Effect of MTTP -493G/T, I128T, Q95H and Q244E polymorphisms on hepatic steatosis in patients with chronic hepatitis

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HIGHLIGHTS

- Important etiologies of chronic liver disease are viral hepatitis.
- Viral hepatitis B and C causes 1.1 million deaths per year.
- Hepatic steatosis (liver fat accumulation) is a metabolic complication of hepatitis C.
- The underlying mechanisms involving steatosis include genetic polymorphisms.
- -493G/T and I128T polymorphisms in the MTTP gene are relevant in hepatic steatosis.

ARTICLE INFO

Background: Chronic hepatitis C is characterized by a progressive deterioration of liver function and is involved in metabolic complications, such as hepatic steatosis.
Objective: The aim of this study was to investigate the role of host and viral characteristics associated with -493G/T (rs1800591), I128T (rs3816873), Q95H (rs61733139), and Q244E (rs17599091) Single Nucleotide Polymorphisms (SNPs) in the Microsomal Triglyceride Transfer Protein (MTTP) gene on hepatic steatosis in chronic hepatitis C.
Methods: SNPs were genotyped by PCR-RFLP and analyzed in combination with host and viral characteristics by multiple logistic regression in different genetic models of inheritance.
Results: The authors analyzed 236 patients with chronic hepatitis C, and 53% had hepatic steatosis. The mutated allele frequencies were >5%, and the genotypes were in Hardy-Weinberg equilibrium (p ≥ 0.05). It was observed that patients with HCV genotype 3 infection (OR = 2.74, 95% CI 1.24–6.06, p = 0.013), female sex (OR = 2.28, 95% CI 1.21–4.28, p = 0.011) and moderate- and high-intensity liver inflammatory activity (A2-A3) (OR = 3.61, 95% CI 1.86–7.01, p < 0.001) alone exhibited a higher risk of steatosis. The results of multiple logistic regression analysis for interaction showed that for the -493G/T SNP, when the GT/TT genotype (dominant model) and the GT genotype (codominant model) were each combined with HCV genotype 3 infection, an 11.51-fold (95% CI 2.08–63.59, p = 0.005) and a 15.69-fold (95% CI 2.46–99.85, p = 0.004) increased risk of steatosis, respectively, was observed. For the I128T SNP, when both the IT/TT genotype (dominant model) and the IT genotype (codominant model) were combined with HCV genotype 3 infection, an 8.51-fold (95% CI 1.59–45.54, p = 0.012) and an 8.40 fold (95% CI 1.51–46.91, p = 0.015) increased risk of steatosis, respectively, was observed.
Conclusion: The present study showed that the viral genotype combined with the -493G/T and I128T SNPs in the MTTP gene influences hepatic steatosis.

Keywords: Chronic hepatitis C, Genetic models of inheritance, Hepatic steatosis, Microsomal Triglyceride Transfer Protein (MTTP), Single Nucleotide Polymorphisms (SNPs)

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Introduction

Chronic liver disease is characterized by a progressive deterioration of liver function for more than six months, and this process is related to the persistent inflammation, destruction, and regeneration of the liver parenchyma. The most common etiologies are the presence of alcoholic liver disease, autoimmune or genetic causes, drugs, Nonalcoholic Fatty Liver Disease (NAFLD)/Nonalcoholic Steatohepatitis (NASH), and chronic viral hepatitis. Regarding viral hepatitis, hepatitis C virus (HCV) causes inflammation in the liver that is caused by Hepatitis C Virus (HCV). According to the World Health Organization (WHO), an estimated 58 million people are chronically infected with HCV worldwide. Viral hepatitis (Hepatitis B and C) causes 1.1 million deaths per year and 3.0 million new infections. Among hepatitis C patients, approximately 62% of those diagnosed receive specific treatment. In addition, hepatitis C is involved in several metabolic complications, such as insulin resistance, hepatic steatosis, hyperlipidemia, and metabolic syndrome.

The development of hepatic steatosis influences disease progression. In addition, persistence and even an increase in the degree of steatosis are observed in patients who achieve a sustained virologic response (SVR) after specific treatment with Direct-Acting Antiviral (DAA) drugs one year after treatment ends. Interestingly, diabetes mellitus was reported to be a factor that independently affects liver stiffness after DAA treatment despite SVR. The underlying mechanisms involving the presence of steatosis, especially before treatment, can be alcohol consumption, being overweight, obesity, diabetes type 2 mellitus, HCV genotype, Human Immunodeficiency Virus (HIV) coinfection, and genetic polymorphisms in genes, such as Transmembrane six SuperFamily member 2 (TM6SF2), Patatin-Like Phospholipase Domain Containing 3 (PNPLA3) and Microsomal Triglyceride Transfer Protein (MTTP).

In this study, we aimed to evaluate the role of host and viral characteristics associated with genetic polymorphisms in liver fat accumulation in chronic hepatitis C patients. The inclusion criteria were patients who presented positive anti-HCV antibody and HCV-RNA results for more than six months and underwent histopathological analysis after liver biopsy, and were older than 18 years. The exclusion criteria were patients who were coinfected with HIV or Hepatitis B Virus (HBV), patients who received previous HCV treatment, and the presence of liver conditions of other etiologies, such as autoimmune liver disease, primary biliary cirrhosis, and Wilson's disease. The patients were included in the study and had biological samples collected before initiating any hepatitis C treatment.

A total of 236 patients with chronic hepatitis C infection under follow-up at the outpatient clinic of infectious diseases in the HCFMUSP who met the criteria described were included from 2010 to 2012. The calculation of the minimum sample size required was performed considering the following parameters: the alpha value of 5%; a beta value of 20% and, consequently, a power of 80% and standard error type 2. The recommended equation for this type of study was used and was as follows: \( n = \frac{\pi^2}{(1-\pi)/e^2} \). This calculation was performed considering the frequency of 27.5% of the recessive allele (T) for the -493G/T SNP in the MTTP gene as described by Mirandola et al. The minimum sample size was calculated to be 225 patients. At the time of enrollment, all patients with chronic hepatitis C attending this outpatient clinic underwent liver biopsy. After histopathological analysis of the liver fragment, the patients were divided into two groups as follows: patients with hepatic steatosis (\( n = 125 \)) and patients without hepatic steatosis (\( n = 111 \)).

Data collection

Data regarding epidemiological and demographic factors and laboratory tests were collected from medical records. The reference values of biochemical tests were as follows: Alanine Aminotransferase (ALT) levels: \( \geq 41 \) U/L, Aspartate Aminotransferase (AST) levels: \( \geq 37 \) U/L, Gamma Glutamyl Transeptidase (GGT) levels: \( \geq 61 \) U/L, insulin levels: \( \geq 25 \) µU/mL, glucose levels: \( >99 \) mg/dL, triglyceride levels: \( \geq 200 \) mg/dL, total cholesterol levels: \( \geq 200 \) mg/dL, High-Density Lipoprotein (HDL) levels: \( \geq 60 \) mg/dL, Low-Density Lipoprotein (LDL) levels: \( \geq 130 \) mg/dL, and Very-Low-Density Lipoprotein (VLDL) levels: \( \geq 40 \) mg/dL. HOMA-IR index (homeostasis model assessment of insulin resistance) was calculated as fasting insulin levels (µU/mL) \( \times \) fasting glucose levels (mmol/L)/22.5, and its reference value was \( \geq 0.85 \). The reference value for HCV viral load was \( <850,000 \) IU/mL, and that for alcohol consumption was \( \geq 20 \) g/day. Metabolic syndrome was defined by the appearance of three or more of the following alterations: high triglyceride levels (\( \geq 150 \) mg/dL), low HDL levels (\( \leq 40 \) mg/dL), diagnosis of diabetes or fasting blood glucose levels \( \geq 100 \) mg/dL, and the diagnosis of high blood pressure or systolic blood pressure \( \geq 130 \) mmHg and/or diastolic blood pressure \( \geq 85 \) mmHg.

Histopathological analysis after liver biopsy of all patients was performed according to Kleiner et al. Classification for the assessment of hepatic steatosis (graded as 0 to 3), according to the METAVIR classification for the assessment of hepatic fibrosis (graded as F0 to F4) and hepatic inflammatory activity (graded as A0 to A4), and according to Perls’ staining criteria (graded as 0 to 4) for the assessment of hepatic siderosis.

Single nucleotide polymorphism genotyping

Peripheral blood (10 mL) was collected from patients with chronic hepatitis C from 2010 to 2012 and was stored at -80°C until processing. To genotype the -493G/T (rs1800599), I128T (rs3816873), Q95H (rs61733139), and Q244E (rs17599091) Single Nucleotide Polymorphisms (SNPs) in the MTTP gene were linked to the presence of hepatic steatosis in patients with chronic hepatitis C.

Materials and methods

Patient selection

Patients with chronic hepatitis C were selected from the Clinical Hospital of the School of Medicine at the University of Sao Paulo (HCFMUSP) in Brazil. The present study was approved by the Ethics Committee (Ethics Committee for Analysis of Research Projects) of HCFMUSP. The protocol followed the guidelines of the 1975 Declaration of Helsinki, and informed consent was obtained from all participants. The actual research did not conflict with any treatment or medical advice. The present study is part of a sequence of previously published studies.
envelope according to the concentration used. The temperature and incubation time varied depending on the enzyme and the manufacturer’s instructions. The restriction enzymes that were utilized had been previously described by Karpe et al.\textsuperscript{19} and Ledmyr et al.\textsuperscript{20} Enzymatic digestion was confirmed under UV light by 3% agarose gel electrophoresis. The genotypes expected for each \textit{MTTP} SNP were as follows: -493G/T SNP, GG: 89 bp and 20 bp, GT: 109 bp, 89 bp, and 20 bp, TT: 109 bp; I128T SNP, II: 167 bp, IT: 167 bp, 138 bp, and 29 bp, TT: 138 bp and 29 bp; Q595 SNP, QQ: 148 bp and 35 bp, QH: 183 bp, 148 bp, and 35 bp, HH: 183 bp; Q244E SNP, QQ: 201 bp, QE: 201 bp, 149 bp, and 52 bp, EE: 149 bp and 52 bp. Quality control was used to verify the reproducibility of the results.

\textbf{Statistical analysis}

For statistical analysis, IBM-SPSS version 20 software (IBM Corp., USA) and Hosmer; Lemeshow\textsuperscript{21} were used. The analysis of the Hardy-Weinberg equilibrium was performed using the Chi-Square test (p \geq 0.05). All variables were categorized, and their frequencies were described. Bivariate and multiple logistic regression was used to determine the individual variables that could influence the presence of steatosis in patients with chronic hepatitis C. The Odds Ratio (OR) of each variable with the presence of hepatic steatosis was estimated with the respective 95\% Confidence Interval (95\% CI). The associations were evaluated in three different genetic models (codominant, dominant and recessive models) because the optimal genetic model of inheritance in genes in complex diseases has not been well established.

A bivariate and a multiple logistic regression model were used to determine which host and viral characteristics combined with each SNP (-493G/T, I128T, Q595H and Q244E) to influence the presence of steatosis in patients with chronic hepatitis C according to different genetic models. All variables independently associated with hepatic steatosis (p < 0.05) in multiple logistic regression and interactions that presented a significance level of 0.20 (p < 0.2) in the bivariate tests were included in the final multiple logistic regression model. In the multiple logistic regression model, the significance level was 0.05 (p < 0.05).

\textbf{Results}

The study group consisted of 236 patients with chronic hepatitis C infection, 56.4\% of whom were females, and the most frequent infection was by genotype non 3 (81.8\%). Additionally, the most frequent self-reported ethnicity was white (81.0\%). The distribution of patients with chronic hepatitis C considering the Body Mass Index (BMI) class is shown in Table 1.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
Characteristic & Hepatic steatosis & \multicolumn{2}{|c|}{Total (n)} \\
\hline & No (n, \%) & Yes (n, \%) & n \\
\hline n & 111 (47.0) & 125 (53.0) & 236 (100) \\
Sex & & & \\
Male & 56 (54.4) & 47 (45.6) & 103 (43.6) \\
Female & 55 (41.4) & 78 (58.6) & 133 (56.4) \\
Age & & & \\
< 50 years & 40 (58.8) & 28 (41.2) & 68 (28.8) \\
\geq 50 years & 71 (42.3) & 97 (57.7) & 168 (71.2) \\
BMI & & & \\
< 25 kg/m\textsuperscript{2} & 45 (48.9) & 47 (51.1) & 92 (39.8) \\
\geq 25 kg/m\textsuperscript{2} & 64 (46.0) & 75 (54.0) & 139 (60.2) \\
Ethnicity & & & \\
White & 85 (44.5) & 106 (55.5) & 191 (81.2) \\
No White & 25 (56.8) & 19 (43.2) & 44 (18.8) \\
HOMA-IR & & & \\
< 3 & 80 (52.6) & 72 (47.4) & 152 (65.5) \\
\geq 3 & 30 (37.5) & 50 (62.5) & 80 (34.5) \\
Alcohol consumption & & & \\
< 20 g/day & 73 (49.3) & 75 (50.7) & 148 (62.7) \\
\geq 20 g/day & 38 (43.1) & 50 (56.9) & 88 (37.3) \\
Hypertension & & & \\
No & 76 (50.7) & 74 (49.3) & 150 (63.6) \\
Yes & 35 (40.7) & 51 (59.3) & 86 (36.4) \\
Diabetes mellitus & & & \\
No & 95 (47.5) & 105 (52.5) & 200 (84.7) \\
Yes & 16 (44.4) & 20 (55.6) & 36 (15.3) \\
HCV genotype 3 & & & \\
No & 99 (51.3) & 94 (48.7) & 193 (81.8) \\
Yes & 12 (27.9) & 31 (72.1) & 43 (18.2) \\
HCV viral load & & & \\
< 850,000 IU/ml & 42 (45.2) & 52 (54.2) & 94 (41.2) \\
\geq 850,000 IU/ml & 64 (47.8) & 70 (52.2) & 134 (58.8) \\
ALT & & & \\
< 41 U/L & 54 (54.0) & 46 (46.0) & 100 (42.4) \\
\geq 41 U/L & 57 (41.9) & 79 (58.1) & 136 (57.6) \\
AST & & & \\
< 37 U/L & 67 (58.3) & 48 (41.7) & 115 (48.7) \\
\geq 37 U/L & 44 (36.4) & 77 (63.6) & 121 (51.3) \\
GGT & & & \\
8–61 U/L & 70 (53.0) & 62 (47.0) & 132 (55.9) \\
> 61 U/L & 41 (39.4) & 63 (60.6) & 104 (44.1) \\
Total cholesterol & & & \\
< 200 mg/dl & 84 (46.7) & 96 (53.3) & 180 (76.3) \\
\geq 200 mg/dl & 27 (48.2) & 29 (51.8) & 56 (23.7) \\
LDL & & & \\
< 130 mg/dl & 91 (46.0) & 107 (54.0) & 198 (83.9) \\
\geq 130 mg/dl & 20 (52.6) & 18 (47.4) & 38 (16.1) \\
HDL & & & \\
\geq 60 mg/dl & 33 (44.0) & 42 (56.0) & 75 (31.8) \\
\leq 60 mg/dl & 78 (48.4) & 83 (51.6) & 161 (68.2) \\
VLDL & & & \\
< 40 mg/dl & 104 (47.3) & 116 (52.7) & 220 (93.2) \\
\geq 40 mg/dl & 7 (43.8) & 9 (56.2) & 16 (6.8) \\
Triglyceride & & & \\
< 200 mg/dl & 104 (47.1) & 117 (52.9) & 221 (93.6) \\
\geq 200 mg/dl & 7 (46.7) & 8 (53.3) & 15 (6.4) \\
Hepatic fibrosis & F0–F2 & 97 (51.9) & 90 (48.1) & 187 (79.2) \\
F3–F4 & 14 (28.6) & 35 (71.4) & 49 (20.8) \\
Hepatic inflammatory activity & A0–A1 & 57 (71.3) & 23 (28.8) & 80 (33.9) \\
A2–A3 & 54 (34.6) & 102 (65.4) & 156 (66.1) \\
Hepatic siderosis & No & 106 (48.0) & 115 (52.0) & 221 (93.6) \\
Yes & 5 (33.3) & 10 (66.7) & 15 (6.4) \\
Metabolic syndrome & No & 111 (47.5) & 123 (52.5) & 234 (99.1) \\
Yes & 0 (0.0) & 2 (100.0) & 2 (0.9) \\
\hline
\end{tabular}
\caption{General characteristics of patients with chronic hepatitis C included in the study (total) and split according to the presence of hepatic steatosis, n (%).}
\end{table}

ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; CI, Confidence Interval; GGT, Gamma Glutamyl Transpeptidase; HCV, Hepatitis C Virus; HDL, High-Density Lipoprotein; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LDL, Low-Density Lipoprotein; OR, Odds Ratio; VLDL, Very Low-Density Lipoprotein.
higher risk of hepatic steatosis than patients without or with low-inten-
tivity (A2 and A3) exhibited a higher risk of hepatic steatosis than patients without or with low-intensity inflammatory activity (A2 and A3). Additionally, patients with moderate- and high-intensity liver inflammatory activity in Table 2 – 5. The character-istics combined with SNPs that had a significant statistical association with hepatic steatosis in patients with chronic hepatitis C (Supplementary Tables S1 and S2). The multivariate analysis indicated that HCV genotype 3 infection (OR = 2.74, 95% CI 2.14–6.06, p = 0.013) and female sex (OR = 2.28, 95% CI 1.21–4.28, p = 0.011) were associated with a higher risk of hepatic steatosis. Additionally, patients with moderate- and high-intensity liver inflammatory activity (A2 and A3) exhibited a higher risk of hepatic steatosis than patients without or with low-intensity inflammatory activity (A0 and A1) (OR = 3.61, 95% CI 1.86–7.01, p < 0.001). The variables that had a significant p-value were included in the interaction analysis (HCV genotype 3, female sex, and inflammatory activity A2-A3). In addition to these variables, HOMA-IR was included in the bivariate and multivariate analyses to assess the association of SNPs in the MTTP gene combined with different characteristics in the presence of hepatic steatosis (interaction analysis) due to its relevance to steatosis in chronic hepatitis C.

The results of the bivariate analysis of the characteristics combined with SNPs in the MTTP gene are presented in Tables 2–5. The character-istics combined with SNPs that had a significant statistical association with the presence of hepatic steatosis in this analysis were also included in the multiple logistic regression analysis for the MTTP -493G/T, -1128T, Q95H and Q244E SNPs, and the results are described in Tables 6–9. Therefore, all variables associated alone with hepatic steatosis (p < 0.05) in multiple logistic regression (Tables S2) and variables combined with SNPs (Tables 2–5) presented a significance level of 0.20 (p < 0.2) in the bivariate tests were included in the final multiple logistic regression model (interaction analysis) according to different genetic models (dominant, codominant and recessive models).

In the final multiple logistic regression model used to determine which characteristics combined with each SNP to influence the presence of hepatic steatosis in patients with chronic hepatitis C, it was observed that in the dominant genetic model (GG × GT/TT), the GT/TT genotype with the presence of hepatic steatosis was confirmed in the analysis.
Table 3
Result of bivariate tests for interactions of the I128T SNP in the MTPP gene in different genetic models with characteristics of interest that influence the presence of hepatic steatosis in patients with chronic hepatitis C.

| Parameter | OR   | 95% CI  | p*  |
|-----------|------|---------|-----|
| **Dominant model** |      |         |     |
| I128T SNP | 1.07 | 0.64–1.78 | 0.800 |
| I128T SNP Sex (female) | 0.79 | 0.28–2.23 | 0.657 |
| I128T SNP Age (≥ 50 years) | 1.51 | 0.46–4.96 | 0.495 |
| I128T SNP BMI (≥ 25 kg/m²) | 0.88 | 0.31–2.54 | 0.818 |
| I128T SNP HOMA-IR (≥ 3) | 0.93 | 0.31–2.82 | 0.897 |
| I128T SNP Hypertension | 1.42 | 0.48–4.20 | 0.532 |
| I128T SNP Diabetes mellitus | 1.12 | 0.26–4.73 | 0.881 |
| I128T SNP HCV genotype 3 | 7.90 | 1.67–37.29 | 0.009 |
| I128T SNP ALT (≥ 41 U/L) | 0.97 | 0.34–2.76 | 0.960 |
| I128T SNP AST (≥ 37 U/L) | 1.20 | 0.42–3.43 | 0.732 |
| I128T SNP GGT (≥ 61 U/L) | 2.39 | 0.83–6.82 | 0.105 |
| I128T SNP Total cholesterol (≥ 200 mg/dL) | 0.44 | 0.13–1.48 | 0.194 |
| I128T SNP HDL (≤ 60 mg/dL) | 0.74 | 0.24–2.22 | 0.588 |
| I128T SNP LDL (≥ 130 mg/dL) | 0.44 | 0.11–1.78 | 0.246 |
| I128T SNP VLDL (≥ 40 mg/dL) | 2.67 | 0.32–21.94 | 0.362 |
| I128T SNP Triglyceride (≥ 200 mg/dL) | 2.19 | 0.26–18.52 | 0.474 |
| I128T SNP Fibrosis (F3–F4) | 1.90 | 0.48–7.51 | 0.363 |
| I128T SNP Inflammatory activity (A2–A3) | 1.52 | 0.46–4.98 | 0.488 |
| I128T SNP Siderosis | 0.92 | 0.10–8.73 | 0.940 |

| Codomominate model |      |         |     |
| I128T SNP II x IT | 0.90 | 0.53–1.54 | 0.699 |
| I128T SNP II x TT | 2.45 | 0.90–6.69 | 0.081 |
| I128T SNP (II x IT) Sex (female) | 0.84 | 0.28–2.49 | 0.747 |
| I128T SNP (II x TT) Sex (female) | 0.44 | 0.05–3.61 | 0.443 |
| I128T SNP (II x IT) Age (≥ 50 years) | 1.40 | 0.40–5.01 | 0.592 |
| I128T SNP (II x TT) Age (≥ 50 years) | 2.11 | 0.20–22.74 | 0.538 |
| I128T SNP (II x IT) BMI (≥ 25 kg/m²) | 0.94 | 0.91–2.86 | 0.914 |
| I128T SNP (II x TT) BMI (≥ 25 kg/m²) | 0.832 | 0.106–6.50 | 0.861 |
| I128T SNP (II x IT) HOMA-IR (≥3) | 0.71 | 0.22–2.59 | 0.569 |
| I128T SNP (II x TT) HOMA-IR (≥3) | 3.35 | 0.28–48.06 | 0.342 |
| I128T SNP (II x IT) Hypertension | 1.06 | 0.85–3.37 | 0.894 |
| I128T SNP (II x TT) Hypertension | 0.67 | 0.54–8.26 | 0.136 |
| I128T SNP (II x IT) Diabetes mellitus | 1.12 | 0.25–5.07 | 0.884 |
| I128T SNP (II x TT) Diabetes mellitus | 1.09 | 0.07–16.48 | 0.949 |
| I128T SNP (II x IT) HCV genotype 3 & | 7.45 | 1.53–36.23 | 0.013 |
| I128T SNP (II x TT) HCV genotype 3 & | 1.99 | 0.28–2.74 | 0.712 |
| I128T SNP (II x IT) ALT (≥ 41 U/L) | 1.82 | 0.22–14.77 | 0.576 |
| I128T SNP (II x TT) ALT (≥ 41 U/L) | 1.08 | 0.36–32.86 | 0.887 |
| I128T SNP (II x IT) AST (≥ 37 U/L) | 1.35 | 0.17–10.95 | 0.780 |
| I128T SNP (II x TT) AST (≥ 37 U/L) | 1.97 | 0.66–5.89 | 0.227 |
| I128T SNP (II x IT) ALT (≥ 41 U/L) & | 0.81 | 0.27–2.43 | 0.712 |
| I128T SNP (II x TT) ALT (≥ 41 U/L) & | 1.08 | 0.36–32.86 | 0.887 |
| I128T SNP (II x IT) Total cholesterol (≥ 200 mg/dL) | 0.45 | 0.12–1.63 | 0.222 |
| I128T SNP (II x TT) Total cholesterol (≥ 200 mg/dL) | 0.33 | 0.03–3.22 | 0.342 |
| I128T SNP (II x IT) LDL (≥ 130 mg/dL) | 0.42 | 0.10–1.85 | 0.251 |
| I128T SNP (II x TT) LDL (≥ 130 mg/dL) | 0.61 | 0.04–9.96 | 0.727 |
| I128T SNP (II x IT) HDL (≤ 60 mg/dL) | 0.61 | 0.19–1.97 | 0.407 |
| I128T SNP (II x TT) HDL (≤ 60 mg/dL) | 2.27 | 0.29–17.93 | 0.438 |
| I128T SNP (II x IT) VLDL (≥ 40 mg/dL) | 3.18 | 0.31–32.67 | 0.331 |
| I128T SNP (II x TT) VLDL (≥ 40 mg/dL) | 0.61 | 0.05–22.00 | 0.969 |
| I128T SNP (II x IT) Triglyceride (≥ 200 mg/dL) | 2.33 | 0.21–25.63 | 0.489 |
| I128T SNP (II x TT) Triglyceride (≥ 200 mg/dL) | 1.06 | 0.05–22.00 | 0.969 |
| I128T SNP (II x IT) Fibrosis (F3–F4) | 2.27 | 0.54–9.57 | 0.265 |
| I128T SNP (II x TT) Fibrosis (F3–F4) | 0.88 | 0.06–12.14 | 0.923 |
| I128T SNP (II x IT) Inflammatory activity (A2–A3) | 1.60 | 0.44–5.48 | 0.784 |
| I128T SNP (II x TT) Inflammatory activity (A2–A3) | 2.24 | 0.23–21.35 | 0.484 |
| I128T SNP (II x IT) Siderosis | 0.81 | 0.08–8.36 | 0.862 |
| I128T SNP (II x TT) Siderosis & | >0.999 |

| Recessive model |      |         |     |
| I128T SNP (II x IT) | 2.57 | 0.97–6.82 | 0.058 |
| I128T SNP (II x TT) Sex (female) | 0.48 | 0.06–3.69 | 0.476 |
| I128T SNP (II x IT) Age (≥ 50 years) | 1.93 | 0.19–19.87 | 0.579 |
| I128T SNP (II x TT) BMI (≥ 25 kg/m²) | 0.85 | 0.12–6.26 | 0.877 |
| I128T SNP (II x IT) HOMA-IR (≥ 3) | 3.94 | 0.35–44.92 | 0.269 |
Table 5
Result of bivariate tests for interactions of the Q244E SNP in the MTP gene with characteristics of interest that influence the presence of hepatic steatosis in patients with chronic hepatitis C.

| Parameter | OR   | 95% CI | p   |
|-----------|------|--------|-----|
| Q244E SNP (Q1 × E1) | 1.33 | 0.59–3.01 | 0.487 |
| Q244E SNP × Sex (female) | 3.52 | 0.58–21.56 | 0.174 |
| Q244E SNP × Age (>250 years) | 3.39 | 0.46–24.87 | 0.229 |
| Q244E SNP × BMI (>25 kg/m²) | 1.06 | 0.21–5.45 | 0.947 |
| Q244E SNP × HOMA-IR (>3) | 0.81 | 0.15–4.44 | 0.810 |
| Q244E SNP × Hypertension | 0.90 | 0.17–4.83 | 0.905 |
| Q244E SNP × Diabetes mellitus | 0.92 | 0.11–7.65 | 0.935 |
| Q244E SNP × HCV genotype 3 & | | | |
| Q244E SNP × ALT (>41 U/L) | 1.08 | 0.19–6.24 | 0.936 |
| Q244E SNP × AST (>37 U/L) | 1.10 | 0.20–5.92 | 0.912 |
| Q244E SNP × GGT (>51 U/L) | 0.41 | 0.07–2.28 | 0.309 |
| Q244E SNP × Total cholesterol (≥200 mg/dL) | 7.88 | 0.73–85.12 | 0.089 |
| Q244E SNP × LDL (≥130 mg/dL) | 3.39 | 0.27–42.14 | 0.342 |
| Q244E SNP × HDL (≥50 mg/dL) | 0.63 | 0.12–3.40 | 0.595 |
| Q244E SNP × VLDL (≥40 mg/dL) & | | | |
| Q244E SNP × Triglyceride (≥200 mg/dL) & | | | |
| Q244E SNP × Fibrosis (F4-F3) | 0.74 | 0.10–5.44 | 0.763 |
| Q244E SNP × Inflammatory activity (A2-A3) | 0.73 | 0.13–4.23 | 0.726 |
| Q244E SNP × Siderosis & | | | |

* Bivariate test. Significance level of p < 0.20.

b No patients presented the QQ genotype. Indicates that the estimation was not possible.ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; BMI, Body Mass Index; CI, Confidence Interval; GGT, Gamma Glutamyl Transpeptidase; HCV, Hepatitis C Virus; HDL, High-Density Lipoprotein; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LDL, Low-Density Lipoprotein; OR, Odds Ratio; SNP, Single Nucleotide Polymorphism; VLDL, Very Low-Density Lipoprotein.

Table 6
Result of multivariate tests for interactions of the −493G/T SNP in the MTP gene (in different genetic models) with characteristics of interest that influence the presence of hepatic steatosis in patients with chronic hepatitis C.

| Parameter | OR   | 95% CI | p   |
|-----------|------|--------|-----|
| −493G/T SNP (GT/TT) | 0.66 | 0.35–1.24 | 0.193 |
| −493G/T SNP (GT/TT) × HCV genotype 3 | 11.51 | 2.08–63.59 | 0.005 |

a Multiple logistic regression. When interaction is placed, only the interaction should be interpreted. The significance level of p < 0.05 is marked in bold font. CI, Confidence Interval; HCV, Hepatitis C Virus; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; OR, Odds Ratio; SNP, Single Nucleotide Polymorphism.

In the dominant genetic model (II × IT/TT), the IT/TT genotype of the 1128T SNP in the MTP gene combined with HCV genotype 3 infection presented an 8.51-fold higher risk of hepatic steatosis than that observed in carriers of the II genotype without HCV genotype 3 infections (95% CI 1.59–45.54, p = 0.012). In the codominant model (II × IT × TT), carriers of the IT genotype of the 1128T SNP combined with HCV genotype 3 infection presented an 8.40-fold higher risk of hepatic steatosis than that observed in carriers of the II genotype without HCV genotype 3 infections (95% CI 1.51–46.91, p = 0.015) (Table 7). The Q95H and Q244E SNPs in the MTP gene did not that observed in carriers of the GG genotype without HCV genotype 3 infections (95% CI 2.46–99.85, p = 0.004) (Table 6).

Table 7
Result of multivariate tests for interactions of the I128T SNP in the MTP gene (in different genetic models) with characteristics of interest that influence the presence of hepatic steatosis in patients with chronic hepatitis C.

| Parameter | OR   | 95% CI | p   |
|-----------|------|--------|-----|
| I128T SNP (IT/II) | 8.40 | 1.51–46.91 | 0.015 |
| I128T SNP (IT/II) × HCV genotype 3 | 8.40 | 1.51–46.91 | 0.015 |
| I128T SNP (IT/II) × HCV genotype 3 | 8.40 | 1.51–46.91 | 0.015 |

a Multiple logistic regression. When interaction is placed, only the interaction should be interpreted. The significance level of p < 0.05 is marked in bold font. CI, Confidence Interval; HCV, Hepatitis C Virus; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; OR, Odds Ratio; SNP, Single Nucleotide Polymorphism.

Table 8
Result of multivariate tests for interactions of the Q95H SNP in the MTP gene (in different genetic models) with characteristics of interest that influence the presence of hepatic steatosis in patients with chronic hepatitis C.

| Parameter | OR   | 95% CI | p   |
|-----------|------|--------|-----|
| Q95H SNP (Q1/H1) | 7.89 | 1.44–43.12 | 0.017 |
| Q95H SNP (Q1/H1) × BMI (≥25) | 0.15 | 0.02–1.03 | 0.053 |

a Multiple logistic regression. When interaction is placed, only the interaction should be interpreted. The significance level of p < 0.05.

b Because only one patient presented the HH genotype, it was not possible to perform the analyses in other genetic models. BMI, Body Mass Index; CI, Confidence Interval; HCV, Hepatitis C Virus; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; OR, Odds Ratio; SNP, Single Nucleotide Polymorphism.
influence the presence of hepatic steatosis in this group of patients when combined with other variables (Tables 6 and 9).

Discussion

Hepatitis C is a frequent liver disease found worldwide. Even in the era of DAAAs, the determination of genetic markers is important to identify individuals at higher risk of severe disease and to guide therapeutic decisions prior to therapy in patients with chronic hepatitis C. In the present study, the authors analyzed the effect of four candidate SNPs in the MTTP gene combined with host and viral characteristics on hepatic steatosis in a group of chronic hepatitis C patients. Multiple logistic regression analysis for interaction showed an 11.51-fold higher risk of steatosis in patients with the GT/TT genotype of the -493G/T SNP and HCV genotype 3 infections. The same results were observed when another genetic model, the codominant model, was analyzed, in which carriers of the GT genotype combined with HCV genotype 3 infection presented a 15.69-fold higher risk of steatosis. The present study also investigated the I128T SNP of the MTTP gene, and the risk of steatosis was 8.51-fold higher in patients with the IT/TT genotype of the I128T SNP when combined with HCV genotype 3 infections. In another genetic model, the codominant model, carriers of the IT genotype combined with HCV genotype 3 infection presented an 8.40-fold higher risk of steatosis.

Several studies have explored the association between the -493G/T and I128T SNPs of the MTTP gene and NAFLD. However, the effect of these polymorphisms on NAFLD remains uncertain due to the inconsistent results of different studies. A recent meta-analysis evaluated these SNPs under different genetic models on NASH and NAFLD, demonstrating that the -493G/T SNP is associated with NASH susceptibility (determined by liver biopsy).

The role of the -493G/T SNP in the MTTP gene associated with hepatitis C has been thoroughly studied, but different results have been reported. Akgöllü and Akkız evaluated the relationship of the -493G/T SNP with hepatic steatosis in a Turkish population with HCV genotype 1 infection. Despite finding a statistically significant association between levels of triglycerides, total cholesterol, LDL, and VLDL and the number of patients with steatosis, these researchers reported that the studied polymorphism is not associated with the presence of steatosis in individuals who exhibited HCV genotype 1 infection. A previous Swiss cohort study, including 443 patients infected with HCV genotype non 3 and 183 patients infected with HCV genotype 3, reported that the -493G/T SNP is associated with the presence of steatosis in multivariate analysis only in patients with HCV genotype non 3. However, in another study evaluating 102 treatment-naive patients for hepatitis C it was reported that patients infected with HCV genotype 3 and with the T (mutated) allele of the -493G/T SNP are associated with hepatic steatosis, which corroborates the present findings. Furthermore, these patients also present a higher grade of inflammation in the liver and more liver fibrosis as well as higher HCV-RNA serum levels than those observed in carriers of the wild-type allele (G).

Regarding the I128T SNP in the MTTP gene, previous multivariate analyses conducted in a Han Chinese population have indicated that this SNP is not associated with NAFLD. However, this polymorphism has been associated with the presence of central obesity, elevated liver enzymes, and alcoholic fatty liver disease in Koreans. Hashemi et al. investigated the association between the I128T and Q95H SNPs in the MTTP gene in a sample of Iranian patients with NAFLD, and they observed that the IT genotype and the IT + TT genotype of the I128T SNP increase susceptibility to NAFLD. In addition, it has been reported that there is no association between the Q95H and NAFLD. Hepatic steatosis is a frequent histological feature among patients with chronic hepatitis C, and it significantly affects disease progression. In the present study, hepatic steatosis was observed in 53% of the patients, which was similar to the mean prevalence of 55% previously described by Asselah et al. Host and viral factors are involved in the development and severity of HCV-associated steatosis. Recently, several SNPs have been reported to be associated with alterations in hepatic fibrosis and steatosis even after DAA therapy for HCV infection, indicating that they may have prognostic value for the assessment of post-SVR evolution.

The present study had several limitations. The sample size was limited, and the study was conducted in a single center. To validate the present findings, a study including a larger number of samples should be performed in the future. However, the present results may aid in the establishment of genetic markers to predict hepatic steatosis.

Conclusions

In summary, the present study highlighted the significant role of MTTP SNPs in the pathogenesis of hepatic steatosis in hepatitis C. The association analysis of the group of patients with chronic hepatitis C provides useful information on the effect of HCV genotype 3 infections combined with the -493G/T and I128T SNPs in the MTTP gene on hepatic steatosis. These findings may have prognostic importance in patients with chronic hepatitis C and help guide decision-making for appropriate follow-up and treatment for those at increased risk of liver disease progression.

Conflicts of interest

The authors declare no conflicts of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.clinsp.2022.100094.

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