# No effect of MTP polymorphisms on PNPLA3 in HCV-correlated steatosis

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## SUMMARY

PNPLA3 and MTP genes have been associated with liver steatosis and chronic hepatitis C.
We studied the influence of MTP and PNPLA3 polymorphisms in 114 Italian patients with chronic hepatitis C, evaluating the histological and clinical presentation of liver disease.
The study confirmed the association of PNPLA3 polymorphisms with liver steatosis (p=0.041), but did not show any additive effect of MTP polymorphisms in the development of steatosis. MTP polymorphisms do not seem to influence PNPLA3 in the development of liver steatosis. Further studies with a larger number of patients are required.

*Keywords*: MTP, PNPLA3, chronic hepatitis C, liver steatosis.

## INTRODUCTION

Hepatic steatosis is a well-known characteristic in chronic hepatitis by the hepatitis C virus (HCV), facilitating the progression of liver damage and fibrosis [1]. Fatty liver disease and the correlated glycometabolic abnormalities are involved in the severity of liver disease, leading to higher fibrosis levels [2,3]. Several studies have shown that HCV requires host lipoproteins for its life because, by using the lipoprotein mechanism of production, the virus promotes its replication and escapes the immune system [4].
For this reason, host factors that might influence the lipid metabolism, hepatic steatosis development and consequently the viral life cycle were investigated; particularly, polymorphisms of genes involved in the lipid metabolism, such as microsomal triglyceride transfer protein (MTP) gene and patatin-like phospholipase domain-containing 3 gene (PNPLA3), were studied in relation to liver steatosis in chronic hepatitis C (CHC) [5-11].
PNPLA3 is predominantly expressed in human liver and adipose tissue and exerts both lipolytic and lipogenic activity *in vitro*; its rs738409 G/G variant has been associated with triglyceride accumulation related to a loss of hydrolysing function, and with liver steatosis and fibrosis progression in patients with CHC [7-12].
MTP is a heterodimeric lipid transfer protein that has a fundamental role in the assembly and secretion of VLDL; HCV decreases MTP RNA expression, facilitating steatosis development, and the modulating effect of HCV on the MTP gene may help its life [5]. The polymorphism in the promoter region of MTP gene, –493G/T, has been associated with liver steatosis; particularly, the GG

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genotype of MTP gene seems to favor steatosis in non-alcoholic fatty liver disease, while the TT genotype favors steatosis in CHC genotype 3 [6]. No study evaluated the role of both polymorphisms on the degree of liver steatosis in CHC patients.
The aim of the present study was to evaluate the role of MTP and PNPLA3 polymorphisms on the histological and clinical presentation in Italian patients with CHC.

## PATIENTS AND METHODS

Enrolled in the study were 114 consecutive Italian patients with histology proven chronic hepatitis C, anti-HCV/HCV-RNA-positive and persistently abnormal serum alanine aminotransferase (ALT) levels for 18-36 months. The patients, all naive for antiviral treatment, underwent liver biopsy from July 2009 to December 2011.
The patients were tested for viral markers (HBV, HCV, HDV, HIV), autoimmunity, iron overload and α-1 antitrypsin, and those with a presence of other causes of liver disease and/or HIV positivity were excluded. For all patients the body mass index (BMI: kg/m$^2$), waist circumference, liver function tests, triglycerides and cholesterol levels were recorded and a liver ultrasound scan was performed.
The histological evaluation of specimens obtained by liver biopsy was determined as previously described [8]. Samples of serum and whole blood were obtained on the day of the liver biopsy and stored at –80°C.
All procedures in the study were in accordance with the international guidelines, with the standards on human experimentation of the Ethics Committee of the Second University of Naples (now University of Campania "L. Vanvitelli") and with the Helsinki Declaration of 1975, revised in 1983.
The HCV viral load was determined and HCV genotype and PNPLA3 polymorphisms were sought as previously described [6, 8]. MTP polymorphism was genotyped by real time PCR utilizing TaqMan SNP Genotyping Assay with TaqMan Genotyping Master Mix (Thermo Fisher Scientific), according to the manufacturer's instructions.
Statistical analyses were performed on prospectively collected data; subjects were subsequently split according to the type of gene polymorphism. The statistical significance of differences observed with respect to the presence or absence of polymorphism variants was evaluated by Chi-squared/Fisher exact test for categorical variables and Mann-Whitney U/Kruskal-Wallis test for numeric variables.
All analyses were performed with SPSS 24 software, with a 5% significance level and two-sided test.

## RESULTS

In the study population, HCV genotypes non-3 were prevalent and most of the patients presented overweight, but blood glucose, cholesterol and triglyceride levels were within the normal range. About 25% of patients reported past alcohol abuse and 11.6% past drug addiction.
The demographic, biochemical, virological and histological data according to the PNPLA3 and MTP polymorphisms are shown in Table 1. The presence of steatosis, liver steatosis score and HOMA-IR were significantly higher in patients with the p. PNPLA3 rs738409 G/G genotype compared to homozygous patients for the p. rs738409 C/C allele (p=0.026, 0.041 and 0.029, respectively). No association was observed with other parameters.
The MTP polymorphisms were not significantly associated with any of the histological parameters (Table 1). A trend (p=0.06) was observed between heterozygotes and wild-type in relation to the degree of steatosis, while a significant difference was seen in relation only to waist circumference (p=0.015).
By stratifying patients according to carriers of the double PNPLA3 variant (rs738409 G/G)/MTP (493 GT or TT) and absence of variant, MTP variant was associated with a significantly higher waist circumference than the double variant and PNPLA3 variant (p=0.012). Conversely, cholesterol was significantly higher among those with the double variant (p=0.026). Finally, steatosis and HOMA-IR index were significantly higher among patients with PNPLA3 variant (p=0.025 and p=0.042, respectively; Table 2).
MTP did not show an additive influence on PNPLA3, particularly regarding liver steatosis (Table 2).

## DISCUSSION

In this study, we evaluated the role of PNPLA3 and MTP polymorphisms on the clinical and histological presentation in Italian CHC patients; because PNPLA3 and MTP polymorphisms have been associated with liver steatosis in previous studies we hypothesized that an association of their variants that further predispose to liver steatosis might have an additive effect in determining liver steatosis [5-8, 13].

In this study, we confirm the association of PNPLA3 with liver steatosis, but we found no role of MTP polymorphisms, either alone or in asso-

**Table 1** - General characteristics of patients stratified by PNPLA3 and MTP variants in patients with chronic hepatitis C.

| *Parameter* | *PNPLA3** | | | *MTP*** | | |
|---|---|---|---|---|---|---|
| | *rs738409 C/C (n=42)* | *rs738409 C/G (n=56)* | *rs738409 G/G (n=16)* | *493G/G (n=54)* | *493G/T (n=47)* | *493T/T (n=13)* |
| Age (yrs), median [IQR] | 53 [43.2–59.7] | 50.5 [41.7–56] | 58 [41–61] | 55 [40.5–59] | 51.5 [42.2–56] | 55 [49.2–63.5] |
| Sex, n (%)<br>*M*<br>*F* | <br>19 (45.2)<br>23 (54.8) | <br>38 (67.9)<br>18 (32.1) | <br>7 (43.8)<br>9 (56.2) | <br>31 (57.4)<br>23 (42.6) | <br>25 (53.2)<br>22 (46.8) | <br>8 (61.5)<br>5 (39.5) |
| BMI (kg/m$^2$), median [IQR] | 26 [24–30.5] | 25.6 [23.7–28.4] | 25.8 [24.3–29.9] | 25 [23.4–27.7] | 26 [24– 29.2] | 29.2 [23.9–32.8] |
| Waist circumference (cm), median [IQR] | 90 [86–102] | 92.7 [85.5–100] | 90 [81–105] | 87.5 [82–95] | 94.2 [88.2–104] | 100 [97–109.7] |
| ALT (n.v. x), median [IQR] | 1.3 [0.8–2.6] | 1.9 [1.3–2.7] | 1.7 [0.8–2.4] | 1.9 [0.7–3] | 1.6 [1–2.4] | 1.9 [1.5–2.2] |
| AST (n.v. x), median [IQR] | 1 [0.2–1.8] | 1.1 [0.5–1.8] | 1.05 [0.48–1.6] | 1.1 [0.4–1.8] | 1 [0.4–1.6] | 1.2 [0.2–1.9] |
| GGT, median [IQR] | 40 [26.2–97] | 49.5 [24.2–92.5] | 49.5 [25–92.5] | 48 [28–90.7] | 45 [25.5–93] | 32 [21.5–93.5] |
| Cholesterol (mg/dL), median [IQR] | 160 [139.2–188.2] | 177 [146–200.5] | 164.5 [130.7–191.5] | 174 [141.7–198] | 159 [144–193.5] | 168 [137–213.5] |
| Triglycerides (mg/dL), median [IQR] | 87 [77–110] | 84.5 [66.7–129.7] | 79.5 [63.5–118] | 85 [70.7–114.7] | 86 [65.5–117] | 96 [59.7–167.7] |
| Glucose (mg/dL), median [IQR] | 86 [78.7–97.2] | 92 [84–97] | 91 [85–98] | 87.5 [82–96.5] | 90 [83.5–98] | 95 [87–98] |
| HOMA-IR, median [IQR] | 3.2 [1.9–4] | 3.1 [2.1–5.1] | 4.6 [3.4–15.2] | 3.2 [1.9–4.8] | 3.5 [2.3–5.6] | 3.6 [3–4.3] |
| Genotype, n (%)<br>*Non-3*<br>*3* | <br>31 (91.2)<br>3 (8.8) | <br>42 (85.7)<br>7 (14.3) | <br>13 (92.9)<br>1 (7.1) | <br>42 (91.3)<br>4 (8.7) | <br>33 (84.6)<br>6 (15.4) | <br>11 (91.7)<br>1 (8.3) |
| HAI score, median [IQR] | 5 [3–10] | 5 [3.7–8] | 6 [4–10] | 5 [3–9] | 5 [3–9.7] | 5 [2.5–8] |
| Fibrosis score, median [IQR] | 2 [1–3] | 2 [1–3] | 2.5 [1–4] | 2 [1–3] | 1.5 [1–2.7] | 3 [1–4] |
| Presence of steatosis, n (%)<br>*Absence*<br>*Steatosis 1-2*<br>*Steatosis 3-4* | <br>14 (38.9)<br>16 (44.4)<br>6 (16.7) | <br>14 (26.4)<br>29 (54.7)<br>10 (18.9) | <br>4 (26.7)<br>3 (20)<br>8 (53.3) | <br>14 (29.2)<br>27 (56.3)<br>7 (14.6) | <br>15 (34.1)<br>15 (34.1)<br>14 (31.8) | <br>3 (25)<br>6 (50)<br>3 (25) |
| Steatosis score, median [IQR] | 1 [0–2] | 1 [0–2] | 3 [0–4] | 1 [0–2] | 1 [0–3] | 1.5 [0.25–2.75] |

* PNPLA3: PCC-GG< 0.05 for HOMA-IR, presence of steatosis and steatosis score (p=0.029, 0.026 and 0.041, respectively) - PGC-GG <0.05 for presence of steatosis (p=0.016).

** MTP: PGG-TT<0.05 for waist circumference (p=0.007).

**Table 2** - Comparison of general characteristics of patients stratified by double variant PNPLA3 (rs738409 G/G)/MTP (493 GT or TT) and absence of variant in patients with chronic hepatitis C.

| *Parameter* | *Double Variant (n=42)* | *PNPLA3 (rs738409 G/G) (n=7)* | *MTP 493T/T – G/T (n=18)* | *No variant (n=47)* | P |
|---|---|---|---|---|---|
| Age (yrs), median [IQR] | 54 [47.2 – 60.7] | 58 [39 – 59] | 48 [41.5 – 75] | 51 [40 – 59] | 0.468 |
| Sex, n (%)<br>*M*<br>*F* | <br>23 (54.8)<br>19 (45.2) | <br>3 (42.9)<br>4 (57.1) | <br>10 (55.6)<br>8 (44.4) | <br>28 (59.6)<br>19 (40.4) | 0.859 |
| BMI (kg/m$^2$), median [IQR] | 26.3 [24 – 29] | 25.8 [24.8 – 30] | 27 [24.1 – 31.1] | 25 [23.1 – 27.6] | 0.367 |
| Waist circumference (cm), median [IQR] | 94.4 [88.5 – 102.5] | 90 [84 – 105] | 102 [90 – 105] | 86 [82 – 92] | 0.012 |
| ALT (n.v. x), median [IQR] | 1.6 [1.2 – 2.3] | 1.9 [1.3 – 3] | 1.2 [0.85 – 2.25] | 1.9 [0.7 – 2.97] | 0.705 |
| AST (n.v. x), median [IQR] | 1 [0.4 – 1.6] | 1.1 [0.6 – 1.6] | 1.2 [0.35 – 1.9] | 1.1 [0.25 – 1.8] | 0.997 |
| GGT, median [IQR] | 41 [22.5 – 81.7] | 52 [35 – 64.5] | 56 [27.7 – 119.5] | 48 [25 – 100] | 0.804 |
| Cholesterol, mg/dL, median [IQR] | 186 [146 – 201] | 175 [136 – 188] | 147.5 [116 – 161.2] | 173 [141 – 204] | 0.026 |
| Triglycerides (mg/dL), median [IQR] | 86.5 [69.2 – 127.5] | 67 [61.5 – 108.5] | 86 [55 – 119] | 87 [74.5 – 119.5] | 0.483 |
| Glucose median (mg/dL), median [IQR] | 92 [85.7 – 96.7] | 93 [86 – 147.25] | 86 [78.25 – 101.5] | 85.5 [81.75 – 95.5] | 0.161 |
| HOMA-IR, median [IQR] | 3.6 [2.8 – 5.6] | 9.98 [3.8 – 15.8] | 2.98 [1.9 – 3.7] | 2.9 [1.6 – 3.9] | 0.042 |
| Genotype<br>*Non-3 (%)*<br>*3 (%)* | <br>31 (86.1)<br>5 (13.9) | <br>6 (100)<br>0 (-) | <br>13 (86.7)<br>2 (13.3) | <br>36 (90)<br>4 (10) | 0.770 |
| HAI score, median [IQR] | 5 [3.25 – 8] | 6 [4 – 9] | 6 [3 – 9.5] | 5 [3 – 9] | 0.995 |
| Fibrosis score, median [IQR] | 2 [1 – 3] | 2.5 [1 – 4.25] | 2 [1 – 3.5] | 2 [1 – 3] | 0.796 |
| Steatosis score, median [IQR] | 1 [0 – 3] | 4 [1 – 4] | 2 [0 – 3.5] | 1 [0 – 1] | 0.025 |

ciation with PNPLA3. Recently, Mirandola et al hypothesized a mechanism regarding the role of MTP in related CHC steatosis; in the early stages of HCV infection, MTP activity might be enhanced to facilitate assembly and secretion of HCV particles, while later HCV may decrease MTP expression through an up-regulation of some MTP suppressors, thus increasing lipogenesis, lipid accumulation and hepatic steatosis [13]. This mechanism might create a safe environment for HCV latency.

Accordingly, with this supposed role of MTP in the mechanisms leading to liver steatosis, we might consider that our patients were a homogeneous group in which we cannot know the precise stage of chronic infection or the time of infection; however, the median fibrosis score was 2 in the whole population, giving the idea that disease was not so advanced [13]. Indeed, patients were relatively young, had median low steatosis scores and very similar HOMA-IR, so no specific differences could be detected. In addition, the low number of patients, particularly of those with MTP G/T polymorphism, contributed to preventing any definitive conclusions to be drawn; in detail, only 1 patient with both rs73,8409 G/G PNPLA3 and 493G/T MTP homozygote polymorphisms was found.

The only significant association was observed between MTP polymorphisms and waist circumference. MTP polymorphisms might indirectly increase a steatosis risk through visceral obesity,

which is another important factor inducing steatosis [3].
The maintenance of the ideal weight is a fundamental target in CHC patients. Obviously, this recommendation should be pursued also in patients treated with direct-acting antiviral agents to protect the liver from fat damage also after viral clearance.
Larger studies are needed to clarify the possible role of PNPLA3 and MTP polymorphisms in CHC-associated steatosis.

**Conflict of interest**
All authors have no conflicts of interest.

## REFERENCES

[1] Adinolfi L.E., Gambardella M., Andreana A., Tripodi M.F., Utili R., Ruggiero G. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. *Hepatology* 33, 1358-1364, 2001.
[2] Rao H., Wu E., Fu S., et al. The higher prevalence of truncal obesity and diabetes in American than Chinese patients with chronic hepatitis C might contribute to more rapid progression to advanced liver disease. *Aliment. Pharmacol. Ther*. 46, 731–740, 2017.
[3] Lingala S., Ghany M.G. Natural history of hepatitis C. *Gastroenterol. Clin. North Am.* 44, 717-734, 2015.
[4] Miyanari Y., Atsuzawa K., Usuda N., et al. The lipid droplet is an important organelle for hepatitis C virus production. *Nat. Cell. Biol* 9, 1089-1097, 2007.
[5] Mirandola S., Realdon S., Iqbal J., et al. Liver microsomal triglyceride transfer protein is involved in hepatitis C liver steatosis. *Gastroenterology* 130, 1661-1669, 2006.
[6] Zampino R., Ingrosso D., Durante-Mangoni E., et al. Microsomal triglyceride transfer protein (MTP) -493G/T gene polymorphism contributes to fat liver accumulation in HCV genotype 3 infected patients. *J. Viral Hepat.* 15, 740-746, 2008.
[7] Valenti L., Rumi M.G., Galmozzi E., et al. Patatin-Like Phospholipase Domain-Containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 53, 791-799, 2011.
[8] Zampino R., Coppola N., Cirillo G., et al. Abdominal fat interacts with PNPLA3 I148M, but not with the APOC3 variant in the pathogenesis of liver steatosis in chronic hepatitis C. *J. Viral Hepat.* 20, 517-523, 2013.
[9] Núñez-Torres R., Macías J., Mancebo M., et al. The PNPLA3 genetic variant rs738409 influences the progression to cirrhosis in HIV/Hepatitis C Virus Coinfected Patients. *PLoS One* 11, e0168265, 2016.
[10] Manchiero C., Nunes A.K.D.S., Magri M.C., et al. The rs738409 polymorphism of the PNPLA3 gene is associated with hepatic steatosis and fibrosis in Brazilian patients with chronic hepatitis C. *BMC Infect Dis.* 17, 780, 2017.
[11] Jiménez-Sousa M.Á., Gómez-Moreno A.Z., Pineda-Tenor D., et al. PNPLA3 rs738409 polymorphism is associated with liver fibrosis progression in patients with chronic hepatitis C: A repeated measures study. *J. Clin. Virol*. 103, 71-74, 2018.
[12] He S., McPhaul C., Li J.Z., et al. A Sequence Variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J. Biol. Chem.* 285, 6706-6715, 2010.
[13] Mirandola S., Bowman D., Hussain M.M., Alberti A. Hepatic steatosis in hepatitis C is a storage disease due to HCV interaction with microsomal triglyceride transfer protein (MTP). *Nutr. Metab.* (Lond) 7, 13, 2010.