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Short communication

PNPLA3 rs738409 polymorphism is associated with liver fibrosis progression in patients with chronic hepatitis C: A repeated measures study

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ABSTRACT

Background: Host genetic background has been associated with liver fibrosis progression.
Objective: To analyze the association between the patatin-like phospholipase domain-containing 3 (*PNPLA3*) rs738409 polymorphism and liver fibrosis progression in hepatitis C virus (HCV)-infected patients.
Study design: In this retrospective cohort study, 187 patients with chronic HCV infection were included, who had at least two liver stiffness measurements (LSM) by transient elastography during the follow-up. Results were expressed in kilopascals (kPa). The analysis of genetic association was carried out according to additive model by using Generalized Linear Models.
Results: No patients had advanced fibrosis/cirrhosis at baseline. During a median follow-up time of 47.9 months, 15 patients developed advanced fibrosis and 17 cirrhosis. In multivariate analysis adjusted by the main clinical and epidemiological covariates, the rs738409 G allele was related to higher increase of LSM values during the follow-up (adjusted arithmetic mean ratio (aAMR) = 1.16 (95%CI = 1.04; 1.29); p = .006) and higher odds of having progression to advanced fibrosis [aOR = 2.03 (95%CI = 1.01; 4.06); p = .045], and progression to cirrhosis [aOR = 3.03 (95%CI = 1.26; 7.30); p = .014].
Conclusions: *PNPLA3* rs738409 polymorphism appears to be related to the increased progression of liver fibrosis in HCV infected patients.

1. Background

The natural course of chronic hepatitis C (CHC) varies widely among individuals [1], and the early recognition of patients at risk for developing liver fibrosis and cirrhosis is essential to take preventive measures that may affect the course of CHC [2]. Several risk factors have been associated with liver fibrosis progression, including age at infection, sex, route of infection, hepatitis C virus (HCV) genotype, and obesity among others [3]. In this regard, single nucleotide polymorphisms have been also associated with liver disease progression [3,4]. Among them, the patatin-like phospholipase domain-containing 3 (*PNPLA3*) rs738409 polymorphism (G allele) promotes nonalcoholic steatohepatitis, liver fibrosis progression and possibly other outcomes, such as hepatocellular carcinoma [5–8] in Caucasian CHC patients. However, the vast majority of these studies had a cross-sectional or case-control design [5,6], which may have introduced inherent biases.

2. Objectives

In this study, we aimed to analyze the association between *PNPLA3* rs738409 polymorphism and liver fibrosis progression in HCV-infected patients.

3. Study design

We carried out a retrospective cohort study with a longitudinal design of repeated measures and follow-up over a prolonged period in 187 HCV-infected patients from Hospital Virgen de la Salud (Toledo, Spain) between 2008 and 2015. The study ran from the day of the first LSM recorded to the last follow-up visit with LSM data, or the initiation

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Table 1
Clinical and epidemiological characteristics of HCV-infected patients stratified by *PNPLA3* rs738409 genotypes.

Characteristic	All Patients	*PNPLA3* rs738409 polymorphism			
		CC	CG	GG	p-value
No.	187	101	72	14	
Male sex	102 (54.5%)	53 (52.5%)	40 (55.6%)	9 (64.3%)	0.691
Age (years)	46.4 (40.9; 55.8)	47.8 (42.1; 56.1)	46.1 (40.6; 55.9)	44.9 (40.9; 49.2)	0.255
Time of HCV infection (years)	7.5 (2.9; 12.9)	6.5 (2.7; 12.9)	8.7 (2.4; 12.5)	11.4 (4.2; 13.2)	0.401
High alcohol intake	25 (13.4%)	13 (12.9%)	9 (12.5%)	3 (21.4%)	0.653
Prior injection drug use	20 (10.7%)	7 (6.9%)	10 (13.9%)	3 (21.4%)	0.138
HCV genotype (n = 216)					
1	154 (83.7%)	84 (84.8%)	59 (83.1%)	11 (78.6%)	0.645
3	14 (7.6%)	9 (9.1%)	4 (5.6%)	1 (7.1%)	–
4	15 (8.2%)	5 (5.1%)	8 (11.3%)	2 (14.3%)	–
5	1 (0.5%)	1 (1%)	0 (0%)	0 (0%)	–
Prior peg-IFN-α/RBV therapy failed	42 (22.5%)	21 (20.8%)	18 (25%)	3 (21.4%)	0.804
Baseline LSM (kPa)	6.1 (5.3; 8.4)	5.8 (4.9; 6.8)	6 (5; 7.3)	6.2 (5.6; 7.3)	0.394
F0-F1 (< 7.1 kPa)	149 (79.7%)	85 (84.2%)	54 (75.0%)	10 (71.4%)	0.245
F2 (7.1–9.4 kPa)	38 (20.3%)	16 (15.8%)	18 (25.0%)	4 (28.6%)	–
Follow-up time (months)	47.9 (29.2; 62.7)	50.5 (30.5; 63.8)	45.8 (27.4; 61.5)	44 (34.4; 60.6)	0.503
Final LSM (kPa)	6.7 (5.3; 8.6)	6.5 (5.3; 7.9)	7.1 (5.5; 8.7)	7.4 (5.9; 14.6)	0.190
F0-F1 (< 7.1 kPa)	108 (57.8%)	66 (65.3%)	36 (50.0%)	6 (42.9%)	0.043
F2 (7.1–9.4 kPa)	47 (25.1%)	23 (22.8%)	20 (27.8%)	4 (28.6%)	–
F3 (9.5–12.4 kPa)	15 (8.0%)	7 (6.9%)	8 (11.1%)	0 (0%)	–
F4 (≥12.5 kPa)	17 (9.1%)	5 (5.0%)	8 (11.1%)	4 (28.6%)	–

Values expressed as absolute numbers (%) and median (percentile 25; percentile 75). p-values were estimated with nonparametric Mann-Whitney *U* test for continuous variables and Chi-square test for categorical variables.
Abbreviations: HCV, hepatitis C virus; LSM, liver stiffness measure; kPa, kilopascal; peg-IFN-α/RBV, peg-interferon-alpha/ribavirin; PNPLA3, patatin-like phospholipase domain-containing protein 3.

of antiviral treatment for HCV in responder patients, who clearance HCV infection.

This work was conducted in accordance with the 1975 Declaration of Helsinki. The Institutional Review Board of the Instituto de Salud Carlos III approved the study, and all patients signed the consent.

Patient selection criteria were: 1) detectable plasma HCV RNA during the follow-up; 2) availability of DNA sample; 3) availability of baseline liver stiffness measurement (LSM) and final LSM with a separation of at least 12 months. The exclusion criteria were: 1) advanced fibrosis/cirrhosis at baseline (F ≥ 3 (LSM ≥ 9.5)); 2) co-infection with hepatitis B virus or human immunodeficiency virus.

Patients received conventional CHC management during the follow-up and consequently, patients could have been treated before or after entering the study with HCV therapy according to clinical guidelines [9,10]. However, in the case of patients who were treated before, we only included those patients who were non-responders; and in the case of patients who were treated after entering the study and achieved sustained virological response (SVR), their follow-ups were truncated at the time of starting HCV therapy. High alcohol intake was considered as > 20 g/day in women and ≥60 g/day in men. Time since HCV diagnosis was defined as the time between HCV diagnosis and the first LSM (LSM1, baseline of study). Time of follow-up was defined as the time between the last LSM (LSM2, the end of study) and the first LSM (LSM1).

DNA samples were genotyped for *PNPLA3* rs738409 polymorphism at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/). Genotyping was performed by using Agena Bioscience's MassARRAY platform (San Diego, CA, USA) using the iPLEX® Gold assay design system [11].

LSM was assessed by transient elastography (FibroScan®, Echosens, Paris, France) using a single machine. Results were expressed in kilopascals (kPa) with a range of 2.5–75 kPa [12]. Transient elastography was performed in our unit by a trained hepatologist, and measurements were considered reliable 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 XL probe. The following cut-offs of LSM were used to stratify patients [13]: < 7.1 kPa (F0-F1 – no or portal/periportal fibrosis), 7.1–9.4 kPa (F2 – septal fibrosis), 9.5–12.4 kPa (F3 - bridging fibrosis), and ≥12.5 kPa (F4 - cirrhosis).

The primary outcome variable was the change in LSM values during follow-up (continuous variable). We calculated the LSM variation during the follow-up (ratio LSM2/LSM1) and whether the LSM increase (ΔLSM = LSM2-LSM1) was higher than 5 kPa (ΔLSM ≥ 5 kPa). Furthermore, we evaluated the progression to F ≥ 3, which is a dichotomous variable that may have values of +1 [if F ≤ 2 (F0, F1 or F2) change to F ≥ 3 (F3 or F4)] or 0 [if F ≤ 2 (F0, F1 or F2) remains)]. The progression to cirrhosis (F4) may also have values of +1 [if F ≤ 2 (F0, F1, or F2) change to F4] or 0 (if F ≤ 2 (F0, F1 or F2) remains), since none patients had advanced fibrosis/cirrhosis at baseline (F ≥ 3 (LSM ≥ 9.5)).

Generalized Linear Model (GLM) was used to analyze the genetic association of *PNPLA3* rs738409 polymorphism with the outcome variables. GLM with a gamma distribution (log-link) was used for continuous variables. This test gives the differences between groups and the arithmetic mean ratio (AMR). Moreover, GLM with binomial distribution (logit-link) was used to investigate the association with dichotomous variables (ΔLSM ≥ 5 kPa, progression to F ≥ 3, and progression to F4). This test gives the differences between groups and the odds ratio (OR). Each regression test was adjusted by age, gender, time since HCV diagnosis, HCV genotype, injection drug use, high alcohol intake, diabetes, HCV antiviral therapy prior to baseline and during follow-up (patients who failed therapy), baseline of LSM, and time of follow-up. All statistical tests were performed with the Statistical Package for the Social Sciences (SPSS) 21.0 software (IBM Corp., Chicago, USA). All p-values were two-tailed and statistical significance was defined as $p < 0.05$.

4. Results

Table 1 shows the baseline characteristics of 187 HCV-infected patients without advanced fibrosis/cirrhosis. We did not find any significant differences at baseline between patients with different *PNPLA3*