ANNALS of Hepatology

ORIGINAL ARTICLE

November-December, Vol. 13 No. 6, 2014: 788-795

The role of *Interleukin 28B* gene polymorphism in Turkish patients with hepatocellular carcinoma

Hikmet Akkïz,* Sedef Kuran,* Ersin Akgöllü,* Oğuz Üsküdar,* Aynur Bekar,* Süleyman Bayram**

* Çukurova University, Faculty of Medicine, Department of Gastroenterology, Adana, Turkey.
**Adıyaman University, Adıyaman School of Health, Department of Nursing, Adiyaman, Turkey.

ABSTRACT

Background and aim. Multiple risk factors lead to hepatocellular carcinoma (HCC) including viral infections, mutation and single nucleotide polymorphisms (SNPs). *Interleukin 28B (IL28B)* gene rs12979860 polymorphism has been shown to be associated with HCC in the different populations, but its association with HCC has not been investigated in the Turkish population. We investigated whether the rs12979860 polymorphism of *IL28B* gene affects the risk of HCC. **Material and method.** We performed genotyping analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in a hospital-based case-control study of 187 confirmed HCC patients and 208 healthy subjects (cancer and viral infection negative) in the Turkish population. **Results.** The allele and genotype analysis showed no significant differences between the risk of HCC and *IL28B* gene rs12979860 polymorphism (OR = 1.10; 95% 0.59-2.08 P = 0.76 for genotype). However, in the HBV-related HCC subgroup, the TT genotype increased a 1.46-fold the risk of developing HCC, but not statistically significant (OR = 1.46; 95% 0.71-2.97 P = 0.30). Furthermore, no significant differences were found between clinical findings, and sex in comparison with the IL28B genotypes in HCC group (P > 0.05). **Conclusion.** Our results suggest, for the first time, that no significant association were found between *IL28B* rs12979860 genotypes with the risk of developing HCC in Turkish patients. Further independent investigations are required to clarify the possible role of *IL28B* gene rs12979860 polymorphism on the risk of developing HCC in a larger series and also in patients of different ethnic origins.

Key words. Hepatitis B. Hepatitis C. Genetic susceptibility.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer throughout the world and the third cause of death among cancers.[1,2] The incidence and mortality rates of HCC are approximately same because it has high fatality rates.[2] It is known that multiple risk factors lead to hepatocarcinogenesis, including chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infections, carcinogen exposure (such as aflatoxin B1), oxidative stress, excessive alcohol consumption, and multiple genetic alterations.[3,4] Despite the fact that HBV and HCV infections are the main cause of HCC, only a portion of infected patients develop HCC throughout their lifetime. The identification of other risk factors (such as mutations, single nucleotide polymorphisms, etc) is important so that high-risk populations can be followed-up more closely.[5]

Interferons (IFNs; IFN-α/β/λ) are the natural human antiviral proteins. IFNs play a crucial role in antiviral immune response pathways.[6] IFNs trigger this antiviral response that is activated through the activation of the janus kinase-signal transducer and activator of transcription (JAK-STAT) and regulation of interferon-stimulated genes *(ISGs)*.[6,7] Several single nucleotide polymorphisms (SNPs) (rs12979860, rs12980275, rs8099917) were shown near the *interleukin 28B* gene *(IL28B*, also known as interferon lambda 3 [IFNλ3] which is a member of the type III IFN family) on chromosome 19.[8-10] One of these SNPs was identified by Ge, *et al.* as a genetic polymorphism cytosine → thymine (C/T)

Correspondence and reprint request: Ersin Akgöllü, Ph.D.
Çukurova University, Faculty of Medicine, Department of Gastroenterology, 01330 Adana, Turkey.
Ph.: +90 322 3386060 (Ext. 3167). Fax:+90 322 3386900
E-mail: ersin0571@gmail.com

Manuscript received: March 04, 2014.
Manuscrip accepted: May 12, 2014.

(rs12979860) 3181 bp upstream of the *IL28B* gene on chromosome 19q13.13.11 Carriers of an rs12979860 C allele have higher IFNλ3 serum level than subjects with a T allele.[8] Currently it remains unclear why the rs12979860 C/T polymorphism affects IFN lambda levels.[8] Several experimental studies in cell lines and in animal models showed that the activation of type III IFN triggers apoptosis[12] and antitumor activities.[6,13,14] These features of IFNλ3 may reduce the risk of developing HCC. A few studies have already reported that the *IL28B* polymorphism is related to HCC occurrence.[15-17]

According to our recent knowledge, no research has been conducted to evaluate *IL28B* rs12979860 (C/T) polymorphism and the risk of HCC in the Turkish population specifically. The aim of this study was to analyze the correlation between *IL28B* rs12979860 (C/T) polymorphism and the hepatocellular carcinoma in the Turkish.

MATERIAL AND METHODS

Patients

The study was approved by the Committee for Ethics of Medical Experiment on Human Subjects, Faculty of Medicine, Çukurova University, Adana. Submission of the individuals to the study was conditioned by an obtained written informed consent form regarding the use of their blood samples for research studies. The study proceeded in agreement with the Helsinki Declaration approved on the World Medical Association Meeting in Edinburgh. 187 consecutively diagnosed patients with HCC were enrolled in the case group. The patients with HCC were stratified as follows: HBV-related HCC, n: 110; HCV-related HCC, n: 49; other causes-related HCC, n: 28. During the same time, 208 healthy subjects were enrolled as the control group from the same hospital. Healthy subjects (who entered the hospital for health check-ups) were negative in terms of cancer and hepatitis viruses. We performed genotyping analysis using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay in a hospital-based case-control study of 187 confirmed HCC patients and 208 cancer-free controls of Turkish citizens.

The HCC diagnostic criteria were based on the guidelines proposed by European Association for the Study of the Liver (EASL).[18] Cirrhosis was diagnosed with liver biopsy, abdominal sonography and biochemical evidence of parenchymal damage plus endoscopic oesophageal or gastric varices.[19] Patients with cirrhosis were classified into three Child-Pugh grades based on their clinical status.[20] Serum HBsAg and Anti-HCV were assessed using an immunoassay (Abbott Laboratories, Abbott Park, IL, USA). Serum α-fetoprotein (AFP) concentration was measured by microparticle enzyme immunoassay (Abbott Laboratories, Chicago, IL, USA). Heavy alcohol intake was defined as a daily minimum consumption of 160 g alcohol for at least 8 years. Technicians who performed the blood tests were blinded to the identity and disease status of participants. Peripheral blood samples were taken from patients and controls in the Department of Gastroenterology between September 2005 and December 2012. Blood specimens were stored at +4 ºC and serum specimens were frozen at -20 ºC until analysis.

IL28B genotype detection

Genotyping for the *IL-28B* rs12979860 C/T polymorphism was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. Genomic DNA was extracted from peripheral whole blood using High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's protocol. A 429 base pair (bp) fragment encompassing the C to T polymorphic site in *IL-28B* region was amplified with the forward primer 5'-GCTTATCGCATACGGCTAGG-3' and the reverse primer 5'-AGGGACCGCTACGTAAGTCA-3', which were designed using the NCBI Primer-Blast Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Amplification was performed in GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Singapore). The 20 μL PCR mixtures contained approximately 250 ng DNA with 0.25 μM of both primers, 0.1 mM of each dNTP, 1 x PCR buffer, 1.5 mM $MgCI_2$ and 1 U Taq polymerase (Promega, Madison, WI, USA). The following cycling conditions were used: 95 ºC for 5 min, followed by 35 cycles of 94 ºC for 60 s, 63 ºC for 60 s and 72 ºC for 60 s, with a final extension at 72 ºC for 10 min. After confirmation of successful PCR amplification by 1.5% agarose gel electrophoresis, each PCR product was digested overnight with 5 units Hpy166II (recognizing the sequence 5'-GTN^{-}NAC-3') restriction endonuclease enzyme at 37 ºC (New England Biolabs Inc., Beverly, MA) and electrophoresed on 3% agarose gel containing 0.5 μg/mL ethidium bromide and visualized under UV illumination. The digest fragments were 322 and 107 bp for the C allele and 292, 107 and 30 bp for the T allele. Samples yielding

322 and 107 bp fragments were scored as CC, and those with 322, 292, 107, 30 bp fragments as CT, and 292, 107 and 30 bp as TT.

Statistical analysis

Data analysis was performed using the computer software Statistical Package for Social Sciences < (version 10.0). Continues variables are presented as the mean (standard deviation, SD) or median (min-max) for abnormal distributions and categorical variables are presented as frequencies (%). Comparisons in the distributions of demographical characteristics between the patients with hepatocellular carcinoma and control subjects were evaluated using the Student's t-test or Mann-Whitney U test for continuous variables (each when adequate) depending on their Gaussian distribution and chi-square test for categorical variables. The observed genotype frequencies were compared with expected values calculated from Hardy-Weinberg equilibrium theory. Significant variables after univariate regression analysis were entered into a stepwise logistic regression analysis to identify factor of HCC risk. Statistical analyses of genotypes were analyzed using the website for SNP Statistics: http://bioinfo.iconcologia.net/snpstats/start.htm. Logistic regression analysis was used to analyze the association of genotypes in inheritance models (codominant, dominant, recessive, over dominant and log-additive) in the case and control groups. Results are expressed as odds ratios with 95% confidence

Table 1. Distribution of selected characteristics in patients with hepatocellular carcinoma and controls.

Characteristic	Patients (n =187) (%)	Controls (n =208) (%)	P-value*
Age (years) mean (± SD) (range)	61.34 ± 10.83 (20-87)	59.80 ± 10.26 (20-87)	NS
Male sex	147 (78.6)	173 (83.2)	NS
Female sex	40 (21.4)	35 (16.8)	NS
Smoking status			NS
Ever	97 (51.9)	108 (51.9)	
Never	90 (48.1)	100 (48.1)	
Alcohol status			NS
Drinker	53 (28.3)	60 (28.8)	
Nondrinker	134 (71.7)	148 (71.2)	
Viral Infection			
HBsAg positive	110 (58.8)	-	
Anti-HCVAb positive	49 (26.2)	-	
Both negative	28 (15)	208 (100)	
Liver cirrhosis			
Present	155 (82.9)	-	
Absent	32 (17.1)	-	
Tumor size (cm)			
≤ 5	109 (58.3)	-	
> 5	78 (41.7)	-	
Child-Pugh classification			
A	38 (24.5)	-	
B	60 (38.7)	-	
C	57 (36.8)	-	
α-fetoprotein (ng/mL)			
≤ 400	127 (67.9)	-	
> 400	60 (32.1)	-	

NS: not significant. n: total number of case patients or control subjects. * P-values were derived from Pearson χ^2 test except age. Student's t-test was used for age. All P-values are two-sided.

interval (CI). All tests were two-sided and P value < 0.05 was considered significant.

Power calculations

%80 Power in the present study depends on the sample size, the level of type I error, and the genotype frequencies in patients and controls. The frequencies of genotype depend on the population frequency of the allele of interest and its effect on the risk of HCC. Effective sample sizes for case-control study, and for obtaining 80% power was calculated by Quanto (version 1.1.) software (http://hydra.usc.edu/gxe) using minor allele frequency data from HapMap (http://hapmap.ncbi.nlm.nih.gov/).

RESULTS

The clinical and demographic characteristics of the 395 Turkish subjects are shown table 1. The characteristics of patients with HCC and control subjects did not differ with regard to age, sex, smoking status and alcohol consumption.

The allele and genotype frequencies of *IL28B* gene rs12979860 polymorphism

The overall allelic frequencies of *IL28B* gene rs12979860 polymorphism were 65.6% and 34.4% for C and T allele, respectively. There were no association between the control group and the HCC group for allele and genotype frequencies of rs12979860 polymorphism (P = 0.74, P = 0.95, respectively). The allele frequencies of patients and controls were in Hardy-Weinberg equilibrium and no selection bias. The allele and genotype frequencies are shown in table 2.

The association between *IL28B* gene rs12979860 genotypes and HCC risk

To identify whether there was a statistically significant increased risk of HCC in terms of the *IL28B* rs12979860 genotypes, we performed logistic regression analysis (Table 2). For the rs12979860 polymorphism, logistic regression analysis showed that subjects homozygous for the TT genotype had a

Table 2. Alleles frequency and models inheritance for IL28B gene rs12979860 polymorphism among cases and controls as well as the association with HCC.

	Cases (%), n = 187	Control (%), n = 208	OR (95% CI)	P-value[a,d]	AIC[b]	BIC[c]
rs12979860						
Allele frequency						
C	243 (65)	275 (66.1)	1.00 (Reference)		-	-
T	131 (35)	141 (33.9)	1.05 (0.78-1.41)	0.74	-	-
Codominant					552.4	564.3
CC	80 (42.8)	92 (44.2)	1.00 (Reference)			
CT	83 (44.4)	91 (43.8)	1.05 (0.69-1.60)	0.95		
TT	24 (12.8)	25 (12)	1.10 (0.58-2.08)	0.76		
Dominant					550.4	558.3
CC	80 (42.8)	92 (44.2)	1.00 (Reference)			
CT+TT	107 (57.2)	116 (55.8)	1.06 (0.71-1.58)	0.77		
Recessive					550.4	558.4
CC+CT	163 (87.2)	183 (88)	1.00 (Reference)			
TT	24 (12.8)	25 (12)	1.08 (0.59-1.96)	0.81		
Overdominant					550.5	558.4
CC+ TT	104 (55.6)	117 (56.2)	1.00 (Reference)			
CT	83 (44.4)	91 (43.8)	1.03 (0.69-1.53)	0.90		
Log-additive	- -		1.05 (0.79-1.40)	0.74	550.4	558.3

[a] Data were calculated by logistic regression analysis. [b] AIC: Akaike's information criterion. [c] BIC: Bayesian information criterion. HCC: hepatocellular carcinoma. [d] Adjusted for age, sex, smoking and drinking status.

Table 3. Comparison of frequency distribution of alleles and genotypes of IL28B gene rs12979860 polymorphism based on HCC aetiology as well as the association with hepatocellular cancer risk.

Subgroups	Allele/genotype	Cases, n (%)	Control, n (%)	OR (95% CI)	P-value[a,b]
HBV-related HCC		110	208		
	C	136 (61.8)	275 (66.1)	1.00 (reference)	
	T	84 (38.2)	141 (33.9)	1.21 (0.86-1.69)	0.28
	CC	43 (39)	92 (44.2)	1.00 (reference)	
	CT	50 (45.5)	91 (43.8)	1.17 (0.71-1.94)	0.53
	TT	17 (15.5)	25 (12)	1.46 (0.71-2.97)	0.30
HCV-related HCC		49	208		
	C	66 (67.3)	275 (66.1)	1.00 (reference)	
	T	32 (32.7)	141 (33.9)	1.04 (0.75-1.42)	0.82
	CC	21 (42.9)	92 (44.2)	1.00 (reference)	
	CT	24 (49)	91 (43.8)	1.16 (0.60-2.22)	0.67
	TT	4 (8.1)	25 (12)	0.70 (0.22-2.23)	0.55

[a] Data were calculated by logistic regression analysis. HBV: hepatitis B, HCV: hepatitis C. HCC: hepatocellular carcinoma. [b] Adjusted for age, sex, smoking and drinking status.

1.10-fold increased the risk of developing HCC compared those with CC genotype but not statistically significant associated with the risk of HCC (OR = 1.10; 95% 0.59-2.08 P = 0.76). Similarly, the heterozygous TC genotype had also increased risk but not statistically significant associated with the risk of HCC (OR =1.05; 95% 0.69-1.60 P = 0.95). Furthermore, we next investigated whether HCC aetiology (viral infections) influenced the effects of rs12979860 genotypes in patients with HCC (Table 3). In the HBV-related HCC subgroup, logistic regression analysis showed that subjects homozygous for the TT genotype had a 1.46-fold increased risk of developing HCC compared with those with CC genotype but not significantly associated with the risk of HCC (Table 3). Moreover, in HCV-related HCC subgroup no relationship was found between HCC cases and healthy controls (Table 3). Additionally, no significant differences were found between clinical findings and sex in comparison with the *IL28B* gene rs12979860 genotypes in HCC group (Table 4).

DISCUSSION

Single nucleotide polymorphisms in many functionally critical genes have been suggested as a risk factor for a variety of cancers, including HCC.[21,22] The prevention of HCC may be ensured in HBV carriers and in HCV infected patients via detection of genetic biomarkers.[23] The present study investigated the effect of *IL28B* rs12979860 polymorphism on the risk of developing hepatocellular carcinoma in a Turkish population. Several genome-wide association studies (GWAS) independently revealed that having homozygous genotype rs12979860 CC which is in powerful linkage disequilibrium, strongly increases the probability to obtain sustained virological response after antiviral therapy for hepatitis C infection.[9,24,25] *IL28B* rs12979860 region contains a CpG dinucleotide on an immune transcription factor site, which is necessary for DNA methylation. Methylated DNA likely response to reduced expression of *IL28B* and may lead to down-regulation of ISGs. Thus, in the homozygous *IL28B* rs12979860 CC variant, the reduced ISG expression may provide an explanation for the increased IFN response.[26,27] In addition to this, IFNλ1 and IFNλ2 induce JAK-STAT signaling and antitumor effects in cancer cell lines including oesophageal, neuroendocrine BON1 and colon[26] cancer cells.[28-30] Therefore, *IL28B* rs12979860 polymorphism was selected as the candidate polymorphism.

Although the association between *IL28* gene rs12979860 C/T polymorphism and the response to PEG-IFN and RBV standard therapy for hepatitis C has been clearly demonstrated, its association with HCC is controversial. In the present study, the allele frequencies of rs12979860 polymorphism were 65.6 and 34.4% for C and T allele, respectively. According to the previous results, the frequencies of C and T allele of rs12979860 polymorphism among the populations are distributed as follows; 55 and 45% in German, 67 and 33% in Italian, 68 and 32% in Moroccan, 63 and 37% in Spanish, 85 and 15% in Chinese, respectively.[16,17,31-33] In this study, the distribution of *IL28B* gene rs12979860 polymorphism genotypes and alleles were not different between HCC cases and healthy controls. Statistical analysis

Table 4. Association between clinical findings, alcohol consumption, and sex according to the IL28B gene rs12979860 genotypes in HCC group.

Valuables	IL28B rs12979860 Genotypes, n=187 (%) TT	CT	CC	P value
Sex				0.52[b]
Male	21 (14.3)	64 (43.5)	62 (42.2)	
Female	3 (7.5)	19 (47.5)	18 (45)	
Alcohol status				0.28[b]
Drinker	10 (18.9)	23 (43.4)	20 (37.7)	
Non-drinker	14 (10.4)	60 (44.8)	60 (44.8)	
Smoking status				0.67[b]
Ever	13 (13.4)	40 (41.2)	44 (45.4)	
Never	11 (12.2)	43 (47.8)	36 (40)	
Tumor size(cm)				0.98[b]
≤ 5	14 (12.8)	49 (45)	46 (42.2)	
>5	10 (12.8)	34 (43.6)	34 (43.6)	
Child-Pugh classification (n=155)				0.74b
A	5 (13.2)	15 (39.4)	18 (47.4)	
B	7 (11.7)	28 (46.7)	25 (41.6)	
C	10 (17.5)	20 (35.1)	27 (47.4)	
α-fetoprotein (ng/mL)				0.70[b]
≤ 400	16 (12.6)	59 (46.5)	52 (40.9)	
> 400	8 (13.3)	24 (40)	28 (46.7)	
ALT(U/L)[†]	51.5 (19-421)	47.5 (11-324)	52 (7-216)	0.44[a]
AST(U/L)[†]	85.5 (23-285)	71.5 (16-380)	77 (20-530)	0.29[a]
Albumin(g/L)[†]	2.6 (2.0-4.3)	2.9 (1.8-4.7)	3.1 (1.6-4.7)	0.26[a]
Ca(mg/dL)*	8.59 ± 0.50	8.84 ± 0.71	8.76 ± 0.72	0.35[c]
PT(s)[†]	15.6 (13-29)	15.6 (11.5-28)	15.3 (11.7-39)	0.98[a]
INR[†]	1.30 (1.10-2.35)	1.32 (0.90-2.30)	1.32 (0.95-1.92)	0.99[a]

[a] P value were calculated by Kruskal-Wallis test. [b] P value were calculated by χ^2 square test. [c] P value were calculated by One-Way ANOVA test. [†] Median (min-max). * Mean ± standard deviation. Ca: calcium. PT: prothrombin time. INR: international normalized ratio. ALT: alanine transaminase. AST: aspartate aminotransferase. HCC: hepatocellular carcinoma.

revealed that there were not a significant relationship between the risk of HCC and *IL28B* gene rs12979860 polymorphism. This result in the present study is in agreement with the results from other studies.[15,32,34] On the contrary, a few studies reported an association between the risk of HCC and rs12979860 polymorphism.[16,17,31,33] Ezzikouri, *et al.* and Ren, *et al.* reported that subjects with the IL28B gene rs12979860 TT genotype have a 4.27 and 6.05 fold to the risk of developing HCC, respectively.[31,33] Additionally, Fabris, *et al.* reported that carriage of rs12979860 T allele seems to augment the risk of developing HCC (85 patients with cirrhosis complicated by HCC *vs.* 171 patients with cirrhosis not complicated by HCC, $P < 0.005$).[17] Also, Chen, *et al.* indicated that carriage of rs12979860 T allele appears to be more prevalent in patients with HCC than in LC ($P = 0.046$), but no significant differences between HCC cases and healthy controls.[15] The significant differences in the populations may be due to discrepancies of ethnicity. Furthermore, when patients with HCC were stratified by their HCC etiology, there was no association among the rs12979860 genotypes. The sample sizes of viral-related HCC subgroups were too small for a reliable result (HBV-related HCC, n: 110; HCV-related HCC, n: 49; other causes-related HCC, n: 28). In the current study, the clinical findings were analyzed according to IL28B rs12979860 genotypes in HCC group, but there were not significant association among the rs12979860 genotypes. As reported previously,[18,19] when the AFP levels and tumor size were catego-

rized in the current study, the AFP levels and tumor size did not display significant differences according to the rs12979860 genotypes. The results presented in our study are similar to results reported by Eurich, *et al.,* except for AFP levels.[16] Eurich, *et al*. revealed that the number and size of the tumor were unrelated to rs12979860 genotypes ($P > 0.05$), but AFP levels were significantly associated with TT genotype ($P = 0.042$).[16] Briefly, according to our findings, IL28B gene rs12979860 polymorphism has no major effect on the risk of HCC in the Turkish population.

There are a number of limitations to this study. Firstly, patients were selected at a single institution (Çukurova University Balcali Hospital in Adana from South Turkey) and thus may have been unrepresentative of HCC patients in the general population. In addition, it should be noted that the control subjects were recruited at the same hospital. Furthermore, the frequencies of patients and controls were also in Hardy-Weinberg equilibrium and there was no selection bias according to Hardy-Weinberg model. Secondly, we limited our study to a small Turkish population owing to variation in allele frequencies between different ethnic groups which have been observed for the *IL28B* gene rs12979860 polymorphism. Thirdly, this study had only focused on a single locus on a single gene without taking into consideration gene-environment, gene-gene interactions, *IL28B* gene expression and interactions between different locuses on the *IL28B* gene, which may affect risk of HCC. Fourthly, although the desired power of our study is set at 80%, this study is limited by the relatively small number of the subgroups analysis. Fifthly, the data of patients was not suitable according to smoking index and degree of alcohol intake status. Therefore, the analysis was performed according to smoking and alcohol habit of patients. Seventhly, a regression analysis to evaluate the independent effect of *IL28B* polymorphism on HCC could not be adjusted for obesity and diabetes due to lack of patients' data.

CONCLUSION

Our results demonstrate for the first time that *IL28B* gene rs12979860 polymorphism has no major influence on the risk of developing HCC in the Turkish population. Further investigations are required to uncover the possible role of *IL28B* gene rs12979860 polymorphism on the risk of developing HCC in a larger series and also in patients of different ethnic origins. Moreover, further investigations should include expression of *IL28B* gene and its genetic polymorphisms.

ABBREVIATIONS

- **AFP:** α-fetoprotein.
- **bp:** base pair.
- **C/T:** cytosine/thymine.
- **CI:** confidence interval.
- **EASL:** European Association for the Study of the Liver.
- **GWAS:** genome-wide association studies.
- **HBV:** hepatitis B virus.
- **HCC:** hepatocellular carcinoma.
- **HCV:** hepatitis C virus.
- **IFNs:** interferons.
- **IFNλ3:** interferon lambda 3.
- **IL28B:** interleukin 28B.
- **ISGs:** interferon-stimulated genes.
- **JAK-STAT:** the activation of the janus kinase-signal transducer and activator of transcription.
- **PCR-RFLP:** polymerase chain reaction–restriction fragment length polymorphism.
- **SD:** standard deviation.
- **SNPs:** single nucleotide polymorphisms.

FUNDING

This study was funded by Çukurova University Research Fund TF2011BAP14.

ACKNOWLEDGEMENT

The authors thank all the subjects who participated in this study.

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
2. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; 55: 74-108.
3. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007; 132: 2557-76.
4. Farazi PA, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer* 2006; 6: 674-87.
5. Akkız H, Bayram S, Bekar A, Akgöllü E, Ulger Y. A functional polymorphism in pre-microRNA-196a-2 contributes to the susceptibility of hepatocellular carcinoma in a Turkish population: a case-control study. *J Viral Hepat* 2011; 18: e399-e407.
6. Li M, Liu X, Zhou Y, Su SB. Interferon-lambdas: the modulators of antivirus, antitumor, and immune responses. *J Leukoc Biol* 2009; 86: 23-32.

7. Marcello T, Grakoui A, Barba-Spaeth G, Machlin ES, Kotenko SV, MacDonald MR, Rice CM. Interferons alpha and lambda inhibit hepatitis C virus replication with distinc signal transduction and gene regulation genetics. *Gastroenterology* 2006; 131: 1887-98.
8. Langhans B, Kupfer B, Braunschweiger I, Arndt S, Schulte W, Nischalke HD, Nattermann J, et al. Interferon-lambda serum levels in hepatitis C. *J Hepatol* 2011; 54: 859-65.
9. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; 461: 798-801.
10. Sugiyama M, Tanaka Y, Nakanishi M, Mizokami M. Novel findings for the development of drug therapy for various liver diseases: genetic variation in IL-28B is associated with response to the therapy for chronic hepatitis C. *J Pharmacol Sci* 2011; 115: 263-9.
11. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment induced viral clearance. *Nature* 2009; 461: 399-401.
12. Li W, Lewis-Antes A, Huang J, Balan M, Kotenko SV. Regulation of apoptosis by type III interferons. *Cell Prolif* 2008; 41: 960-79.
13. Numasaki M, Tagawa M, Iwata F, Suzuki T, Nakamura A, Okada M, Iwakura Y, et al. IL-28 elicits antitumor responses against murine fibrosarcoma. *J Immunol* 2007; 178: 5086-98.
14. Maher SG, Sheikh F, Scarzello AJ, Romero-Weaver AL, Baker DP, Donnelly RP, Gamero AM, et al. IFNalpha and IFNlambda differ in their antiproliferative effects and duration of JAK/STAT signaling activity. *Cancer Biol Ther* 2008; 7: 1109-15.
15. Chen J, Wang L, Li Y, Cai B, Fu Y, Liao Y, Zhang J. Association analysis between SNPs in IL-28B gene and the progress of hepatitis B infection in Han Chinese. *PLoS One* 2012; 7: e50787.
16. Eurich D, Boas-Knoop S, Bahra M, Neuhaus R, Somasundaram R, Neuhaus P, Neumann U, et al. Role of IL28B polymorphism in the development of hepatitis C virus-induced hepatocellular carcinoma, graft fibrosis, and post-transplant antiviral therapy. *Transplantation* 2012; 93: 644-9.
17. Fabris C, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, Cmet 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: 716-22.
18. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; 35: 421-30.
19. Tsai JF, Chang WY, Jeng JE, Ho MS, Lin ZY, Tsai JH. Hepatitis B and C virus infection as risk factors for liver cirrhosis and cirrhotic hepatocellular carcinoma: a case-control study. *Liver* 1994; 14: 98-102.
20. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the esophagus for bleeding esophageal varices. *Br J Surg* 1973; 60: 646-9.
21. Kim YJ, Lee HS. Single nucleotide polymorphisms associated with hepatocellular carcinoma in patients with chronic hepatitis B virus infection. *Intervirology* 2005; 48: 10-5.
22. Kato N, Ji G, Wang Y, Baba M, Hoshida Y, Otsuka M, Taniguchi H, et al. Large-scale search of single nucleotide polymorphisms for hepatocellular carcinoma susceptibility genes in patients with hepatitis C. *Hepatology* 2005; 42: 846-53.
23. Ludwig JA, Weinstein JN. Biomarkers in cancer staging, prognosis and treatment selection. *Nat Rev Cancer* 2005; 5: 845-56.
24. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, et al. IL28B is associated with response to chronic hepatitis C interferon-a and ribavirin therapy. *Nat Genet* 2009; 41: 1100-4.
25. Honda M, Sakai A, Yamashita T, Nakamoto Y, Mizukoshi E, Sakai Y, Yamashita T, et al. Hepatic ISG expression is associated with genetic variation in interleukin 28B and the outcome of IFN therapy for chronic hepatitis C. *Gastroenterology* 2010; 139: 499-509.
26. Sakamoto N, Nakagawa M, Tanaka Y, Sekine-Osajima Y, Ueyama M, Kurosaki M, Nishida N, et al. Association of IL28B variants with response to pegylated-interferon alpha plus ribavirin combination therapy reveals intersubgenotypic differences between genotypes 2a and 2b. *J Med Virol* 2011; 83: 871-8.
27. Fischer J, Böhm S, Scholz M, Müller T, Witt H, George J, Sarrazin C, et al. Combined effects of different interleukin-28B gene variants on the outcome of dual combination therapy in chronic hepatitis C virus type 1 infection. *Hepatology* 2012; 55: 1700-10.
28. Li Q, Kawamura K, Ma G, Iwata F, Numasaki M, Suzuki N, Shimada H, et al. Interferon-lambda induces G1 phase arrest or apoptosis in oesophageal carcinoma cells and produces anti-tumour effects in combination with anti-cancer agents. *Eur J Cancer* 2010; 46: 180-90.
29. Zitzmann K, Brand S, Baehs S, Göke B, Meinecke J, Spöttl G, Meyer H, et al. Novel interferon-lambdas induce antiproliferative effects in neuroendocrine tumor cells. *Biochem Biophys Res Commun* 2006; 344: 1334-41.
30. Sato A, Ohtsuki M, Hata M, Kobayashi E, Murakami T. Antitumor activity of IFN-lambda in murine tumor models. *J Immunol* 2006; 176(12): 7686-94.
31. Ezzikouri S, Alaoui R, Rebbani K, Brahim I, Fakhir FZ, Nadir S, Diepolder H, et al. Genetic variation in the interleukin-28B gene is associated with spontaneous clearance and progression of hepatitis C virus in Moroccan patients. *PLoS One* 2013; 8: e54793.
32. Agúndez JA, García-Martin E, Maestro ML, Cuenca F, Martínez C, Ortega L, Carballo M, et al. Relation of IL28B gene polymorphism with biochemical and histological features in hepatitis C virus-induced liver disease. *PLoS One* 2012; 7: e37998.
33. Ren S, Lu J, Du X, Huang Y, Ma L, Huo H, Chen X, et al. Genetic variation in IL28B is associated with the development of hepatitis B-related hepatocellular carcinoma. *Cancer Immunol Immunother* 2012; 61: 1433-9.
34. Lee DH, Cho Y, Seo JY, Kwon JH, Cho EJ, Jang ES, Kwak MS, 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: 84-90.