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The role of FABP2 gene polymorphism in alcoholic cirrhosis

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Abstract

Hypertriglyceridemia and dietary lipids have been suggested to modulate the severity of alcoholic liver disease and the progression to alcoholic cirrhosis (AC). The intestinal fatty acid binding protein (IFABP) is the main transporter of dietary fatty acids into the enterocyte and has a genetic polymorphism, FABP2 A54T that has been associated with hypertriglyceridemia. We determined the frequency of the FABP2 gene polymorphism using PCR-RFLP and measured serum triglycerides, HDL, LDL, total lipids and cholesterol in 67 patients with AC and in 124 unrelated healthy individuals. Frequencies of genotypes and alleles were similar between the two groups. The healthy subjects, who were homozygous for the Thr54 genotype had significantly higher mean triglyceride serum concentrations than those homozygous for the Ala54 genotype ($P < 0.05$). However, AC patients who were homozygous for the Thr54 genotype, had lower mean triglyceride serum concentrations ($P < 0.01$), and had a significantly longer period of continued alcohol abuse prior to the diagnosis of liver cirrhosis compared to the AC patients homozygous for the Ala54 genotype ($P < 0.05$).

Our data suggests that the polymorphism Thr54 of the FABP2 gene is associated with a later onset of AC in the lower economic status Mexican population studied.

Keywords: FABP2; Alcohol; Cirrhosis; Mexican; Lipid; Economic status

1. Introduction

Alcoholic cirrhosis (AC) is the end result of the chronic inflammatory and oxidative effects of alcohol consumption [1–3]. The incidence of AC varies worldwide, ranging from 6 to 40% of people drinking more than 160 g of alcohol every day for at least 5 years [4–19]. This data indicate that not every person abusing alcohol will develop the disease, suggesting that genetics, in combination with environmental factors, plays an important role in the development of AC.

The first evidence supporting the role of genetic factors in AC susceptibility comes from a 1981 twin study [20]. Since then, and with the aid of molecular techniques, polymorphisms in genes encoding immunoregulatory proteins [21], proinflammatory cytokines [22–24], ethanol metabolizing enzymes, and antioxidant enzymes [25] have been studied and associated with the progression of AC.

Lipid alterations play an important role in AC development; however, polymorphisms of the genes regulating lipid metabolism have been barely studied in AC [26]. Lipid accumulations are a major characteristic in the onset of alcoholic liver disease (i.e. steatosis) and lipids are directly involved in the formation of fibrosis [27]. In AC, hepatocytes are replaced with a fibrous scar through activation of hepatic stellate cells

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(HSC). The activation of HSC is multifactorial, although lipid peroxidation, through the activation of the immune system, plays a crucial role [28,29]. Acetaldehyde is the major product of alcohol metabolism and, in conjunction with alcohol, significantly induces lipid peroxidation [30,31]. For lipid peroxidation to take place, polyunsaturated fatty acids (PUFAs) have to be available and the major source of fatty acids (FAs) is dietary intestinal absorption [32].

The intestinal fatty acid binding protein (IFABP) is found only in the enterocytes of the proximal small intestine [33]. The IFABP binds and transports preferentially long-chain saturated FAs from the diet into the enterocytes, where FAs are transported through the cytosol into the endoplasmic reticulum to be sterified as triglycerides and incorporated into newly formed chylomicrons [34,35]. The gene FABP2, which codifies the IFABP, has a polymorphism A54T, which translates an amino acid change in the active protein from Alanine (Ala54) to Threonine (Thr54) at residue 54. Thr54 has been shown to have a two fold higher affinity for FAs in vitro, in cultured cells, and in vivo, when compared to the wild type, Ala54 [33,36,37]. In many populations, the Thr54 polymorphism has been associated with elevated body mass index (BMI), insulin resistance, insulin secretion deficiencies, regional adiposity, increased postprandial lipemia, and increased triglyceride levels [38–42]. Lipids and insulin variations are part of the metabolic alterations found in AC; however, the Thr54 polymorphism has not been studied in liver diseases.

In this study, we analyzed FABP2 genetic polymorphism in patients with AC in a western Mexican population.

2. Patients and methods

2.1. Subjects

Over the period of 1 year, we studied 67 unrelated Mexican patients with alcoholic cirrhosis (54 males and 13 females, 45 ± 12 years old) from our Gastroenterology Inpatient Department and 124 unrelated age-matched disease-free controls (68 males and 56 females, 43 ± 18 years old). All procedures were approved by the Review Board and Ethical Committee of Human Experimentation at the University of Guadalajara and were conducted in accordance with the Helsinki Declaration of 1975. The study protocol was explained to all subjects and an informed consent was obtained. Patients who consumed any lipid-lowering drug or that tested positive for hepatitis virus B antigen and/or hepatitis virus C antibodies were excluded.

The clinical presentation of the patient with AC is quite variable. Some may be asymptomatic, whereas others present with jaundice, ascites, bleeding varices, or hepatic encephalopathy. The diagnosis of AC is based on a history of excessive alcohol intake (160 g/day for at least 5 years), negative serologies for other causes of liver disease, and, if possible, a liver biopsy showing micronodular cirrhosis [19,43]. Therefore, we collected data on physical signs and/or laboratory evidence of liver disease, followed by an interview concerning the history of habitual alcohol intake, including length and intensity. However, we were concerned that patients usually minimize or deny alcohol abuse, physical signs may not be present, and laboratory evidence may be non-specific [44]. To address this problem, a complete clinical history was taken, including interrogation of two relatives for each patient. Also, diagnosis was confirmed with a liver biopsy in all patients except those with ascites or those with abnormalities in the blood coagulation system. The age of drinking initiation showed no significant differences, and all patients drank more than 160 g of alcohol every day for more than 5 years, had advanced liver disease at the time of this study and were Child–Pugh classes B and C. These patients are from a population that typically seeks medical attention only in advanced disease states.

2.2. Lipids

All venous blood samples were drawn after an overnight 12 h fast. Serum total cholesterol (CHOD-PAP) and triglycerides (GPO-PAP) were determined with Merck enzymatic spectrophotometric reagent kits, and lipoproteins were separated by ultracentrifugation and HDL precipitation following the manufacturer's instructions (Merck, Germany).

2.3. FABP2 genotyping

Genomic DNA was extracted from 5 ml of peripheral blood as reported previously [45]. FABP2 A54T polymorphism was investigated using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique similar to Baier et al., except that smaller volumes of DNA were used [46]. Ten microliters of the 180 bp PCR products were digested with 1 U of endonuclease HhaI at 37 °C for 3 h and fractioned on a 3% agarose gel stained with ethidium bromide. Bands were visualized using indirect UV illumination. PCR products with substitution of G by A at codon 54 lacked the HhaI site, whereas the wild type products were cleaved into fragments of 99 and 81 bp. Photographic results are shown in Fig. 1. Since this substitution of G at codon 54 leads to a change in the encoded amino acid from alanine (Ala54) to threonine (Thr54), the subjects were classified into three groups: homozygous for Ala54, heterozygous for Ala54 and Thr54, and homozygous for Thr54.

All samples were blinded as to clinical status and were analyzed three times to insure reproducibility of results.

2.4. Statistical analysis

The results for continuous variables are given as means ± standard deviation and for gene frequencies as percentages. The differences between allele frequencies as well as Hardy–Weinberg equilibrium were assessed by the χ^2-test.