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Association of hepatitis C virus infection and liver fibrosis severity with the variants alleles of *MBL2* gene in a Brazilian population

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ABSTRACT

Mannose binding lectin (MBL) is a molecule of the innate immunity, which activates the complement system and modulates inflammation. We investigated the association of the polymorphisms in the exon 1 and promoter region of the MBL gene (*MBL2*) with the susceptibility to hepatitis C virus (HCV) infection and the degree of liver fibrosis in Brazilian patients chronically infected with HCV. The study was performed in 232 healthy control subjects and 186 patients, 157 of whom underwent liver biopsy after histopathology analysis and classification of fibrosis according to Metavir score. Exon 1 was genotyped by melting temperature assay and the promoter region by Taqman real-time polymerase chain reacation. The frequency of genotypes related to low production of MBL was higher in patients with HCV than in controls ($p_c = 0.0001$, odds ratio = 3.52; confidence interval = 1.86–6.71). In addition, the frequency of variant haplotype, HYO was higher in patients with the severe fibrosis stage F4 (10.7%) than in patients with the mild/moderate fibrosis stage F1/F2 (3.4%), when compared with the HYA haplotype ($p_c = 0.04$, odds ratio = 5.25, confidence interval = 1.11–23.62). We conclude that MBL variant alleles expressing low levels of MBL are associated with the susceptibility to HCV infection and that the inheritance of HYO haplotype could be associated with fibrosis severity.

1. Introduction

Hepatitis C virus (HCV) infection is the major cause of chronic liver disease worldwide. Recent studies suggest that liver inflammation in chronic HCV infection is controlled by several mechanisms, including host regulatory immune responses and viral polypeptides interaction with cells of the host innate and adaptive immunity [1]. It is believed that an effective immune response will clear HCV infection in 20%–40% of cases. However, the failure of an adequate immune response will tolerate continuous viral replication, with chronic recruitment of inflammatory cellular infiltration to the liver [2]. An important factor in the pathogenesis of HCV chronic infection is the liver damage sustained by the chronic inflammation and tissue fibrosis [2–4], which could lead to cirrhosis and hepatocellular carcinoma in 50%–80% of patients [2].

Mannose binding lectin (MBL) is a C-type lectin of the innate immunity, which binds to carbohydrate on the surface of certain microorganisms and activates the lectin pathway of the complement system (CS), resulting in opsonization and defense against various bacterial, viral, fungal, and protozoan infections [5,6].

The relative sufficiency of MBL levels and function for any given individual is largely determined by polymorphisms within the *MBL2* gene, on chromosome 10. There are three missense mutations within exon 1 of the *MBL2* that significantly affect MBL function and serum levels; the variant alleles that occur in the exon 1 are B (codon 54), C (codon 57), and D (codon 52). These coding variants are collectively designated as allele O, and the wild type is represented by allele A. Varying levels of MBL expression is due, at least in part, to other polymorphisms which occur in the promoter region at positions −550 (alleles H/L), −221 (alleles X/Y), and +4 (alleles P/Q) [7].

When inherited in the context of a coding allele A, the promoter region haplotypes HY, LY, and LX are associated with high, intermediate, and low serum MBL concentrations, respectively. The genotypes O/O, A/O, and LXA/LXA are all associated with low levels of MBL as well as binding dysfunction [7]. Genotype O/O is correlated with the most extreme MBL deficit [8]. These mutations reduce the proportion of higher-order oligomers formation in circulation and consequently decrease the MBL molecules able to bind

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targets through the carbohydrate domain region. In addition, the interference in the formation of high oligomers seems to inhibit the association of MBL-associated serine protease (MASPs) resulting in a reduced capacity to activate complement [9,10]. Therefore MBL allelic variants are associated with increased susceptibility to infections and disease progression, especially in immune-compromised individuals [6,11,12].

The individual variability of patients to progression of hepatic fibrosis has been attributed to age, gender, and environmental factors [13]. The different genetic polymorphisms may indicate that variants of genes encoding immunoregulatory proteins as well as pro- and anti-inflammatory cytokines determine liver fibrosis in patients with chronic hepatitis C virus infection [13,14].

The *MBL2* polymorphism has been associated with HCV infection susceptibility in some clinical investigations [15,16]. By contrast, the association of *MBL2* variant alleles with fibrosis has shown conflicting results. Alves Pedroso et al. [16], investigating Euro-Brazilian subjects, suggested that MBL polymorphism related to low levels may be a protection factor for severe fibrosis (stage F4) when compared with moderate fibrosis (stage F2) in HCV-infected patients. Brown et al. [17] also observed a correlation between MBL/MASP-1 complex activity and severe fibrosis in HCV patients. Conversely, Koutsounaki et al. [18] studying Greek patients found that low levels of serum MBL and the polymorphism of the exon 1 of *MBL2* gene was associated to progression of HCV clinical infection regarding the liver inflammation and fibrosis.

Our aim was to investigate the possible association between the polymorphisms in the exon 1 and promoter region of the *MBL2* gene with susceptibility to infection and degree of fibrosis in patients chronically infected with HCV from the northeastern region of Brazil, in which the structural polymorphism has been previously associated with the susceptibility to HCV infection [15].

2. Subjects and methods

2.1. Subjects and samples

A total of 186 patients (97 men and 89 women; mean age 53 years, range 22–73 years) from the Gastrohepatology Service of the Oswaldo Cruz University Hospital of the University of Pernambuco (Recife, northeastern Brazil) were included. Among them, 60 patients were previously analyzed only for structural polymorphism of *MBL2* [15] and were followed up in this study regarding the promoter polymorphism; of these, 30 patients were further included in analyzes for fibrosis severity. All patients were diagnosed with chronic hepatitis C through serologic analysis (enzyme linked immunosorbent Assay [ELISA] and recombinant immunoblot assay [RIBA]) and reverse transcriptase–polymerase chain reaction (RT-PCR). Presence of hepatitis A, hepatitis B, and human immunodeficiency virus (HIV) antibodies were considered as exclusion criteria. The majority of patients followed in this study likely acquired the disease through surgery or blood transfusion.

Among the 186 patients, 157 underwent liver biopsy before the antiviral treatment. Liver histopathology was assessed according Metavir classification; 48 patients (30.6%) had stage F1 liver fibrosis, 55 (35.0%) had F2, 27 (17.2%) had stage F3, and 27 (17.2%) had stage F4 liver fibrosis or cirrhosis. Men and women with alcohol consumption of more than 40 and 20 g/day, respectively, were excluded from the analysis. A total of 232 blood donors who were negative for anti-HCV, anti-HBV, and anti-HIV were included as healthy controls.

The study was approved by the Ethical Committee in Research of the *University of Pernambuco* under Protocol 074/07 CAAE: 0074.0.097.000-07.

2.2. MBL2 genotyping

DNA extraction from whole blood was done using Wizard Genomic DNA Purification Kit (Promega, Madison, WI) following the protocol as described by the manufacturer.

The *MBL2* SNPs −550 and −221, and exon 1 were genotyped using Rotor Gene-6000 apparatus (Corbett Research, Sydney, Australia). The primers used for exon 1 genotyping were as follows: forward primer: 5′-AGGCATCAACGGCTTCCCA-3′; reverse primer: 5′-CAGAACAGCCCAACACGTACCT-3′. Melting curve profiles were obtained using the dissociation software of the Rotor Gene-6000. All three variant alleles of exon 1 were grouped as allele O, whereas the wild-type allele was called A [19,20].

The promoter polymorphisms were detected using Taqman real time PCR probes technology (Applied Biosystems, Foster City, CA), the primers and probes used were as follows: (−550) 5′-CCAACG-TAGTAAGAAATTTCCAGAGA-3′ forward; 5′-CAACCCAGCCCAGAAT-TAACTG-3′ reverse; (−221) 5′-GCACGGTCCCATTTGTTCTCA-3′ forward; 5′-GCGTTGCTGCTGGAAGACTATAAA-3′ reverse; and (−550): 5′VIC- CCTGTCTAAAACACC-MGB -3′ (allele L), 5′FAM-AGCCTGTGTA-AAAC -MGB-3′ (allele H) and (−221) 5′FAM-CATGCTTTCCGTGGCAG-MGB-3′ (allele Y), 5′VIC-CATGCTTTCGGTGGCAG-MGB-3 (allele X), respectively. Protocol conditions are available on the SNP500 Cancer Web site [21].

2.3. Statistical analysis

Allele frequencies were calculated by Arlequin software, and differences in frequencies were analyzed by χ^2 probability test with the Yates correction and Fisher exact test when appropriate using 2×2 contingency tables. The difference was considered statistically significant when the p value was <0.05. Odds ratios (ORs) and 95% confidence intervals (CIs) were used, and analyses were performed with the Epi Info software, version 6. To avoid false-positive results, p values were calculated with the Bonferroni correction (p_C) for multiple comparisons.

3. Results

Table 1 shows the characterization of control and HCV-infected patient groups. The patients were further classified in relation to the severity of fibrosis according to Metavir score. Neither the mean age nor virus distribution genotype differed among the patients in the four groups classified by stages of fibrosis. All groups assessed in this study were found to be in Hardy–Weinberg equilibrium.

Table 2 summarizes the *MBL2* (−550, −221, and exon 1 SNPs) allelic, genotypic and haplotypic frequencies found between HCV patients and controls. Regarding the frequencies of alleles Y/X and A/O, the X and O alleles showed higher frequency in the patients infected with HCV when compared with controls (X vs Y: $p = 0.01$, OR = 1.58, CI = 1.11–2.26; O vs A: $p = 0.01$, OR = 1.49, CI = 1.06–2.08). Also, the frequency of Y/X + X/X genotypes was significantly higher in patients infected with HCV compared with the controls (Y/X + X/X vs Y/Y: $p = 0.002$, OR = 1.90, CI = 1.24–2.91), as

Table 1
Characterization of the patients with HCV infection stratified by Metavir fibrosis classification and controls

	Fibrosis (Metavir score)				Controls $n = 232$
	F1 $n = 48$	F2 $n = 55$	F3 $n = 27$	F4 $n = 27$	
Gender (male/female)	27/21	29/26	14/13	12/15	135/97
Age (mean ± SD)	53 ± 11.1	53 ± 11.2	52 ± 11.2	53 ± 11.1	33 ± 8.3
HCV genotype 1	31 (64.5%)	35 (63.6%)	19 (70.3%)	16 (59.2%)	—
HCV genotype 2	1 (2.2%)	2 (3.7%)	—	—	—
HCV genotype 3	16 (33.3%)	18 (32.7%)	8 (29.7%)	11 (40.8%)	—

n = number of individuals.