The allele 4 of neck region liver-lymph node-specific ICAM-3-grabbing integrin variant is associated with spontaneous clearance of hepatitis C virus and decrease of viral loads

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Abstract

L-SIGN is a C-type lectin expressed on liver sinusoidal endothelial cells involved in the capture of hepatitis C virus and *trans*-infection of adjacent hepatocyte cells. The neck region of L-SIGN is highly polymorphic, with three to nine tandem repeats of 23 residues. This polymorphism is associated with a number of infectious diseases, but has not been explored in HCV. We therefore investigated the impact of L-SIGN neck region length variation on the outcome of HCV infection. We studied 322 subjects, 150 patients with persistent HCV infection, 63 individuals with spontaneous clearance and 109 healthy controls. In healthy subjects, we found a total of nine genotypes, with the 7/7 genotype being the most frequent (33%) followed by the 7/6 (22.9%) and the 7/5 (18.3%). The frequencies of the alleles were as follows: 7-LSIGN (56.4%), 6-LSIGN (20.2%), 5-L-SIGN (18.3%) and 4-L-SIGN (5%). The frequency of the 7/4 genotype was higher in spontaneous resolvers (14.3%) as compared with the persistent group (4%) (OR = 0.25, 95% CI = 0.07–0.82, p 0.022). In addition, we found that 4-L-SIGN was associated with spontaneous resolution of HCV infection (OR = 0.30, 95%CI, 0.12–0.74, p 0.005). Interestingly, patients with 4-L-SIGN had lower viral loads when compared with carriers of the 5 (p 0.001), 6 (p 0.021) and 7-alleles (p 0.048). The results indicate that neck region polymorphism of L-SIGN can influence the outcome of HCV infection and the four-tandem repeat is associated with clearance of HCV infection.

Keywords: Chronic hepatitis C, DC-SIGNR, gene polymorphism, outcome, viral loads
Original Submission: 14 May 2013; **Revised Submission:** 29 August 2013; **Accepted:** 15 September 2013
Editor: G. Antonelli
Article published online: 6 November 2013
Clin Microbiol Infect 2014; **20:** O325–O332
10.1111/1469-0691.12403

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Introduction

Chronic hepatitis C virus (HCV) infection is a leading cause of end-stage liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC), with 170 million of the world's population infected [1,2]. Spontaneous eradication of the virus is achieved in only 15–50% of newly infected individuals, while the rest develop chronic infection [2]. The ability of the virus to persist within a host is attributed to its efficient ability to evade the adaptive and innate components of the host's immune system [3,4]. Liver-lymph node-specific ICAM-3-grabbing integrin (L- SIGN), also known as dendritic cell-specific intracellular adhesion molecular-3-grabbing nonintegrin related (DC-SIGNR or CD209L), is a C-type lectin expressed on endothelial cells in the liver and lymph node sinusoids, and might promote spread of viruses that target liver and lymph nodes [5–7]. Several studies have demonstrated that L-SIGN binds soluble HCV E2 and mediates *trans*-infection of liver cells by HCV pseudoparticles (HCVpp) [8–12]. Thus it may concentrate HCV in the liver and enable captured virus to

cross the endothelial barrier, thereby facilitating infection of adjacent hepatocytes [9,13]. These findings raise the hypothesis that interaction of HCV with this molecule in liver and lymph nodes may have important consequences for HCV infection [9].

L-SIGN is a type II transmembrane protein composed of three domains: an N-terminal cytoplasmic domain, a neck region made up of a highly polymorphic domain, and a C-terminal extracellular C-type carbohydrate recognition domain (CRD) involved in pathogen binding [5]. The neck region is responsible for the homo-oligomerization that brings the CRDs into proximity for high-affinity ligand binding [14]. The L-SIGN gene, located on chromosome 19p13, is highly polymorphic in the neck region based on the number of repeats in exon 4. There are between three and nine repeats (3- to 9- alleles) of a 69-base-pair segment [5,15,16]. Each repeat encodes a hydrophobic motif characterized by α-helical coiled coils [14,15]. Disease association studies have shown that polymorphism of the L-SIGN repeat region is associated with protection against SARS coronavirus infection [17] and also HIV infection [18,19]. The high expression levels of L-SIGN on hepatic sinusoidal endothelial cells suggests it may be involved in the capture and concentration of HCV in the liver and hence the establishment of persistent infection [8,20]. In addition, *in vitro* studies have shown that length variation in the L-SIGN neck region could influence the establishment of HCV infection [20]. To our knowledge, the association between L-SIGN neck region length polymorphism and spontaneous clearance of HCV has not been explored. In this study, we tested the hypothesis that this variant may be associated with persistent/spontaneous clearance of HCV infection in a Moroccan population that has been well characterized in terms of the natural outcome of HCV infection.

Material and Methods

Study population

After giving written informed consent for genetic testing, every participant was interviewed and completed a structured questionnaire on demographic data and selected risk factors. The study protocol was evaluated and 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 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee. A total of 322 Moroccan subjects were enrolled in this study. Blood samples from chronic hepatitis C patients and individuals with spontaneous clearance were collected at the Medical Center of Biology at the Pasteur Institute of Morocco and Service of Medicine B CHU Ibn Rochd Hospital, Casablanca, from November 2010 to September 2012. One hundred and fifty patients had persistent HCV infection (90 women, 60 men), among them 40 patients with cirrhosis according to fibrosis stage. All subjects were persistently positive for anti-hepatitis C virus (anti-HCV) antibodies and HCV ribonucleic acid (HCV-RNA) over a period of more than 6 months. Sixty-three individuals had spontaneously resolved HCV infection (45 women, 18 men). All were positive for HCV-specific antibodies and negative for serum HCV RNA by qRT-PCR from at least two measurements more than 6 months apart. To obtain information on the frequency of alleles in the Moroccan population, a set of 109 unrelated anti-HCV and HBsAg-negative healthy subjects (56 women, 53 men) of mixed Berberic and Arabic ethnicity, with normal serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were recruited from the Pasteur Institute of Morocco. All patients were HBsAg and 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 using COBAS AmpliPrep/COBAS TaqMan (Roche Diagnostics, Mannheim, Germany). Genotyping of HCV was determined by PCR-sequencing as described previously [21].

Genotyping of L-SIGN neck region length polymorphism

The genomic DNA samples were obtained from peripheral whole blood using the QIAamp Blood kit (Qiagen, CA, USA) according to the manufacturer's protocols. Genomic DNA concentration was assessed using a NanoVue spectrophotometer (GE Healthcare, Freiburg, Germany) and adjusted to 10 ng/μL in H_20.

The L-SIGN repeat region in exon 4 was amplified from genomic DNA with the following pair of primers: L32: 5′-CCACTTTAGGGCAGGAC-3′ and L28: 5′-AGCAAA CTCA-CACCACA AA-3′, as reported previously [5,16]. PCR was carried out in a final volume of 25 μL, containing 50 ng of genomic DNA, 20 pmol/μL of each primer, 0.5 units of Platinum Taq DNA Polymerase (Invitrogen, Foster City, CA, USA), 200 μM of each dNTP and 1.5 mM MgCl2. The cycle conditions were 5 min at 94°C followed by 30 cycles of 5 s at 95°C, 1 min at 70°C, and then a single cycle of 10 min at 72°C. Allele 9 yielded 716 bp, allele 8 yielded 647 bp, allele 7 yielded 578 bp, allele 6 yielded 509 bp, allele 5 yielded 440 bp, allele 4 yielded 371 bp and allele 3 yielded 302 bp PCR products. Alleles products were resolved on 3% agarose gel and