# The *IL7RA* rs6897932 polymorphism is associated with progression of liver fibrosis in patients with chronic hepatitis C: Repeated measurements design

María Ángeles Jiménez-Sousa[1◉], **Ana Zaida Gómez-Moreno**[2◉], **Daniel Pineda-Tenor**[3], **Luz Maria Medrano**[1], **Juan José Sánchez-Ruano**[2], **Amanda Fernández-Rodríguez**[1], **Tomas Artaza-Varasa**[2], **José Saura-Montalbán**[4], **Sonia Vázquez-Morón**[1], **Pablo Ryan**[5,6,7], **Salvador Resino**[1]*
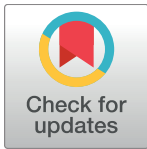
1 Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Majadahonda, Spain, 2 Servicio de Digestivo, Hospital Virgen de la Salud, Toledo, Spain, 3 Servicio de Laboratorio Clínico, Hospital Universitario de Fuenlabrada, Madrid, Spain, 4 Servicio de Laboratorio Clínico, Hospital Virgen de la Salud, Toledo, Spain, 5 Servicio de Medicina Interna, Hospital Universitario Infanta Leonor, Madrid, Spain, 6 Facultad de Medicina, Universidad Complutense de Madrid (UCM), Madrid, Spain, 7 Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain

◉ These authors contributed equally to this work.
* sresino@isciii.es

Abstract

The polymorphisms at the α-chain of the IL-7 receptor (*IL7RA*) have been related to T-cell homeostasis and development and may contribute to immune system deregulation. In the present study, we analyzed the association between *IL7RA* polymorphisms and the progression of liver fibrosis in patients infected with HCV. We carried out a retrospective study with a design consisting of repeated measurements in 187 HCV-infected patients, to study the risk prediction of liver fibrosis progression using genetic factors. We genotyped the rs6897932, rs987106 and rs3194051 *IL7RA* polymorphisms using the Agena Bioscience's MassARRAY. Transient elastography was used to measure liver stiffness. The used cut-offs were: <7.1 kPa (F0-F1), 7.1–9.4 kPa (F2; significant fibrosis), 9.5-12.4 kPa (F3; advanced fibrosis), and ≥12.5 kPa (F4; cirrhosis). All HCV genotypes were analyzed. The median of follow-up time was 47.9 months. Baseline liver stiffness measurement (LSM) values did not show significant statistical differences for *IL7RA* genotypes (p>0.05). In univariate analysis, the rs6897932 T allele had a positive relationship with an increase in LSM (arithmetic mean ratio (AMR) = 1.21 (95%CI = 1.08; 1.36); p = 0.001), progression to advanced fibrosis (F≥3) (odds ratio (OR) = 2.51 (95%CI = 1.29; 4.88); p = 0.006) and progression to cirrhosis (F4) (OR = 2.71 (95%CI = 0.94; 5.03); p = 0.069). In multivariable analysis, the rs6897932 T allele was related to a higher increase of LSM values during follow-up (adjusted AMR = 1.27 (95%CI = 1.13; 1.42); p<0.001) and higher odds of progression to advanced fibrosis [adjusted OR = 4.46 (95%CI = 1.87; 10.62); p = 0.001], and progression to cirrhosis [adjusted OR = 3.92 (95%CI = 1.30; 11.77); p = 0.015]. Regarding *IL7RA* rs987106 and rs3194051 polymorphisms, we did not find significant results except for the relationship between *IL7RA* rs987106 and the increase in LSM values [adjusted OR = 1.12

(95%CI = 1.02; 1.23); p = 0.015]. The *IL7RA* rs6897932 polymorphism seems to be related to increased risk of liver fibrosis progression in HCV-infected patients. Thus, the rs6897932 polymorphism could be related to the physiopathology of CHC and might be used to successfully stratify the risk of CHC progression.

Introduction

Hepatitis C virus (HCV) infection results in chronic hepatitis C (CHC) in about 80% of the subjects exposed. HCV infection is related to the development of liver fibrosis, cirrhosis and end-stage liver disease [1–3], and is a major cause for liver transplantation [4,5]. However, CHC progression presents a great deal of variability, from reduced rates of liver fibrosis progression to massive fibrosis and cirrhosis in a short time [6]. The clinical management of CHC patients depends on the disease stage [7], since patients with cirrhosis have a shorter median survival time than those without cirrhosis [6]. Thus, the appropriate identification of patients at risk of liver fibrosis progression and cirrhosis is essential to take preventive measures that may affect the course of CHC [8]. In this regard, transient elastography may accurately predict the presence of fibrosis/cirrhosis in patients with CHC [9]. However, the recent introduction of direct-acting antivirals (DAAs) has revolutionized HCV treatment with sustained virological response rates greater than 90% [10]. HCV eradication has been associated with a reduction in the risk of liver-related events such as hepatic decompensation, hepatocellular carcinoma and death due to liver disease [11]. However, patients with fibrosis bridges (F3) or cirrhosis (F4), despite the eradication of HCV, remain at risk of progression of the disease and of hepatocellular carcinoma at least 8–10 years after the virological cure [12,13].

Interleukin-7 (IL-7) plays a role in homeostasis and development of T cells [14]. IL-7 is also a critical factor in T-cell-mediated antiviral response [15]. The IL-7 receptor is made up of a heterodimer consisting of the α-chain of the IL-7 receptor (IL7RA or CD127) and the gamma chain of the common cytokine receptor (CD132) [14]. In this way, HCV clearance is related to the early expression of IL7RA on HCV-specific T cells during the acute phase of HCV infection [16]. Additionally, IL-7 released by hepatocytes during HCV infection eventually leads to viral clearance [17]. Moreover, IL-7 and IL7RA levels decrease during CHC, which leads to impaired reactivity of HCV-specific cytotoxic T cells [18,19].

IL7RA polymorphisms have been related to the homeostasis and development of T-cells, so they may contribute to the deregulation of the immune system [20,21]. Furthermore, *IL7RA* polymorphisms are related to autoimmune diseases [22,23] and CD4+ T cell recovery in human immunodeficiency virus (HIV)-infected patients on antiretroviral therapy [24–26]. Among HCV-infected patients, *IL7RA* polymorphisms have been associated with the response to peg-interferon (IFN)-α/ribavirin therapy against HCV and severe liver fibrosis in HIV/HCV-coinfected patients [27,28].

We aimed to analyze the relationship between *IL7RA* polymorphisms and the progression of liver fibrosis in patients infected with HCV in a study with repeated measurements.

Materials and methods

Study population

We performed a retrospective cohort study (repeated measurements design) in 187 HCV-infected patients in the Hospital Virgen de la Salud (Toledo, Spain) between 2008 and 2015.

This study was developed according to the ethical principles of the 1975 Declaration of Helsinki. The study was approved by the Institutional Review Board of the Instituto de Salud Carlos III ("Comités de Ética de la Investigación y Bienestar Animal"– 04/04/2013). Patients included in this study signed a written informed consent. This study has been reported in accordance with the FibroSTARD guidelines.

We considered as inclusion criteria: 1) detectable HCV RNA in plasma during follow-up; 2) data availability of liver stiffness measurement (LSM) for a range of at least 12 months; 3) availability of a DNA sample. The exclusion criteria were: 1) advanced fibrosis or cirrhosis at baseline (F3 or F4; LSM≥9.5); 2) co-infection with hepatitis B virus or HIV; 3) autoimmune liver disease such as primary biliary cholangitis and autoimmune hepatitis.

From around 1500 HCV-infected patients attended to at the Hospital Virgen de la Salud (Toledo, Spain), we had 608 blood samples available from May 2013 to May 2015. Of them, 92 patients were discarded due to incomplete clinical data, leaving 516 patient samples that were sent for DNA genotyping. These 516 patients constitute our study cohort for this and future studies. Of them, 77 patients were discarded due to unavailable LSM data, 178 patients due to unavailable LSM2 data at least 12 months after baseline LSM, 37 patients for being responders to HCV treatment before the last LSM, and 37 patients because the baseline LSM was ≥9.5 ([Fig 1](#)).

Clinical data

Medical records were used to obtain clinical and epidemiological data. We considered a high alcohol intake to be levels above 60 grams/day for men and above 20 grams/day for women, which has been described as a risk factor for the development of cirrhosis [29]. Time since HCV diagnosis was defined as the time between HCV diagnosis and the first LSM (LSM1, the baseline of study).

The management of the patients during follow-up was conventional. For this reason, HCV therapy could be used before or after the inclusion into the study, following the recommendations of clinical guidelines [30,31]. However, we only included those patients who were non-responders from patients undergoing HCV treatment. In the case of patients who achieved a sustained virological response (SVR), their follow-ups were truncated at the time of HCV therapy initiation, because elimination of HCV may cause the regression of liver fibrosis and cirrhosis [32]. The time of follow-up was the time between the first LSM (LSM1) and the last LSM (LSM2, the end of study). The administrative censoring date was March 31, 2016.

Genotyping of *IL7RA* polymorphisms

Three single nucleotide polymorphisms (SNPs) located at the *IL7RA* gene [rs987106 (intronic region), rs6897932 (exon 6) and rs3194051 (exon 8)] were evaluated in all patients who met the inclusion criteria regardless of LSM value. Our group has previously analyzed these polymorphisms in HIV-coinfected patients in two recent papers [27,28]. These *IL7RA* polymorphisms are located at a putative regulatory region, but their effects still are unclear. Their minor allelic frequencies were greater than 20% in white Europeans.

QIAsymphony DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract DNA from 200μl of peripheral blood. The Spanish National Genotyping Center (CeGen; http://www.cegen.org/) genotyped the samples by Agena Bioscience's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system.

The 1000 Genomes Project website (http://www.1000genomes.org/home) were used to obtain the frequencies of alleles and genotypes represented in the general population. This database contains a broad representation of common human genetic variation from multiple
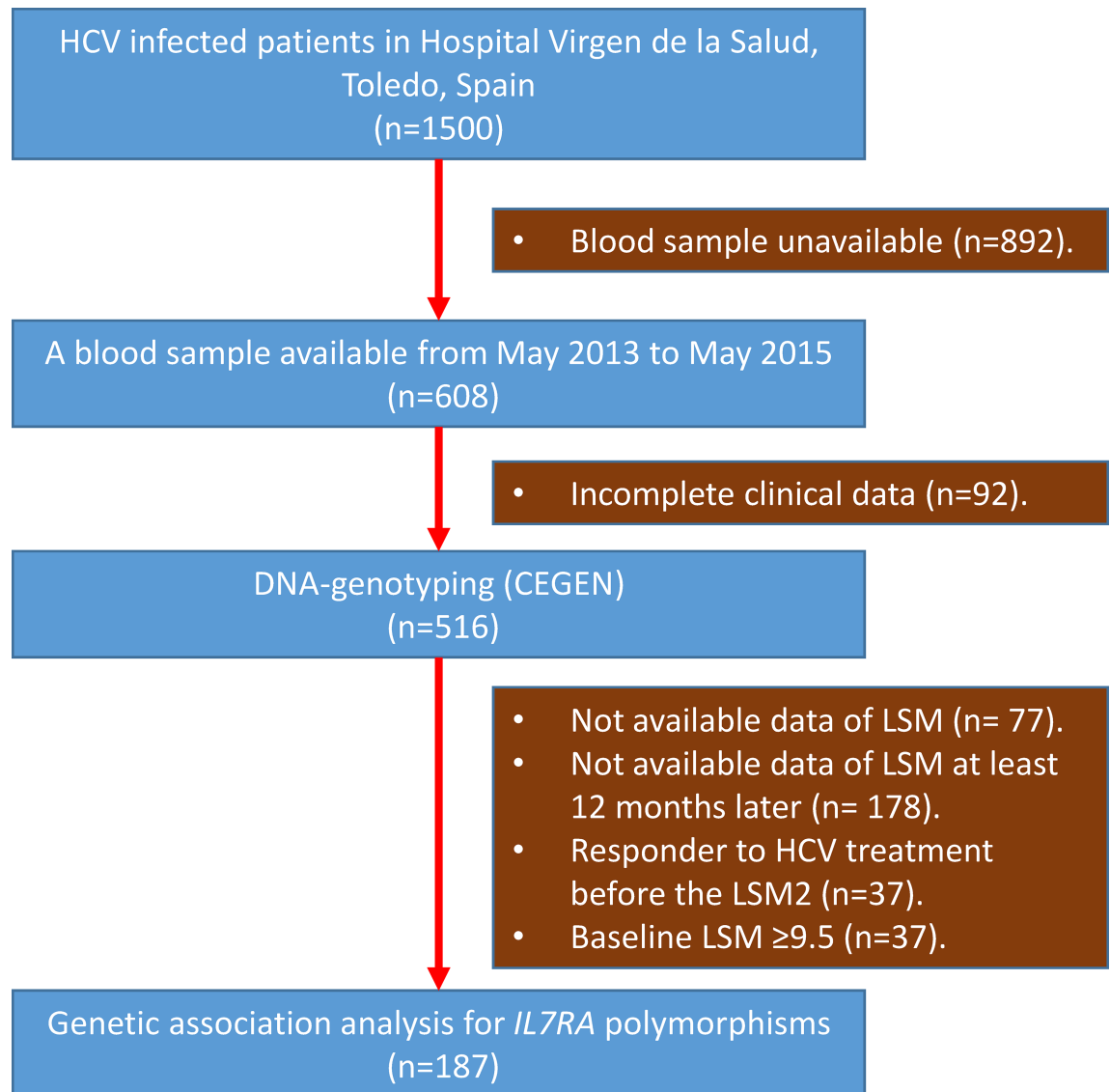
Fig 1. Flow chart of patients included in the genetic association analysis for *IL7RA* polymorphisms. Abbreviations: HCV, hepatitis C virus; *IL7RA*, gene of α-chain of the IL-7 receptor; LSM, liver stiffness measurement (LSM).

https://doi.org/10.1371/journal.pone.0197115.g001

populations [33], including the Iberian population in Spain (IBS) and Utah residents with Northern and Western European ancestry (CEU), which were included in the study as a comparative control.

Liver stiffness measurement

One trained hepatologist evaluated liver stiffness using a single machine of transient elastography (FibroScan®, Echosens, Paris, France). The results were expressed in kilopascals (kPa), with a range of 2.5 to 75 kPa [34]. We considered the measurements appropriate when the interquartile-range-to-median ratio for at least ten successful measurements was lower than 0.30. All LSM measurements were performed with at least four hours of fasting. Advanced obese patients were not included in this study because we did not have access to an XL probe, and the M probe was used in all cases. The cut-offs used were: <7.1 kPa (F0-F1), 7.1–9.4 kPa

(F2; significant fibrosis), 9.5-12.4 kPa (F3; advanced fibrosis), and ≥12.5 kPa (F4; cirrhosis) [35]. In this regard, many LSM cut-offs have been reported recently for stratifying patients according to liver fibrosis. However, we used the LSM cut-offs described by Castera et al (2005) [35], because these have been validated by a large number of scientific articles and have been recently endorsed by the American Gastroenterological Association [36].

Outcome variable

The primary outcome variable was the change in LSM values during follow-up (continuous variable). We calculated the LSM variation between the last LSM (LSM2) and the first LSM (LSM1) during follow-up (ratio LSM2/LSM1). Furthermore, we evaluated the progression to advanced fibrosis, which is a dichotomous variable that may have values of +1 [if F≤2 (F0, F1 or F2) changes to F≥3 (F3 or F4)] or 0 [if F ≤2 (F0, F1 or F2) remains)]. The progression to cirrhosis may also have values of +1 [if F≤2 (F0, F1, or F2) changes to F4] or 0 (if F≤2 (F0, F1 or F2) remains).

Statistical analysis

We evaluated the genetic association according to dominant, recessive and additive models of inheritance. The best model was chosen according to the goodness of fit evaluated by Akaike information criterion (AIC) value and Bayesian information criterion (BIC).

The Chi-square test or Fisher's exact test (categorical variables) and Mann-Whitney U test (continuous variables) were used to evaluate the differences between *IL7RA* genotypes. The genetic association of *IL7RA* polymorphisms with the outcome variables was evaluated using a Generalized Linear Model (GLM). When we analyzed continuous variables, a GLM with a gamma distribution (log-link) was employed. This test provides the differences between groups and the arithmetic mean ratio (AMR) that indicates the ratio by which the arithmetic mean of the original outcome should be multiplied. In this way, a GLM with a binomial distribution (logit-link) was employed to investigate dichotomous outcome variables (progression to F≥3 and progression to F4). This test provides the odds ratio (OR) for dichotomous outcome variables. Each multivariable analysis was adjusted by the most significant co-variables associated with each one of the outcome variables, avoiding the over-fitting of the model. At each step, covariables were considered for removal with a p-value for exit greater than 0.50. The factors used were age, gender, diabetes, time since HCV diagnosis, HCV genotype, injection drug use (IDU), high alcohol intake, HCV antiviral therapy before baseline and during follow-up (patients who failed therapy), the baseline of LSM, and the time of follow-up. Although IDU does not have a direct association with liver fibrosis progression, we included IDU as a covariable because these subjects generally have a worse lifestyle, which can negatively affect the progression of liver disease [36].

The Statistical Package for the Social Sciences (SPSS) 21.0 software (IBM Corp., Chicago, USA) was used to perform the statistical tests. Haploview 4.2 software was used to evaluate the pairwise linkage disequilibrium (LD). The frequencies of haplotypes were estimated considering the Expectation-Maximization algorithm. PLINK software (http://zzz.bwh.harvard.edu/plink/) was used to analyze the association between haplotypes and fibrosis progression. All p-values were two-tailed and statistical significance was defined as p<0.05.

Results

Study population

The baseline characteristics of 187 HCV-infected patients are summarized in **Table 1**.

Table 1. Clinical and epidemiological characteristics of HCV-infected patients.

Characteristic	All Patients
No.	187
Male sex	102 (54.5%)
Age (years)	46.4 (40.9; 55.8)
Time of HCV infection (years)	7.5 (2.9; 12.9)
High alcohol intake	25 (13.4%)
Prior injection drug use	20 (10.7%)
HCV genotype (n = 184)	
1	154 (83.7%)
3	14 (7.6%)
4	15 (8.2%)
5	1 (0.5%)
Prior peg-IFN-α/RBV therapy failed	42 (22.5%)
Baseline LSM (kPa)	6.1 (5.3; 8.4)
F0-F1 ($<$7.1 kPa)	149 (79.7%)
F2 (7.1–9.4 kPa)	38 (20.3%)
Follow-up time (months)	47.9 (29.2; 62.7)
Final LSM (kPa)	6.7 (5.3; 8.6)
F0-F1 ($<$7.1 kPa)	108 (57.8%)
F2 (7.1–9.4 kPa)	47 (25.1%)
F3 (9.5–12.4 kPa)	15 (8.0%)
F4 ($\geq$12.5 kPa)	17 (9.1%)

Values expressed as absolute numbers (%) and median (percentile 25; percentile 75). High alcohol intake was considered as levels above 60 grams/day for men and above 20 grams/day for women.

Abbreviations: Abbreviations: HCV, hepatitis C virus; LSM, liver stiffness measurement; kPa, kilopascal; IFN, interferon; peg-IFN-α/RBV, peg-interferon-alpha/ribavirin.

https://doi.org/10.1371/journal.pone.0197115.t001

We observed that 38 patients presented significant fibrosis (LSM$\geq$7.1 kPa). Male patients made up 54.5% of the total, and the median age was 46.4 years, with a high intake of alcohol rate of 13.4%. Nearly eleven percent of patients were prior injection drug users, 83.7% were infected with HCV genotype 1, and 22.5% had previously failed antiviral therapy (peg-IFN-α/ribavirin). The median LSM in the baseline was 6.1 kPa and the follow-up time was 47.9 months.

Characteristics of *IL7RA* polymorphisms

The *IL7RA* polymorphisms showed a strong linkage disequilibrium (LD) (non-random association of alleles at different loci) (D´ = 1.0). Nevertheless, the r-square among SNPs was low (r-square$<$0.40) (**Fig 2**).

The allelic and genotypic frequencies of the *IL7RA* polymorphisms are shown in **Table 2.** These frequencies were compared to those for healthy subjects extracted from the 1000 genomes database, and no significant differences between groups were found. All SNPs had a minimum allele frequency $>$5% and were in Hardy-Weinberg equilibrium (p$>$0.05). We did not obtain any missing value for any *IL7RA* polymorphisms.

IL7RA polymorphisms and liver fibrosis progression

Baseline LSM values did not show significant differences among *IL7RA* genotypes (p$>$0.05). We only obtained significant results for the *IL7RA* rs6897932 polymorphism. Additionally, the additive model was the one that best fit our data.
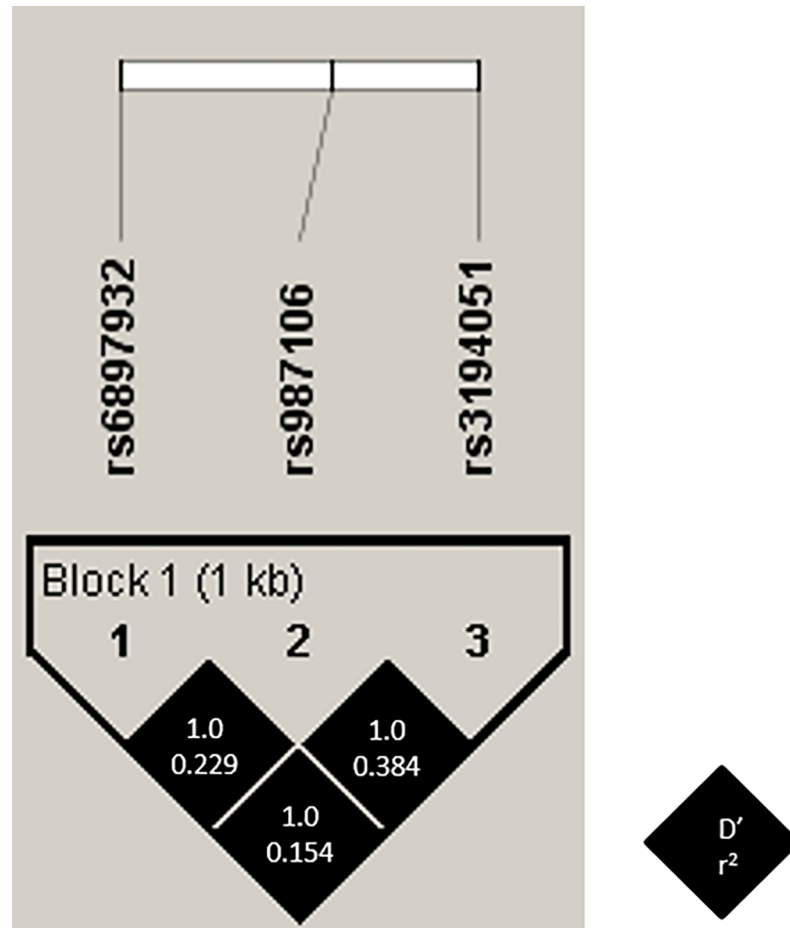
Fig 2. Pairwise linkage disequilibrium (LD) pattern for *IL7RA* polymorphisms. Each diagonal represents a different SNP, with each square representing a pairwise comparison between two SNPs.

https://doi.org/10.1371/journal.pone.0197115.g002

Table 2. Summary of Hardy Weinberg Equilibrium and frequencies of alleles and genotypes for *IL7RA* polymorphisms in HCV-infected patients compared to Iberian populations in Spain (IBS) and Utah residents with Northern and Western European ancestry (CEU) from 1000 genomes project data (http://browser.1000genomes.org/index.html).

SNPs	HCV group (n = 187)						IBS group (n = 107)						χ^2 test(a) p-value	χ^2 test (b) p-value	CEU group (n = 99)						χ^2 test(a) p-value	χ^2 test (b) p-value
	HWE	Alleles		Genotypes			HWE	Alleles		Genotypes					HWE	Alleles		Genotypes				
rs6897932	0.216	C	76.7%	CC	56.7%		0.551	C	74.8%	CC	57.0%		0.714	0.225	0.507	C	75.8%	CC	58.6%		0.865	0.255
		T	23.3%	CT	40.1%			T	25.2%	CT	35.5%					T	24.2%	CT	34.3%			
				TT	3.2%					TT	7.5%							TT	7.1%			
rs987106	0.605	A	43.0%	AA	19.8%		0.308	A	49.5%	AA	27.1%		0.283	0.308	0.456	A	42.4%	AA	16.2%		0.922	0.592
		T	57.0%	AT	46.5%			T	50.5%	AT	44.9%					T	57.6%	AT	52.5%			
				TT	33.7%					TT	28.0%							TT	31.3%			
rs3194051	0.255	A	66.3%	AA	46.5%		0.937	A	74.8%	AA	56.1%		0.130	0.100	0.646	A	66.7%	AA	43.4%		0.946	0.446
		G	33.7%	AG	39.6%			G	25.2%	AG	37.4%					G	33.3%	AG	46.5%			
				GG	13.9%					GG	6.5%							GG	10.1%			

Statistical: p-values were calculated by Chi-squared test; (a), differences between allele frequencies; (b), differences between genotype frequencies.

Abbreviations: HWE, Hardy Weinberg Equilibrium; HCV, hepatitis C virus; IL7RA, α-chain of the interleukin 7 receptor.

https://doi.org/10.1371/journal.pone.0197115.t002

In univariate analysis, the rs6897932 T allele had a significant positive relationship with an increase in LSM values (AMR = 1.21 (95%CI = 1.08; 1.36); p = 0.001; **Fig 3A1**), progression to advanced fibrosis (OR = 2.51 (95%CI = 1.29; 4.88); p = 0.006; **Fig 3B1**) and progression to cirrhosis (OR = 2.71 (95%CI = 0.94; 5.03); p = 0.069; **Fig 3C1**). These trends are maintained in the multivariable analysis adjusted by the main clinical and epidemiological covariates (**Fig 3D1**). The rs6897932 T allele was related to a higher increase in LSM values during follow-up (adjusted AMR = 1.27 (95%CI = 1.13; 1.42); p<0.001), and higher odds of progression to advanced fibrosis [adjusted OR = 4.46 (95%CI = 1.87; 10.62); p = 0.001], and progression to cirrhosis [adjusted OR = 3.92 (95%CI = 1.30; 11.77); p = 0.015] (**Fig 3D1**).

In analysis of the *IL7RA* rs987106 and rs3194051 polymorphisms, we did not find significant results except for the relationship between *IL7RA* rs987106 and the increase in LSM values [adjusted OR = 1.12 (95%CI = 1.02; 1.23); p = 0.015] (**Fig 3D2**). In addition, **S1 Fig** shows data for all *IL7RA* polymorphisms according to recessive and dominant models.

Discussion

In this study, we stratified our HCV-infected patients by *IL7RA* genotypes, and we evaluated the progression of liver fibrosis, which constitutes a critical element of the management of
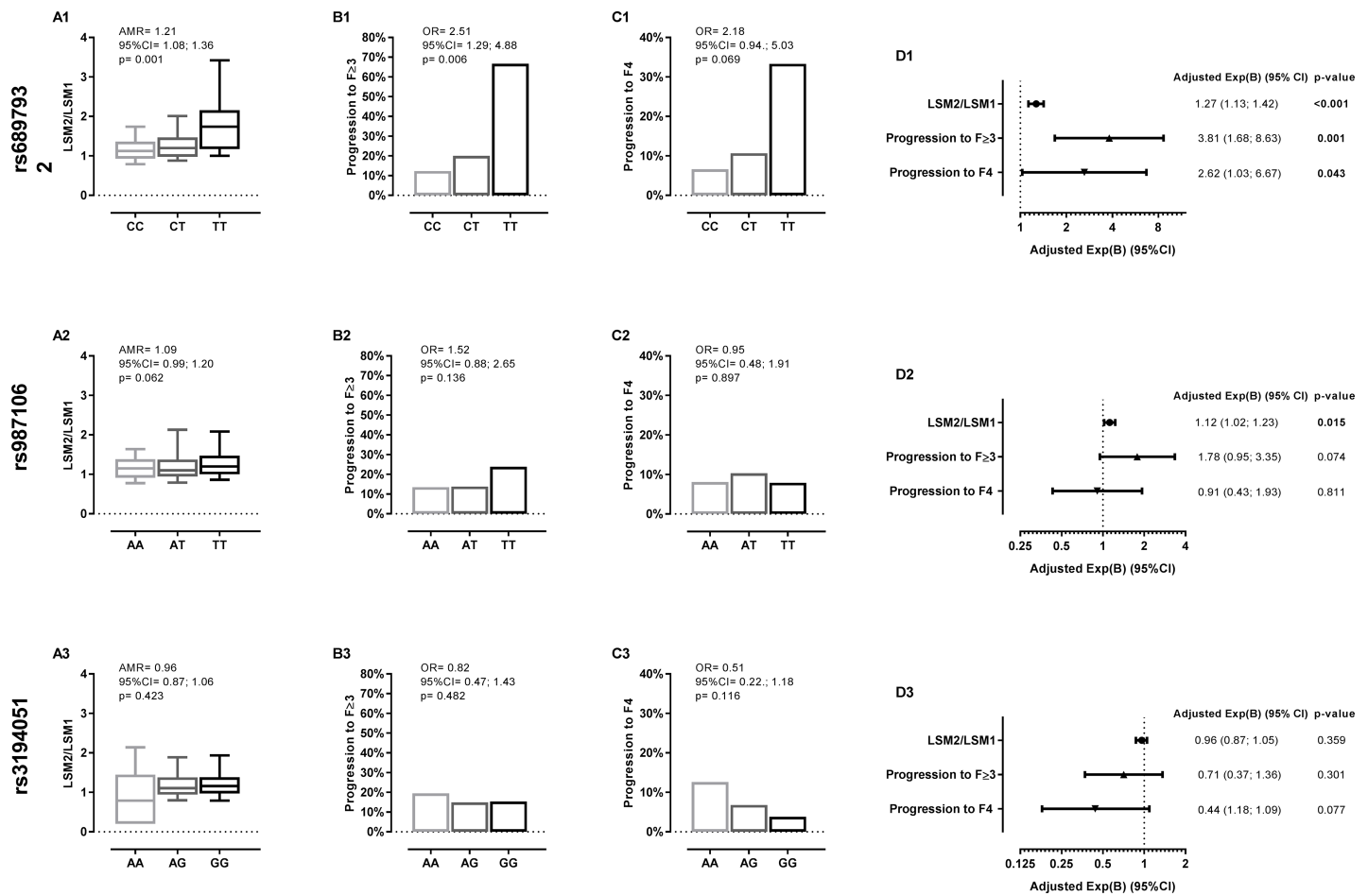

Fig 3. Summary of the relationship between *IL7RA* polymorphisms and variation of liver stiffness measurements (LSM) and fibrosis stages in HCV-infected patients with an additive inheritance model. Statistical: p-values were calculated by univariate and multivariable regression adjusted by the most important clinical and epidemiological characteristics (see statistical analysis section). Significant differences are shown in bold. **Abbreviations**: LSM, liver stiffness measure; kPa, kilopascal; LSM1, baseline LSM; LSM2, final LSM; Exp(B), arithmetic mean ratio (AMR) for continuous variable and odds ratio (OR) for categorical variables; 95%CI, 95% of confidence interval; p-value, level of significance; F≥3, advanced fibrosis; F4, cirrhosis; IL7RA, α-chain of the interleukin 7 receptor.

https://doi.org/10.1371/journal.pone.0197115.g003

patients with CHC by providing a useful prognostic tool and helping in treatment decisions [7,37]. The major finding of our study was that the rs6897932 T allele was associated with a higher risk of liver fibrosis progression in HCV-infected patients. However, in our study rs987106 showed a weaker association with liver disease progression and rs3194051 showed none. We must emphasize that in addition to the additive model we also found a significant association with liver fibrosis progression when we used the recessive and dominant models, but with a slightly lower statistical significance. To our knowledge, this is the first article that relates *IL7RA* polymorphisms with liver fibrosis progression in HCV-monoinfected patients. Additionally, our study has a longitudinal design of repeated measurements with a follow-up over a prolonged period of time. This dynamic design is more valid for detecting changes at the individual level, which is a novel aspect of our results in comparison with previous findings.

The *IL7RA* rs6897932 polymorphism constitutes a replacement of a threonine by an isoleucine in the IL7RA (also called CD127) transmembrane domain. This fact could affect the levels of the soluble IL7RA isoform (sIL7RA) and membrane-bound IL7RA isoform by putatively disrupting an exonic splicing motif [22]. The rs6897932 T allele, in contrast to the rs6897932 C allele, has been associated with lower plasma levels of sIL7RA [22,38–40], without limiting the effect of circulating IL-7 [39,41]. By contrast, the rs6897932 C allele has been related to elevated plasma concentrations of sIL7RA [22,38–40], which is able to bind to circulating IL-7 and reduce the bioavailability of IL-7, limiting its effects [39,41]. Moreover, it has been described that the "TT" genotype has increased signal transduction and proliferation in response to IL-7 among HIV-infected individuals [42]. This fact may explain the positive effect of the rs6897932 T allele on CD4+ T cell recovery in HIV-infected patients on antiretroviral therapy [24,25,40]. In our study, patients with the rs6897932 T allele had a higher risk of liver fibrosis progression during follow-up. Therefore, it would be plausible that the rs6897932 T allele, versus C allele, may confer lower levels of sIL7RA, higher IL-7 bioavailability [41] and higher immune response against HCV infection [17]. Thus, it may lead to a greater destruction of hepatocytes and liver injury, which favors the progression of liver fibrosis during CHC [43].

Moreover, in a previous cross-sectional study, we observed that both rs987106 and rs3194051 polymorphisms were associated with advanced fibrosis in HIV/HCV-coinfected patients [28]. However, in the current study, we found a weak association between rs987106 and liver disease progression and none for rs3194051 polymorphism. The discrepancy observed for these two SNPs may be due to multiple reasons, among which we should highlight the study design (cross-sectional vs. longitudinal) and the type of patients included (HIV/HCV-coinfected vs. HCV-monoinfected). Moreover, in our study, a high LD was observed, but the r-square among SNPs was low (r-square<0.40), meaning that the *IL7RA* polymorphisms provide different information and cannot be substituted one for the other, supporting the differential association observed among SNPs in this study.

From now going forward with the new DAAs to treat HCV infection, all HCV-infected patients will be treated in the short or medium term, resulting in a virological cure for 90–95% of them. However, it has recently been reported that disease progression and the incidence of hepatocellular carcinoma have both increased in patients who achieved SVR [44–47]. Under this new context, further studies will be needed to investigate whether the association between the rs6897932 T allele and fibrosis progression is maintained after SVR, which could shed light on the importance of the *IL7RA* polymorphism on the clinical evolution of patients with HCV clearance.

This study has several limitations that must be considered. Firstly, we used a retrospective design that included patients who came to the hospital and had a sufficient follow-up. However, the repeated measurements design provides robustness to this study. Secondly, the

number of samples was small and the reduced number of patients with some *IL7RA* genotypes may have made the detection of associations more difficult, as well as, the accuracy of the risk estimation. Therefore, independent replication studies with a larger sample size, including different ethnic groups, may be necessary. Thirdly, we did not have access to plasma specimens, so the concentration in plasma was missing for both IL-7 and sIL7RA. Fourthly, we performed the analysis on European patients only, and studies would benefit if they were performed on different ethnic groups. Fifthly, around 23% of patients were previously treated with IFN therapy, but they maintained the HCV infection. This treatment failure does not affect the natural course of CHC during long-term follow up [48,49], so these non-responders were not excluded. Moreover, we did not find any significant effect of IFN-failed therapy on the statistical analysis of liver fibrosis progression. Sixthly, we could not study the association between *IL7RA* polymorphisms and hepatocellular carcinoma because only a few patients developed hepatocellular carcinoma in our cohort. Additionally, we did not have enough data of some relevant clinical variables (e.g., HCV viral load, ALT levels), which were therefore not considered in the multivariable analysis. Finally, regarding multiple testing, there is a considerable controversy about adjusting the "p-value" in clinical-orientated studies [24,25]. In our case, we were not literally doing a random search for a meaningful result because we have a hypothesis supported by theory and previous articles that have shown relationships of *IL7RA* polymorphisms to viral infection outcomes [24–26], including HCV infection [27,28]. Thus, our results should not be affected by carrying out a high number of statistical tests.

Conclusions

In conclusion, the *IL7RA* rs6897932 polymorphism seems to be related to increased risk of liver fibrosis progression in HCV-infected patients. Thus, the rs6897932 polymorphism could play a crucial role in the physiopathology of CHC and could be used to stratify the risk of CHC progression.

Supporting information

S1 Fig. Summary of the relationship between *IL7RA* polymorphisms and variation of liver stiffness measurements (LSM) and fibrosis stages in HCV-infected patients with recessive and dominant inheritance model. Statistical: p-values were calculated by multivariable regression adjusted by the most important clinical and epidemiological characteristics (see statistical analysis section). Significant differences are shown in bold.
Abbreviations: LSM, liver stiffness measure; LSM1, baseline LSM; LSM2, final LSM; Exp(B), arithmetic mean ratio (AMR) for continuous variable and odds ratio (OR) for categorical variables; 95%CI, 95% of confidence interval; p-value, level of significance; F≥3, advanced fibrosis; F4, cirrhosis; IL7RA, α-chain of the interleukin 7 receptor.
(TIF)

Acknowledgments

Author Contributions

Conceptualization: María Ángeles Jiménez-Sousa, Ana Zaida Gómez-Moreno.

Data curation: Ana Zaida Gómez-Moreno, Daniel Pineda-Tenor, Luz Maria Medrano, Juan José Sánchez-Ruano, Tomas Artaza-Varasa, José Saura-Montalbán, Sonia Vázquez-Morón.

Formal analysis: María Ángeles Jiménez-Sousa, Daniel Pineda-Tenor, Luz Maria Medrano, Salvador Resino.

Investigation: María Ángeles Jiménez-Sousa, Ana Zaida Gómez-Moreno, Daniel Pineda-Tenor, Juan José Sánchez-Ruano, Amanda Fernández-Rodríguez.

Resources: María Ángeles Jiménez-Sousa.

Supervision: Salvador Resino.

Writing – original draft: María Ángeles Jiménez-Sousa, Ana Zaida Gómez-Moreno, Salvador Resino.

Writing – review & editing: Daniel Pineda-Tenor, Luz Maria Medrano, Amanda Fernández-Rodríguez, Tomas Artaza-Varasa, Pablo Ryan.

References

1. Ghany MG, Kleiner DE, Alter H, Doo E, Khokar F, Promrat K, et al. (2003) Progression of fibrosis in chronic hepatitis C. Gastroenterology 124: 97–104. https://doi.org/10.1053/gast.2003.50018 PMID: 12512034

2. Shepard CW, Finelli L, Alter MJ (2005) Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 5: 558–567. https://doi.org/10.1016/S1473-3099(05)70216-4 PMID: 16122679

3. Asrani SK, Larson JJ, Yawn B, Therneau TM, Kim WR (2013) Underestimation of liver-related mortality in the United States. Gastroenterology 145: 375–382 e371-372. https://doi.org/10.1053/j.gastro.2013.04.005 PMID: 23583430

4. Razavi H, ElKhoury AC, Elbasha E, Estes C, Pasini K, Poynard T, et al. (2013) Chronic Hepatitis C Virus (HCV) Disease Burden and Cost in the United States. Hepatology (Baltimore, Md) 57: 2164–2170.

5. Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, et al. (2012) Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). J Hepatol 57: 675–688. https://doi.org/10.1016/j.jhep.2012.04.015 PMID: 22609307

6. Westbrook RH, Dusheiko G (2014) Natural history of hepatitis C. J Hepatol 61: S58–68. https://doi.org/10.1016/j.jhep.2014.07.012 PMID: 25443346

7. (2015) EASL Recommendations on Treatment of Hepatitis C 2015. J Hepatol 63: 199–236. https://doi.org/10.1016/j.jhep.2015.03.025 PMID: 25911336

8. Tsochatzis EA, Bosch J, Burroughs AK (2014) Liver cirrhosis. Lancet 383: 1749–1761. https://doi.org/10.1016/S0140-6736(14)60121-5 PMID: 24480518

9. Castera L (2015) Noninvasive Assessment of Liver Fibrosis. Dig Dis 33: 498–503. https://doi.org/10.1159/000374097 PMID: 26159265

10. Liang TJ, Ghany MG (2014) Therapy of hepatitis C—back to the future. N Engl J Med 370: 2043–2047. https://doi.org/10.1056/NEJMe1403619 PMID: 24795199

11. Veldt BJ, Heathcote EJ, Wedemeyer H, Reichen J, Hofmann WP, Zeuzem S, et al. (2007) Sustained virologic response and clinical outcomes in patients with chronic hepatitis C and advanced fibrosis. Ann Intern Med 147: 677–684. PMID: 18025443

12. Aleman S, Rahbin N, Weiland O, Davidsdottir L, Hedenstierna M, Rose N, et al. (2013) A risk for hepatocellular carcinoma persists long-term after sustained virologic response in patients with hepatitis C-associated liver cirrhosis. Clin Infect Dis 57: 230–236. https://doi.org/10.1093/cid/cit234 PMID: 23616492

13. Forner A, Reig M, Bruix J (2018) Hepatocellular carcinoma. Lancet.

14. Capitini CM, Chisti AA, Mackall CL (2009) Modulating T cell Homeostasis with IL-7: Preclinical and Clinical Studies. Journal of internal medicine 266: 141–153. PMID: 19623690

15. Pellegrini M, Calzascia T, Toe JG, Preston SP, Lin AE, Elford AR, et al. (2011) IL-7 engages multiple mechanisms to overcome chronic viral infection and limit organ pathology. Cell 144: 601–613. https://doi.org/10.1016/j.cell.2011.01.011 PMID: 21295337

16. Shin EC, Park SH, Nascimbeni M, Major M, Caggiari L, de Re V, et al. (2013) The frequency of CD127 (+) hepatitis C virus (HCV)-specific T cells but not the expression of exhaustion markers predicts the outcome of acute HCV infection. J Virol 87: 4772–4777. https://doi.org/10.1128/JVI.03122-12 PMID: 23388706

17. Hou L, Jie Z, Desai M, Liang Y, Soong L, Wang T, et al. (2013) Early IL-17 production by intrahepatic T cells is important for adaptive immune responses in viral hepatitis. J Immunol 190: 621–629. https://doi.org/10.4049/jimmunol.1201970 PMID: 23233727

18. Larrubia JR, Lokhande MU, Garcia-Garzon S, Miquel J, Gonzalez-Praetorius A, Parra-Cid T, et al. (2013) Persistent hepatitis C virus (HCV) infection impairs HCV-specific cytotoxic T cell reactivity through Mcl-1/Bim imbalance due to CD127 down-regulation. J Viral Hepat 20: 85–94. https://doi.org/10.1111/j.1365-2893.2012.01618.x PMID: 23301543

19. Larrea E, Riezu-Boj JI, Aldabe R, Guembe L, Echeverria I, Balasiddaiah A, et al. (2014) Dysregulation of interferon regulatory factors impairs the expression of immunostimulatory molecules in hepatitis C virus genotype 1-infected hepatocytes. Gut 63: 665–673. https://doi.org/10.1136/gutjnl-2012-304377 PMID: 23787026

20. Broux B, Hellings N, Venken K, Rummens JL, Hensen K, Van Wijmeersch B, et al. (2010) Haplotype 4 of the multiple sclerosis-associated interleukin-7 receptor alpha gene influences the frequency of recent thymic emigrants. Genes Immun 11: 326–333. https://doi.org/10.1038/gene.2009.106 PMID: 20072142

21. Jager J, Schulze C, Rosner S, Martin R (2013) IL7RA haplotype-associated alterations in cellular immune function and gene expression patterns in multiple sclerosis. Genes Immun 14: 453–461. https://doi.org/10.1038/gene.2013.40 PMID: 23985573

22. Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A, et al. (2007) Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. Nat Genet 39: 1083–1091. https://doi.org/10.1038/ng2103 PMID: 17660817

23. Santiago JL, Alizadeh BZ, Martinez A, Espino L, de la Calle H, Fernandez-Arquero M, et al. (2008) Study of the association between the CAPSL-IL7R locus and type 1 diabetes. Diabetologia 51: 1653–1658. https://doi.org/10.1007/s00125-008-1070-4 PMID: 18563381

24. Guzman-Fulgencio M, Berenguer J, Jimenez-Sousa MA, Micheloud D, Garcia-Alvarez M, Bellon JM, et al. (2015) IL7RA polymorphisms predict the CD4+ recovery in HIV patients on cART. Eur J Clin Invest 45: 1192–1199. https://doi.org/10.1111/eci.12539 PMID: 26402121

25. Hartling HJ, Thorner LW, Erikstrup C, Harritshoj LH, Kronborg G, Pedersen C, et al. (2014) Polymorphism in interleukin-7 receptor alpha gene is associated with faster CD4(+) T-cell recovery after initiation of combination antiretroviral therapy. AIDS 28: 1739–1748. https://doi.org/10.1097/QAD.0000000000000354 PMID: 24911352

26. Rajasuriar R, Booth D, Solomon A, Chua K, Spelman T, Gouillou M, et al. (2010) Biological determinants of immune reconstitution in HIV-infected patients receiving antiretroviral therapy: the role of interleukin 7 and interleukin 7 receptor alpha and microbial translocation. J Infect Dis 202: 1254–1264. https://doi.org/10.1086/656369 PMID: 20812848

27. Guzman-Fulgencio M, Berenguer J, Pineda-Tenor D, Jimenez-Sousa MA, Garcia-Alvarez M, Aldamiz-Echevarria T, et al. (2015) Association between IL7RA polymorphisms and the successful therapy against HCV in HIV/HCV-coinfected patients. Eur J Clin Microbiol Infect Dis 34: 385–393. https://doi.org/10.1007/s10096-014-2245-1 PMID: 25236396

28. Guzman-Fulgencio M, Berenguer J, Jimenez-Sousa MA, Pineda-Tenor D, Aldamiz-Echevarria T, Garcia-Broncano P, et al. (2015) Association between IL7R polymorphisms and severe liver disease in HIV/HCV coinfected patients: a cross-sectional study. J Transl Med 13: 206. https://doi.org/10.1186/s12967-015-0577-y PMID: 26123260

29. O'Shea RS, Dasarathy S, McCullough AJ (2010) Alcoholic liver disease. Hepatology 51: 307–328. https://doi.org/10.1002/hep.23258 PMID: 20034030

30. Calvaruso V, Craxì A (2012) 2011 European Association of the Study of the Liver hepatitis C virus clinical practice guidelines. Liver International 32: 2–8.

31. (2014) EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 60: 392–420. https://doi.org/10.1016/j.jhep.2013.11.003 PMID: 24331294

32. Mallet V, Gilgenkrantz H, Serpaggi J, Verkarre V, Vallet-Pichard A, Fontaine H, et al. (2008) Brief communication: the relationship of regression of cirrhosis to outcome in chronic hepatitis C. Ann Intern Med 149: 399–403. PMID: 18794559

33. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. (2015) A global reference for human genetic variation. Nature 526: 68–74. https://doi.org/10.1038/nature15393 PMID: 26432245

34. Sandrin L, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, et al. (2003) Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 29: 1705–1713. PMID: 14698338

35. Castera L, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, et al. (2005) Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. Gastroenterology 128: 343–350. PMID: 15685546

36. Smith DJ, Combellick J, Jordan AE, Hagan H (2015) Hepatitis C virus (HCV) disease progression in people who inject drugs (PWID): A systematic review and meta-analysis. Int J Drug Policy 26: 911–921. https://doi.org/10.1016/j.drugpo.2015.07.004 PMID: 26298331

37. Foucher J, Chanteloup E, Vergniol J, Castera L, Le Bail B, Adhoute X, et al. (2006) Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. Gut 55: 403–408. https://doi.org/10.1136/gut.2005.069153 PMID: 16020491

38. Kreft KL, Verbraak E, Wierenga-Wolf AF, van Meurs M, Oostra BA, Laman JD, et al. (2012) Decreased systemic IL-7 and soluble IL-7Ralpha in multiple sclerosis patients. Genes Immun 13: 587–592. https://doi.org/10.1038/gene.2012.34 PMID: 22914435

39. Lundstrom W, Highfill S, Walsh ST, Beq S, Morse E, Kockum I, et al. (2013) Soluble IL7Ralpha potentiates IL-7 bioactivity and promotes autoimmunity. Proc Natl Acad Sci U S A 110: E1761–1770. https://doi.org/10.1073/pnas.1222303110 PMID: 23610432

40. Rajasuriar R, Booth DR, Gouillou M, Spelman T, James I, Solomon A, et al. (2012) The role of SNPs in the alpha-chain of the IL-7R gene in CD4+ T-cell recovery in HIV-infected African patients receiving suppressive cART. Genes Immun 13: 83–93. https://doi.org/10.1038/gene.2011.65 PMID: 21938017

41. Crawley AM, Faucher S, Angel JB (2010) Soluble IL-7R alpha (sCD127) inhibits IL-7 activity and is increased in HIV infection. J Immunol 184: 4679–4687. https://doi.org/10.4049/jimmunol.0903758 PMID: 20304824

42. Hartling HJ, Ryder LP, Ullum H, Odum N, Nielsen SD (2017) Gene variation in IL-7 receptor (IL-7R) alpha affects IL-7R response in CD4+ T cells in HIV-infected individuals. Sci Rep 7: 42036. https://doi.org/10.1038/srep42036 PMID: 28181541

43. Shin EC, Sung PS, Park SH (2016) Immune responses and immunopathology in acute and chronic viral hepatitis. Nat Rev Immunol 16: 509–523. https://doi.org/10.1038/nri.2016.69 PMID: 27374637

44. Conti F, Buonfiglioli F, Scuteri A, Crespi C, Bolondi L, Caraceni P, et al. (2016) Early occurrence and recurrence of hepatocellular carcinoma in HCV-related cirrhosis treated with direct-acting antivirals. J Hepatol 65: 727–733. https://doi.org/10.1016/j.jhep.2016.06.015 PMID: 27349488

45. van der Meer AJ, Feld JJ, Hofer H, Almasio PL, Calvaruso V, Fernandez-Rodriguez CM, et al. (2017) Risk of cirrhosis-related complications in patients with advanced fibrosis following hepatitis C virus eradication. J Hepatol 66: 485–493. https://doi.org/10.1016/j.jhep.2016.10.017 PMID: 27780714

46. Janjua NZ, Chong M, Kuo M, Woods R, Wong J, Yoshida EM, et al. (2017) Long-term effect of sustained virological response on hepatocellular carcinoma in patients with hepatitis C in Canada. J Hepatol 66: 504–513. https://doi.org/10.1016/j.jhep.2016.10.028 PMID: 27818234

47. Reig M, Marino Z, Perello C, Inarrairaegui M, Ribeiro A, Lens S, et al. (2016) Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol 65: 719–726. https://doi.org/10.1016/j.jhep.2016.04.008 PMID: 27084592

48. Shiffman ML, Sterling RK, Contos M, Hubbard S, Long A, Luketic VA, et al. (2014) Long term changes in liver histology following treatment of chronic hepatitis C virus. Ann Hepatol 13: 340–349. PMID: 24927604

49. Poynard T, Moussalli J, Munteanu M, Thabut D, Lebray P, Rudler M, et al. (2013) Slow regression of liver fibrosis presumed by repeated biomarkers after virological cure in patients with chronic hepatitis C. J Hepatol 59: 675–683. https://doi.org/10.1016/j.jhep.2013.05.015 PMID: 23712051