



RESEARCH ARTICLE



Effect of *Peroxisome Proliferator-Activated Receptor- γ Coactivator-1 Alpha* Variants on Spontaneous Clearance and Fibrosis Progression during Hepatitis C Virus Infection in Moroccan Patients

Raouia ElFihry^{1,2} · Mohcine Elmessaoudi-Idrissi¹ · Fatima-Zahra Jadid¹ · Imane Zaidane¹ · Hajar Chihab¹ · Mohamed Tahiri³ · Mostafa Kabine² · Wafaa Badre³ · Isabelle Chemin⁴ · Agnes Marchio⁵ · Pascal Pineau⁵ · Sayeh Ezzikouri¹ · Soumaya Benjelloun¹

Received: 4 October 2019 / Accepted: 8 February 2020
© Wuhan Institute of Virology, CAS 2020

Abstract

Hepatitis C virus (HCV) is still one of the main causes of liver disease worldwide. Metabolic disorders, including non-alcoholic fatty liver disease (NAFLD), induced by HCV have been shown to accelerate the progression of fibrosis to cirrhosis and to increase the risk of hepatocellular carcinoma. An optimal peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) activity is crucial to prevent NAFLD installation. The present study aims to investigate the associations between two common *PPARGC1A* polymorphisms (rs8192678 and rs12640088) and the outcomes of HCV infection in a North African context. A series of 592 consecutive Moroccan subjects, including 292 patients with chronic hepatitis C (CHC), 100 responders and 200 healthy controls were genotyped using a TaqMan allelic discrimination assay. *PPARGC1A* variations at rs8192678 and rs12640088 were not associated with spontaneous clearance of HCV infection (adjusted ORs = 0.76 and 0.79 respectively, $P > 0.05$, for both). Furthermore, multivariable logistic regression analysis showed that both SNPs were not associated with fibrosis progression (OR = 0.71; 95% CI 0.20–2.49; $P = 0.739$; OR = 1.28; 95% CI 0.25–6.54; $P = 0.512$, respectively). We conclude that, in the genetic context of South Mediterranean patients, rs8192678 and rs12640088 polymorphisms of *PPARGC1A* are neither associated with spontaneous clearance nor with disease progression in individuals infected with HCV.

Keywords Chronic hepatitis C · Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A) · Polymorphisms · Disease progression

Introduction

Chronic hepatitis C virus (HCV) infection is a major global health problem. Chronically infected patients may develop cirrhosis and liver cancer responsible for a substantial mortality (Lauer and Walker 2001). The World Health Organization estimates that approximately 71 million people are infected with HCV worldwide. HCV causes not only severe liver problems but also general metabolic manifestations, such as insulin resistance (IR) and type 2 diabetes mellitus (T2DM) (Jacobson *et al.* 2010; WHO 2017).

In case of chronic hepatitis C (CHC), a large inter-individual variability in the risk of liver disease progression is observed. A subset of infected persons develops progressive hepatic fibrosis and subsequent cirrhosis, two

✉ Sayeh Ezzikouri
sayeh.ezzikouri@pasteur.ma

✉ Soumaya Benjelloun
soumaya.benjelloun@pasteur.ma

¹ Virology Unit, Viral Hepatitis Laboratory, Institut Pasteur du Maroc, 20360 Casablanca, Morocco

² Laboratoire Santé et Environnement, département de Biologie, Faculté des Sciences Ain Chock, University Hassan II of Casablanca, 20360 Casablanca, Morocco

³ Service d'Hépatogastro-Entérologie, CHU Ibn Rochd, 20360 Casablanca, Morocco

⁴ Centre de Recherche en Cancérologie de Lyon, UMR INSERM 1052, CNRS 5286, Lyon Cedex 03, France

⁵ Unité "Organisation Nucléaire et Oncogénèse", INSERM U993, Institut Pasteur, 75015 Paris, France

conditions associated with a higher risk of hepatocellular carcinoma (HCC) (Thomas *et al.* 2000). In addition, the activation of pro-inflammatory mediators, such as nuclear factor-kappa-B and tumor necrosis factor α , caused by the chronic infection state, interferes with insulin signaling and contributes both to hepatic and peripheral insulin resistance (Maeno *et al.* 2003; Knobler and Schattner 2005). In agreement with the evidence suggesting a central role for reactive oxygen species in the development of insulin resistance, HCV infection is known to promote cellular oxidative stress through multiple mechanisms, including chronic inflammation, and iron overload. Some of the HCV proteins such as Core protein and NS5A (Nonstructural protein 5A) were reported to directly contribute to this process (Choi and Ou 2006; Houstis *et al.* 2006).

Peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PPARGC1A) is one of the key regulators of various metabolic pathways through the modulation of adaptive thermogenesis, mitochondrial biogenesis, fatty acid oxidation, gluconeogenesis, and lipogenesis, all of which are to some extent perturbed in the development of non-alcoholic fatty liver disease (NAFLD) (Soyal *et al.* 2006). Thus, dysfunctions of this transcription factor are likely to contribute to the onset and progression of obesity and related metabolic disorders (Ramos-Lopez *et al.* 2018).

PPARGC1A co-activates several transcription factors, including hepatocyte nuclear factor-4 α (HNF-4 α) and forkhead box O1 (FOXO1), controlling, therefore, the transcription of rate-limiting gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase (PEPCK), pyruvate dehydrogenase kinase isoenzyme 4 (PDHK4) and glucose-6-phosphatase (G6Pase) (Finck and Kelly 2006).

PPARGC1A expression is dramatically elevated in HCV-infected cells, and accompanied by an upregulated expression of PEPCK and G6Pase (Li *et al.* 2007; Qadri *et al.* 2012). In addition, the HCV nonstructural protein 5A induces metabolic dysregulation and IR in human hepatoma cells, a process to which PPARGC1A could participate (Parvaiz *et al.* 2014). Furthermore, a recent study has reported that HCV infection induces PPARGC1A expression and endoplasmic reticulum (ER) stress. Moreover, pharmacological induction of ER stress upregulates PPARGC1A expression, and pharmacological inhibition of HCV-induced ER stress prevents PPARGC1A upregulation (Yao *et al.* 2014). Finally, treatment of HCV replicon cells with the antioxidant N-acetylcysteine can attenuate PPARGC1A induction, suggesting that HCV-promoted PPARGC1A expression is mediated by oxidative stress and inflammation (Shlomai *et al.* 2012).

Several studies reported an association between PGC-1 α polymorphism at position +1564G/A (rs8192678) with NAFLD, T2DM and metabolic syndrome, which is associated with the substitution of Gly with Ser (Gly482Ser)

(Ek *et al.* 2001; Kunej *et al.* 2004; Vohl *et al.* 2005; Yoneda *et al.* 2008; Burgueno *et al.* 2013; Saremi *et al.* 2019). In addition, rs12640088 had a significant interaction with body mass index (BMI) (Barroso *et al.* 2006; Villegas *et al.* 2014). These studies have been conducted in Japan, Europe, Argentina and Iran i.e. in countries rather distant from Morocco. North Africa is known for the high prevalence of overweight and obesity that affect its populations, we were still curious to explore HCV and PPARGC1A in the Moroccan genetic context. These two polymorphisms in the Moroccan population have not yet been studied and needs to be explored. In this context, the aim of the current study was to investigate the effects of two polymorphisms of PPARGC1A gene in patients from a North Africa infected with HCV: a G to A transition at position + 1564 in exon 8 (that predicted a glycine to serine amino acid substitution at position 482, and therefore referred to as Gly482Ser: rs8192678) and intron variant rs12640088 A > C. These two SNPs have been previously associated with several human diseases, including type 2 diabetes, and obesity (Barroso *et al.* 2006; Villegas *et al.* 2014).

Materials and Methods

Patients and Healthy Controls

A total of 592 Moroccan subjects were enrolled in this case-control study at the Medical Center of Biology at the Pasteur Institute of Morocco and in the department of Medicine B at the Ibn Rochd university hospital, Casablanca from May 2012 to January 2016. This study included 292 patients with persistent HCV infection, 100 individuals who spontaneously cleared the virus, and 200 healthy controls. Each participant completed a structured questionnaire on demographic data, medical history, lifestyle features and other characteristics. Peripheral blood from the study subjects was collected on EDTA-containing tubes.

CHC are defined as patients persistently positive both for anti-hepatitis C virus (anti-HCV) antibodies and for HCV RNA by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) for at least 6 months. In these patients, histological features were assessed noninvasively using FibroTest-ActiTest combining α -2-macroglobulin, GGT, apolipoprotein A1, haptoglobin, total bilirubin, age and gender (Biopredictive, Paris, France). Patients were stratified into two groups according to fibrosis stage. Among chronic HCV patients, 92 had mild chronic hepatitis C (mCHC i.e. patients with F0–F2 fibrosis stages), 200 patients had advanced liver disease (AdLD i.e. patients with F3–F4 or HCV-related-HCC).

The HCV-spontaneous clearance group was positive for anti-HCV and negative for HCV RNA by qRT-PCR according to at least two measurements performed more than 6 months apart. Healthy controls were negative for hepatitis serological markers and with normal serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) with no current or past history of liver disease. Patients with evidence of co-infection with human immunodeficiency virus, hepatitis B virus infection or presence of autoimmune liver disease were excluded from the study.

Serological and Molecular Analyses

Serological markers were tested for HBsAg, anti-HCV (AxSYM/Architect, Abbott Diagnostics, Wiesbaden-Delkenheim, Germany) and anti-HIV (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). HCV RNA level below the detection threshold (15 IU/mL) was scored as negative for HCV RNA. ALT, AST, gamma-glutamyltransferase (GGT), bilirubin, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides were collected at time of blood sampling by two separate interviews.

Genomic DNA Isolation and PPARGC1A Polymorphisms Genotyping

Genomic DNA was isolated from the peripheral blood mononuclear cells as described previously (Ezzikouri *et al.* 2018). Genotyping for *PPARGC1A* rs8192678 and rs12640088 was undertaken using the TaqMan SNP genotyping allelic discrimination method (Thermo Fisher Scientific, Foster City, CA, USA) using a Light Cycler[®] 480 Real-Time PCR System (Roche diagnostics, Mannheim, Germany). Genomic DNA was amplified in a 10 µl reaction volume containing 1 × SensiFAST Genotyping Lo-Rox Mix (Bioline, London, UK), SNP Genotyping Assay, genomic DNA (20 ng), and completed with DNase-free water.

Statistical Analysis

Clinical and biochemical data are presented as arithmetic mean ± standard deviation, and differences between groups were assessed with the Student's *t* test for quantitative variables with a normal distribution. Otherwise, Wilcoxon-Mann-Whitney test was applied for non-normally distributed quantitative traits. Hardy-Weinberg equilibrium (HWE) was assessed using SNP-HWE program. Genotype frequencies were compared between

groups by regression analysis under dominant, co-dominant, recessive and log-additive models of inheritance. Results were expressed as p-value, odds ratio (OR), and 95% confidence interval (CI). All statistical procedures were performed with R software for Windows and the effect of genetic polymorphism on spontaneous clearance was examined with the SNPAssoc R package (<https://www.r-project.org>). *P*-value < 0.05 was considered a significant difference. All statistical tests were two-sided.

Results

Clinical and Demographical Features

In the present case-control study, a total of 592 volunteers were enrolled, among these 292 individuals with persistent HCV infection including 92 patients with mild chronic hepatitis C and 200 with advanced liver disease (AdLD) including liver cirrhosis and hepatocellular carcinoma. In addition, 100 subjects who spontaneously cleared HCV and 200 healthy controls were recruited. The demographic, biochemical, viral characteristics of the study population are shown in Table 1.

Patients with advanced liver fibrosis were older ($P < 0.0001$) than the others. Serum aminotransferases and bilirubin levels were very significantly increased in the AdLD group as compared to the mCHC group ($P < 0.0001$). Moreover, AdLD patients exhibited significantly higher values of fasting serum glucose ($P = 0.003$) but lower total cholesterol and LDL-cholesterol values compared to the mCHC group ($P < 0.05$). No significant difference was observed regarding creatinine, triglyceride, HDL-cholesterol and HCV viral load between AdLD and mCHC groups (Table 2). An analysis of biological parameters and viral characteristic of patients with CHC infection according to HCV genotypes was also performed. We observed that patients infected with HCV genotype 1 have higher serum triglyceride ($P = 0.001$) and lower LDL-cholesterol levels ($P = 0.01$) compared to genotype 2-infected patients. There was no difference regarding fasting serum glucose, total cholesterol and HDL-cholesterol levels between genotypes 1 and 2 ($P > 0.05$, data not shown). Likewise, serum aminotransferase level was significantly higher in HCV genotype 1-infected group compared to the genotype 2-infected subset ($P = 0.01$, Data not shown). However, no significant difference between HCV genotypes regarding viral loads was observed ($P > 0.05$, data not shown). These results confirm that the progression of the liver disease due to HCV includes both features of liver injury and metabolic traits with genotype 1 apparently capable to more profoundly disturb liver function than genotype 2.

Table 1 Baseline characteristics of healthy subjects, chronic HCV patients and HCV-spontaneous clearance group.

	Healthy controls (<i>n</i> = 200)	Chronic HCV infection (<i>n</i> = 292)	HCV-spontaneous clearance (<i>n</i> = 100)
Age (years)	55 [13–93]	63 [20–88]	58 [20–76]
Gender (%)			
Male	67 (33.5)	108 (36)	41 (41)
Female	133 (66.5)	192 (64)	59 (59)
<i>Biochemical variables (mean ± SD)</i>			
Alanine aminotransferase (IU/L)	35.33 ± 21.52	78.43 ± 55.23	46.44 ± 56.25
Aspartate aminotransferase (IU/L)	29.35 ± 16.39	68.85 ± 46.25	34.7 ± 21.43
Bilirubin (μmol/L)	NA	15.19 ± 6.08	15.16 ± 11.17
Creatinine (μmol/L)	NA	108.54 ± 199.72	82.34 ± 34.55
Fasting serum glucose (g/L)	0.95 ± 0.19	1.06 ± 0.41	1.23 ± 0.54
Total cholesterol (g/L)	1.91 ± 0.38	1.53 ± 0.36	1.72 ± 0.38
Triglycerides (g/L)	1.31 ± 0.68	1.04 ± 0.37	1.37 ± 1.33
HDL-cholesterol (g/L)	0.54 ± 0.36	0.52 ± 0.17	0.48 ± 0.10
LDL-cholesterol (g/L)	1.11 ± 0.38	0.86 ± 0.6	0.94 ± 0.36
Median viral load (IU/mL)	NA	2.8 E + 06	–
[range]		[0.9 E + 03 – 64.5 E + 06]	
<i>Viral genotypes (%)</i>			
Genotype 1	–	59.90	–
Genotype 2	–	39.06	–
Genotype 3	–	0.52	–
Genotype 4	–	0.52	–
<i>Disease stages (n)</i>			
mCHC	–	92	–
AdLD	–	127	–
HCC	–	73	–

SD standard deviation, Na non applicable, HDL high density lipoprotein, LDL low density lipoprotein, mCHC mild chronic hepatitis C, AdLD advanced liver disease, HCC hepatocellular carcinoma.

Frequency of *PPARGC1A* Genotypes

To estimate the frequencies of the *PPARGC1A* polymorphisms in Moroccan population, rs8192678 and rs12640088 SNPs were genotyped in 200 healthy controls. The distributions of both SNPs in healthy control group complied with Hardy–Weinberg equilibrium ($P > 0.05$). The minor allele (A) frequency (MAF) in Moroccan population was 0.175 for rs8192678 and 0.115 for rs12640088, respectively (Table 3).

Association between *PPARGC1A* Polymorphisms and Spontaneous Clearance of HCV Infection

Outcomes regarding the impact of *PPARGC1A* variant on spontaneous HCV clearance are displayed in Table 3. Prevalence of homozygous A/A genotype at rs8192678 was 8.0% in the spontaneously recovered group and 7.2% in the HCV persistent group, while homozygous C/C genotype at rs12640088 was 3.0% in the spontaneous

recovery group compared to 2.4% in the persistence group. There were no significant differences in the distribution of the genotype frequencies between groups ($P > 0.05$).

When the rs8192678 G/G and rs12640088 A/A genotypes were used as the reference group, none of the G/A, A/A and A/C, C/C genotypes were associated with chronicity (adjusted ORs = 0.69, 95% CI 0.43–1.11; 0.76, 95% CI 0.32–1.82 and 0.99, 95% CI 0.57–1.71; 0.79, 95% CI 0.20–3.14, respectively). The A/A and C/C genotypes were not associated either with spontaneous resolution of HCV infection.

Association between the rs8192678 and rs12640088 Polymorphisms and Disease Progression of HCV Infection

In order to analyze the effect of the two polymorphisms on liver disease progression in the Moroccan population, we tested the association of rs8192678 and rs12640088 with fibrosis stage by multiple logistic regression analysis

Table 2 Baseline characteristics of mild and advanced liver diseases groups.

	Mild chronic hepatitis (<i>n</i> = 92)	Advanced liver disease (<i>n</i> = 200)	<i>P</i> value
Mean age ± SD (years)	57.13 ± 14.09	64.14 ± 9.47	< 0.0001
<i>Gender (%)</i>			
Male	26 (27.37)	41 (31.30)	0.558
Female	69 (72.63)	90 (68.70)	
<i>Biochemical variables (mean ± SD)</i>			
Alanine aminotransferase (IU/L)	53.63 ± 39.77	96.69 ± 58.73	< 0.0001
Aspartate aminotransferase (IU/L)	42.91 ± 24.91	87.94 ± 51.96	< 0.0001
Bilirubin (µmol/L)	13.13 ± 5.54	16.76 ± 6.01	< 0.0001
Creatinine (µmol/L)	87.91 ± 165.39	130.56 ± 249.41	0.148
Fasting serum glucose (g/L)	0.97 ± 0.19	1.14 ± 0.53	0.003
Total cholesterol (g/L)	1.60 ± 0.36	1.47 ± 0.31	0.004
Triglycerides (g/L)	1.03 ± 0.38	1.06 ± 0.40	0.570
HDL-cholesterol (g/L)	0.54 ± 0.19	0.51 ± 0.15	0.186
LDL-cholesterol (g/L)	0.91 ± 0.68	0.74 ± 0.31	0.012
Median viral load (IU/mL)	2.7 E + 06	2.9 E + 06	0.818
[range]	[1.7 E + 03–31.8 E + 06]	[0.9 E + 03–64.5 E + 06]	
<i>Viral genotypes (%)</i>			
Genotype 1	53.85	64.03	0.114
Genotype 2	46.15	34.21	–
Genotype 3	–	0.88	–
Genotype 4	–	0.88	–

SD standard deviation, *HDL* high density lipoprotein, *LDL* low density lipoprotein.

adjusted for age, gender and viral load. CHC patients were stratified according to fibrosis stages as absent/mild (Mild group) or significant (AdLD group). The result indicated that age, male sex, cholesterol, GGT, ALT and AST were associated with advanced fibrosis ($P < 0.05$). On the other hand, we observed an absence of impact of both rs8192678 AA and rs12640088 CC genotypes on progression of liver disease (OR = 0.71; 95% CI 0.20–2.49; $P = 0.739$; OR = 1.28; 95% CI 0.25–6.54; $P = 0.512$, respectively Table 4).

Biochemical and Metabolic Data in Patients with CHC Infection According to PPARGC1A Polymorphisms

No significant associations were found between rs8192678 and rs12640088 SNPs with biochemical features available (Fig. 1).

Discussion

HCV-related liver disease is known to progress gradually from chronic hepatitis to liver cirrhosis and to hepatocellular carcinoma (HCC) in a significant subset of infected subjects (Niederau *et al.* 1998). It has been shown that both

viral and host factors are involved in HCV spontaneous clearance and disease progression (Mosley *et al.* 2005). Metabolic factors may affect the course of chronic hepatitis C (CHC). Obesity and the metabolic syndrome are conditions associated with liver manifestations, including steatosis and fibrosis. In individuals with chronic hepatitis C, obesity is associated with an exacerbation of inflammation, insulin resistance, steatosis, progression of fibrosis. Furthermore, these patients are considered as poor responders to treatment with IFN- α (Hui *et al.* 2003; White *et al.* 2008; Lazo *et al.* 2017). In the Middle-East and North Africa (MENA) region, metabolic impact of HCV is suspected to synergize with the pathological burden due to a widespread prevalence of overweight, obesity and T2D (Tazi *et al.* 2003; Musaiger 2011).

The peroxisome proliferator-activated receptor gamma coactivator 1-alpha is a novel transcriptional co-activator playing an important role in lipid and glucose metabolisms. Genetic variations in this gene were associated with obesity, type 2 diabetes, and some other related diseases (Mirzaei *et al.* 2012). It is known to regulate lipid metabolism and long-chain fatty acid oxidation by upregulating the expression of several genes of the tricarboxylic acid cycle and the mitochondrial fatty acid oxidation pathway (Cheng *et al.* 2018). *PPARGC1A* gene is a transcriptional

Table 3 Effect of PPARGC1A polymorphisms on the outcomes of HCV infection.

PPARGC1A	Healthy controls <i>n</i> = 200 (%)	Spontaneous clearance <i>n</i> = 100 (%)	Persistent infection <i>n</i> = 292 (%)	Subjects with persistence versus subjects with spontaneous clearance OR (95% CI)	<i>P</i> value
<i>rs8192678</i>					
G/G	142 (71)	48 (48)	166 (56.85)	1	
G/A	46 (23)	44 (44)	105 (35.96)	0.69 [0.43–1.11]	0.126
A/A	12 (6)	8 (8)	21 (7.19)	0.76 [0.32–1.82]	0.536
G allele	0.825 ± 0.021	0.700 ± 0.032	0.748 ± 0.018	1	
A allele	0.175 ± 0.021	0.300 ± 0.032	0.252 ± 0.018	0.78 [0.55–1.12]	0.181
Dominant model		48/52	166/126	0.70 [0.44–1.10]	0.125
Recessive model		92/8	271/21	1.12 [0.48–2.62]	0.789
<i>rs12640088</i>					
A/A	154 (77)	75 (75)	221 (75.68)	1	
A/C	46 (23)	22 (22)	64 (21.92)	0.99 [0.57–1.71]	0.963
C/C	0 (0)	3 (3)	7 (2.40)	0.79 [0.20–3.14]	0.739
A allele	0.885 ± 0.015	0.860 ± 0.026	0.866 ± 0.014	1	
C allele	0.115 ± 0.015	0.140 ± 0.026	0.134 ± 0.014	0.95 [0.59–1.51]	0.818
Dominant model		75/25	221/71	0.96 [0.57–1.63]	0.890
Recessive model		97/3	285/7	1.26 [0.32–4.97]	0.745

Table 4 Factors associated with disease progression of HCV infection.

	Mild group (<i>n</i> = 92)	Advanced group (<i>n</i> = 200)	OR	95% CI	<i>P</i> value
Age (years)	58.5 [51–67]	65 [58–73]	1.06	1.04–1.09	< 0.001
Male sex (%)	23 (25.0%)	81 (41.3%)	2.1	1.23–3.71	0.011
Total cholesterol (g/L)	1.64 [1.36–1.85]	1.42 [1.27–1.68]	0.23	0.07–0.75	0.015
Triglycerides (g/L)	0.96 [0.81–1.27]	0.98 [0.78–1.21]	1.12	0.41–3.06	0.874
HDL (g/L)	0.52 [0.42–0.66]	0.51 [0.38–0.60]	0.14	0.01–1.73	0.232
LDL (g/L)	0.89 [0.60–1.15]	0.80 [0.50–1.00]	0.42	0.10–1.68	0.200
Gamma-glutamyltransferase (IU/L)	27.0 [19.0–40.0]	63.5 [38.2–119]	1.02	1.01–1.03	< 0.001
ALT (IU/L)	40.5 [28.2–61.8]	82.0 [54.0–123]	1.02	1.01–1.04	< 0.001
AST (IU/L)	33.5 [27.0–44.2]	76.0 [51.5–116]	1.04	1.02–1.06	< 0.001
PPARGC1A rs8192678 AA genotype	4 (4.35%)	8 (6.30%)	0.71	0.20–2.49	0.739
PPARGC1A rs12640088 CC genotype	3 (2.36)	3 (2.36)	1.28	0.25–6.54	0.512

Factors associated with progression of liver disease in chronic hepatitis C patients.

HDL high density lipoprotein, *LDL* low density lipoprotein, *ALT* alanine aminotransferase, *AST* Aspartate aminotransferase, *PPARGC1A* Peroxisome proliferator-activated receptor gamma coactivator 1 alpha, *OR* Odds ratio, *CI* confidence interval.

coactivator of the nuclear receptor PPAR γ that exerts powerful effects on hepatic glucose and fat metabolism and may be involved in the development of NAFLD (Estall *et al.* 2009). A protective role of rs8192678 against hepatic steatosis has been found both *in vivo* and *in vitro* (Chen *et al.* 2013; Tai *et al.* 2016). Furthermore, recent evidence of a direct role for *PPARGC1A* inactivation in the pathogenesis of steatosis and HCC has been obtained in a mouse model combining HCV core transgene with *PPARGC1A* knockout where a liver steatosis was induced (Tanaka *et al.*

2008). Meanwhile, HCV core protein has been shown to reduce mitochondrial long chain fatty acid β -oxidation by impairing the expression and function of *PPARGC1A* (Cheng *et al.* 2005; Dharancy *et al.* 2005).

As no data was available concerning the association of *PPARGC1A* variants and liver disease progression so far, we set out to explore the role of *PPARGC1A* gene polymorphisms rs8192678 and rs12640088 in liver disease progression of Moroccan patients chronically infected with HCV. In the population studied, the MAF frequency for

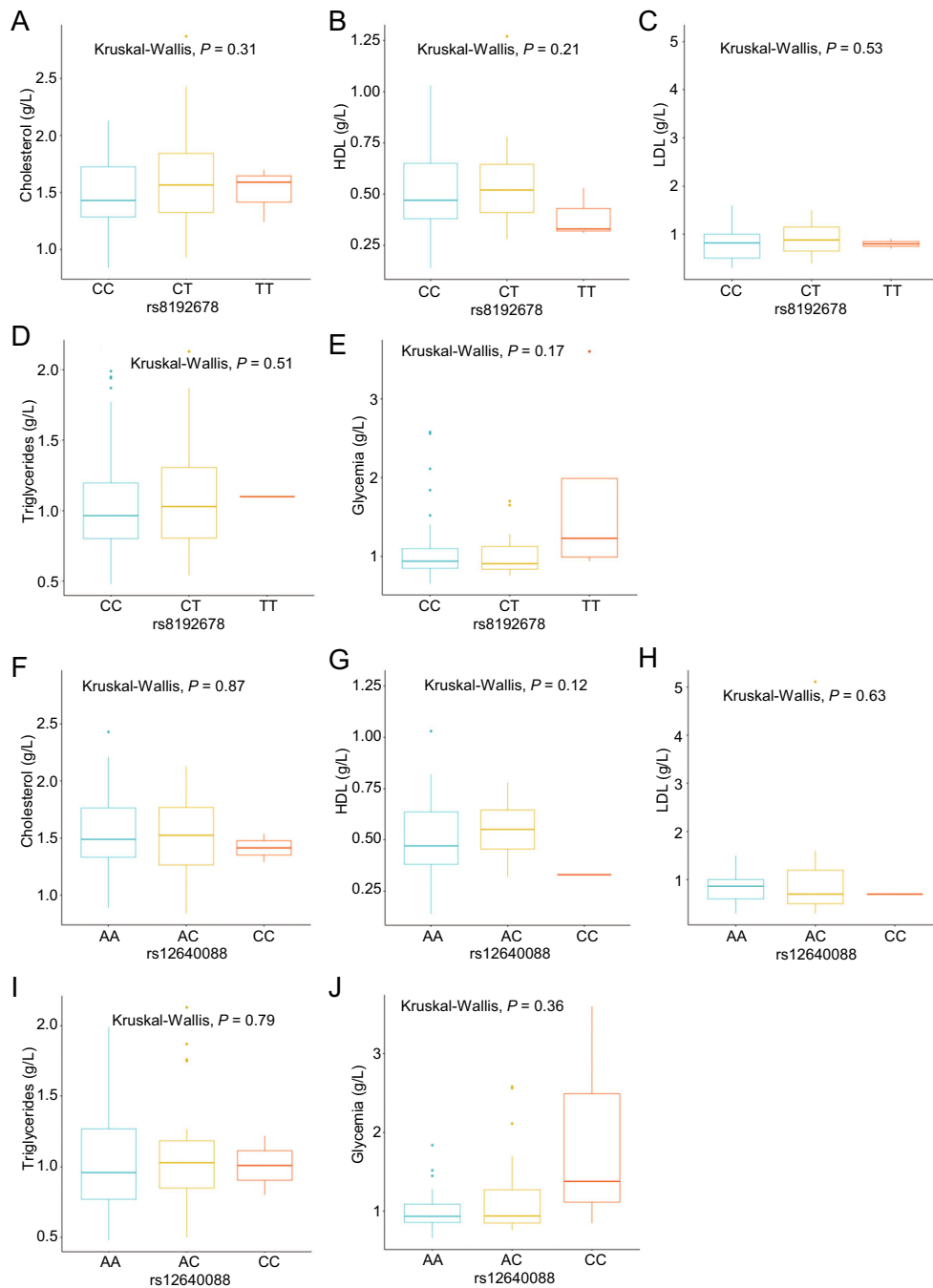


Fig. 1 Association of rs8192678 and rs12640088 genotypes with lipid profile and blood glucose in CHC patients. Expression of serum cholesterol (A), HDL (B), LDL (C), Triglycerides (D) and Glycemia (E) according to genotypes of PPARGC1A rs8192678. Expression of

serum cholesterol (F), HDL (G), LDL (H), Triglycerides (I) and Glycemia (J) according to genotypes of PPARGC1A rs12640088. Data are expressed as the mean and standard deviation. Statistical analyses were performed using Kruskal–Wallis.

rs8192678 was 0.175, which is lower than frequencies reported in both Caucasians and Asian population (Tai *et al.* 2016). However, regarding the rs12640088, the MAF was 0.115 in line with those reported in Caucasians and Asian population (Tai *et al.* 2016).

To our knowledge, this is the first description of the relationship between PPARGC1A gene rs8192678 and

rs12640088 polymorphisms and the clearance of HCV infection. The major finding of our study was a lack of association of both SNPs in PPARGC1A and spontaneous resolution of HCV infection. Furthermore, in the present study, we found that PPARGC1A rs8192678 and rs12640088 are not associated with the risk of liver disease progression in CHC. Our data are, thus, at odd with

previous studies that found a significant association between *PPARGC1A* rs8192678 (Gly482Ser) polymorphism and HCC development, in a primarily HBV-infected Eastern Chinese Han population (Zhang *et al.* 2018). Moreover, Liu and colleagues found that a decreased expression of *PPARGC1A* plays an important role in the formation and development of HCC, while the overexpression of *PPARGC1A* in HCC cell lines promotes apoptosis, suggesting that *PPARGC1A* may be a tumor suppressor and potential therapeutic target for HCC (Liu *et al.* 2017).

Regarding the metabolic aspects of the disease, our data did not reveal any significant associations with clinical characteristics such as liver enzymes, carbohydrates metabolism and lipid profile in CHC patients. These observations suggest that genetic variations affecting *PPARGC1A* are not crucial determinants in the genetic architecture and the pathological spectrum of North African and possibly Middle Eastern populations.

In conclusion, the *PPARGC1A* variants are neither associated with spontaneous resolution of HCV infections nor with progression of the chronic liver disease. Our study has some limitations, including missing data on the *PPARGC1A* expression in HCV-infected liver biopsies. Moreover, we are fully aware that another limitation of the current study is the relatively small size of the cohort examined. Further surveys combining the recruitment of a larger number of subjects and analyses of additional polymorphisms in *PPARGC1A* gene will help to clarify its role in HCV infections and progression. Further studies on North African patients with non-virus induced NAFLD, are warranted to definitively refute or confirm an effect of rs8192678 and rs12640088 on liver diseases progression.

Acknowledgements The authors would like to acknowledge all patients for their participation in this study.

Author Contributions SB, SE, RE conceived, designed the experiments and wrote the paper; SB, SE, MK and PP contributed to the drafting and revision of the article; IZ, FZJ and HC helped in the collection of samples; WB and MT contributed to the clinical data; MEI helped in the statistical analysis; IC and AM helped in the interpretation of data; SB contributed to the final approval of the version to be submitted. All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of interest Authors declare that they have no conflict of interest.

Animal and Human Rights Statement The study was approved by the Ethics Committee of the Faculty of Medicine of Casablanca in accordance with the ethical guidelines of the Declaration of Helsinki. For study participation, written informed consent for genetic testing was obtained from all subjects.

References

- Barroso I, Luan J, Sandhu MS, Franks PW, Crowley V, Schafer AJ, O'Rahilly S, Wareham NJ (2006) Meta-analysis of the Gly482Ser variant in *PPARGC1A* in type 2 diabetes and related phenotypes. *Diabetologia* 49:501–505
- Burgueno AL, Cabrerizo R, Gonzales Mansilla N, Sookoian S, Pirola CJ (2013) Maternal high-fat intake during pregnancy programs metabolic-syndrome-related phenotypes through liver mitochondrial DNA copy number and transcriptional activity of liver *ppargc1a*. *J Nutr Biochem* 24:6–13
- Chen Y, Mu P, He S, Tang X, Guo X, Li H, Xu H, Woo SL, Qian X, Zeng L, Wu C (2013) Gly482ser mutation impairs the effects of peroxisome proliferator-activated receptor gamma coactivator-1alpha on decreasing fat deposition and stimulating phosphoenolpyruvate carboxykinase expression in hepatocytes. *Nutr Res* 33:332–339
- Cheng Y, Dharancy S, Malapel M, Desreumaux P (2005) Hepatitis C virus infection down-regulates the expression of peroxisome proliferator-activated receptor alpha and carnitine palmitoyl acyl-CoA transferase 1a. *World J Gastroenterol* 11:7591–7596
- Cheng CF, Ku HC, Lin H (2018) PGC-1alpha as a pivotal factor in lipid and metabolic regulation. *Int J Mol Sci* 19:3447
- Choi J, Ou JH (2006) Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis c virus. *Am J Physiol Gastrointest Liver Physiol* 290:G847–G851
- Dharancy S, Malapel M, Perlemuter G, Roskams T, Cheng Y, Dubuquoy L, Podevin P, Conti F, Canva V, Philippe D, Gambiez L, Mathurin P, Paris JC, Schoonjans K, Calmus Y, Pol S, Auwerx J, Desreumaux P (2005) Impaired expression of the peroxisome proliferator-activated receptor alpha during hepatitis C virus infection. *Gastroenterology* 128:334–342
- Ek J, Andersen G, Urhammer SA, Gaede PH, Drivsholm T, Borch-Johnsen K, Hansen T, Pedersen O (2001) Mutation analysis of peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to type ii diabetes mellitus. *Diabetologia* 44:2220–2226
- Estall JL, Kahn M, Cooper MP, Fisher FM, Wu MK, Laznik D, Qu L, Cohen DE, Shulman GI, Spiegelman BM (2009) Sensitivity of lipid metabolism and insulin signaling to genetic alterations in hepatic peroxisome proliferator-activated receptor-gamma coactivator-1alpha expression. *Diabetes* 58:1499–1508
- Ezzikouri S, Elfihiy R, Chihab H, Elmessaoudi-Idrissi M, Zaidane I, Jadid FZ, Karami A, Tahiri M, Elhabazi A, Kabine M, Chair M, Pineau P, Benjelloun S (2018) Effect of MBOAT7 variant on hepatitis B and C infections in moroccan patients. *Sci Rep* 8:12247
- Finck BN, Kelly DP (2006) PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. *J Clin Invest* 116:615–622
- Houstis N, Rosen ED, Lander ES (2006) Reactive oxygen species have a causal role in multiple forms of insulin resistance. *Nature* 440:944–948
- Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, McCaughan GW, George J (2003) Insulin resistance is associated with chronic hepatitis c virus infection and fibrosis progression [corrected]. *Gastroenterology* 125:1695–1704
- Jacobson IM, Cacoub P, Dal Maso L, Harrison SA, Younossi ZM (2010) Manifestations of chronic hepatitis C virus infection beyond the liver. *Clin Gastroenterol Hepatol* 8:1017–1029
- Knobler H, Schattner A (2005) TNF- α , chronic hepatitis c and diabetes: a novel triad. *QJM* 98:1–6
- Kunej T, Globocnik Petrovic M, Dovc P, Peterlin B, Petrovic D (2004) A Gly482Ser polymorphism of the peroxisome proliferator-activated receptor-gamma coactivator-1 (PGC-1) gene is

- associated with type 2 diabetes in caucasians. *Folia Biol (Praha)* 50:157–158
- Lauer GM, Walker BD (2001) Hepatitis C virus infection. *N Engl J Med* 345:41–52
- Lazo M, Nwankwo C, Daya NR, Thomas DL, Mehta SH, Juraschek S, Willis K, Selvin E (2017) Confluence of epidemics of hepatitis C, diabetes, obesity, and chronic kidney disease in the united states population. *Clin Gastroenterol Hepatol* 15(1957–1964):e1957
- Li X, Monks B, Ge Q, Birnbaum MJ (2007) Akt/PKB regulates hepatic metabolism by directly inhibiting PGC-1 α transcription coactivator. *Nature* 447:1012–1016
- Liu R, Zhang H, Zhang Y, Li S, Wang X, Wang X, Wang C, Liu B, Zen K, Zhang CY, Zhang C, Ba Y (2017) Peroxisome proliferator-activated receptor gamma coactivator-1 alpha acts as a tumor suppressor in hepatocellular carcinoma. *Tumour Biol* 39:1010428317695031
- Maeno T, Okumura A, Ishikawa T, Kato K, Sakakibara F, Sato K, Ayada M, Hotta N, Tagaya T, Fukuzawa Y, Kakumu S (2003) Mechanisms of increased insulin resistance in non-cirrhotic patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 18:1358–1363
- Mirzaei K, Hossein-nezhad A, Emamgholipour S, Ansari H, Khosrofar M, Tootee A, Alatab S (2012) An exonic peroxisome proliferator-activated receptor-gamma coactivator-1 α variation may mediate the resting energy expenditure through a potential regulatory role on important gene expression in this pathway. *J Nutrigenet Nutrigenomics* 5:59–71
- Mosley JW, Operskalski EA, Tobler LH, Andrews WW, Phelps B, Dockter J, Giachetti C, Busch MP (2005) Viral and host factors in early hepatitis C virus infection. *Hepatology* 42:86–92
- Musaiger AO (2011) Overweight and obesity in eastern mediterranean region: prevalence and possible causes. *J Obes* 2011:407237
- Niederer C, Lange S, Heintges T, Erhardt A, Buschkamp M, Hurter D, Nawrocki M, Kruska L, Hensel F, Petry W, Haussinger D (1998) Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 28:1687–1695
- Parvaiz F, Manzoor S, Iqbal J, McRae S, Javed F, Ahmed QL, Waris G (2014) Hepatitis C virus nonstructural protein 5a favors upregulation of gluconeogenic and lipogenic gene expression leading towards insulin resistance: a metabolic syndrome. *Arch Virol* 159:1017–1025
- Qadri I, Choudhury M, Rahman SM, Knotts TA, Janssen RC, Schaack J, Iwahashi M, Puljak L, Simon FR, Kilic G, Fitz JG, Friedman JE (2012) Increased phosphoenolpyruvate carboxykinase gene expression and steatosis during hepatitis C virus subgenome replication: role of nonstructural component 5a and CCAAT/enhancer-binding protein beta. *J Biol Chem* 287:37340–37351
- Ramos-Lopez O, Riezu-Boj JI, Milagro FI, Goni L, Cuervo M, Martinez JA (2018) Association of the Gly482Ser PPARGC1A gene variant with different cholesterol outcomes in response to two energy-restricted diets in subjects with excessive weight. *Nutrition* 47:83–89
- Saremi L, Lotfpanah S, Mohammadi M, Hosseinzadeh H, Hosseini-Khah Z, Johari B, Saltanatpour Z (2019) Association between ppargc1a single nucleotide polymorphisms and increased risk of nonalcoholic fatty liver disease among iranian patients with type 2 diabetes mellitus. *Turk J Med Sci* 49:1089–1094
- Shlomai A, Rechtman MM, Burdelova EO, Zilberberg A, Hoffman S, Solar I, Fishman S, Halpern Z, Sklan EH (2012) The metabolic regulator PGC-1 α links hepatitis C virus infection to hepatic insulin resistance. *J Hepatol* 57:867–873
- Soyal S, Krempler F, Oberkofler H, Patsch W (2006) Pgc-1 α : a potent transcriptional cofactor involved in the pathogenesis of type 2 diabetes. *Diabetologia* 49:1477–1488
- Tai CM, Huang CK, Tu HP, Hwang JC, Yeh ML, Huang CF, Huang JF, Dai CY, Chuang WL, Yu ML (2016) Interactions of a PPARGC1A variant and a PNPLA3 variant affect nonalcoholic steatohepatitis in severely obese taiwanese patients. *Medicine (Baltimore)* 95:e3120
- Tanaka N, Moriya K, Kiyosawa K, Koike K, Gonzalez FJ, Aoyama T (2008) Ppar α activation is essential for HCV core protein-induced hepatic steatosis and hepatocellular carcinoma in mice. *J Clin Invest* 118:683–694
- Tazi MA, Abir-Khalil S, Chaouki N, Cherqaoui S, Lahmouz F, Srairi JE, Mahjour J (2003) Prevalence of the main cardiovascular risk factors in Morocco: results of a national survey, 2000. *J Hypertens* 21:897–903
- Thomas DL, Astemborski J, Rai RM, Anania FA, Schaeffer M, Galai N, Nolt K, Nelson KE, Strathdee SA, Johnson L, Laeyendecker O, Boitnott J, Wilson LE, Vlahov D (2000) The natural history of hepatitis C virus infection: host, viral, and environmental factors. *JAMA* 284:450–456
- Villegas R, Williams SM, Gao YT, Long J, Shi J, Cai H, Li H, Chen CC, Tai ES, Consortium A-TD, Hu F, Cai Q, Zheng W, Shu XO (2014) Genetic variation in the peroxisome proliferator-activated receptor (PPAR) and peroxisome proliferator-activated receptor gamma co-activator 1 (PGC1) gene families and type 2 diabetes. *Ann Hum Genet* 78:23–32
- Vohl MC, Houde A, Lebel S, Hould FS, Marceau P (2005) Effects of the peroxisome proliferator-activated receptor-gamma co-activator-1 Gly482Ser variant on features of the metabolic syndrome. *Mol Genet Metab* 86:300–306
- White DL, Ratziu V, El-Serag HB (2008) Hepatitis C infection and risk of diabetes: a systematic review and meta-analysis. *J Hepatol* 49:831–844
- WHO (2017) Guidelines on hepatitis b and c testing, p 204, Licence: CC BY-NC-SA 3.0 IGO, February 2017 edn
- Yao W, Cai H, Li X, Li T, Hu L, Peng T (2014) Endoplasmic reticulum stress links hepatitis C virus RNA replication to wild-type PGC-1 α /liver-specific PGC-1 α upregulation. *J Virol* 88:8361–8374
- Yoneda M, Hotta K, Nozaki Y, Endo H, Uchiyama T, Mawatari H, Iida H, Kato S, Hosono K, Fujita K, Yoneda K, Takahashi H, Kirikoshi H, Kobayashi N, Inamori M, Abe Y, Kubota K, Saito S, Maeyama S, Wada K, Nakajima A (2008) Association between PPARGC1A polymorphisms and the occurrence of nonalcoholic fatty liver disease (NAFLD). *BMC Gastroenterol* 8:27
- Zhang S, Jiang J, Chen Z, Wang Y, Tang W, Chen Y, Liu L (2018) Relationship of PPARG, PPARGC1A, and PPARGC1B polymorphisms with susceptibility to hepatocellular carcinoma in an Eastern Chinese Han population. *Oncol Targets Ther* 11:4651–4660