PNPLA3 I148M polymorphism and progressive liver disease

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Abstract

The 148 Isoleucine to Methionine protein variant (I148M) of patatin-like phospholipase domain-containing 3 (PNPLA3), a protein is expressed in the liver and is involved in lipid metabolism, has recently been identified as a major determinant of liver fat content. Several studies confirmed that the I148M variant predisposes towards the full spectrum of liver damage associated with fatty liver: from simple steatosis to steatohepatitis and progressive fibrosis. Furthermore, the I148M variant represents a major determinant of progression of alcohol related steatohepatitis to cirrhosis, and to influence fibrogenesis and related clinical outcomes in chronic hepatitis C virus hepatitis, and possibly chronic hepatitis B virus hepatitis, hereditary hemochromatosis and primary sclerosing cholangitis. All in all, studies suggest that the I148M polymorphism may represent a general modifier of fibrogenesis in liver diseases. Remarkably, the effect of the I148M variant on fibrosis was independent of that on hepatic steatosis and inflammation, suggesting that it may affect both the quantity and quality of hepatic lipids and the biology of non-parenchymal liver cells besides hepatocytes, directly promoting fibrogenesis. Therefore, PNPLA3 is a key player in liver disease progression. Assessment of the I148M polymorphism will possibly inform clinical practice in the future, whereas the determination of the effect of the 148M variant will reveal mechanisms involved in hepatic fibrogenesis.

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Key words: Alcoholic liver disease; Chronic hepatitis C virus hepatitis; Fibrogenesis; Genetics; Hepatocellular carcinoma; Liver disease; Nonalcoholic fatty liver disease; Patatin-like phospholipase domain-containing 3; Single nucleotide polymorphism; Steatosis

Core tip: The 148 Isoleucine to Methionine protein variant (I148M) of patatin-like phospholipase domain-containing 3 (PNPLA3) has recently been identified as a major determinant of liver fat content. Several studies conducted in different ethnicities confirmed that I148M influences the full spectrum of liver damage: from simple steatosis to nonalcoholic steatohepatitis and progressive fibrosis to hepatocellular carcinoma. Furthermore, I148M turned out to represent a major determinant of progression of alcohol related steatohepatitis, and to influence fibrosis progression and related clinical outcomes in chronic hepatitis C virus hepatitis, as well as other in liver diseases. All in all, studies suggest that the PNPLA3 I148M polymorphism may represent a general modifier of fibrogenesis in liver diseases.
Dongiovanni et al. PNPLA3 and liver disease

INTRODUCTION
The clinical evolution of chronic liver diseases is highly variable, and genetic factors play a key role in determining the inter-individual susceptibility towards end-stage liver disease and hepatoellular carcinoma. It is now established that all common diseases, including type 2 diabetes, atherosclerosis, and nonalcoholic fatty liver disease (NAFLD) among liver diseases [3], exhibit a heritable component of susceptibility accounting for 30%-50% of risk. Indeed, also liver diseases can be considered complex traits that result from environmental exposures, e.g., diet and physical inactivity for NAFLD, excessive alcohol intake for alcoholic liver disease (ALD), or infection for chronic viral hepatitis, acting on a susceptible polygenic background comprising multiple independent modifiers [3]. These are generally represented by common genetic variants with a mild effect or rare variants associated with a more marked phenotype [3].

Despite initial hypothesis-driven, case-control studies identified some genetic loci associated with the progression of liver damage, the genetic determinants of NAFLD remained obscure until recently [4]. By 2008, the first genomewide association studies in the field of hepatic steatosis allowed to identify the rs738409 variant, by an hypothesis free drive approach, as the single major genetic determinant of hepatic fat content [4, 5]. This sequence variation is a C > G single nucleotide change, encoding for the 148 Isoleucine to Methionine protein variant (1148M) of Patatin-like phospholipase domain-containing 3 (PNPLA3). The main purpose of this review is to provide an overview of the current knowledge of the PNPLA3 I148M polymorphism role in the progression of liver disease.

NONALCOHOLIC FATTY LIVER
Ectopic fat accumulation in the liver related to systemic insulin resistance represents the prototypical manifestation of NAFLD. With a prevalence of 20%-34% and rising, NAFLD is now the most frequent liver disease in industrialized countries [6, 7]. In a minority of susceptible individuals, steatosis, that is excessive fat accumulation in the liver (> 5%), is associated with oxidative hepatoellular damage, inflammation and activation of fibrogenesis, i.e., nonalcoholic steatohepatitis (NASH) [8], which can progress to cirrhosis and hepatocellular carcinoma [9, 10].

NAFLD is epidemiologically associated with obesity and metabolic syndrome. The pathogenesis is related to adipose tissue insulin resistance [11], leading to an increased flux of free fatty acids to the liver [12], increased lipogenesis induced by hyperinsulinemia, abnormal intra-hepatic lipid metabolism and dietary factors. Hepatic fat accumulation, in turn, worsens insulin resistance and liver damage, determining an increased risk of both cardiovascular and liver related mortality [13-15].

Besides hepatocytes, two cell types play a key role in the pathogenesis of NASH. Kupffer cells are the hepatic macrophages that under basal conditions are involved in the maintenance of immune homeostasis, but during NASH become activated by intestinal bacterial products and oxidized lipids via Toll-like receptor-4 and secrete reactive oxygen species, chemokines and several cytokines, thereby orchestrating inflammation [16]. Hepatic stellate cells are hepatic pericytes localized between sinusoidal endothelial cells and the hepatocytes, which in quiescent conditions store lipids and retinoids, secrete extracellular matrix, and regulate blood flow. Upon activation in NASH, stellate cells release retinoids, undergo myofibroblast transition, secrete type 1 collagen and a variety of fibrogenic mediators thereby initiating the process of fibrogenesis [17].

GENETIC PREDISPOSITION TO FATTY LIVER
The risk of NAFLD is highly variable even in individuals with obesity and type 2 diabetes. Furthermore, even if the majority of obese subjects with metabolic syndrome develop simple steatosis, only about one third has NASH, and a minority progresses to more severe forms of the disease.

Epidemiological, familial and twin studies provide evidence for a component of hereditability of liver fat content and NAFLD [21, 22]. Indeed, NAFLD is more prevalent in Hispanics compared to Europeans, and less common in African-Americans, and this difference is not explained by diabetes and obesity [22, 23]. Family studies demonstrated a strong heritability of NASH [23, 24, 25]. Accordingly, twin studies shown that, in subjects without viral hepatitis and alcohol abuse, alanine aminotransferases (ALT) levels and liver fat content are strongly heritable traits, with genetic factors explaining up to 60% of variability [26, 27]. Overall, evidences indicate that about half of steatosis variability, determined by biochemical indices or noninvasive assessment of liver fat, is inherited [1, 3]. Therefore, several hypothesis-driven studies tried to evaluate the role of candidate genetic variants in the susceptibility to NAFLD and progressive NASH, with the goal of identifying disease markers or potential drug targets, but with inconsistent results [1].

PNPLA3 I148M IS A MAJOR DETERMINANT OF FATTY LIVER
In 2008, two independent genome-wide association studies linked the common rs738409 polymorphism of PNPLA3 (I148M) with hepatic fat content and ALT levels [18, 19]. In particular, a genomewide scan of the associa-
Equilibrium: Hepatocellular fat content
0%–5%

Equilibrium: Hepatocellular fat content
6% →

Macrovesicular steatosis

Figure 1  Hypothetical mechanism of hepatic fat accumulation associated with the 148 Isoleucine to Methionine protein variant patatin-like phospholipase domain-containing 3 polymorphism. ER: Endoplasmic reticulum; LPA: Lyso-phosphatidic acid; PA: Phosphatidic acid; Tg: Triglycerides; ?: To be confirmed; PNPLA3: Patatin-like phospholipase domain-containing 3.

ASSOCIATION OF PNPLA3 I148M WITH PROGRESSIVE FIBROSIS IN STEATOHEPATITIS

Even if steatosis severity is a risk factor for NASH and progressive disease in NAFLD [58], the association is not invariable, and hepatocellular fat is believed to represent a critical factor determining hepatic fat accumulation when stressing factors such as increased flux of free fatty acids related to adipose tissue insulin resistance in visceral obesity, increased lipogenesis stimulated by hyperinsulinemia and carbohydrates, or altered lipid metabolism intervene.

FUNCTION OF WILD-TYPE AND MUTANT PNPLA3

PNPLA3, also called adiponutrin, encodes a 481 amino acid protein with a molecular mass of approximately 53 kDa that in humans is mainly expressed in intracellular membrane fractions in hepatocytes [48], and is induced in the liver after feeding and during insulin resistance by the master regulator of lipogenesis, Steroid Regulatory Element Binding Protein-1c [49].

Wild-type (148I) PNPLA3 has lipolytic activity towards triglycerides [48,50]. The I148M mutation determines a critical aminoacidic substitution next to the catalytic domain, likely reducing the access of substrates and reducing the PNPLA3 enzymatic activity towards glycerolipids, thereby leading to the development of macrovesicular steatosis [48,50]. However, other reported a gain of lipogenic function associated with the I148M variant, which would acquire the ability to synthesize phosphatidic acid from lysophosphatidic acid [51]. In addition, results deriving from murine models gave contradictory results [52-55]. The issue of the functional consequences of the I148M polymorphism is therefore still intensively debated, and it may be hypothesized that PNPLA3 has additional physiological substrates. Human studies have also suggested a possible direct or indirect influence of PNPLA3 genotype on adipose tissue biology [56,57], which however awaits replication.

A model depicting hypothetical mechanisms of hepatic fat accumulation associated with the I148M PNPLA3 polymorphism is shown in Figure 1.
Table 1. Studies evaluating the association between the 148 Isoleucine to Methionine protein variant of patatin-like phospholipase domain-containing 3 polymorphism and liver fibrosis in patients affected by chronic hepatitis C virus infection, a leading cause of liver related mortality in Western countries

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Patients</th>
<th>Outcome</th>
<th>OR 95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valentí et al</td>
<td>Cross-sectional</td>
<td>819</td>
<td>Cirrhosis</td>
<td>1.5 1.2-1.9</td>
</tr>
<tr>
<td>Müller et al</td>
<td>Cross-sectional</td>
<td>605</td>
<td>Cirrhosis</td>
<td>2.8 1.2-6.2</td>
</tr>
<tr>
<td>Trépo et al</td>
<td>Cross sectional</td>
<td>537</td>
<td>Fibrosis stage</td>
<td>3.1 1.5-6.5</td>
</tr>
<tr>
<td></td>
<td>prospective</td>
<td></td>
<td>Fibrosis progression</td>
<td>2.6 1.2-5.7</td>
</tr>
</tbody>
</table>

more a epiphenomenon of insulin resistance and altered lipid metabolism than the key driver underpinning liver damage progression. Indeed, accumulation of neutral lipids in cytoplasmic droplets is now retained to represent a protective response towards the increased burden of hepatotoxic free fatty acids and other lipids[69]. Therefore, the first question arising after the discovery of PNPLA3 genotype as the major determinant of hepatic fat content, was whether the I148M polymorphism decreased liver damage favoring accumulation of fatty acids in lipid droplets or conversely increased the susceptibility to develop progressive NASH and fibrogenesis.

The answer came soon, as the I148M allele was linked with NASH[65], and our group first reported that homozygosity for the 148M allele was associated with an 3.3-fold increased risk of both NASH and liver fibrosis in two independent cohorts of European subjects with histological NAFLD[35]. The association between PNPLA3 I148M and the severity of fibrosis in NAFLD was almost contemporarily replicated by independent groups in adults[34,35] and in the pediatric population[36], and confirmed by a recent meta-analysis[37].

ALD shares many pathophysiological features with NAFLD[38], most notably steatohepatitis being the key driver of fibrogenesis and liver damage progression. Indeed, candidate gene studies demonstrated that the I148M polymorphism is also strongly associated with the risk of developing ALD and with the susceptibility towards cirrhosis in alcohol abusers of different ethnic groups[60-62]. In one study in German subjects, the I148M polymorphism alone explained as much as 26% of cirrhosis variability in alcohol abusers[39]. Furthermore, the earlier the age of the increase at risk alcohol intake the stronger the effect of the PNPLA3 148M mutation has on the cirrhosis susceptibility[33].

PNPLA3 I148M AND CHRONIC HCV DISEASES

The next natural question was clearly whether PNPLA3 genotype represents a modifier of progression of other liver diseases in which steatosis plays a key role in the pathogenesis. Chronic hepatitis C virus (CHC), a leading cause of end stage liver disease and hepatocellular carcinoma in many Western countries[40], is frequently characterized by steatosis, occurring in more than half of patients. The presence of steatosis has been associated with more aggressive histological features, faster progression of fibrosis, and poorer response to therapy[30,31]. Hepatic steatosis favors hepatitis C virus (HCV) life-cycle[32], and both viral and host factors are believed to contribute to its pathogenesis[76,77,78]. It became soon clear that the I148M polymorphism is a major determinant of the susceptibility to steatosis in also CHC, in particular in patients not infected by genotype 3 strains that per se strongly induces steatosis by altering very low density lipoproteins export[74,75]. This model is also consistent with the recent finding of a nonsense mutation of APOB in humans causing hypo-beta-alipoproteinemia and a massive history of severe steatosis associated with development of hepatocellular carcinoma in carriers of this mutation[79].

Studies that specifically evaluated the association between the I148M PNPLA3 variant and fibrosis progression in CHC are reported in Table 1. As in NAFLD and ALD, the effect of the 148M mutation was not limited to predisposition to steatosis, but extended towards progressive fibrosis and cirrhosis development[76,77,78]. Interestingly, the size effect of the association of the I148M polymorphism with fibrosis appeared larger in subjects with at risk alcohol intake (> 30 g/d in males/females)[76,78], suggesting the existence of an interaction between different triggering factors and PNPLA3 genotype in fibrogenesis. Furthermore, genetic factors influencing immunological response towards HCV, i.e., IL28B region polymorphisms, may influence the association between PNPLA3 and steatosis[80].

We could speculate that when steatosis inducing stressors such as obesity and insulin resistance, excess alcohol intake, and HCV infection stress the liver, in the presence of the “normal” 148I PNPLA3 allele the damage will result in simple uncomplicated steatosis, whereas the 148M “at risk” allele will favor steatohepatitis and fibrogenesis, with progression towards cirrhosis and its complication in susceptible individuals[80].

Finally, some studies indicate that during treatment with peg-interferon plus ribavirin the I148M polymorphism may affect sustained virological response i.e., cure rate[76] and viral kinetics[80], especially in difficult to cure CHC patients with advanced fibrosis[79]. However, the clinical impact of PNPLA3 on the response to therapy will likely be modest in the new era of direct antiviral agents[79].

PNPLA3 I148M IN OTHER LIVER DISEASES

Having established that the I148M polymorphism is a modifier of the natural history of liver diseases associated with steatosis, i.e., NAFLD, ALD and CHC, the possible role of I148M in determining the susceptibility to steatohepatitis and progressive liver damage in other liver diseases is becoming the subject of investigation.

As it affects more than 350 million people worldwide
and is a leading cause of liver-related mortality \cite{84}, chronic hepatitis B virus (CHB) infection represented the next disease in which the role of PNPLA3 genotype had to be understood. Steatosis is indeed commonly observed also in CHB, and overall evidence suggests that it contributes to fibrosis progression \cite{85-88}. Recent data from our group obtained in a relatively large cohort of European CHB patients with histological evaluation of liver damage indicate that the 148M variant predisposes to steatosis \cite{89}. In patients with overweight or a positive history of alcohol intake, the I148M polymorphism predisposes also to severe steatosis, which in this population was associated with more severe fibrosis \cite{89}. Additional studies will be required to test the interaction between the I148M genetic variant and acquired risk factors in the pathogenesis of progressive fibrosis and on related clinical outcomes also in CHB infection.

Hereditary hemochromatosis represents another interesting disease model. In fact, in a homogeneous genetic background in subjects homozygous for the C282Y mutation of the HFE gene, activation of fibrogenesis is caused by progressive hepatocellular iron overload via generation of oxidative stress \cite{90,91} in the presence of precipitating factors, among which steatosis has a major role \cite{92}. In a large series of Italian HFE C282Y homozygous patients with hemochromatosis, we showed that the I148M polymorphism is a strong predictor of the presence of steatosis and higher liver enzymes levels, and it is also associated with the severity of fibrosis \cite{93}. A possible interaction with other genetic forms of liver disease (i.e., Wilson disease) may also be hypothesized and studies on this topic would help understanding the whole picture of PNPLA3 gene interaction with liver stressors.

Finally, it has been reported that in primary sclerosing cholangitis, an autoimmune cholestatic liver disease characterized by inflammatory changes of major bile ducts, the 148M PNPLA3 variant is associated with increased mortality \cite{94}. The effect of PNPLA3 genotype was evident in the subgroup of patients with severe disease, i.e., males with stenosis of the main duct, but unfortunately it could not be determined whether the association was mediated by steatosis and faster progression of fibrosis.

Although much work has clearly yet to be done in these and many other forms of liver damage, collectively these initial studies suggest that PNPLA3 I148M is a promising candidate general modifier of fibrogenesis in liver diseases.

**ROLE OF PNPLA3 IN FIBROGENESIS**

Intriguingly, the association between the I148M polymorphism and NASH appears to be independent of the severity of steatosis \cite{31}, thus suggesting that this genetic variant not only influences the overall amount of hepatocellular fat, but by impacting on the concentration and subcellular localization of specific lipid species directly
modulates inflammation. It should not be forgotten that several lipids behave as inflammatory mediators acting through specific receptors[5]. Alternatively, if the I148M mutation slows down triglycerides kinetics between cell compartments, it could be speculated that renders them more susceptible to lipoperoxidation, leading to oxidative stress, and in turn to hepatocellular damage and inflammation[6]. These hypothetical mechanisms linking the PNPLA3 I148M mutation with hepatic fibrogenesis are shown in Figure 2. In the aforementioned scenario, shown in panel A, hepatocellular damage and the release of inflammatory mediators would lead to secondary activation of Kupffer cells amplifying the inflammatory cascade and cell death, and of hepatic stellate cells with initiation of fibrogenesis.

Even more striking is though the observation that patients the I148M polymorphism is associated in NAFLD with advanced fibrosis independently of NASH[7], and in CHC with cirrhosis independently of steatosis, ALT levels, and hepatic necroinflammatory activity[8]. It could therefore be envisioned that the I148M mutation also directly influences the activation of non-parenchymal hepatic cells in response to hepatotoxic insults, as shown in Figure 2B. This hypothesis needs to be addressed and the potential mechanisms investigated by experimental studies.

PNPLA3 I148M AND HEPATOCELLULAR CARCINOMA

Last but not least, evidence is also accumulating that the I148M polymorphism predisposes to hepatic carcinoma, a common complication of cirrhosis and the fifth cause of cancer worldwide, with a clinically significant increment in risk, thereby representing a potentially useful biomarker[9-11]. We have recently reviewed elsewhere the clinical studies supporting such an association and the potential mechanisms involved[12]. To summarize, data indicate that the I148M PNPLA3 mutation favors hepatic carcinogenesis in steatohepatitis as well as in other liver diseases, and the mechanism is partly independent of the predisposition towards fibrogenesis and cirrhosis.

CONCLUSION

In conclusion, PNPLA3 is a novel key player in liver disease progression. Assessment of the I148M polymorphism will possibly inform clinical practice in the future, whereas the determination of the physiological role of wild-type PNPLA3 and the I148M variant will reveal mechanisms involved in hepatic fibrogenesis and carcinogenesis and hopefully identify novel therapeutic targets.

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