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The Correlation Between Interferon Lambda 3 Gene Polymorphisms and Susceptibility to Hepatitis B Virus Infection

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Abstract

Background: Cytokines are proteins that mediate innate and adaptive immunity responses. It is hypothesized that interferon lambda 3 (IFNL3) levels can influence the outcome of chronic hepatitis B virus (HBV) infection. Polymorphisms in IFN genes have been associated with response to infection.

Objectives: This study was carried-out to investigate the association of IFNL3 gene polymorphisms (rs12979860 and rs8099917) with HBV susceptibility, in chronic HBV-infected patients.

Patients and Methods: In this case-control study, we determined IFNL3 single nucleotide polymorphisms (SNPs) (rs12979860 and rs8099917) in 221 individuals, with chronic HBV infection, and 200 healthy individuals, who were voluntary blood donors, with negative test for HBV. Alleles and genotypes analyses were performed by amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods.

Results: The frequencies of the rs12979860 and rs8099917 genotypes were not significantly different between the HBV-infected and the control groups (CC:CT:TT of 30.3%:48.0%:21.7% vs. 33.0%:49.0%:18.0%, P > 0.05, and GG:GT:TT of 5.8%:39.4%:54.8% vs. 5.0%:41.0%:54.0%, P > 0.05, respectively). Also, the frequencies of the alleles were not significantly different between both groups (C:T of 54.3%:45.7% vs. 57.5%:42.5%, P > 0.05, and G:T of 25.6%:74.4% vs. 25.5%:74.5%, P > 0.05, respectively) and the chronic HBV infection. There were no significant differences between patients, with at least one rs12979860C and or rs8099917T alleles compared to the healthy controls (rs12979860: CT + CC:TT, OR = 1.26, 95%CI = 0.78 - 2.04, P = 0.341 and rs8099917: GT + TT:GG, OR = 1.03, 95%CI = 0.70 - 1.51, P = 0.877, respectively).

Conclusions: Our study showed no correlation between rs12979860 and rs8099917 SNPs and chronic HBV infection. Further studies, with larger sample sizes and different ethnicities, are necessary to validate our findings.

Keywords: Genetic Polymorphism, Chronic Hepatitis B, Interferon Lambda 3 (IFNL3)

1. Background

Chronic hepatitis B virus (HBV) infection has become a major health issue. It is one of the most common liver diseases. The HBV can cause both acute and chronic diseases and is the main factor for HBV-related cirrhosis and HCC (1, 2). The occurrence rates of HBV infection have a various regional distribution and prevalence in developing Asian-Pacific countries (3) and its prevalence is rising worldwide, with the greatest expansion in the Asia-pacific region (4, 5). The prevalence of HBV in Iranian population has been found to be of about 2% (6). The clinical outcomes of HBV infection vary from spontaneous clearance to chronic carriers or death from HCC (7). There is evidence that host genetic factors have a role in determining the progression of HBV infection. Also, twin studies show that genetic is the key point influencing the HBV infection (8, 9). Studies suggest that cytokines control the host response. These compounds play an important role in determining HBV infection outcome (10, 11). Cytokines are proteins that mediate innate and adaptive immunity responses. Allelic variants in the genome can affect the disease progression and therapeutic effect of treatment, after HBV infection. The evidence demonstrated that genetic variants of cytokine genes were associated with chronic HBV infection (11-15).

At the first step, an association between single nucleotide polymorphism (SNP) near the interferon lambda 3 (IFNL3) gene and the sustained virological response rate with pegylated IFN (PEG-IFN) plus ribavirin treatment was discovered, for chronic hepatitis C (16). Further studies indicated that there was an association between IFNL3 and spontaneous HCV clearance (17). It is founded that IFN exhibits antitumoral functions, in several animal models and cell lines (18-21). Also, certain genotypes might lead to

lower risk of HCC development in HCV patients (22).

Studies that have been conducted on murine and human hepatocyte cell lines show that IFNL3 inhibits HBV viral replication and plays a vital role in antiviral immunity against hepatotropic viruses (HBV and HCV) (18, 23-27). The results show that rs8099917 and rs12979860 have been associated with the response of individuals to viral infections (28, 29). The results of racial and geographical studies on the prevalence of polymorphisms of IFNL3 show that the rs12979860 C allele frequency in patients with hepatitis C is high in Asia (65% - 100 %), average in Europe (53% - 85%) and low in Africa (23% - 54%) (29).

It has been shown that IFN-λ is active against HBV. On the other hand, HBV is an IFN-λ-responsive chronic viral illness (30). Considering that HBV and HCV share a similar natural history, modes of transmission and levels of pathogenesis, and, moreover, IFN-λ has been reported to induce antiviral activity against viruses, it is possible that genetic variants of IFNL3 play a functional role in chronic HBV infection.

2. Objectives

The objective of our research was to study the association between the two upstream SNPs of IFNL3 (rs8099917 and rs12979860) and susceptibility to chronic HBV infection, in the Iranian population.

3. Patients and Methods

3.1. Patient Population

This work has been approved by the institutional ethics committee of the Zahedan University of Medical Sciences, Zahedan, Iran, and carried out in the infectious diseases and tropical medicine research center, Zahedan, Iran. All the patients agreed to participate in the study and provided written informed consent.

A total of 221 chronic active HBV infected patients were enrolled. All subjects were inpatients in the blood transfusion organization clinics in Zahedan, Iran, between July and December 2014. The diagnosis of chronic hepatitis B was based on persistent increase of serum alanine aminotransferase (ALT) concentration, during a minimum of 6 months, in the presence of HBsAg, by enzyme-linked immunosorbent assay (ELISA) and HBV-DNA with real-time polymerase chain reaction (RT-PCR).

Patients were excluded from the study if they were known to be infected with HCV, hepatitis E virus, hepatitis A virus and HIV. Also, patients who suffered from other significant medical conditions (diabetes, pregnancy, immune diseases) were excluded.

A group of 200 healthy individuals who were voluntary blood donors, with negative test for HBV, HCV and HIV serology and with normal values for ALT, served as controls. Subjects with active alcohol abuse over the last 3 months, presence of sepsis or hepatorenal syndrome, ALT levels twice the upper limit of normal and individuals who suffered from other significant concurrent medical conditions or who were taking hepatotoxic drugs, were excluded from the control group.

A sample of whole blood was obtained from each subject. These samples were stored at -80°C, until they were used for the study.

3.2. Interferon Lambda 3 Genotyping

Whole-blood samples were tested for IFNL3 genotype. Human DNA was extracted from 500 μL of whole blood, using the salting-out method.

The rs12979860 polymorphisms of IFNL3 were identified using specific primers, by PCR-RFLP assay. Primers for PCR amplification were as follows: rs12979860: 5'-GCTTATCGCATACGGCTAGG-3' (forward); 5'-AGGCTCAGGGTCAATCACAG-3' (reverse). A 242 base pair (bp) product was obtained. The PCR amplification was carried out in a total volume of 20 μL containing 1 μL of each primer, 100 ng of template DNA and 10 μL of 2X prime taq premix (Genet Bio, Daejeon, South Korea) and 7 μL double-distilled water (ddH_2O). Samples were subjected to 35 cycles of initial denaturation, 5 minutes at 95°C denaturation, 30 seconds at 95°C annealing, 30 seconds at 60°C extension, 30 seconds at 72°C final extension, 5 minutes at 72°C. An amount of 10 μL of the amplicons were digested with one unit of the BstU-I restriction enzyme (Fermentas, Vilnius, Lithuania), at 60°C, overnight. The IFNL3-C allele gives three fragments of 135 + 82 + 25 bp, and IFNL3-T allele, with two fragments of 160 + 82 bp. The fragments were resolved by electrophoresis in 4% agarose gel, after staining with ethidium bromide.

When genotyping the IFNL3, rs8099917 polymorphism was determined by employing tetra-primer amplification refractory mutation system-PCR (T-ARMS-PCR) method, as described previously by specific primers (31). Primers for PCR amplification were as follows: rs8099917 5'-CATCACCTATAACTTCACCATCCTCCTC-3' (forward outer); 5'-GGTATCAACCCCACCTCAAATTATCCTA-3' (reverse outer); 5'-CTTTTGTTTTCCTTTCTGTGAGCAGTG-3' (forward inner); 5'-TATACAGCATGGTTCCAATTTGGGTAAA-3' (reverse inner). Product sizes were 197 bp, for G allele and 295 bp, for T allele, and 437 bp, for the two outer primers (control band). Amplification was performed in a volume of 25 μL, containing 1 μL (10 μM) of each primer, 100 ng of template DNA and 10 μL of 2X prime taq premix and 10 μL ddH_2O. The PCR were run for 30 cycles: initial denaturation for

5 minutes at 95°C, denaturation for 30 seconds at 95°C, annealing for 30 seconds at 58°C, extension for 30 seconds at 72°C and final extension for 10 minutes at 72°C. Each reaction was verified on a 2% agarose gel containing ethidium bromide.

3.3. Statistical Analysis

Statistical analyses were performed by SPSS 20.0 (SPSS Inc., Chicago, IL, USA). Computing the odds ratio (OR) and 95% confidence intervals (95%CI) from logistic regression analysis were employed to assess the relationship between genotypes and HBV. The distributions of genotypes were analyzed with the chi-square test. Data are expressed as mean ± SD. A $P < 0.05$ indicated statistical significance.

4. Results

A total of 221 chronic HBV infected patients (122 male, 99 female, mean 29.81 ± 8.05 years) and 200 healthy individuals (109 male, 91 female, mean 31.11 ± 7.54 years) were enrolled. Demographical characteristics of the study subjects have been described in Table 1. There were no statistical differences in gender, age and ethnicity between these two groups ($P > 0.05$).

Table 1. Demographic Parameters of Chronic Hepatitis B Virus Patients and Control Group[a]

Parameters	HBV	Control	P Value
Age, y	29.81 (8.05)	31.11 (7.54)	0.091
Sex			0.885
Male	122 (55.2)	109 (54.5)	
Female	99 (44.8)	91 (45.5)	
Ethnicities			0.817
Sistani	92 (41.6)	86 (43.0)	
Baluch	59 (26.7)	48(24.0)	
Others	70 (31.7)	66 (33.0)	

Abbreviations: HBV, hepatitis B virus; IFNL3, interferon lambda 3; OR, odds ratio; 95%CI, 95% confidence interval.
[a]Values are exressed as No. (%) or mean (SD).

Genotype and allele frequencies of IFNL3 rs12979860 C/T and rs8099917 G/T polymorphism in HBV and healthy subjects are shown in Table 2.

In analyzing the polymorphisms rs12979860 and rs8099917, both groups were within the Hardy-Weinberg equilibrium ($P > 0.05$).

The frequencies of the rs12979860 and rs8099917 genotypes were not significantly different between the HBV infected and the control groups (CC:CT:TT, 30.3%:48.0%:21.7% vs. 33.0%:49.0%:18.0%, $P > 0.05$ and GG:GT:TT, 5.8%:39.4%:54.8% vs. 5.0%:41.0%:54.0%, $P > 0.05$ respectively). Also, the frequencies of the alleles were not significantly different between both groups (C:T, 54.3%:45.7% vs. 57.5%:42.5%, $P > 0.05$ and G:T, 25.6%:74.4% vs. 25.5%:74.5%, $P > 0.05$, respectively, and the chronic HBV infection. There were no significant differences between patients with at least one rs12979860C and/or rs8099917T alleles, compared to the healthy controls (rs12979860: CT + CC:TT, OR = 1.26, 95%CI = 0.78 - 2.04, P = 0.341 and rs8099917: GT + TT:GG, OR = 1.03, 95%CI = 0.70 - 1.51, P = 0.877). Analysis of the genotype combination of the polymorphisms did not show any significant differences between HBV infected patients and the control group (P = 0.295) (Table 3).

5. Discussion

The present study showed that the rs12979860 and rs8099917 SNPs were not associated with susceptibility to HBV infection, in a sample of Iranian population. Studies have shown that IFNL inhibits HBV viral replication, in human hepatocyte cell lines (18, 23, 29, 32). Certain studies have revealed an association between the outcome of treatment for HCV and genetic variation in IFNL3 locus and reported the precise OR for associations between several IFNL3 polymorphisms and spontaneous HCV clearance (16). In other words, IFNL3 variants have a vital role in response to therapy in these patients. For the first time, in 2010, several studies reported an association between IFNL3 and HBV infection (33). In the studies conducted in 2011, an association between genotype, allele and haplotype frequencies of IFNL3 and ALT, AST and HBV DNA was reported (28, 34). Data shows that the 12979860 C allele and CC genotype occurred frequently in the viral clearance group (29) and suggest that the C allele may be useful for the inhibition of HCV replication. On the other hand, an analysis in Han Chinese revealed that 12979860 T allele appeared to be more prevalent in patients with HCC than in those with liver cirrhosis (35). Lampertico et al. reported a positive association between rs12979860 and HBV infection. The findings indicated that IFNL3 polymorphism is a good predictor in HBeAg-negative patients, chronically infected by genotype D HBV (36).

In several studies, the frequencies of the T and C alleles and the genotypes of the rs12979860 SNP have been compared between subjects who were chronically infected by HBV and healthy controls. The allelic and genotypic frequencies of the rs12979860 polymorphism were not significantly different between HBV infected and healthy subjects. In a number of previous studies, the predominant genotype was CC (33, 37), whereas in our study, the CT genotype was predominant in HBV-infected group. Also, the

Table 2. The Frequency of Genotypes and Alleles of Interferon Lambda 3 (rs12979860 and rs8099917) Polymorphisms Gene[a]

IFNL3 Polymorphisms	HBV	Control	OR (95%CI)	P Value
rs12979860C/T				
CC	67 (30.3)	66 (33.0)	1.31 (0.76 - 2.27)	0.331
CT	106 (48.0)	98 (49.0)	1.23 (0.74 - 2.05)	0.432
TT	48 (21.7)	36 (18.0)	1	NA
CT + CC	173 (78.3)	164 (82.0)	1.26 (0.78 - 2.04)	0.341
Allele				
C	240 (54.3)	230 (57.5)	0.88 (0.67 - 1.15)	0.350
T	202 (45.7)	170 (42.5)	1	NA
rs8099917G/T				
GG	13 (5.8)	10 (5.0)	0.86 (0.36 - 2.04)	0.736
GT	87 (39.4)	82 (41.0)	1.05 (0.71 - 1.57)	0.788
TT	121 (54.8)	108 (54.0)	1	NA
GT + TT	100 (45.5)	92 (46.0)	1.03 (0.70 - 1.51)	0.877
Allele				
G	113 (25.6)	102 (25.5)	1.00 (0.73 - 1.37)	0.983
T	329 (74.4)	298 (74.5)	1	NA

Abbreviations: HBV, hepatitis B virus; IFNL3, interferon lambda 3; NA, not available; OR, odds ratio; 95%CI, 95% confidence interval.
[a]Values are exressed as No. (%).

Table 3. Genotype Combination Frequencies in Chronic Hepatitis B (Case) and Normal Subjects (Control)[a,b]

Genotype Combination	HBV	Control
CC/GT	9 (4.1)	9 (4.5)
CC/TT	58 (26.2)	57 (28.5)
CT/GT	46 (20.8)	47 (23.5)
CT/TT	60 (27.1)	46 (23.0)
TT/GG	13 (5.9)	10 (5.0)
TT/GT	32 (14.5)	21 (10.5)
TT/TT	3 (1.4)	10 (5.0)
TOTAL	221 (100.0)	200 (100.0)

Abbreviation: HBV, hepatitis B virus.
[a]Values are expressed as No. (%).
[b]$\chi^2 = 7.283$, P = 0.295.

data reported by Martin-Carbonero et al. (38) and Martin et al. (33) indicated no differences between the allelic and genotypic frequencies of the rs12979860 polymorphism of two groups. Consistent with our study, in the studies that have been conducted in Brazil (39) and USA (33), a slight predominance of the C allele was found. Also, no correlation was found between CC genotype and clinical outcome in Asian chronic hepatitis B patients, both HBeAg-positive and negative (40). Sonneveld et al. (41) determined that IFNL3 genotype was significantly associated with HBeAg seroconversion, in patients treated with PEG-IFN ($P < 0.01$). The IFNL3 genotype was also associated with HBsAg clearance. Therefore, it seems that polymorphisms near IFNL3 were associated with serologic response in patients with chronic hepatitis B. In one study that was conducted in a Chinese Han population (42), a possible association between IFNL3 and HBeAg-positive chronic hepatitis B was demonstrated. In another research that was conducted in Korea (43), three polymorphisms near the IFNL3 gene, rs8099917T, rs12979860C and rs12980275, were identified. No significant association was identified between these polymorphisms and the natural courses of chronic HBV infection, including the HBV clearance and HCC occurrence. However, results derived from certain studies are conflicting. In a study that was carried out by Peng et al. they found that rs12979860 polymorphism has no association with spontaneous HBV clearance (37). On the other hand, the genotype and allele frequencies of the rs12979860 and rs8099917 were reported to be associated with the HBV viral loads (28).

It seems that viral genotypes have different geographical distributions and susceptibilities to infection. In addition, differences among ethnic groups have suggested a

genetic contribution in susceptibilities to HBV infection. As mentioned above, there is still controversy regarding the magnitude association between rs12979860 polymorphism and chronic HBV infection. Therefore, discrepancies in these studies are reasonable.

In the field of rs8099917 polymorphism, there are several studies that have shown no significant data for allelic and genotypic frequencies, although they identified a predominance of the T allele between chronic HBV, HCC, self-limiting infections and healthy control groups (28, 44). We have not found significant differences in the rs8099917 TT genotype distribution between the two groups. In this study, we showed that there were no significant differences between rs8099917 genotype frequencies. The relationship between HBV outcomes and these polymorphisms have been analyzed in several studies, but they did not identify any relationships (37). The effect of rs8099917 in IFNL3 gene, on HBV recurrence, in liver transplant patients, indicated that in HBV-related liver transplant recipients, rs8099917 was associated with aminotransferase concentrations. The higher aminotransferase levels were associated with allele G in recipients. These subjects have higher HBV related liver damage. No association was found between IFNL3 polymorphisms with HBV recurrence in the liver transplant recipients. It seems that the allele G of rs8099917 was associated with hepatocyte injury caused by HBV. In addition, the frequency of the minor allele (G allele) was not superior in HBV subjects compared to healthy subjects and was not statistically significant (45). It seems that IFNL is a cure for hepatitis, as the rs8099917 G allele has been associated with lower expression levels of the IFNL3 gene (17, 46). The G allele increases the risk of chronic hepatitis in subjects. Presence of G allele causes little chance of treatment in HCV patients (17). Also, Li et al. founded that the G allele carrier HBV infected patients had higher ALT level and HBV viral load. On the other hand, they found that the genotype and allele frequencies of IFNL3 polymorphisms associate with HBV viral load and serum ALT level (28).

In our study, rs12979860 and rs8099917 were not associated with susceptibility to HBV infection and more evidence is required to obtain a conclusion, so that other SNPs should be included in the future studies. The strong point of the current study was the large sample size of subjects. Moreover, the study was conducted in south east of Iran, where the prevalence of hepatitis B infection is higher than in other parts of Iran. Therefore, our study population may be a good representative of the whole population. The findings of this study might be limited by a number of factors. Inconsistent results may be due to the different inclusion criteria, different sample sizes and genotyping methods, in different studies. Because of these limitations, which may affect the results, we recommend further studies in HBV patients, with a larger sample size and different ethnicities, to validate our results. Also, due to interference of other cytokines in HBV infection, the effect of other IFNs should also be studied. There is a new IFN gene, IFNL4, that has a special polymorphism (rs368234815). This polymorphism is associated with hepatitis clearance. It was in linkage disequilibrium with IFNL3 (47). We recommend further studies regarding IFNL4 polymorphism and its possible involvement in the HBV infection.

In summary, our study shows that IFNL3 gene polymorphisms do not have an influence on the natural history of chronic hepatitis B virus infection, in a sample of Iranian population.

In conclusion, our study showed no correlation between rs12979860 and rs8099917 SNPs and chronic hepatitis B virus infection. Our findings indicate that SNPs in the IFNL3 gene have no role in the susceptibility to chronic hepatitis B infection. Further studies, with larger sample sizes and different ethnicities, are needed to validate our findings.

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Footnotes

Authors' Contribution: The authors contributed equally to this research.

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References

1. Dienstag JL. Hepatitis B virus infection. *N Engl J Med.* 2008;**359**(14):1486–500. doi: 10.1056/NEJMra0801644. [PubMed: 18832247].
2. Te HS, Jensen DM. Epidemiology of hepatitis B and C viruses: a global overview. *Clin Liver Dis.* 2010;**14**(1):1–21. doi: 10.1016/j.cld.2009.11.009. [PubMed: 20123436].
3. Chen CJ, Wang LY, Yu MW. Epidemiology of hepatitis B virus infection in the Asia-Pacific region. *J Gastroenterol Hepatol.* 2000;**15 Suppl**:E3–6. [PubMed: 10921373].
4. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat.* 2004;**11**(2):97–107. [PubMed: 14996343].
5. Margolis HS, Alter MJ, Hadler SC. Hepatitis B: evolving epidemiology and implications for control. *Semin Liver Dis.* 1991;**11**(2):84–92. doi: 10.1055/s-2008-1040427. [PubMed: 1832236].

6. Poorolajal J, Majdzadeh R. Prevalence of chronic hepatitis B infection in Iran: a review article. *J Res Med Sci.* 2009;**14**(4):249–58. [PubMed: 21772891].
7. Shepard CW, Simard EP, Finelli L, Fiore AE, Bell BP. Hepatitis B virus infection: epidemiology and vaccination. *Epidemiol Rev.* 2006;**28**:112–25. doi: 10.1093/epirev/mxj009. [PubMed: 16754644].
8. Lin CL, Kao JH. Hepatitis B viral factors and clinical outcomes of chronic hepatitis B. *J Biomed Sci.* 2008;**15**(2):137–45. doi: 10.1007/s11373-007-9225-8. [PubMed: 18058038].
9. Yu MW, Chang HC, Liaw YF, Lin SM, Lee SD, Liu CJ, et al. Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst.* 2000;**92**(14):1159–64. [PubMed: 10904089].
10. Liu YZ, Hou FQ, Ding P, Ren YY, Li SH, Wang GQ. Pegylated interferon alpha enhances recovery of memory T cells in e antigen positive chronic hepatitis B patients. *Virol J.* 2012;**9**:274. doi: 10.1186/1743-422X-9-274. [PubMed: 23158844].
11. Ben-Ari Z, Mor E, Papo O, Kfir B, Sulkes J, Tambur AR, et al. Cytokine gene polymorphisms in patients infected with hepatitis B virus. *Am J Gastroenterol.* 2003;**98**(1):144–50. doi: 10.1111/j.1572-0241.2003.07179.x. [PubMed: 12526950].
12. Singh R, Kaul R, Kaul A, Khan K. A comparative review of HLA associations with hepatitis B and C viral infections across global populations. *World J Gastroenterol.* 2007;**13**(12):1770–87. [PubMed: 17465466].
13. Deng G, Zhou G, Zhai Y, Li S, Li X, Li Y, et al. Association of estrogen receptor alpha polymorphisms with susceptibility to chronic hepatitis B virus infection. *Hepatology.* 2004;**40**(2):318–26. doi: 10.1002/hep.20318. [PubMed: 15368436].
14. Block TM, Mehta AS, Fimmel CJ, Jordan R. Molecular viral oncology of hepatocellular carcinoma. *Oncogene.* 2003;**22**(33):5093–107. doi: 10.1038/sj.onc.1206557. [PubMed: 12910247].
15. Juran BD, Lazaridis KN. Genomics and complex liver disease: Challenges and opportunities. *Hepatology.* 2006;**44**(6):1380–90. doi: 10.1002/hep.21453. [PubMed: 17133459].
16. 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].
17. 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**(4):1338–45. doi: 10.1053/j.gastro.2009.12.056. [PubMed: 20060832].
18. Li M, Liu X, Zhou Y, Su SB. Interferon-lambdas: the modulators of antivirus, antitumor, and immune responses. *J Leukoc Biol.* 2009;**86**(1):23–32. doi: 10.1189/jlb.1208761. [PubMed: 19304895].
19. Li W, Lewis-Antes A, Huang J, Balan M, Kotenko SV. Regulation of apoptosis by type III interferons. *Cell Prolif.* 2008;**41**(6):960–79. doi: 10.1111/j.1365-2184.2008.00558.x. [PubMed: 19040572].
20. Numasaki M, Tagawa M, Iwata F, Suzuki T, Nakamura A, Okada M, et al. IL-28 elicits antitumor responses against murine fibrosarcoma. *J Immunol.* 2007;**178**(8):5086–98. [PubMed: 17404291].
21. Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP, et al. IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol Ther.* 2008;**7**(7):1109–15. [PubMed: 18698163].
22. 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].
23. Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. *J Virol.* 2005;**79**(6):3851–4. doi: 10.1128/JVI.79.6.3851-3854.2005. [PubMed: 15731279].
24. Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. *J Allergy Clin Immunol.* 2008;**121**(5):1108–11. doi: 10.1016/j.jaci.2008.02.026. [PubMed: 18405958].
25. Uze G, Monneron D. IL-28 and IL-29: newcomers to the interferon family. *Biochimie.* 2007;**89**(6-7):729–34. doi: 10.1016/j.biochi.2007.01.008. [PubMed: 17367910].
26. Witte K, Witte E, Sabat R, Wolk K. IL-28A, IL-28B, and IL-29: promising cytokines with type I interferon-like properties. *Cytokine Growth Factor Rev.* 2010;**21**(4):237–51. doi: 10.1016/j.cytogfr.2010.04.002. [PubMed: 20655797].
27. Li MC, Wang HY, Wang HY, Li T, He SH. Liposome-mediated IL-28 and IL-29 expression in A549 cells and anti-viral effect of IL-28 and IL-29 on WISH cells. *Acta Pharmacol Sin.* 2006;**27**(4):453–9. doi: 10.1111/j.1745-7254.2006.00292.x. [PubMed: 16539846].
28. Li W, Jiang Y, Jin Q, Shi X, Jin J, Gao Y, et al. Expression and gene polymorphisms of interleukin 28B and hepatitis B virus infection in a Chinese Han population. *Liver Int.* 2011;**31**(8):1118–26. doi: 10.1111/j.1478-3231.2011.02507.x. [PubMed: 21745278].
29. 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**(7265):798–801. doi: 10.1038/nature08463. [PubMed: 19759533].
30. Ank N, West H, Bartholdy C, Eriksson K, Thomsen AR, Paludan SR. Lambda interferon (IFN-lambda), a type III IFN, is induced by viruses and IFNs and displays potent antiviral activity against select virus infections in vivo. *J Virol.* 2006;**80**(9):4501–9. doi: 10.1128/JVI.80.9.4501-4509.2006. [PubMed: 16611910].
31. Hashemi M, Moazeni-Roodi A, Bahari A, Taheri M. A tetra-primer amplification refractory mutation system-polymerase chain reaction for the detection of rs8099917 IL28B genotype. *Nucleosides Nucleotides Nucleic Acids.* 2012;**31**(1):55–60. doi: 10.1080/15257770.2011.643846. [PubMed: 22257210].
32. Zhu H, Butera M, Nelson DR, Liu C. Novel type I interferon IL-28A suppresses hepatitis C viral RNA replication. *Virol J.* 2005;**2**:80. doi: 10.1186/1743-422X-2-80. [PubMed: 16146571].
33. Martin MP, Qi Y, Goedert JJ, Hussain SK, Kirk GD, Hoots WK, et al. IL28B polymorphism does not determine outcomes of hepatitis B virus or HIV infection. *J Infect Dis.* 2010;**202**(11):1749–53. doi: 10.1086/657146. [PubMed: 20977343].
34. Tseng TC, Yu ML, Liu CJ, Lin CL, Huang YW, Hsu CS, et al. Effect of host and viral factors on hepatitis B e antigen-positive chronic hepatitis B patients receiving pegylated interferon-alpha-2a therapy. *Antivir Ther.* 2011;**16**(5):629–37. doi: 10.3851/IMP1841. [PubMed: 21817184].
35. Chen J, Wang L, Li Y, Cai B, Fu Y, Liao Y, et al. Association analysis between SNPs in IL-28B gene and the progress of hepatitis B infection in Han Chinese. *PLoS One.* 2012;**7**(12):e50787. doi: 10.1371/journal.pone.0050787. [PubMed: 23227209].
36. Lampertico P, Vigano 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**(3):890–6. doi: 10.1002/hep.25749. [PubMed: 22473858].
37. Peng LJ, Guo JS, Zhang Z, Shi H, Wang J, Wang JY. IL28B rs12979860 polymorphism does not influence outcomes of hepatitis B virus infection. *Tissue Antigens.* 2012;**79**(4):302–5. doi: 10.1111/j.1399-0039.2011.01835.x. [PubMed: 22239156].
38. Martin-Carbonero L, Rallon NI, Benito JM, Poveda E, Gonzalez-Lahoz J, Soriano V. Short communication: Does interleukin-28B single nucleotide polymorphisms influence the natural history of hepatitis B?. *AIDS Res Hum Retroviruses.* 2012;**28**(10):1262–4. doi: 10.1089/AID.2011.0365. [PubMed: 22324878].
39. Conde SR, Rocha LL, Ferreira VM, Monteiro JC, Filgueiras NK, Lins PA, et al. Absence of correlation between IL-28B gene polymorphisms and the clinical presentation of chronic hepatitis B in an Amazon Brazilian population. *Dis Markers.* 2014;**2014**:534534. doi:

10.1155/2014/534534. [PubMed: 24817780].
40. Holmes JA, Nguyen T, Ratnam D, Heerasing NM, Tehan JV, Bonanzinga S, et al. IL28B genotype is not useful for predicting treatment outcome in Asian chronic hepatitis B patients treated with pegylated interferon-alpha. *J Gastroenterol Hepatol.* 2013;**28**(5):861–6. doi: 10.1111/jgh.12110. [PubMed: 23301835].
41. Sonneveld MJ, Wong VW, Woltman AM, Wong GL, Cakaloglu Y, Zeuzem S, et al. Polymorphisms near IL28B and serologic response to peginterferon in HBeAg-positive patients with chronic hepatitis B. *Gastroenterology.* 2012;**142**(3):513–520 e1. doi: 10.1053/j.gastro.2011.11.025. [PubMed: 22108195].
42. Wu X, Xin Z, Zhu X, Pan L, Li Z, Li H, et al. Evaluation of susceptibility locus for response to interferon-alpha based therapy in chronic hepatitis B patients in Chinese. *Antiviral Res.* 2012;**93**(2):297–300. doi: 10.1016/j.antiviral.2011.12.009. [PubMed: 22209781].
43. Lee DH, Cho Y, Seo JY, Kwon JH, Cho EJ, Jang ES, et al. Polymorphisms near interleukin 28B gene are not associated with hepatitis B virus clearance, hepatitis B e antigen clearance and hepatocellular carcinoma occurrence. *Intervirology.* 2013;**56**(2):84–90. doi: 10.1159/000342526. [PubMed: 23343781].
44. 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**(9):1433–9. doi: 10.1007/s00262-012-1203-y. [PubMed: 22310928].
45. Li Y, Shi Y, Chen J, Cai B, Ying B, Wang L. Association of polymorphisms in interleukin-18 and interleukin-28B with hepatitis B recurrence after liver transplantation in Chinese Han population. *Int J Immunogenet.* 2012;**39**(4):346–52. doi: 10.1111/j.1744-313X.2012.01097.x. [PubMed: 22325058].
46. 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].
47. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. *Nat Genet.* 2013;**45**(2):164–71. doi: 10.1038/ng.2521. [PubMed: 23291588].