



Liver, Pancreas and Biliary Tract

## PNPLA3 rs738409 and TM6SF2 rs58542926 variants increase the risk of hepatocellular carcinoma in alcoholic cirrhosis



Edmondo Falletti, Annarosa Cussigh, Sara Cmet, Carlo Fabris, Pierluigi Toniutto\*

Department of Medicine and Pathology Clinical and Experimental, Medical Liver Transplantation Unit, Internal Medicine, University of Udine, Italy

## ARTICLE INFO

## Article history:

Received 29 April 2015

Accepted 19 September 2015

Available online 28 September 2015

## Keywords:

Genetic polymorphisms  
Hepatocellular carcinoma  
Lipids  
Steatosis

## ABSTRACT

**Background:** *PNPLA3* rs738409 polymorphism is associated with fatty liver disease, alcoholic or non-alcoholic (NAFLD) and hepatocellular carcinoma (HCC). *TM6SF2* rs58542926 is clearly associated with NAFLD, but it is not clearly associated with HCC. The relationship between *TM6SF2* rs58542926 and HCC and the potential synergistic effect of *TM6SF2* and *PNPLA3* variants in modifying the risk of HCC are not known.

**Aim:** This study assessed the interaction between *PNPLA3* rs738409 and *TM6SF2* rs58542926 variants in the conditioning of HCC development.

**Methods:** A total of 511 cirrhotic patients (44% alcohol-related, 56% viral, 57.5% liver transplanted) were retrospectively investigated for HCC occurrence. *PNPLA3* rs738409 and *TM6SF2* rs58542926 were genotyped using restriction fragment length polymorphism and real-time allelic discrimination polymerase chain reaction methods.

**Results:** Patients with HCC were more likely to be *PNPLA3* rs738409 G/G homozygotes (41/150 vs. 60/361,  $p=0.009$ ) or *TM6SF2* rs58542926 C/T-T/T (27/150 vs. 41/361,  $p=0.044$ ). The presence of either *PNPLA3* G/G or *TM6SF2* \*T identified high-risk genotypes for HCC, which were strongly associated with HCC (64/150 vs. 93/361,  $p=0.0002$ ). This association was evident in alcohol-related ( $p=0.0007$ ) but not in viral cirrhosis.

**Conclusion:** *TM6SF2* C/T or T/T in conjunction with *PNPLA3* G/G variants may be potential genetic risk factors for developing HCC in alcohol-related cirrhosis.

© 2015 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third cause of cancer-related mortality worldwide [1]. The majority of HCCs develop in patients with liver cirrhosis in Western countries with an incidence ranging from 1 to 8% annually. The aetiology of liver cirrhosis complicated with the occurrence of HCC is predominantly related to chronic viral infection (hepatitis B or hepatitis C) or alcohol consumption [2]. A growing incidence of HCC was reported in patients with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steato-hepatitis (NASH) [3]. Male gender and older age are also the main demographic independent predictors of HCC development in cirrhosis [4]. Inherited factors

were implicated in the pathogenesis of HCC, and these factors contribute to the greater inter-individual variability in disease susceptibility [5].

Genome-wide association studies failed to identify a single genetic pattern that accounts for hepato-carcinogenesis in cirrhosis, but several reports demonstrated a strong association between the presence of 148 methionine variants of patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) and the occurrence of HCC, particularly in patients with alcoholic or NASH-related liver cirrhosis [6–8]. *PNPLA3* encodes a protein that is distributed between liver lipid droplets and membranes, and it exhibits a predominantly hydrolase activity [9,10]. The effect of the 148 methionine (rs738409 G) variant on loss or gain of function remains controversial despite numerous studies on *PNPLA3* enzymatic activity. The link between *PNPLA3* variants and liver lipid-related diseases is strong, and this link is no longer questionable. However, a simple mechanism that involves the increase in fatty acid formation and/or impairment of fatty acid depletion is not known. The *PNPLA3* 148Met variant favours hepatocellular fat accumulation

\* Corresponding author at: Department of Medical Sciences Experimental and Clinical, Internal Medicine, Medical Liver Transplantation Section, University of Udine, Italy. Tel.: +39 0432559801; fax: +39 0432559490.

E-mail address: [pierluigi.toniutto@uniud.it](mailto:pierluigi.toniutto@uniud.it) (P. Toniutto).

in the presence of triggering factors, such as obesity, alcohol consumption and insulin resistance, which leads to liver fibrosis progression and HCC development. A direct carcinogenic effect of the *PNPLA3* 148Met variant was proposed [11], but the vast majority of the available data suggest that this variant enhances HCC susceptibility in patients with established liver cirrhosis [6].

The transmembrane 6 superfamily 2 (*TM6SF2*) is the putative functional gene in the 19p12 locus that is responsible for lipid metabolism in the liver [12–14]. *TM6SF2* encodes a protein of 351 amino acids with 7–10 predicted transmembrane domains, and it is predominantly expressed in the liver and intestine. *TM6SF2* siRNA inhibition is associated with a reduced secretion of triglyceride-rich lipoproteins and increased cellular triglyceride concentrations and lipid droplet content, and *TM6SF2* overexpression is associated with reduced liver cell steatosis. The genetic variant encoding Glu167Lys (rs58542926 C>T) is associated with decreased protein expression, which contributes to the enhancement of liver triglyceride content and NAFLD development [14].

These data suggest that both genetic variants strongly influence lipid metabolism in the liver and favour the development of steatosis. This aspect highlights recent observations of the strict relationship between lipid accumulation in the liver and the risk of fibrosis progression and cancer development. Therefore, a synergistic or complementary effect of *PNPLA3* and *TM6SF2* genetic variants in modifying the risk of HCC development in patients with liver cirrhosis may be hypothesized. This effect may also occur in patients in whom steatosis progresses to cirrhosis, which originated from alcohol-related liver damage. This hypothesis has not been carefully investigated.

This study assessed the role of *PNPLA3* rs738409 and *TM6SF2* rs58542926 genetic variants in the conditioning of HCC development in patients with alcoholic- compared to viral-related liver cirrhosis.

## 2. Materials and methods

### 2.1. Patients

The study included 511 Italian patients of Caucasian ethnicity who were diagnosed with cirrhosis. Most patients ( $N=294$ , 57.5%) received transplantation for end-stage liver disease. Histological evaluation of the explanted liver confirmed the diagnoses of liver cirrhosis and HCC in this first group. Cirrhosis was diagnosed clinically in the remaining patients ( $N=217$ , 42.5%), who were not referred for transplantation, based on signs of portal hypertension, pertinent imaging features and laboratory findings. HCC in this second group was diagnosed using dynamic imaging studies, in accordance with current practice guidelines, and a liver biopsy was performed in select cases when imaging studies were not conclusive. Table 1 reports the main demographic and clinical characteristics of patient population.

A total of 228 healthy Italian blood donors of Caucasian ethnicity, who were age- and sex-matched with patients, served as controls. This group included 165 males (72.4%) and 63 females (27.6%) with a median age of 55 (interquartile range 51–59) years. Control subjects did not have any clinical and/or laboratory evidence of liver disease or other major pathological conditions, such as diabetes mellitus. Informed consent to participate in the study was obtained from each subject, in accordance with the declaration of Helsinki and following local ethical committee indications. All study participants approved the storage of their frozen DNA specimens for research purposes in our laboratory.

**Table 1**

Primary demographic and clinical characteristics of the studied population ( $N=511$ ). Continuous variables are reported as medians (interquartile range), and categorical variables are reported as frequencies (%).

	N=511
Male gender, N	365 (71.4%)
Age (years)	57.0 (50.0–62.9)
Body mass index (kg/m <sup>2</sup> )	25.0 (22.8–27.2)
Presence of diabetes mellitus, N	129 (25.2%)
Aetiology of liver disease, N	
Alcohol	226 (44.2%)
HCV	209 (40.9%)
HBV	76 (14.9%)
Presence of HCC, N	150 (29.3%)
Child–Pugh score	7 (5–9)
<i>PNPLA3</i> rs738409	C/C = 176–C/G = 234–G/G = 101
<i>TM6SF2</i> rs58542926	C/C = 443–C/T = 66–T/T = 2

HCV: hepatitis C virus; HBV: hepatitis B virus; HCC: hepatocellular carcinoma.

### 2.2. Histology

All hepatectomy specimens were sectioned at intervals of approximately 1 cm in the search for suspicious focal hepatic lesions. Standard histological staining techniques were used to confirm the presence of HCC.

### 2.3. Genotyping

The *PNPLA3* non-synonymous (Ile148Met) single nucleotide polymorphism, rs734809 C>G localized in exon 3 of the gene encoding *PNPLA3*-adiponutrin, was genotyped as previously described [15]. Exonic *TM6SF2* rs58542926 C>T (Glu167Lys) genotyping was performed using a real-time allelic discrimination PCR method with locked nucleic acids (LNA) probes. The forward primer was (f) 5'-AGGGGATGGTGAGGAAGAAG-3', and the reverse was (r) 5'-ACAGATGTCAGCAGGGTTC-3'. Dual-labelled LNA hybridization probes were 5'-Fam-GATCTCGGAGCTGT-BH1-3' for the C allele and 5'-Hex-CTGATCTTGGAGCTGT-BH1-3' for the T allele (underlined nucleotides denote LNA bases). Real-time PCR was performed using a BIO-RAD IQ5 Real-Time PCR System (Bio-Rad Laboratories, Milan, Italy). The thermal profile was 35 cycles of 95 °C for 10 s (denaturation) and 64 °C for 60 s for annealing, elongation and reading. The genomic region encompassing the *TM6SF2* rs58542926 C>T (Glu167Lys) polymorphism was sequenced in 50 patients, and these results confirmed the results of the real-time LNA assay. The calling rate for the two polymorphisms was 100% of samples tested.

### 2.4. Statistical analysis

Statistical analyses of data were performed using the BMDP dynamic statistical software package 7.0 (Statistical Solutions, Cork, Ireland) and Stata 11.1 for the generalized linear model (StataCorp LP, USA). Continuous variables are presented as medians (interquartile range), and categorical variables are expressed as frequencies (%). The chi-square G test ("Goodness of Fit") was used to verify whether the genotype frequencies of the *PNPLA3* rs738409 C>G and *TM6SF2* rs58542926 C>T polymorphisms were distributed in controls and patients in accordance with the Hardy–Weinberg equation. The existence of differences in allele and genotype frequencies between different groups was assessed using the Pearson chi-square test (chi-square test for linear trend when appropriate), and the odds ratios were calculated with the 95% confidence intervals. The interactions between polymorphic loci were examined with the aid of PLINK and BOOST epistasis software routines. Multiple locus analysis was performed using the PLINK (ver. 1.03) multiple locus tool and with the aid of Hapstat software. A generalized linear model was used to select the better genetic model.

Download English Version:

<https://daneshyari.com/en/article/3261244>

Download Persian Version:

<https://daneshyari.com/article/3261244>

[Daneshyari.com](https://daneshyari.com)