DOI: 10.34172/mejdd.2022.264

Association of Interleukin-28B Polymorphisms (rs12979860 C/T, rs12980275 A/G, rs8099917 T/G) and Risk of Hepatocellular Carcinoma in an Iranian Population

Leila Hajizadeh[1], Nader Mansour Samaei[1,*], Afsaneh Bazgir[1], Mehdi Agha Gholizadeh[2], Ayoob Khosravi[1]

[1] Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[2] Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

[*] **Corresponding Author:**
Nader Mansour Samaei, MD, PhD
Human Genetics
Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran

Telefax: +98 1732430563
Email: samaei@goums.ac.ir, n_samaei@yahoo.com

Received: 26 Oct. 2020
Accepted: 18 Apr. 2021
Publieshed: 30 Jan. 2022

ABSTRACT

BACKGROUND:
Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world. The association of interleukin 28B (IL-28B) polymorphisms and HCC has been investigated in several populations. However, the findings are controversial. This study aimed to address the association between IL-28B polymorphisms (rs 8099917 T/G, rs12979860 C/T, rs12980275 A/G) and the risk of HCC in an Iranian population.

METHODS:
We have evaluated the association between IL-28B polymorphisms (rs 8099917 T/G, rs12979860 C/T, rs12980275 A/G) and HCC in 180 Iranian individuals (60 patients with HCC and 120 healthy matched controls) using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method. Single nucleotide polymorphism (SNP) association analysis and also haplotypes were estimated using the SNPstats online software.

RESULTS:
There was no significant association between these three polymorphisms of IL-28B and HCC ($P>0.05$). Moreover, haplotype analysis showed no significant association between the haplotypes and HCC.

CONCLUSION:
There was no association between IL-28B polymorphisms and HCC in an Iranian population.

KEYWORDS:
Hepatocellular carcinoma; Interleukin 28B; Polymorphism

Please cite this paper as:
Hajizadeh L, Mansour Samaei N, Bazgir A, Agha Gholizadeh M, Khosravi A. Association of Interleukin-28B Polymorphisms (rs12979860 C/T, rs12980275 A/G, rs8099917 T/G) and Risk of Hepatocellular Carcinoma in an Iranian Population. *Middle East J Dig Dis* 2022;**14**:118-123. doi: 10.34172/mejdd.2022.264.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third common cause of cancer-related mortality worldwide.[1] It is generally considered that a variety of environmental and genetic factors are involved

in the pathogenesis of HCC. Hepatitis B and C viruses are believed to be the major causes of chronic hepatitis, which can progress to HCC.[2] Genetic factors including cytokines and interleukins may play a critical role in the development of HCC in HCV infection by influencing the differentiation, maturation, and immune responses.[3] One of the most important genetic factors is interferons (IFNs; IFN-α/β/λ), which are the natural human antiviral proteins. IFNs play a crucial role in immune systems and trigger some critical pathways. One of the most important IFNs in this way is IL-28B that is activated through the JAK-STAT pathway. Interleukin 28B gene (IL28B, also known as interferon lambda 3 [IFNλ3], which is a member of the type III IFN family) is located on chromosome 19. Several single nucleotide polymorphisms (SNPs) in IL-28B can affect mRNA stability and, therefore, can disrupt the durability and function of this gene. So, the immune system can be disturbed and be prone to some complex diseases such as HCC.[1] Recent studies have demonstrated that SNP of the IL28B gene plays an important role in the diversity of immune responses to HCV infection.[4] It was reported that genetic variants of IL28B, including rs12979860, rs 12980275, rs8099917 were strongly associated with the natural clearance of HCV and also played an important role in anti-HCV therapy as a strong prognostic value.[5,6] Studies suggest that interferon-13 (IFN-13) encoded by the IL28B gene has been involved in the defense mechanisms against several viruses, including HBV and HCV. Therefore, IL28B polymorphisms may affect the risk of developing HCC.[7,8] The association between IL28B polymorphism and the risk of development of HCV-associated HCC remains unclear.[9,10] Therefore, we evaluated the association between genetic variants at SNP rs12979860, rs 12980275, rs8099917 with the risk of HCC among individuals with HCV infection in an Iranian population.

MATERIALS AND METHODS

Patients

180 Iranians, including 60 patients with HCC and 120 healthy individuals as control group, were investigated for genetic variations in the IL28B gene polymorphisms. The patients with HCC were recruited from the hospitals in Mazandaran, Tehran, Shiraz, Isfahan, Sistan and Baluchestan, and Gorgan Provinces, Iran. The controls, randomly recruited from Mazandaran, were matched with patients with HCC for age, sex, and ethnic origin. The diagnosis of patients with HCC was confirmed with pathological and histopathological criteria.[11]All patients were positive for HCV, and they did not have any other types of liver diseases such as autoimmune liver diseases, alcoholic liver diseases, or metabolic liver diseases. The patients' and the control group's average ages were 50 and 57.5, respectively. 42% of the patients with HCC were female, and 57% were male. In the control group, 49% were female, and 51% were male. Written informed consent was obtained from all the subjects.

DNA extraction

DNA was extracted from paraffin-embedded tissues of the patients, using Cinna Pure DNA kit (sinaclon, Iran) according to manufacturer's instructions and from the peripheral blood cells of the controls, using the boiling procedure. DNA concentration was determined by Picodrop UV/Vis spectrophotometer (Picodrop Ltd, UK).

IL28B genotyping

Genotyping of IL28B SNP rs12979860 C/T, rs12980275 A/G, rs8099917 G/T was performed by restriction fragment length polymorphisms (RFLP) analysis. Briefly, the region was amplified by polymerase chain reaction (PCR), as previously described.[12] The primers used are shown in table 1. The PCR was performed using the following conditions for three primers: an initial denaturation step at 95°C for 5 min followed by 35 cycles of 30s at 95°C; 40s at 72°C, and a final extension step of at 72°C for 5 min. Annealing condition for rs12979860 C/T is 30s 66°, rs12980275 A/G is 30s 56°, rs8099917 G/T is 30s 66°. The PCR products were digested overnight (18-20 hours) with BseMl for rs8099917 G/T, BstUl for rs12979860 C/T, and Bsl I for rs12980275 A/G at 37°C, separated by electrophoresis in a 3% agarose gel in Tris base EDTA (TBE) buffer stained with ethidium bromide and visualized by Gel Doc Imaging System (E-Box VILBER, Japanise). BseMl digestion produced 552 bp for TT genotype, 322 and 230 bp for GG genotype, and 522, 322, and 230 bp for GT genotype for rs8099917. BstUl digestion produced 196 and 45 bp for CC genotype, 241 bp for TT genotype, and 241, 196, and 45 bp for CT genotype for rs12979860. Bsl I digestion produced 320

Table 1: Primer sequences were used in PCR amplification

rs numbers	Primer sequences
rs12979860 C/T	5′-GCGGAAGGAGCAGTTGCGCT-3′(Forward) 5′-GGGGCTTTGCTGGGGGAGTG-3′(Reverse)
rs12980275 A/G	5′-GAG AGC AAG AGG AGG GAA GGA A -3′(Forward) 5′-GTG TGC CAT TAG CCA GTC AGA T -3 (Reverse)
rs8099917 G/T	5′-CCCACTTCTGGAACAAATCGTCCC-3′(Forward) 5′-TCTCCTCCCCAAGTCAGGCAACC-3′(Reverse)

and 121 bp for AA genotype, 290, 121, and 30 bp for GG genotype, and 320, 290, 121, and 30 bp for AG genotype for rs12980275 (Figure 1).

Statistical analysis

Case and control groups were determined by standard χ^2 test for genotyping and allele frequency. Hardy-Weinberg equilibrium (HWE) was tested by using the χ^2 test. Association was determined by calculating odds ratios (OR) and 95% confidence intervals (CIs). The SNPstats software analyses the associations by linear or logistic regression according to the response variable.[13] This web-based software uses the multiple inheritance models (co-dominant, dominant, recessive, overdominant, and log-additive) for SNP association analysis, and also haplotypes were estimated using the SNPstats online software.

RESULTS

Our study included 60 patients with HCC and 120 controls aged over 57.5 years. The genotype distribution was in Hardy-Weinberg equilibrium in two groups. For rs8099917, allele frequency for T allele in the control group and HCC patients was 0.76 and 0.68, and for G allele was 0.24 and 0.32. The distribution of this polymorphism was 0.59% TT, 0.33% TG, and 0.08% GG in the control group and in patients with HCC distribution of genotyping was 0.48% TT, 0.40% TG, and 0.12% GG (Table 2). For rs12979860, allele frequency for C allele in the control group and patients with HCC was 0.64 and 0.59, and for T allele was 0.36 and 0.41. Distribution of this polymorphism was 0.31% CC, 0.65% CT, and 0.04% TT in the control group and in patients with HCC distribution of genotyping was 0.23% CC, 0.72% CT, and 0.05% TT (Table 3). For rs12980275, allele frequency for A allele in the control group and patients with HCC was 0.74 and 0.64, and for G allele was 0.26 and 0.36. Distribution of this polymorphism was 0.54% AA, 0.39% AG, and 0.07% GG in the control group and in patients with HCC distribution of genotyping was 0.38% AA, 0.52% AG, and 0.1% GG (Table 4). Our results showed that there was no significant association between the genotype frequencies of these SNPs with the risk of HCC development. There was no significant association between the genotype frequency of patients with HCC and healthy controls under genetic models (Tables 2-4).

Also, haplotype analysis showed that there was no

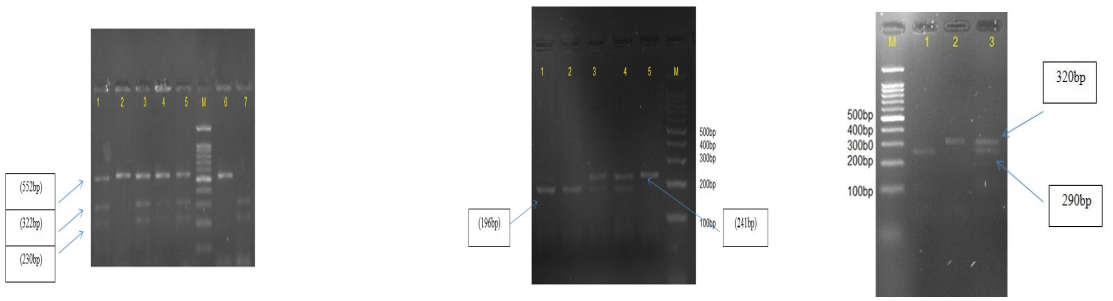

Fig. 1: Electrophoresis digested products with restriction enzymes. The RFLP products of the IL28B amplicons with BseMl for rs8099917 G/T(A), BstUl for rs12979860 C/T(B), and Bsl I for rs12980275(C) is shown on 3% agarose gel. The marker (M) that was used was 100 base pairs.

Table 2: Association of IL28B polymorphism (rs8099917) with HCC (n=140, adjusted by sex)

Model	Genotype	Control (%)	Case (%)	OR (95% CI)	*P* value
Codominant	T/T	59 (59%)	19 (47.5%)	1.00	0.47
	T/G	33 (33%)	16 (40%)	0.67 (0.3-1.48)	
	G/G	8 (8%)	5 (12.5%)	0.53 (0.16-1.84)	
Dominant	T/T	59 (59%)	19 (47.5%)	1.00	0.24
	T/G-G/G	41 (41%)	21 (52.5%)	0.64 (0.31-1.34)	
Recessive	T/T-T/G	92 (92%)	35 (87.5%)	1.00	0.45
	G/G	8 (8%)	5 (12.5%)	0.63 (0.19-2.07)	
Overdominant	T/T-G/G	67 (67%)	24 (60%)	1.00	0.45
	T/G	33 (33%)	16 (40%)	0.74 (0.35-1.59)	
Logg-additive	-	-	-	0.71 (0.41-1.23)	0.22

Table 3: Association of IL28B polymorphism (rs12979860) with HCC (n=140, adjusted by sex)

Model	Genotype	Control (%)	Case (%)	OR (95% CI)	*P* value
Codominant	C/C	31 (31%)	9 (22.5%)	1.00	0.6
	T/C	65 (65%)	29 (72.5%)	0.65 (0.27-1.54)	
	T/T	4 (4%)	2 (5%)	0.65 (0.10-4.19)	
Dominant	C/C	31 (31%)	9 (22.5%)	1.00	0.31
	T/C-T/T	69 (69%)	31 (77.5%)	0.65 (0.28-1.53)	
Recessive	C/C-T/C	96 (96%)	38 (95%)	1.00	0.89
	T/T	4 (4%)	2 (5%)	0.88 (0.15-5.11)	
Overdominant	C/C-T/T	35 (35%)	11 (27.5%)	1.00	0.37
	T/C	65 (65%)	29 (72.5%)	0.69 (0.31-1.56)	
Logg-additive	-	-	-	0.71 (0.35-1.47)	0.36

Table 4: Association of IL28B polymorphism (rs12980275) with HCC (n=140, adjusted by sex)

Model	Genotype	Control (%)	Case (%)	OR (95% CI)	*P* value
Codominant	A/A	54 (54%)	15 (37.5%)	1.00	0.21
	A/G	39 (39%)	21 (52.5%)	0.541(0.23-1.12)	
	G/G	7 (7%)	4 (10%)	0.52 (0.13-2.05)	
Dominant	A/A	54 (54%)	15 (37.5%)	1.00	0.078
	A/G-G/G	46 (46%)	25 (62.5%)	0.51 (0.24-1.09)	
Recessive	A/A-A/G	93 (93%)	36 (90%)	1.00	0.64
	G/G	7 (7%)	4 (10%)	0.73 (0.20-2.67)	
Overdominant	A/A-G/G	61 (61%)	19 (47.5%)	1.00	0.16
	A/G	39 (39%)	21 (52.5%)	0.56 (0.27-1.19)	
Logg-additive	-	-	-	0.63 (0.35-1.12)	0.11

significant association between haplotypes and the risk of HCC (Table 5). Six haplotypes were inferred from three polymorphisms, and the most common haplotype was T-C-A (Table 6). The frequency of T-C-A was 0.5935, while those of other haplotypes were below 0.2399.

DISCUSSION

HCC is a heterogeneous malignancy. Multiple genetic and also epigenetic alterations play an important role in the pathogenesis of HCC. Dysregulation of IL28B is important for tumor growth and progression. Some

Table 5: Haplotype association with HCC (n=140, adjusted by sex)

	SNP1 rs8099917	SNP2 rs12979860	SNP3 rs12980275	Frequency	OR (95% CI)	*P* value
H1	T	C	A	0.5935	1.00	---
H2	G	T	G	0.2399	0.71 (0.33 - 1.51)	0.37
H3	T	T	A	0.1062	1.20 (0.38 - 3.73)	0.76
H4	T	T	G	0.0324	0.36 (0.08 - 1.61)	0.18
H5	G	C	G	0.0205	0.67 (0.10 - 4.46)	0.68
H6	G	C	A	0.0074	0.35 (0.02 - 6.81)	0.48

Table 6: Haplotype frequencies estimation (n=140)

	SNP1 rs8099917	SNP2 rs12979860	SNP3 rs12980275	Total	group.Control	group.Case	Cumulative frequency
H1	T	C	A	0.5935	0.6124	0.5464	0.5935
H2	G	T	G	0.2399	0.2224	0.2839	0.8334
H3	T	T	A	0.1062	0.1174	0.0777	0.9396
H4	T	T	G	0.0324	0.0252	0.0509	0.9721
H5	G	C	G	0.0205	0.0174	0.0277	0.9926
H6	G	C	A	0.0074	0.0052	0.0134	1

studies showed that IL28B polymorphisms could have an association with allergies in children and liver cancer due to the hepatitis B virus.[14] Association between Il28B polymorphisms and HCC susceptibility has been reported in recent years, but the results remain inconclusive because the effects of SNPs in different populations and ethnic groups can be different.[15-18] The aim of this study was to analyze the association between IL28B polymorphisms (rs8099917 T/G, rs12979860 C/T, rs12980275 A/G) and the development of HCV-related HCC. In the present study, we observed that IL28B polymorphisms (rs 8099917 T/G, rs12979860 C/T, rs12980275 A/G) did not present a risk factor for the development of HCC in an Iranian population (Tables 2-4). This finding is opposite to some studies that reported IL28B polymorphisms (rs8099917 T/G, rs12979860 C/T, rs12980275 A/G) were significantly associated with the increased risk of HCC. For example, Zhang and colleagues, in their study in 2016, showed the significant effect of IL28B SNP (rs8099917 T/G) with HCC.16,19 Our study is in line with others, as H Akkiz and colleagues in 2014 showed no significant relation between IL28B SNP (rs12979860 C/T) and HCC.[15,17] So we can state that IL28B and the increased risk of HCC might be dependent on the population and different ethnic origins. However, it is necessary to replicate the study with a larger sample size in different populations, and of course, understanding more functions of IL28B can help us in understanding the genetics of phenotype variation, especially the genetic basis of HCC as a complex disease. The genotypic analysis also showed that there was no significant association between the frequency of the IL28B polymorphisms (rs 8099917 T/G, rs12979860 C/T, rs12980275 A/G) among the patients with HCC and healthy controls under genetic models. Also, haplotype analysis showed that there was no association between haplotypes and the risk of HCC development (Table 6). This finding is in line with some studies.[20] Therefore, further investigation with a larger sample size and haplotype analysis should be done in other ethnic populations.

CONCLUSION

Our study shows that IL28B polymorphisms (rs 8099917 T/G, rs12979860 C/T, rs12980275 A/G) were not a risk factor for the development of HCC in an Iranian population. Also, haplotype analysis showed that there was no association between IL28B polymorphisms. Further investigations with a larger sample size with

different ethnic origins should be conducted to clarify the association between IL28B polymorphisms and HCC in the Iranian population.

ACKNOWLEDGMENTS

We are grateful to research deputy of Golestan University of medical sciences and all the subjects who participated in this study.

ETHICAL APPROVAL

There is nothing to be declared.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

REFERENCES

1. Al-Qahtani A, Al-Anazi M, Abdo AA, Sanai FM, Al-Hamoudi W, Alswat KA, et al. Correlation between genetic variations and serum level of interleukin 28B with virus genotypes and disease progression in chronic hepatitis C virus infection. J Immunol Res 2015;2015:768470. doi: 10.1155/2015/768470
2. Bouchard MJ, Navas-Martin S. Hepatitis B and C virus hepatocarcinogenesis: lessons learned and future challenges. Cancer Lett2011;305:123-43. doi: 10.1016/j.canlet.2010.11.014
3. Wan L, Kung YJ, Lin YJ, Liao CC, Sheu JJ, Tsai Y, et al. Th1 and Th2 cytokines are elevated in HCV-infected SVR (−) patients treated with interferon-α. Biochem Biophys Res Commun 2009;379:855-60. doi: 10.1016/j.bbrc.2008.12.114
4. Kimkong I, Chankaew J, Kunanopparat A, Hirankarn N, Tangkijvanich P. Gene polymorphisms of interleukin 28B and the risk to chronic hepatitis B virus infection in Thai. Tissue Antigens 2015;85:177-81. doi: 10.1111/tan.12517
5. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'hUigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461:798. doi: 10.1038/nature08463
6. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-α and ribavirin therapy. Nature Genet 2009;41:1100-4. doi: 10.1038/ng.447
7. Balagopal A, Thomas DL, Thio CL. IL28B and the control of hepatitis C virus infection. Gastroenterology 2010;139:1865-76. doi: 10.1053/j.gastro.2010.10.004
8. Lampertico P, Viganò M, Cheroni C, Facchetti F, Invernizzi F, Valveri V, et al. IL28B polymorphisms predict interferon-related hepatitis B surface antigen seroclearance in genotype D hepatitis B e antigen–negative patients with chronic hepatitis B. Hepatology 2013;57:890-6. doi: 10.1002/hep.25749
9. Chang K-C, Tseng P-L, Wu Y-Y, Hung H-C, Huang C-M, Lu S-N, et al. A polymorphism in interferon L3 is an independent risk factor for development of hepatocellular carcinoma after treatment of hepatitis C virus infection. Clin Gastroenterol hepatol 2015;13:1017-24. doi: 10.1016/j.cgh.2014.10.035
10. Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138:1338-45.e7. doi: 10.1053/j.gastro.2009.12.056
11. International Consensus Group for Hepatocellular Neoplasia The International Consensus Group for Hepatocellular Neoplasia. Pathologic diagnosis of early hepatocellular carcinoma: a report of the international consensus group for hepatocellular neoplasia. Hepatology 2009;49:658-64. doi: 10.1002/hep.22709
12. Scott JA, Brogdon WG, Collins FH. Identification of single specimens of the Anopheles gambiae complex by the polymerase chain reaction. Am J Trop Med Hyg 1993;49:520-9. doi: 10.4269/ajtmh.1993.49.520
13. Solé X, Guinó E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. Bioinformatics 2006;22:1928-9. doi: 10.1093/bioinformatics/btl268
14. Bellanti F, Vendemiale G, Altomare E, Serviddio G. The impact of interferon lambda 3 gene polymorphism on natural course and treatment of hepatitis C. Clin Dev Immunol 2012;2012:849373. doi: 10.1155/2012/849373
15. Akkiz H, Kuran S, Akgöllü E, Üsküdar O, Bekar A, Bayram S. The role of Interleukin 28B gene polymorphism in Turkish patients with hepatocellular carcinoma. Ann Hepatol 2014;13:788-95.
16. Zhang Y, Zhu S-L, Chen J, Li L-Q. Meta-analysis of associations of interleukin-28B polymorphisms rs8099917 and rs12979860 with development of hepatitis virus-related hepatocellular carcinoma. Onco Targets Ther 2016;9:3249-57. doi: 10.2147/OTT.S104904
17. Trung N, Giang D, Quyen D, Binh M, Bang M. Relationship between Il28b Gene Polymorphisms and the Risk of Hepatocellular Carcinoma Development within Vietnamese Hepatitis B Virus Carriers. J Clin Microbiol Biochem Technol 2017;3:035-9. doi:10.17352/jcmbt.000024
18. Qin S, Wang J, Zhou C, Xu Y, Zhang Y, Wang X, et al. The influence of interleukin 28B polymorphisms on the risk of hepatocellular carcinoma among patients with HBV or HCV infection: An updated meta-analysis. Medicine (Baltimore) 2019;98:e17275. doi: 10.1097/MD.0000000000017275
19. Teama SH, Agwa SH, El Sayed OA, Sayed MM, El Samee AA, El Nakeep S. Assessment of interleukin-28B (interferon λ3) rs12979860 C/T gene polymorphism and the risk for hepatocellular carcinoma in chronic hepatitis C cirrhotic patients. Egyptian Liver J 2016;6:48-53. doi: 10.1097/01.ELX.0000515930.52529.6c
20. Ren S, Lu J, Du X, Huang Y, Ma L, Huo H, et al. Genetic variation in IL28B is associated with the development of hepatitis B-related hepatocellular carcinoma. Cancer Immunol Immunother 2012;61:1433-9. doi: 10.1007/s00262-012-1203-y