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Review article

PNPLA3 I148M variant and hepatocellular carcinoma: A common genetic variant for a rare disease

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ABSTRACT

Hepatocellular carcinoma (HCC) is highly associated with chronic liver disease. The rs738409 genetic variant in the patatin-like phospholipase domain-containing 3 (*PNPLA3*, adiponutrin) gene has been implicated as a genetic determinant of the entire spectrum of liver diseases, ranging from steatosis, chronic hepatitis, cirrhosis and ultimately to HCC. In this review, first we will examine the current genetic theories of disease susceptibility. Next, we will analyze the evidences for the association between *PNPLA3* I148M variant and HCC. Moreover, we will exploit this association to propose a new paradigm in human genetics: a common genetic variant contributing to a rare disease. Finally, we will examine the molecular genetics of PNPLA3 and, specifically, the theories that have been proposed to explain the function of PNPLA3 in health and disease.

1. Introduction

Hepatocellular carcinoma (HCC) is mostly found in subjects with cirrhosis caused by chronic liver diseases. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection account for 54% and 31% of cases, respectively, and thus the incidence and geographical prevalence of HCC presently mirror those of infection with hepatitis viruses [1,2]. Excessive alcohol intake, liver steatosis associated with obesity and insulin resistance, hereditary hemochromatosis, and other inherited diseases such as alpha-1-antitrypsin deficiency account for the majority of the non-viral-related cases [3–6].

Family history, gender, diabetes mellitus and ethnicity influence the risk of HCC, and several studies have demonstrated a role of genetic mutations in the predisposition to HCC [7,8]. Here we focus on the rs738409 genetic variant in the patatin-like

phospholipase domain-containing 3 (*PNPLA3*, adiponutrin) gene, which has been implicated as a genetic determinant of HCC susceptibility (Table 1). This variation is the major genetic determinant of hepatic fat content and liver enzyme serum levels in the general population identified to date [9–11], and is a risk factor for HCC independently of its effect on the progression of liver fibrosis [12–17].

In this review, we present the current model explaining the influence of genetic variations on determining human disease, the evidence in support of an association of the *PNPLA3* I148M variant with HCC, and the hypothesized mechanism linking this genetic variant with hepatic carcinogenesis.

2. Current genetic theories of disease susceptibility

Three models explaining the contribution of genetic variants to human diseases have been proposed (Fig. 1). These models are mostly based on the frequency of the genetic variant and the disease that are simplistically defined as common or rare.

2.1. A rare genetic variant for a rare disease

Rare deleterious alleles that cause a major modification in the expression/activity of the encoded protein result in monogenic forms of disease. This is exemplified by mutations in lipoprotein

Table 1
Studies reporting an association between the *PNPLA3* I148M variant and HCC.

Author	Design	Setting	Aetiology	Subjects evaluated	Genetic model	HCC risk OR (95% CI)
Valenti et al. [17]	Retrospective	CHC patients	CHC	50 HCC, 275 CHC	Recessive	2.2 (1.3–3.6)
Ginanni Corradini et al. [33]	Retrospective	Cirrhotic patients	CHC	90 HCC, 131 UC	Recessive	2.2 (1.4–3.5)
Nischalke et al. [13]	Case–control	Cirrhotic patients	CHC	80 HC, 80 UC	Recessive	1.7 (0.5–5.3)
			ALD	81 HCC, 81 UC	Recessive	2.8 (1.2–6.4)
Falleti et al. [14]	Retrospective	Cirrhotic patients	Mixed	141 HCC, 342 UC	Recessive	1.8 (1.1–2.9)
Trepo et al. [12]	Retrospective	Cirrhotic patients	ALD	145 HCC, 426 UC	Recessive	4.7 (2.6–8.4)
Burza et al. [15]	Prospective	Swedish Obese Subjects study	Severe obesity	4047 obese	Recessive	16.0 (2.3–111)
Guyot et al. [34]	Prospective	Cirrhotic patients	CHC	93 HCC, 160 UC	Recessive	1.0 (0.6–1.9)
			ALD	66 HCC, 213 UC	Recessive	1.9 (1.3–2.8)

Abbreviations: PNPLA3, patatin like phosphplipase domain-containing; HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval; CHC, chronic hepatitis; UC, uncomplicated cirrhosis; and ALD, alcoholic liver disease.

lipase that are responsible for rare cases of primary hypertriglyceridemia [18,19].

2.2. A rare genetic variant for a common disease

Circulating levels of triglycerides are also strongly influenced by many environmental factors (diabetes mellitus, obesity and alcohol), and therefore they may be considered an example of a common (*i.e.*, multifactorial) trait. At a general population level, circulating triglycerides are influenced by rare (minor allele frequency (MAF) < 0.01) mutations in the angiopoietin-like protein family members (3–5), providing an example of a rare genetic variant influencing a common trait.

2.3. A common genetic variant for a common disease

The Human Genome Project [20], which started at 1990, built a catalogue of all common genetic variants (MAF > 0.05) during 13 years. Based on the hypothesis that common genetic variants would explain the susceptibility to common disease [21] in 2005 the genome wide association study era started [22].

In terms of liver disease, two studies that examined two different but closely related traits (hepatic triglyceride content and aminotransferase levels) simultaneously pointed to the influence of the rs738409 genetic variation in *PNPLA3* on these common traits [9,10]. *PNPLA3* rs738409 is a common genetic variant (MAF ranges

0.18–0.30 in individuals of European descent) [23,24] consisting of a guanine to cytosine substitution that results in an isoleucine to methionine substitution at position 148 of the PNPLA3 protein. The deleterious mutated allele in the homozygous state (148M) is present in 5–8% individuals of European descent. The *PNPLA3* 148M allele associates with the entire spectrum of liver disease, from simple hepatic fat content variation to increases in aminotransferase levels [9,10] and advanced liver fibrosis/cirrhosis [17,25–31], as well as to metabolic traits such as insulin resistance and susceptibility to diabetes mellitus [23].

3. A new paradigm in human genetics: "A common genetic variant for a rare disease"

We have recently shown that individuals who are homozygous for *PNPLA3* 148M and have long-standing advanced liver disease and cirrhosis have a 2–16-fold increase risk of developing HCC [12–17]. In Northern Europe, the average incidence of HCC is lower than 3 cases per 100,000 inhabitants [2], and from this perspective HCC can be considered a rare disease at a general population level. The association between the *PNPLA3* I148M genetic variant and HCC thus represents a new paradigm within the models explaining the contribution of genes to human diseases, namely a common genetic variant explaining a rare disease (dashed arrow in Fig. 1). This model is also supported by the recent observation of a common genetic variant (rs2305089) substantially increasing the susceptibility of chordoma, a rare primary bone cancer [32].

3.1. Association between PNPLA3 I148M and HCC: the clinical evidence

The clinical studies supporting an association between the *PNPLA3* I148M genotype and HCC are shown in Table 1. We reported for the first time a predisposing effect of the 148M *PNPLA3* variant on HCC in a retrospectively evaluated cohort of 325 Northern Italian patients with chronic hepatitis C, showing that homozygosity for the 148M allele was associated with a 2.2-fold higher risk of HCC at the end of follow-up, independently of other risk factors [17]. These results were confirmed in a retrospective series of Italian patients with chronic hepatitis C-related cirrhosis [33] and also in patients with cirrhosis of mixed etiologies [14].

In a case–control study where the genetic background of patients with HCC was compared with that of patients with uncomplicated cirrhosis matched for age and sex, 148M homozygosity conferred a 2.8-fold higher risk of HCC in patients with alcoholic liver disease (ALD) [13]. In contrast, the association was not significant in a similar number of subjects with cirrhosis and chronic hepatitis C, although the wide confidence interval was consistent with previous results [17,33]. An even stronger predisposing effect of *PNPLA3* genotype on HCC risk in ALD (4.7-fold higher risk for 148M homozygosity) was reported in a retrospectively evaluated
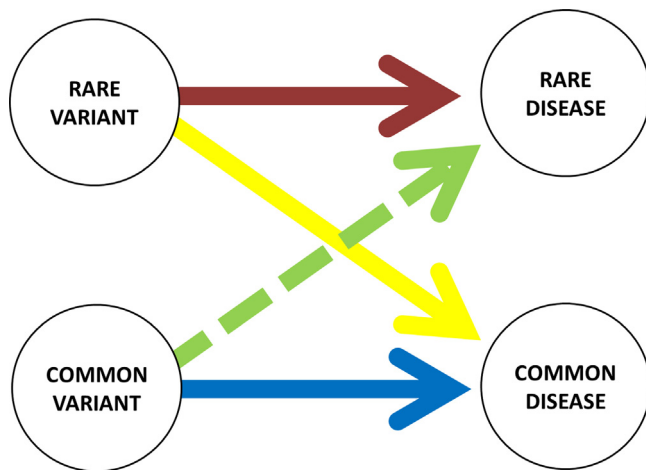

Fig. 1. Genetic models explaining the contribution of genetic variants to human diseases. Three different models explaining the contribution of genetic variants to human disease have been proposed (solid arrows) in the past: rare genetic variants determine rare diseases/traits (red arrow); rare variants determine a proportion of the common diseases/traits (yellow arrow); common genetic variants determine common diseases/traits (blue arrow). The susceptibility conferred by the PNPLA3 I148M variant to hepatocellular carcinoma is the first example of common genetic variants determining a rare disease/trait (dashed green arrow).
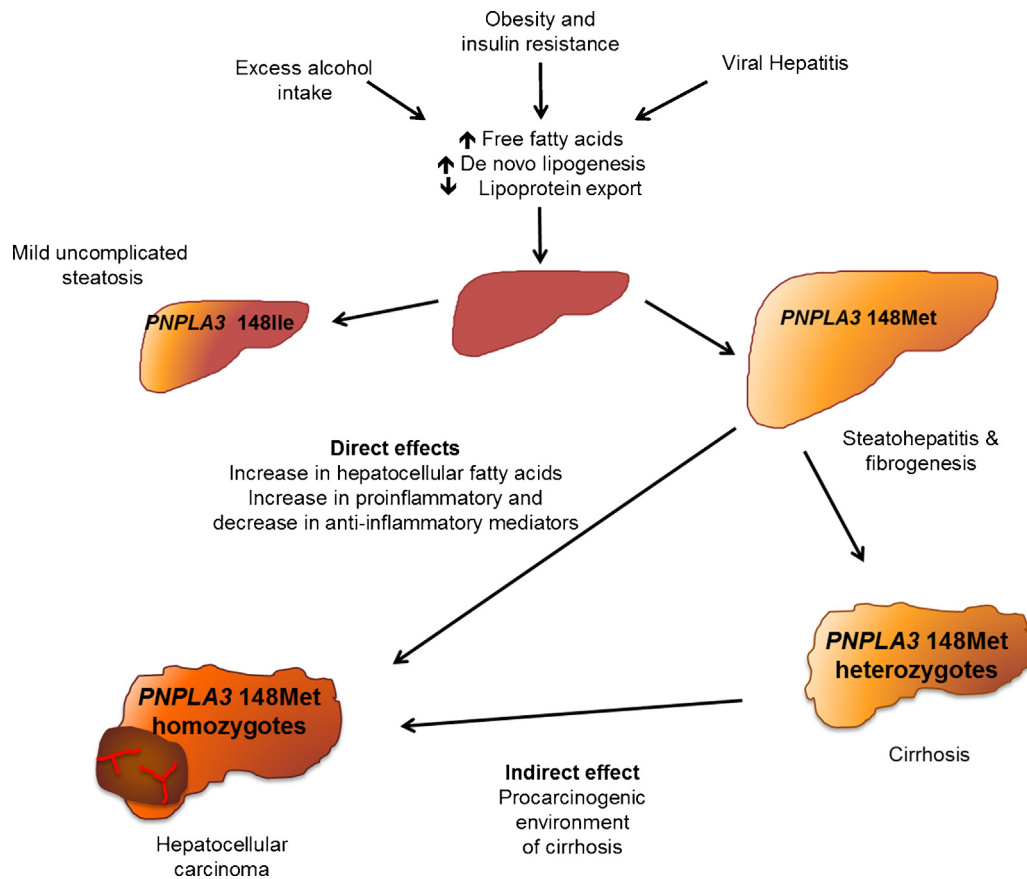
Fig. 2. The natural history of liver disease. Several different environmental stressors contribute to an overload of lipids in the liver. In carriers of the *PNPLA3* 148I wildtype allele, this results in a mild and uncomplicated accumulation of fat in the liver. Conversely, in heterozygotes for the *PNPLA3* 148M mutant allele, an increase in hepatic fat progresses to inflammation and usually ends in cirrhosis. In individuals who are homozygous for the *PNPLA3* 148M mutant allele, hepatocellular carcinoma occurs more frequently either because of a more accelerated course of the disease or through a direct effect of PNPLA3.

cohort of individuals with cirrhosis [12]; this finding was recently confirmed in a prospective study [34].

We also examined the association between the I148M polymorphism and HCC incidence in a prospective study of obese subjects included in the Swedish Obese Subjects study (SOS) cohort, consisting of matched individuals who underwent either bariatric surgery or conventional treatment, and have been followed up for a median of 15 years [35]. A higher incidence of HCC was observed in obese subjects in whom weight loss was not observed, and 148M homozygosity was associated with a 16-fold higher risk of HCC [15]. This very high relative risk may be explained by the inclusion of an unselected population of severely obese patients, as *PNPLA3* genotype not only is a likely risk factor for HCC in patients with cirrhosis, but also predisposes to earlier stages of liver disease [9,17,25–27].

In principle, the *PNPLA3* genotype could perform as a reliable noninvasive biomarker of HCC risk at early stages of liver damage and in the general population, which is generally at very low risk of HCC. In contrast, the specificity in detecting the disease risk is likely to be reduced in patients with chronic viral hepatitis and cirrhosis because of the many coexistent carcinogenic factors, particularly cirrhosis itself. A recently reported association between the *PNPLA3* I148M variant and HCC related to nonalcoholic fatty liver and obesity in Japanese patients also lends support to the hypothesis of an interaction between increased fat body mass and *PNPLA3* in the pathogenesis of HCC [36], as demonstrated for hepatic fat accumulation [37]. Thus, despite current limitations of the studies, we conclude that *PNPLA3* I148M variant is a risk factor for HCC, in particular in subjects without chronic liver disease related to HBV and HCV infections.

4. PNPLA3 role in health and hepatocellular carcinoma

4.1. PNPLA3 expression in humans and mice

PNPLA3 expression has been examined in detail in mouse models, where *Pnpla3* mRNA is mostly expressed in adipose tissue [38]. By contrast, *PNPLA3* is expressed mostly in the liver in humans [39,40]. *Pnpla3* mRNA levels in mice have been shown to decrease after fasting and are promptly rescued by refeeding [41]. Consistently, obese rats show higher *Pnpla3* mRNA levels than their congenic lean controls [42]. These findings have been confirmed in humans by measuring the *PNPLA3* mRNA expression in subcutaneous adipose tissue of obese and non-obese women after a very low calorie diet and subsequent refeeding [40]. The nutritional regulation of *PNPLA3* was further clarified when the gene was found to be under the control of the sterol regulatory element binding protein 1c (SREBP-1c) [43], which responds in turn to insulin and the liver X receptor (LXR).

4.2. PNPLA3 physiological role: lipase or acyltransferase activity?

Human PNPLA3 consists of an N-terminal patatin (calcium-independent phospholipase A2, iPLA2) homology domain with triglyceride lipase and acyl-CoA independent transacylase activities [44]. The lipase activity of PNPLA3 has also been shown in independent studies by purification of the recombinant protein from insect (Sf9) cells and by overexpression in human hepatoma (HuH7) cells [43,45]. In contrast, a recent study found the purified recombinant PNPLA3 to have a predominant lysophosphatidic

acid-acyltransferase activity [46]. A possible explanation of these findings is that the purified protein is able to catalyze both reactions, depending on the concentration of substrates.

4.3. PNPLA3 I148M variant: gain or loss of function?

The effect of the isoleucine to methionine substitution at position 148 on the function of PNPLA3 is still controversial. Experiments on purified human PNPLA3 show that the variant promotes a decrease in the protein activity, suggesting loss of function [47]. However, a very recent study showed that this variant induces a gain of function in the lysophosphatidic acid-acyltransferase activity, leading to increased hepatic triglyceride synthesis [46].

Genetically modified mouse models have not resolved the conflicting results of *in vitro* studies on the enzymatic function of the protein. Indeed, the adenoviral overexpression of the human protein in mice does not affect the liver fat content [45], suggesting that the lipase activity is not present *in vivo*. Furthermore, two *Pnpla3* knockout mouse models do not show any differences in triglyceride lipase activity or in the extent of fat accumulation in the liver or adipose tissue [48,49]. However, it is worth noting that human and mouse proteins differ at their C termini (mouse pnpla3 is shorter than the human protein) and the mRNA expression pattern differs considerably between human and mice [40]. These differences suggest that the human and mouse PNPLA3 might play different physiological roles.

We recently presented an *in vitro* model in which stable transfection of the human PNPLA3 mutant protein determines a loss of function in the export of triglycerides from the liver [50]. Our data suggest that wildtype PNPLA3 hydrolyzes triglycerides stored in lipid droplets to release fatty acids, which are then available for new synthesis of triglycerides and their subsequent incorporation into very low-density lipoproteins (VLDL). These data are consistent with a study performed in humans after administration of a high fructose diet for two weeks in which the direct relationship between changes in *de novo* lipogenesis and serum triglycerides is abolished in homozygotes for the 148M variant [51].

We propose that the I148M substitution interferes with this function, thus reducing intracellular lipid turnover and export pathways in hepatocytes [50]. These data are consistent with the triglyceride lipase activity hypothesis, but a lysophosphatidic acid-acyltransferase activity cannot be excluded.

Furthermore, several alternative models of PNPLA3 protein action have been proposed [46,50,52], and additional studies are required to clarify which of these is closest to human physiology.

4.4. PNPLA3 I148M variant: direct hepatic carcinogenic activity?

The association of the *PNPLA3* I148M variant with steatosis has been confirmed in several studies [9,10,24,27,29,53–59], but the effect of the variant is not limited to a modulation of hepatic fat content (Fig. 2). Indeed, it became evident early on that the 148M allele predisposes to the development of non-alcoholic steatohepatitis and liver fibrosis independently of the presence of other cofactors of liver damage, leading to advanced liver disease [29,56,60]. Moreover, clinical studies have shown that the *PNPLA3* I148M variant influences HCC risk independently of the presence of cirrhosis [12–17], raising the question as to whether this genetic variant determines other key biological alterations involved in hepatic carcinogenesis.

Interestingly, the predisposing effect of the *PNPLA3* I148M variant on liver disease progression is also independent of the severity of liver fat accumulation thus suggesting that it influences the release of molecules that directly regulate inflammation and fibrogenesis [28,61,62]. Indeed, the *PNPLA3* I148M variant has been associated with increased circulating levels of the proinflammatory

mediator intercellular adhesion molecule 1 (ICAM-1) [63], and reduced levels of the adipokine adiponectin [64], which has anti-inflammatory, anti-fibrotic, and oncosuppressive activities [65]. When the hepatic microenvironment is then altered by steatohepatitis, the liver could become cancer prone even in the absence of frank cirrhosis [6,66]. Potential mechanisms behind the carcinogenic effect of the *PNPLA3* I148M variant include low-grade hepatic inflammation, with increased release of tumour necrosis factor α and interleukin-6 [67], altered release of adipokines influencing insulin resistance and inflammation [68], increased lipogenesis and cellular availability of fatty acids supporting energy for rapidly growing cells [69,70], lipotoxicity influencing intracellular signalling pathways [71], and oxidative stress related to lipid peroxidation and mitochondrial damage [72].

5. Conclusion

The *PNPLA3* I148M variant has been associated with HCC clinical presentation, and in particular with a more advanced disease and a poorer prognosis [36,73]. Thus, although these results need to be confirmed, it is conceivable that PNPLA3 regulates a specific pathway involved in HCC pathogenesis, and may represent a future noninvasive marker of a specific HCC subtype and a possible therapeutic target.

In conclusion, we propose for the first time a genetic model of a common genetic variant (*i.e.*, *PNPLA3* I148M) influencing the susceptibility to a rare disease (*i.e.*, HCC). Further studies are warranted to assess the potential of using *PNPLA3* genotype as a marker to increase clinical surveillance and whether modulation of PNPLA3 expression/activity could be used to modify susceptibility to hepatocellular carcinoma.

Conflict of interest
Declared no conflicts of interest.

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