It is Patatin-like Phospholipase Domain-containing 3 Gene (PNPLA3)—All the Way

Abstract 1

Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of non-alcoholic fatty liver disease. *Hepatology* 2011;53:1883–94.

Our objective was to estimate the strength of the effect of the I148M (rs738409 C/G) patatin-like phospholipase domain containing three (PNPLA3) variants on non-alcoholic fatty liver disease (NAFLD) and disease severity across different populations. We performed a systematic review by a meta-analysis; literature searches identified 16 studies. Our results showed that rs738409 exerted a strong influence not only on liver fat accumulation (GG homozygous showed 73% higher lipid fat content when compared with CC ones, data from 2937 subjects; $P < 1 \times 10^{-9}$), but also on the susceptibility of a more aggressive disease (GG homozygous had 3.24-fold greater risk of higher necro-inflammatory scores and 3.2-fold greater risk of developing fibrosis when compared with CC homozygous; $P < 1 \times 10^{-9}$; data from 1739 and 2251 individuals, respectively). Non-alcoholic steatohepatitis (NASH) was more frequently observed in GG than in CC homozygous (odds ratio [OR] 3.488, 95% confidence interval [CI] 1.859–6.545, random model; $P < 2 \times 10^{-4}$; data from 2124 patients). Evaluation of the risk associated with heterozygosity for the variant suggests that the additive genetic model best explains the effect of rs738409 on the susceptibility to develop NAFLD. Nevertheless, carrying two G alleles does not seem to increase the risk of severe histological features. Meta-regression showed a negative correlation between male sex and the effect of rs738409 on liver fat

content (slope: -2.45 ± 1.04 ; $P < 0.02$). The rs738409 GG genotype vs the CC genotype was associated with a 28% increase in serum alanine aminotransferase levels. **Conclusion:** By summarizing the amount of evidence, this study provided unequivocal evidence of rs738409 as a strong modifier of the natural history of NAFLD in different populations around the world.

Abstract 2

Valenti L, Rumi M, Galmozzi E, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011;53:791–9.

Steatosis has been reported to negatively influence the natural history of chronic hepatitis C (CHC), but controversy remains over its causal role due to the confounding effect of adiposity, insulin resistance, and diabetes. The rs738409 C>G patatin-like phospholipase domain-containing 3 (PNPLA3) single nucleotide polymorphisms (SNP), encoding for the I148M protein variant, influences liver fat without affecting insulin resistance and body composition. The aim of this study was to evaluate the effect of the rs738409 CG genotype on liver fat and fibrosis in patients with CHC. We also explored the possible effect of PNPLA3 genotype on other steatosis-related complications, namely treatment failure and hepatocellular carcinoma (HCC) development. To this end, we considered two independent series of 325 and 494 patients with CHC with available DNA and liver biopsy followed at tertiary referral centers in northern Italy. The rs738409 genotype was determined by a Taqman assay. The rs738409 GG genotype, observed in 10% of patients, was associated with steatosis independently of age, sex, body mass index (BMI), diabetes, alcohol intake, and viral genotype (odds ratio [OR] 1.90, 95% confidence interval [CI] 1.4–2.7; $P < 0.001$). The association with rs738409 genotype was confirmed for severe steatosis, was independent of alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) values, and was observed in all viral genotypes but the 3. The rs738409 GG genotype was associated with fibrosis stage and cirrhosis (OR 1.47, 95% CI 1.2–1.9; $P = 0.002$), treatment response ($n = 470$; OR 0.63, 95% CI 0.4–0.8; $P = 0.006$), and HCC occurrence ($n = 325$; OR 2.16, 95% CI 1.3–3.6; $P = 0.002$), independently of confounders. **Conclusion:** The rs738409 PNPLA3 genotype influences steatosis development in CHC and is independently associated with cirrhosis and other steatosis-related clinical outcomes, such as lack of response to antiviral treatment and possibly HCC.

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Abbreviations: ALT: alanine aminotransferase; CHC: chronic hepatitis C; GGT: gamma-glutamyl transferase; NAFLD: non-alcoholic fatty liver disease; PNPLA3: patatin-like phospholipase domain-containing 3 gene; SNP: single nucleotide polymorphisms

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Abstract 3

Nischalke HD, Berger C, Luda C, et al. The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. *PLoS One* 2011; 6:e27087. Epub 2011 Nov 7.

Background: An isoleucine > methionine mutation at position 148 in the PNPLA3 gene (p.I148M, rs738409) has recently been identified as a susceptibility factor for liver damage in steatohepatitis. Here, we studied whether the PNPLA3 rs738409 polymorphism also affects predisposition to hepatocellular carcinoma (HCC). **Method:** We compared distributions of PNPLA3 genotypes in 80 and 81 Caucasian patients with alcoholic and hepatitis C virus (HCV)-associated HCC to 80 and 81 age- and sex-matched patients with alcohol-related and HCV-related cirrhosis without HCC, respectively. PNPLA3 genotypes in 190 healthy individuals from the same population served as reference. Potential confounders obesity, diabetes, HCV genotype, and HBV co-infection were controlled by univariate and multivariate logistic regression with forward variable selection. **Results:** PNPLA3 genotypes were in Hardy-Weinberg equilibrium for all study groups. The frequency of the 148M allele was significantly ($P < 0.001$) increased in alcoholic cirrhosis with (53.7%) and without HCC (36.2%) but was not different between healthy controls (22.9%) and patients with cirrhosis (25.3%; $P = 0.545$) and HCC (30.2%; $P = 0.071$) due to hepatitis C. The HCC risk was highest in 148M/M homozygous patients with alcoholic liver disease (odds ratio (OR) 16.8 vs healthy controls; 95% confidence interval (CI) 6.68–42.43, $P < 0.001$). Finally, multivariate regression confirmed 148M/M homozygosity (OR 2.8; 95% CI 1.24–6.42; $P = 0.013$) as HCC risk factor in alcoholic cirrhosis. In HCV-related cirrhosis, only HCV genotype 1 was confirmed as a HCC risk factor (OR 4.2; 95% CI 1.50–11.52; $P = 0.006$). **Conclusion:** The PNPLA3 148M variant is a prominent risk factor for HCC in patients with alcoholic cirrhosis, while its effects are negligible in patients with cirrhosis due to HCV. This polymorphism provides a useful tool to identify individuals with particularly high HCC risk in patients with alcoholic liver disease that should be taken into account in future HCC prevention studies.

COMMENT

Patatin-like phospholipase domain-containing protein three (PNPLA3), also known as adiponutrin, is an enzyme that in humans is encoded by the PNPLA3 gene. The PNPLA3 is a triacylglycerol lipase that mediates triacylglycerol hydrolysis in adipocytes. The encoded protein, which appears to be membrane bound, may be involved in the balance of energy usage/storage in adipocytes. The mature protein is 481 amino acids in length and the predicted molecular weight is 52.865 kDa. The PNPLA3 is expressed in the endoplasmic reticulum and at the surface of lipid droplets in

hepatocytes and adipocytes and is induced in liver after feeding and during insulin resistance. The mechanism by which PNPLA3 variant influences the hepatic fat is unresolved but could depend on the hydrolytic or lipogenic action of this enzyme.

The PNPLA3 variants and NAFLD relationship are straight forward and clear with almost all studies favoring a link between the two even though the strength of association varies in different ethnicities with highest association in Hispanics and lowest in African Americans.¹ The genome-wide association studies revealed that PNPLA3 rs738409 C>G SNP encoding for isoleucine to methionine variant (I148M) was a major determinant of differences in hepatic fat content among patients of different races independent of insulin resistance, body mass, and lipid levels.¹ The studies also revealed that the genetic variation rs738409 (I148M) in PNPLA3 not only influences NAFLD but also serum levels of liver enzymes and hepatic fibrosis.² The results of meta-analysis (Abstract 1) show that rs738409 nonsynonymous I148M polymorphism exerts a strong influence not only on liver fat accumulation (GG carriers have 73% higher lipid content in comparison to CC carriers) but also on the histological disease severity (GG homozygous subjects have 3.24-fold more risk of necro-inflammatory scores and 3.2-fold higher risk of fibrosis than CC homozygotes).³ The meta-analysis provided unequivocal evidence of rs738409 of PNPLA3 as a strong modifier of the natural history of NAFLD in different populations around the world.³ Data are now also available with a strong association of PNPLA3 variant and NAFLD starting early in life in children, both in obese and in non-obese and in patients with diabetes and non-diabetes.^{4–6} What is required now is not only to look at the presence of hepatic fat in patients with PNPLA3 variants but using this association for better diagnostic and therapeutic purposes. Data need to be generated if PNPLA3 variants can help in making a diagnostic algorithm in predicting disease severity in patients with NAFLD. This may be more helpful in children, where prediction about disease severity may be made early in life and preventive measures taken at appropriate time. Similarly, understanding the PNPLA3 biology may help in designing some novel treatment strategies.

Patients with chronic hepatitis C (CHC) are at high-risk of liver fibrosis, and up to 20% develop cirrhosis and its complications. The rate of progression of fibrosis is variable among patients with several factors determining the progression. Studies have shown that factors like old age, male sex, elevated body mass index, insulin resistance, chronic alcohol consumption, viral factors, or co-infection with human immunodeficiency virus are associated with a greater risk of fibrosis progression. Steatosis occurs in around half of patients with CHC and has been associated with more aggressive histological disease, faster progression

of fibrosis, and poorer response to therapy. A direct cytopathic effect by induction of steatosis by viral proteins of HCV has been proposed in genotype 3 infection and host factors including overweight and type 2 diabetes have been linked to steatosis and fibrosis progression in non-genotype-3 patients. Data on PNPLA3 in patients with NAFLD have suggested a genetic basis for hepatic steatosis. Valenti et al⁷ (Abstract 2) have shown in a large series of Italian patients with CHC that the rs738409 PNPLA3 genetic variant not only affects steatosis development but is also independently associated with fibrosis and cirrhosis, and may influence treatment response and HCC occurrence in patients with CHC. This association was independent of known confounding factors and the selective association between PNPLA3 and cirrhosis in younger patients is consistent with an expected relatively greater effect of inherited factors on fibrosis progression in patients with a shorter duration of disease. The data suggest that genetically determined nonviral hepatic steatosis has a causal role in determining fibrosis progression in patients with CHC. In addition, authors have suggested that PNPLA3 variant may influence treatment response and HCC development in patients with CHC.

Though another recent study⁸ on the effect of PNPLA3 variant on CHC disease severity has supported the findings of Valenti et al,⁷ the relationship with occurrence of HCC needs to be studied further as Nischalke et al⁹ (Abstract 3) found a strong relationship of PNPLA3 variant and HCC in patients with alcoholic cirrhosis but not in patients with HCV cirrhosis.

Further, the evaluation of PNPLA3 variant and its interaction with other genetic factors, such as IL28B SNPs,

may help in better prediction of treatment outcome in patients with CHC.

REFERENCES

1. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40:1461–5.
2. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. *Hepatology* 2010;51:1209–17.
3. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011;53:1883–94.
4. Valenti L, Alisi A, Galmozzi E, et al. I148M patatin-like phospholipase domain-containing 3 gene variant and severity of pediatric nonalcoholic fatty liver disease. *Hepatology* 2010;52:1274–80.
5. Wang CW, Lin HY, Shin SJ, et al. The PNPLA3 I148M polymorphism is associated with insulin resistance and nonalcoholic fatty liver disease in a normoglycaemic population. *Liver Int* 2011;31:1326–31.
6. Cox AJ, Wing MR, Carr JJ, et al. Association of PNPLA3 SNP rs738409 with liver density in African Americans with type 2 diabetes mellitus. *Diabetes Metab* 2011;37:452–5.
7. Valenti L, Rumi M, Galmozzi E, et al. Patatin-like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. *Hepatology* 2011;53:791–9.
8. Trépo E, Pradat P, Potthoff A, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. *Hepatology* 2011;54:60–9.
9. Nischalke HD, Berger C, Luda C, et al. The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. *PLoS One* 2011;6:e27087.