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ORIGINAL ARTICLE

Interleukin 28A.rs12980602 and interleukin 28B.rs8103142 genotypes could be protective against HCV infection among Egyptians

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Abstract

Previous studies showed that interleukin (IL)-28B gene polymorphisms were associated with hepatitis C Virus (HCV) infection and treatment outcomes. We tested whether single-nucleotide polymorphisms (SNPs) in IL-28A and IL-28B are associated with HCV infection among Egyptians with HCV genotype 4 infections. We enrolled 144 chronic HCV patients, 72 spontaneously resolved HCV subjects, and 69 healthy controls. Four SNPs in IL-28A and IL-28B genes (IL-28A.rs12980602, IL-28B.rs12979860, IL-28B.rs8099917, and IL-28B.rs8103142) were genotyped. The most frequent IL-28B haplotype "TCT" was significantly more frequent in HCV-infected subjects than in HCV negative subjects (62.2% vs. 48.6%, respectively; $p = 0.005$). The frequency of IL-28A.rs12980602 "T" allele was significantly higher than the "C" allele in healthy controls compared to HCV-infected subjects ($p < 0.001$) with the "TT" genotype significantly higher in healthy controls compared to HCV-infected subjects ($p < 0.001$) with no association with viral load ($p = 0.11$) among chronically infected subjects. The results, also, confirmed the previous role of IL-28B SNPs in predicting HCV infection outcome. Importantly, IL-28B.rs8099917 "TT" genotype was significantly associated with low viral load in HCV-infected subjects, while the remaining three SNPs did not. The three IL-28B SNPs were in linkage disequilibrium ($D' > 0.68$; $r^2 > 0.43$) for all comparisons in HCV patients, while there was no linkage disequilibrium of IL-28A polymorphisms and the three IL-28B SNPs. In conclusion, IL-28A.rs12980602 and IL-28B.rs8103142 TT genotype could be protective against HCV infection. Also, IL-28B.rs12979860, IL-28B.rs8099917, and IL-28B.rs8103142 SNPs predicted the outcome of HCV infection among genotype-4-infected Egyptians. Moreover, IL-28B.rs8099917 SNP affected the viral load in chronic HCV patients.

Keywords HCV · Single-nucleotide polymorphism · IL-28A · IL-28B · IFN-λ

Introduction

Hepatitis C virus (HCV) infection is one of the leading causes of chronic liver disease and has emerged as a global public health concern affecting between 70 million and 100 million persons worldwide [1] with > 399,000 individuals dying yearly from HCV-related liver diseases [2]. Egypt has one of the highest worldwide HCV prevalence with an antibody positivity rate of 6.3% among individuals aging 1–59 years; of whom 4.4% are HCV-RNA positive [3, 4]. About 90% of Egyptian HCV isolates belong to HCV genotype 4a [5–8]. Recent epidemiological studies to assess the HCV disease

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burden have suggested that the viremic prevalence has decreased from 9.7% in the Demographic Health Survey (DHS) of 2008 [9, 10] to 4.5% of the population (7% in the age groups 15–59 years and 0.2% in those aged < 15 years) [11] estimated by the Ministry of Health and Population in 2015 [12]. This is mainly due to the death of older populations (from the study in 2008) who had the highest prevalence of infection in 2008 or being excluded from the 2015 survey as they became older than 59 years and due to a significant decrease in new infections in those aged 15–19 years. Starting form 2016, direct-acting antiviral agents (DAAs) were introduced to the hepatitis treatment protocol in Egypt. To date, more than 850,000 patients received DAA treatment [13] with the goal of achieving a national chronic infection prevalence of < 2% by 2025 [14] and HCV elimination by 2030 [15].

Several epidemiological, host, and viral factors contribute to HCV clearance or disease progression. Host factors such as gender, co-infections, and genetics are known to affect the likelihood of clearance or persistence and consequently the heterogeneity of the outcome in different populations [16]. A strong and broad immune response during acute infection is associated with viral clearance [17]. However, the strongest genetic factor associated with spontaneous clearance described to date is single-nucleotide polymorphisms (SNPs) around the interferon (IFN) lambda 3 (IFN-λ3) gene (also known as the interleukin; IL-28B gene), in particular rs8099917 and rs12979860 [18]. The mechanism underlying this association was recently examined [19].

IFN-λs comprise three distinct genes: IFN-λ1 (IL-29), IFN-λ2 (IL-28A), and IFN-λ3 (IL-28B) [20, 21]. IL-28A and IL-28B proteins are 95% identical while IL-29 shares only 80% amino acid identity with IL-28A or IL-28B. Structurally, IFN-λs are related to IL-10 and other members of the IL-10-like family such as IL-22 [22], which were shown to confer hepato-protection [23, 24]. IFN-λs functionally resemble type I IFNs (IFN-α/β), which exhibit activity against a broad range of viruses such as encephalomyocarditis virus or vesicular stomatitis virus [20, 21], human immunodeficiency virus [25], Apeu virus [26], cytomegalovirus [27], and herpes simplex virus [28]. It has, also, been shown that IFN-λs inhibit hepatic HCV replication [29, 30], induce expression of antiviral proteins, and are upregulated during viral infection [31]. Several studies demonstrated that the activation of type III IFN induces apoptosis and that polymorphisms of the IL28B gene (IFN-λ3) showed a powerful association with response to treatment in HCV patients. This effect was influenced by ethnicity [32, 33].

Among the genetic factors possibly involved, the stronger genetic association with HCV treatment response was observed in IFN-λ3 gene, located on chromosome 19 (19q13.13) and encoding for IL-28B. More specifically, IL-28B.rs12979860 and IL-28B.rs8099917 gene polymorphisms have been associated with response to drug treatment in HCV-infected patients [32, 34]. Although several studies examined the association of IL-28B.rs12979860 among Egyptians with HCV infection [35–39] and those with genotype 4 infection [40], there have been scarce data regarding association of IL-28A (IFN-λ2) and IL-28B (IFN-λ3) with the outcome of HCV infection among Egyptians. We, therefore, investigated the distribution and effect of these variants on resolution of HCV infection among an Egyptian cohort well characterized for the natural outcome of HCV infection. We show that IL-28A.rs12980602 and IL-28B.rs8103142 TT genotype may be protective against HCV infection and that IL-28B.rs12979860, IL-28B.rs8099917, and IL-28B.rs8103142 SNPs predict the outcome of HCV infection among genotype-4-infected Egyptians.

Subjects and methods

Study population

We enrolled 144 patients with chronic HCV infection, 72 subjects with spontaneously resolved infection, and 69 healthy controls. Individuals with HBV co-infection were not included in the study. All subjects were enrolled in the period from 2010 to 2013 and were treatment-naïve at the time of enrolment. The subjects were either patients attending the National Liver Institute (NLI), Menoufia University, or healthy healthcare workers at the same institute. The study included 181 males (63.5%) and 104 females (36.5%). The average age of the subjects was 39.9 ± 11.6 years. None of the HCV-infected subjects had received interferon-α and ribavirin or any other HCV treatment before participation in this study. Based on the clinical history of the subjects, HCV antibody-positive subjects who tested negative for HCV-RNA were considered spontaneous clearers of HCV infection. The study protocol was approved by the NLI-IRB committee and a written informed consent was obtained from each subject enrolled in the study.

Laboratory testing

Serum alanine (ALT) and aspartate (AST) aminotransferases were measured using commercial kits. Antibodies against HCV were tested using a third generation ELISA (Murex anti-HCV; version 4.0; USA) as recommended by the supplier's instructions. Hepatitis B virus surface antigen (HBsAg) was tested by ELISA as per the manufacturer's instructions (Murex). Serum levels of HCV-RNA were quantified by reverse transcriptase polymerase chain reaction (RT-PCR) using a Stratagene

mx3005p qPCR following extraction of RNA by means of a viral RNA extraction kit (QIAgen, USA) as reported [8]. Also, HCV genotype was tested as described [8].

DNA extraction and IL-28 A/B genotyping

Two hundred microliters of whole blood was used for obtaining genomic DNA via extraction by the QIAamp DNA blood Mini kit as described in the manufacturer's protocol (Qiagen, USA). IL-28B genotyping was determined by SYBR green real time PCR and specific primers as follows: **IL-28B.rs12979860**: 5′-GCTTATCGCATACGGCTAGGC-3′, 5′-GCAATTCAACCCTGGTTC G, and GCAATTCA ACCCTGGTTCA-3′ [41]; **IL-28B.rs8099917**: 5′-CTCC TTTTGTTTTCC TTTCTG-3′, 5′-CATGGTTCCAATTT GGGTGAA-3′, and 5′-CATGGTTCCAATTTGGGTG AC-3′; **IL-28B.rs8103142**: 5′-TGAGCAGGGCTGGGAGG-3′, 5′-CTTCTGAAGGACTGC AG-3′, and 5′-CTTC TGAAGGACTGCAA-3′ [42]; **IL-28A.rs12980602**: 5′-ATTACAGGT GTGGGCCACTG-3′, 5′-CATA TAACAATATGAAAGCCAGAGAT-3′, and 5′-CATATA ACAATATGAAAGCCAGAGAC-3′ (all primers were designed by Dr. S. Knapp). Genotyping was examined on a Stratagene mx3005p qPCR machine using 96-well plates and 10–100 ng genomic DNA with 0.5 μmol/l of each primer in a 20 μL reaction mixture. The PCR protocol consisted of an initial denaturation step at 95 °C for 10 min, followed by 40 two-step amplification cycles of 95 °C for 20 s and 60 °C for 20 s.

Statistical analysis

Patients and laboratory data were recorded in a Microsoft Access database (Redmond, WA). Data were entered twice for quality control. Data analysis was performed using SPSS package version 17.0 (SPSS Inc., Chicago, IL, USA). Categorical data were compared by chi-square test and the Student's *t* test (or Mann-Whitney's *U* test when appropriate) was used to compare continuous data. Categorical variables of the groups (genotype allele distributions and gender) were compared by Pearson's chi-square test or Fisher's exact test. Continuous data (age) of the groups were compared by the Mann–Whitney *U* test. Adjusted "*p*" values and odds ratio (OR) including variables associated with the outcome of HCV infection (genotype, age, and gender) were examined by multivariate logistic regression analysis. A multivariate regression model was calculated for any two strongly associated genetic markers to avoid the effects of co-linearity. Two-tailed *p* values are presented and a *p* value of < 0.05 pointed out a statistically significant departure from the null hypothesis. Haploview software version 4.2 was used to calculate pair-wise linkage disequilibrium (LD; D0, r^2).

Results

Characteristics of the studied populations

The demographic and laboratory characteristics of the study subjects are shown in Table 1. The average ages at admission of the total (males and females) chronic patients, resolvers, and the healthy controls were 42.8 ± 11.6, 41.7 ± 10.7, and 32.1 ± 8.5 years, respectively ($p < 0.017$). The chronic, resolved and healthy controls included 59.7%, 65.3%, and 69.6% males, respectively; with remaining subjects being females ($p = 0.429$). The rural residence for the chronic, resolved, and healthy controls groups were 53.5%, 58.3%, and 44.9%, respectively ($p = 0.23$). The average ALT and AST levels of the chronic group were significantly higher than that of the resolved and healthy controls ($p < 0.001$, Table 1).

Impact of IL-28A and IL-28B gene polymorphisms on susceptibility to HCV infection

The association of each genetic variant of IL-28A and IL-28B on the protection from HCV infection among this Egyptian cohort is shown in Table 2. In this regard, only the frequency of the beneficial IL-28B.rs8103142 TT genotype and IL-28A.rs12980602 TT genotypes were significantly higher than the CT and TT genotypes in healthy controls compared to HCV-infected subjects including chronic and resolved subjects ($p < 0.001$). Meanwhile, the frequency of the "T" allele was significantly higher than the "C" allele for both IL-28B.rs8103142 and IL-28A.rs12980602 in healthy controls compared to HCV-infected subjects ($p < 0.001$; OR = 2.05; 95% CI = 1.27–3.31 and $p < 0.001$; OR = 2.72; 95% CI = 1.70–4.35, respectively). On the other hand, there were no significant differences in the frequency of different genotypes in healthy controls compared to HCV-infected subjects for both IL-28B.rs12979860 and IL-28B.rs8099917 ($p > 0.05$).

Impact of IL-28A and IL-28B gene polymorphisms on the outcome of HCV infection

HCV-infected subjects were then stratified as spontaneous resolvers of HCV infection and chronically infected subjects and compared to healthy controls. The factors associated with each genetic variant using multivariate logistic regression analysis on the outcome of HCV infection among this Egyptian cohort are shown in Table 3. As expected, analysis of IL-28B.rs12979860 genotypes revealed that subjects with the CC genotype is significantly higher in resolved subjects in compression to chronic subjects ($p = 0.011$, OR = 0.48, 95% CI = 0.269–0.850), and significantly higher in healthy control subjects in compression to chronic subjects ($p = 0.042$, OR = 1.82, 95% CI = 1.01–3.258), while this higher prevalence was

Table 1 Characteristics of the studied populations

Characteristic	Total ($n = 285$)	Chronic ($n = 144$)	Resolved ($n = 72$)	Healthy controls ($n = 69$)	p value
Age (years ± SD)	39.9 ± 11.6	42.8 ± 11.6	41.7 ± 10.7	32.1 ± 8.5	0.017
Male; no. (%)	181 (63.5%)	86 (59.7%)	47 (65.3%)	48 (69.6%)	0.429
Rural residence; no. (%)	150 (52.6%)	77 (53.5%)	42 (58.3%)	31 (44.9%)	0.230
ALT level ± SEM (IU/l)	48.0 ± 3.2	63.5 ± 5.7	35.1 ± 3.8	28.2 ± 1.7	< 0.001
AST level ± SEM (IU/l)	66.9 ± 6.1	70.5 ± 5.3	43.3 ± 4.5	32.9 ± 3.3	< 0.001

not established when comparing resolved subjects with healthy control subjects (Table 3).

In the same line, the frequency of subjects with IL-28B.rs8103142 TT genotype was significantly higher in healthy controls compared to chronic subjects ($p < 0.001$, OR = 0.26, 95% CI = 0.126–0.540), and to resolved subjects ($p = 0.034$, OR = 0.42, 95% CI = 0.189–0.941). On the other hand, no significant difference was found between chronic and resolved subjects in this genotype (Table 3).

Likewise, the frequency of subjects with IL-28B.rs8099917 TT genotype was significantly higher in resolved subjects compared to chronic subjects ($p < 0.001$, OR = 0.35, 95% CI = 0.175–0.698), and significantly higher in healthy controls in compression to chronic subjects ($p = 0.02$, OR = 0.47, 95% CI = 0.245–0.907). This higher prevalence was not established when comparing resolved subjects with healthy control subjects (Table 3).

In addition, there was a highly significant difference for IL-28A.rs12980602 between healthy control subjects in comparison to chronic subjects ($p < 0.001$) and resolved subjects ($p = 0.005$). IL-28A.rs12980602 CC genotype was significantly lower in healthy control subjects in comparison to resolved subjects ($p = 0.01$, OR = 4.78, 95% CI = 0.995–23.011) and chronic subjects ($p = 0.005$,

Table 2 IL-28B and IL-28A polymorphism: allele, genotype, and haplotype frequencies (and counts) in studied population classified as HCV antibody-positive and HCV antibody-negative

Genotype frequency	HCV positive no. = 216 (%)	Healthy control no. = 69 (%)	p	OR	95% CI
IL-28B.rs12979860					
CC	91 (42.1%)	35 (50.7%)	0.21[a]	0.71	0.41–1.22
TT	28 (13.0%)	4 (5.8%)	0.10[b]	2.42	0.82–7.16
CT	97 (44.9%)	30 (43.5%)	0.19[c]		
C alleles	279 (64.6%)	100 (72.5%)	0.09[d]	0.69	0.45–1.06
T alleles	153 (35.4%)	38 (27.5%)			
IL-28B.rs8103142					
CC	4 (15.9%)	27 (8.0%)	0.16[a]	2.17	0.72–6.53
TT	24 (22.4%)	38 (48.0%)	0.00[b]	0.31	0.16–0.60
CT	105 (61.8%)	22 (44.0%)	0.00[c]		
C alleles	159 (46.8%)	30 (30.0%)	0.00[d]	2.05	1.27–3.31
T alleles	181 (53.2%)	70 (70.0%)			
IL-28B.rs8099917					
GG	1 (6.1%)	13 (1.4%)	0.12[a]	4.44	0.57–34.59
TT	53 (67.9%)	144 (76.8%)	0.16[b]	0.64	0.34–1.20
GT	55 (25.9%)	15 (21.7%)	0.20[c]		
G alleles	81 (19.1%)	17 (12.3%)	0.07[d]	1.68	0.96–2.95
T alleles	343 (80.9%)	121 (87.7%)			
IL-28A.rs12980602					
CC	2 (15.0%)	32 (2.9%)	0.01[a]	5.92	1.38–25.40
TT	45 (37.6%)	80 (65.2%)	0.00[b]	0.32	0.18–0.57
CT	101 (47.4%)	22 (31.9%)	0.00[c]		
C alleles	165 (38.7%)	26 (18.8%)	0.00[d]	2.72	1.70–4.35
T alleles	261 (61.3%)	112 (81.2%)			

a = comparison for the indicated genotype, b = comparison for the indicated genotype, c = comparison for the association between the three genotypes, d = comparison for the indicated alleles

Table 3 IL-28B and IL-28A polymorphism: allele, genotype and haplotype frequencies (and counts) in studied population classified into HCV chronic, resolved, and healthy controls

Genotype frequency	Chronic no. (%)	Resolved no. (%)	Healthy controls no. (%)	Chronic/resolved			Chronic/healthy controls			Resolved/healthy controls		
				p	OR	95% CI	*p*	OR	95% CI	*p*	OR	95% CI
IL-28B.rs12979860												
CC	52 (36.1%)	39 (54.2%)	35 (50.7%)	0.011[a]	0.48	0.269–0.850	0.042[a]	1.82	1.01–3.258	0.682[a]	0.86	0.449–1.688
TT	25 (17.4%)	3 (4.2%)	4 (5.8%)	0.006[b]	4.83	1.407–16.56	0.021[b]	0.29	0.09–0.878	0.656[b]	1.42	0.305–6.568
CT	67 (46.5%)	30 (41.7%)	30 (43.5%)	0.005[c]			0.028[c]			0.862[c]		
C alleles	171 (59.4%)	108 (75.0%)	100 (72.5%)	0.001[d]	0.49	0.312–0.760	0.008[d]	1.80	1.158–2.80	0.628[d]	0.88	0.516–1.492
T alleles	117 (40.6%)	36 (25.0%)	38 (27.5%)									
IL-28B.rs8103142												
CC	24 (21.2%)	3 (5.3%)	4 (8.0%)	0.01[a]	4.84	1.394–1.689	0.04[a]	3.1	1.015–9.473	0.57[a]	0.64	0.135–3.003
TT	22 (19.5%)	16 (28.1%)	24 (48.0%)	0.204[b]	0.62	0.295–1.301	0.000[b]	0.26	0.126–0.540	0.034[b]	0.42	0.189–0.941
CT	67 (59.3%)	38 (66.7%)	22 (44.0%)	0.021[c]			0.005[c]			0.016[c]		
C alleles	115 (50.9%)	44 (38.6%)	30 (30.0%)	0.032[d]	1.65	1.042–2.607	0.000[d]	2.42	1.464–3.989	0.187[d]	1.47	0.29–2.594
T alleles	111 (49.1%)	70 (61.4%)	70 (70.0%)									
IL-28B.rs8099917												
GG	12(8.5%)	1 (1.4%)	1 (1.4%)	0.04[a]	6.51	0.829–51.12	0.05[a]	6.33	0.805–49.68	0.98[a]	0.97	0.0596–15.4
TT	86(61.0%)	58 (81.7%)	53 (76.8%)	0.000[b]	0.35	0.175–0.698	0.02[b]	0.47	0.245–0.907	0.48[b]	1.35	0.592–3.06
GT	43(30.5%)	12(16.9%)	15 (21.7%)	0.006[c]			0.033[c]			0.767[c]		
G alleles	67(23.8%)	14(9.9%)	17 (12.3%)	0.000[d]	0.85	1.538–5.276	0.005[d]	2.22	1.245–3.949	0.51[d]	0.78	0.367–1.64
T alleles	215 (76.2%)	128 (90.1%)	121 (87.7%									
IL-28A.rs12980602												
CC	23 (16.3%)	9 (12.5%)	2 (2.9%)	0.461[a]	1.36	0.595–3.126	0.005[a]	6.53	1.492–28.56	0.034[a]	4.78	0.995–23.011
TT	51 (36.2%)	29 (40.3%)	45 (65.2%)	0.56[b]	0.84	0.469–1.505	0.001[b]	0.31	0.165–0.552	0.003[b]	0.36	0.181–0.712
CT	67 (47.5%)	34 (47.2%)	22 (31.9%)	0.713[c]			0.000[c]			0.005[c]		
C alleles	113 (40.1%)	52 (36.1%)	26 (18.8%)	0.42[d]	1.18	0.781–1.791	0.000[d]	2.88	1.766–4.695	0.001[d]	2.43	1.410–4.201
T alleles	169 (59.9%)	92 (63.9%)	112 (81.2%)									

a = comparison for the indicated genotype, b = comparison for the indicated genotype, c = comparison for the association between the three genotypes, d = comparison for the indicated alleles.

OR = 6.53, 95% CI = 1.492–2.56). Subjects with IL-28A.rs12980602 TT genotype was significantly higher in healthy control subjects in comparison to chronic subjects ($p = 0.001$, OR = 0.31, 95% CI = 0.165–0.552), and resolved subjects ($p = 0.003$, OR = 0.36, 95% CI = 0.181–0.712). On the other hand, IL-28A.rs12980602 CC and TT genotypes were not significantly higher in resolved subjects in comparison to chronic subjects. IL-28A.rs12980602 "C" allele is significantly lowered in healthy control subjects in compression to chronic subjects ($p < 0.001$, OR = 2.33, 95% CI = 1.766–4.695), and resolved subjects ($p = 0.001$, OR = 2.43, 95% CI = 1.410–4.201; Table 3).

IL-28 gene polymorphisms frequencies distributions were in Hardy-Weinberg equilibrium in all groups and subgroups analyzed. When IL-28B and IL-28A polymorphism frequencies were compared between the totalities of HCV-infected individuals and HCV negative subjects, some significant differences in allelic, genotype and haplotype frequency were observed. The three IL-28B polymorphisms were in linkage disequilibrium ($D' > 0.68$; $r^2 > 0.43$) for all comparisons in HCV patients (Fig. 1, Table 4) forming one block and combined to form four major haplotypes (TCT, CTT, CTG, and CCT; frequency = 94.6%) and other minor haplotypes (frequency = 3.9%; Table 5). There was no linkage disequilibrium for IL-28A polymorphisms in comparison to the three IL-28B polymorphisms in HCV patients (Fig. 1, Table 4). The most frequent haplotype; TCT was significantly more frequent in HCV-infected subjects than in negative HCV subjects (62.2% vs 48.6%, respectively; $p = 0.005$), while the frequency of the other haplotypes; CTT, CTG, and CCT was similar to HCV negative subjects (Table 5).

Association between IL-28B and IL-28A polymorphism and HCV viral load

The association between IL-28B SNPs (rs12979860, rs8103142, rs8099917) and IL-28A.rs12980602 and viral load in chronic patients was evaluated and represented in Table 6. Viral load was quantified in individuals with

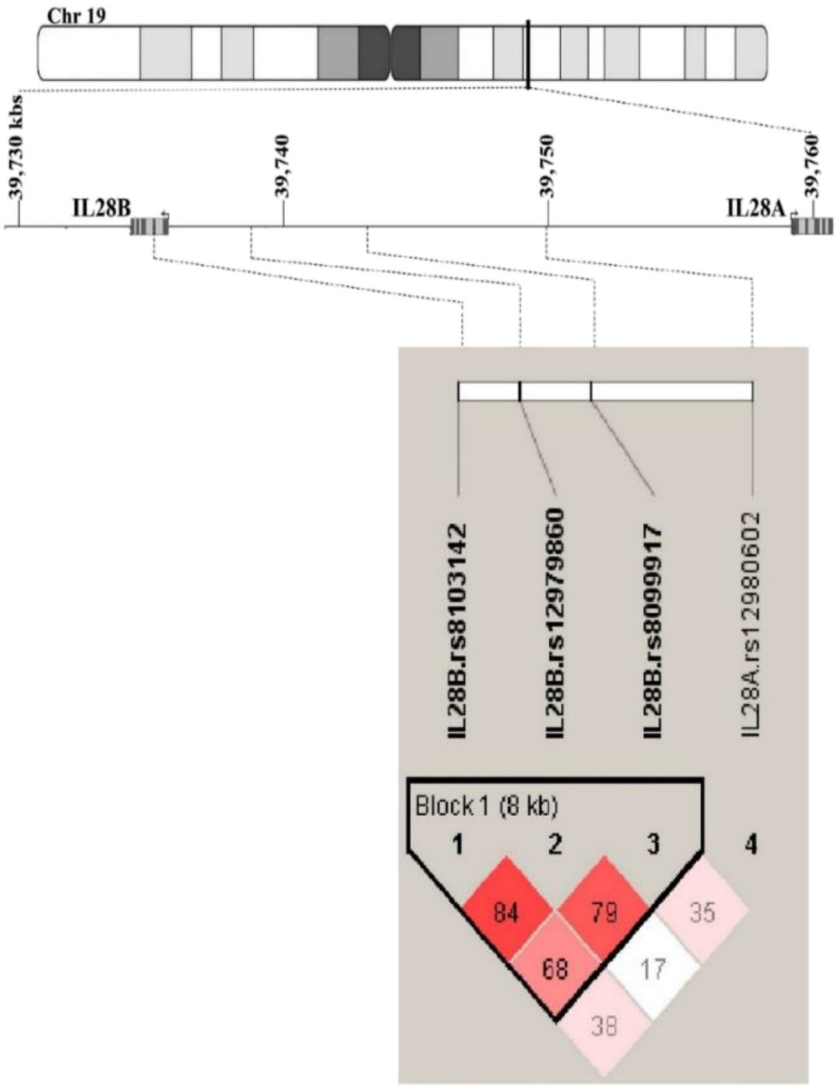

Fig. 1 Linkage disequilibrium between IL-28B and IL-28A SNPs among an Egyptian cohort of 216 subjects infected with HCV and 69 healthy seronegative aviremic subjects (Haploview)

persistent infection ($n = 144$) with different IL-28B and IL-28A genotypes. The average (± SEM) HCV viral loads for those subjects with viral load more than 10^5 IU/ml ($n = 90$) was $1.9 \times 10^6 \pm 559{,}368$ IU/ml while the viral load of those subjects with viral load less than 10^5 IU/ml ($n = 54$) was $35 \times 10^3 \pm 4191$. No significant association was observed between viral load and IL-28B.rs12979860 ($p = 0.728$); rs8103142 ($p = 0.76$); and IL-28A.rs12980602 ($p = 0.11$) genotypes. However, there was an association between IL-28B.rs8099917 and viral load ($p = 0.046$). The viral load was higher in subjects with the "favorable" rs8099917 TT genotype and was

Table 4 Linkage disequilibrium for the studied IL-28A and IL-28B polymorphisms

Locus 1	Locus 2	*D′*	LOD	r^2	CI low	CI hi
IL-28B.rs12979860	IL-28B.rs8103142	0.844	24.39	0.424	0.75	0.91
IL-28B.rs12979860	IL-28B.rs8099917	0.799	20.78	0.27	0.68	0.88
IL-28B.rs8099917	IL-28B.rs8103142	0.68	7.21	0.127	0.49	0.81
IL-28A.rs12980602	IL-28B.rs12979860	0.175	1.7	0.029	0.06	0.28
IL-28A.rs12980602	IL-28B.rs8099917	0.358	3.74	0.052	0.21	0.49
IL-28A.rs12980602	IL-28B.rs8103142	0.381	4.35	0.111	0.24	0.5

D′, the value of *D* prime between the two loci; LOD, log of likelihood odd ratio; r^2, correlation coefficient between the two loci; CI low, 95% confidence lower pound on *D′*, CI hi, 95% confidence upper pound on *D′*

Table 5 Haplotypes frequencies for the studied polymorphisms

Haplotype	Frequency (%)	HCV positive (frequency) (%)	HCV negative (frequency) (%)	chi-square	*p* value
TCT	51.9	62.2	48.6	7.801	0.005
CTT	17.1	16.3	17.4	0.081	0.776
CTG	13.0	8.6	14.4	3.079	0.079
CCT	12.6	8.6	13.9	2.679	0.102
TTG	2.2	2.1	2.2	0.007	0.934
CCG	1.7	1.5	1.8	0.056	0.813

lowest in those with the "GG" genotype ($p = 0.02$) with percentage of 48.1% and 68.5% in subjects with viral load $> 10^5$ IU/ml and subjects with viral load $< 10^5$ IU/ml, respectively. On the other hand, the viral load was higher in the subjects with the "favorable" "T" allele and was lowest in those with the "G" allele ($p = 0.03$) with percentage of 69.2% and 80.3% in subjects with viral load $> 10^5$ IU/ml and subjects with viral load $< 10^5$ IU/ml, respectively. However, there was no statistically significant difference between HCV viral load and genotype of other polymorphisms ($p > 0.05$; Table 6).

Discussion

To our knowledge, this is the first study designed to investigate the role of four different IFN-λ gene SNPs: 3 SNPs in the IL-28B gene, (rs12979860, rs8103142 and rs8099917), and one IL-28A.rs12980602, as predictors of HCV outcome in HCV genotype-4 mono-infected patients of Egyptian ethnicity. Our data show that IL-28A.rs12980602 and IL-28B.rs8103142 TT genotype could be protective against HCV infection. Also, IL-28B SNPs (rs12979860, rs8099917, and rs8103142) predicted the outcome of HCV

Table 6 IL-28B and IL-28A polymorphism: allele, genotype and haplotype frequencies (and counts) in chronic subjects classifies as subjects with viral load more than 10^5 IU/ml and subjects with viral load less than 10^5 IU/ml

Genotype frequency	$> 10^5$ IU/ml no. (%)	$< 10^5$ IU/ml no. (%)	*p*	OR	95% CI
IL-28B.rs12979860					
CC	18 (33.3%)	34 (37.8%)	0.59[a]	0.82	0.41–1.67
TT	11 (20.4%)	14 (15.6%)	0.46[b]	1.39	0.58–3.33
CT	25 (46.3%)	42 (46.7%)	0.728[c]		
C alleles	61 (56.5%)	110 (61.1%)	0.44[d]	0.83	0.51–1.34
T alleles	47 (43.5%)	70 (38.9%)			
IL-28B.rs8103142					
CC	11 (24.4%)	13 (19.1%)	0.50[a]	1.37	0.55–3.40
TT	9 (20.0%)	13 (19.1%)	0.91[b]	1.06	0.41–2.73
CT	25 (55.6%)	42 (61.8%)	0.76[c]		
C alleles	47 (52.2%)	68 (50.0%)	0.74[d]	1.09	0.64–1.86
T alleles	43 (47.8%)	68 (50.0%)			
IL-28B.rs8099917					
GG	5 (9.6%)	7 (7.9%)	0.72[a]	1.25	0.37–4.15
TT	25 (48.1%)	61 (68.5%)	0.02[b]	0.43	0.21–0.86
GT	22 (42.3%)	21 (23.6%)	*0.046*[c]		
G alleles	32 (30.8%)	35 (19.7%)	0.03[d]	1.82	1.04–3.17
T alleles	72 (69.2%)	143 (80.3%)			
IL-28A.rs12980602					
CC	13(24.5%)	10 (11.4%)	0.04[a]	2.54	1.02–6.29
TT	16 (30.2%)	35 (39.8%)	0.25[b]	0.65	0.32–1.35
CT	24 (45.3%)	43 (48.9%)	*0.11*[c]		
C alleles	50 (47.2%)	63 (35.8%)	0.06[d]	1.60	0.98–2.61
T alleles	56 (52.8%)	113 (64.2%)			

a = comparison for the indicated genotype, b = comparison for the indicated genotype, c = comparison for the association between the three genotypes, d = comparison for the indicated alleles

infection among genotype-4-infected Egyptians. In addition, IL-28B.rs8099917 SNP affects the viral load in chronic HCV patients.

HCV infection is one of the most important factors of chronic or serious hepatic diseases, and affects ~ 70–100 million of the world's population [1]. Host genetic factors have been identified to be associated with infectious disease, including HBV, HCV, and HIV infection [43, 44]. Many SNPs have been reported to be associated with HCV infection, viral clearance, and treatment effect [45, 46], albeit with some controversies. The IL-28A, IL-28B, and IL-29 genes belonged to a new interferon family, interferon III (or IFN-λ). The members in IFN-λ family were reported to inhibit HCV amplification or were overexpressed in chronic HCV patients [47, 48]. Several GWAS suggested that SNPs in the IL-28B gene could affect the viral spontaneous clearance and response to pegylated interferon-α and ribavirin therapy [16, 32, 34, 49]. All these studies indicated that the IFN-λ genes play important roles in HCV infection. In vitro, IL-28B can inhibit HCV replication through the Janus kinase/signal transducer and activator of transcription pathway in a time and dose-dependent manner [50, 51]. Additionally, an association between the IL-28B genotype and differential expression of intrahepatic interferon-stimulated genes (ISGs) was found in patients chronically infected with HCV [52].

Regarding IL-28A.rs12980602, which is located about 6 kb upstream of the IL-28A gene, few reports demonstrated the association with HCV infection. IL-28A.rs12980602 TT genotype is one of the SNPs which showed a significant association with the stage of liver fibrosis and spontaneous or interferon-induced viral clearance in thalassemia patients with HCV infection [53]. By contrast, another study found no association between genotypes and alleles in IL-28A.rs12980602 with HCV infection in Han Chinese, China [54]. On the other hand, IL-28A.rs12980602 CT and CC genotypes were significantly higher in hepatitis B responders in Chinese patients treated with lamivudine than patients with the TT genotype [55]. Importantly, there has been no report concerning the relationship between rs12980602 variation and HCV diseases among Egyptians. Our data indicated that the frequency of the "T" allele was significantly higher than the "C" allele in healthy controls compared to HCV-infected subjects ($p < 0.001$). Also, IL-28A.rs12980602 TT genotype was significantly higher in healthy controls compared to HCV-infected subjects ($p < 0.001$). Our results concerning this SNP could not indicate any significant difference between chronic and resolved subjects; ($p > 0.5$) or any association with viral load ($p = 0.11$). Taken together, our data indicate that the "T" allele and especially IL-28A.rs12980602 TT genotype may be a protective SNP against HCV infection.

The IL-28B SNPs (rs12979860, rs8103142, and rs8099917) near the IL-28 gene on chromosome 19 were chosen for genotyping because they were previously reported in three independent studies including mostly Caucasian, Asian, and African patients infected with HCV [32, 34, 56]. Many studies examined the impact of IL-28B SNPs (rs12979860, rs8099917, and rs8103142) on HCV infection among Egyptians with genotype 4 infections [35–38]. For instance, one report studied eight IL-28B SNPs in Egyptian population and showed that the LD block of the IL-28B SNP cluster associated with HCV clearance was shorter than that in European populations and that the core association depended on two SNPs; rs12979860 and rs8103142 [38]. Another study confirmed that the IL-28B.rs12979860, IL-28B.rs8099917, and IL-28B.rs11881222 SNPs are the strongest pre-treatment predictors of SVR in patients with genotype 4 HCV infections [37]. These studies and ours confirmed the previous role of IL-28B SNPs in predicting HCV infection outcome; this would allow determining which of them is most suitable for establishing a diagnostic test for predicting treatment outcome [57]. Our data show that the frequency of the IL-28B.rs8103142 "T" allele was significantly higher than the "C" allele in healthy controls compared to HCV-infected subjects ($p = 0.00$). Also, IL-28B.rs8103142 TT genotype was significantly higher in healthy controls compared to HCV-infected subjects ($p < 0.001$). Thus, these data indicate that the "T" allele and especially IL-28B.rs8103142 TT genotype could be a protective SNP for HCV infection. Polymorphism of IFN-λ3 showed a powerful association with response to treatment in HCV patients and anticipation of SVR in regimens using DAAs either in combination with IFN [58–62] or alone [63, 64].

We used the Haploview software to confirm that IL-28B.rs12979860, IL-28B.rs8099917, and IL-28B.rs8103142, and IL-28A.rs12980602 are not in linkage disequilibrium in HCV patients. The three IL-28B SNPs were in linkage disequilibrium ($D' > 0.68$; $r^2 > 0.43$) for all comparisons in HCV patients forming one block and combined to form four major haplotypes (TCT, CTT, CTG, and CCT; frequency = 94.6%). The TCT was significantly more frequently found in HCV-infected subjects in comparison to HCV negative subjects at rates of 62.2%; 48.6%, respectively; $p = 0.005$, while the other haplotypes; CTT, CTG, and CCT; were higher in HCV negative subjects. However, this was not statistically significant.

In our study, IL-28.rs8099917 TT genotype was significantly associated with low viral load in HCV-infected subjects, while no significant associations were observed between viral load and IL-28B SNPs rs12979860, rs8103142, or IL-28A.rs12980602 genotypes. This was confirmed by a previous study which demonstrated that IL-28B.rs12979860 SNP does not affect the viral load in chronically infected Egyptian patients with HCV genotype 4 [65]. Another study showed that there is no association between IL-28B.rs8099917 TT or IL-28B.rs12979860 CC genotypes and SVR according to HCV viral load in HCV

genotype 1 patients [66]. On the other hand, another study indicated that IL-28B SNP has been associated with viral load decline during therapy [67].

In conclusion, IL-28A.rs12980602 TT genotype could be protective against HCV infection while, the IL-28B SNPs (rs12979860, rs8099917, and rs8103142) predicted the outcome of HCV infection among genotype-4-infected Egyptians. Also, the IL-28B.rs8099917 SNP affected the viral load in chronically infected patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committees and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study protocol was approved by the NLI Institutional Review Board.

Informed consent Informed consent was obtained from all participants included in the study.

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