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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.