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The adiponutrin I148M variant is a risk factor for HCV-associated liver cancer in North-African patients

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ABSTRACT

Recent reports revealed an association between variation in the PNPLA3 gene and alcohol-induced hepatocellular carcinoma among Europeans. We have assessed whether the PNPLA3 rs738409 (I148M) polymorphism may also affect the resolution and/or the progression of hepatitis C in a Moroccan cohort.

Genotype and allele frequencies at rs738409 were determined using a TaqMan 5′ allelic discrimination assay in 437 individuals. Among them, 230 patients had a persistent infection with hepatitis C virus (HCV) with 129 patients affected by a chronic hepatitis and 101 patients by a hepatocellular carcinoma (HCC). In addition, we analyzed 75 individuals who naturally cleared HCV and 132 healthy subjects.

Variation at rs738409 was not associated with significant changes in resolution rate of hepatitis C. By contrast, M/M genotype, present at higher frequencies (22.8%) in HCC patients than in patients with chronic hepatitis C (8.5%, $P = 0.004$) or control individuals (9.1%, $P = 0.005$) was associated with a 3-fold increase of liver cancer risk.

In North African subjects, the PNPLA3 I148M variant apparently stimulates liver cancer development without interfering on the HCV clearance process. This polymorphism may, therefore, represent a valuable genetic marker to monitor liver cancer risk in populations from the Southern bank of the Mediterranean.

1. Introduction

Chronic hepatitis C is a major health burden with 2–3% of the world population persistently infected (Shepard et al., 2005). Primo-infection with hepatitis C virus (HCV) results in a chronic hepatitis in more than 80% of the patients, the remaining clear the virus spontaneously (Thomas and Seeff, 2005). Patients with chronic hepatitis C (CHC) display a great variability in the risk of liver disease progression. Many infected persons develop progressive hepatic fibrosis and subsequent cirrhosis, two conditions associated with a higher risk of hepatocellular carcinoma (HCC). In addition, accumulation of lipid droplets in infected cells and liver steatosis are common features in the course of chronic hepatitis C suggesting that an interaction between virus life cycle and lipid metabolism may contribute to disease progression. Actually, a positive impact of triglyceride (TG) levels on HCV infection is plausible even more so that the virus is associated with TG in the blood and that virions entry into hepatocytes fluctuates with TG levels (Monazahian et al., 1999). Besides, host-related factors clearly associated with spontaneous viral clearance or a more frequent progression of liver disease have been described (Chen and Morgan, 2006). It has been proposed, therefore, that genetic polymorphisms affecting lipid metabolism and resulting in increased TG levels may act as modulators of HCV clearance (Marzouk et al., 2007; Ryder, 2007).

Recently, genome-wide screens of nonsynonymous SNPs in a mixed American population identified a strong association of a variant in the adiponutrin/patatin-like phospholipase domain-containing 3 (PNPLA3, rs738409C/G, p.I148M) and high liver fat content (Romeo et al., 2008), or a risk to develop severe fibrosis

Abbreviations: HCV, hepatitis C virus; HCC, hepatocellular carcinoma; CHC, chronic hepatitis C; TG, triglyceride; NAFLD, nonalcoholic fatty liver disease; PNPLA3, adiponutrin/patatin-like phospholipase domain-containing 3; PBMCs, peripheral blood mononuclear cells; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; mCHC, mild chronic hepatitis C; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C virus; PegIFN-α, peginterferon-alpha; RVB, ribavirin; SVR, sustained virological response.

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in patients with nonalcoholic fatty liver disease (NAFLD) (Valenti et al., 2010). Adiponutrin, encoded by PNPLA3, a transmembrane protein expressed in human adipose tissue and liver, is a multifunctional enzyme with triacylglycerol lipase and acylglycerol O-acyltransferase activities (Jenkins et al., 2004). However, the precise role of PNPLA3 in liver fat metabolism remains to be determined. Based on an in vitro structural model of the patatin-like domain of PNPLA3 protein, PNPLA3-148M is thought to promote triglyceride accumulation by limiting TG hydrolysis (He et al., 2010). This initial finding was further validated in several populations confirming that the M allele is significantly associated with an increased risk of hepatic triglyceride accumulation and fatty liver disease (Sookoian and Pirola, 2011). Finally, the presence of I148M PNPLA3 variant correlates with more severe histological features in case of metabolic, toxic, or viral insult of liver tissue (Valenti et al., 2012b).

Currently, no genetic data assessing the relationship between the PNPLA3 I148M genetic variant and spontaneous clearance of HCV infection is available. In addition, we have at disposal concerning rs738409 association with HCC data coming mostly from European patients (Burza et al., 2012; Corradini et al., 2011; Falleti et al., 2011; Guyot et al., 2013; Trepo et al., 2012; Valenti et al., 2011). The aim of this study was, therefore, to examine the role of PNPLA3 I148M in patients from Morocco, a North-African country engaged in a nutritional transition process in a context of an obesity epidemic (Batnitzky, 2008; Belahsen et al., 2004). Two manifestations present at opposite ends of HCV infection spectrum i.e., spontaneous clearance and HCC were explored as major outcomes.

2. Materials and methods

2.1. Patients and controls

For study participation, written informed consent for genetic testing was obtained from all individuals. Each participant completed a structured questionnaire on demographic data. The protocol was approved by the ethics Committee of the Faculty of Medicine of Casablanca and the study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki. Both plasma and peripheral blood mononuclear cells (PBMCs) were stored for all patients. A total 437 Moroccan individuals were enrolled in this study at the Medical Center of Biology at the Pasteur Institute of Morocco and Service of Medicine B CHU Ibn Rochd hospital, Casablanca from January 2008 to January 2013. This study included 230 subjects with persistent HCV infection, 75 individuals who spontaneously cleared the virus, and 132 healthy controls. The persistent infection group, included 104 male and 126 female, were positive for anti-hepatitis C virus (anti-HCV) antibodies and HCV RNA by a quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) for at least 6 months. Patients were stratified into two groups according to fibrosis stage. One hundred and twenty nine patients had mild chronic hepatitis C (mCHC) (patients with F0 and F1) and 101 with HCV-related-HCC. The group with spontaneous viral clearance comprised 23 men and 52 women who were positive for HCV-specific antibodies and negative for HCV RNA in patient's sera by qRT-PCR from at least two measurements more than 6 months apart. The healthy controls included 62 men and 70 women who had no medical history of any liver disease and were considered as representative of the local mixed berberic and arabic ethnicity. They were negative for viral hepatitis markers and had normal serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). All patients were hepatitis B surface antigen (HBsAg) and human immunodeficiency virus (HIV) negative. Serological markers for HBsAg, anti-HCV and anti-HIV were tested with commercially available kits (Axsym, Abbott Diagnostics, Wiesbaden-Delkenheim, Germany and Genscreen Ag/Ab HIV Ultra, Biorad, Marnes La Coquette, France). Plasma HCV-RNA was measured by qPCR using COBAS AmpliPrep/COBAS TaqMan (Roche Diagnostics, Germany). Viral loads were determined with reference to internal controls in international units/mL. HCV RNA level below the detection threshold (50 IU/mL) were scored as negative for HCV RNA. Hepatitis C virus genotypes were determined by sequencing as described previously (Brahim et al., 2012).

2.2. Isolation of genomic DNA and PNPLA3 polymorphism genotyping

Genomic DNA was isolated from PBMC using QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Genomic DNA concentration was assessed using a NanoVue plus spectrophotometer (GE Healthcare, US). The rs738409 I148M polymorphism was genotyped using a predesigned TaqMan SNP Genotyping Assay (Applied Biosystems; assay ID C_7241_10). For 10% of the samples, genotyping was repeated for quality control. The results were 100% concordant.

2.3. Statistical analysis

Continuous variables are presented as mean ± standard deviation or median (range) while categorical variables are expressed as frequencies (%). Differences between continuous variables were analyzed with one-way analysis of variance (ANOVA), when necessary after logarithmic transformation in order to create a normal distribution, whilst those between categorical variables were evaluated using the Pearson chi-square test. Departures from Hardy–Weinberg equilibrium were determined by comparing the observed genotype frequencies with expected genotype frequencies in all groups calculated using observed allele frequencies by chi-square G test "Goodness of Fit" with 1 degree of freedom. The existence of differences in allelic and genotypic frequencies between different groups was assessed by means of chi-square test for linear trend when appropriate and calculating the odds ratio (OR) with the 95% confidence intervals (CI). *P*-value <0.05 was considered a significant difference. Statistical analysis of data was performed using the SPSS software package 10.0 (SPSS Inc., USA). All tests were two-sided.

3. Results

The demographic, biochemical, virological characteristics of the population studied are shown in Table 1. A total of 230 individuals with HCV infection including 162 (70.4%) patients infected by HCV genotype 1 and 68 (29.6%) by genotype 2 were analyzed. In addition, 75 subjects who spontaneously cleared HCV and 132 healthy controls were recruited. No patients with HBV or HIV co-infections were included.

3.1. Frequency of PNPLA3 genotype

Out of 132 healthy subjects, 66 (50%) were homozygous for the wild type I/I, 54 (40.9%) were heterozygous, and 12 (9.1%) were homozygous for M/M, with a M allele frequency of 29.5%. No significant deviations in standard genotype distribution according to the Hardy–Weinberg equilibrium ($P = 0.835$) were noted (Table 2).