Impact of PNPLA3 variants on the liver histology of 168 patients with HIV infection and chronic hepatitis C

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Introduction

In HIV/HCV coinfection, 40–75% of patients show liver steatosis, associated with HCV-genotype 3, alcohol abuse, diabetes, and intravenousdrug use (IVDU). A genome-wide association study recently identified a non-synonymous sequence variation (rs738409 C to G) encoding an isoleucine-to-methionine substitution at position 148 in the adiponutrin/patatin-like phospholipase-3 (PNPLA3) gene, which appears to be the strongest determinant of human steatosis. The I148M variant of PNPLA3 was found to be independently associated with liver steatosis and disease progression in NAFLD, alcoholic liver disease, CHC and HCC. Adiponutrin, expressed in the liver and adipose tissue, has both triacylglycerol hydrolase and acylglycerol transacetylase activity. The 148M allele encodes for a loss-of-function variant that predisposes to steatosis by decreasing triglyceride hydrolysis in hepatocytes. The data on the impact of PNPLA3 on liver steatosis in HIV/HCV confection published are far from conclusive because fragmentary or obtained applying methods less sensitive than the gold standard liver histology. The present study analyzes the influence of PNPLA3 polymorphisms on liver histology of 168 consecutive patients with biopsy proven CHC and HIV infection.

Methods

One-hundred and sixty-eight consecutive anti-HIV-positive patients with anti-HCV/HCVRNA-positive asymptomatic chronic hepatitis for 18-36 months were enrolled at the time they underwent their first liver biopsy (LB), from 1993 to 2008. All patients enrolled were Caucasian of European ancestry and anti-HCV treatment naïve, HBsAg-negative, stated no active alcohol abuse (>40g/day for males, >30g/day for females for at least 5 years) or statin administration and had no evidence of autoimmune or genetic disorders that induce liver disease. None of the 168 patients had impaired fasting glycemia or diabetes. Ultrasound or serological signs of HCC were never detected. At the first observation, each patient signed the informed consent to undergo LB, for collection and storage at −80°C samples of serum and whole blood and for the use in clinical research of the data obtained, as established by the Ethics Committee of Second University of Naples (protocol number 112/15 March 2013). Percutaneous LB was performed for all patients under US guidance. An experienced pathologist unaware of the clinical and laboratory data used the Ishak's scoring system to grade necroinflammation (HAI) and fibrosis. Liver steatosis was assessed as follows: score 1 for a fatty deposition in 1-10% of hepatocytes, score 2 in 11-30%, score 3 in 31-60% and score 4 in >60%. Genomic DNA was extracted from whole blood by the QIAamp DNA Mini Kit (Qiagen, Germany) and analyzed for the PNPLA3 rs738409 C to G variant underlying I148M substitution, applying in all cases both direct sequencing and restriction enzyme analysis. The following primers were used, F:5'-GCCCTGCTCACTTGGAGAAA-3' and R:5'-TGAAAGGCAGTGAGGCATGG-3'. The *Fok*I restriction enzyme was used to identify the variant, since the G allele eliminates a *Fok*I restriction site.

Results

Of the 168 HIV/HCV-coinfected patients, 72.6% were males, 42% were infected with HCV-genotype 3 and 38.9% with genotype 1, 61.9% had a history of IVDU. The median age of the 168 patients was 40.6 years (IQR:37.6-44.1), with 506.0 (397.5-668.5) CD4+ cells/mm3 and a nadir of 258.0 (163.5-404.5) cells/mm3. Thirty-five (20.8%) patients were naive for antiretroviral therapy and 133 (79.2%) were receiving HAART; the therapy-naïve patients showed a CD4+ cell count higher than that observed in those under HAART (654.1±79.7 (M±SD) vs. 524.3±234.9 cells/mm3, p=0.006). The PNPLA3 p.148I/I variant was detected more frequently (53%) than p.148I/M (39.9%) and p.148M/M (7.1%) variants, the minor allele frequency was 0.27 and genotypes were distributed according to the Hardy–Weinberg equilibrium (p=0.2). Thirty-eight (22.6%) patients showed moderate or severe necroinflammation (HAI >9), 30 (28.1%) moderate or severe fibrosis (score>3) and 56 (33.3%) severe steatosis (score >3).

The 89 subjects with PNPLA3 p.148I/I variant more frequently than the 79 with PNPLA3 p.148I/M or M/M variants had a history of IVDU (70.8% vs. 51.9%, p=0.01) and less frequently showed severe steatosis (scores 3-4) (43% vs. 24.7%, p=0.001) (Table 1).

Table 1: Initial characteristics of 168 HIV/HCV coinfected patients, according to the PNPLA3 variants.

	p.148 I/I	p.148 I/M and p.148 M/M	p-value
N° of patients	89	79	
Age years, median (IQR)	40.0 (36.4-44.1)	41.3 (38.4-44.2)	ns
Males, N° (%)	65 (73.0)	57 (72.1)	ns
Alcohol abusers (> 30g/die), N° (%)	0	0	--
IVDU, N° (%)	63 (70.8)	41 (51.9)	0.01
BMI kg/m2, median (IQR)	22.8 (21.3-24.8)	23.4 (21.9-25.2)	ns
Glucose mg/dL, median (IQR)	86.5 (80.3-94.0)	89.0 (81.0-99.0)	ns
Bilirubin mg/dL, median (IQR)	0.7 (0.5-1.0)	0.7 (0.5-1.0)	ns
AST IU/L, median (IQR)	55.0 (39.0-103.0)	63.5 (44.3-101.8)	ns
ALT IU/L, median (IQR)	76.0 (44.0-131.8)	98.0 (55.0-140.0)	ns
Cholesterol mg/dL, median (IQR)	160.5 (134.5-181.0)	168.0 (133.0-197.0)	ns
Triglycerides mg/dL, median (IQR)	115.0 (75.0-157.0)	137.0(98.0-190.0)	ns
GGT IU/mL, median (IQR)	71.0 (36.0-151.3)	76.5 (44.3-168.5)	ns
ALP IU/mL, median (IQR)	173.0 (120.8-231.0)	198.0 (167.0-259.0)	ns
HCV RNA IU/mL, median (IQR)	636000.0 (202848.3-1365000.0)	601550.0 (182797.0-1665730.0)	ns
Nadir of CD4⁺ cells/mm³, median (IQR)	271.0 (164.0-407.0)	257.5 (166.3-399.8)	ns
HIV RNA cps/mL, median (IQR)	83.0 (49.0-7365.5)	79.0 (49.0-7455.5)	ns
CD4⁺ cell/mmc, median (IQR)	514.0 (390.5-673.0)	500.0 (399.0-627.0)	ns
HAART, N°(%): treated	70 (78.7)	63 (79.7)	ns
PI/r-NRTI	24 (36.4)	21 (33.9)	
PI-NRTI	2 (3.0)	0	
PI/r-NRTI-NNRTI	2 (3.0)	4 (6.5)	
NRTI	20 (30.3)	23 (37.1)	
NRTI-NNRTI	18 (27.3)	14 (22.2)	
Missing, N°	4	1	
naïve-HAART	19 (21.3)	16 (20.3)	
Duration of HAART, years, median (IQR)	8.2 (6.4-11.5)	7.8 (4.6-10.8)	ns
HCV Genotype, N° (%): 1	34 (38.2)	29 (38.7)	ns
2	5 (5.6)	3 (4.0)	ns
3	38 (42.7)	30 (40.0)	ns
4	10 (11.3)	13 (17.3)	ns
Missing	2	4	
HAI score, Mean ± SD	5.85 ± 3.14	6.1 ± 3.04	ns
Degree of HAI, N° (%): score 0-8	67 (75.2)	63 (79.7)	ns
score 9-18	22 (24.7)	16 (20.3)	
Fibrosis score, Mean ± SD	2.2 ± 1.6	2.4 ± 1.6	ns
Degree of fibrosis, N° (%): 0	10 (11.2)	3 (3.8)	ns
1	28 (31.5)	22 (27.8)	
2	17 (19.1)	19 (24.05)	
3	21 (23.4)	17 (21.5)	
4	3 (3.4)	8 (10.3)	
5	5 (5.6)	3 (3.8)	
6	5 (5.6)	7 (10.4)	
Steatosis score, Mean ± SD	1.44 ± 1.29	1.97 ± 1.27	0.007
Degree of steatosis, N° (%): 0	32 (36)	16 (20.5)	*0.01
1	12 (13.5)	10 (12.7)	
2	23 (25.8)	19 (24.0)	
3	18 (20.2)	28 (35.4)	
4	4 (4.5)	6 (7.6)	

*: patients with steatosis score higher than 2 in p.148 I/I vs p.148 I/M and M/M

Compared with the 112 with a lower degree of steatosis, the 56 patients with severe steatosis had a higher BMI p=0.03, a higher serum level of AST p<0.0001, ALT p<0.0001 and GGT p=0.04 (Table 2). They also showed a lower CD4+ cell count p=0.002, and a higher prevalence of patients with HCV-genotype 3 p=0.01, PNPLA3-p.148I/M or M/M p=0.01, severe HAI p<0.02 and severe fibrosis p<0.01. The mean scores of HAI and fibrosis were also higher in patients with severe steatosis, p=0.003 and p=0.001, respectively.

Table 2: Initial characteristics of 168 HIV/HCV coinfected patients, according to the degree of liver steatosis.

	Steatosis score 0-2	Steatosis score 3-4	p-value
N° of patients	112	56	
Age, years, median (IQR)	40.2 (37.3-44.2)	41.8 (38.4-44.0)	n.s.
Males, N° (%)	80 (22)	42 (10)	n.s.
IVDU, N° (%)	73 (39)	28 (28)	n.s.
BMI kg/m2, median (IQR)	22.7 (21.3-24.5)	23.9 (21.9-25.6)	0.03
Glucose mg/dL, median (IQR)	86.0 (79.5-94.0)	92.5 (82.0-102.0)	0.007
Bilirubin mg/dL, median (IQR)	0.6 (0.4-1.0)	0.8 (0.6-1.1)	0.04
AST IU/L, median (IQR)	54.0 (38.5-79.0)	92.0 (55.8-142.3)	<0.0001
ALT IU/L, median (IQR)	67.0 (43.0-114.5)	129.0 (78.8-201.5)	<0.0001
Cholesterol mg/dL, median (IQR)	167.5 (141.0-196.5)	147.0 (120.0-174.0)	0.004
Triglycerides mg/dL, median (IQR)	130.0 (84.0-188.5)	123.0 (87.0-156.5)	n.s.
GGT IU/L, median (IQR)	70.0 (35.0-142.5)	97.0 (46.5-229.5)	0.04
ALP IU/L, median (IQR)	181.5 (136.8-240.3)	196.0 (147.5-265.0)	n.s.
HCV RNA IU/ml, median (IQR)	520000.0 (151362.5-1433192.5)	747593.0 (243750.0-1400000.0)	n.s.
Nadir of CD4⁺ cells/mm³, median (IQR)	258.0 (161.5-420.0)	257.5 (172.8-360.5)	n.s.
HIV RNA cps/mL, median (IQR)	79.0 (49.0-4847.0)	708.0 (49.0-21471.0)	n.s.
CD4⁺ cells/mm³, median (IQR)	531.0 (426.5-720.5)	463.0 (373.0-542.8)	0.002
Duration of HAART, years, median (IQR)	8.2 (6.2-11.5)	7.6 (4.4-10.8)	n.s.
HAART, N°(%): treated	89 (79.5)	44 (78.6)	n.s.
untreated	23 (20.5)	12 (21.4)	
HCV Genotype, N°(%):			
1	45 (41.7)	18 (33.3)	0.01*
2	5 (4.6)	3 (5.6)	
3	38 (35.2)	30 (55.6)	
4	20 (18.5)	3 (5.6)	
missing	4	2	
PNPLA3, N° (%): p.148I/I	67 (59.8)	22 (39.3)	0.01**
p.148I/M	38 (33.9)	29 (51.8)	
p.148M/M	7 (6.2)	5 (8.9)	
HAI score, Mean ± SD	5.5 ± 3.0	6.9 1± 3.0	0.003
Degree of HAI, N° (%): 0-8	92 (82.1)	37 (66.1)	
9-18	20 (17.8)	19 (33.9)	0.02
Fibrosis score, Mean ± SD	2.05 ± 1.6	2.89 ± 1.6	0.001
Degree of fibrosis, N° (%): 0-3	97 (86.6)	40 (71.4)	0.01
4-6	15 (13.3)	16 (28.8)	

*: genotype 3 vs. non-3; ** p.148 I/I vs I/M or M/M

In a multivariate logistic regression analysis was performed. PNPLA3-p.148I/M or M/M polymorphisms (p=0.007) were found to be independently associated with severe steatosis. (Table 3)

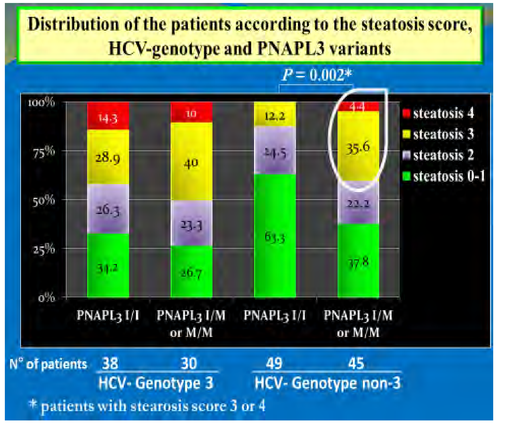
Table 3: Logistic Regression Analysis for severe liver steatosis.

Independent variables	Coefficient	Standard error	Chi-square	p-value	OR	C.I.
Constant	-1.74	1.64				
BMI	0.15	0.06	5.02	0.02	1.16	1.031-1.33
CD4⁺ count	-0.002	0.00	5.33	0.02	0.99	0.995-0.997
Total Cholesterol	-0.01	0.004	5.34	0.02	0.98	0.97-0.99
γGT	0.004	0.001	6.46	0.01	1	1.002-1.007
PNPLA3*	1.08	0.41	7.28	0.007	2.96	1.3-6.7
HCV genotype**	-1.19	0.43	7.98	0.005	0.3	0.12-0.761
HAART	-0-05	0.55	0.008	0.92	0.95	0.31-2,84

Model R-squared =20,2; p = 0.00001 ; *: patients with p.148 I/I vs. p.148 I/M and M/M; **: genotype 3 vs. non-3

In the 68 HCV-genotype 3 patients, the prevalence of patients with severe steatosis was similar in the 30 with PNPLA3-p.148I/M or M/M variants and in the 38 with PNPLA3-p.148I/I variant (50% and 43.2%, respectively), whereas in the 94 patients with HCV-genotype non-3, the 45 with PNPLA3-p.148I/M or M/M variants showed severe steatosis more frequently than the 49 with PNPLA3-p.148I/I variant (40% vs. 12.2%,p=0.002) (Figure 1).

Figure 1



A multivariate logistic regression analysis was separately performed for patients with HCV-genotype 3 and for those with HCV-genotype non-3, including the PNPLA3 variants (I/M+M/M vs. I/I), BMI, age, sex and CD4+ cell count. In HCV-genotype 3 patients, BMI was the only predictor of severe steatosis (OR:1.19, CI:1.1-1.4, p=0.037), whereas for HCV-genotype non-3 patients, PNPLA3 variants (I/M+M/M vs. I/I) and the CD4+ cell count at LB were identified as independent predictors of severe steatosis (OR:3,CI:1.2-12.1, p<0.012; and OR:0.99, CI:0.98-0.99, p<0.004, respectively).

Of the 89 patients with the PNPLA3 p.148I/I variant enrolled in the present study, 53 were treated with Pegylated Interferon (Peg-IFN) or Peg-IFN+ribavirin, according to the current international guidelines, and 23 (43.3%) of them achieved a sustained virological response (SVR). The response to Peg-IFN or Peg-IFN+ribavirin treatment was similar for the 55 patients with the PNPLA3-p.148I/M or M/M variants who received the same antiviral therapy (36.4%,p=0.63), i.e., 37.8% for the 45 with PNPLA3-p.148I/M and 30% for the 10 with PNPLA3-p.148 M/M variant. No patient stated active alcohol abuse, 141 (83.9%) were occasional or social drinkers and 27 (16.1%) abstainers. There were no significant differences for the PNPLA3 variants regarding alcohol intake.

Conclusion

The PNPLA3-p.148I/M and M/M variants, HCV-genotype 3, the CD4+ cell count and BMI were identified as independent predictors of severe liver steatosis in patients with HIV infection and CHC. However, when separate multivariate logistic regression analyses were performed for patients with HCV-genotype 3 and for those with HCV-genotype non-3, BMI was identified as the only predictor of severe steatosis for HCV-genotype 3 patients, whereas PNPLA3 variants (I/M+M/M vs. I/I) and the CD4+ cell count were identified as the only predictors of severe steatosis for those with HCV-genotype non-3.

The present investigation shows that HCV-genotype 3 is independently associated with severe steatosis, an observation in agreement with the data of a previous study on 97 HIV/HCV coinfected patients [27] and with those of other investigations on HCV-monoinfected patients[7,8,28].

A low CD4+ cell count was independently associated with severe steatosis in the present study, an observation in agreement with another investigation showing an independent association between the CD4+ cell count and a progressive reduction in liver steatosis[29].

No PNPLA3 variant influenced the response to Peg-IFN or Peg-IFN+ribavirin treatments since the percentage of treated patients who obtained an SVR was similar for the three PNPLA3 variants.

The present study presents some limitations. In fact, to our best knowledge it is the second study on this topic, but the technique we used to detect liver steatosis (liver histology) is more sensitive than that used in the previous study (non-contrast computed tomography). A sample of 168 patients might be considered a small number for a genetic association study, a limitation we think compensated by the gold-standard method we used to detect liver steatosis.

In conclusion, the data from the present study demonstrate that the PNPLA3-p.148I/M and M/M variants are independent predictors of severe liver steatosis in patients with HIV-infection and HCV-genotype non-3 chronic hepatitis, an association of clinical value that warrants further investigation to better define its role in clinical practice.

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