IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Medical Sciences

2016; 13(7): 556-561. doi: 10.7150/ijms.15853

Research Paper

Association between Interleukin-18 Polymorphisms and Hepatocellular Carcinoma Occurrence and Clinical Progression

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Received: 2016.04.15; Accepted: 2016.07.01; Published: 2016.07.05

Abstract

We investigated the association between interleukin-18 (IL-18) polymorphisms and the susceptibility and clinicopathological state of hepatocellular carcinoma (HCC). In total, 901 participants, including 559 healthy controls and 342 patients with HCC, were recruited. The allelic discrimination of −607A/C (rs1946518) and −137G/C (rs187238) polymorphisms of *IL-18* was assessed through real-time polymerase chain reaction by performing the TaqMan assay. The *IL-18* −137G/C polymorphism but not the −607A/C polymorphism showed a significant association with the risk of HCC. Participants carrying the *IL-18* −137 polymorphism with heterozygous G/C and homozygous CC genotypes showed a 1.987-fold increase (95% CI = 1.301–3.032; $p = 0.001$) in the risk of HCC compared with those homozygous for wild-type G/G. The 342 patients with HCC carrying the *IL-18* −137G/C polymorphism were positive for hepatitis B virus (HBV) infection with an adjusted odds ratio of 1.668. Moreover, the 142 HBV positive patients with HCC and the *IL-18* −137 polymorphism were positive for at least one C genotype and showed significant vascular invasion ($p = 0.018$). Furthermore, the level of α-fetoprotein was high in the patients carrying the *IL-18* −137 polymorphism with GC+CC alleles ($p = 0.011$). In conclusion, the *IL-18* −137G/C polymorphism with a GC+CC genotype could be a factor that increases the risk of HCC. Furthermore, the correlation between the *IL-18* −137G/C polymorphism and HCC-related HBV infection is a risk factor for vascular invasion and has a synergistic effect that can further enhance HCC prognosis.

Key words: hepatocellular carcinoma; interleukin-18; hepatitis B virus

Introduction

Hepatocellular carcinoma (HCC) is a primary malignancy of the liver; it is a major threat to human health and has poor prognosis. HCC is the fifth and seventh most frequent cancer in men and women worldwide, respectively, and is the third and second leading cause of cancer related deaths worldwide and

in Taiwan, respectively [1, 2]. Major risk factors for HCC include infections of hepatitis B virus (HBV) and hepatitis C virus (HCV), exposure to substances (e.g., alphatoxins) that are toxic to the liver, and alcohol and tobacco consumption. Moreover, immune system–mediated chronic inflammation of the liver can lead to HCC [3-5]. In addition, various inflammatory cytokines, such as Interleukins (IL)-1β, -18, -6 [3-5], and -17 [3, 6, 7], participate in chronic hepatic inflammation, leading to tumorigenesis. Tangkijvanich et al. [8] reported that the serum level of IL-18 is a useful biological marker of tumor invasiveness and an independent prognostic factor for survival among patients with HCC. Shiraki et al. [9] reported that the serum level of IL-18 increased in patients with HCV-related stage IV HCC compared with patients with earlier-stage HCC. Although genetic predisposition is one of the factors critical for HCC progression, few studies have focused on *IL-18* single nucleotide polymorphisms (SNPs) in patients with HCC. Moreover, research on the combined effect of *IL-18* SNPs and HBV infection on the risk and clinicopathologic development of HCC remain scant.

IL-18, an 18-kDa cytokine, originally known as interferon-γ (IFN-γ)-inducing factor, shares structural and functional properties with IL-1. This cytokine is mainly produced by activated macrophages and Kupffer cells and can promote IFN-γ production [10, 11]. *IL-18* is located on chromosome 11q23. 1 and contains six exons spanning over 20.8 kb and five introns [12, 13]. Several SNPs in the promoter region and two polymorphisms in the 5′-nontranslated region of *IL-18* have been identified [12, 14-16]. Among these, two functional polymorphisms in the promoter region at loci −607A/C and −137G/C are the most studied. The structure of *IL-18* promoter suggests that transcription factor PU.1 might be a critical regulator of its activity [17]. Serum levels of IL-18 may influence the risk of coronary artery disease and asthma and is a crucial event in oral carcinoma cells for oncogenesis [10, 18]. Thompson et al. [17] revealed the influence of serum levels of IL-18 on the risk of diseases and reported that variations in *IL-18* may influence IL-18 synthesis. Moreover, Lebel-Binay et al. [19] reported that improper synthesis of IL-18 contributes to cancer pathogenesis and may influence the clinical outcome in patients. In addition, SNP is the most common type of DNA sequence variation, influencing the occurrence and progression of gene-related hepatocarcinogenesis. Our previous studies reported that SNPs in CD44, fibroblast growth factor receptor 4, intercellular adhesion molecule-1, metallothionein-1, reversion-inducing-cysteine-rich protein with Kazal motifs, and CCR2 genes may predict the risk of HCC [20-25]. However, few studies have focused on *IL-18* SNPs in patients with HCC who are HBV positive. Therefore, the aim of this study was to identify *IL-18* polymorphisms specifically in patients showing HBV-related susceptibility and clinicopathological status of HCC.

Materials and Methods

Patients

In this study, we recruited 342 patients with HCC at the Chung Shan Medical University Hospital, Taiwan. A diagnosis of HCC was made according to the criteria specified in the national guidelines for HCC detection. All 559 control subjects were recruited at the same hospital and these control groups had neither self-reported history of cancer of any sites. Personal information and characteristics collected from the study subjects using interviewer-administered questionnaires contained questions involving demographic characteristics. All the subjects in the study were Han Chinese with the same ethnicity. The blood samples which obtained from the controls and HCC patients were stored in EDTA tubes, centrifuged immediately and stored at −80°C. The Institutional Review Board of Chung Shan Medical University Hospital approved this study (CSMUH No: CS15099), and informed written consent was obtained from each participant.

Selection of *IL-18* Polymorphisms

A total of two SNPs in IL-18 were selected from the International HapMap Project data for this study. We included -607A/C (rs1946518) and IL-18 -137G/C (rs187238) in the promoter region which were selected in this study since these 2 SNPs were found to modify the binding affinities [17].

DNA extraction and quantitative real-time PCR

Genomic DNA was extracted from EDTA anti-coagulated venous blood using a QIAamp DNA blood mini kit based on the manufacturer's protocol as described in detail previously [26]. Allelic discrimination of *-607A/C* (rs1946518) and *-137G/C* (rs187238) polymorphisms of the *IL-18* gene was assessed with the ABI StepOne™ Real-Time PCR System (Applied Biosystems, Foster City , CA , USA) and analyzed using SDS vers. 3.0 software (Applied Biosystems), with the TaqMan assay [18].

Statistical analysis

The distributions of demographic characteristics and genotype frequencies for different genotypes between the study participants and controls were analyzed using the chi-square test for certain categories of variables. Student's *t*-test was used to

evaluate the differences in the laboratory findings between the 2 groups. The odds ratios (ORs) and their 95% confidence intervals (CIs) of the association between the genotype frequencies and HCC were estimated using multiple logistic regression models by controlling for covariates. A p value of less than 0.05 was considered statistically significant. The data were analyzed using SPSS 12.0 statistical software.

Results

Demographic characteristics and clinical parameters of the 559 healthy controls and 342 patients with HCC are summarized in Table 1. The mean age (SD) in the control and patient groups was 51.86 ± 14.71 and 62.96 ± 11.67 years, respectively. The patients with HCC were predominantly older males (71.3%). The genotypic distributions and associations between patients with HCC and healthy controls carrying the *IL-18* polymorphisms, −607A/C (rs1946518) and −137G/C (rs187238), are listed in Table 2. In our recruited control group, the frequencies of *IL-18* −607A/C (χ2 value: 0.080, p=0.777) and −137G/C (χ2 value: 0.803, p=0.370) were in Hardy-Weinberg equilibrium, respectively.

After adjusting for the covariates of age, sex, and alcohol consumption, the *IL-18* −137G/C (rs187238) polymorphism genotypes, GC+CC and GG, in the healthy controls and patients with HCC differed significantly (adjusted odds ratio [AOR] = 1.987; 95 % confidence interval [CI] = 1.301–3.032; $p < 0.001$).

Table 3 shows the distribution frequency of the *IL-18* −137 polymorphism among the 342 patients with HCC along with the clinical status. The patients with HCC were evaluated to understand the influence of *IL-18* −137 polymorphism genotypes, GC+CC and GG, on the clinical TNM stage, primary tumor size, lymph node involvement, distant metastasis, vascular invasion, Child–Pugh grade, presence of an HBV or HCV infection, and liver cirrhosis. No significant differences were observed in the influence of the aforementioned *IL-18* −137 polymorphism genotypes on the clinical TNM stage and clinical variables, except for HBV infection (GC+CC vs GG; AOR = 1.668; 95 % CI = 1.001–2.786; $p < 0.05$).

In addition, we explored the potential association between the *IL-18* −137 polymorphism genotypes, GC+CC and GG, in the patients with HCC who were HBV positive (Table 4). Among the 142 patients with HCC who were HBV positive, significant vascular invasion was observed in those carrying *IL-18* −137 polymorphism genotype GC+CC compared with those carrying GG genotype (AOR = 2.825; 95% CI = 1.168–6.833; p = 0.018). As shown in Table 5, the level of α-fetoprotein differed significantly ($p < 0.05$) in the patients with HCC under the influence of *IL-18* −137G/C polymorphism genotypes GG and GC+CC. The other clinicopathological statuses for HCC, including ALT, AST, and AST/ALT ratio, showed no significant differences.

Table 1. The distributions of demographical characteristics and clinical parameters in 559 controls and 342 patients with HCC.

Variable	Controls (N=559)	Patients (N=342)	p value
Age (yrs)	**Mean ± S.D.**	**Mean ± S.D.**	
	51.86 ± 14.71	62.96 ± 11.67	p<0.001
Gender	**n (%)**	**n (%)**	
Male	456 (81.6%)	244 (71.3%)	
Female	103 (18.4%)	98 (28.7%)	p <0.001
Alcohol consumption			
No	345 (61.7%)	217 (63.5%)	
Yes	214 (38.3%)	125 (36.5%)	p=0.602
Tobacco consumption			
No	339 (60.6%)	205 (59.9%)	
Yes	220 (39.4%)	137 (40.1%)	p =0.834
Stage			
I+II		224 (65.5%)	
III+IV		118 (34.5%)	
Tumor T status			
≤T2		228 (66.7%)	
>T2		114 (33.3%)	
Lymph node status			
N0		330 (96.5%)	
N1+N2		12 (3.5%)	
Metastasis			
M0		324 (94.7%)	
M1		18 (5.3%)	
vascular invasion			
No		282 (82.5%)	
Yes		60 (17.5%)	

Table 2. Distribution frequency of *IL-18* genotypes in 559 controls and 342 patients with HCC.

Variable	Controls (N=559) n (%)	Patients (N=342) n (%)	OR (95% CI)	AOR (95% CI)
IL-18 -607A/C (rs1946518)				
AA	148 (26.5%)	88 (25.7%)	1.00	1.00
AC	276 (49.4%)	167 (48.8%)	1.018 (0.734-1.410)	0.978 (0.670-1.427)
CC	135 (24.1%)	87 (25.5%)	1.084 (0.743-1.580)	1.274 (0.818-1.982)
AC+CC	411 (73.5%)	254 (74.3%)	1.039 (0.765-1.412)	1.065 (0.747-1.520)
IL-18 -137G/C (rs187238)				
GG	476 (85.2%)	266 (77.8%)	1.00	1.00
GC	78 (13.9%)	73 (21.3%)	1.675 (1.177-2.383)*	2.066 (1.337-3.191)*
CC	5 (0.9%)	3 (0.9%)	1.074 (0.255-4.528)	1.054 (0.201-5.524)
GC+CC	83 (14.8%)	76 (22.2%)	1.639 (1.160-2.315)*	1.987 (1.301-3.032)*

The odds ratios (ORs) and with their 95% confidence intervals (CIs) were estimated by logistic regression models. The adjusted odds ratios (AORs) with their 95% confidence intervals (CIs) were estimated by multiple logistic regression models after controlling for age, gender, alcohol and tobacco consumption.

* p value < 0.05 as statistically significant.

Table 3. Odds ratio (OR) and 95% confidence interval (CI) of clinical status and *IL-18* -137G/C genotypic frequencies in 342 HCC patients.

Variable	Genotypic frequencies			
	GG (N=266)	GC+CC (N=76)	OR (95% CI)	*p* value
Clinical Stage				
Stage I/II	176 (66.2%)	48 (63.2%)	1.00	*p*=0.627
Stage III/IV	90 (33.8%)	28 (36.8%)	1.141 (0.671-1.940)	
Tumor size				
≦ T2	180 (67.7%)	48 (63.2%)	1.00	*p*=0.462
> T2	86 (32.3%)	28 (36.8%)	1.221 (0.717-2.079)	
Lymph node metastasis				
No	256 (96.2%)	74 (97.4%)	1.00	*p*=0.637
Yes	10 (3.8%)	2 (2.6%)	0.692 (0.148-3.228)	
Distant metastasis				
No	253 (95.1%)	71 (93.4%)	1.00	*p*=0.560
Yes	13 (4.9%)	5 (6.6%)	1.371 (0.473-3.974)	
Vascular invasion				
No	222 (83.5%)	60 (78.9%)	1.00	*p*=0.362
Yes	44 (16.5%)	16 (21.1%)	1.345 (0.710-2.550)	
Child-Pugh grade				
A	206 (77.4%)	54 (71.1%)	1.00	*p*=0.250
B or C	60 (22.6%)	22 (28.9%)	1.399 (0.789-2.481)	
HBsAg				
Negative	163 (61.3%)	37 (48.7%)	1.00	*p*=0.049*
Positive	103 (38.7%)	39 (51.3%)	1.668 (1.001-2.786)	
Anti-HCV				
Negative	136 (51.1%)	42 (55.3%)	1.00	*p*=0.525
Positive	130 (48.9%)	34 (44.7%)	0.847 (0.507-1.413)	
Liver cirrhosis				
Negative	53 (19.9%)	15 (19.7%)	1.00	*p*=0.971
Positive	213 (80.1%)	61 (80.3%)	1.012 (0.534-1.919)	

The ORs with analyzed by their 95% CIs were estimated by logistic regression models.

> T2: multiple tumor more than 5 cm or tumor involving a major branch of the portal or hepatic vein(s)

* *p* value < 0.05 as statistically significant.

Table 4. Odds ratio (OR) and 95% confidence interval (CI) of clinical status and *IL-18* -137G/C genotypic frequencies in 142 HCC patients with HBsAg positive.

Variable	Genotypic frequencies			
	GG (N=103)	GC+CC (N=39)	OR (95% CI)	*p* value
Clinical Stage				
Stage I/II	68 (66.0%)	20 (51.3%)	1.00	*p*=0.106
Stage III/IV	35 (34.0%)	19 (48.7%)	1.846 (0.873-3.902)	
Tumor size				
≦ T2	70 (68.0%)	20 (51.3%)	1.00	*p*=0.066
> T2	33 (32.0%)	19 (48.7%)	2.015 (0.950-4.275)	
Lymph node metastasis				
No	99 (96.1%)	37 (94.9%)	1.00	*p*=0.742
Yes	4 (3.9%)	2 (5.1%)	1.338 (0.235-7.614)	
Distant metastasis				
No	96 (93.2%)	35 (89.7%)	1.00	*p*=0.491
Yes	7 (6.8%)	4 (10.3%)	1.567 (0.432-5.682)	
Vascular invasion				
No	89 (86.4%)	27 (69.2%)	1.00	*p*=0.018*
Yes	14 (13.6%)	12 (30.8%)	2.825 (1.168-6.833)	
Child-Pugh grade				
A	81 (78.6%)	25 (64.1%)	1.00	*p*=0.075
B or C	22 (21.4%)	14 (35.9%)	2.062 (0.921-4.618)	
Anti-HCV				
Negative	88 (85.4%)	33 (84.6%)	1.00	*p*=0.902
Positive	15 (14.6%)	6 (15.4%)	1.067 (0.382-2.981)	
Liver cirrhosis				
Negative	14 (13.6%)	9 (23.1%)	1.00	*p*=0.171
Positive	89 (86.4%)	30 (76.9%)	0.524 (0.206-1.334)	

The ORs with analyzed by their 95% CIs were estimated by logistic regression models.

> T2: multiple tumor more than 5 cm or tumor involving a major branch of the portal or hepatic vein(s)

* *p* value < 0.05 as statistically significant.

Table 5. Association of *IL-18* genotypic frequencies with HCC laboratory status.

Characteristic	α-Fetoprotein [a] (ng/mL)	AST [a] (IU/L)	ALT [a] (IU/L)	AST/ALT ratio [a]
IL-18 -607A/C (rs1946518)				
AA	4630.2 ± 2184.6	121.1 ± 22.6	101.7 ± 16.8	1.40 ± 0.10
AC+CC	3082.9 ± 925.3	142.2 ± 19.6	120.7 ± 15.6	1.51 ± 0.10
p value	0.446	0.557	0.502	0.551
IL-18 -137G/C (rs187238)				
GG	2279.7 ± 732.6	137.1 ± 19.2	118.7 ± 15.1	1.46 ± 0.10
GC+CC	7685.5 ± 3024.9	135.6 ± 21.9	105.6 ± 16.9	1.58 ± 0.15
p value	0.011*	0.967	0.659	0.526

Mann-Whitney U test was used between two groups.

a Mean ± S.E.

* *p* value < 0.05 as statistically significant.

Discussion

Compared with healthy people, the serum level of IL-18 in patients with HCC is controversial. Tangkijvanich et al. [8] and Mohran et al. [27] reported that IL-18 levels in patients with HCC were significantly higher than those in healthy controls. However, Bao et al. [12] showed that the level of serum IL-18 were significantly lower in patients with HCC than in healthy people. In addition, they reported that the *IL-18* −137G/C polymorphism was significantly correlated with the risk of HCC; however, *IL-18* SNPs were not associated with the serum concentration of IL-18. Thus, we suggested that serum levels of IL-18 may be distinct in various diseases and cancer stages. In addition, we revealed that the genetic of patients profile is one of the key factors for the serum level of IL-18 to regulate the development of HCC. In our study, the presence of *IL-18* −607A/C (rs1946518) and −137G/C (rs187238)

polymorphisms was analyzed in 559 healthy controls and 342 patients with HCC. The number of patients with HCC carrying the *IL-18* −137G/C (rs187238) polymorphism genotypes GC+CC and GG differed significantly ($p < 0.001$). Our results confirmed that patients carrying the *IL-18* −137G/C polymorphism genotype GC+CC have a high risk of HCC, similar to the results obtained by Bao et al [12], reporting a significant association between the *IL-18* −137G/C polymorphism and the risk of HCC, where a high frequency of the G allele was associated with an increased risk of HCC.

No association between the *IL-18* −137 polymorphism genotypes GC+CC and GG and the aforementioned clinical parameters except HBV infection was observed in our study. We found that among the patients with HCC who were HBV positive, the risk of HCC was higher in those who were carrying the *IL-18* −137 polymorphism genotype GC+CC (39/76 = 51.3%) than those who were carrying the GG genotype (103/266 = 38.7%) with an AOR of 1.668 ($p < 0.05$). This result is similar to the results of a study performed by Bouzgarrou et al. [28], reporting that a polymorphism in C allele at position −607 (CC + C/A) was associated with an increased risk of cirrhosis and HCC in patients who were HBV positive. Because HBV infection can lead to severe liver diseases, such as chronic hepatitis, cirrhosis, and HCC [29, 30], the natural course of HBV infection is probably associated with host immune factors, and IL-18 is crucial in immune defense. Kim et al. [31] showed that the −148C, +8925G, and +13925C alleles of *IL-18* are associated with the development of HCC, and the −148G>C SNP was functionally essential in determining the disease outcome. However, the association between *IL-18* SNP and HBV-related HCC has been studies seldom. Karra et al. [32] reported that polymorphisms in the *IL-18* promoter region at positions −607 and −137 combining with HBV infection can be associated with various outcomes, including spontaneous recovery, chronic hepatitis, liver cirrhosis, and HCC. Therefore, as reported by Thio et al. [33], the genetic background of an individual might influence the clinical outcome of HBV infection. Interestingly, in a meta-analysis, Yang et al. suggested that *IL-18* - 137G/C polymorphism, but not -607C/A polymorphism, was associated with chronic hepatitis C virus infections [34]. Coordinating innate and adaptive humoral and cell-mediated immunities, various cytokines, such as IL-18, and associations with SNPs may reveal some clues in some spontaneous clearance, while others develop cirrhosis and liver cancer; these results can provide new clues to explain the mechanism of HBV infection [35, 36].

An advanced statistics on the 142 patients with HCC who were HBV positive revealed that the GC genotype and C allele of the *IL-18* −137 SNP were associated with a significantly increased risk of vascular invasion compared with the GG genotype and G allele. Migita et al. [37] reported that the polymorphisms at −607 and −137 in the *IL-18* promoter region may affect the development and progression of HBV-related liver diseases; we obtained similar results with an AOR of 2.825 ($p = 0.018$). Moreover, Kim et al. [31] confirmed that the −148C, +8925G, and +13925C alleles of *IL-18* in patients with HBV infection are associated with the presence of HCC, and the 148G>C SNP is functionally crucial in determining the disease outcome. However, Chung et al. [38] reported that *IL-18* and *IL-18R* polymorphisms may contribute to the development and lymph node metastasis of papillary thyroid carcinoma. We revealed that the *IL-18* −137C polymorphism can be a crucial factor for the risk of HBV-related HCC outcome of vascular invasion. Moreover, *IL-18* and *IL-18R* polymorphisms in various cancers have different influence on the outcomes and specific characteristic phenomena. Perrella et al. [39] reported no significant difference among patients with HCC and no correlation between cytokines and other evaluated variables, such as HCV infection, RNA, α-fetoprotein, genotype, and demographic characteristics of patients with HCC. Our results suggest that the clinicopathological statuses for HCC, including ALT, AST, and AST/ALT ratio, showed no significant differences except the α-fetoprotein level. The level of α-fetoprotein was significantly associated with the presence of the *IL-18* −137G/C polymorphism genotypes GG and GC+CC in the patients with HCC ($p < 0.05$). Therefore, the *IL-18* −137G/C polymorphism might be crucial for the pathogenesis of HCC, and the GC+CC genotype in the *IL-18* −137 SNP combining with α-fetoprotein levels can be utilized as markers for an early diagnosis of HCC.

In conclusion, our results confirmed that the *IL-18* −137G/C (rs187238) SNP can be a factor that increases the risk of HCC. Moreover, the association of the GC genotype of the *IL-18* −137 SNP with HBV-related HCC can increase the risk of vascular invasion. In addition, the α-fetoprotein level combined with the *IL-18* −137 SNP can be utilized as a marker for the early diagnosis of HCC.

Acknowledgment

This study was supported by research grants from Chung Shan Medical University Hospital (CSH-2016-C-019).

Competing Interests

The authors have declared that no competing interest exists.

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