Jundishapur J Microbiol. 2017 January; 10(1):e39178. doi: 10.5812/jjm.39178.

Published online 2016 December 18. **Research Article**

Genetic Variation in Interleukin-28B and Response to Peg-IFNα-2a/RBV Combination Therapy in Patients with Hepatitis C Virus Infection

Farah Bokharaei-Salim,[1,2] Mostafa Salehi-Vaziri,[3] Farzin Sadeghi,[4,5] Khadijeh Khanaliha,[6] Maryam Esghaei,[1] Seyed Hamidreza Monavari,[1] Seyed Moayed Alavian,[7,8] Shahin Fakhim,[9] and Hossein Keyvani[1,*]

[1]Department of Virology, School of Medicine, Iran University of Medical Sciences, Tehran, IR Iran
[2]HIV Laboratory of National Center, Deputy of Health, Iran University of Medical Sciences, Tehran, IR Iran
[3]Department of Arboviruses and Viral Hemorrhagic Fevers (National Ref Lab), Pasteur Institute of Iran, Tehran, IR Iran
[4]Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, IR Iran
[5]Virology Department, Babol University of Medical Sciences, School of Medicine, Babol, Iran
[6]Research Center of Pediatric Infectious Diseases, Rasoul-e-Akram Hospital, Iran University of Medical Sciences, Tehran, IR Iran
[7]Middle East Liver Disease Center, Tehran, IR Iran
[8]Iran Hepatitis Network, Tehran, IR Iran
[9]Department of Civil Engineering, Faculty of Engineering, Payame Noor University, Karaj, Irann

[*]*Corresponding author*: Hossein Keyvani, Department of Virology, Iran University of Medical Sciences, Tehran, IR Iran. Tel/Fax: +98-2188602205, E-mail: keyvanlab@yahoo.com

Received 2016 June 12; **Revised** 2016 October 23; **Accepted** 2016 December 06.

Abstract

Background: The hepatitis C virus (HCV) infection is one of the major causes of progressive liver diseases worldwide. Despite the new treatments for HCV infection, antiviral therapy with a combination of pegylated interferon-α 2a plus ribavirin (Peg-IFNα-2a/RBV) is still used in developing countries.

Objectives: The aim of the current study was to determine the relationship between rs12979860 polymorphism in the interleukin 28B gene (IL28B) and response to Peg-IFNα-2a/RBV combination therapy in Azerbaijani patients with chronic HCV infection.

Methods: A total of 72 Azerbaijani patients with established chronic HCV-1b took part in this cross-sectional study between January 2010 and September 2015. Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs) of the patients and the rs12979860 single nucleotide polymorphism was diagnosed by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP).

Results: The mean age of the patients was 36.9 $\pm$ 12.4 (range of 27 - 57 years) and 42 (58.3%) out of 72 were male. Concerning the *IL28B* polymorphism in rs12979860, the results indicated the presence of CC, CT, and TT genotypes in 24 (33.3%), 42 (58.3%), and 6 (8.3%) patients, respectively. There was a significant association between *IL28B* genotypes and response to HCV combination therapy with peg-IFNα-2a/RBV (P = 0.001).

Conclusions: The results of this study indicate that the rs12979860 CC genotype of *IL28B* was associated with a response to Peg-IFNα-2a/RBV combination therapy in Azerbaijani patients with HCV-1b infection. Therefore, host genetics may be useful in predicting the response to hepatitis C treatment.

Keywords: Hepatitis C Virus, Interferon, Ribavirin, *IL28B* Polymorphisms

1. Background

Hepatitis C virus (HCV) infection is a major global public health challenge, affecting an estimated 3% (120 to 180 million) of people worldwide (1). Approximately, 70 to 80% of HCV infected patients become chronic hepatitis C carriers and may progress to chronic liver diseases including hepatocellular carcinoma (HCC), cirrhosis of the liver and liver failure (2, 3). Although effective antiviral therapy with a combination of pegylated interferon-α 2a plus ribavirin (Peg-IFNα-2a/RBV) may prevent these complications, treatment outcomes vary. Different sustained virologic response (SVR) rates (50%) have been observed in different populations (4-6).

Viral factors, including pre-treatment HCV RNA load, HCV genotype, liver cirrhosis, and host factors, including gender, age, innate immunity, and genetic variation in Interleukin-28B (IL28b) are beneficial in predicting response to interferon (7, 8). Several viral factors may predict the outcome of Peg-IFNα-2a/RBV combination therapy; for instance, 40 amino acids in the non-structural 5A (NS5A) region (9, 10), and amino acid substitutions in the core region have been reported as predictors of hepatitis C therapy outcome (11, 12).

Numerous common polymorphisms have been found in the human genome that may be correlated with the ef-

fectiveness of interferon therapy. For instance, two single nucleotide polymorphisms (SNPs) were observed in the IL28B locus. The rs12979860 (located 3 kb upstream of the *IL28B* gene) and the rs8099917 (located 8.9 kb upstream of the *IL28B* gene) SNPs are the strongest SVR predictors in patients infected with chronic HCV (13, 14). One of the most acceptable, reliable, and fastest methods to determine the rs12979860 polymorphism is PCR-RFLP assay. This method can be used by every molecular diagnostic laboratory with PCR-RFLP assay capability. Determining this polymorphism will be clinically useful in predicting treatment response in participants with HCV infection (15, 16).

Recently, a meta-analysis indicated that the rs12979860 allele is more important than the rs8099917 allele and more accurately predicts SVR (17). Furthermore, the prevalence of SVR and these SNPs varies among ethnicities and HCV genotypes (18). Thus, more studies are warranted to identify the mechanisms. Unfortunately, there are currently limited reports in literature about the role of rs12979860 polymorphisms in Azerbaijani patients with established chronic hepatitis C. The patients taking part in this study had come from the Republic of Azerbaijan to Iran for medical treatment by Peg-IFNα-2a/RBV combination therapy.

2. Objectives

We aimed to investigate the influence of rs12979860 polymorphism on the rate of response to Peg-IFNα-2a/RBV combination therapy in Azerbaijani patients with HCV infection.

3. Methods

3.1. Study Population

This cross-sectional study was conducted on a total of 102 Azerbaijani patients with established chronic hepatitis C between January 2010 and September 2015. The patients had come from the Republic of Azerbaijan to Iran for medical treatment while all of them were anti-HCV Abs and HCV-RNA positive. They referred to hospitals affiliated to Iran University Medical Sciences and Tehran hepatitis centre. Informed consent was obtained from all of the participants. The exclusion criteria included (i) having other hepatitis infection [HBV (HBV-DNA and hepatitis B surface antigen negative), and HDV (HDV-RNA and anti-HDV antibody negative)], (ii) using immunosuppressive drugs, (iii) coexistent autoimmune liver diseases (negativity for antimitochondrial and antinuclear antibodies, etc.), (iv) diabetes mellitus, (v) genetic disorders, (vi) alcohol intake, (vii) drug toxicity, and (viii) having any other disease that suppress the immune system for instance human immunodeficiency virus infection (anti-HIV Abs negative). The study was approved by the local ethics committee of Gastrointestinal and liver disease research center (GILDRC) at Iran University of Medical Sciences, Tehran, Iran.

3.2. The Treatment Protocol

The treatment protocol with Peg-IFNα-2a/RBV combination therapy was based on standard protocols for hepatitis C treatment (19): Peg-IFNα-2a (Pegasys, Roche, Basel, Switzerland) 180 mg per week and ribavirin (RBV) (Copegus, Roche) 1,000 - 1,200 mg per day for 48 weeks. Determination of the response to anti-HCV treatment has been described previously in detail (20).

3.3. Sample Preparation

A 5 ml blood specimen was collected in an EDTA-containing vacutainer tube from each patient. Following plasma separation, peripheral blood mononuclear cells (PBMCs) were isolated by using a standard method of Ficoll-Hypaque gradient centrifugation (Lymphoprep, Oslo, Norway). After washing 3 times with phosphate-buffered saline (pH = 7.3 $\pm$ 0.1), the achieved PBMCs were counted and resuspended in 250 μL RNALater (Ambion Inc., Austin, TX, USA), and frozen at -70°C until use.

3.4. Detection of HCV-RNA and HCV-Genotyping

Viral RNA was extracted from 140 μL of plasma by using QIAamp viral RNA extraction kit (Qiagen GmbH, Hilden, Germany) according to manufacturer's instructions. For the detection of genomic HCV RNA, the extracted viral RNAs were subjected to reverse-transcription-nested polymerase chain reaction (RT-nested PCR), as described before (20). Polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP) was employed to determine HCV genotypes (21).

3.5. Single Nucleotide Polymorphism (SNP) of the IL28B Genotype

Genomic DNA was extracted from a pellet of about 3 - 5 $\times 10^6$ PBMC specimens using a QIAamp DNA blood mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. rs12979860 SNP genotyping was performed using PCR-RFLP assay.

The oligonucleotide primers were as follows: 5′-GCT TAT CGC ATA CGG CTA GC-3′ (sense) and 5′-AGG CTC AGG GTC AAT CAC AG-3′ (the primers were synthesized by metabion company, Germany). Polymerase chain reaction amplification was performed (6) and PCR products (252 base pairs) were digested with 1 unit of the BstU-I restriction endonuclease (New England Biolabs, Inc.) at 60°C for 16 hours.

BstU-I digestion of allele CC yields the fragments of 135, 82, and 25 base pairs, and DNA containing the allele TT polymorphism yields the fragments of 160 and 82 base pairs. Restriction digestion products of specimens with undigested products and positive and negative controls and DNA size marker were visualized using 3.5% agarose gel electrophoresis (6). In 10 patients, the genomic region surrounding the *IL28B* rs12979860 C/T polymorphism was also confirmed by sequencing.

3.6. Statistical Analysis

Statistical analysis was performed using SPSS software version 16 (SPSS, Inc., Chicago, IL). To evaluate the normality of data, Kolmogorov-Smirnov test was used. Analysis of continuous variables was conducted by one-way ANOVA and Kruskal-Wallis tests. The chi-square test was applied to assess associations between categorical variables. A P value of ≤ 0.05 was considered statistically significant.

3.7. Ethical Approval

The ethical approval of the present research was received from the ethics committee of Iran University of Medical Sciences (No.2083 at 2015-04-28) before starting this study.

4. Results

4.1. Clinical and Demographic Features

The current prospective study was conducted on a total of 102 Azerbaijani patients chronically infected with HCV. The mean age of the patients was 36.5 $\pm$ 12.8 (ranged between 8 and 71 years). Out of 102 patients, 58 (56.9%) were male. The HCV genotypes in the study population were determined as follows: subtype 1b in 72 (70.6%) patients, subtype 3a in 17 (16.7%), genotype 2 in 7 (6.9%), subtype 1a in 5 (4.9%), and mixed infection in 1 (1.0%) patient. The patients with HCV-1b infection took part in the present study. The data on demographic, laboratory, and epidemiological characteristics of the Azerbaijani patients with HCV infection and different types of response to anti-viral therapy with RBV/PEG-IFN2α are summarised in Table 1.

4.2. Frequency of IL28B Genotypes

The genotyping of *IL28B* rs12979860 showed the presence of CC, CT, and TT genotypes in 41 (40.2%), 52 (51.0%), and 9 (8.8%) out of 102 individuals, respectively. There was a significant association between *IL28B* genotypes and response to HCV combination therapy with peg-IFNα-2a/RBV (Table 2) (P = 0.018).

4.3. Clinical and Demographic Features of Patients with HCV-1b Infection

The mean age of patients with HCV-1b infection was 36.9 $\pm$ 12.4 (ranged between 27 and 57 years). Out of 72 patients, 42 (58.3%) were male. Table 1 represents the demographic characteristics, laboratory parameters, and different responses to combination therapy with peg-IFNα-2a/RBV (SVR [n = 48, 66.7%], NR [n = 11, 15.2%], and relapsers [n = 13, 18.1%]). In the current investigation, no significant associations were observed between gender (P = 0.234), age (P = 0.248), viral load (P = 0.204), ALT level (P = 0.893), and AST level (P = 0.940) of Azerbaijani patients with hepatitis C virus infection and outcome of HCV combination therapy (Table 3).

4.4. Frequency of IL28B Genotypes Among Patients With HCV-1b Infection

The genotyping of *IL28B* rs12979860 showed the presence of CC, CT, and TT genotypes in 24 (33.3%), 42 (58.3%) and 6 (8.3%) out of 72 patients, respectively. There was a significant correlation between *IL28B* rs12979860 genotypes and response to HCV combination therapy with peg-IFNα-2a/RBV (Table 4) (P = 0.001).

4.5. Association of IL28B Genotypes with HCV Genotype and Viral Load

The association between *IL28B* rs12979860 with HCV genotype and viral load was investigated using linear regression analysis. No significant correlation was observed between *IL28B* rs12979860 polymorphism and HCV genotype (P = 0.263), while there was a significant association between *IL28B* rs12979860 genotypes and viral load, as the mean viral load was significantly lower in patients with CT and TT genotypes than those with CC genotype (P = 0.001).

4.6. Factors Associated with SVR

A multivariate logistic regression analysis was performed to determine the correlated factors with SVR. After adjusting for potential confounder variables, the results showed that age and IL28B polymorphism could be significant predictors of SVR. The results showed that by a unit increase in the age, the chance of SVR occurrence decreases 0.949 times (95% CI 0.909 - 0.992, P = 0.021). Also, the chance of SVR occurrence in IL28B polymorphism of CT and TT genotypes compared to CC genotype (as a reference group) was 0.111 (95% CI: 0.026 - 0.465, P = 0.003) and 0.110 (95% CI: 0.014 - 0.843, P = 0.034) times, respectively, which means that the chance of SVR occurrence was significantly more in CC type of IL28B polymorphism than two others types (Table 5).

Table 1. Demographic and Laboratory parameters of the Azerbaijani Patients with Hepatitis C Virus Infection and Different Types of Response to Anti-viral Therapy with Ribavirin/PEG-IFN2α[a]

Patients	Sustained Virological Responders	Non-Responders	Relapsers	Total	P Value
No. of patients	71 (69.6)	17 (16.7)	14 (13.7)	102 (100)	
Gender Male/Female	38/33	10/7	10/4	22/29	0.458
Age	34.7 ± 13.2 (8 - 71)	38.7.7 ± 11.3 (20 - 56)	39.6 ± 11.6 (22 - 57)	36 ± 12.8 (8 - 71)	0.270
Laboratory parameters					
Viral Load, IU/mL	3,138,045 (10,065 - 23,800,950)	4,420,864 (47050 - 15,584,670)	721,119 (14100 - 2,583,880)	801,990 (10,065 - 23,800,950)	0.098
ALT, IU/L	70.1 (14 - 269)	73.3 (27 - 187)	47.8 (17 - 180)	71.8 (14.269)	0.480
AST, IU/L	59.8 (15 - 171)	58.9 (18 - 148)	71.3 (20 - 156)	61.3 (15 - 171)	0.702
HCV genotype					0.933
1a	3 (75)	1 (25)	0	4 (100)	
1a/1b	2 (100)	0	0	2 (100)	
1b	48 (66.7)	11 (15.3)	13 (18.1)	72 (100)	
2	5 (71.4)	1 (14.3)	1 (14.3)	7 (100)	
3a	11 (68.8)	4 (25)	1 (6.2)	16 (100)	
ND	1 (100)	0	0	1 (100)	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ND, not determined.
[a] Values are expressed as No. (%) or mean ± SD.

Table 2. Correlation between IL28B Polymorphisms (rs12979860) of HCV and Response to Peg-IFNα-2a/RBV Combination Therapy in Azerbaijani Patients[a]

		Response			Total	P Value
		SVR (n = 70)	NR (n = 17)	Relapse (n = 15)		
IL28B	CC	34 (48.6)	5 (29.4)	2 (13.3)	41 (40.2)	0.018
	CT	30 (42.9)	9 (52.9)	13 (86.7)	52 (51.0)	
	TT	6 (8.6)	3 (17.6)	0	9 (8.8)	
Total		70 (100)	17 (100)	15 (100)	102 (100.0)	

[a] Values are expressed as No. (%).

Table 3. Demographic and Laboratory Parameters of the Azerbaijani Patients with Hepatitis C Virus 1b Infection and Different Types of Response to Anti-Viral Therapy with Peg-IFNα-2a/RBV[a]

Patients		Sustained Virological Responders	Non-Responders	Relapsers	Total	P Value
No. of patients		48 (66.7)	11 (15.2)	13 (18.1)	72 (100)	
Gender	Female	19 (39.6)	7 (63.6)	4 (30.8)	30 (41.7)	0.234
	Male	29 (60.4)	4 (36.4)	9 (69.2)	42 (58.3)	
Age, Mean		35.2 ± 12.8 (8 - 60)	39.6 ± 11.7 (20 - 56)	40.9 ± 10.8 (27 - 57)	36.9 ± 12.4 (27 - 57)	0.248
Laboratory parameters						
Viral Load, IU/mL[b]		672447.5 (10065 - 23800950)	1032875 (47050 - 11015400)	391180 (14100 - 2853880)	659865 (10065 - 23800950)	0.204
ALT, IU/L		63.5 (21 - 269)	56 (27 - 157)	49 (17 -180)	60.5 (17 - 269)	0.893
AST, IU/L		52.2 (17 - 171)	50 (50 - 148)	45 (20 - 156)	50.5 (17 - 171)	0.940

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase;
[a] Values are expressed as No. (%) or mean ± SD.
[b] These parameters had not a normal distribution.

5. Discussion

Combination therapy with Peg-IFNα-2a/RBV is still the standard treatment protocol in Azerbaijani patients with chronic HCV infection. New, direct-acting antiviral agents are unavailable. The most frequent HCV genotype in Azerbaijani patients is subtype 1b, which is one of the most difficult-to-treat genotypes (22). Therefore, study of effective pre-treatment predictors of therapeutic outcome is unnecessary. Recent studies have suggested that polymor-

Table 4. Genetic Variation in Interleukin-28B and Response to Peg-IFNα-2a/RBV Combination Therapy in Azerbaijani Patients with Hepatitis C Virus Subtype-1b Infection[a]

Parameters		Response to Peg-IFNα-2a/RBV Combination Therapy				P Value
		SVR (n = 48)	NR (n = 11)	Relapse (n = 13)	Total (n = 72)	
IL28B	CC	23 (47.9)	1 (9.1)	0	24 (33.3)	0.001
	CT	21 (43.8)	8 (72.7)	13 (100)	42 (58.3)	
	TT	4 (8.3)	2 (18.2)	0	6 (8.3)	
Total		48 (100)	11 (100)	13 (100)	72 (100)	

[a] Values are expressed as No. (%).

Table 5. Factors Predictive of Sustained Virologic Response Obtained by Multivariate Logistic Regression Analysis

Variables	SVR			
		Odds Ratio	95% CI	P Value
Age		0.949	0.909 - 0.992	0.021
Gender	Female	1	-	-
	Male	0.417	0.150 - 1.158	0.093
Genotype	1a	1	-	-
	1b	0.505	0.039 - 6.465	0.600
	2	0.738	0.025 - 21.15	0.860
	3a	0.265	0.014 - 4.764	0.368
Viral Load, IU/mL		0.999	0.999 - 1	0.236
IL28	CC	1	-	-
	CT	0.111	0.026 - 0.465	0.003
	TT	0.110	0.014 - 0.843	0.034
ALT, IU/L		1.003	0.979 - 1.028	0.771
AST, IU/L		0.998	0.969 - 1.028	0.940

phisms near the *IL28B* locus are associated with the response to Peg-IFNα-2a/RBV combination therapy (13, 14, 17).

Identification of SNPs including rs12979860 and rs8099917 in the *IL28B* locus provides an extremely effective pre-treatment predictor of response to interferon therapy. Although both SNPs are independent pre-treatment predictors of response to anti-HCV therapy, recent investigations have suggested that rs12979860 contributes directly to therapeutic responses (13, 17). The rs12979860 CC genotype was associated with higher chances of SVR. Hence, the facts mentioned above motivated us to investigate the prevalence of rs12979860 genotypes in Azerbaijani patients who were chronically infected with HCV subtype 1b and evaluate the influence of rs12979860 genotypes in anti-HCV treatment response.

In the present study, the PCR-RFLP assay was used to determine the rs12979860 polymorphism. It is noteworthy that PCR-RFLP assay has been utilized to determine this polymorphism in several studies such as those conducted by Sharafi et al. (23), Irman et al. (24), Fateh et al. (16), and Nakamoto et al. (25). These studies have reported that this assay is simple, fast, inexpensive, and valid. This method is applicable in every molecular diagnostic laboratory with PCR-RFLP assay capability (16).

Among 102 Azerbaijani patients with chronic HCV infection, CC, CT, and TT genotypes were present in 41 (40.2%), 52 (51.2%), and 9 (8.8%) cases, respectively (Table 2). On the other hand, because the most studied patients were infected with HCV genotype 1b, this polymorphism was analyzed separately in these participants. The genotype frequencies of rs12979860 polymorphisms in these patients with CC, CT, and TT genotypes were 33.3%, 58.3%, and 8.3%, respectively, which are close to the corresponding frequencies in Iranian and Turkish patients (26-28).

The results of the current study demonstrated a significant association between rs12979860 CC genotype and SVR rate. The CC genotype of rs12979860 occurred in 48.6% of patients who achieved SVR, and 47.9% of patients who were infected with HCV subtype 1b, which were significantly higher than the corresponding values of CT and TT genotypes. These findings are consistent with the results of other studies in different ethnic groups (29). Based on these findings, the rs12979860 CC genotype could constitute an effective pre-treatment predictor in Azerbaijani chronic hepatitis C patients, especially when associated with HCV subtype 1b. In contrast, a significant association between rs12979860 CT genotype and non-response or relapse was observed. Frequencies of CT genotype in NR

group and relapsers were 72.7% and 100%, respectively. This finding is consistent with those of previous studies (16, 28, 30).

In this study, the rs12979860 CC genotype of *IL28B* was associated with response to Peg-IFNα-2a/RBV combination therapy in Azerbaijani patients with chronic HCV infection. The results of this study may help identify the patients for whom the treatment may be successful. Further studies with larger sample sizes seem necessary to evaluate the present results.

Acknowledgments

We really appreciate all the volunteers who participated in the present study.

Footnotes

Author's Contribution: Farah Bokharaei-Salim, Hossein Keyvani, Mostafa Salehi-Vaziri, and Farzin Sadeghi designed the study and were responsible for the overall study management. Farah Bokharaei-Salim, Mostafa Salehi-Vaziri, and Farzin Sadeghi organized the analysis of the study. Farah Bokharaei-Salim, Mostafa Salehi-Vaziri, Farzin Sadeghi, Khadijeh Khanaliha, Maryam Esghaei, Seyed Hamidreza Monavari, Seyed Moayed Alavian, and Hossein Keyvani prepared the manuscript. The statistical analyses have been conducted by Farah Bokharaei-Salim, Khadijeh Khanaliha, Shahin Fakhim. All the authors contributed to the final revision of the manuscript.

Financial Disclosure: The authors have no financial disclosures to express and no conflicts of interest to report.

Funding/Support: This study was supported by research deputy of Iran University of Medical Sciences, Grant number 25660.

Conflict of Interest: The authors declare no conflict(s) of interest.

References

1. Jahanbakhsh Sefidi F, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Bokharaei-Salim F. Distribution of hepatitis C virus genotypes in Iranian chronic infected patients. *Hepat Mon.* 2013;**13**(1):ee7991. doi: 10.5812/hepatmon.7991. [PubMed: 23550108].
2. Tomimatsu M, Ishiguro N, Taniai M, Okuda H, Saito A, Obata H, et al. Hepatitis C virus antibody in patients with primary liver cancer (hepatocellular carcinoma, cholangiocarcinoma, and combined hepatocellular-cholangiocarcinoma) in Japan. *Cancer.* 1993;**72**(3):683–8. [PubMed: 8192727].
3. Bastani MN, Bokharaei-Salim F, Keyvani H, Esghaei M, Monavari SH, Ebrahimi M, et al. Prevalence of occult hepatitis C virus infection in Iranian patients with beta thalassemia major. *Arch Virol.* 2016;**161**(7):1899–906. doi: 10.1007/s00705-016-2862-3. [PubMed: 27132015].
4. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet.* 2001;**358**(9286):958–65. [PubMed: 11583749].
5. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FJ, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med.* 2002;**347**(13):975–82. doi: 10.1056/NEJMoa020047. [PubMed: 12324553].
6. Fabris C, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, et al. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol.* 2011;**54**(4):716–22. doi: 10.1016/j.jhep.2010.07.019. [PubMed: 21146242].
7. Matsuyama N, Mishiro S, Sugimoto M, Furuichi Y, Hashimoto M, Hijikata M, et al. The dinucleotide microsatellite polymorphism of the IFNAR1 gene promoter correlates with responsiveness of hepatitis C patients to interferon. *Hepatol Res.* 2003;**25**(3):221–5. [PubMed: 12697242].
8. Sadeghi F, Bokharaei-Salim F, Salehi-Vaziri M, Monavari SH, Alavian SM, Salimi S, et al. Associations between human TRIM22 gene expression and the response to combination therapy with Peg-IFNalpha-2a and ribavirin in Iranian patients with chronic hepatitis C. *J Med Virol.* 2014;**86**(9):1499–506. doi: 10.1002/jmv.23985. [PubMed: 24889558].
9. Bokharaei-Salim F, Keyvani H, Salehi-Vaziri M, Sadeghi F, Monavari SH, Mehrnoush L, et al. Mutations in the NS5A gene of hepatitis C virus subtype 1b and response to peg-IFNalpha-2a/RBV combination therapy in Azerbaijani patients. *Arch Virol.* 2014;**159**(11):2893–9. doi: 10.1007/s00705-014-2133-0. [PubMed: 25139545].
10. Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med.* 1996;**334**(2):77–81. doi: 10.1056/NEJM199601113340203. [PubMed: 8531962].
11. Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol.* 2007;**46**(3):403–10. doi: 10.1016/j.jhep.2006.09.019. [PubMed: 17126448].
12. Bokharaei-Salim F, Salehi-Vaziri M, Sadeghi F, Esghaei M, Monavari SH, Alavian SM, et al. The Association of Substitutions in the Hepatitis C Virus Subtype 1b Core Gene and IL28B Polymorphisms With the Response to Peg-IFNalpha-2a/RBV Combination Therapy in Azerbaijani Patients. *Hepat Mon.* 2016;**16**(5):ee35597. doi: 10.5812/hepatmon.35597. [PubMed: 27313635].
13. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature.* 2009;**461**(7262):399–401. doi: 10.1038/nature08309. [PubMed: 19684573].
14. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet.* 2009;**41**(10):1105–9. doi: 10.1038/ng.449. [PubMed: 19749757].
15. Fateh A, Aghasadeghi MR, Keyvani H, Mollaie HR, Yari S, Hadizade Tasbiti AR, et al. High resolution melting curve assay for detecting rs12979860 IL28B polymorphisms involved in response of Iranian patients to chronic hepatitis C treatment. *Asian Pac J Cancer Prev.* 2015;**16**(5):1873–80. [PubMed: 25773839].
16. Fateh A, Aghasadeghi M, Siadat SD, Vaziri F, Sadeghi F, Fateh R, et al. Comparison of Three Different Methods for Detection of IL28 rs12979860 Polymorphisms as a Predictor of Treatment Outcome in Patients with Hepatitis C Virus. *Osong Public Health Res Perspect.* 2016;**7**(2):83–9. doi: 10.1016/j.phrp.2015.11.004. [PubMed: 27169005].

17. Li S, Hu P, Zhang QQ, Liu YH, Hu HD, Zhang DZ, et al. Single nucleotide polymorphisms of the IL28B and sustained virologic response of patients with chronic hepatitis C to PEG-interferon/ribavirin therapy: A meta-analysis: Meta-analysis of IL28B. *Hepat Mon.* 2011;**11**(3):163–72. [PubMed: 22087138].
18. Ahlenstiel G, Booth DR, George J. IL28B in hepatitis C virus infection: translating pharmacogenomics into clinical practice. *J Gastroenterol.* 2010;**45**(9):903–10. doi: 10.1007/s00535-010-0287-4. [PubMed: 20635099].
19. Alavian SM, Tabatabaei SV, Keshvari M, Behnava B, Miri SM, Elizee PK, et al. Peginterferon alpha-2a and ribavirin treatment of patients with haemophilia and hepatitis C virus infection: a single-centre study of 367 cases. *Liver Int.* 2010;**30**(8):1173–80. doi: 10.1111/j.1478-3231.2010.02296.x. [PubMed: 20629950].
20. Bokharaei-Salim F, Keyvani H, Monavari SH, Alavian SM, Madjd Z, Toosi MN, et al. Occult hepatitis C virus infection in Iranian patients with cryptogenic liver disease. *J Med Virol.* 2011;**83**(6):989–95. doi: 10.1002/jmv.22044. [PubMed: 21503911].
21. Pohjanpelto P, Lappalainen M, Widell A, Asikainen K, Paunio M. Hepatitis C genotypes in Finland determined by RFLP. *Clin Diagn Virol.* 1996;**7**(1):7–16. [PubMed: 9077432].
22. Bokharaei-Salim F, Keyvani H, Monavari SH, Alavian SM, Fakhim S, Nasseri S. Distribution of hepatitis C virus genotypes among azerbaijani patients in capital city of iran-tehran. *Hepat Mon.* 2013;**13**(9):ee13699. doi: 10.5812/hepatmon.13699. [PubMed: 24282427].
23. Sharafi H, Pouryasin A, Alavian SM, Behnava B, Keshvari M, Mehrnoush L, et al. Development and Validation of a Simple, Rapid and Inexpensive PCR-RFLP Method for Genotyping of Common IL28B Polymorphisms: A Useful Pharmacogenetic Tool for Prediction of Hepatitis C Treatment Response. *Hepat Mon.* 2012;**12**(3):190–5. doi: 10.5812/hepatmon.849. [PubMed: 22550527].
24. Imran M, Manzoor S, Azam S, Resham S. Genetic variant of IL28B rs12979860, as predictive marker of interferon-based therapy in Pakistani population. *APMIS.* 2015;**123**(4):342–9. doi: 10.1111/apm.12365. [PubMed: 25703417].
25. Nakamoto S, Kanda T, Imazeki F, Wu S, Arai M, Fujiwara K, et al. Simple assay based on restriction fragment length polymorphism associated with IL28B in chronic hepatitis C patients. *Scand J Gastroenterol.* 2011;**46**(7-8):955–61. doi: 10.3109/00365521.2011.574731. [PubMed: 21529139].
26. Sharafi H, Pouryasin A, Alavian SM, Behnava B, Keshvari M, Salimi S, et al. Distribution of IL28B Genotypes in Iranian Patients with Chronic Hepatitis C and Healthy Individuals. *Hepat Mon.* 2012;**12**(12):ee8387. doi: 10.5812/hepatmon.8387. [PubMed: 23550102].
27. Taheri S, Aygen B, Korkmaz K, Yildiz O, Zararsiz G, Canatan H. Characterization of the Interleukin-28B Gene rs12979860 C/T Polymorphism in Turkish Chronic Hepatitis C Patients and Healthy Individuals. *Balkan Med J.* 2015;**32**(2):147–55. doi: 10.5152/balkanmedj.2015.15156. [PubMed: 26167338].
28. Behnava B, Sharafi H, Keshvari M, Pouryasin A, Mehrnoush L, Salimi S, et al. The Role of Polymorphisms Near the IL28B Gene on Response to Peg-Interferon and Ribavirin in Thalassemic Patients With Hepatitis C. *Hepat Mon.* 2016;**16**(1):ee32703. doi: 10.5812/hepatmon.32703. [PubMed: 27110259].
29. Chen Y, Xu HX, Wang LJ, Liu XX, Mahato RI, Zhao YR. Meta-analysis: IL28B polymorphisms predict sustained viral response in HCV patients treated with pegylated interferon-alpha and ribavirin. *Aliment Pharmacol Ther.* 2012;**36**(2):91–103. doi: 10.1111/j.1365-2036.2012.05131.x. [PubMed: 22591106].
30. Sedighimehr P, Irani S, Sakhaee F, Vaziri F, Aghasadeghi M, Sadat SM, et al. IL28B rs12980275 and HLA rs4273729 genotypes as a powerful predictor factor for rapid, early, and sustained virologic response in patients with chronic hepatitis C. *Arch Virol.* 2016 doi: 10.1007/s00705-016-3095-1. [PubMed: 27714501].