-10, IL-12, and IL-18 levels are predictive of the response to HCV treatment of HCV-genotype 1 (Yoneda et al., 2011). Few studies have investigated the relationship between cytokines and response to treatment in genotype 4. The interferon-c inducible protein 10 (IP-10, CXCL10) has been proven to be a valid biomarker associated with HCV fibrosis (Romero et al., 2006). IP-10 is secreted into the blood by HCV infected hepatocytes and therefore can be used as a direct indicator of ongoing inflammation in the liver (Li et al., 2012). The impact of IP-10 on the elimination of HCV RNA in genotype 4 Egyptian patients during therapy under IL28B genetic variant conditions is not known.

The aim of this study was to assess the effect of four different IL28B polymorphisms (rs12979860, rs8099917, rs11881222 and rs.81803142) on the sustained virological response (SVR) to genotype 4 and the effect of haplotype combination of these polymorphisms on viral clearance. The rational for choosing the SNPs rs12979860, rs8099917, rs11881222 and rs.81803142, were based on previous published reports of their relation to SVR by Ito et al. (2011), Tanaka et al. (2009), Ge et al. (2009b), Shi et al. (2012). Furthermore, we assessed plasma IP-10 in relation to genetic variants and their impact on viral clearance in response to treatment.

Results

Clinical and biochemical data of HCV patients (Table 1, Fig. 1)

Among 159 patients, 98 patients achieved the SVR with a ratio of 61.6%. The mean age of all patients was 46.47 ± 8.83 years, and 91% were males. The non-responders had significantly higher levels of pre-treatment AST, viral load at 4 weeks of treatment and stages of inflammation (mild and severe) compared to the responders (SVR) group. No significant difference was found between SVR and NR for age, BMI, pre-ALT, pre-albumin, pre-total bilirubin, white blood cell and platelet count before the treatment and the degree of fibrosis, as shown in Table 1.

In addition, overall the SNP genotypes and allelic frequencies were determined in 159 individuals. Filtering the SNPs in this study was performed by a call rate $<90.0\%$ and if the Hardy-Weinberg Equilibrium (HWE) was <0.005 among populations.

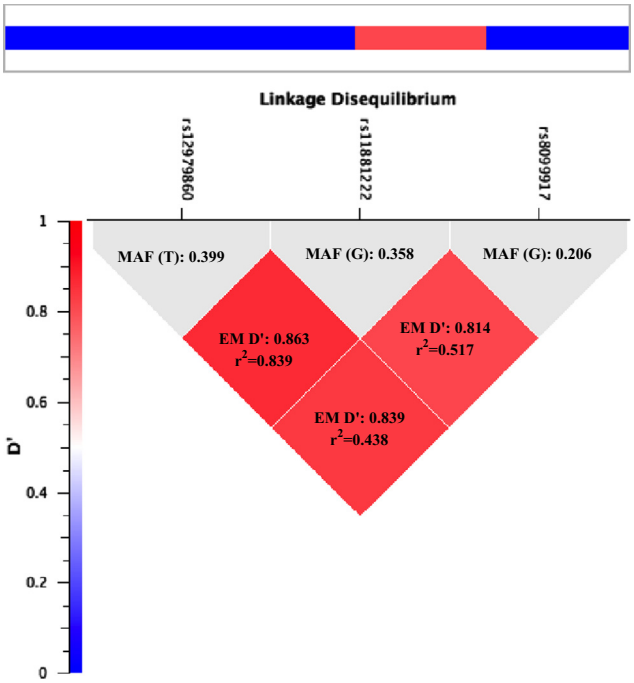

Fig. 1. Linkage Disequilibrium of the rs12979860, rs11881222 and rs8099917 of IL-28 B gene in chronic hepatitis patients C under treatment. *Footnote*: Red diamonds represents pairwise LD "EM D" values between rs12979860, and rs11881222 (upper left), rs11881222 and rs8099917 (upper right), and rs12979860, and rs8099917 (lower), respectively.

Table 1
Demographic and biochemical data among all study patients and on basis of their response to treatment by the antiviral therapy as sustained virological responders (SVR) and non-responders (non-SVR and relapse).

Variables	All study subjects	Responders (N=**98**)	Non-responders (N=61)	P value
Age (years)	46.47 (8.83)	45.81(8.88)	47.52 (8.69)	**0.237**
Gender (Males)/(Females)	144/15 (91.0%/9.0%)	86/12 (12.24/87.76%)	3/58 (4.92/95.08%)	**0.124**
BMI (kg/m^2)	30.18 (5.05)	30.53 (5.74)	29.62 (3.63)	**0.289**
LFT (pre-treatment)				
Pre-ALT (U/L)*	51.00 (34.00–87.00)	47.50 (30.00–87.00)	56.00(40.00–90.00)	0.089
Pre-AST (U/L)*	38.00 (27.00–50.75)	35.00 (26.00–47.25)	42.00 (32.00–63.50)	**0.021**
Pre-treatment viral load (copies/ml)*	449165.50 (21861.50–2116677.50)	174991.0 (3842.0–719013.0)	710476.0 (463008.0–2835489.0)	< **0.001**
Viral load at 4 weeks (copies/ml)	148001.99 (508930)	21253.98 (10139.84)	588602.19 (95126.63)	< **0.001**
Pre-total WBC (10^3 /μl)*	2.80 (1.78–3.60)	2.60(1.70–3.70)	2.85 (1.80–3.60)	0.679
Pre- platelet count (10^5/μl)	190.00 (155.50–228.00)	193.00 (152.50–231.50)	188.90 (158.50–226.50)	0.688
Degree of fibrosis (n, %)				
Mild	109 (68.55%)	71 (72.45%)	38 (62.29%)	0.214
Severe	50 (31.45%)	27 (17.55%)	23 (37.71%)	
Inflammation grades (n, %)				
Mild	112 (70.44%)	77 (78.57%)	35 (57.38%)	**0.017**
Severe	47 (29.56%)	21 (21.43%)	26 (42.62%)	
rs12979860				
CC	57 (35.8)	46 (46.9)	11 (18.0)	0.0001
CT	77 (48.4)	45 (45.9)	32 (52.5)	
TT	25 (15.8)	7 (7.2)	18 (29.5)	
C	0.6	0.69	0.44	
rs11881222				
AA	64 (40.2)	49 (48.9)	15 (22.0)	0.0001
AG	75 (47.2)	43 (41.8)	32 (54.3)	
GG	20 (12.6)	6 (4.3)	14 (23.7)	
A	0.64	0.72	0.5	
rs8099917				
GG	8 (5.0)	3 (3.1)	5 (8.2)	0.004
GT	55 (34.6)	26 (26.5)	29 (47.5)	
TT	96 (60.4)	69 (70.4)	27 (44.3)	
T	0.78	0.84	0.68	

Continuous data are presented as means (SD) for normally—distributed data and as *median and interquartile for non-distributed data. Categorical data are presented as number and (percentage). Two-tailed P value is significant <0.05. *Abbreviations*: BMI, body mass index; LFT, liver function tests; Pre, pre-treatment; Pre-ALT, pre-treatment Alanine Aminotransferase; Pre-AST, pre-treatment Aspartate Aminotransferase; and **WBC**, White Blood Cells count.

All examined *Il-28B* gene polymorphisms were in HWE ($p>0.005$), except for rs8103142, where the HWE was 1.399×10^{-13} and 0.0003 among the control (SVR) and active cases (NR), respectively. Therefore, rs8103142 was not included in this study. A significant difference was observed in the genotypic distribution of rs12979860, rs11881222 and rs8099917 between the SVR and NR subjects as shown in Table 1. The frequency (%) of the major alleles for all studied SNPs is presented in Table 1. Utilizing the logistic regression analysis, the odds ratio and 95% CI, after multiple test corrections for FDR and Bonferroni adjustments of SNPs numbers, between SVR and NR was 2.92 (1.83–4.68, $p=2.01^{-5}$), 2.89 (1.79–4.70, $p=2.53^{-5}$) and 2.73 (0.21–0.65, $p=0.0007$) for the major allele "C" of rs12979860, the "A" allele of rs11881222, and the "T" allele of rs8099917, respectively.

Genotype distribution of rs12979860 (C/T), rs11881222 (A/G), and rs8099917 (G/T) of the IL-28B gene between responders [R] and non-responders [NR] during (RVR), (EVR) and (ETR) in chronic hepatitis C patients genotype 4 under treatment (Table 2)

A significant difference was observed in the genotypic distribution of rs12979860, and rs11881222 between responders and non-responders for RVR, EVR and ETR with P values <0.005. In addition, the homozygous CC and AA genotypes are the most frequent genotype observed in responders during RVR, EVR and ETR.

The homozygous TT genotype is the most frequent genotype among RVR, EVR and ETR for rs8099917 polymorphism. A significant difference was observed in the genotypic distribution of rs8099917 between responders and non-responders for EVR and ETR with P values <0.005. Of note, the heterozygote genotype CT, AG and GT are the most frequent observed among non –responders during RVR, EVR and ETR for rs12979860 (C/T), rs11881222 (A/G), and rs8099917 (G/T), respectively. In addition, the likelihood ratios between RVR and SVR was 7.087, $p=0.008$ by chi-square test.

Table 2
Genotype distribution among rs12979860, rs11881222, and rs8099917 at 4 weeks (RVR), 12 weeks (EVR) and 48 weeks (ETR) of treatment.

Genotype	RVR		EVR		ETR	
	R	NR	R	NR	R	NR
rs12979860	**(N=84)**	**(N=75)**	**(N=93)**	**(N=66)**	**(N=95)**	**(N=64)**
CC	39 (46.4)	18 (31.6)	45 (48.4)	12 (18.2)	44 (46.3)	13 (20.3)
CT	32 (38.1)	45 (58.4)	41 (44.1)	36 (54.5)	44 (46.3)	33 (51.6)
TT	13 (15.5)	12 (16.0)	7 (7.5)	18 (27.3)	7 (7.4)	18 (28.1)
P value	0.008		0.0001		0.0001	
rs11881222	**(N=84)**	**(N=75)**	**(N=93)**	**(N=66)**	**(N=95)**	**(N=64)**
AA	41 (48.8)	21 (28.0)	43 (46.2)	19 (28.8)	44 (46.3)	17 (26.6)
AG	31 (36.9)	44 (58.7)	41(44.1)	34 (51.5)	43 (45.3)	32 (50.0)
GG	12 (14.3)	10 (13.3)	9 (9.7)	13 (19.7)	8 (8.4)	15 (23.4)
P value	0.004		0.033		0.0008	
rs8099917	**(N=84)**	(N=75)	**(N=93)**	(N=66)	**(N=95)**	(N=64)
GG	5 (6.0)	5 (6.7)	5 (5.4)	5 (7.6)	4 (4.2)	7 (10.9)
GT	27 (31.1)	26 (34.7)	23 (24.7)	32 (48.5)	24 (25.3)	30 (46.9)
TT	52 (61.9)	44 (58.7)	65 (69.9)	29 (43.9)	67 (70.5)	27 (42.2)
P	0.879		0.005		0.0003	

Categorical data are presented as number and (percentage). Two-tailed P value is significant <0.05.

Table 3
Relapse rate in count and in percentage and its genotype distribution among HCV patients at 12 (EVR), 48 (ETR) and 72 (SVR) weeks of treatment.

	EVR relapse	ETR relapse	TVR
Relapse rate	(*N*=28, 18.0%)	(*N*=25, 16.0%)	(*N*=11, 6.92%)
rs12979860			
CC	5 (17.9)	7 (28.0)	3(27.3)
CT	15 (53.6)	15 (60.0)	5(45.4)
TT	8 (28.5)	3 (12.0)	3(27.3)
P value	0.031	0.449	0.571
rs11881222			
AA	7.0 (25.0)	7.0 (28.0)	4.0 (36.2)
AG	14.0 (50.0)	14.0 (56.0)	5.0 (45.4)
GG	7.0 (25.0)	4.0 (16.0)	2.0 (18.2)
P value	0.185	0.671	0.795
rs8099917			
GG	1.0 (3.6)	3.0 (12.0)	2.0 (18.2)
GT	15.0 (53.6)	10.0 (40.0)	4.0 (36.2)
TT	12.0 (42.8)	12.0 (48.0)	5.0 (45.4)
P value	0.048	0.077	0.795

Categorical data are presented as number and (percentage). Two-tailed *P* value is significant < 0.05.

Relapse rate and genotype distribution among HCV patients during early virological response, end of treatment response and transient virological response (Table 3)

Table 3 highlights the relapse rate and the genotype distribution among HCV patients who received pegylated interferon and oral ribavirin for 48 weeks. Of note the relapse rate decreases from 18.0% in EVR to 16.0% in ETR, while TVR was 6.92% among the study subjects. Furthermore, the genotype distribution among patients who had EVR and ETR shows that the heterozygous groups of rs12989860 (CT), and rs11881222 (AG) are the most frequent pattern seen. Among TVR subjects, the most frequent genotype seen among these patients are CT, AG and TT for rs12979860, rs11881222 and rs8099917, respectively. No significant difference was observed among the genotype distribution for rs12979860, rs11881222 and rs8099917 among EVR, ETR and TVR ($p > 0.05$)

Association between SVR and Il-28 B gene polymorphisms (rs12979860, rs11881222 and rs8099917) based on genetic models (Table 4)

Using the genetic model (CC+TC/TT) for rs12979860, the carriers of all C alleles (CC+CT) had an increase of ~15-fold (OR, (95% CI)=15.05 (2.83–22.92), $p=0.0001$) in the odds of the clearance rate of HCV in response to treatment compared with T allele carriers. Moreover, using the genetic model (CC/TC+TT), the CC genotype had a marked significant effect on treatment response events by odds of 5.13-fold (95% CI=2.07–12.75, $p=0.000$), as shown in Table 2. Using the genetic model (AA +AG/GG) for rs11881222, carriers of all A alleles (AA+AG) had an increase of ~15-fold (95% CI=3.76–59.298, $p=0.000$) in the clearance rate of HCV in response to treatment compared with G allele carriers. Additionally, using the genetic models (AA/AG+GG), the AA genotype clearly had a significant effect on treatment response events by ~5-fold (95% CI=2.13–11.29, $p=0.000$) after adjustment of the OR, as shown in Table 2. Using the genetic model (GG+GT/TT) for rs8099917, carriers of all G alleles (GG+GT) had an increase of ~13-fold (OR 12.80, 95% CI=1.87–227.44, $p=0.014$) in the clearance rate of HCV in response to treatment compared with T allele carriers. Furthermore, using the genetic model (GG/GT+TT), the GG genotype had a remarkably significant effect on treatment response events by odds of 4.15-fold (95% CI=1.88–9.19), $p=0.000$), as shown in Table 2.

Table 4
Odds ratio (OR), 95% confidence interval with *P* value of Il-28 polymorphisms; rs12979860, rs11881222, and rs8099917 of IL-28 B gene among sustained virological responders (SVR) and non-responders (NR) in chronic hepatitis patients C-genotype 4 under treatment.

Polymorphism	SVR Vs. NR Genotype (models)	Adjusted odds ratio (95% CI)	*P* value
rs12979860	CC+TC/TT	15.05 (2.83–22.92)	0.000
	CC/TC+TT	5.13 (2.07–12.75)	0.000
	CC/TT	18.24 (5.17–64.42)	0.000
	CC/CT	3.75 (1.37–9.22)	0.009
	CT/TT	5.13 (1.72–15.28)	0.003
rs11881222	AA+AG/GG	14.97 (3.76–59.298)	0.000
	AA/AG+GG	4.90 (2.13–11.29)	0.000
	AA/GG	31.87 (7.03–144.38)	0.000
	AA/AG	3.80 (1.46–8.35)	0.005
	AG/GG	9.11(2.21–37.62)	0.002
rs8099917	GG+GT/TT	12.80 (1.87–227.44)	0.014
	GG/ GT+TT	4.15 (1.88–9.19)	0.000
	GG/TT	12.48 (3.08–505.99)	0.005
	GG/GT	3.86 (1.59–8.11)	0.002
	GT/TT	11.0 (0.86–141.07)	0.065

Adjusted odds ratios (aORs), 95% Confidence Intervals (CIs), and *P* values were tested using logistic regression. aORs was adjusted for age, BMI, pre-treatment viral load, fibrosis and pre-ALT, using the recessive, dominant and additive genetic models for rs12979860, rs11881222 and rs8099917, respectively). SVR=sustained virological response and NR=non-responders. Two-tailed *P value* is significant □0.05.

Table 5
The positive predictive values for the major allele and the homozygous of the major alleles for better treatment response (SVR) versus non-SVR after follow up of complete treatment.

Polymorphism	Major allele **PPV 95% confidence intervals & +LR**	Major homozygous allele **PPV 95% confidence intervals& +LR**
rs12979860 C/T	71.73% (64.77%–77.99%)	78.69% (66.32–88.14%)
+LR	1.58	1.95
rs11881222 A/G	70.26% (63.31%–76.85%)	68.42% (54.76%—80.09%)
+LR	1.5	1.25
rs8099917 G/T	57.23% (49.49%-64.71%)	32.14% (15.88%-52.35%)
+LR	0.81	0.86

Data are evaluated by 2×2 tables with fisher exact test and presented as %. PPV=probability that the disease is present when the test is positive, and +LR, positive likelihood ratio. The major alleles are C, A and T for rs12979860, rs11881222 and rs8099917, respectively. *Abbreviations*: PPV, positive predictive value; LR, positive likelihood ratio (see statistical analysis).

The positive predictive value of rs12979860, rs11881222 and rs8099917 among treated HCV patients in response to therapy with higher clearance rates (Table 5).

The ***positive predictive value*** (PPV) of the major alleles for SVR with enhanced response to therapy were in the order of 71.73%, 70.26%, and 57.23% with positive likelihood ratios (+LR) of 1.58, 1.50, and 0.81 for rs12979860, rs11881222 and rs8099917, respectively. The PPV of the major homozygous alleles for SVR with enhanced response to therapy were in the order of 78.69%, 68.42%, and 32.14% with positive likelihood ratios (+LR) of 1.95, 1.25, and 0.86 for rs12979860, rs11881222 and rs8099917, respectively (Table 3).

Linkage disequilibrium (LD) of the three Il-28 B gene polymorphism SNPs (rs12979860, rs11881222 and rs8099917) in HCV patients (Fig. 1)

Pairwise LD between the 3 polymorphisms was evaluated using data from all subjects. Using the expectation-maximization (EM), the results of LD and the r2 for all study subjects are presented in

Table 6
Haplotype frequency of the rs12979860, rs11881222 and rs8099917polymorphisms of *IL-28B* gene and its associations with the poor treatment response in HCV patients for (NR) Vs. (SVR) outcome.

rs12979860 C	rs12979860/Tr	rs11881222/Ar	rs11881222/G	rs8099917/G	rs8099917/T	Haplotype	EM frequency (SVR)	EM frequency (NR)	Adjusted odds ratio, 95% Cl	*P* value
C	T	A	G	G	T	CAG	0.667	0.395	0.33 (0.19–0.53)	9.58^{-6}
C	T	A	G	G	T	TGT	0.112	0.287	3.27 (1.75–6.12)	0.0001
C	T	A	G	G	T	TGG	0.111	0.202	2.08 (1.08–4.05)	0.027
C	T	A	G	G	T	TAT	0.053	0.059	1.70 (0.42–3.28)	0.765
C	T	A	G	G	T	CGT	0.028	0.018	0.62 (0.12–3.27)	0.575
C	T	A	G	G	T	CAT	0.020	0.013	0.66(0.09–4.55)	0.667

Data are presented as frequency for SVR and NR for each haplotype with the odds ratio, lower and upper 95% confidence intervals and *P* value. The data were analyzed by logistic regression analysis. aORs was adjusted for age, BMI, pre-treatment viral load, fibrosis and pre-ALT. SVR=sustained virological response and NR=non-responders. Two-tailed *P* valueis significant □ 0.05.

Fig. 1. The results of the LD of the three pairs had modest to highest LD in the study subjects.

Haplotype frequency and its association with poor therapy response among HCV patients under treatment (Table 6)

The alleles (rs12979860, rs11881222 and rs8099917) of the Il-28 B gene formed several frequent haplotypes, as shown in Table 4. Only the CAT haplotype has a significant protective effect against poor response to therapy by 67%, and its frequency in SVR is nearly 1.7 times its frequency in NR (66.7% vs. 39.5%, $p=9.58^{-6}$). Alternatively, the two haplotypes TGG and TGT significantly increase the odds of a poor therapy response by 3.27- (95% CI=1.75–6.12) and 2.08-fold (95% CI=1.08–4.05) in HCV patients receiving the treatment, respectively. Their frequencies in NR were 28.7% and 20.2%, which were nearly double its frequency in SVR (11.2% and 11.1%) for TGG and TGT, respectively.

Multi-logistic regression analysis to assess the factors involved in the efficacy of treatment response (Table 7)

The multinomial regression analysis was performed to discriminate the significant effect of the following independent variables: age above 45 years, body mass index above 30 kg/m^2, pre-ALT, liver fibrosis, basic level of the viral load, and the three SNPs (rs12979860, rs11881222 and rs8099917) in their additive models on the dependent factor, which is the rate of the clearance response (SVR vs. NR). After normalizing for these factors, only the rs12979860 SNP and baseline viral load were still significantly associated with SVR. The odds of having a better response and clearance of the HCV after treatment was 2.41- and 2.2-fold for rs12979860 and pre-treatment viral load with *P*-values of 0.036 and 0.034, respectively, as shown in Table 5.

IP-10 and its association with genotype distribution and response to treatment (Fig. 2)

The median and the interquartile values for IP-10, based on the genotype distribution and response to treatment, are presented in Fig. 2a–d. Patients carrying the T allele of rs12979860, the G allele of rs11881222 and the G allele of rs8099917 exhibited greater significant values compared to C, A and T allele patient carriers, respectively (Fig. 2a). The median and the interquartile values for *IP-10 based on the treatment response* (SVR and non-SVR) are presented in Fig. 2d. Non-responders to treatment (NR) had significantly higher values of IP-10 than responder patients (SVR), with a *P*-value $<=0.001$ (Fig. 2d).

Table 7
Logistic regression analysis of the factors involved in the efficacy of therapy response (SVR Vs. NR) in all chronic hepatitis C patients.

Regressor	*B* Coefficient	Std. error	*P* value	Odds ratio	Lower conf. interval	Upper conf. interval
Age	0.011	0.021	0.607	1.01	0.968	1.055
BMI	0.038	0.078	0.627	1.039	0.486	1.763
Fibrosis	1.031	0.704	0.138	2.804	0.705	11.151
Pre-ALT	0.007	0.009	0.429	1.007	0.989	1.026
Viral load-basic	0.793	0.397	0.043	2.212	1.014	4.823
rs12979860	0.880	0.447	0.036	2.413	1.015	5.793
rs11881222	0.575	0.426	0.177	1.771	0.771	4.100
rs8099917	0.249	0.432	0.562	1.283	0.550	2.994

Data are presented as *b*- coefficient, standard error, the odds ratio, lower and upper 95% confidence intervals and *P* value. The data were analyzed by logistic regression analysis. SVR=sustained virological response and NR=non-responders. Two-tailed *P value* is significant □0.05.

Discussion

Pegylated-interferon-a and ribavirin still maintain pivotal roles in the management of chronic HCV-Genotype 4. Thus, the prediction of a patient's response is important to reduce adverse effects and expenses.

Genome-wide association studies have identified polymorphisms located near the gene encoding IL28B, which was discovered to be the best predictor of patient response to pegylated-interferon plus ribavirin for chronic hepatitis C virus (HCV) genotype 1 infection.

In support of the current data that CC genotype of rs12979860 was the predominant allele among our Egyptian study group, a recent study by El-Awady et al. (2012) demonstrated the Genotype C/C is the prominant genotype . These data may explain the higher response rate among Egyptians Genoptype4 compared to western reports. El-Ewady screened his patients for rs12979860 polymorphism only and this may explain higher prevelance of the favroble TT genotype of SNP rs8099917 among our patient

In 2009, Ge et al. (2009b), along with subsequent studies in European and American cohorts, demonstrated a stronger association between SVR rates and the C/C genotype of rs12979860 in HCV-genotype 1 (McCarthy et al., 2010). However, the IL-28B genotype did not predict the outcomes in individual groups based on prior treatment response and only plays a minor role when on-treatment viral responses are taken into consideration (Liu et al., 2012). Additionally, an rs8099917 T/T variant has been reported to be an independent predictor of SVR in Genotype-1 patients (Miyamura et al., 2012).

In five studies, patients reported to be monoinfected with HCV were studied: 102 cases from Austria (97% of them were Egyptians) (Stattermayer et al., 2011), 103 patients from Milan, Italy

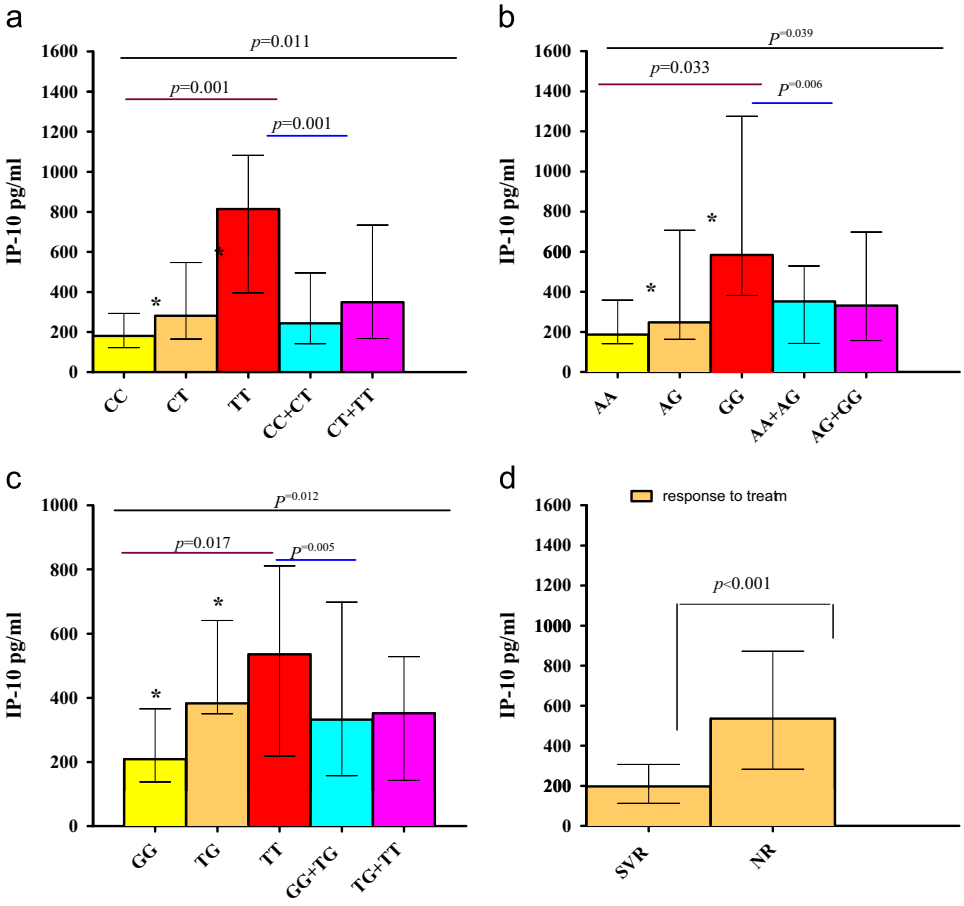

Fig. 2. Serum IP-10 level based upon genotype distribution, of rs12979860 (3 A), rs11881222 (3B) and rs8099917 (3c) of IL-28 B gene, and upon sustained response to treatment (SVR vs. NR), (3d) in chronic hepatitis patients C under treatment. (a) IP-10 and genotypes of rs12979860, (b) IP-10 and genotypes of rs11881222, (c) IP-10 and genotype of rs8099917 (d) IP-10 and sustained response to treatment,degree of fibrosis and degreeof inflammation. *Footnote*: data are presented as median and interquartile values of IP-10. Two-tailed *P* value is significant □ 0.05.

(68% from Egypt) (Ge et al., 2009a), 82 patients from different ethnic groups (51% Egyptians, 34% Europeans and 13.4% Sub-Saharan Africans) (Asselah et al., 2012), 201 cases from Qatar (Derbala et al., 2012) and 182 from Syria (Antaki et al., 2013), were all analyzed for a single SNP, *rs12979860* (except for Antaki et al., who studied *rs8099917* and *rs12979860*). *The current study provides* the largest study group to date with patients of the same ethnic group with genotype 4 and studies 3 SNPs in correlation with the cytokine IP-10.

To our knowledge, this is the first study designed to investigate the role of 3 SNPs of the IL28B gene, the rs12979860, rs11881222 and rs8099917 genotypes, in association with IP-10, as predictors of SVR to PegIFN plus ribavirin in HCV-4 monoinfected patients of Egyptian ethnicity.

Similar to Genotype 1, we found that rs12979860 and rs8099917 are strong pre-treatment predictors of SVR in patients with genotype 4 HCV infections. Moreover, we found a similar association with the rs11881222 SNP, which was not previously investigated in Genotype 4. A Marked linkage disequilibrium was noted between rs12979860 and rs11881222, which is in agreement with a previous study by Rauch et al. (2010), Rauch et al. (2010). We confirm the association of the following C, A and T alleles of 12979860, rs8099917 and rs11881222 with SVR in Genotype 4, respectively. Among Egyptians with genotype 4, the CC genotype appears to be a clear predictor of drug-induced viral clearance events by 5-fold compared to the two- to three-fold increase in the SVR rate in Genotype 1 (Thompson et al., 2010). *Furthermore*, the AA and GG genotypes of rs8099917 and rs11881222, respectively, are associated with a 4 to 5-fold increase in achieving SVR. Similar to genotype 1, the major alleles of rs12979860 and rs11881222 had higher predictive values for SVR compared with rs8099917. In support of our finding, with the role of C, A, and T alleles of rs12979860, rs11881222 and rs8099917, respectively, in achieving better response to treatment by univariate analysis, the frequency of the CAT haplotype of these SNPs was double in our patients who achieved SVR compared with NR. Additionally, the current study revealed that the haplotype TGG, which had a significant risk of poor response, had the following risk alleles: T of rs12979860, G of rs11881222 and G of rs8099917. Although only a few SNPs were studied, we attempted to calculate the LD, which shows medium to highest LD among the studied SNPs in this block. More SNPs within this region could be included in further studies among our HCV patients to clarify this LD association. A recent study by Pedergnana et al. (2012) studied the LD for six SNPs of the IL-28B gene in Egyptian populations and showed that the *r*2 value was 0.41 between rs12979860 and rs8099917, which is congruent with our findings.

In agreement with previous reports on genotype 4 that investigated the rs12979860 SNP (Asselah et al., 2012; Cieśla et al., 2012; El-Awady et al., 2012), we found that the tested IL28B SNPs rs12979860 and rs8099917 were independent predictors of SVR. The polymorphism rs12979860 had the highest predictive value of SVR compared with rs11881222 and rs8099917, and the CC genotype of rs12979860 had the highest predictive value in genotype 4. Moreover, the multivariate regression analysis showed that the rs12979860 SNP was the only SNP associated with clearance, whereas the other SNPs ceased, confirming that this association was based on a single signal (Papastergiou et al., 2012).

The strong association between IL-28B polymorphisms and response to antiviral therapy in patients with chronic HCV infection

seems to be mediated by IFN-λ3, which is encoded by the IL-28B gene (Balagopal et al., 2010). IFN-λ3 is a type III IFN that is involved in host antiviral immunity and plays a role in immune-mediated and treatment-associated clearance of HCV (Rallon et al., 2012). The role of IFN-γ in inducing response to treatment was reconfirmed by Zhang et al. (2011), who reported on the absence of correlations between the levels of Th1/Th2 cytokines in the serum before and after IFN therapy, while increasing serum IFN-γ levels induced by IFN treatment are associated with a sustained response (Zhang et al., 2011). Alternatively, Askarieh et al. (2010) suggested that IL28B variants are linked to antiviral effectiveness by blocking the production or release of infectious virions, rather than by clearing HCV infected cells.

The favorable TT genotype of rs8809917 was the predominant among TVR, so extended course of therapy in these patients may be helpful as, IFN-stimulated genes appear to be initially down-regulated in patients with the rs8099917 TT genotype, which may help to prevent desensitization and promote maximal induction of IFN-stimulated genes (Honda et al., 2010).

Whereas, our findings showed that IL28B polymorphisms and rapid viral clearance, as in genotype 1 (Chayama et al., 2011) remain one of the strongest predictors of SVR in genotype 4, but the current study highlights the importance and sensitivity of IP-10 as a biomarker of poor response and its inverse correlation with the treatment response.

Similar to Genotype 1, considering the high SVR rates among HCV genotype-4 infected homozygous carriers of C/C at rs12979860, G/G at rs11881222, and T/T at rs8099917 with low IP-10 levels, these patients have the best chance of achieving treatment-induced viral clearance (Lagging et al., 2011).

Recent research has shown that in patients with chronic HCV infection, the majority of plasma IP-10 exists in a 2 amino-acid truncated antagonist form, which inhibits the recruitment of T cells and thus is responsible for the lack of desired antiviral effects of IP- 10 and could play an important role in pathology (Charles and Dustin, 2011).

Because of limitation in sample size, which may affect the results, we recommend further studies in Genotype 4 including larges sample to validate our results.

Conclusions

In genotype 4, the IL28B rs12979860, rs8099917, and rs11881222 SNPs may have added value in the treatment algorithm because they are the strongest predictors of response. We think that our study provides additional evidence that IP-10 is a strong negative predictor of response to Peg-IFN/Ribavirin therapy in HCV-4 monoinfected patients, highlights the need to consider this factor in the individualization of treatment, and augments the level of predictiveness of IL28B polymorphisms for final treatment outcome.

Considering the high SVR rates among HCV genotype-4 infected homozygous carriers of C/C at rs12979860, G/G at rs11881222, and T/T at rs8099917 with low IP-10 levels, these carriers have the best chance of achieving treatment-induced viral clearance. The predominance of favorable rs8099917 genotypes TT, rs11881222 genotypes AA and rs12979860 genotype CC may explain the higher SVR among Egyptians.

Patients and methods

Study design

This study was a retrospective-prospective cohort study. All Egyptian, chronic HCV genotype 4 patients, who were followed in the Hamad Medical Corporation outpatient clinic in the state of Qatar, received treatment between January and December 2010 and completed the 24-week post-treatment follow-up, were included in the study (retrospective aspect). All patients were treated subcutaneously, with 180 lg of Peginterferon -2a (Pegasys; Hoffmann-LaRoche, Basel, Switzerland) once weekly and Ribavirin (Copegus-; Hoffmann-La Roche, Basel, Switzerland) given at an oral daily dose of 1000 mg (body weight £75 kg) or 1200 mg (body weight ‡75 mg) for 48 weeks.

Polymorphisms (rs12979860, rs8099917, rs11881222 and rs.81803142) in the IL28B genes were investigated in all patients. The patients were classified according to the response to treatment into responders (control group) and non-responders (case group). All patients provided written informed consent according to the Declaration of Helsinki of 1979.

Patient selection

The study recruited 159 chronic HCV-genotype 4 patients. Nationalities other than Egyptian were excluded to avoid ethnic-specific variability of response to treatment. Chronic HCV infection was confirmed by positive serology for anti-HCV, active viral replication by the detection of HCV-RNA in the serum and histological findings of chronic active hepatitis according to the Scheuer Score. The necroinflammatory and fibrosis scores were assigned based on the Scheuer scoring system from 0 to 4. Patients were further subdivided into mild fibrosis (stages I & II) and cirrhosis (stages III & IV). None of the patients in our study exhibited serological evidence of autoimmune liver disease or inheritable disorders, such as hemochromatosis, Wilson's disease, a history of alcoholism or drug abuse. Hepatocellular carcinoma was excluded using α-fetoprotein testing and ultrasound scanning. Anti-HCV tests were performed using a commercial ELISA kit (Axsym 3.0; Abbott Laboratories, Chicago, IL, USA). All patients were confirmed to be HCV-G4 using the Inno-LiPA HCV II assay (Innogenetics, Inc., Alpharetta, GA, USA). The monitoring of serum HCV RNA levels was performed using Amplicor (version 2.0, Hoffmann-La Roche) with a minimum detection limit of 50 IU/mL. All patients were treated with pegylated interferon once a week and oral ribavirin at a daily dose of 1000 mg (body weight >75 kg) or 1200 mg (body weight <75 kg) for 48 weeks. Patients with positive serology for schistosomiasis were treated with praziquantel (biltricide) prior to antiviral therapy.

The primary efficacy outcome was a sustained virological response (SVR), which was defined as undetectable HCV RNA 24 weeks after the end of the treatment. Relapse was defined as the reappearance of HCV RNA during follow-up in subjects with previous end-of-treatment response (ETR), which is undetectable serum HCV RNA at the end of therapy, whereas Rapid Virological Response (RVR) is undetectable hepatitis C virus after 4 weeks of treatment, Early Virological Response(EVR) is undetectable or dropped viral load by 2 log after 12 weeks of HCV treatment and a transient viral response (TVR) was defined as a reappearance of HCV RNA in serum after treatment was discontinued in a patient who had undetectable HCV RNA during the therapy or on completion of the therapy. A non-viral response (NVR) was defined as cases with detectable viremia after and during treatment.

Genotyping of 4 polymorphism variants (SNPs) of IL28B

The SNP selection was performed following the work of Ito et al. (2011), and genotyping was performed as published previously by Rizk and Derbala (2012). Genomic DNA was extracted from EDTA whole blood samples using the QiaAmp DNA Blood Mini Kit #51166 (Qiagen GmbH, Hilden, Germany) followed by assessment of DNA concentration using a Nanodrop spectrophotometer. Polymorphisms of the studied SNPs were analyzed by the

5′ nuclease assay using a TaqMan MGB probe assay. The primers and TaqMan MGB probes of these polymorphisms were provided by the assay on-demand ™ service by Applied Biosystems. The 5′ nuclease assay was performed using 20 ng genomic DNA, 1X TaqMan Universal PCR Master Mix (Applied Biosystems), and 1X primer/probe mix using the appropriate conditions for amplification according to the manufacturer's instructions. Negative controls and non-template controls were included in each run. The reaction was performed using an ABI 7500 (Applied Biosystems, Foster City, CA) in the Biomedical Labs-Health Sciences Department-CAS-at University of Qatar, Doha-Qatar.

IP-10 Assay

For the detection of serum IP-10, the human cytokine multiplex immunoassay kit (MPXHCYTO-60K-06) was used according to the manufacturer's instructions from Millipore (Merck Millipore, Billerica, MA, USA). The percentage intra- and inter-assay CV of the assay was 4.6–13.8 and 3.7–17.2, respectively. The assay was performed using a Luminex 200 (Austin, TX, USA).

Statistical analysis

Continuous data are expressed as the mean and standard deviation for normalized distributed variables and by the median and interquartile for non-normalized distributed variables. Categorical data are presented in the form of number and percentage. All statistical analyses were performed using the SPSS program for Windows (version 20 statistical software; Texas instruments, IL, USA), and the Golden Helix SNP and Variation Suite (SVS 7.6 software; Bozeman, MT, USA) were used for genetic analysis. GraphPad Prism (version 6, for Mac, GraphPad Software, La Jolla California USA) was used for graphic representations. Genotype distributions and allele frequencies between the study groups were compared by constructing 2×2 contingency tables, x^2, and/or Fisher exact test corrected for Bonferroni adjustment for the number of SNPs. The Hardy-Weinberg equilibrium (HWE) was calculated using the chi-squared test to determine the genotype distribution in all study subjects. The odds ratios (ORs), 95% confidence intervals (CIs), and corresponding P values for the samples were analyzed by logistic regression analysis. The ORs were computed using the major homozygous allele as the reference group unless otherwise stated. The positive predictive value (PPV) was calculated for the major allele for good therapy response where $\mathrm{PPV}=(n_{\text{true positive}}/n_{\text{true positive}}+n_{\text{false positive}})$. Pairwise linkage disequilibrium coefficients (D') and haplotype frequency were estimated using the SVS 7.6 software. A two-tailed P-value < 0.05 was considered statistically significant.

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References

Antaki, N., Bibert, S., Kebbewar, K., Asaad, F., Baroudi, O., Alideeb, S., Hadad, M., Abboud, D., Sabah, H., Bochud, P.Y., Negro, F., 2013. IL28B polymorphisms predict response to therapy among chronic hepatitis C patients with HCV genotype 4. J. Viral. Hepatitis 20, 59–64.

Askarieh, G., Alsio, A., Pugnale, P., Negro, F., Ferrari, C., Neumann, A.U., Pawlotsky, J.M., Schalm, S.W., Zeuzem, S., Norkrans, G., Westin, J., Soderholm, J., Hellstrand, K., Lagging, M., Ditto, H.C.V., Groups, N.O.S., 2010. Systemic and intrahepatic interferon-gamma-inducible protein 10 kDa predicts the first-phase decline in hepatitis C virus RNA and overall viral response to therapy in chronic hepatitis C. Hepatology 51, 1523–1530.

Asselah, T., De Muynck, S., Broet, P., Masliah-Planchon, J., Blanluet, M., Bieche, I., Lapalus, M., Martinot-Peignoux, M., Lada, O., Estrabaud, E., Zhang, Q., El Ray, A., Vidaud, D., Ripault, M.P., Boyer, N., Bedossa, P., Valla, D., Vidaud, M., Marcellin, P., 2012. IL28B polymorphism is associated with treatment response in patients with genotype 4 chronic hepatitis C. J. Hepatol. 56, 527–532.

Balagopal, A., Thomas, D.L., Thio, C.L., 2010. IL28B and the control of hepatitis C virus infection. Gastroenterology 139, 1865–1876.

Charles, E.D., Dustin, L.B., 2011. Chemokine antagonism in chronic hepatitis C virus infection. J. Clin. Invest. 121, 25–27.

Chayama, K., Hayes, C.N., Abe, H., Miki, D., Ochi, H., Karino, Y., Toyota, J., Nakamura, Y., Kamatani, N., Sezaki, H., 2011. IL28B but not ITPA polymorphism is predictive of response to pegylated interferon, ribavirin, and telaprevir triple therapy in patients with genotype 1 hepatitis C. J. Infect. Dis. 204, 84–93.

Cieśla, A., Bociąga-Jasik, M., Sobczyk-Krupiarz, I., Głowacki, M.K., Owczarek, D., Cibor, D., Sanak, M., Mach, T., 2012. IL28B polymorphism as a predictor of antiviral response in chronic hepatitis C. World J. Gastroenterol.: WJG 18, 4892.

Derbala, M., Rizk, N., Al-Kaabi, S., Amer, A., Shebl, F., Al Marri, A., Aigha, I., Alyaesi, D., Mohamed, H., Aman, H., Basem, N., 2009. Adiponectin changes in HCV-Genotype 4: relation to liver histology and response to treatment. J. Viral. Hepatitis 16, 689–696.

Derbala, M., Rizk, N., Shebl, F., Alkaabi, S., Eldweik, N., John, A., Sharma, M., Yaqoob, R., Almohanadi, M., Butt, M., Alejji, K., 2012. Interleukin-28 and hepatitis C virus genotype-4: treatment-induced clearance and liver fibrosis. World J. Gastroenterol. 18, 7003–7008.

El-Awady, M.K., Mostafa, L., Tabll, A.A., Abdelhafez, T.H., Bader El Din, N.G., Zayed, N., Shenawy, R.E., El Abd, Y., Hasan, R.M., Zaghlol, H., El Khayat, H., Abdel Aziz, A.O., 2012. Association of IL28B SNP with progression of Egyptian HCV Genotype 4 patients to end stage liver disease. Hepatitis Mon. 12, 271–277.

El-Shamy, A., Shoji, I., El-Akel, W., Bilasy, S.E., Deng, L., El-Raziky, M., Jiang, D.P., Esmat, G., Hotta, H., 2012. NS5A sequence heterogeneity of hepatitis C virus genotype 4a predicts clinical outcome of pegylated-interferon-ribavirin therapy in Egyptian patients. J. Clin. Microbiol. 50, 3886–3892.

Fernandez-Arcas, N., Lopez-Siles, J., Trapero, S., Ferraro, A., Ibanez, A., Orihuela, F., Maldonado, J., Alonso, A., 2006. High prevalence of hepatitis C virus subtypes 4c and 4d in Malaga (Spain): phylogenetic and epidemiological analyses. J. Med. Virol. 78, 1429–1435.

Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., Shianna, K.V., Urban, T.J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., 2009a. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461, 399–401.

Ge, D., Fellay, J., Thompson, A.J., Simon, J.S., Shianna, K.V., Urban, T.J., Heinzen, E.L., Qiu, P., Bertelsen, A.H., Muir, A.J., Sulkowski, M., McHutchison, J.G., Goldstein, D.B., 2009b. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 461, 399–401.

Honda, M., Sakai, A., Yamashita, T., Nakamoto, Y., Mizukoshi, E., Sakai, Y., Yamashita, T., Nakamura, M., Shirasaki, T., Horimoto, K., 2010. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. Gastroenterology 139, 499–509.

Ito, K., Higami, K., Masaki, N., Sugiyama, M., Mukaide, M., Saito, H., Aoki, Y., Sato, Y., Imamura, M., Murata, K., Nomura, H., Hige, S., Adachi, H., Hino, K., Yatsuhashi, H., Orito, E., Kani, S., Tanaka, Y., Mizokami, M., 2011. The rs8099917 polymorphism, when determined by a suitable genotyping method, is a better predictor for response to pegylated alpha interferon/ribavirin therapy in Japanese patients than other single nucleotide polymorphisms associated with interleukin-28B. J. Clin. Microbiol. 49, 1853–1860.

Kamal, S.M., Nasser, I.A., 2008. Hepatitis C genotype 4: What we know and what we don't yet know. Hepatology 47, 1371–1383.

Lagging, M., Askarieh, G., Negro, F., Bibert, S., Soderholm, J., Westin, J., Lindh, M., Romero, A., Missale, G., Ferrari, C., Neumann, A.U., Pawlotsky, J.M., Haagmans, B.L., Zeuzem, S., Bochud, P.Y., Hellstrand, K., Group, D.-H.S., 2011. Response prediction in chronic hepatitis C by assessment of IP-10 and IL28B-related single nucleotide polymorphisms. PLoS One 6, e17232.

Li, K., Li, N.L., Wei, D., Pfeffer, S.R., Fan, M., Pfeffer, L.M., 2012. Activation of chemokine and inflammatory cytokine response in hepatitis C virus-infected hepatocytes depends on Toll-like receptor 3 sensing of hepatitis C virus double-stranded RNA intermediates. Hepatology 55, 666–675.

Liu, C.H., Liang, C.C., Liu, C.J., Tseng, T.C., Lin, C.L., Yang, S.S., Su, T.H., Lin, J.W., Chen, J.H., Chen, P.J., Chen, D.S., Kao, J.H., 2012. Interleukin 28B genetic polymorphisms play a minor role in identifying optimal treatment duration in HCV genotype 1 slow responders to pegylated interferon plus ribavirin. Antiviral Therap. 17, 1059–1067.

McCarthy, J.J., Li, J.H., Thompson, A., Suchindran, S., Lao, X.Q., Patel, K., Tillmann, H.L., Muir, A.J., McHutchison, J.G., 2010. Replicated association between an < i > IL28B < /i > gene variant and a sustained response to pegylated interferon and ribavirin. Gastroenterology 138, 2307–2314.

Miyamura, T., Kanda, T., Nakamoto, S., Wu, S., Jiang, X., Arai, M., Fujiwara, K., Imazeki, F., Yokosuka, O., 2012. Roles of ITPA and IL28B genotypes in chronic hepatitis C patients treated with peginterferon plus ribavirin. Viruses 4, 1264–1278.

Papastergiou, V., Dimitroulopoulos, D., Skorda, L., Lisgos, P., Ketikoglou, I., Kostas, N., Karatapanis, S., 2012. Predictors of sustained virological response in Greek and

Egyptian patients with hepatitis C genotype 4: does ethnicity matter? J. Med. Virol. 84, 1217–1223.
Pedergnana, V., Abdel-Hamid, M., Guergnon, J., Mohsen, A., Le Fouler, L., Theodorou, I., Mohamed, M.K., Fontanet, A., Plancoulaine, S., Abel, L., 2012. Analysis of IL28B variants in an Egyptian population defines the 20 kilobases minimal region involved in spontaneous clearance of hepatitis C virus. PLoS One 7, e38578.
Rallon, N.I., Soriano, V., Naggie, S., Restrepo, C., McHutchison, J., Vispo, E., Benito, J.M., 2012. Impact of IL28B gene polymorphisms on interferon-lambda3 plasma levels during pegylated interferon-alpha/ribavirin therapy for chronic hepatitis C in patients coinfected with HIV. J. Antimicrob. Chemother. 67, 1246–1249.
Rauch, A., Kutalik, Z., Descombes, P., Cai, T., Di Iulio, J., Mueller, T., Bochud, M., Battegay, M., Bernasconi, E., Borovicka, J., Colombo, S., Cerny, A., Dufour, J.F., Furrer, H., Gunthard, H.F., Heim, M., Hirschel, B., Malinverni, R., Moradpour, D., Mullhaupt, B., Witteck, A., Beckmann, J.S., Berg, T., Bergmann, S., Negro, F., Telenti, A., Bochud, P.Y., 2010. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 138 (1338–1345), 1345 e1331–1337.
Rizk, N.M., Derbala, M.F., 2012. Genetic polymorphisms of ICAM 1 and IL28 as predictors of liver fibrosis severity and viral clearance in hepatitis C genotype 4. Clin. Res. Hepatol. Gastroenterol.
Romero, A.I., Lagging, M., Westin, J., Dhillon, A.P., Dustin, L.B., Pawlotsky, J.M., Neumann, A.U., Ferrari, C., Missale, G., Haagmans, B.L., Schalm, S.W., Zeuzem, S., Negro, F., Verheij-Hart, E., Hellstrand, K., Group, D.-H.S., 2006. Interferon (IFN)-gamma-inducible protein-10: association with histological results, viral kinetics, and outcome during treatment with pegylated IFN-alpha 2a and ribavirin for chronic hepatitis C virus infection. J. Infect. Dis. 194, 895–903.
Shalaby, S.M., Radwan, M.I., Abdelazim, S., Nafee, A.M., 2012. Interleukin-4 polymorphisms and response to combination therapy in Egyptian chronic hepatitis C patients. Cell. Immunol. 276, 110–113.
Shi, X., Pan, Y., Wang, M., Wang, D., Li, W., Jiang, T., Zhang, P., Chi, X., Jiang, Y., Gao, Y., Zhong, J., Sun, B., Xu, D., Jiang, J., Niu, J., 2012. IL28B genetic variation is associated with spontaneous clearance of hepatitis C virus, treatment response, serum IL-28B levels in Chinese population. PLoS One 7, e37054.
Stattermayer, A.F., Stauber, R., Hofer, H., Rutter, K., Beinhardt, S., Scherzer, T.M., Zinober, K., Datz, C., Maieron, A., Dulic-Lakovic, E., Kessler, H.H., Steindl-Munda, P., Strasser, M., Krall, C., Ferenci, P., 2011. Impact of IL28B genotype on the early and sustained virologic response in treatment-naive patients with chronic hepatitis C. Clin. Gastroenterol. Hepatol. 9 (344–350), e342.
Tanaka, Y., Nishida, N., Sugiyama, M., Kurosaki, M., Matsuura, K., Sakamoto, N., Nakagawa, M., Korenaga, M., Hino, K., Hige, S., Ito, Y., Mita, E., Tanaka, E., Mochida, S., Murawaki, Y., Honda, M., Sakai, A., Hiasa, Y., Nishiguchi, S., Koike, A., Sakaida, I., Imamura, M., Ito, K., Yano, K., Masaki, N., Sugauchi, F., Izumi, N., Tokunaga, K., Mizokami, M., 2009. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat. Genet. 41, 1105–1109.
Thompson, A.J., Muir, A.J., Sulkowski, M.S., Ge, D., Fellay, J., Shianna, K.V., Urban, T., Afdhal, N.H., Jacobson, I.M., Esteban, R., 2010. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. Gastroenterology 139 (120–129), e118.
Yoneda, S., Umemura, T., Katsuyama, Y., Kamijo, A., Joshita, S., Komatsu, M., Ichijo, T., Matsumoto, A., Yoshizawa, K., Ota, M., Tanaka, E., Nagano Interferon Treatment Research, G., 2011. Association of serum cytokine levels with treatment response to pegylated interferon and ribavirin therapy in genotype 1 chronic hepatitis C patients. J. Infect. Dis. 203, 1087–1095.
Zhang, L., Miao, L., Han, F., Dou, X.G., 2011. Cytokine levels in serum of patients with chronic hepatitis C and its significance. Xi bao yu fen zi mian yi xue za zhi=Chinese J. Cell. Mol. Immunol. 27, 301–303.