European Journal of Internal Medicine 25 (2014) 566–570

Contents lists available at ScienceDirect

European Journal of Internal Medicine

journal homepage: www.elsevier.com/locate/ejim

Original Article

Non-alcoholic fatty liver disease, metabolic syndrome and patatin-like phospholipase domain-containing protein3 gene variants

M. Del Ben [a], L. Polimeni [a], M. Brancorsini [a], A. Di Costanzo [a], L. D'Erasmo [a], F. Baratta [a], L. Loffredo [a], D. Pastori [a], P. Pignatelli [a], F. Violi [a], M. Arca [a], F. Angelico [b,*]

[a] Department of Internal Medicine and Medical Specialties, Sapienza University, Rome, Italy
[b] Department of Public Health and Infectious Disease, Sapienza University, Rome, Italy

ARTICLE INFO

Article history:
Received 30 March 2014
Received in revised form 10 May 2014
Accepted 23 May 2014

Keywords:
Non-alcoholic fatty liver disease
Metabolic syndrome
PNPLA3

ABSTRACT

Background & aims: Non-alcoholic fatty liver disease was traditionally interpreted as a condition which may progress to liver-related complications. However, the increased mortality is primarily a result of cardiovascular diseases. It has been suggested that fatty liver can be considered as the hepatic consequence of the metabolic syndrome. The aim was to describe the different clinical presentations of non-alcoholic fatty liver disease on the basis of the patatin-like phospholipase domain-containing protein3 (PNPLA3) rs738409 gene variant.
Methods: Fatty liver was defined by ultrasonographic Hamaguchi's criteria in 211 consecutive subjects with non-alcoholic fatty liver disease. The rs738409 polymorphism was determined by TaqMan assays. Metabolic syndrome was defined according to ATPIII modified criteria.
Results: Prevalence of PNPLA3-148II, PNPLA3-148IM, and PNPLA3-148MM genotypes was 45.0%, 40.7%, and 14.3% respectively. Prevalence of metabolic syndrome progressively increased with the severity of liver steatosis (from 52.5% to 65.2%, and 82.3% respectively, $p < 0.01$). The PNPLA3-148MM group had significantly lower mean serum triglycerides ($p < 0.001$), Framingham cardiovascular risk score ($p < 0.01$) and lower prevalence of metabolic syndrome ($p < 0.05$) and its components. Age and HOMA-IR were positive independent predictors of metabolic syndrome, while a negative independent association was found between metabolic syndrome and the homozygotes PNPLA3 I148M variant.
Conclusions: We suggest a lower prevalence of MetS and reduced cardiovascular risk in NAFLD patients with PNPLA3MM genotype.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common and emerging liver disease in Western countries [1,2]. Fatty liver includes a wide spectrum of histologic alterations ranging from simple steatosis, to non-alcoholic steatohepatitis (NASH), which is characterized by inflammation and fibrosis. Moreover, NAFLD has been traditionally interpreted as a condition, which may eventually progress to liver related complications such as cirrhosis, liver cancer and liver mortality [3–5].

However, the pathogenesis of NAFLD is multifactorial and many mechanisms that cause fatty liver infiltration, inflammation, oxidative stress and fibrosis have been proposed.

The association of liver steatosis with a number of common metabolic conditions and cardiovascular risk factors has been extensively reported. Notably, increased mortality of NAFLD patients is primarily a result of cardiovascular diseases and, to a lesser extent, to liver-related diseases [6–12]. It has been suggested that fatty liver can be considered as the hepatic consequence of the metabolic syndrome (MetS), a cluster of metabolic disorders including central obesity, hyperglycemia, arterial hypertension, hypertriglyceridemia and low HDL-cholesterol. Thus, a strong bidirectional association between NAFLD and MetS has been proposed. Insulin resistance, the key feature of MetS, is considered to play a central role in the first stages of fatty liver infiltration [13–15]. However, whether insulin resistance and hyperinsulinemia are components of MetS promoting fatty liver or whether NAFLD itself induces chronic hyperinsulinemia by impaired insulin degradation is still under debate. In addition, not all subjects with MetS will develop NAFLD and not all subjects with NAFLD will develop MetS.

Several lines of evidence clearly indicated that also genetic factors may predispose to NAFLD and among the others a variant (I148M) located at the patatin-like phospholipase domain-containing protein 3

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; PNPLA3, patatin-like phospholipase domain-containing protein3; ALT, alanine aminotransferase; HOMA-IR, homeostasis model of insulin resistance; MetS, metabolic syndrome; US, ultrasonographic scanning; BMI, body mass index; APN, adiponectin.

* Corresponding author at: I Clinica Medica, Policlinico Umberto I, Viale del Policlinico 155, 00161 Rome, Italy. Tel./fax: +39 9649972249.
E-mail address: francesco.angelico@uniroma1.it (F. Angelico).

http://dx.doi.org/10.1016/j.ejim.2014.05.012
0953-6205/

(PNPLA3) gene appears to show the strongest effect. Based on the presence of I148M genotype, two different clinical presentations of NAFLD have been recently proposed [16–23]. The first one is associated to the presence of common I148I allele and characterized by a high prevalence of obesity and possibly high cardiovascular risk, whereas the other one is associated to the I148M allele presenting an higher susceptibility to more severe liver histology and liver disease progression.

The aim of the study was to evaluate whether PNPLA3 gene variants may predict different clinical phenotypes in a large series of Italian patients with NAFLD.

2. Materials and methods

2.1. Study patients

The study was performed in 211 consecutive patients referred to our metabolic outpatient clinic who had evidence of fatty liver disease at a liver ultrasonographic scanning (US) performed as part of routine clinical examination.

To be eligible for the study, patients had to have fulfilled the following inclusion criteria: no history of current or past excessive alcohol drinking as defined by an average daily consumption of alcohol >20 g; negative tests for the presence of hepatitis B surface antigen and antibody to hepatitis C virus; absence of history and clinical, biochemical and US findings consistent with cirrhosis and other chronic liver diseases. None of the subjects was taking amiodarone and other drugs known to promote fatty liver disease. Subjects underwent routine clinical and biochemical evaluation. Waist circumference, height and weight were recorded and body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Blood pressure was recorded following standard procedures.

Diabetes was diagnosed according to the WHO criteria [24]. Subjects taking insulin or oral antidiabetic drugs were considered to have diabetes. According to the modified criteria of the ATP III Expert Panel of the US National Cholesterol Education Program [25], metabolic syndrome was diagnosed on the concomitant presence of at least three of the following five clinical features: waist circumference (central obesity) >102 cm in men and >88 cm in women, fasting blood glucose ≥100 mg/dl, triglycerides ≥150 mg/dl, HDL-cholesterol <40 mg/dl in men and <50 mg/dl in women, and arterial systolic/diastolic blood pressure ≥130/≥85 mm Hg. A metabolic score was calculated for each patient based on the number of the discrete components of MS identified. Cardiovascular risk was expressed as Framingham score determined by using the National Cholesterol Education Program (NCEP) calculator, available on-line at http://hp2010.nhlbihin.net/atpiii/calculator.asp.

Written informed consent was obtained from all patients before the study. The study was approved by the hospital Ethics Committee and conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.2. Laboratory measurements

Serum total cholesterol, HDL-cholesterol and triglycerides were measured by an Olympus AN 560 apparatus using an enzymatic colorimetric method. LDL-cholesterol levels were calculated according to the Friedewald formula. Plasma insulin levels were assayed by commercially available radioimmunoassay. The homeostasis model of insulin resistance (HOMA-IR), based on serum fasting glucose and insulin levels, was used as a measure of IR [26].

Adiponectin (APN) serum levels were measured with a commercial immunoassay (Tema Ricerca, Italy). Intra-assay and inter-assay coefficients of variation were 6 and 8%, respectively. Serum levels of cytokeratin 18-M30 were measured as marker of liver damage with a commercial immunoassay (Tema Ricerca, Italy) and expressed as mIU/ml. Intra-assay and inter-assay coefficients were 6% and 7% respectively.

2.3. Ultrasonographic examination

Liver US was performed to assess the degree of steatosis. All US were performed by the same operator who was blinded to laboratory values using a GE Vivid S6 apparatus equipped with a convex 3.5 MHz probe. Liver steatosis was defined according to Hamaguchi criteria based on the presence of abnormally intense, high level echoes arising from the hepatic parenchyma, liver–kidney difference in echo amplitude, echo penetration into deep portion of the liver and clarity of liver blood vessel structure [27,28]. Steatosis was assessed semi-quantitatively on a scale of 0–6: 0, absent; 1–2, mild; 3–4, moderate; 5–6, severe.

2.4. Genetic analysis

DNA was extracted from peripheral blood and purified by the Wizard® Genomic DNA Purification Kit following the manufacturing protocol. Fluorogenic 5′-nucleotidase assays were developed to genotype the *PNPLA3* rs738409 C to G non-synonymous sequence variant, encoding I148M, in all subjects. The assay was performed using the TaqMan C7241_10 assay (Applied Biosystems, Foster City, CA) on ABI PRISM 7900 HT Sequence Detection Systems (Applied Biosystems). The plate was run using standard condition at 95 °C for 10 min, 95 °C for 15 s then 60 °C for 1 min for 40 cycles. Allele frequencies were in Hardy–Weinberg equilibrium. The TaqMan assay was validated by direct sequencing of the SNP (rs738409) in representative samples of DNA on ABI PRISM 3130 XL Genetic Analyzer, and both methods gave identical results.

2.5. Statistical analysis

Statistical analysis was performed using the SPSS statistical software version 18.0 for Windows (SPSS, Inc., Chicago. Illinois). Student's t-test for unpaired data was used for the comparison of mean values. Distribution of continuous variables was tested for normality using the Kolmogorov–Smirnov test. Data are expressed as the mean ± SD for normally distributed variables and as median and interquartile range for non-normally distributed data. Group comparisons were performed by use of analysis of variance (ANOVA) and unpaired Student's t-test when appropriate. Non-normally distributed variables were tested by Mann–Whitney test and Kruskall–Wallis test. Proportions and categorical variables were tested by the χ^2-test and by the 2-tailed Fisher's exact method when appropriate. All tests were two-tailed; a p value of less than 0.05 was considered to indicate statistically significance. Multivariate analyses were performed using the stepwise logistic regression analysis testing for the dichotomous response variable presence or absence of MetS after controlling for possible clinical and biochemical confounders. The predictor variables entered in the different models were age, gender, PNPLA3-148MM, Hamaguchi score, HOMA-IR, serum lipid levels, serum ALT, adiponectin and cytokeratin-18 values, and positive family history for diabetes and cardiovascular disease.

3. Results

Mean age was 54.5 + 11.9 years. Among the 211 subjects with fatty liver, US examination revealed mild steatosis in 40, moderate steatosis in 90 and severe steatosis in 81. Clinical and laboratory characteristics of subjects (mean age 54.5 ± 11.9 years) with different severity of fatty liver according to Hamaguchi's classification of steatosis are reported in Table 1. A progressive, statistically significant increase in the mean values of the indexes of central obesity (BMI, waist and hip circumferences), glucose metabolism (fasting blood glucose, insulin, HOMA-IR and glycosylated hemoglobin) serum alanine aminotransferase (ALT) and cytokeratin-18 was observed from the group with mild steatosis to the groups with moderate and severe steatosis; conversely, an opposite trend was observed for serum HDL cholesterol and adiponectin levels.