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ORIGINAL ARTICLE

Interleukin 10 promoter haplotype is associated with alcoholic liver cirrhosis in Taiwanese patients

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Abstract Alcoholic liver cirrhosis is a severe form of alcohol-related liver damage. More than 95% of heavy drinkers develop a fatty liver, but only 35% of them develop cirrhosis. We postulate that genetic factors may play a role in this difference. Genetic polymorphisms of the cytokine genes may influence Kupffer cells cytokine genes expression. In this study, we evaluated the promoter polymorphisms of interleukin (IL) 1β, IL 6, IL 10, and tumor necrosis factor alpha (TNFα) and aimed to clarify the association between the polymorphisms and the disease. Forty alcoholic patients with liver cirrhosis and 64 healthy volunteers were included in our investigation. Genotyping on IL 1β −511 T>C, IL 6 −572 G>C, IL 10 −819 C>T, IL 10 −1082 G>A, and TNFα −308 G>A was done. Another 36 patients with recurrent alcoholic pancreatitis were included as an additional control group. Genotyping on IL 10 −819 C>T and IL 10 −1082 G>A was done. The polymorphisms on IL 1 and IL 6 showed no significant association. The *p* value for TNFα −308 G>A was 0.028 in comparison with healthy volunteers. Although the *p* value was less than 0.05, it did not reach significance after Bonferroni correction. The *p* values for IL 10 −819 C>T and IL 10 −1082 G>A were respectively 0.031 and 0.026 in healthy volunteers and 0.028 and 0.023 in the alcoholic pancreatitis group. The results also did not reach significance after Bonferroni correction. Among the participants with the GCC haplotype, healthy volunteers had $p = 0.027$ ($p < 0.05$) and an odds ratio (OR) of 0.124 [confidence interval (95%) CI, 0.015–0.997], whereas the alcoholic pancreatitis group had $p = 0.023$ ($p < 0.05$) and an OR of 0.106 (95% CI, 0.012–0.912). The odds ratio of people having one ATA haplotype

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was 6.233 (95% CI, 0.739—52.547) in healthy volunteers and 6.588 (95% CI, 0.727—59.679) in the alcoholic pancreatitis group; the corresponding rate was 10.521 (95% CI, 1.252—88.440) and 12.833 (95% CI 1.408—117.008) for people with two ATA haplotypes. The p values in these groups were 0.031 ($p < 0.05$) and 0.028 ($p < 0.05$), respectively. The presence of a GCC haplotype could have protective effect against alcoholic liver disease, whereas the presence of an ATA haplotype could predispose carriers to the disease. The IL 10 promoter haplotype is associated with alcoholic liver cirrhosis in Taiwanese patients.

Introduction

Alcoholism is a major cause of chronic liver disease worldwide [1] and contributes up to 48% of cirrhosis-related deaths in the United States [2]. The spectrum of alcohol-related liver injury ranges from simple fatty liver to more severe forms of liver damage such as liver fibrosis and cirrhosis, and even superimposed with hepatocellular carcinoma.

More than 95% of heavy drinkers develop a fatty liver, but only up to 35% of these individuals develop more severe forms of alcoholic liver disease [3]. This indicates that other factors may be involved. Several risk factors for alcoholic liver disease have been identified: sex, obesity, drinking patterns, dietary factors, genetic factors, and cigarette smoking [4—6]. Factors such as female gender, obesity, and drinking patterns have been well documented [7,8]. Genetic factors may also influence susceptibility to advanced alcoholic liver disease; however, only scanty data are available on this topic.

The mechanism of alcohol liver damage initiates from the activation of innate immunity in the sinusoid by endotoxins such as lipopolysaccharides from portal circulation. The toxin is produced by Gram-negative bacteria in the intestine and translocates to portal circulation owing to increased intestinal permeability after excess alcohol consumption [9]. This stimulation initiates and promotes oxidative stress and inflammatory process via interaction with Kupffer cells in the sinusoid [10]. The proinflammatory cytokines released from Kupffer cells may further activate stellate cells and cause liver fibrosis. Therefore, inflammatory responses originating from Kupffer cells may play an important role in the process of alcoholic liver cirrhosis [11]. Hence, the different inflammatory responses of immune cells and following cytokine expression in the liver may be critical to the pathogenesis of alcoholic liver cirrhosis.

Therefore, we postulate that the gene expression of these cytokines of the Kupffer cells may be responsible for the immune response and subsequent fibrosis. Because the major cytokines secreted by Kupffer cell are *TNFα*, *IL 1*, *IL 6*, *IL 10*, *PDGF*, *MCP 1*, and *TGF-β* [10,11], we selected four cytokines with reported promoter polymorphisms for further genotyping. In this study, we evaluated the association of promoter polymorphisms on *IL 10: −1082 G>A* (rs1800896), *−819 C>T* (rs180871), *−592 C>A* (rs1800872), *IL 6: −572 G>C* (rs1800796), *IL 1β: −511 T>C* (rs 1143627), as well as *TNFα: −308 G>A* (rs1800629) in patients with alcoholic liver cirrhosis.

Methods

Patients

We recruited 40 ethnically Taiwanese Han patients with continuous alcohol consumption and liver cirrhosis and 64 adult healthy volunteers without regular alcohol consumption. To represent heavy drinkers without liver cirrhosis, another group of 36 patients with continuous alcohol consumption who experienced episodes of acute pancreatitis more than twice but without liver cirrhosis were included as an additional control group; in this group, interleukin (IL) 10 promoter polymorphisms were genotyped.

People with positive hepatitis C antibody, hepatitis B surface antigen, and any other liver diseases were excluded. The definition of liver cirrhosis called for patients with (1) cirrhotic liver parenchyma on ultrasonography and (2) endoscopically confirmed esophageal or gastric varices, or (3) with splenomegaly on ultrasonography or computed tomography. The definition of continuous alcohol drinking was ingestion of >60 g/day of alcohol for more than 10 years in men and >20 g/day for more than 10 years in women [12].

The information on the amount of alcohol consumption was acquired by individual history taking. This study was approved by the Institutional Review Board of En Chu Kong Hospital, New Taipei City, Taiwan, and a written informed consent was obtained from each participant.

Genotyping

About 2 mL of peripheral blood was drawn from patients with alcoholic liver cirrhosis, recurrent acute pancreatitis, and healthy controls. Genomic DNA was extracted from whole blood using the standard spin-column method (NucleoSpin Blood, Macherey-Nagel, Düren, Germany).

Genotyping of *IL 1β −511 T>C*, and *IL 6 −f572 G>C* was carried out using polymerase chain reaction (PCR) with restriction fragment length polymorphism [13,14]. Genotyping of *IL 10 -819 C>T* and *TNFα -308 G>A* was carried out using ambulatory refractory mutation system-PCR [15]. Genotyping of *IL 10 −1082 G>A* was done using bidirectional allele-specific amplification [16]. Because *IL 10 −819 C>T* is in complete linkage disequilibrium with *IL 10 −592 C>A*, genotyping was only done on *IL 10 −819 C>T* [17]. The methods of genotyping are summarized in Table 1.

Table 1 Methods of genotyping.

Loci	Method	Primers and restriction enzyme
IL 1B −511 T>C (rs1143627)	PCR-RFLP	5′-TGGCAT TGATCT GGT TCATC-3′ 5′-GTTTAGGAATCTTCCCACTT-3′ Restriction enzyme: *Ava*I
IL 6 −572 G>C (rs1800796)	PCR-RFLP	5′-GAGACGCCTTGAAGTAACTG-3′ 5′-AACCAAAGATGTTCTGAACTGA-3′ Restriction enzyme: *Bsr*BI
IL 10 −819 C>T (rs1800871)	ARMS-PCR	5′-AGGATGTGTTCCAGGCTCCT-3′ 5′-CCCTTGTACAGGTGATGTAAC-3′ 5′-ACCCFFGTACAGGTGATGTAAT-3′
IL 10 −1082 G>A (rs1800896)	Bidirectional allele specific amplification	5′-TTTCCAGATATCTGAAGAAGTCCTG-3′ 5′-AACACTACTAAGGCTTCTTTGGGTA-3′ 5′-TCTAAAGTTTAAAAGATGGGGTGGA-3′ 5′-CTCTTACCTATCCCTACTTCCCGC-3′
TNFα −308 G>A (rs1800629)	ARMS-PCR	5′-TCTCGGTTTCTTCTCCATCG-3′ 5′-ATAGGTTTTGAGGGGCATGG-3′ 5′-AATAGGTTTTGAGGGGCATGA-3′

Internal control primers for ARMS-PCR: 5′-GCCTTCCCAACCATTCECTTA-3′ 5′-TCACGGATTTCAGTTGTGTTTC-3′.
ARMS-PCR = ambulatory refractory mutation system-polymerase chain reaction; PCR-RFLP = polymerase chain reaction with restriction fragment length polymorphism.

Statistical analysis

The genotype distribution in the controls was compatible with the Hardy–Weinberg equilibrium. For the comparison of the genotype difference between patients and controls, Chi square test was used, and Fisher's exact test was used if any predicted value was <5.

Results

Polymorphism frequency

The case–control comparison of the genotype of *IL 1β −511 T>C* and *IL 6 −572 G>C* showed $p = 0.44$ and $p = 0.94$, respectively, in comparison to the healthy control group. Both p values are far greater than 0.05.

On *IL 10 −819 C>T*, the cirrhotic-healthy control comparison $p = 0.031$ (Fisher's exact test). Using the CC genotype as the reference genotype, the cirrhotic-healthy control odds ratio (OR) of the CT genotype was 6.233 [95% confidence interval (CI), 0.739–52.547] and was 10.521 (95% CI, 1.252–88.440) in the TT genotype. In the alcoholic pancreatitis control group, the cirrhotic-pancreatitis comparison $p = 0.028$ (Fisher's exact test). The cirrhotic-pancreatitis control OR of the CT genotype was 7.0 (95% CI, 0.775–63.206) and was 12.83 (95% CI, 1.408–117.01) in the TT genotype.

On *IL 10 -1082 A>G*, the AA genotype was 82.8%, AG was 17.2%, and GG was 0% in healthy controls; in cirrhotic patients, the corresponding values were 97.5%, 2.5%, and 0%, respectively. The cirrhotic-healthy control comparison $p = 0.026$ (Fisher's exact test). The cirrhotic-healthy control OR of having a G allele was 0.124 (95% CI, 0.015–0.997). In the alcoholic pancreatitis control group, there was also no patient with a GG genotype. The cirrhotic-pancreatitis comparison $p = 0.023$ (Fisher's exact test). The cirrhotic-pancreatitis control OR of having a G allele was 0.106 (95% CI, 0.013–0.912). The results of alcoholic pancreatitis in this locus were similar to those of healthy controls.

As far as *TNFα-308 G>A* is concerned, the GG genotype was 87.5%, the GA genotype was 12.5%, and AA was 0% in healthy controls; in patients, the corresponding values were 70%, 30%, and 0%, respectively. The p value was 0.028 (Chi square test). The case–control odd's ratio of having an A allele was 3.0 (95% CI, 1.100–8.179).

Among the polymorphisms tested, polymorphisms on *IL 1β* and *IL 6* were not statistically significant. The cirrhotic-healthy control comparison p values of polymorphisms on *IL 10* and *TNFα* were all less than 0.05 and so was the cirrhotic-pancreatitis control comparison p value on *IL 10*. However, because five polymorphisms were tested, Bonferroni correction (a multiple testing correction) had to be considered, and consequently the threshold of significance became 0.01. Therefore, these three polymorphisms were not considered significant despite the raw $p < 0.05$. These data are summarized in Table 2.

IL 10 haplotype analysis

In further haplotype analysis, because of the marked linkage disequilibrium, there were three haplotypes of the *IL 10 −1082 A>G*, *−819C>T*, and *−592C>A*: ACC (H1), ATA (H2), and GCC (H3). The haplotypes could be easily identified in each participant without ambiguous biphasing [17]. As in Table 3, we presumed that each haplotype was dominant separately and found that the presence of H3 and H2 haplotypes was statistically significant.

In people with one H3 haplotype (the H3/H3 diplotype is not found in this study), the cirrhotic-healthy control comparison $p = 0.027$ ($p < 0.05$) and the cirrhotic-healthy control OR was 0.124 (95% CI, 0.015–0.997), whereas the cirrhotic-pancreatitis control comparison $p = 0.023$ ($p < 0.05$) and the cirrhotic-pancreatitis control OR was

Table 2 Analysis of the cytokine promoter polymorphism.

Loci		Alcoholic liver cirrhosis ($n = 40$)	Healthy control ($n = 64$)	Alcoholic acute pancreatitis ($n = 36$)	p (χ^2 or Fisher's exact test)	Referent allele, OR (95% CI)
IL 1β −511	Genotype					
T>C	TT	5 (12.5)	4 (6.25)	—	0.44	1
	TC	12 (30)	17 (26.6)			0.565 (0.125—2.552)
	CC	23 (57.5)	43 (67.15)			0.428 (0.105—1.751)
	Allele, frequency					
	T	0.275	0.195	—	0.233	1.559 (0.765—3.17)
	C	0.725	0.805			1
IL 6 −572	Genotype					
G>C	GG	2 (5)	3 (4.7)		0.94	1
	GC	15 (37.5)	22 (35.11)	—		1.023 (0.152—6.877)
	CC	23 (57.5)	39 (60.19)			0.885 (0.137—5.693)
	Allele, frequency					
	G	0.238	0.219	—	0.865	1.112 (0.538—2.265)
	C	0.762	0.781			1
IL 10 −819	Genotype				Healthy 0.031	1
C>T	CC	1 (2.5)	11 (17.2)	7 (19.5)		6.233 (0.739—52.547)
						10.521 (1.252—88.440)
	CT	17 (42.5)	30 (46.9)	17 (47.2)	Pancreatitis 0.028	1
	TT	22 (55)	23 (35.9)	12 (33.3)		7.0 (0.775—63.206)
						12.83 (1.408—117.01)
	Allele, frequency				Healthy 0.016	0.457 (0.230—0.883)
	C	0.238	0.406	0.431		1
	T	0.762	0.594	0.569	Pancreatitis 0.015	0.412 (0.206—0.825)
						1

IL 10 −1082	Genotype				Healthy 0.026	
A>G	AA	39 (97.5)	53 (82.8)	29 (80.6)		1
						0.124
						(0.015−0.997)
	AG	1 (2.5)	11 (17.2)	7 (19.4)	Pancreatitis 0.023	1
	GG	0	0	0		0.106
						(0.013−0.912)
	Allele, frequency				Healthy 0.032	7.377
						(1.035−323.21)
	A	0.988	0.914	0.917		1
	G	0.012	0.086	0.083	Pancreatitis 0.027	8.508
						(1.02−70.938)
						1
TNFA −308	Genotype				0.040	
G>A	GG	28 (70)	56 (87.5)			1
	AG	12 (30)	8 (12.5)	—		3 (1.1−8.179)
	AA	0	0			—
	Allele, frequency				0.052	
	G	0.85	0.938	—		0.38
						(0.128−1.068)
	A	0.15	0.062			1

CI = confidence interval; OR = odds ratio.

0.106 (95% CI, 0.012−0.912). This result indicated that people with H3 haplotype may be less likely to develop alcoholic liver disease. As far as H2 is concerned, in cirrhotic-healthy control comparison, the case−control OR of people without any H2 haplotype to people having one H2 haplotype was 6.233 (95% CI, 0.739−52.547), and the corresponding value was 10.521 (95% CI, 1.252−88.440) in people with two H2 haplotypes. Also, the p value in this group was 0.031 ($p < 0.05$). In cirrhotic-pancreatitis control comparison, the OR of people without an H2 haplotype to people with one H2 haplotype was 10.521 (95% CI, 1.252−88.44), and this was 12.833 (95% CI, 1.408−117.008) in people with two H2 haplotypes; $p = 0.028$ ($p < 0.05$). This result suggests that people with H2 haplotypes may be prone to develop alcoholic liver cirrhosis. As shown in Table 4, H2/H2 diplotypes accounted for 55% in alcoholic liver cirrhosis patients but only 34.38% in healthy controls and 33.3% in alcoholic pancreatitis. In patients with alcoholic liver cirrhosis, only 2.5% had H3 haplotype, but this figure goes up to 17.15% in healthy controls and 19.4% in alcoholic pancreatitis control. As for haplotype frequencies, the presence of H1 was 22.5% in cirrhotic patients, 32% in healthy controls, and 33.3% in alcoholic pancreatitis controls. The presence of H2 was 76.25% in cirrhotic patients, 59.4% in healthy controls, and 57% in alcoholic pancreatitis controls. Finally, the presence of H3 accounted for 1.25% in cirrhotic patients, 8.6% in healthy controls, and 9.7% in alcoholic pancreatitis control (Table 3). The distribution of haplotype frequencies was also statistically significant in both healthy controls and alcoholic pancreatitis controls ($p = 0.014$ and $p = 0.011$, respectively). These results implied that the presence of the H3 haplotype could have a protective effect against alcoholic liver disease, whereas the H2 haplotype could predispose carriers to the disease. The results were consistent in both control groups, suggesting that the polymorphism of the haplotype was unique to alcoholic liver cirrhosis, and did not reflect the stimulation of alcohol.

Discussion

Alcoholism-induced problems are more serious than liver diseases. It may relate to several types of cancer, unintentional injuries both in the workplace and on the road, domestic and social violence, broken marriages, and damaged social and family relationships [18]. Although abstinence has been proved to be the best method of treatment [19], the 1-year relapse rate was found to range from 67% to 81%, which shows the high possibility of recurrence [20]. As far as treatment is concerned, medical treatments for alcoholic liver disease and alcoholism itself are suboptimal. Given the high recurrent rate of alcoholism and lack of adequate medical treatment, a thorough understanding of the disease mechanism and risk factors merits more efforts.

Among the complications of alcoholism, advanced liver disease is frequently encountered in the clinical setting and management of this disease is difficult. So far, the mechanism of the severe form of alcoholic liver disease has been proposed as a "gut−liver axis" theory: excess alcohol consumption may increase the permeability of the intestines,

Table 3 Analysis of IL 10 haplotype.

Haplotype	Diplotype	Alcoholic liver cirrhosis	Healthy control	Alcoholic acute pancreatitis	*p* (Fisher's exact test)	Referent allele OR (95% CI)
Haplotype 1 (ACC)	*other/other*	23 (57.5%)	30 (46.9%)	18 (50.0%)	Healthy 0.25	1
	other/H1	16 (40.0%)	27 (42.2%)	12 (33.3%)		0.773 (0.339, 1.760)
	H1/H1	1 (2.5%)	7 (10.9%)	6 (16.7%)		0.186 (0.021, 1.623)
					Pancreatitis 0.126	1
						1.044 (0.396, 2.752)
						0.13 (0.014, 1.181)
Haplotype 2 (ATA)	*other/other*	1 (2.5%)	11 (27.5%)	7 (19.5%)	Healthy 0.031	1
	other/H2	17 (42.5%)	30 (46.9%)	17 (47.2%)		6.233 (0.739, 52.547)
	H2/H2	22 (55.0%)	23 (25.6%)	12 (33.3%)		10.521 (1.252, 88.44)
					Pancreatitis 0.028	1
						6.588 (0.727, 59.679)
						12.833 (1.408, 117.008)
Haplotype 3 (GCC)	*other/other*	39 (97.5%)	53 (82.8%)	29 (80.6%)	Healthy 0.027	1
	other/H3	1 (2.5%)	11 (17.2%)	7 (19.4%)		0.124 (0.015, 0.997)
	H3/H3	0	0	0	Pancreatitis 0.023	1
						0.106 (0.012, 0.912)
						–
Haplotype frequency						
ACC (H1)		18 (22.5%)	41 (32.0%)	24 (33.3%)	Healthy 0.014	1
ATA (H2)		61 (76.3%)	76 (59.4%)	41 (57.0%)		1.828 (0.956, 3.497)
GCC (H3)		1 (1.25%)	11 (8.6%)	7 (9.7%)		0.207 (0.025, 1.727)
					Pancreatitis 0.011	1
						1.984 (0.958, 4.109)
						0.191 (0.022, 1.689)

**p* values and odds ratios with significance are in bold type.

and endotoxins generated by Gram-negative bacteria translocate to the portal vein and activate an immune response in the sinusoid [9]. According to this theory, the innate immune response of the Kupffer cells is the starting point of the subsequent inflammatory cascade. Kupffer cells may generate several sorts of cytokines and mediate the response by them. In the initial stage of this study, we only included healthy individuals as the control population. However, a question was raised as to whether the variation in cytokine expression between normal individuals and alcoholic cirrhotic patients may reflect the stimulation of alcohol or may be regarded as secondary to histological liver damage. To clarify this point, we added a group of patients with continuous alcohol consumption and recurrent pancreatitis but had no liver cirrhosis. Because only *IL 10* haplotype was statistically significant in this study, we genotyped *IL 10* promoter polymorphism on these patients. The results of this additional test showed that the allele frequency of *IL 10* promoter polymorphism in alcoholic pancreatitis patients was very similar to that in healthy controls. This may reflect that the haplotype polymorphism may be unique in alcoholic cirrhotic patients.

Among the cytokines, TNFα plays a pivotal role in inflammation; it induces the expression of other proinflammatory molecules, chemotactic cytokines, and adhesion factors that cause liver damage [21]. Moreover, TNFα has been reported to be important in the pathogenesis of certain diseases such as asthma, systemic lupus erythematosus, and rheumatoid arthritis [22—24]. A meta-analysis published in 2009 concerning the association of *TNFα*

Table 4 Analysis of IL 10 diplotype.

Diplotype	Alcoholic liver cirrhosis	Healthy control	Alcoholic pancreatitis	*p* (Fisher's exact test)	Referent diplotype OR (95% CI)	
					Healthy	Pancreatitis
H1/H1	1 (2.5)	7 (10.9)	6 (16.7)	Healthy 0.054	1	1
H1/H2	16 (40)	24 (37.5)	11 (30.5)		4.667 (0.523—1.64)	8.727 (0.918—82.959)
H1/H3	0	4 (6.3)	1 (2.8)		NA	NA
H2/H2	22 (55)	22 (34.4)	12 (33.3)	Pancreatitis 0.012	7 (0.794—61.741)	11 (1.182—102.38)
H2/H3	1 (2.5)	7 (10.9)	6 (16.7)		1 (0.052—19.36)	1 (0.05—19.963)
H3/H3	0	0	0		NA	NA

Data are presented as *n* (%), unless otherwise indicated.
H1 = ACC; *H2* = ATA; *H3* = GCC.

promoter *−308G>A* polymorphism with alcoholic liver disease, claimed no significant association [25] despite the A allele having been reported to be with higher *TNFα* expression [26]. However, while reviewing the meta-analysis, we found that most results of the previous studies were conflicting. As shown in our study, although the *p* value was low, it was still statistically nonsignificant. Despite the conflicting result, two anti-TNF α agents had been studied for therapy of alcoholic hepatitis: infliximab and etanercept. Despite the encouraging results in several preliminary studies [27−29], subsequent large-scale studies showed no significant benefit of both agents and cautioned that they should not be used clinically [30,31].

By contrast, stimulation of Kupffer cells may also produce hepatoprotective cytokine IL 6 and anti-inflammatory cytokine IL 10 [32]. It has been reported that IL 6 may ameliorate alcoholic liver damage through the activation of STAT3 (signal transducer and activator of transcription 3), and downregulate lipogenic genes and upregulate β-oxidation genes in the liver [33]. However, no association between *IL 6* promoter polymorphism and alcoholic liver damage was found in the literature. Among the promoter polymorphisms in *IL 6*, *IL 6 −174 G>C* (rs1800795) has been widely studied; however, this polymorphism does not seem to exist in Asians in the dbSNP database. We tested another polymorphism on *IL 6 −572 G>C*, which is more frequently found among Asians, but still found no significant association. We also tested *IL 1β −511 T>C* polymorphism because it has been reported to be associated with reflux esophagitis and may be helpful in maintaining the integrity of the intestine [13]. As shown in the result, no association was found.

IL 10 was originally described as cytokines synthesis inhibitory factor because of its ability to inhibit the production of *IL 2*, *IL 3*, *interferon gamma* (*INF-γ*), and *granulocyte-macrophage colony stimulating factor (GM-CSF)* by Th1 clones [34]. An elevated IL 10 level has been described to be related to systemic lupus erythematosus, asthma, and susceptibility to infectious diseases [35]. A liver protection effect has been reported with elevated IL 10 level through the activation of STAT3 in Kupffer cells/macrophages and the subsequent inhibition of liver inflammation [32]. The frequently studied polymorphisms in the promoter region of *IL 10* are *−1082 G>A*, *−819 C>T*, and *−592 C>A*. Because *IL 10 −819 C>T* and *IL 10 −592 C>A* are in complete linkage, we did not genotype the latter. Our results showed that both *IL 10 −1082 G>A* and *IL 10 −819 C>T* had considerably low *p* values ($p = 0.026$ and $p = 0.031$, respectively) in comparison with the healthy control group, and the *p* values in comparison to pancreatitis controls were also low. Although the *p* value did not reach statistical significance ($p < 0.01$), the case−control OR of people with G allele in −1082 G>A was 0.124 (95% CI, 0.015−0.997) in healthy controls and 0.106 (95% CI, 0.013−0.912) in pancreatitis controls; this implied that people carrying a G allele in this locus were less likely to develop alcoholic liver cirrhosis. By contrast, with the presence of T allele in IL 10 −819 C>T, the reference OR in healthy controls became 6.233 (95% CI, 0.739−52.547) in the CT genotype and 10.521 (95% CI, 1.252−88.440) in the TT genotype; a similar trend was also noted in pancreatitis controls. This result implied that people with T alleles were prone to develop alcoholic liver cirrhosis. A study conducted by Suárez et al. [33] found that IL 10 mRNA expression is higher in *IL 10 −1082* G allele, and the serum *IL 10* is higher in GG genotype. However, no definite promoter activity studies regarding *IL 10 −819 C>T* were found, and the studies on its complete linkage locus *IL 10 −592 C>A* showed controversial results [36,37]. In comparison with our result, we supposed that people with G allele in *IL 10 −1082* and C allele in *IL 10 −819/−592* may have a good defense against alcoholic liver cirrhosis and the gene expression may be higher.

Because of linkage disequilibrium, only three haplotypes were found on *IL 10* promoter polymorphisms: ACC (H1), ATA (H2), and GCC (H3) [17]. A previous study using luciferase reporting assay concluded that the H3 construct conferred a stronger transcriptional activity than H2 [38]. There was a study carried out in Northern Spain to quantify by reverse transcription-PCR the mRNA extracted from 128 healthy Caucasian individuals [37]. The study showed significantly increased levels of mRNA expression for individuals carrying the H3/H3 diplotype, compared to H2/H2 ($p = 0.016$) or H2/H1 ($p = 0.01$). As far as liver disease is concerned, one *IL 10* haplotype (ACC, H1 haplotype) has been reported to be associated with increased risk of hepatocellular carcinoma progression in patients with chronic hepatitis B [17]. However, the haplotype reported above is not significant in the current study. In our study, as shown in Table 3, the *p* value of carrying an H1 haplotype or not was not significant in both control groups. However, in participants carrying an H3 haplotype or not, the *p* value between case and healthy controls was 0.027 ($p < 0.05$) and the OR was 0.124 (95% CI, 0.015−0.997), and the rate was even lower in pancreatitis controls. By contrast, in participants with zero, one, and two H2 haplotypes, $p = 0.031$ ($p < 0.05$) in healthy controls and $p = 0.028$ ($p < 0.05$) in pancreatitis controls. Using people without H2 haplotype as the reference diplotype, the OR of other/H2 diplotype was 6.233 (95% CI, 0.739−52.547) and the OR of H2/H2 was 10.521 (95% CI, 1.252−88.440) in healthy controls, and this was 6.588 (95% CI, 0.727−59.679) in pancreatitis controls with other/H2 diplotype and 12.833 (95% CI, 1.408−117.008) in pancreatitis controls with H2/H2 diplotype. Our results implied that people with H3 haplotype were less likely to develop alcoholic liver cirrhosis and people who have H2 haplotype were prone to develop alcoholic liver cirrhosis. Because the H3 haplotype may be with the strongest transcriptional activity and H2 may be the weakest, higher *IL 10* concentrations in the liver may confer a protective effect against alcohol-related damage. Our result suggested that *IL 10* promoter haplotype polymorphisms were associated with alcoholic liver cirrhosis.

The mechanism of alcoholic liver damage has been widely studied, and several factors have been proposed. However, the differences in genetic background between individuals are rarely discussed. In this study, we found *IL 10* promoter haplotypes to be associated with alcoholic liver cirrhosis. Nevertheless, we believe that other genetic factors may influence the pathogenesis of the disease. We reasoned that a genome-wide association study will be beneficial. A better understanding of the genetic background of alcoholic liver cirrhosis may advance the prevention and treatment of the disease.

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