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ORIGINAL ARTICLE

Basic Study

TM6SF2 E167K variant predicts severe liver fibrosis for human immunodeficiency/hepatitis C virus co-infected patients, and severe steatosis only for a non-3 hepatitis C virus genotype

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Abstract

AIM

To evaluate the impact of the Glu167Lys (E167K) transmembrane 6 superfamily member 2 (TM6SF2) variant on the biochemical and morphologic expression of liver lesions in human immunodeficiency virus (HIV)/hepatitis C virus (HCV) co-infected patients.

METHODS

The study comprised 167 consecutive patients with HIV/HCV coinfection and biopsy-proven chronic hepatitis. A pathologist graded liver fibrosis and necroinflammation

using the Ishak scoring system, and steatosis using Kleiner's scoring system. Patients were genotyped for TM6SF2 E167K (rs58542926) by real-time Polymerase chain reaction. The 167 patients, 35 therapy-naive and 132 receiving ART, were prevalently males (73.6%), the median age was 40.7 years and the immunological condition good (median $CD4^+$ cells/mm^3 = 505.5).

RESULTS

The 17 patients with the TM6SF2 E167K variant, compared with the 150 with TM6SF2-E/E, showed higher AST (P = 0.02) and alanine aminotransferase (P = 0.02) and higher fibrosis score (3.1 ± 2.0 *vs* 2.3 ± 1.5, P = 0.05). In a multivariate analysis, TM6SF2 E167K was independently associated with severe fibrosis. The same analysis showed that HCV-genotype 3, present in 42.2% of patients was an independent predictor of severe steatosis. The association of TM6SF2 E167K with severe steatosis, absent for the whole group of 167 patients, was re-evaluated separately for HCV-genotype 3 and non-3 patients: No factor was independently associated with severe steatosis in the HCV-genotype-3 subgroup, whereas an independent association was observed between severe steatosis and TM6SF2 E167K in non-3 HCV genotypes. No association between the TM6SF2 E167K variant and severe liver necroinflammation was observed.

CONCLUSION

In HIV/HCV coinfection the TM6SF2 E167K variant is an independent predictor of severe fibrosis, but appears to be independently associated with severe steatosis only for patients with a non-3 HCV genotype.

Key words: Human immunodeficiency virus/hepatitis C virus co-infection; TM6SF2; Liver histology; Liver steatosis; Liver biopsy

Core tip: The transmembrane 6 superfamily member 2 (TM6SF2) E167K variant has been identified as an independent predictor of severe liver steatosis in patients with hepatitis C virus (HCV)-related chronic hepatitis (CH) lacking human immunodeficiency virus (HIV) infection. We analyzed the impact of the TM6SF2 E167K variant on the liver histology of 167 HIV/HCV co-infected patients with CH. The TM6SF2 E167K variant was found to be an independent predictor of severe fibrosis, while an independent association with severe steatosis was demonstrated only for patients with a non-3 HCV genotype.

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INTRODUCTION

The non-synonymous sequence variation (rs58542926 C to T, NP_001001524.2: p. E167K) in the transmembrane 6 superfamily member 2 (TM6SF2) gene encodes for an adenine-to-guanine substitution at nucleotide 499 that replaces glutamate at residue 167 with lysine[1].

The *TM6SF2* gene is considered a regulator of liver fat metabolism since it influences the secretion of triglyceride-rich lipoproteins and the hepatic triglyceride content[2]. In patients with non-alcoholic fatty liver disease (NAFLD), the TM6SF2 E167K variant induces a reduction in very-low-density-lipoprotein secretion and a predisposition to retain triglycerides in lipid droplets in liver cells[1-3], conditions associated with higher alanine aminotransferase (ALT) serum levels, lower lipoprotein plasma concentrations and advanced hepatic fibrosis[1,3-5].

In addition, the TM6SF2 E167K variant has been identified as an independent predictor of severe liver steatosis in patients with hepatitis C virus (HCV)-related chronic hepatitis (CH) lacking human immunodeficiency virus (HIV) infection[6,7], whereas controversial results have been published on the impact of this polymorphism on liver fibrosis[8-10].

As no data were available on this topic for HIV/HCV coinfection, we studied the impact of the TM6SF2 E167K variant on the biochemical and morphologic expression of liver lesions in 167 HIV/HCV coinfected patients with CH.

MATERIALS AND METHODS

Patients

This investigation comprised 167 consecutive anti-HIV-positive patients with CH (defined by positive HCV RNA) first observed from 1996 to 2008 who underwent a diagnostic liver biopsy at one of the two participating Units of Infectious Diseases after an observation period of nearly 18 mo. The two participating Units have been cooperating for years in clinical investigations and apply the same clinical and laboratory approach[11-14].

At the time the liver biopsy was performed, all patients were naive for anti-HCV treatment, were HBsAg-negative and had no evidence of autoimmune or genetic disorders inducing liver disease. No patient declared alcohol abuse (a daily intake of > 40 g/d for males and > 30 g/d for females for at least 5 years). No patient had serological or ultrasound evidence of HCC.

The patients received ART or were left untreated

according to the current DHHS guidelines at the time of liver biopsy[15]. For each patient, a serum sample obtained at liver biopsy was stored at -80 ℃ and thawed for this investigation.

The standards of human experimentation of the local Ethics Committees and with the Helsinki Declaration of 1975, revised in 1983. The present study was designed as retrospective and was approved by the Azienza Ospedaliera Universitaria - Second University of Naples in 2013 (protocol number 112/15 March 2013). Each patient signed their informed consent to undergo liver biopsy, for the collection and storage of biological material and for the use in clinical research of the data obtained.

Liver biopsy

Percutaneous liver biopsy was performed under US guidance using a modified disposable Menghini needle (16 gauge - external diameter 1.65 mm). A liver specimen of more than 1.5 cm in length was always obtained and at least 11 portal tracts were examined in each sample. Specimens were fixed in formalin, embedded in paraffin and stained with hematoxylin-eosin and Masson's trichrome stain[16]. A skilled pathologist unaware of the clinical and laboratory data examined the liver biopsies and used the Ishak scoring system to grade liver necroinflammation and fibrosis[17]. To assess liver steatosis, a home-made scoring system obtained with a partial modification of Kleiner's scoring system for NAFLD was used[6,18]: score 1 identifies the presence of fatty deposition in 1%-10% of hepatocytes, score 2 in 11%-31%, score 3 in 31%-60% and score 4 in > 60%[12-14,16,19].

Serological determinations

Serum HBsAg was sought using a commercial immunoenzymatic assay (Abbott Laboratories, North Chicago, IL, United States). The anti-HCV antibody was sought using a 3rd generation commercial immunoenzymatic assay (Ortho Diagnostic Systems, Neckargemund, Germany). Antibodies to HIV 1 and 2 were sought using a commercial ELISA (Abbott Lab., North Chicago, IL, United States) and positive results were confirmed by Western blot (Genelabs Diagnostics, Science Park Drive, Singapore).

HCV RNA was quantified by performing a real-time polymerase chain reaction (PCR) in a Light cycler 1.5 (Roche Diagnostics, Branchburg, NJ, United States), with the detection limit in plasma samples estimated at around 40 IU/mL. HCV genotyping was performed by a Line-Probe assay (INNO-LIPA HCV-Ⅱ; Innogenetics, Zwigndrecht, Belgium). The HIV viral load was assessed using the Amplicor HIV Monitor 1 test (Roche Molecular Systems Inc., Branchburg, New Jersey) with the lowest detection limit of 400 copies/mL.

Lymphocyte subsets ($CD4^+$, $CD8^+$) were evaluated by flow cytofluorimetry using monoclonal antibodies and a fluorescence-activated cell sorter scan (Becton Dickinson, Mountain View, CA, United States). Liver function tests, serum triglycerides, cholesterol and routine analyses were performed applying standard procedures.

Detection of TM6SF2 and PNPLA3 polymorphisms

A blood sample obtained from each patient was frozen (-80 ℃) and never thawed until used for this investigation.

Genomic DNA was extracted from peripheral whole blood with a DNA extraction kit (Promega, Madison WI, United States). Patients were genotyped for TM6SF2 E167K (rs58542926) and PNPLA3 I148M (rs738409) polymorphisms by Taqman allelic discrimination assay on an ABI 7900HT real-time PCR system, as previously described[6,14,19].

The genotype distributions of TM6SF2 and PNPLA3 polymorphisms were in the Hardy-Weinberg equilibrium, $P = 0.4$ and $P = 0.3$, respectively.

Statistical analysis

Continuous variables not normally distributed were summarized as median and interquartile range (IQR), and categorical variables as absolute and relative frequencies. Differences in the continuous data were evaluated by a non-parametric test (Kruskal-Wallis), and the χ^2 test was applied to categorical variables. A generalized linear model analysis was applied to perform multivariate analysis. All independent variables with a significant effect on liver steatosis, fibrosis or necroinflammation at univariate analysis or with biological plausibility were included in the multivariate analysis. A P value < 0.05 was considered to be statistically significant. Statistical analysis was performed using StatGraph, version 3.0 (Adalta, Arezzo, Italy).

RESULTS

The 167 patients enrolled were prevalently males (72.5%), with a median age of 40.7 years (IQR: 37.6-44.1) and a good immunological condition shown by the $CD4^+$ cell count (median 505.5 cells/mm^3, IQR: 397.3-665.5) at the time of liver biopsy and by the nadir of $CD4^+$count (median 258.0 cells/mm^3, IQR: 166.5-407.3). At the time of the liver biopsy, 35 (21.0%) patients were left untreated because they showed a high $CD4^+$cell count (median 507.5 cells/mm^3, IQR: 398.0-662.0), whereas 132 (79.0%) had been receiving ART for a median duration of 8.0 years (IQR: 6.0-11.0). HCV-genotype 3 was detected in 42.2% of the cases, HCV-genotype 1 in 38.5%, HCV-genotype 4 in 14.3% and HCV-genotype 2 in 5.0%.

Of the 167 patients investigated, 89.8% were TM6SF2 167E homozygous and 11.2% heterozygous for the TM6SF2 E167K variant. The PNPLA3 I/I, I/M and M/M variants at codon 148 were observed respectively in 52.7%, 40.1% and 7.2% of patients.

The 167 patients showed a mean fibrosis score of 2.3 ± 1.46, a mean histological activity index (HAI) score of 5.9 ± 3.0 and a mean steatosis score of 1.7 ± 1.3. Thirty-seven (22.2%) patients showed a HAI of 9-18, 58 (34.7%) a fibrosis score 4-6 and 56 (33.5%) a steatosis score 3-4.

The demographics and initial biochemical, virological and histological data according to the TM6SF2 variants are shown in Table 1 (Table 1). Compared with the 150 patients with the TM6SF2 E167E variant, the 17 with the TM6SF2 E167K variant were older (P = 0.04), showed higher AST (P = 0.02) and ALT (P = 0.02) serum values and a higher fibrosis score (P = 0.05), whereas no difference was observed in the degree of steatosis or liver necroinflammation.

The 31 patients with a liver fibrosis score 4-6, compared with the 136 with a lower score (0-3), were older (P = 0.05) and showed higher bilirubin (P = 0.01), AST (P = 0.0008), ALT (P = 0.01) and ALP (P = 0.01) serum values (Table 2). They had a lower $CD4^+$/mL cell count (P = 0.01), a higher HAI score (P = 0.0001), a higher percentage of cases with an HAI score > 9 (P = 0.0001), a higher steatosis score (P = 0.02), a higher percentage of patients with a steatosis score 3-4 (P = 0.0001) and a greater frequency of cases with the TM6SF2 E167K variant (P = 0.03) (Table 2). Other differences shown in Table 2 were not significant to the statistical analysis or of low or no clinical impact.

To identify factors independently associated with a severe fibrosis, a multivariate analysis was performed, including the TM6SF2 variants (p. 167 E/K *vs* p. 167 E/E), the PNPLA3 variants (p. 148 I/I *vs* p. 148 I/M + M/M), HCV-genotype 3 *vs* other genotypes, BMI and other potential confounding factors (sex, nadir of $CD4^+$ cell count, HIV viral load, ART, AST, ALT, GGT, total bilirubin, triglyceride and cholesterol values. The TM6SF2 E167K variant (P = 0.0272) and the total cholesterol serum value (P = 0.0203) were the only factors independently associated with the severity of liver fibrosis.

Compared with the 111 with a lower score, the 56 patients with severe steatosis (score 3-4) showed a higher BMI (P = 0.03), higher bilirubin (P = 0.01), AST (P = 0.0004), ALT (P < 0.0001), GGT (P = 0.04) and glucose serum values (P = 0.004), and lower cholesterol serum levels (P < 0.01). In addition, they had a lower $CD4^+$/mL cell count (P = 0.002), a higher mean HAI score (P = 0.002), a higher percentage of cases with an HAI score > 9 (P = 0.01), a higher mean fibrosis score (P = 0.003), a higher percentage of patients with a fibrosis score 4-6 (P = 0.001) and a higher percentage of patients with HCV-genotype 3 (P = 0.04) (Table 3). Other differences shown in Table 3 were not significant to the statistical analysis or of low or no clinical impact.

To identify factors independently associated with severe steatosis, a multivariate analysis was performed, including the TM6SF2 variants (p. 167 E/K *vs* p. 167 E/E), the PNPLA3 variants (p. 148 I/I *vs* p. 148 I/M + M/M), HCV genotype (3 *vs* other genotypes), BMI and other potential confounding factors (sex, nadir of $CD4^+$ cell count, HIV viral load, duration of HIV infection, ART, GGT, AST, ALT, total bilirubin, glucose). The only factors independently associated with severe steatosis were HCV-genotype 3 (P = 0.0227), the PNPLA3 p. 148 I/M plus M/M variants (P = 0.0321) and the glucose serum values (P = 0.0441).

Due to the high percentage of patients with HCV-genotype 3 and the association of this genotype with severe steatosis in this and other investigations[6,8-10,20], the initial characteristics of 161 of the HIV/HCV coinfected patients (HCV genotype was not available in 6 cases) were analyzed according to the presence or absence of HCV-genotype 3. Compared to the 93 patients with a non-3 HCV genotype, the 68 with HCV-genotype 3 showed higher AST (P = 0.004) and ALT (P = 0.0002) serum values, a higher HAI score (P = 0.03), higher steatosis scores (P = 0.002), a higher rate of patients with severe steatosis (P = 0.03) and lower serum levels of GGT (P = 0.004), triglycerides (P = 0.003) and cholesterol (P = 0.0006) (Table 4). The association between the TM6SF2 variants and severe steatosis was investigated separately for patients with HCV-genotype 3 and for those with a non-3 HCV genotype in multivariate analyses, including the TM6SF2 variants (p. 167 E/K *vs* E/E), the PNPLA3 variants (p. 148 I/I *vs* p. 148 I/M + M/M), BMI, sex, the nadir of $CD4^+$ cell count, HIV viral load, triglyceride, cholesterol, GGT and ART at the time of the liver biopsy. None of the factors considered was identified as an independent predictor of severe steatosis in the HCV-genotype-3 subgroup, whereas the TM6SF2 E167K variant (P = 0.0339), the PNPLA3 I/I variant (P = 0.0263), BMI (P = 0.0348) and GGT (P = 0.0049) were independently associated with severe steatosis in patients with a non-3 HCV genotype.

We admit that the present study has some limitations considering the relatively small number of patients, barely sufficient for a genetic association study. These limitations are offset by the gold-standard method used to detect liver lesions (liver biopsy examination by a skilled pathologist) and by the new information regarding HIV/HCV coinfection.

DISCUSSION

This is the first investigation, to our best knowledge, to demonstrate that the TM6SF2 E167K variant is independently associated with severe liver fibrosis in HIV/HCV coinfected patients with CH.

This association was previously identified in HCV-mono-infected patients with CH or cirrhosis[10,20] and in patients with NAFLD[5,10].

In a cross-sectional cohort of 815 Italian therapy-naïve HCV-mono-infected patients, the TM6SF2 E167K variant was independently associated with histological cirrhosis[10], and in a subset of 645 Swiss/German CHC patients in the same study, it was associated with a fibrosis Metavir stage F2-F4[10]. In a cross-sectional

Table 1 Initial characteristics of 167 patients with human immunodeficiency virus infection and chronic hepatitis C, according to the *TM6SF* variants

	TM6SF2 p. 167 E/K	TM6SF2 p. 167 E/E	*P* value
Patients, *n*	17	150	
Age (yr)	42.6 (40.4-47.8)	40.3 (37.4-44.0)	0.04
Males	12 (70.6)	109 (72.7)	0.8
IVDU	8 (61.5)	95 (74.8)	0.2
BMI (kg/m^2)	24.0 (22.0-25.0)	23.0 (21.3-25.0)	0.3
Glucose (mg/dL)	81.0 (75.0-87.0)	88.0 (81.0-98.0)	0.002
Bilirubin (mg/dL)	0.9 (0.7-1.2)	0.7 (0.5-1.0)	0.06
AST (IU/L)	108.0 (52.0-134.0)	59.0 (40.0-94.0)	0.02
ALT (IU/L)	141.0 (122.0-172)	81.5 (46.0-131.0)	0.02
Cholesterol (mg/dL)	144.0 (123.0-194.0)	164.0 (135.5-191.0)	0.4
Triglycerides (mg/dL)	136.0 (118.0-154.0)	125.5 (83.5-182.3)	0.6
GGT (IU/mL)	65.0 (46.0-95.0)	79.0 (39.0-165.0)	0.6
ALP (IU/mL)	192.0 (156.3-238.3)	186.5 (139.3-252.0)	0.9
HCV RNA (IU/mL), median (IQR)	598679.0 (140698.5-997750.0)	601550.0 (202000.0-1432735.0)	0.7
Nadir of CD4$^+$ cells/mm^3	214.0 (153.0-380.0)	266.0 (176.3-413.3)	0.5
HIV RNA (cps/mL)	7638.0 (4112.5-19215.5)	10870.0 (3100.5-35374.4)	0.7
HIV RNA < 50 cps/mL	6 (35.3)	66 (44.0)	
CD4$^+$ cell/mmc	435.0 (380.0-650.0)	508.0 (399.3-670.0)	0.2
ART, treated	14 (82.3)	118 (78.7)	0.6
PI/r-NRTI-NNRTI	0	6 (5.3)	
PI/r-NRTI	6 (46.1)	40 (35.1)	
NRTI-NNRTI	2 (15.4)	30 (26.3)	
PI-NRTI	0	7 (6.1)	
NRTI	5 (38.5)	31 (27.2)	
Therapy missing, *n*	1	4	
Therapy-naïve	3 (17.6)	32 (21.3)	
Duration of ART (yr)	7.7 (6.4-12.8)	8.0 (5.7-10.8)	0.3
Duration of HIV infection (yr)	17.4 (12.5-21.4)	14.0 (7.7-17.9)	0.05
HCV Genotype			
1	5 (29.4)	57 (39.6)	0.3
2	1 (5.9)	7 (4.9)	
3	6 (35.3)	62 (43.1)	
4	5 (29.4)	18 (12.5)	
Missing	0	6	
HAI score	6.8 ± 2.9	5.8 ± 3.0	0.2
HAI: score 0-8	13 (76.5)	117 (78.0)	0.2
score 9-18	4 (23.5)	33 (22.0)	
Fibrosis score	3.1 ± 2.0	2.3 ± 1.5	0.05
Degree of fibrosis			
0	2 (11.8)	11 (7.3)	0.1
1	2 (11.8)	48 (32.0)	
2	2 (11.8)	33 (22.0)	
3	6 (35.3)	32 (21.3)	
4	0	11 (7.3)	
5	2 (11.8)	6 (4.0)	
6	3 (17.6)	9 (6.0)	
Steatosis score, mean ± SD	1.9 ± 1.2	1.7 ± 1.3	0.4
Degree of steatosis			0.8
0	3 (17.6)	45 (30.0)	
1	3 (17.6)	19 (12.7)	
2	4 (23.5)	37 (24.7)	
3	6 (35.3)	40 (26.7)	
4	1 (5.8)	9 (6.0)	
PNPLA3			
p. 148 I/I	9 (52.9)	79 (52.7)	0.7
p. 148 I/M	6 (35.3)	61 (40.7)	
p. 148 M/M	2 (11.8)	10 (6.7)	

Data represent as *n* (%), mean ± SD, or median (IQR). HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; BMI: Body mass index; HAI: Histological activity index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ glutamyl transferase; ART: Antiretroviral therapy; NRTIs: Nucleoside/nucleotide analogue reverse transcriptase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PIs: Protease inhibitors; TM6SF2: Transmembrane 6 superfamily member 2; PNPLA3: Patatin-like phospholipase domain-containing 3 gene; IQR: Interquartile range.

Table 2 Initial characteristics of 167 patients with human immunodeficiency virus infection and chronic hepatitis C, according to the fibrosis score

	Fibrosis score 0-3	Fibrosis score 4-6	*P* value
Patients, *n*	136	31	
Age (yr)	40.3 (37-44)	42.0 (39.5-46.7)	< 0.05
Males	96 (70.6)	25 (80.6)	0.1
BMI (kg/m^2)	23 (21.2-24.9)	22.8 (21.7-26.5)	0.5
Glucose (mg/dL)	87.0 (80.0-95.0)	91.0 (81-102)	0.6
Bilirubin (mg/dL)	0.68 (0.4-1.0)	0.97 (0.7-1.5)	< 0.01
AST (IU/L)	55.0 (39-91)	96.0 (63.0-143.0)	0.0008
ALT (IU/L)	79.0 (44-131)	120.0 (67-180)	< 0.01
Cholesterol (mg/dL)	162 (137-194.0)	158 (116.8-177)	0.3
Triglycerides (mg/dL)	122.0 (84.0-164)	147.5 (55-227)	0.1
GGT (IU/mL)	70.0 (36.0-150)	108 (60.0-227.0)	0.2
ALP (IU/mL)	178.0 (136.0-239)	221 (182-269)	< 0.01
HCV RNA (IU/mL)	5.2e5 (1.8e5-1.4e6)	7e5 (2.3e5-1.7e6)	0.4
Nadir of CD4$^+$ cells/mm^3	271 (175.5-429.5)	238.0 (145.0-347.5)	0.1
HIV RNA (copies/mL)	10914.5 (4402.3-31052.0)	4500.0 (784.0-36959.0)	0.1
CD4$^+$ cells/mm^3	510.0 (403-708)	454.0 (338-586)	< 0.01
ART, Treated	105 (77.2)	27 (87.1)	0.4
PI/r-NRTI-NNRTI	6 (5.9)	0	
PI/r-NRTI	35 (33.6)	11 (42.3)	
PI-NRTI	6 (5.9)	1 (3.8)	
NRTI-NNRTI	25 (24.8)	7 (26.9)	
NRTI	29 (28.7)	7 (26.9)	
Therapy missing, *n*	4	1	
Therapy-naïve	31 (22.8)	4 (12.9)	
Duration of ART (yr)	7.8 (5.4-10.9)	9.6 (6.3-12)	0.2
Duration of HIV infection (yr)	14.4 (7.5-18.1)	13.9 (8.4-17.4)	0.6
HCV Genotype			
1	51 (38.9)	11 (3.7)	0.4
2	7 (5.3)	1 (0.4)	
3	54 (41.2)	14 (46.7)	
4	19 (14.5)	4 (13.4)	
Missing, *n*	5	1	
HAI score	5.0 ± 2.7	8.6 ± 2.3	< 0.0001
HAI			
score 0-8	115 (84.6)	15 (48.4)	< 0.0001
score 9-18	21 (15.4)	16 (51.6)	
Steatosis score	1.4 ± 1.2	1.9 ± 1.3	< 0.02
Degree of steatosis			
0	44 (32.4)	4 (12.9)	0.0001
1	18 (13.2)	4 (12.9)	
2	34 (25.0)	7 (22.6)	
3	32 (23.55)	14 (45.2)	
4	8 (5.9)	2 (6.5)	
TM6SF			
p. 167 E/K	12 (8.8)	5 (16.1)	< 0.03
p. 167 E/E	124 (91.2)	26 (83.9)	
PNPLA3,			
p. 148 I/I	75 (55.1)	13 (41.9)	0.2
p. 148 I/M	49 (36.1)	18 (58.6)	
p. 148 M/M	12 (8.8)	0	

Data represent as *n* (%), mean ± SD, or median (IQR). e: Elevated; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; BMI: Body mass index; HAI: Histological activity index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ glutamyl transferase; ART: Antiretroviral therapy; NRTIs: Nucleoside/nucleotide analogue reverse transcriptase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PIs: Protease inhibitors; TM6SF2: Transmembrane 6 superfamily member 2; PNPLA3: Patatin-like phospholipase domain-containing 3 gene; IQR: Interquartile range.

analysis of 2023 HCV-mono-infected patients with a chronic liver disease recently performed by Eslam *et al*[8], TM6SF2-E rs58542926 was only marginally associated with the severity of liver fibrosis in a univariate analysis, but not in a multivariate regression analysis. In the same study, the Authors evaluated the association between the rs58542926 variants and fibrosis progression in 1174 of the 2023 patients with a chronic liver disease: Despite a marginal association in the univariate analysis, after adjustment for other variables, the T allele was not independently associated with fibrosis. Instead, no association with the severity

Table 3 Initial characteristics of 167 patients with human immunodeficiency virus infection and chronic hepatitis C, according to the steatosis score

	Steatosis score 0-2	Steatosis score 3-4	*P* value
Patients, *n*	111	56	
Age (yr)	40.3 (37.4-44.2)	41.8 (38.4-44.0)	0.3
Males	79 (71.2)	42 (75)	0.6
BMI (kg/m^2)	22.8 (21.3-24.5)	23.9 (21.9-25.6)	0.03
Glucose (mg/dL)	85.5 (79.3-94.0)	92.5 (82.0-102.0)	0.004
Bilirubin (mg/dL)	0.6 (0.4-1.0)	0.8 (0.6-1.1)	0.01
AST (IU/L)	54.0 (38.3-79.0)	92.0 (55.8-142.3)	0.0004
ALT (IU/L)	67.0 (43.0-114.5)	129.0 (78.8-201.5)	0.00006
Cholesterol (mg/dL)	168.0 (141.0-196.5)	147.0 (120.0-174.0)	0.01
Triglycerides (mg/dL)	130.5 (84.0-188.5)	123.0 (87.0-156.0)	0.5
GGT (IU/mL)	70.0 (34.5-142.5)	97.0 (46.5-229.5)	0.04
ALP (IU/mL)	185.0 (138-240.3)	196.0 (147.5-265.0)	0.5
HCV RNA (IU/mL)	5.2e5 (1.5e5-1.4e6)	7.4e5 (2.4e5-1.4e6)	0.7
Nadir of CD4$^+$ cells/mm^3	272.0 (166.5-425.8)	257.5 (172.8-360.5)	0.2
HIV RNA copies/mL	7484.0 (2925.3-18994.0)	18918.0 (3991.0-37219.0)	0.1
HIV RNA < 50 copies/mL	61 (54.9)	25 (44.6)	
CD4$^+$ cell/ mm^3	527.0 (425.3-720.5)	463.0 (373.0-542.8)	0.002
ART, Treated	88 (79.3)	44 (78.6)	0.91
PI/r-NRTI-NNRTI	5 (5.9)	1 (2.4)	
PI/r-NRTI	33 (38.8)	13 (30.2)	
PI-NRTI	3 (3.5)	4 (9.5)	
NRTI-NNRTI	21 (24.7)	11 (26.2)	
NRTI	23 (27.1)	13 (30.9)	
Therapy missing, *n*	3	2	
Therapy-naïve	23 (41.1)	12 (21.4)	
Duration of HAART (yr)	8.2 (6.2-11.5)	7.6 (4.5-10.8)	0.3
Duration of HIV infection (yr)	14.1 (7.8-17.8)	14.2 (8.1-18.1)	0.8
HCV-genotype			
1	44 (41.1)	18 (33.3)	0.04
2	5 (4.7)	3 (5.6)	
3	38 (35.5)	30 (55.6)	
4	20 (18.7)	3 (5.6)	
Missing, *n*	4	2	
HAI score	5.4 ± 2.9	6.9 ± 3.0	0.002
Degree of HAI			
score 0-8	98 (88.3)	38 (67.8)	0.01
score 9-18	19 (17.1)	18 (32.1)	
Fibrosis score	2.1 ± 1.6	2.9 ± 1.6	0.003
Degree of fibrosis			
0	12 (10.8)	1 (1.8)	0.001
1	40 (36.0)	10 (17.9)	
2	21 (18.9)	14 (25)	
3	23 (20.7)	15 (26.3)	
4	5 (4.5)	6 (10.7)	
5	3 (2.7)	5 (8.9)	
6	7 (6.3)	5 (8.9)	
TM6SF			
p. 167 E/K	10 (0.9)	7 (12.5)	0.48
p. 167 E/E	101 (90.9)	49 (87.5)	
PNPLA3			
p. 148 I/I	66 (59.4)	22 (39.3)	0.06
p. 148 I/M	38 (38.3)	29 (51.8)	
p. 148 M/M	7 (6.3)	5 (8.9)	

Data represent as *n* (%), mean ± SD, or median (IQR). e: Elevated; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; BMI: Body mass index; HAI: Histological activity index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ glutamyl transferase; ART: Antiretroviral therapy; NRTIs: Nucleoside/nucleotide analogue reverse transcriptase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PIs: Protease inhibitors; TM6SF2: Transmembrane 6 superfamily member 2; PNPLA3: Patatin-like phospholipase domain-containing 3 gene; IQR: Interquartile range.

of fibrosis was observed for 694 Caucasian patients with CHC due to HCV-genotype 1[9].

Some studies on the association between the TM6SF2 rs58542926 variants and the severity of liver fibrosis in patients with NAFLD have provided interesting information. Using two histologically characterized cohorts including steatosis and steatohepatitis, and fibrosis and cirrhosis, Liu *et al*[5]

Table 4 Initial characteristics of 161 patients with human immunodeficiency virus infection and chronic hepatitis C, according to the hepatitis C virus genotype

	HCV genotype 3	non- 3 HCV genotype	*P* value
Patients, *n*	68	93	
Age (yr)	40.6 (36.7-44.0)	40.8 (37.8-44.1)	0.3
Males	43 (63.2)	69 (74.2)	0.07
IVDU	44 (64.7)	57 (61.3)	0.8
BMI (kg/m^2)	22.7 (21.2-25.2)	23.1 (21.7-24.9)	0.5
Glucose (mg/dL)	87.0 (79.0-94.0)	89.0 (82.0-98.0)	0.1
Bilirubin (mg/dL)	0.7 (0.6-1.0)	0.7 (0.5-1.0)	0.5
AST (IU/L)	77.5 (53.3-124.0)	55.0 (38.0-85.0)	0.004
ALT (IU/L)	120.5 (62.5-187.5)	66.0 (40.0-117.0)	0.0002
Cholesterol (mg/dL)	143.5 (115.3-173.0)	171.0 (147.5-197.0)	0.0006
Triglycerides (mg/dL)	99.0 (69.5-158.0)	139.0 (103.5-216.8)	0.003
GGT (IU/mL)	61.0 (32.8-106.0)	96.0 (49.5-226.5)	0.004
ALP (IU/mL)	196.0 (143.0-260.5)	192.0 (150.0-246.0)	0.9
HCV RNA (IU/mL)	477624.0 (124539.0-1050000.0)	730500.0 (233250.0-1941008.0)	0.07
Nadir of CD4$^+$ cells/mm^3	247.7 (155.3-380.0)	252.0 (175.0-404.0)	0.6
HIV RNA copies/mL	5607.5 (1972.3-18918.0)	12130.0 (1806.8-37226.5)	0.7
HIV RNA < 50 copies/mL	30 (44.0)	42 (45.2)	
CD4$^+$ cell/mm^3	506.5 (397.8-638.0)	494.0 (398.0-697.5)	0.7
ART, Treated	50 (73.5)	79 (84.9)	0.4
PI/r-NRTI-NNRTI	1 (2.0)	5 (6.6)	
PI/r-NRTI	22 (44.9)	24 (31.6)	
PI-NRTI	3 (6.1)	4 (5.3)	
NRTI-NNRTI	13 (26.5)	18 (23.7)	
NRTI	10 (20.4)	25 (32.9)	
Therapy missing, *n*	1	3	
Therapy-naïve	18 (26.47)	14 (15.0)	
Duration of ART (yr)	9.0 (4.3-12.6)	7.7 (6.1-10.4)	0.4
Duration of HIV infection (yr)	14.0 (7.7-18.5)	14.1 (8.1-17.1)	0.8
HAI score	6.5 ± 3.1	5.5 ± 2.9	0.03
HAI			
score 0-8	48 (70.6)	76 (81.7)	0.09
score 9-18	20 (14.7)	17 (18.3)	
Fibrosis score	2.5 ± 1.6	2.2 ± 1.6	0.2
Degree of fibrosis			
0	4 (5.9)	9 (9.7)	0.7
1	16 (23.5)	32 (34.4)	
2	16 (23.5)	17 (18.3)	
3	18 (26.5)	19 (20.4)	
4	4 (5.9)	5 (5.3)	
5	5 (7.3)	4 (4.3)	
6	4 (5.9)	7 (7.5)	
Steatosis score	2.0 ± 1.3	1.4 ± 1.2	0.002
Degree of steatosis			
0	14 (20.6)	31 (33.3)	0.03
1	7 (10.3)	14 (15.0)	
2	16 (23.5)	20 (21.5)	
3	23 (33.8)	22 (23.7)	
4	7 (10.1)	2 (2.1)	
TM6SF			
p. 167 E/K	6 (8.8)	11 (11.8)	0.5
p. 167 E/E	62 (91.2)	82 (88.2)	
PNPLA3			
p. 148 I/I	38 (55.9)	48 (51.6)	0.5
p. 148 I/M	24 (35.3)	40 (43.0)	
p. 148 M/M	6 (8.8)	5 (5.4)	

HCV genotype missing in 6 cases. Data represent as *n* (%), mean ± SD, or median (IQR). e: Elevated; HCV: Hepatitis C virus; HIV: Human immunodeficiency virus; BMI: Body mass index; HAI: Histological activity index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ glutamyl transferase; ART: Antiretroviral therapy; NRTIs: Nucleoside/nucleotide analogue reverse transcriptase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; PIs: Protease inhibitors; TM6SF2: Transmembrane 6 superfamily member 2; PNPLA3: Patatin-like phospholipase domain-containing 3 gene; IQR: Interquartile range.

demonstrated that the TM6SF2 E167K rs58542926 variant enhances hepatic fibrosis progression in patients with NAFLD. Eslam *et al*[8] investigated 502 Caucasian patients with NAFLD and demonstrated that the TM6SF2 E167K rs58542926 variant is independently associated with the risk of a > F2 Metavir score.

Concluding on this point, an independent association between the TM6SF2 E167K variant and severe liver fibrosis has been demonstrated in HIV/HCV co-infected patients with CH and in NALFD patients, whereas contrasting results have been observed for HCV-monoinfected CH patients.

In the present study, the TM6SF2 E167K variant has been identified as an independent predictor of severe steatosis in HIV/HCV coinfected patients with HCV-genotype other than 3, an association not detected in those with HCV-genotype 3 most probably because this genotype favors lipid droplet deposition and storage in hepatocytes, possibly obscuring other associations.

In accordance with this, in HCV-mono-infected CH patients, Milano *et al*[10] observed a marginal association between the E167K variant and severe steatosis, but more interestingly, an independent association was found for patients with a non-3 HCV genotype and no association for those with HCV-genotype 3[10]. These data were confirmed by Eslam *et al*[8], who observed a marginal but independent association between the TM6SF2 rs58542926 T allele and the severity of steatosis, but stratifying this cohort by HCV-genotype (3 *vs* non-3), they demonstrated that the association persisted only for the subgroup of patients with a non-3 HCV genotype. They concluded that the TM6SF2 E167K substitution may promote lipid abnormalities and steatosis by altering TM6SF2 and the microsomal triglyceride transfer protein expression[8]. Petta *et al*[9], however, found no association between the *TM6SF2* gene and the severity of steatosis in HCV-genotype-1 mono-infected CH patients.

The TM6SF2 E167K variant was not identified as an independent predictor of severe necroinflammation in the HIV/HCV co-infected CH patients enrolled in the present study. In accordance with this, in HCV-mono-infected CH patients the association between the TM6SF2 E167K variant and severe liver necroinflammation was marginal in the study by Milano *et al*[10] and absent in the study by Eslam *et al*[8].

In conclusion, the data from the present study show that in HIV/HCV coinfected CH patients the TM6SF2 E167K variant plays an important role in steatosis severity, as demonstrated in patients infected with a non-3 HCV genotype and not found in those harboring HCV-genotype 3, most probably due to the strong action of this viral genotype on fat deposition in liver cells. This phenomenon seems to be independent of HIV infection, since it has been observed both in anti-HIV-positive (the present study) and anti-HIV-negative patients[10,20].

The data from the present study also demonstrate that the TM6SF2 E167K variant is an independent predictor of severe fibrosis in HIV/HCV coinfected CH patients, an association identified in anti-HIV-negative subjects by some Authors[10,20] and not found by others[9].

The role of the TM6SF2 E167K variant as a predictor of severe necroinflammation remains uncertain, since it was not observed in the HIV/HCV CH patients in the present study and not or only marginally observed in HCV-monoinfected CH patients[9,10,20].

COMMENTS

Background

The transmembrane 6 superfamily member 2 (*TM6SF2*) gene is a regulator of liver fat metabolism since it influences the secretion of triglyceride-rich lipoproteins and the hepatic triglyceride content. In addition, it has been demonstrated that in patients with non-alcoholic fatty liver disease (NAFLD), the TM6SF2 E167K variant induces a reduction in very-low-density-lipoprotein secretion and a predisposition to retain triglycerides in lipid droplets in liver cells, conditions associated with increased alanine aminotransferase serum levels, lower lipoprotein plasma concentrations and advanced hepatic fibrosis. Previous studies identified the TM6SF2 E167K variant as an independent predictor of severe liver steatosis in patients with hepatitis C virus (HCV)-related chronic hepatitis (CH) lacking human immunodeficiency virus (HIV) infection, whereas controversial results have been published on the impact of this polymorphism on liver fibrosis.

Research frontiers

As no data were available on this topic for HIV/HCV coinfection, the authors studied the impact of the TM6SF2 E167K variant on the biochemical and morphologic expression of liver lesions of 167 HIV/HCV coinfected patients with CH.

Innovations and breakthroughs

This is the first study investigating the association of TM6SF2 E167K variant with the entity of liver lesions in patients with HIV/HCV coinfection and biopsy-proven CH. Considering the whole group of patients, the TM6SF2 E167K variant was not associated with severe steatosis. However, nearly 40% of patients in this study had HCV-genotype 3, which resulted to be independently associated with severe steatosis. An independent association was found between severe steatosis and TM6SF2 E167K in the non-3 HCV-genotype subgroup, whereas no association was found in the HCV-genotype-3 subgroup, most probably because this viral genotype favors fat deposition in liver cells. This phenomenon seems to be independent of HIV infection, since it was observed both in anti-HIV-positive patients in the present study and in anti-HIV-negative patients in other investigations. The data from the present study also demonstrate that the TM6SF2 E167K variant is an independent predictor of severe fibrosis in HIV/HCV coinfected CH patients, an association identified in anti-HIV-negative subjects by some Author but denied by others. No association was found between the TM6SF2 E167K variant and severe liver necroinflammation.

Applications

As the TM6SF2 E167K variant is an independent predictor of severe liver steatosis and severe liver fibrosis in HIV/HCV coinfected CH patients, its detection has a marked diagnostic and clinical value. The authors believe that their article will be a stimulus for other clinicians to start testing patients with CH C and in particular those with HIV infection for the TM6SF2 E167 variants.

Peer-review

This is a well-conducted research paper that illustrates the potential specialty for using HIV/HCV patients in NAFLD study. The authors results showed that 167 consecutive patients with HIV/HCV coinfection and biopsy-proven CH and showed A pathologist graded liver fibrosis and necroinflammation. The

authors reported that the TM6SF2 E167K variant on the liver histology of HIV/HCV co-infected patients with CH and independent predictor of severe fibrosis with severe steatosis was demonstrated only for patients with a non-3 HCV genotype. There are no major criticisms of the work and I would support acceptance of the manuscript. The paper is able to publish to World Journal of Gastroenterology.

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