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
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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

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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 $H_2 0$.

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

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ethidium-bromide staining. For 30% of the samples, PCR was repeated to ensure reproducibility of the assay. The results of the PCRs were 100% concordant.

Genotyping for rs12979860 was performed as described previously [22] by using a TaqMan 5′ allelic discrimination assay custom-designed primers and probes as follows: forward primer TGCCTGTCGTGTACTGAACCA-3′ and reverse 5′-GAGC GCGGAGTGCAATTC-3′, and probes TGGTTCGCGCCTTC (VIC) and CTGGTTCACGCCTTC (FAM) (Applied Biosystems, Foster City, CA, USA). The genotype of each sample was analysed by using SDS 1.1 software for allelic discrimination (ABI7000, Applied Biosystems).

Statistical analysis

Comparisons of continuous variables between groups were assessed with the Mann–Whitney *U*-test and a post hoc correction for multiple comparisons was made for an ANOVA test. For categorical variables the χ^2 test or Fischer's exact test was used. Allele and genotype frequencies were obtained by direct counting. Departures from Hardy–Weinberg equilibrium (HWE) were determined by comparing the observed genotype frequencies with expected genotype frequencies using Arelquin software. The Fisher's exact test or χ^2 test was used to determine differences in allele/genotype frequencies. A p value of <0.05 was considered statistically significant. All tests were two-sided. Logistic regression was performed using the multinomial logistic regression analysis tool of SPSS v20. Only genotypes represented in both groups were included in the analysis and the 7/7 genotype or 7/7 and C/C genotypes were used as the reference group.

Results

Table 1 illustrates the main demographic and clinical characteristics. There were no significant differences between groups in terms of age and gender (p >0.05). As shown in Table 2, overall, the most common genotype in our population was 7/7 (33%), followed by 7/6 (22.9%) and 7/5 (18.3%). Allele 7 has the highest frequency at 56.4%, followed by allele 6 at 20.2%, allele 5 at 18.3% and allele 4 at 5%. The genotype frequencies in the control population did not deviate from the Hardy-Weinberg equilibrium (p 0.315).

We next examined whether the frequency distributions of L-SIGN neck region genotypes and alleles were significantly different between the HCV-infected and resolved groups. Logistic regression analysis of the association of genotype with outcome demonstrated that the 7/4 genotype was the only genotype significantly associated with resolution of HCV (p 0.022, OR = 0.25, 95% CI = 0.07–0.82) (Table 2). The 7/4 genotype frequency was over-represented in the resolved group (14.3%) as compared with the chronic patients (4%) (p 0.015) (Table 3).

TABLE 1. Demographic and clinical characteristics of the study subjects

	Chronic hepatitis C (*n* = 150)	Spontaneous clearance (*n* = 63)	Healthy controls (*n* = 109)
Mean age ± SD, years	63.09 ± 12.06	59.81 ± 12.81	56.45 ± 10.86
Gender (male/female)	60/90	18/45	53/56
ALT (IU/L)	74.96 ± 43.10	29.81 ± 14.61	27.49 ± 6.02
AST (IU/L)	77.25 ± 6.11	29.11 ± 18.36	25.21 ± 9.37
Median viral load (IU/mL)	868 500		
HCV genotype 1/2	99/51		

A previous study showed that the medium length 6- or 7-tandem repeats form a rigid neck region that projects the CRDs away from the membrane, ensuring strong multivalent adhesion to the target membrane, thus promoting the access to pathogens [23]. Therefore, we grouped these alleles and the frequency of 6/6 + 7/6 + 7/7 genotypes as higher in the chronic group (82/150, 54.7%) than the spontaneous resolvers (24/61, 39.3%) (OR = 1.86, 95% CI = 0.97–3.57, p 0.04) (Table 2).

As for allelic frequencies, we found that allele 4 was associated with spontaneous clearance of HCV infection (OR = 0.30, 95% CI = 0.12–0.74). Analysis between spontaneous clearance group with allele 4 and chronic patients group showed that this allele is most prevalent in the spontaneous subjects (11.1% vs. 3.7%, respectively, p 0.005).

In order to explore the contribution of L-SIGN neck region polymorphism to HCV replication and necroinflammatory activity, we analysed viral loads in infected cases according to the various L-SIGN alleles (Fig. 1a). Patients carrying allele 4-had lower viral loads (median = 121 266 UI/mL) than patients with allele 5- (median = 862 317 UI/mL) (p 0.001), allele 6-(median = 842 000 UI/mL) (p 0.021) and allele 7- (median = 785 324 UI/mL) (p 0.048). In contrast, no significant correlation was found between L-SIGN alleles and mean ALT (Fig. 1b, p >0.05) or AST levels (Fig. 1c, p >0.05). To test the association between the L-SIGN variants and liver damage, we genotyped this polymorphism in 40 patients with cirrhosis then compared them with 109 healthy subjects. The distribution of allelic frequencies was not significant between patients and healthy subjects (Fig. 1d, p >0.05).

In addition, when chronic HCV patients were stratified by their viral genotypes, the frequencies of L-SIGN genotypes were not different between patients with genotype 1 (G-1) and patients with genotype 2 (G-2) (Fig. 2a).

Finally, we checked the response of patients with shorter and longer tandem repeat to PegIFN-α plus RBV treatment.

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TABLE 2. L-SIGN neck-region polymorphism among Moroccan healthy controls, spontaneous clearance subjects and the HCV-infected group

	Moroccan healthy group (*n* = 109) (%)	Spontaneous clearance (*n* = 63) (%)	Chronic hepatitis C (*n* = 150) (%)	Spontaneous clearance vs. chronic hepatitis C OR (95%CI)	p-value (Pc)
Genotypes					
4/4	2 (1.8)	1 (1.6)	0 (0)	na	1.000
5/4	1 (0.9)	1 (1.6)	2 (1.3)	0.74 (0.06–8.77)	0.809
6/4	0 (0)	2 (3.1)	3 (2)	0.55 (0.08–3.66)	0.539
7/4	6 (5.5)	9 (14.3)	6 (4)	0.25 (0.07–0.82)	0.022 (0.264)
5/5	7 (6.4)	2 (3.1)	4 (2.7)	0.74(0.12–4.48)	0.740
6/5	5 (4.6)	7 (11.1)	23 (15.3)	1.21 (0.42–3.44)	0.720
7/5	20 (18.3)	16 (25.4)	28 (18.7)	0.65 (0.27–1.54)	0.321
8/5	0 (0)	0 (0)	1 (0.7)	na	1.000
6/6	7 (6.9)	4 (6.3)	15 (10)	1.38 (0.39–4.88)	0.616
7/6	25 (22.9)	7 (11.1)	29 (19.3)	1.53 (0.54–4.27)	0.420
7/7	36 (33)	14 (22.2)	38 (25.3)	1.00	
9/7	0 (0)	0 (0)	1 (0.7)	na	1.000
Homozygotes	52 (47.7)	21 (33)	57 (38)		
Heterozygotes	57 (52.3)	42 (67)	93 (62)		
	***n* = 218**	***n* = 126**	***n* = 300**		
L-SIGN alleles					
4	11 (5)	14 (11.1%)	11 (3.7)	0.30 (0.12–0.74)	0.005 (0.030)
5	40 (18.3)	28 (22.2%)	62 (20.7)	0.91 (0.53–1.56)	0.795
6	44 (20.2)	24 (19%)	85 (28.3)	1.68 (0.98–2.89)	0.052
7	123 (56.4)	60 (47.7%)	140 (46.7)	0.96 (0.62–1.49)	0.915
8	0 (0)	0 (0%)	1 (0.3)	na	1.000
9	0 (0)	0 (0%)	1 (0.3)	na	1.000

na, not applicable.
Pc, Bonferroni correction was applied.

TABLE 3. Risk of chronicity for combinations of *L-SIGN* and *IL28B* genotypes

L-SIGN polymorphism	IL28B rs12979860	Chronic hepatitis C (*n* = 150)	Spontaneous clearance (*n* = 63)	OR (95%CI)	p-value
7/7	CC	14 (9.3%)	8 (12.7%)	1.00	
7/7	CT	10 (6.7%)	3 (4.8%)	1.93 (0.40–9.02)	0.413
7/7	TT	14 (9.3%)	3 (4.8%)	2.67 (0.583–12.18)	0.198
7/6	CC	11 (7.3%)	3 (4.8%)	2.09 (0.45–9.81)	0.343
7/6	CT	12 (8%)	4 (6.3%)	1.71 (0.41–7.14)	0.457
7/6	TT	6 (4%)	0 (0%)	7.62 (0.38–152.83)	0.080
6/6	CC	5 (3.3%)	2 (3.2%)	1.43 (0.22–9.14)	0.705
6/6	CT	8 (5.3%)	2 (3.2%)	2.28 (0.39–13.50)	0.355
6/6	TT	2 (1.3%)	0 (0%)	2.93 (0.12–68.55)	0.296
6/5	CC	13 (8.7%)	5 (7.9%)	1.49 (0.39–5.72)	0.564
6/5	CT	8 (5.3%)	1 (1.6%)	4.57 (0.48–43.50)	0.160
6/5	TT	2 (1.3%)	1 (1.6%)	1.14 (0.09–14.68)	0.918
7/5	CC	9 (6%)	9 (14.3%)	0.57 (0.16–2.03)	0.385
7/5	CT	15 (10%)	2 (3.2%)	4.29 (0.77–23.74)	0.081
7/5	TT	4 (2.7%)	5 (7.9%)	0.46 (0.09–2.21)	0.325
7/4	CC	3 (2%)	8 (12.7%)	0.21 (0.04–1.05)	0.048
7/4	CT	1 (0.7%)	0 (0%)	1.75 (0.06–48.19)	1.000
7/4	TT	2 (1.3%)	1 (1.6%)	1.14 (0.09–14.68)	0.918
5/5	CC	1 (0.7%)	2 (3.2%)	0.29 (0.02–3.67)	0.315
5/5	CT	3 (2%)	0 (0%)	4.10 (0.19–89.44)	0.205
6/4	CC	2 (1.3%)	2 (3.2%)	0.57 (0.07–4.87)	0.606
6/4	CT	1 (0.7%)	0 (0%)	1.75 (0.06–48.19)	1.000
5/4	CC	2 (1.3%)	1 (1.6%)	1.14 (0.09–14.68)	0.918
4/4	CC	0 (0%)	1 (1.6%)	0.19 (0.01–5.35)	0.202
8/5	CC	1 (0.7%)	0 (0%)	1.75 (0.06–48.19)	0.455
9/7	CC	1 (0.7%)	0 (0%)	1.75 (0.06–48.19)	0.455

We analysed this polymorphism with viral clearance after treatment in patients infected with genotype 1b. Frequencies of 4-, 5-, 6- and 7- were 4%, 12%, 48% and 35% in In non-responders group and were 8%, 15%, 46% and 31% in sustained virological response (SVR) group, respectively ($p > 0.05$) (Fig. 2b).

Given that *L-SIGN* and *IL28B* genes are located on chromosome 19, we hypothesized that clearance could be detected when favourable genotypes are combined in a given subject. Consequently, statistical gene–gene interactions between the L-SIGN and IL28B polymorphisms were examined (Table 3). We observed that cleared subjects carrying the 7/4 genotype had a higher frequency of the *IL28B*-CC genotype than chronic patients (12.7% vs. 2%, p 0.003). Further, a significant spontaneous clearance of HCV infection among *L-SIGN 7/4* and IL28B-CC genotype carriers with OR of 0.21 (95% CI = 0.04–

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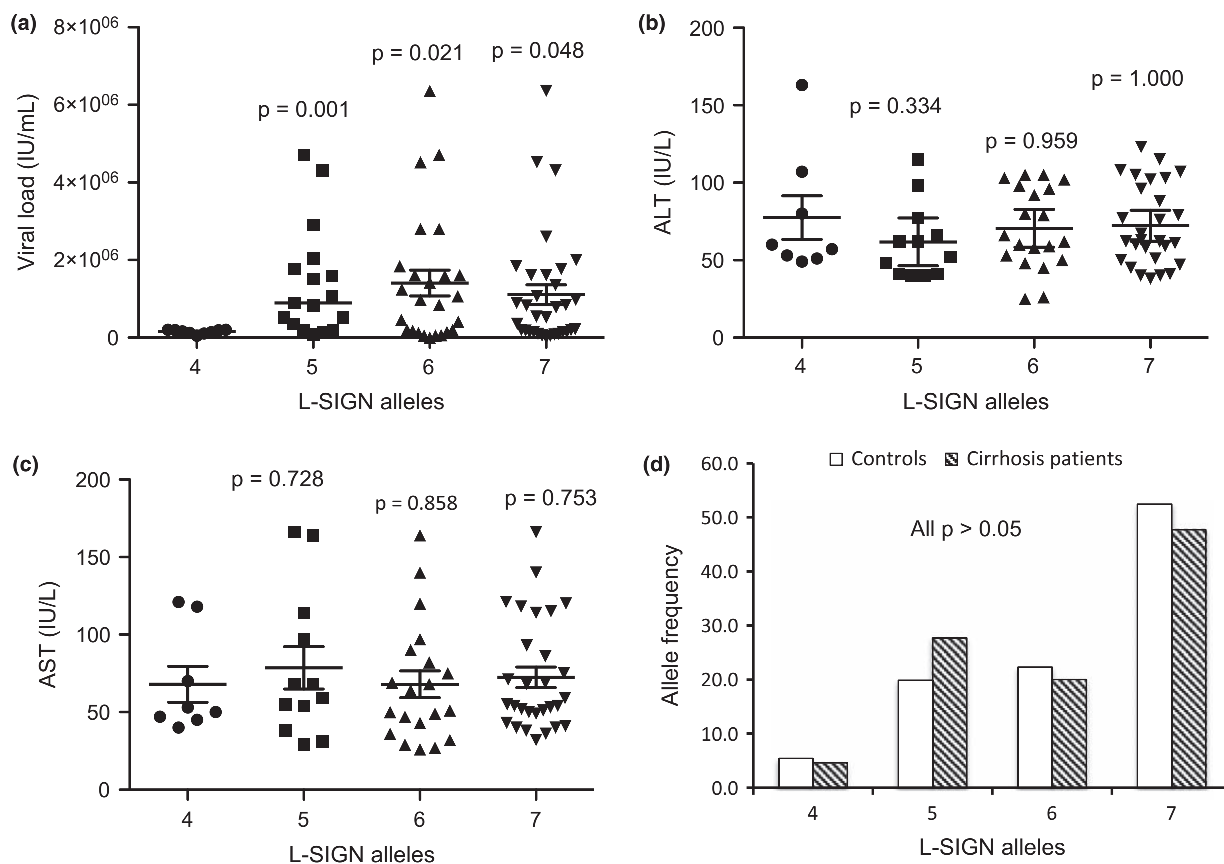

FIG. 1. Analysis of virological, biochemical parameters and stage of disease in relation to L-SIGN. (a) Scatter plot and association of viral loads. ALT and alleles in HCV-infected patients. (b) The mean AST levels stratified with respect to L-SIGN alleles and the results are shown as mean and SEM. (c) AST levels according to L-SIGN and the results are shown as mean and SEM. (d) Distribution of L-SIGN variants in cirrhosis patients and healthy controls.

1.05, p 0.048) was observed, suggesting a possible link between *L-SIGN* and *IL28B* genotypes in clearance of the virus.

Finally, to check the role of L-SIGN genotype 7/4 in the prevalence of spontaneous HCV clearance, we stratified subjects according to L-SIGN- 7/4 and IL28B-CC genotypes (Fig. 2c). The result indicated that the prevalence of spontaneous clearance is higher (52.4%) among individuals with the CC genotype without 7/4, followed by those carrying both CC and 7/4 genotypes (12.7%).

Discussion

L-SIGN is a calcium-dependent carbohydrate-binding protein, specialized in the recognition of carbohydrate structures present on cellular and viral proteins and implicated in several processes such as cell adhesion and antigen presentation [15,24]. L-SIGN has been shown to act as a receptor with high-affinity-binding to hepatitis C virus glycoprotein E2 and may facilitate transmission into adjacent hepatocytes [8–12]. The neck region of the L-SIGN region plays a crucial role in the tetramerization of the receptor and the support of CRDs, thus influencing the pathogen-binding properties of these receptors. The neck region of L-SIGN repeats is highly variable and varies with ethnic groups [25]. Therefore, we first examined the frequency of this polymorphism in a Moroccan population characterized by mixed Berberic and Arabic ethnicity as determined by their geographical location between the Mediterranean Sea, the Atlantic Ocean and the Sahara desert. The frequency of heterozygosity for L-SIGN alleles was similar in our Moroccan population (52.3%) to that reported in East Asian populations (52%), Caucasian European populations (61.5%) and Middle-Eastern populations (60.8%) [25], and the frequency of heterozygotes was higher than that reported in Chinese (45%) [17] and Japanese (37.4%) populations [26]. Allelic distribution of repeats in Moroccan individuals was mostly represented by the wild-type 7-repeat form (56.4%), followed by the 6- (20.2%), 5- (18.3%) and 4- alleles (5%). This

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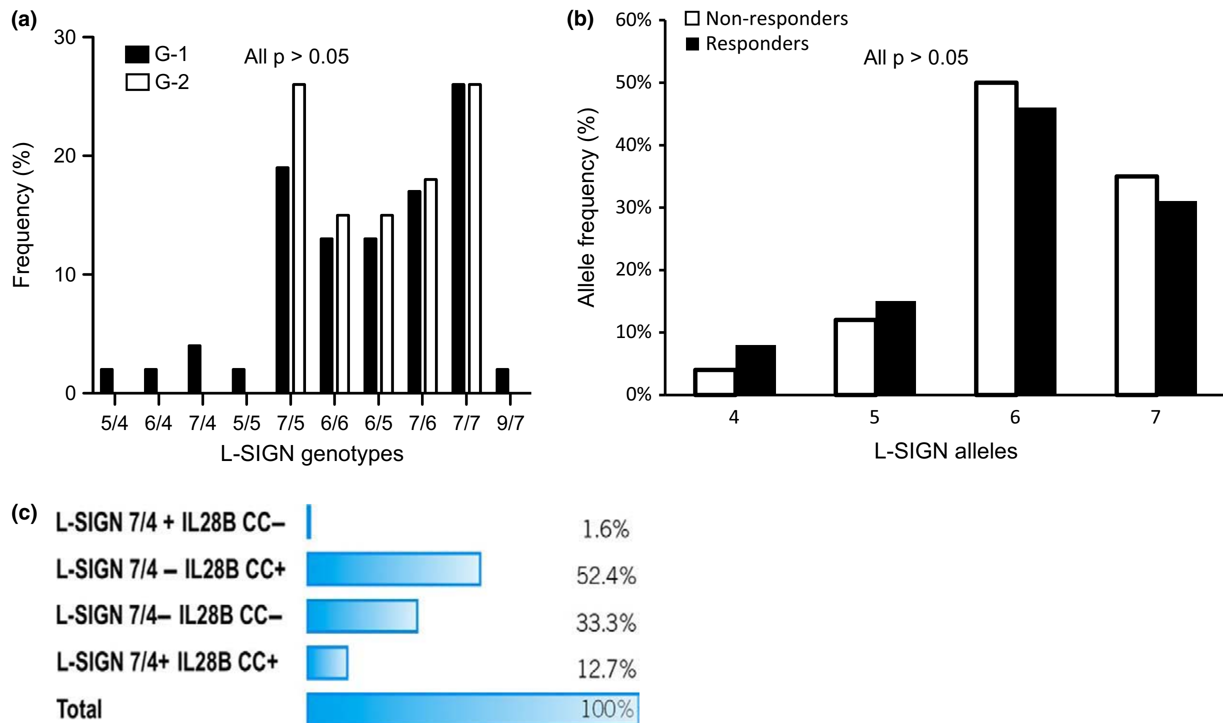

FIG. 2. Association of L-SIGN alleles and HCV infection. (a) This figure shows L-SIGN genotype distribution and HCV genotypes (G-1, Genotype 1; G-2, genotype 2). (b) Frequency of L-SIGN alleles in HCV genotype 1-infected patients according to outcomes after treatment with PEG-IFN/ribavirin. (c) Prevalence of spontaneous HCV clearance according to L-SIGN and IL28B rs12979860 genotypes (+, presence of genotype; −, absence of genotype).

distribution is closer to that reported in Caucasian populations [5,25,27]. The diversity of L-SIGN polymorphisms is due to balancing selection in non-African populations [25]. However, a recent study showed that demographic migration of different tribes might also explain L-SIGN diversity [28].

In a large group of individuals from two combined cohorts it has been demonstrated that the homozygous 7/7 genotype was significantly associated with an increased risk of HIV-1 infection (OR = 1.87, p 0.0015), with the heterozygous 7/5 genotype correlating with resistance to HIV-1 infection (OR = 0.69, p 0.029) [18]. This result was further confirmed in additional studies [19,29]. Moreover, a study on SARS patients showed that individuals homozygous for L-SIGN neck-region repeats were less susceptible to SARS-CoV infection (OR = 0.649; p 0.005) [17]. However, these correlations were not found in studies on HIV [30–32] or studies of *Mycobacterium tuberculosis* infections [33]. The discrepancy between these studies may be related to ethnic differences between the different studies. A study, however, explored the possible association between L-SIGN neck-region variation and HCV infection using HCV-infected patients and healthy controls. Although they did not find a difference between these two groups within the HCV population, they found that individuals with the 4 and 9 alleles had lower viral loads, which agrees well with our results [34].

Our study explores, for the first time, the role of L-SIGN neck-region variation in the clearance of HCV infection, particularly the 7/4 genotype and 4- allele. The length variation of this neck region had been proposed to affect the pathogen-binding properties of the CRD of these proteins [15,24]. Moreover, *in vitro* studies show that cells with the L-SIGN-7 allele have higher levels of capture and *trans*-infection decreased with progressive deletions of the tandem repeats [20]. In addition, it has been reported that the number of tandem repeats also influences binding affinity for HIV-1 gp120 [35]. Recently, it has been shown that loss of neck repeats in this variant reduces the extension of the CRDs from the membrane surface, a feature that could reduce access to pathogens [23]. These data indicate that L-SIGN isforms thereby serve to modulate the outcome of HCV infection and disease progression by influencing viral entry [20]. Interestingly, our work suggests that variations in the number of L-SIGN repeats were linked with HCV viraemia. Patients with 5-, 6- and 7-repeat alleles had higher HCV-RNA loads than patients with the 4-repeat allele. This finding is consistent with a previous report [34] highlighting the concept of *trans*-infection

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and capture of the HCV virus to infect adjacent hepatocyte cells and HCV replication efficacy were influenced by the L-SIGN neck-region length. Furthermore, we found that variations in L-SIGN genotypes/alleles were not associated with ALT and AST levels, suggesting that this polymorphism was not involved in hepatic inflammation and also there was no association with viral genotype and treatment response. Indeed, this result was confirmed when we analysed the L-SIGN isoforms with the progression of disease to cirrhosis. These preliminary data indicate that L-SIGN neck region length polymorphism has no affect on liver damage.

In summary, our data suggest that L-SIGN neck region length polymorphism is associated with spontaneous clearance of HCV and the replication efficiency of the hepatitis C virus. Given the relatively small sample size in our study, future studies of L-SIGN polymorphisms are warranted with larger cohorts of individuals with diverse ethnic backgrounds.

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Transparency Declaration

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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