Impact of *Interleukin-28B* gene polymorphism (rs12979860) on Egyptian patients infected with hepatitis C virus genotype-4

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تأثير الانترلوكين B 28 لتعدد أشكال الجين (rs12979860) على المرضى المصريين المصابين بفيروس التهاب الكبد سي النمط الجيني – 4

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الخلاصة: يمكن لتعدد أشكال النكليوتيدات المنفردة في جين انترلوكين B 28، وهي rs12979860، أن تنبئ بالاستجابة للعلاج بالانترفيرون–ألفا–ريبافيرين المضاد للفيروسات في المرضى المصابين بالتهاب الكبد الفيروسي "سي" من النمط الجيني 1. وقد تحقق الباحثون من وجود دور مماثل في دراسة للحالات والشواهد شملت 93 مريضا مصرياً مصاباً بالعدوى المزمنة بفيروس التهاب الكبد "سي" من النمط الجيني– 4، بالمقارنة مع 22 فردا تخلصوا تلقائياً من فيروس الكبد "سي"، و 70 متطوعا من الأصحاء. واتضح للباحثين ارتباط النمط الجيني للأليل "سي" المتماثل الزيجوت باستجابة فيروسية ممتدة للعلاج، مقارنة بالنمط الجيني للأليل "تي" المتماثل الزيجوت، وبالنمط الجيني للأليل "سي" المتغاير الزيجوت. وقد كان معدل الاستجابة في مجموعة الاستجابة الفيروسية الممتدة أعلى كثيراً وبدرجة يعتد بها إحصائيا في النمط الجيني للأليل "سي" المتماثل الزيجوت (58.6٪) مقارنة مع النمط الجيني للأليل "تي" المتماثل الزيجوت، ومع النمط الجيني للأليل "سي" المتغاير الزيجوت (20.3٪). ولم يشاهد تـرابط بين الأنماط الجينية لمرضى الاستجابة الفيروسية الممتدة والاستجابة المبكرة للعلاج أو الخط القاعدي للحمل الفيروسي لفيروس التهاب الكبد "سي". وتصف النتائج التي توصل الباحثون إليها كيف يمكن للتنميط الجيني IL-28B SNP أن يرشد إلى الاختيار المناسب للعلاج بالانترفيرون–ألفا–ريبافيرين للمرضى المصابين بفيروس التهاب الكبد سي-4.

ABSTRACT Single nucleotide polymorphisms (SNPs) in the *Interleukin (IL)-28B* gene, namely rs12979860, could predict response to pegylated interferon-α–ribavirin (PR) therapy in hepatitis C virus genotype 1 (HCV-1)-infected patients. A similar role was investigated in a case–control study conducted on 93 Egyptian patients chronically infected with HCV-4 in comparison to 22 individuals with spontaneous HCV clearance and 70 healthy volunteers. The homozygous C allele genotype (CC) was associated with sustained viral response (SVR) to therapy compared with the homozygous T allele genotype (TT) and the heterozygous genotype (CT). In the SVR group, the response rate was statistically significantly higher in CC genotypes (58.6%) compared with CT/TT (20.3%). There was no correlation between SVR patients' genotypes and early response to therapy or HCV baseline viral load. Our findings describe how *IL-28B* SNP genotyping may guide appropriate selection of HCV-4-infected patients for PR therapy.

Impact du polymorphisme du gène de l'*Interleukine-28B* (rs12979860) chez des patients égyptiens infectés par le virus de l'hépatite C de génotype 4

RÉSUMÉ Les polymorphismes mononucléotidiques du gène de l'*Interleukine (IL)-28B*, en l'occurence le rs12979860, permettent de prédire la réponse au traitement par interféron-α pégylé associé à la ribavirine chez des patients infectés par le virus de l'hépatite C de génotype 1. Un rôle similaire a été examiné au cours d'une étude cas-témoins conduite auprès de 93 patients égyptiens chroniquement infectés par le virus de l'hépatite C de génotype 4 comparés à 22 sujets ayant connu une clairance spontanée du virus de l'hépatite C et à 70 volontaires en bonne santé. Le génotype CC homozygote pour l'allèle C a été associé à une réponse virale soutenue au traitement par rapport au génotype TT homozygote pour l'allèle T et le génotype CT hétérozygote. Dans le groupe ayant obtenu une réponse virale soutenue, le taux de réponse était statistiquement supérieur pour les génotypes CC(58,6 %) par rapport aux génotypes CT/TT (20,3 %) Aucune corrélation n'a été observée entre le génotype des patients ayant obtenu une réponse virale soutenue et une réponse précoce au traitement ou la charge virale initiale du VHC. Nos résultats décrivent comment le génotypage des polymorphismes mononucléotidiques du gène de l'*Interleukine-28B* faciliterait la sélection des profils appropriés de patients infectés par le virus de l'hépatite C de génotype 4 pour qu'ils reçoivent un traitement par interféron-α pégylé associé à la ribavirine. Nous soulignons le rôle du génotypage (*IL)-28B* en tant qu'outil susceptible d'augmenter le rapport coût-avantages de ce traitement en Égypte.

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Received: 24/09/12 ; accepted: 09/12/12

Introduction

Hepatitis C virus (HCV) infection, one of the major causes of liver disease worldwide, is highly prevalent in Egypt with a predominance of HCV genotype 4 (HCV-4) [1]. Infection outcome depends on how people respond to the HCV virus. About 20% of acutely infected persons can clear the virus; this is known as "spontaneous clearance" [2]. Despite significant improvements in HCV treatment, e.g. in treatment of other forms such as HCV-1, the efficacy of pegylated interferon (Peg-IFN)/Ribavirin to induce sustained virological eradication is only expected to be achieved in up to 60% of Egyptian patients infected with HCV-4 [3]. Taking into consideration the side-effects of the treatment and the low compliance, clinicians should have accurate determinants of response to treatment to help them distinguish responders from non-responders [4].

Genetic variants in immune response genes influence disease outcome and the likelihood of achieving spontaneous viral clearance or sustained viral response (SVR) [5]. Recently, *Interleukin-28B* gene (*IL-28B*) polymorphisms were shown to strongly predict viral clearance [5,6]. The rs12979860 is a single-nucleotide polymorphism (SNP) located 3 kb upstream of the *IL28B* gene region [6]. It seemed to be a good predictor for spontaneous clearance and treatment associated resolution of HCV in several genome-wide association studies [7–9]. The distribution of rs12979860 and its relation to treatment response have been studied in HCV-1- and HCV-2-infected patients, and to a lesser extent with HCV-3 [7,10]. To date, one cohort study conducted on Egyptians has reported the association between rs12979860 and HCV spontaneous clearance [11]. In addition, 2 studies have described its frequency in HCV-4-infected patients [12,13].

In the current study, we describe for the first time the distribution of rs12979860 genotype and allele frequency in healthy Egyptian individuals compared with HCV spontaneous clearance patients and HCV-4-infected patients treated with IFN therapy. We also analysed the possible correlation between the pattern of this SNP in HCV patients and the clinical aspects of the disease, including treatment response.

Methods

Patients

This matched case–control study comprised 93 patients chronically infected with HCV-4 followed up in the Gastroenterology and Hepatology Unit, Department of Internal Medicine, Suez Canal University Hospital, Ismailia, Egypt, between July 2011 and January 2012. Seventy three (73) patients (78.5%) finished the treatment regimen, which consisted of 180 μg pegylated interferon-α-2a (pegIFN)/week plus 800–1400 mg Ribavirin (RBV) given orally daily based on body weight for 48 weeks. The rest of the patients (21.5%) were still under treatment. Clinical and laboratory data of HCV patients at the time of diagnosis and prior to therapy were retrieved from their files, including HCV RNA titre, HCV genotype and liver biopsy findings.

HCV patients were further stratified according to their response to treatment into:

- SVR: patients who were HCV RNA negative more than 6 months after the end of therapy (30/93, 32.3%).
- EVR: patients who had undetectable HCV RNA at weeks 4 and 12 during therapy (20/93, 21.5%),
- non-responders (NR): patients who did not respond to treatment or had a relapse after the end of therapy [total: 43 patients (46.2%); non-responders: 25/93 (26.9%); relapsers: 18/93 (19.3%).

Twenty two (22) participants who had ≥ 2 positive HCV antibody tests and ≥ 2 negative HCV RNA results more than 3 months apart were also enrolled in the study as having spontaneous HCV clearance.

Patients co-infected with hepatitis B virus, HIV or schistosomiasis were excluded from the study.

Controls

Seventy apparently healthy, age- and sex-matched volunteers were recruited from the blood bank in the Suez Canal University hospital, Ismailia, Egypt. They were negative for anti-HCV antibody, hepatitis B surface antigen and HIV antibody and had normal levels of liver enzymes. They had negative serum HCV RNA as detected by quantitative real time polymerase chain reaction (RT-PCR).

Ethical considerations

This study was conducted in accordance with the guidelines in the Declaration of Helsinki and was approved by the Review Board of the Faculty of Medicine, Suez Canal University. Informed, written consent was obtained from all participants.

Hepatitis C virus antibody testing:

Positivity for hepatitis C virus antibody in patients with spontaneous HCV clearance and negativity in the control group were confirmed with commercially available, third-generation enzyme-linked immunosorbent assay (ELISA) kits (Abbott Laboratories, North Chicago, Illinois).

Quantification of hepatitis C virus RNA levels

Viral RNA was extracted from participants' sera samples using QIAamp Viral RNA Mini Kit (Cat No. 52904, Qiagen GmbH Corporation, Hilden, Germany). RT-PCR was performed using TaqMan One-Step RT-PCR master mix Reagents Kit [Cat No. 4309169,

Applied Biosystems, Foster City, United States of America (USA)], primers and a FAM labelled HCV probe for the 5′ non-coding region of the HCV genome (Applied Biosystems). HCV RNA titres of samples were determined using ABI prism 7000 software (Applied Biosystems). The detection limit was 50 IU/mL.

IL-28B polymorphism genotyping:

Genomic DNA was extracted from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, Madison, Wisconsin, USA), and stored at –20 °C until genotyping was performed. Detection of rs12979860 C/T polymorphism was performed by PCR and restriction fragment length polymorphism analysis (RFLP).

PCRs were carried out in a total volume of 25 µL with 150 ng genomic DNA, 2.5 U Taq polymerase in 1X Taq polymerase buffer (Promega), 1.5 mmol $MgCl_2$, 0.2 mM of each dNTP, and 10 pmol of the following primers, 5′-AACTCAACGCCTCTTCCTCCT-3′ (forward) and 5′-TTCCCATACACCCGTTCCTGT-3′ (reverse) [14] generating a 402 bp PCR product. PCR amplifications were performed as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles at 94 °C for 30 sec, 58 °C for 30 sec, and 72 °C for 1 min, with a final extension at 72 °C for 10 min.

Ten µL of the amplified product was digested overnight at 37 °C with 2U of *BstUI* restriction enzyme (Fermentas Inc, USA). According to the digestion products visualized on 2% agarose gel, rs12979860 genotypes were categorized into: CC genotype, identified by the presence of 4 bands (170, 118, 89, and 25 bp), and TT genotype, which generated 3 bands (195, 118 and 89 bp).

Statistical analysis

Statistical analysis was performed using *SPSS*, version15.0. Results were presented as mean and standard deviation (SD) or percentage, as appropriate. Hardy–Weinberg equilibrium was determined by the chi-squared test with 1 degree of freedom. Allele frequencies for *IL-28B* SNP were calculated and compared between those with sustained responders to treatment, spontaneous clearance and HCV persistence using *MedCalc* software, version 12.0.1.

The association of polymorphisms with treatment response, viral clearance or persistence was examined for statistical significance using standard Pearson chi-squared test or Fisher exact test, as appropriate. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess treatment response conferred by a specific allele.

Differences were considered statistically significant at $P < 0.05$.

Results

Characteristics of HCV–infected patients

Demographic characteristics and clinco–laboratory data of chronic HCV patients and individuals associated with viral clearance are summarized in Table 1. HCV patients ranged from 23 to 65 years of age; the majority of patients were older than 40 years (87.1%). Patients with HCV spontaneous clearance had significantly lower mean age compared to the total HCV patient group ($P \leq 0.001$) ranging from 18 to 55 years (Table 1). There was no sex difference between HCV patient subgroups or viral clearance individuals (male/female ratio ~ 1.7 and 2:1, respectively).

Genotypes distribution and rs12979860 polymorphism alleles' frequency

The rs12979860 genotypes fitted the Hardy–Weinberg equilibrium in all studied groups and subgroups ($P \geq 0.05$). There was no difference in either genotype distribution or allelic frequencies in healthy controls compared to total HCV-4-infected patients at the rs12979860 loci (Table 2). The favourable C allele frequency was statistically significantly higher in individuals with spontaneous HCV clearance compared to total HCV patients and the control group (75.0% vs. 56.4% and 55.0%, respectively; $P = 0.026$ and 0.02 respectively) (Table 2). Individuals who carried the protective CC genotype were 2.6 (95% CI: 1.02–6.8; $P = 0.04$) times more likely to clear the virus than those who carried the CT/TT genotypes (Table 2).

rs12979860 SNP association with host response to combined interferon therapy

Frequencies of rs12979860 genotypes and alleles were tested for their association with response to PR therapy in treated chronic HCV patient subgroups. The CC genotype was significantly associated with sustained response to combined IFN therapy compared to CT/TT (56.7% vs 13.9% in the NR subgroup) (OR = 8.06; 95% CI: 2.61–24.8; $P = 0.003$) (Table 2). Furthermore, the favourable allele C frequency was significantly higher in the SVR than in the NR subgroup (73.3% vs 44.2% (OR = 3.47; 95% CI: 1.7–7.08; $P = 0.006$) (Table 2).

When the early response to combined IFN therapy was considered, there was no statistically significant difference in genotypes distribution or allelic frequencies in the EVR subgroup compared to NR patients ($P \geq 0.05$) (Table 2). In addition, there was difference in the proportion of CC genotype patients who achieved early viral response compared to CT/TT in the SVR group, although the difference was not statistically significant (41.1% vs 30.7%; $P = 0.2$). Interestingly, EVR was lower in NR patient with CC genotype compared to CT/TT, but again the difference was not significant (16.7% vs 27%; $P = 0.59$).

Table 1 Characteristics of HCV–infected patients in the study population

Characteristic	Total HCV patients (*n* = 93)		PR treated HCV patients						Control Group (*n* = 70)		HCV spontaneous clearance (*n* = 22)	
			Responders[a] (*n* = 30)		Non-responders[b] (*n* = 43)		Early responders (*n* = 20)					
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Sex												
Male	59	63.4	18	60.0	27	62.8	14	70.0	42	60.0	15	68.2
Female	34	36.6	12	40.0	16	37.2	6	30.0	28	40.0	7	31.8
Age group (years)												
< 40	12	12.9	8	26.7[c]	4	9.3	0	0.0	16	22.8	13	59.1[d]
≥ 40	81	87.1	22	73.3	39	90.7	20	100.0	54	77.2	9	40.1
Fibrosis (Metavir score)									NA		NA	
0 and 1	15	16.1	11	36.7[c]	0	0.0	4	20.0	–		–	
2	57	61.3	19	63.3	22	51.2	16	80.0	–		–	
3	21	22.6	0	0.0	21	48.8	0	0.0	–		–	
Baseline viral load distribution												
< 6×10^5 IU/mL	10	10.7	3	10.0	3	7.0	4	20.0	NA		NA	
≥ 6×10^5 IU/mL	83	89.3	27	90.0	40	93.0	16	80.0	–		–	
	Mean (SD)		**Mean (SD)**		**Mean (SD)**		**Mean (SD)**		**Mean (SD)**		**Mean (SD)**	
Age (years)	47.5 (6.9)		45.7 (7.0)		47.8 (6.6)		49.7 (7.0)		47.3 (2.4)		37.7 (10.6)[e]	
AST (U/L)	48.3 (25.3)		33.2 (15.8)[c]		60.5 (28.3)		44.5 (15.3)		27.8 (9.2)		25.8 (7.4)[e]	
ALT (U/L)	51.2 (30.7)		34.2 (17.1)[c]		65.4 (35.4)		45.8 (20.3)		30.8 (6.8)		26.1 (4.1)[e]	
Serum albumin (g/dL)	4.2 (0.48)		4.2 (0.2)		4.2 (0.4)		4.5 (0.5)		4.5 (0.67)		4.7 (0.13)	
Total bilirubin (mg/ dL)	0.8 (0.3)		0.85 (0.29)		0.77 (0.3)		0.7 (0.19)		0.7 (0.4)		0.6 (0.2)	
Platelet count (× 10^9/L)	211.6 (191.3)		213.7.6 (52.3)		278.5 (230.7)		170.1 (56.9)		281.2 (20.8)		274.2 (36.2)[e]	
Baseline viral load (× 10^6 IU/mL)	1.56 (1.0)		1.77 (1.1)		1.55 (0.78)		1.28 (1.1)		NA		NA	

[a]Patients with sustained viral response to peg-IFN/RBV therapy; [b]Non-responder or relapser group; [c]comparison between responders and non-responders (P < 0.05); [d]comparison of age distribution of spontaneous HCV clearance patients with all HCV patients and non-responders (P ≤ 0.001); [e]comparison of spontaneous clearance individuals with total HCV patients (P < 0.01).

ALT = alanine aminotransferase; AST = aspartate aminotransferase; HCV = hepatitis C virus; IU = international unit; NA = not applicable; PR = pegylated interferon-α-2a plus ribavirin therapy; SD = standard deviation.

Table 2 Comparison of genotype distribution and allele frequencies of rs12979860 polymorphism among the various patient groups

Patient group	rs12979860 genotype						*P*-value	Odds ratio (95% CI)	rs12979860 allele				*P*-value	Odds ratio (95% CI)
	CC		CT		TT				C		T			
	No.	%	No.	%	No.	%			No.	%	No.	%		
All HCV (n = 93)	29	31.2	47	50.5	17	18.3	NS[a]	–	105	56.4	81	43.6	NS[a]	–
SVR (n = 30)	17	56.7	10	33.3	3	10.0	0.003[b]	8.06 (2.61–24.8)[b]	44	73.3	16	26.7	0.006[b]	3.47 (1.7–7.08)[b]
NR (n = 43)	6	13.9	26	60.5	11	25.6			38	44.2	48	55.8		
EVR (n = 20)	6	30.0	11	55.0	3	15.0	NS[c]	–	23	57.5	17	42.5	NSc	–
Controls (n = 70)	20	28.6	37	52.8	13	18.6			77	55.0	63	45.0		
Spontaneous clearance (n = 22)	12	54.5	9	41.0	1	4.5	0.04[d]	2.6 (1.02–6.8)[d]	33	75.0	11	25.0	0.026[d]	2.3 (1.1–4.85)[d]
							0.029[e]	3 (1.11–8.04)[e]					0.02[e]	2.45 (1.14–5.2)[e]

CC vs. CT/TT genotypes or C vs. T allele were compared in: [a]total HCV patients group vs control group; [b]SVR group vs NR group, [c]EVR group vs SVR group, and in HCV clearance group vs [d]total HCV group and [e]control group.
CI = confidence interval; EVR = early viral response to combined Interferon therapy; NS = not significant (P > 0.05); NR = transient and no response to combined interferon therapy; SVR = sustained viral response to therapy.

Factors associated with treatment response or viral clearance in relation to the rs12979860 SNP and different clinico–laboratory risk variables

Univariate analysis showed no relationship between viral clearance and the patients' sex (OR = 0.8; 95% CI: 0.3– 2.1; P = 0.6) whereas older age at infection was associated with viral persistence (OR = 9.75; 95% CI: 3.4–27.7; P < 0.001). Analysis of the different clinico–laboratory variables in relation to treatment response showed no significant role of the patient's sex or baseline viral load on treatment outcome. On the other hand, later age at onset of therapy and advanced fibrosis score seemed to have a negative effect on treatment response (Table 3).

In relation to the rs12979860 genotypes, the sustained response rate in the SVR group was significantly higher in CC genotype patients (58.6%) compared to CT/TT (20.3%) (P = 0.0003). Univariate analysis revealed an association between advanced stages of fibrosis (≥ 2) and rs12979860 CT/TT genotypes in the total treated patients group (OR = 5.03; 95% CI: 1.29–19.48; P = 0.019). However, in the SVR subgroup, the stage of liver fibrosis was independent of *IL28B* SNP genotype (CC vs CT/TT) (OR = 1.57; 95% CI: 0.34–7.2; P = 0.55). Moreover, SVR patients with CC genotype had slightly higher mean baseline viral load compared with the NR subgroup and also compared with responders with CT and TT genotypes (mean: 2.1 (SD 1.2); 1.7 (SD 0.86); 1.37 (SD 0.83) and 1.18 (SD 0.56) × 10^6 IU/mL, respectively). The differences were, however, not statistically significant (P = 0.098, 0.075 and 0.079 respectively).

Discussion

The rs12979860 SNP in *IL-28B* gene loci has been identified as the strongest predictor of spontaneous HCV viral clearance [5,6,8] and SVR to IFN therapy [6,7]. The current study investigated the association of rs12979860 SNP with different treatment outcomes in Egyptian patients infected with HCV-4, as well as allele frequencies in the normal population. In spite of the small number of healthy subjects enrolled in the study, the C allele frequency was lower in both controls and total HCV-infected patients, approximately 55%, compared with individuals with spontaneous clearance and SVR, approximately 75%. The C allele frequency in our population is similar to previously reported data in Europeans (52%–79%) in the ALlele FREquency Database [15]. We also demonstrated that the favourable CC genotype is strongly associated with SVR to combined IFN therapy. However, early responders had similar genotype distributions and allelic frequencies compared with non-responders. Surprisingly, the protective genotype was accompanied by a high baseline viral load, even in the SVR subgroup, while sustained response was not associated with an early response to therapy.

A similar protective role of rs12979860 CC genotype has been reported in different racial groups infected with HCV-1 [6,7,16] and in a few studies on HCV-4-infected patients [12,13,17]. In addition, the association between the rs12979860 C allele and natural HCV clearance was confirmed in European cohorts [5,7] and has been

Table 3 Relationship between treatment response and clinical and laboratory risk factors

Variable	Responders[a] (*n* = 30)		Non-responders[b] (*n* = 43)		OR (95% CI)	*P*-value
	No.	%	No.	%		
Sex						
Female	18	60.0	27	62.8	1	
Male	12	40.0	16	37.2	1.1 (0.43–2.9)	0.8
Age group (years)						
< 40	8	26.6	4	9.3	1	
≥ 40	22	73.4	39	90.7	3.5 (0.95–13.1)	0.058
Fibrosis (Metavir score)						
≤ 1	11	36.6	0	0.0	1	
≥ 2	19	63.4	43	100.0	51.3 (2.87–915.3)	0.0007
Baseline viral load (IU/mL)						
$< 6 \times 10^5$	3	10.0	3	7.0	1	
$\geq 6 \times 10^5$	27	90.0	40	93.0	1.48 (0.28–7.9)	0.64

[a]*Patients with sustained viral response to peg-IFN/RBV therapy.*
[b]*Non-responder or relapser group.*
OR = odds ratio; CI = confidence interval.

tested in only 1 Egyptian study [11]. Although the protective genotype frequency in the current study was slightly higher than that reported by Asselah et al. [12] and De Nicola et al. [13] (23% and 26.8%, respectively), the response rates of the CC genotype patients with SVR in these 2 studies were quite high compared with our results (81.8% and 89%, respectively, vs. 58.6%). Several host and viral factors have been identified as predictors of treatment response, yet ethnic differences and the genetic make-up of infected patients is worth further investigations to help selecting putative responders to IFN therapy. For example, rs12979860 TT genotype correlated to non-response and/or relapse in Africans although it had a higher frequency in SVR patients of European ancestry [18].

The exact biological pathways underlining the IL28B gene SNP association with treatment response and viral clearance remain unknown. Host innate immune mechanisms including IFN-λ, a direct product of the *IL28B* gene, control viral infection [19]. Initial binding of IFN-λs to its receptor is followed by several events that end with the induction of interferon-stimulating genes (ISGs) [19]. It is speculated that this mechanism suppresses viral infection. Moreover, IFN-λ has been shown to block HCV replication in human hepatocytes *in vitro* [20]. The role of *IL-28B* variants in relation to INF-λ3 expression could be explained by a number of possibilities:

- *IL-28B* SNP may be a marker of another DNA sequence that modifies the gene expression. Actually, the protective CC genotype of rs12979860 is strongly associated with a non-synonymous *IL-28B* mutation (rs8103142). Since this mutation is distant from the receptor binding site, it has no effect on INF-λ3 antiviral activity [21].
- The upstream location of the rs12979860 SNP in *IL-28B* gene may correlate with the regulation of *IL-28B* transcription [6].
- Alternatively, it can act indirectly through modification of a transcription factor binding site [22].
- Finally, *IL28B* variants might cause an abnormal expression of endogenous IFN- λ3 (non-, weak-, or hyper-functioning variants). This might alter the host response to antiviral therapy by negative feedback [22].

Our data did not show any difference in C allele frequency between early responders and non-responders/relapsers subgroups. In addition, the SVR subgroup was not associated with early response to therapy, even in CC genotype patients. This was unexpected since rs12979860 CC genotype has been reported to be associated with EVR in HCV-1-infected patients [5,7,16], HCV-2 and -3 [23], and HCV-4 [17]. This controversy could be related to the direct effect of variables like ethnicity, liver fibrosis staging and HCV viral load [3]. In fact, the mechanism by which the SNPs in *IL28B* affects the IFN signalling pathways still needs to be further clarified, in particular its role in early response to treatment. In agreement with our findings, Marcello et al. [19] found that IFN-λs activity against HCV *in vivo* was mediated through slow induction of steady increase in ISGs genes, whereas IFN-α resulted into an early activation of ISGs followed by a rapid decline [19]. We can speculate that the effect of endogenous INF-λ is more related to the sustained response to combined IFN therapy than to the

early response. However, the role of CC genotype at the rs12979860 locus in relation to early response to treatment should be confirmed with a larger scale study.

Our results confirm the positive role of *IL-28B* polymorphism in maintaining IFN treatment response in HCV-4-infected Egyptian patients. These findings emphasize the concepts of tailored therapy and pharmacogenetics that should be clinically considered for successful treatment response in HCV-4-infected patients. Given the high association of rs12979860 CC genotype with SVR but not with EVR, this should encourage clinicians to continue the therapy, even in the absence of early response. This is essential in low-income, developing countries with a high prevalence of HCV infection like Egypt where treatment cost is a major concern.

Given the known variable response to IFN therapy and its side-effects, our findings report the priority of *IL-28B* gene variation assessment in the proper selection of patient for therapy. Our data shed the light on the pattern of *IL-28B* SNP in chronic HCV-4 patients and its association with the different variables that can alter the physician's decision prior to therapy. Lastly, we describe an effective, simple and low-cost approach of *IL28B* SNP genotyping using PCR-RFLP procedure. Characterization of rs12979860, in correlation with variables like viral load and liver fibrosis stage, would maximize the cost–benefit of therapy in Egypt and prevent advanced liver disease, including hepatocellular carcinoma.

Funding: None.

Competing interests: None declared.

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