

Original article

Interferon- λ rs12979860CC genotype predicts sustained virological response to therapy in patients with chronic hepatitis C Genotype 4 but enhances hepatic fibrosis

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Abstract:

Recently, several genome wide association studies (GWAS) have revealed that several single nucleotide polymorphisms (SNPs) in proximity to the interleukin (IL)-28B genes can predict spontaneous clearance of hepatitis C virus (HCV) infection as well as sustained virological response (SVR) to Pegylated interferon- α 2a/b plus ribavirin therapy. In HCV-genotype 1 patients, the allele rs12979860 is associated with greater hepatic necro-inflammation and worse clinical outcome, but lower fibrosis progression. Data on patients infected with HCV genotype 4 (G4) is limited. The present study assesses the impact of rs12979860 IL28 SNP on hepatic fibrosis and SVR in patients with chronic HCV G4 infection. A total of 163 patients with chronic HCV G4 infection (123 males and 40 females) who had pre-treatment liver biopsy were included. Both HCV RNA and IL28 rs12979860 genotypes were determined by real time polymerase chain reaction. Hepatic fibrosis, IL28B rs12979860 genotype, and SVR were statistically correlated together and with various clinical, biochemical, and virological parameters. All patients were found to be infected with HCV G4. IL28B rs12979860 genotypes TT, CT, and CC were detected in 29 (17.8%), 107 (56.6%), and 27 (16.6%) patients, respectively. SVR was achieved in 85 (52.1%), severe fibrosis (\geq F3) by METAVIR score was found in 75 (64.0%). The CC allele is associated with nearly twice (OR=1.898) the risk for having severe fibrosis (\geq F3), but nearly 16 times SVR (OR=15.833) than having CT and TT. Contrary to HCV G1, the CC allele at IL28B rs12979860 appears to be associated with higher hepatic fibrosis, but better SVR to therapy in patients with chronic HCV G4 infection.

Keywords:

Chronic hepatitis C

Genotype 4

Interleukin-28B

Hepatic fibrosis

Genetics

Abbreviations: CHC; chronic hepatitis C. GWAS; genome wide association studies. HCV; hepatitis C virus. IL-28B; interleukin-28B. Peg-IFN- α 2; pegylated interferon- α 2. SNPs; single nucleotide polymorphisms. SVR; sustained viral response. G4; genotype 4.

1. Introduction

Chronic hepatitis C virus (HCV) infection represents a serious public health problem, with an estimated worldwide prevalence of 3% [1]. HCV genotypes 1, 2 and 3 are common throughout America and Europe while genotype-4 (G4) is predominant in the Middle East and Africa, where it is responsible for more than 80% of HCV infections [1,2]. In Egypt, the prevalence of HCV is estimated to range from 14.7-22% with a predominance of G4 (90%) followed by genotype 1 [2,3]. Host genetics controlling the immune-inflammatory pathways affects hepatic fibrosis [4-6]. Indeed, many studies reported that single nucleotide polymorphism (SNP) in proximity to interleukin-28B (IL28B) interferon- λ (IFN- λ) region (rs12979860) is a predictor of sustained virological response (SVR) to IFN-based therapy [7-11]. Several reports have showed that the rs12979860 'CC' genotype is associated with greater inflammation grade [12-14]. The relation of rs12979860 'CC' genotype to hepatic fibrosis progression, however, is controversial [12-21]. The present study involved only chronic hepatitis C (CHC)-G4 patients treated in a single center and aimed to clarify the impact of rs12979860 gene polymorphisms on hepatic fibrosis; to explore the interactions of rs12979860 genotype with host and viral factors; and to explore the impact of rs12979860 genotype on SVR to antiviral therapy in the same group of patients.

2. Patients and Methods

2.1. Patients

A total of 163 patients with chronic HCV-infection attending the Egyptian Liver Research Institute and Hospital

(ELRIAH), Mansoura, Egypt were included in this study.

2.2. Approvals

The study protocol conformed to the 1975 Declaration of Helsinki and was approved by the Institutional Review Board Research Ethics Committee in the hospital. All patients gave an informed consent prior to enrolment in the study and signed another informed consent prior to undergoing liver biopsies.

2.3. Methods

2.3.1. Baseline Assessment

Baseline (pre-treatment) clinical, hematological, biochemical, and serological data of all included patients were collected. Percutaneous liver biopsies were obtained according to standard protocols and were interpreted according to the METAVIR Score [22], by a single pathologist who was blinded to all other clinical, virological, molecular, and therapeutic data of the patients.

2.3.2. Therapy and Follow up

All patients were treated either with Peg-IFN α -2a at doses of 180 μ g subcutaneously once weekly respectively in combination with ribavirin 1000 mg (body weight \leq 75 kg) or 1200 mg (body weight \geq 75kg). The complete course of therapy was 48 weeks. Patients were then followed up for another 24 weeks to assess for the development of SVR.

2.4. Definitions

The SVR was defined as undetectable HCV RNA 24 weeks after end of treatment (i.e., at week 72). Non-response was designated when HCV RNA did not drop >2 log₁₀ from the pre-treatment values at 12 weeks of treatment.

Relapse was designated when HCV RNA reappeared within 24 weeks after end of treatment response. Both non-responders and relapsers were grouped as “No-SVR” group in this study.

2.5. Detection of HCV RNA and HCV genotyping

HCV RNA was detected by real time PCR using AmpliprebTaqMan, Roche Diagnostics. HCV RNA genotypes were determined by Real Time PCR (Light Cycler 480, Roche diagnostics).

2.6. IL-28B genotyping

IL28 B polymorphism was determined by Real Time PCR (Slan Real Time PCR detection system, LG Life Sciences, Korea) using Amplisense, Genoscreen-IL28 B FRT PRC kit (Amplisense, Moscow, Russia). Discrimination between wild type and mutant allele was achieved based on labeling the probes with two different dyes namely VIC for wild type of FAM for mutant allele.

2.7. Statistical Analyses

Data were collected initially in a specialized data collection form, then introduced into a Microsoft Excel worksheet, and finally transferred to the Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS 16.0, SPSS Inc., Chicago, IL, USA) for analyses. A P value of <0.05 was considered statistically significant.

Means of continuous variables were compared using Student’s *t*-tests or non-parametric tests (Wilcoxon’s and Mann Whitney), as appropriate. The Chi-Square or Fisher’s exact tests were used to compare frequencies and proportions. Multivariate stepwise logistic regression analysis was performed to determine the role of genetic and bio-clinical characteristics as independent predictors of SVR and advanced hepatic fibrosis. Only variables, that were found to be significant at the 0.05 level at univariate analyses were included in multivariate logistic regression analysis.

3. Results

3.1. Patients’ characteristics

The study comprises 163 patients with CHC, studied and treated in a single center. The mean age was 45.2, 75.5% were males, 90.2% had body mass index (BMI) of ≥ 25 Kg/m², and none had any history of alcohol intake. The rs allele distribution was CC, CT, and TT in 16.6%, 65.5%, and 17.8% of patients, respectively. Regarding pathology, severe fibrosis (METAVIR F \geq 3) and severe inflammation (METAVIR A \geq 3) were found in 64.0% and 90.2% of patients, respectively. SVR was achieved in 85 (52.1%) patients. Detailed baseline demographic, laboratory, and histologic data of the whole cohort are shown in table (1).

Table (1) Characteristics of all chronic HCV-G4 patients and according to hepatic fibrosis and response to therapy

Variable	All (n=163)	Hepatic Fibrosis			Response to Therapy		
		F0-2 (n=99)	F3-4 (n=79)	P value	SVR (n=86)	No SVR (n=78)	P value
Age (Years)	45.2 ± 8.2	43.3 ± 8.1	47.3 ± 7.8	0.002	46.0 ± 8.5	44.3 ± 7.9	0.124
Age group							
- <40 years	38 (23.3)	26 (29.5)	12 (16.0)	0.041	17 (20.0)	21 (26.9)	0.296
- \geq 40 years	125 (76.7)	62 (70.5)	63 (84.0)		68 (80.0)	57 (73.1)	

Gender								
- Male	123 (75.5)	65 (73.9)	58 (73.3)	0.608	66 (77.6)	57 (73.1)	0.498	
- Female	40 (24.5)	33 (26.1)	17 (22.7)		19 (22.4)	21 (26.9)		
BMI (Kg/m²)	30.3 ± 3.9	29.7 ± 3.8	31.2 ± 4.0	0.016	30.3 ± 0.4	30.4 ± 3.9	0.900	
BMI group								
- <25 Kg/m ²	16 (9.8)	10 (11.4)	6 (8.0)	0.472	10 (11.8)	6 (7.7)	0.383	
- ≥25 Kg/m ²	147 (90.2)	78 (88.6)	69 (92.0)		75 (88.2)	72 (92.3)		
ALT (IU/mL)	73.0±59.9	51.3±29.4	98.5±75.0	0.000	73.0±58.5	73.1±61.8	0.742	
AST (IU/mL)	60.0±43.8	39.3±19.7	84.2±51.5	0.000	60.7±44.6	59.2±43.3	0.734	
Albumin (g/L)	4.3±0.3	4.4±0.3	4.2±0.4	0.016	4.3±0.3	4.4±0.3	0.063	
Bilirubin (mg/L)	0.9±0.2	0.9±0.2	0.9±0.2	0.546	0.9±0.2	0.9±0.2	0.881	
ALP (IU/mL)	82.6±17.8	76.9±15.5	89.27±18.2	0.000	82.1±19.8	83.1±15.5	0.769	
HCV RNA (copies/ml)	58E5±32E4	67E5±	5.8E5±5.0 E5	0.447	5.5E5±6.2E5	6.2E5±8.3E5	0.504	
HCV RNA (log₁₀)	5.5±0.6	5.5±	5.4±0.5	0.447	5.4±0.6	5.5±0.5	0.504	
HCV group								
- <400,000/ml	97 (59.5)	48 (54.5)	49 (65.3)	0.162	48 (56.5)	49 (62.8)	0.409	
- ≥400,000/ml	66 (40.5)	40 (45.5)	26 (34.7)		37 (43.5)	29 (37.2)		
HCV group								
- <600,000/ml	106 (65.0)	54 (61.4)	52 (69.3)	0.288	54 (63.5)	52 (66.7)	0.675	
- ≥600,000/ml	57 (35.0)	34 (38.6)	23 (30.7)		31 (36.5)	62 (33.3)		
Grade*								
- 0-2	16 (9.8)	80 (90.9)	15 (20.0)	0.000	52 (61.2)	43 (55.1)	0.434	
- 3-4	147 (90.2)	8 (9.2)	60 (80.0)		33 (38.8)	35 (44.9)		
Stage*								
- 0-2	88 (54.0)	-	-	-	44 (51.8)	44 (56.4)	0.552	
- 3-4	75 (46.0)	-	-	-	41 (48.2)	34 (43.6)		
IL28B SNPs rs12979860								
- CC	27 (16.6)	11 (12.5)	16 (21.3)	0.314	25 (29.4)	2 (2.6)	0.000	
- CT	107 (65.6)	61 (69.3)	46 (61.3)		53 (62.4)	54 (69.2)		
- TT	29 (17.8)	16 (18.2)	13 (17.3)		7 (8.2)	22 (28.2)		
IL28B SNPs rs12979860								
- CC	27 (16.6)	11 (12.5)	16 (21.3)	0.131	25 (29.4)	2 (2.6)	0.001	
- Non-CC	136 (83.4)	77 (87.5)	59 (78.7)		60 (70.6)	76 (97.4)		
IL28B SNPs rs12979860								
- TT	134 (82.2)	16 (18.2)	13 (17.3)	0.888	7 (8.2)	22 (28.2)	0.001	
- Non-TT	29 (17.8)	72 (81.8)	62 (82.7)		78 (91.8)	56 (71.8)		

Data expressed as mean ± SD or n (%) as appropriate. n; number. HCV; hepatitis C virus. BMI; body mass index. SVR; sustained viral response. SNPs; single nucleotide polymorphisms. *Grade (of inflammation) and *Stage (of fibrosis) by METAVIR score. ALT; alanine transaminase. AST; Aspartate transaminase. ALP; alkaline phosphatase.

3.2. Characteristics of the patients' rs12979860 genotypes

Detailed baseline demographic, laboratory, and histologic data of patients with “CC” genotype versus those with “CT/TT” genotype are shown in table (2), and figure (1-a & b). Compared to patients with rs12979860 genotypes CT/TT, patients with the genotype CC were

associated with nearly twice severe fibrosis (P = 0.131; OR: 1.9) and more SVR (P = 0.001; OR: 15.8). SVR was achieved in 25(92.6%) of the 27 patients who have the “CC” allele of the rs12979860 gene, and in only 60 (44.1%) of the 136 patients who have “CT/TT” genotypes (P = 0.001; tab., (2 & 3).

Table (2) Comparison between patients infected with HCV genotype 4 without or with the “CC” allele

<i>Variable</i>	<i>CT/TT (n=136)</i>	<i>CC (n=27)</i>	<i>P value</i>	<i>OR (95% CI)</i>
Age (Years)	44.5 ± 8.3	47.7 ± 6.9	0.016	-
Age group				
- <40 years	35 (25.7)	3 (11.1)	0.101	2.77 (0.79 – 9.78)
- ≥40 years	101 (74.3)	24 (88.9)		
Gender				
- Male	101 (74.3)	22 (81.5)	0.426	1.53 (0.54 – 4.33)
- Female	35 (25.7)	5 (18.5)		
BMI (Kg/m²)	30.4 ± 3.9	30.1 ± 4.8	0.964	-
BMI group				
- <25 Kg/m ²	12 (8.8)	4 (14.8)	0.339	0.56 (1.17-1.88)
- ≥25 Kg/m ²	124 (91.2)	23 (85.2)		
ALT (IU/mL)	74.5 ± 63.4	65.5 ± 37.5	0.899	-
AST (IU/mL)	60.8 ± 44.5	56.0 ± 34.8	0.842	-
Albumin (g/L)	4.3 ± 0.3	4.2 ± 0.4	0.063	-
Bilirubin (mg/L)	0.92 ± 0.16	0.85 ± 0.15	0.015	-
ALP (IU/mL)	83.7 ± 16.9	76.9 ± 21.3	0.096	-
HCV RNA (copies/ml)	5.8E4±7.7E5	6.0E5±5.2E5	0.234	-
HCV RNA (log₁₀)	5.4 ± 0.5	5.5 ± 0.5	0.234	-
HCV group				
- <400,000/ml	85 (62.5)	12 (44.4)	0.081	2.08 (0.90 – 4.80)
- ≥400,000/ml	51 (37.5)	15 (55.6)		
HCV group				
- <600,000/ml	89 (65.4)	17 (63.0)	0.805	1.11 (0.47 – 2.63)
- ≥600,000/ml	47 (34.6)	10 (37.0)		
Grade*				
- 0.2	79 (58.1)	16 (59.3)	0.910	0.95 (0.41 – 2.21)
- 3-4	57 (41.9)	11 (40.7)		
Stage*				
- 0.2	77 (56.5)	11 (40.7)	0.131	1.90 (0.82 – 4.39)
- 3-4	59 (43.5)	16 (59.3)		
Response to therapy				
- SVR	60 (44.1)	25 (92.6)	0.000	15.83 (3.60 – 69.52)
- No-SVR	76 (55.9)	2 (7.4)		

Data expressed as mean ± SD or n (%) as appropriate. n; number. HCV; hepatitis C virus. BMI; body mass index. SVR; sustained viral response. Grade (of inflammation) and stage (of fibrosis) by METAVIR score. OR; Odds ratio; CI; Confidence Interval. ALT; alanine transaminase. AST; Aspartate transaminase. ALP; alkaline phosphatase.

Table (3): Multivariate regression analysis of variables independently affecting SVR in patients with chronic HCV genotype 4 infection (n=163)

<i>Variable</i>	<i>Odds Ratio</i>	<i>P Value</i>	<i>95 % Confidence Interval</i>	
			<i>Lower</i>	<i>Upper</i>
Albumin	0.45	0.123	0.16	1.24
IL28B “CC”* allele	0.07	0.000	0.02	0.29

n; number. *; Interferon-λ rs12979860 genotype CC. SVR; sustained virological response. HCV; hepatitis C virus.

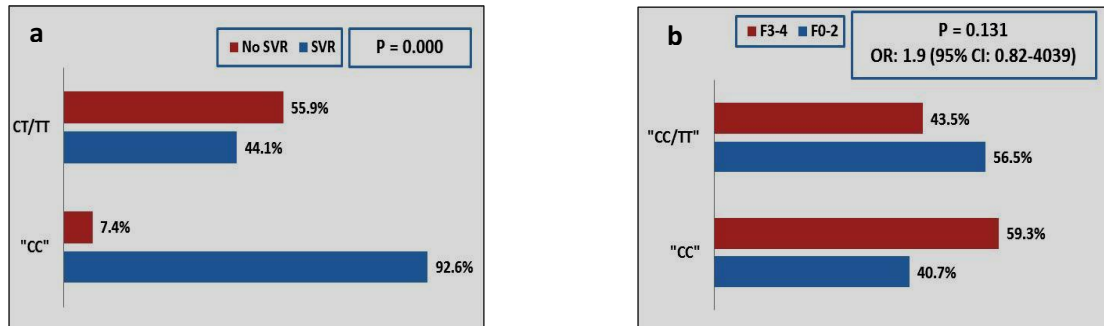


Figure (1) The percentage distribution of rs12979860 in patients with SVR versus No-SVR (A), and patients with severe fibrosis (F_≥3) versus those with F0-3 (B); sustained virological response. "CC", "CT" and "TT" are rs12979860 alleles (genotypes). Test was done by Chi-Square and the Fisher's Exact tests.

3.3. Severe hepatic fibrosis

Compared to patients with non-severe fibrosis stage (F0-2), patients with severe fibrosis (F3-4) were older (P = 0.002), had higher BMI (P = 0.016), higher serum ALT (P = 0.000), higher serum AST (P = 0.000), lower serum albumin (P = 0.016), higher serum alkaline phosphatase (P = 0.000), and more severe hepatic inflammation (P = 0.000). The similar percentages (P = 0.552) of SVR and no-SVR in patients with severe fibrosis (F3-4) and those without (F0-2) are shown in Figure 2. Confirming the importance of inflammation grade in predicting severe hepatic fibrosis (F3-4), severe inflammation (A3-4) was detected in 9.1% and 80% of patients with F0-2 and those with F3-4, respectively (P = 0.000; Figure 3). Using

multivariate logistic regression analysis, the independent predictors of severe fibrosis (F3-4) were age, serum AST, serum albumin, and inflammation grade, table (4).

3.4. Sustained virological response (SVR)

Compared to non-responders, patients who achieved SVR were similar regarding demographic, biochemical, virological, and pathological parameters. A total of 25/85 (29.4%) of patients who achieved SVR and only 2/78 (2.6%) of those who did not had the "CC" allele of the rs12979860 gene (P = 0.001). Using multivariate logistic regression analysis, the independent predictors of SVR was only the CC allele of the rs12979860 gene (P = 0.000; OR: 0.07; CI: 0.02-0.29, table (3).

Table (4) Independent predictors of severe fibrosis (F3-4) by multivariate regression analysis in patients with chronic HCV genotype 4 infection (n=163).

Variable	Odds Ratio	P Value	95 % Confidence Interval	
			Lower	Upper
Age	1.22	0.000	1.10	1.37
BMI	1.15	0.078	0.98	1.35
ALT	0.99	0.766	0.96	1.03
AST	1.05	0.033	1.00	1.10
Albumin	0.01	0.010	0.00	0.35
ALP	0.99	0.582	0.95	1.03
Grade 3-4	0.01	0.000	0.00	0.04

n; number. HCV; hepatitis C virus. BMI; body mass index. ALT; alanine transaminase.

4. Discussion

In the last few years, polymorphism in the IL28B gene has been extensively investigated mainly in HCV genotype 1 infected patients because of its predictive role in HCV treatment outcome and possible association with disease progression [23]. It has been shown that allele distributions of IL28B SNPs are different between races and ethnic backgrounds [24]. This partially explains the variations in response rates to antiviral therapy between different races and among different HCV genotypes. This study was conducted in a single center, with patients infected only with HCV genotype 4 and absence of alcohol intake as a confounder, and with simultaneous testing of the role of the “CC” allele of the rs129798660 gene on both fibrosis and response to anti-viral therapy. These patients criteria makes this study different from previous studies that addressed the role of this gene polymorphism in predicting hepatic fibrosis because other studies included different genotypes, smaller number of HCV genotype 4 patients, patients with concomitant alcohol intake, and patients from different localities and ethnicities. The association of rs12979860 ‘CC’ genotype with greater necro-inflammatory grade has been confirmed by many studies [12-14]. This is not the case with hepatic fibrosis stage and progression, where many controversial reports are published.¹²⁻²¹ In addition, a recent study by Nouredin et al, 2013 in (CHC-G1) patients added to such controversy [13]. They showed that patients with the ‘CC’ genotype of rs12979860 had lower mean Ishak fibrosis scores, worse clinical outcomes, and greater hepatic

inflammation compared to patients with other rs12979860 genotypes (CT/TT), together with no association between the rs12979860CC genotype with fibrosis progression on paired biopsies [13]. These findings are difficult to reconcile because hepatic fibrosis is a consequence of inflammation [1,25]. Also, inflammation grade on initial liver biopsy is the best predictor of fibrosis progression [26,27]. A recent meta-analysis of 28 studies that included 10,024 patients failed to resolve this controversy [28], probably reflecting the inter-study variability in the outcomes. In agreement with our results, a large study published recently by Eslam and colleagues was conducted in 4,172 patients; 3, 129 of them with CHC. They found that CHC patients with rs12979860CC had greater stage-constant and stage-specific fibrosis progression rates [29]. This study utilized both cross-sectional and longitudinal studies and used a detailed validation strategy with multiple analytical approaches in a large cohort of patients. They confirmed that rs12979860 genotype is unequivocally associated with inflammation, fibrosis stage, and faster fibrosis progression. Indeed, this confirms the previous data that both viral- and host-related factors modulate hepatic fibrosis risk, and now provides additional strong evidence for an independent role for the rs12979860 polymorphism in modulating the phenotype and response to therapy. Consistent with the notion from other cohorts with dual liver biopsies [25,26] that inflammation grade is a dominant driver of fibrosis, our study showed that patients with severe fibrosis have significantly

higher inflammation grade in addition to elevated aminotransferases and alkaline phosphatase levels compared to those with less severe fibrosis. The present study confirms the association of the “CC” allele of the rs12979860 gene with more severe hepatic fibrosis. Moreover, the same allele is not only a predictor of HCV clearance and consequently a better response to anti-viral therapy, as shown in other studies on patients infected with HCV genotype 4 [30-33], but also it was found that this allele is the only independent predictor of SVR in the included group. In addition, this study confirms the role of hepatic necro-inflammatory grade as a predictor of more severe hepatic fibrosis. The mechanisms underlying the fibrogenic effect of rs12979860 genotypes are unclear. However, a recent study demonstrated that rs12979860 genotype up regulates cell death-related and survival-related networks in human infected and non-infected hepatocytes in responder patients [34]. Another study by Raglowet, et al. [35] showed a higher level of ISGs in non-infected livers of rs12979860 responder patients. Therefore, it is possible that rs12979860 genotype can render hepatocytes more efficient at responding to therapy, but on the other hand, may lead to more hepatocyte necro-inflammation and subsequent liver fibrosis [29]. So the confirmation from our results represented a milestone for decision to treat with IFN-based regimens, yet its role in predicting response to the directly acting antivirals needs further investigation. The important message is that once a patient’s genotype is rs12979860 “CC”, treatment needs to be given as soon as possible to benefit from

good response to therapy, otherwise more fibrosis progression will be the consequence.

5. Conclusion

In conclusion, the present study that involved only CHC-G4 patients confirms that, contrary to patients with genotype 1, the ‘CC’ allele of rs12979860 is associated with hepatic fibrosis progression, but better response to therapy necessitates early screening of CHC patients and early treatment of those with the ‘CC’ allele to gain the benefit of the greater response to therapy before the progression of hepatic fibrosis.

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