



Patatin-like phospholipase domain containing-3 gene I148M polymorphism, steatosis, and liver damage in hereditary hemochromatosis

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Abstract

AIM: To investigate whether the *patatin-like phospholipase domain containing-3* gene (*PNPLA3*) I148M polymorphism is associated with steatosis, fibrosis stage, and cirrhosis in hereditary hemochromatosis (HH).

METHODS: We studied 174 consecutive unrelated homozygous for the C282Y HFE mutation of HH (C282Y+/+ HH) patients from Northern Italy, for whom the presence of cirrhosis could be determined based on

histological or clinical criteria, without excessive alcohol intake (< 30/20 g/d in males or females) or hepatitis B virus and hepatitis C virus viral hepatitis. Steatosis was evaluated in 123 patients by histology ($n = 100$) or ultrasound ($n = 23$). The *PNPLA3* rs738409 single nucleotide polymorphism, encoding for the p.148M protein variant, was genotyped by a Taqman assay (assay on demand, Applied Biosystems). The association of the *PNPLA3* I148M protein variant (p.I148M) with steatosis, fibrosis stage, and cirrhosis was evaluated by logistic regression analysis.

RESULTS: *PNPLA3* genotype was not associated with metabolic parameters, including body mass index (BMI), the presence of diabetes, and lipid levels, but the presence of the p.148M variant at risk was independently associated with steatosis [odds ratio (OR) 1.84 per p.148M allele, 95% confidence interval (CI): 1.05-3.31; $P = 0.037$], independently of BMI and alanine aminotransferase (ALT) levels. The p.148M variant was also associated with higher aspartate aminotransferase ($P = 0.0014$) and ALT levels ($P = 0.017$) at diagnosis, independently of BMI and the severity of iron overload. In patients with liver biopsy, the 148M variant was independently associated with the severity (stage) of fibrosis (estimated coefficient 0.56 ± 0.27 , $P = 0.041$). In the overall series of patients, the p.148M variant was associated with cirrhosis in lean ($P = 0.049$), but not in overweight patients ($P =$ not significant). At logistic regression analysis, cirrhosis was associated with BMI ≥ 25 (OR 1.82, 95% CI: 1.02-3.55), ferritin > 1000 ng/mL at diagnosis (OR 19.3, 95% CI: 5.3-125), and with the G allele in patients with BMI < 25 (OR 3.26, 95% CI: 1.3-10.3).

CONCLUSION: The *PNPLA3* I148M polymorphism may represent a permissive factor for fibrosis progression in patients with C282Y+/+ HH.

Key words: Fatty liver; Fibrosis; Hemochromatosis; HFE protein; Iron overload; *Patatin-like phospholipase domain containing-3* gene; Steatosis

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INTRODUCTION

Hereditary hemochromatosis (HH) is a genetic disorder of iron metabolism characterized by defective release or activity of hepcidin, the hepatic hormone that inhibits iron absorption by binding and inactivating ferroportin^[1]. HH is most frequently related to hampered hepcidin up-regulation by iron stores as a consequence of homozygosity for the C282Y mutation in the *HFE* gene^[2]. The resultant increase in serum iron leads to progressive accumulation in the liver and other parenchymal organs, however, although hepatic iron overload leads to progressive liver fibrosis and cirrhosis in some affected individuals, the phenotypic expression is unpredictable and highly variable^[3].

Indeed, liver disease is the most frequent clinical manifestation of homozygous for the C282Y *HFE* mutation of HH (C282Y+/+ HH), but it is now clear that only a proportion of subjects carrying this genotype will ever develop hepatic fibrosis^[4]. Most of C282Y +/+ male subjects develop expanded iron stores during life, whereas due to the physiological iron losses during fertile age, the female gender represents a major protective factor. In population based screening studies it has been shown that between 75% and 94% of C282Y+/+ males develop elevated transferrin saturation, and that 64% to 68% will have an increased serum ferritin^[4-8]. However, even in males, the prediction of risk of clinical disease remains uncertain^[9].

The recognition of the incomplete penetrance of HH has led to a search for genetic and other modifiers of clinical expression. HH expression may be influenced at different levels^[9]: (1) by factors affecting iron loading, including sex and genetic factors (genes regulating hepcidin expression, beta-thalassemia trait^[10]); (2) by factors influencing the progression to liver disease, such as hepatic steatosis^[11], viral hepatitis, genes regulating pro-inflammatory cytokines and oxidative injury^[12,13]; and (3) by those regulating both, such as alcohol intake and

hepatitis C virus (HCV) infection^[14,15].

There is established evidence that increased body mass (BMI) and the metabolic syndrome^[16] are strong risk factors for hepatic steatosis^[17] and that steatosis accelerates the progression of liver diseases by favoring oxidative stress and hepatocellular damage. In 214 C282Y +/+ patients^[11], a significant association between steatosis and the presence of fibrosis was detected. This relationship remained significant after adjustment for confounding factors such as alcohol intake and iron loading.

Recently, the rs738409 C > G single nucleotide polymorphism (SNP) of *patatin-like phospholipase domain containing-3* gene (*PNPLA3*), encoding for the I148M protein variant (p.I148M), has been identified as a determinant of liver fat content and of the susceptibility to develop steatohepatitis and progressive fibrosis^[18-23]. Importantly, *PNPLA3* genotype influences liver fat independently of body mass, dyslipidemia, and insulin resistance^[24,25].

Since hepatic steatosis has been reported to influence HH expression, the aim of this study was to determine whether the *PNPLA3* I148M variant predisposes to the development of steatosis, and to progressive liver damage, as evaluated fibrosis stage and the presence of cirrhosis, in patients with pure C282Y+/+ HH stratified according to the presence of overweight.

MATERIALS AND METHODS

Patients

From 232 consecutive unrelated C282Y+/+ HH patients referred to two centers in the Milan area of Northern Italy, we excluded subjects with alcohol intake > 30/20 g per day in male/female, hepatitis B virus (HBV) and/or HCV infections, and other cofactors of liver disease ($n = 30$), and those with an uncertain diagnosis of cirrhosis or incomplete clinical data ($n = 28$), and finally included 174 patients in the analysis (Figure 1). DNA samples were available for all patients.

Diagnosis of cirrhosis was based upon liver histology ($n = 100$) or clinical evidence ($n = 74$): in particular, cirrhosis was diagnosed by liver histology in 26 patients, and by clinical criteria in 6 cases (in the presence of hepatic decompensation or of portal hypertension; liver biopsy was not indicated for ethical reasons), whereas it was excluded by liver histology in 74 cases, and by clinical criteria in the remaining 68 cases (when liver biopsy was not indicated and not performed for ethical reasons).

Tissue sections were stained with hematoxylin and eosin, impregnated with silver for reticulin framework, and stained with trichrome for collagen and Perls for iron. Steatosis was considered present when involving at least 5% of hepatocytes and graded according to Kleiner^[26]. Tissue iron was graded according to Scheuer^[27]. Fibrosis was scored according to Ishak^[28]. The minimum biopsy size was 1.7 cm and the number of portal areas was 10. For data analysis, a fibrosis stage of 6 was attributed to patients with a clinical diagnosis of cirrhosis.

Ultrasonographic diagnosis of steatosis at diagnosis

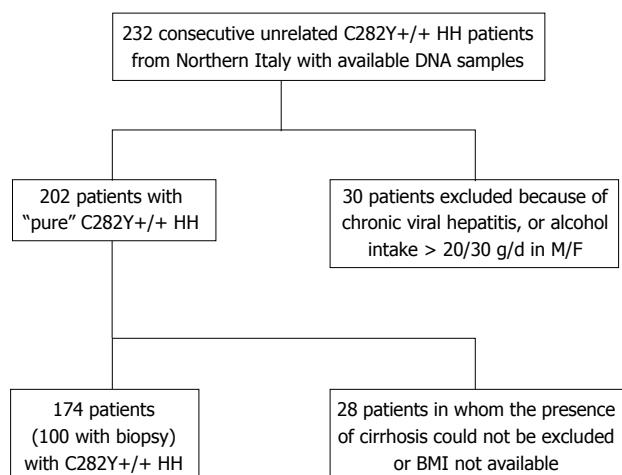


Figure 1 Study flow chart. C282Y+/+ HH: Homozygous for the C282Y HFE mutation of hereditary hemochromatosis; M/F: Male/female; BMI: Body mass index.

by an experienced operator (available in 123) was based on evident ultrasonographic contrast between the hepatic and right renal parenchyma of the right intercostal sonogram in the midaxillary line, or abnormally intense, high-level echoes arising from the hepatic parenchyma, and was graded on a three-grade scale as none, mild, or severe in accordance with intensity^[29].

Cirrhosis was considered clinically absent only if all these conditions were satisfied: (1) age < 40 years; (2) alanine aminotransferase (ALT) within normal levels; and (3) ferritin < 1000 ng/mL. These criteria have been shown to rule out not only cirrhosis, but also advanced fibrosis with high specificity in patients with C282Y+/+ HH without viral hepatitis and excessive alcohol intake^[30].

Overweight was considered present when BMI > 25 kg/m². For each patient we collected data on sex, age, geographical origins, BMI, alcohol consumption, aspartate aminotransferase (AST), ALT and γ -glutamyl transferase (GGT) levels, ferritin, transferrin saturation percentage, total cholesterol, high density lipoprotein cholesterol and triglycerides levels, glucose, and type 2 diabetes^[31]. Clinical features of the patients included are shown in Table 1. Informed written consent was obtained from each patient included. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Institutional Review Board of the Institutions involved.

Genetic analysis

DNA was extracted from peripheral blood by the phenol-chloroform method. Success rate in extracting DNA was 100% for each study group. The PNPLA3 rs738409 SNP was genotyped by a Taqman assay (assay on demand for rs738409, Applied Biosystems, Foster City, CA, United States) by personnel unaware of patients and controls clinical status. Post-polymerase chain reaction allelic discrimination was carried out measuring allele-specific fluorescence on the Opticon2 detection system (MJ Research, Waltham, MA, United States). Random samples

Table 1 Demographic, anthropometric, clinical, and histological features, as evaluated at diagnosis, of 174 Italian patients with homozygous for the C282Y HFE mutation of hereditary hemochromatosis subdivided according to the PNPLA3 I148M genotype

	All patients	PNPLA3 I148M genotype			P value
		I/I	I/M	M/M	
n (%)	174	82 (47)	70 (40)	22 (13)	
Age (yr)	47 ± 13	46 ± 13	47 ± 14	50 ± 11	0.22
Gender F (%)	49 (28)	24 (30)	19 (27)	6 (27)	0.79
DAI (g)	10 (0-20)	10 (0-20)	5 (0-20)	10 (0-20)	0.98
BMI (kg/m ²)	24 ± 3	24 ± 3	24 ± 3	25 ± 2	0.11
Diabetes (%)	16 (9)	7 (9)	6 (9)	3 (14)	0.57
Total cholesterol (mg/dL)	191 ± 44	197 ± 37	186 ± 50	182 ± 44	0.25
HDL cholesterol (mg/dL)	53 ± 16	53 ± 14	53 ± 17	57 ± 15	0.47
Triglycerides (mg/dL)	120 ± 66	123 ± 64	122 ± 74	99 ± 35	0.30
TS %	80 ± 16	80 ± 16	79 ± 15	81 ± 23	0.94
Ferritin (ng/mL)	1000 (509-1800)	1018 (490-1813)	947 (516-1732)	1053 (504-2101)	0.36
AST (IU/mL)	34 ± 21	31 ± 16	35 ± 24	44 ± 29	0.019
ALT (IU/mL)	46 ± 32	41 ± 26	47 ± 34	55 ± 45	0.06
GGT (IU/mL)	24 (16-37)	24 (17-37)	24 (15-38)	23 (14-30)	0.95
Steatosis ¹ (%)	55 (48)	18 (31)	29 (58)	8 (53)	0.014
Advanced fibrosis % (Ishak 4) %	18 (10)	9 (11)	7 (10)	2 (9)	0.95
Cirrhosis (Ishak 5-6) %	32 (18)	13 (16)	14 (20)	5 (23)	0.39

¹Available in 123 patients. I: Isoleucine; M: Methionine; n: Number; F: Female; DAI: Daily alcohol intake; BMI: Body mass index; HDL: High density lipoprotein cholesterol; TS: Transferrin saturation; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ -glutamyl transferase.

were confirmed by direct genotyping which provided concordant results in all cases^[19]. Quality controls were performed to verify the reproducibility of the results. Valid genotypic data were obtained for 100% of subjects analyzed.

Statistical analysis

Values are expressed as mean ± SD or median (interquartile range) according to distribution. Mean values were compared by analysis of variance or Wilcoxon, and frequencies by *F* test and χ^2 test for trend, when appropriate. The study had a > 85% power to detect a two-fold higher risk of cirrhosis in carriers of the 148M allele, but only 33% to detect a 33% increased risk. Independent predictors of AST and ALT levels were analyzed by generalized linear model. The association of the PNPLA3 p.148M variant with fibrosis was evaluated by ordinal logistic regression analysis, and with the presence of steatosis and cirrhosis was evaluated by multivariate logistic regression analysis. *P* values were considered significant when < 0.05 (two-tailed). Analyses were carried out with JMP 6.0 statistical analysis software (SAS Institute Inc., Cary, NC, United States).

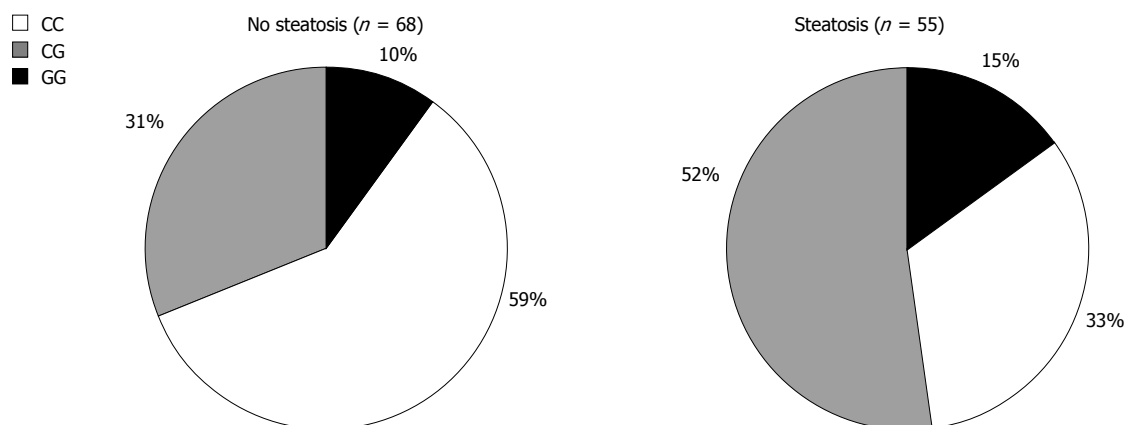


Figure 2 Frequency distribution of the rs738409 C > G single nucleotide polymorphism, encoding for the I148M protein variant, in 123 patients with homozygous for the C282Y HFE mutation of hereditary hemochromatosis subdivided according to the presence of steatosis ($P = 0.015$).

Table 2 Independent predictors of steatosis and cirrhosis in Italian patients with homozygous for the C282Y HFE mutation of hereditary hemochromatosis, as evaluated by logistic regression analysis

	OR	95% CI	P value
Independent predictors of steatosis $n = 123$			
BMI (per kg/m ²)	1.22	1.06-1.42	0.008
ALT (per IU/mL)	1.01	0.99-1.02	0.353
PNPLA3 genotype (per p allele)	1.84	1.05-3.31	0.037
Independent predictors of cirrhosis $n = 174$			
Ferritin (ng/mL)	1.001	1.000-1.002	< 0.0001
Diabetes	0.54	0.26-1.16	0.101
BMI (>/m ²)	1.83	1.02-3.57	0.055
PNPLA3 p allele present (BMI > 25)	0.76	0.37-1.53	0.453
PNPLA3 p allele present (BMI ≤ 25)	3.26	1.28-10.30	0.024

OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; ALT: Alanine aminotransaminase; p.148M: PNPLA3 rs738409 148Met protein variant.

RESULTS

Association of PNPLA3 gene genotype with steatosis

We first sought to confirm the association of the G allele encoding for the p.148M variant with liver fat in C282Y+/+ HH. The frequency distribution of the rs738409 PNPLA3 SNP ($P =$ not significant for Hardy-Weinberg equilibrium testing) in patients subdivided according to the presence of steatosis is shown in Figure 2 ($P = 0.014$). The frequency of the G allele was 0.41 in patients with and 0.26 in those without steatosis ($P = 0.011$), and did not change after the exclusion of patients with ultrasonographic evaluation of the presence of steatosis. Independent predictors of steatosis at logistic regression analysis, considered as independent variables selected by a stepwise mixed regression model, are shown in Table 2. Steatosis was independently associated with BMI ($P = 0.008$) and PNPLA3 genotype [odds ratio (OR) 1.84 per G allele, 95% confidence interval (CI): 1.05-3.31; $P = 0.037$].

Association of PNPLA3 gene genotype with liver enzymes

As expected, PNPLA3 genotype was not significantly asso-

ciated with demographic or anthropometric features, daily alcohol intake, metabolic parameters, including the presence of diabetes, and the severity of iron overload (Table 1). However, we observed an association between PNPLA3 and transaminases, which was significant for AST levels [P for trend (i.e., for increasing levels with increasing number of 148M alleles) = 0.019 for AST and P for trend = 0.06 for ALT], whereas GGT levels were not affected. Independent predictors of AST and ALT levels in the generalized linear model are shown in Table 3; variables included were selected by a stepwise mixed regression model. Both AST and ALT levels were significantly and independently correlated with younger age, higher iron parameters (TS% and ferritin levels), GGT levels, BMI, and the number (0-2) of 148M PNPLA3 alleles ($P = 0.0014$ and $P = 0.017$ for AST and ALT levels, respectively).

Association of PNPLA3 gene genotype with severity of fibrosis and cirrhosis

We next evaluated whether PNPLA3 genotype influences fibrosis stage. At ordinal regression analysis conducted in patients with liver biopsy or clinical diagnosis of cirrhosis ($n = 106$; shown in Table 4), fibrosis stage (0-6) was independently associated with gender, ALT and GGT values, and PNPLA3 p.148M alleles (estimated coefficient of correlation 0.56 ± 0.27 , $P = 0.04$). Possibly due to the relatively low number of patients studied, PNPLA3 genotype was not significantly associated with cirrhosis in the whole cohort (Table 1), although the presence of the 148M allele was nominally significantly associated with cirrhosis in patients with BMI < 25 ($P = 0.05$, $P = 0.1$ after Bonferroni correction; Figure 3). Importantly, positivity for the PNPLA3 148M variant was associated with an increase in the prevalence of steatosis in subjects with BMI < 25, which reached levels similar to those of overweight patients (BMI < 25: 17/37, 46% vs 7/32, 22%, $P = 0.036$; BMI ≥ 25: 22/31, 71% vs 10/26, 38%; $P = 0.017$ for patients positive and negative for the 148M variant, respectively). Independent predictors of cirrhosis are shown in Table 2. At logistic regression analysis, cirrhosis was associated with BMI ≥ 25 (OR 1.82, 95% CI:

Table 3 Independent predictors of aspartate aminotransferase and alanine aminotransaminase levels in 174 Italian patients with homozygous for the C282Y HFE mutation of hereditary hemochromatosis, in the multivariate generalized linear model

	AST			ALT		
	Estimate	95% CI	P value	Estimate	95% CI	P value
Age (yr)	-0.27 ± 0.09	-0.45 - -0.09	0.0034	-0.55 ± 0.14	-0.81 - -0.27	0.0001
TS (%)	0.14 ± 0.07	-0.01 - 0.29	0.063	0.28 ± 0.11	0.05 - 0.50	0.016
Ferritin (ng/mL)	0.01 ± 0.001	0.008 - 0.012	< 0.0001	0.01 ± 0.001	0.009 - 0.014	< 0.0001
GGT (IU/mL)	0.10 ± 0.03	0.03 - 0.16	0.003	0.20 ± 0.05	0.10 - 0.29	< 0.0001
BMI (kg/m ²)	0.86 ± 0.42	0.03 - 1.69	0.042	3.36 ± 0.63	2.12 - 4.60	< 0.0001
PNPLA3 genotype (per p.148M allele)	5.46 ± 1.68	2.15 - 8.67	0.0014	6.05 ± 2.51	1.09 - 11.00	0.017

AST: Aspartate aminotransferase; ALT: Alanine aminotransaminase; CI: Confidence interval; TS: Transferrin saturation; GGT: γ -glutamyl transferase; BMI: Body mass index; p.148M: PNPLA3 rs738409 148Met protein variant.

Table 4 Independent predictors of fibrosis stage in 106 Italian patients with homozygous for the C282Y HFE mutation of hereditary hemochromatosis, at ordinal logistic regression analysis

	Estimate	P value
Age (yr)	-0.03 ± 0.02	0.077
Gender (female)	-0.57 ± 0.26	0.029
BMI (kg/m ²)	-0.08 ± 0.08	0.311
Diabetes	0.57 ± 0.34	0.09
ALT	-0.02 ± 0.008	0.013
GGT (IU/mL)	0.02 ± 0.01	0.035
Ferritin (ng/mL)	0.0001 ± 0.00	0.421
PNPLA3 genotype (per p.148M allele)	0.56 ± 0.27	0.041

BMI: Body mass index; ALT: Alanine aminotransaminase; GGT: γ -glutamyl transferase; p.148M: PNPLA3 rs738409 148Met protein variant.

1.02-3.55), ferritin > 1000 ng/mL at diagnosis (OR 19.3, 95% CI: 5.3-125), and with the G allele in patients with BMI < 25 (OR 3.26, 95% CI: 1.3-10.3).

DISCUSSION

In this study, we evaluated the effect of the PNPLA3 rs738409, encoding for the p.148M variant, on steatosis and liver damage in patients affected by C282Y+/+ HH without other causes of liver damage. Our results confirm the association of the PNPLA3 148M allele with the presence of steatosis and liver enzymes, independently of iron overload, which represents the major cause of progressive liver damage in HH patients. Furthermore, PNPLA3 rs738409 was also associated with fibrosis stage, and with the presence of cirrhosis, albeit only in the presence of normal BMI.

Although data on steatosis were not available in the whole series of patients evaluated, the association of PNPLA3 with steatosis in HH was expected based on data obtained in the general population, in patients with non-alcoholic fatty liver disease (NAFLD), and in other liver diseases. In addition, the magnitude of the observed association was in line with previous reports^[18,19,32]. Due to the retrospective design of the study, insulin resistance evaluation was not available for all patients, however, previous studies have excluded a major effect of PNPLA3

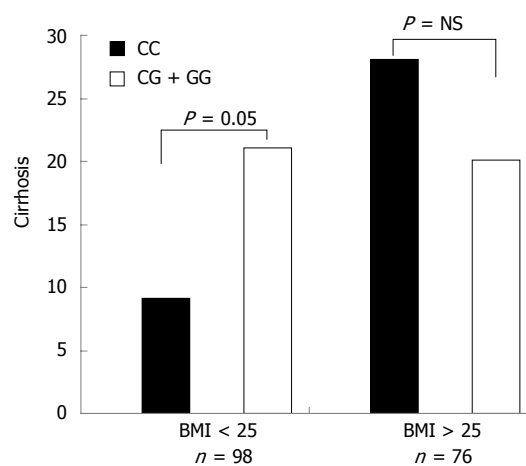


Figure 3 Effect of the rs738409 G allele, encoding for the 148M PNPLA3 variant, on liver cirrhosis in 174 patients with HH subdivided according to the presence of overweight. NS: Not significant; BMI: Body mass index.

genotype on insulin resistance, and the 148M variant was not associated with diabetes in this study.

As steatosis has been reported to influence fibrosis progression in C282Y+/+ patients, independently of alcohol intake and iron loading^[11], the main aim of the present study was to evaluate whether PNPLA3 genotype influences liver damage progression in HH. We found that the PNPLA3 148M allele was a strong predictor of transaminase levels, and in particular of AST levels, which are generally more strongly linked with chronic liver damage (fibrosis stage) than ALT^[11,33]. PNPLA3 polymorphism has been reported to represent a major determinant of transaminase levels in the general population, in patients with NAFLD, and in obese subjects at risk of steatosis^[18,19,34,35]. Our findings indicate, that this is also true for patients with C282Y+/+ HH. It is likely that this association reflects the predisposing effect of the 148M PNPLA3 allele on steatosis. Interestingly, BMI, which was the other determinant of steatosis in our series of patients, was also associated with transaminases. Furthermore, we demonstrated that PNPLA3 genotype was associated with fibrosis stage in patients with HH, consistent with the hypothesis that the 148M allele of PNPLA3 influences the progression of liver damage in

HH, even if this has to be confirmed in a larger series of patients.

The association of the 148M variant with the risk of cirrhosis, a turning point in the natural history of patients with HH due to the frequent progression to hepatocellular carcinoma^[36,37], is also in line with this hypothesis. Interestingly, data obtained by our and other groups indicate that the 148M variant is significantly associated with cirrhosis and hepatocellular carcinoma in patients with HCV-related chronic hepatitis, thus suggesting that this can be true also for liver diseases of different etiology^[32,38].

Based on the previously reported interaction between the effect of the PNPLA3 I148M mutation and body mass^[35], the other major determinant of steatosis in our series of patients, we analyzed the effect of PNPLA3 rs738409 on cirrhosis risk in patients stratified according to the presence of overweight. We found an association between PNPLA3 genotype and cirrhosis, but this was restricted to subjects with normal BMI. There are several possible explanations for this finding. The first is that the result is due to chance. Because of the relatively limited number of subjects included, the study power to detect an association with cirrhosis was relatively low, i.e., > 85% to detect a two-fold higher risk of cirrhosis in carriers of the 148M allele, but only 33% to detect a 33% increased risk. Secondly, this was a retrospective study with data evaluation at the time of diagnosis, therefore we could not exclude the fact that some patients “normalized” their body mass after the development of cirrhosis due to the malnutrition typical of this condition. However, increased BMI was independently associated with steatosis and tended to be associated also with cirrhosis risk, and the shift in body weight should have equally affected patients with and without the 148M allele. Lastly, we cannot exclude that the PNPLA3 148M variant, by favoring steatosis development in subjects with normal BMI, may play a permissive role in the progression of liver damage in lean patients with C282Y+/+ HH, whereas in overweight subjects steatosis is mainly related to metabolic factors, reducing the role played by PNPLA3. Additional studies are required to clarify this issue, and to evaluate whether the 148M variant may predispose to hepatocellular carcinoma development also in HH and in patients carrying HFE mutations^[39-41].

In conclusion, we showed that in Italian patients with C282Y+/+ HH the PNPLA3 I148M polymorphism is associated with the risk of steatosis, increased liver enzymes, higher stage of fibrosis, and possibly with an increased risk of cirrhosis at diagnosis, particularly in subjects with normal body mass. Future studies should evaluate whether PNPLA3 I148M genotype might be clinically useful for selecting HH patients for biopsy, or to determine screening intervals for hepatocellular carcinoma in cirrhotics.

COMMENTS

Background

Hereditary hemochromatosis, characterized by progressive accumulation of

iron in tissues, is a very frequent genetic disease in individuals of European descent. The most frequent clinical manifestation is liver disease, which may lead to liver cancer. However, disease expression is highly variable. Previous work has led to hypothesize that genetic factors and liver fat accumulation (i.e., “steatosis”) are implicated in this process. Recently, the common I148M patatin-like phospholipase domain containing-3 genetic polymorphism has been recognized together with obesity as a key factor regulating fat accumulation in the liver, contributing significantly to the liver disease burden in the general population.

Research frontiers

The identification of genetic factors involved in the penetrance and expression of hereditary hemochromatosis is a very active area of research, as such markers would be helpful to identify subjects at risk during screening and to personalize treatment and follow-up in patients presenting with liver disease.

Innovations and breakthroughs

The key findings of the study are that the PNPLA3 genetic variant, present in 40% of patients, was a key determinant, together with overweight, of hepatic fat accumulation and of alterations of biochemical indices of liver damage. Furthermore, this marker was also associated with chronic fibrotic damage detected by liver biopsy. Importantly, the PNPLA3 variant put at risk of steatosis also normal weight patients, who would be normally protected, allowing the development of progressive liver damage and cirrhosis, with potential clinical complications.

Applications

These results raise new hope to offer better, personalized treatment to patients with hemochromatosis, which will be tested in future studies. In particular, intensified follow-up and preventive treatments could be proposed to subjects at risk of developing liver cancer, the leading cause of death in patients with clinically overt hemochromatosis, which is also favored by steatosis.

Terminology

Hereditary hemochromatosis is a genetic disorder of iron metabolism characterized by defective release or activity of hepcidin, the hepatic hormone that inhibits iron absorption, leading to progressive accumulation in the liver and other parenchymal organs; PNPLA3 is an enzyme with phospholipase activity, which is expressed in the liver, and likely involved in the breakdown triglycerides; genetic polymorphisms: inherited variant of the DNA, which is detected in > 1% of the population and is not associated *per se* with a pathologic phenotype.

Peer review

This is an interesting clinical study, which provides evidence for association between the PNPLA3 148M polymorphism and progression of liver fibrosis. The study is well designed and the data are novel.

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