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The PNPLA3 I148M variant and chronic liver disease: When a genetic mutation meets nutrients

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ABSTRACT

The isoleucine to methionine substitution at position 148 in the patatin-like phospholipase domain containing 3 protein (PNPLA3; I148M variant, rs738409) is associated with liver fat accumulation and an increased risk of chronic liver disease ranging from hepatitis to hepatocellular carcinoma. This review discusses the interaction between the PNPLA3 I148M variant and obesity/intake of specific nutrients in determining the susceptibility to liver disease. We present the results of several studies showing that obesity or alcohol abuse enhances the effect of the PNPLA3 I148M variant on the liver. We also show that specific nutrients interact with the PNPLA3 I148M variant in modulating liver disease susceptibility.

1. Introduction

The accumulation of fat in the liver is a pathologic condition also known as hepatic steatosis. Hepatic steatosis is the most common chronic liver disorder in Western countries affecting up to one third of the population in the United States of America (Browning, Szczepaniak, Dobbins, Nuremberg, Horton, Cohen, et al., 2004). In some individuals, hepatic steatosis is associated with liver inflammation, which may progress to more serious liver disorders such as cirrhosis and hepatocellular carcinoma (HCC) (Adams, Lymp, St Sauver, Sanderson, Lindor, Feldstein, et al., 2005).

In 2008, a genome-wide association study in a population comprising Hispanic, African American and European American subjects showed that a common genetic variant in the patatin-like phospholipase domain containing 3 protein (PNPLA3) gene is associated with liver fat accumulation (Romeo, Kozlitina, Xing, Pertsemlidis, Cox, Pennacchio, et al., 2008). The substitution of isoleucine to methionine at position 148 (I148M, rs738409) in the PNPLA3 protein was the first variant robustly associated with an increased risk for hepatic steatosis. Many other studies in different populations have since confirmed this association and shown an enrichment of the mutant PNPLA3 148M allele in subjects with chronic liver disorders ranging from liver inflammation to cirrhosis and HCC, suggesting that the allele may be responsible for this progression (Corradini, Burza, Molinaro, & Romeo, 2011; Sookoian & Pirola, 2011; Valenti, Al-Serri, Daly, Galmozzi, Rametta, Dongiovanni, et al., 2010).

Excess intake of calories (i.e. obesity), alcohol and specific nutrients (e.g. fructose) are well-established risk factors for hepatic steatosis (Becker, Deis, Sorensen, Gronbaek, BorchJohnsen, Muller, et al., 1996; Bedogni, Miglioli, Masutti, Tiribelli, Marchesini & Bellentani, 2005; Ouyang, Cirillo, Sautin, McCall, Bruchette, Diehl, et al., 2008). However, the individual response to such stimuli is extremely variable (Daly, Ballestri, Carulli, Loria, & Day, 2011). In this review, we examine the interplay between the PNPLA3 148M variant and nutrients, which explains a proportion of the individual susceptibility to diet-induced hepatic steatosis (Table 1) and represents a paradigm of nutrigenetics.

2. Obesity

Obesity is a major risk factor for hepatic steatosis and inflammation (Anstee, Targher, & Day, 2013; Bedogni et al., 2005). In a cohort of 678 obese Italian individuals with a mean body-mass index of 41 kg/m^2, we tested the hypothesis that excess body weight would expose the association of the PNPLA3 I148M variant with liver damage and found that carriers of the 148M allele had higher serum transaminase levels

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Table 1
Risk factors interact with the PNPLA3 I148M variants in determining the risk for chronic liver disease.

Risk factor	Outcome of risk factor — 148M PNPLA3 interaction
Obesity	Higher serum transaminases, nonalcoholic liver disease, HCC
Alcohol	Alcoholic liver disease, HCC
Carbohydrates	Hepatic retention of newly synthesized triglycerides
Fatty acids	Reduced amount of circulating oleic acid
Iron	Higher serum transaminases and liver steatosis in subjects with hemochromatosis

(Romeo, Sentinelli, Dash, Yeo, Savage, Leonetti, et al., 2010). An increase in serum transaminases is a common sign of hepatic steatosis, indicating liver damage and release of liver enzymes into the blood (Amacher, 1998). The hypothesis that obesity enhances the effect of the PNPLA3 I148M variant on liver fat was subsequently supported by other studies showing that obese carriers of the PNPLA3 variant are at higher risk of developing hepatic steatosis (Petit, Guiu, Masson, Duvillard, Jooste, Buffier, et al., 2010; Valenti, Alisi, Galmozzi, Bartuli, Del Menico, Alterio, et al., 2010). In a Chinese cohort with nonalcoholic steatohepatitis, Li et al. showed that body-mass index interacts with the PNPLA3 I148M variant to promote increased transaminase levels (Li, Xing, Tian, & Ku, 2012), confirming the interaction between the PNPLA3 variant and the degree of obesity.

Long-term interaction between chronic stressors and genetic background may promote permanent damage and diseases. When obesity develops during childhood, it is associated with future increased risk of developing obesity-related comorbidities, including chronic liver disease (Berentzen, Gamborg, Holst, Sørensen, & Baker, 2013; Freedman, Khan, Serdula, Dietz, Srinivasan & Berenson, 2004, 2005). Of note, in carriers of the 148M variant, an excess in adiposity in children determines liver damage early in life (Lin, Chang, Hu, Yang, Chang & Ni, 2011; Romeo et al., 2010; Santoro, Kursawe, D'Adamo, Dykas, Zhang, Bale, et al., 2010), suggesting that obese young 148M carriers will be exposed to chronic liver damage throughout their lives.

Body fat distribution between the subcutaneous and visceral compartments is an important determinant of obesity metabolic complications including liver fat accumulation. Waist-to-hip ratio is commonly used in clinical studies as a proxy for abdominal fat. A study in obese Italian children showed that the extent of the association between the PNPLA3 I148M variant and liver damage depends on the abdominal fat accumulation measured as waist-to-hip ratio (Miraglia Del Giudice, Grandone, Cirillo, Santoro, Amato, Brienza, et al., 2011). Moreover, another study performed in a European American population showed that visceral adipose tissue measured by computed tomography interacts with the PNPLA3 I148M variant in determining liver fat accumulation (Graff, North, Franceschini, Reiner, Feitosa, Carr, et al., 2013). Interestingly, the interaction between abdominal fat and PNPLA3 I148M in the pathogenesis of fat accumulation has also been shown in an Italian cohort in a liver disease of different etiology, namely chronic hepatitis C (Zampino, Coppola, Cirillo, Boemio, Pisaturo, Marrone, et al., 2013).

Steatosis of the liver may progress to inflammation and fibrosis with profound disruption of the normal architecture and function of the liver leading to cirrhosis. Results from a cohort of 266 Argentinean subjects have shown that carriers of the 148M allele have higher risk of liver inflammation evaluated by liver biopsy (Sookoian, Castaño, Burgueño, Gianotti, Rosselli & Pirola, 2009). We also examined the hepatic consequences of carrying the 148M allele in adults and children with nonalcoholic fatty liver. We showed that a greater steatosis severity and signs of liver fibrosis and inflammation were present in carriers of the 148M allele (Valenti, Alisi, et al., 2010; Valenti, Al-Serri, et al., 2010).

From a clinical perspective, chronic liver damage may evolve into HCC, which represents the end of the natural history of chronic liver disease. To examine the long-term effect of the interaction between obesity and the PNPLA3 I148M variant, we studied a cohort from the Swedish Obese Subjects (SOS) study at a 15-year follow-up (Burza, Pirazzi, Maglio, Sjöholm, Mancina, Svensson, et al., 2012). The cohort comprised: 1754 individuals who had undergone bariatric surgery and experienced subsequent sustained weight loss and amelioration of metabolic parameters in the follow-up; and 1719 individuals who had not undergone surgery and thus constituted the control group characterized by persistent morbid obesity. At the 15-year follow-up, 5 cases of HCC were observed in the control group and 4 in the surgery group. The PNPLA3 148M allele was associated with increased HCC risk only in the SOS control group (hazard ratio 5.9; 95% confidence interval 1.5–24; P value = 0.01). It is worth nothing that all the subjects who developed HCC in the control group did not have other known risk factors for liver disease and 4 of them did not have a diagnosis of cirrhosis. Weight loss is known to reverse liver fat accumulation and thus prevent the evolution to more serious liver disease. In the SOS surgery group, weight loss suppressed the association between the I148M variant and HCC risk (hazard ratio 1.2; 95% confidence interval 0.2–6.3; P value = 0.80) (Burza et al., 2012). Moreover, we also showed that bariatric surgery in obese adults abolishes the association of the PNPLA3 genetic variant with liver damage (Palmer, Maglio, Pirazzi, Burza, Adiels, Burch, et al., 2012). This is also supported by a greater liver fat reduction (measured by magnetic resonance imaging) after weight loss in individuals homozygous for the 148M allele compared to 148I homozygous subjects (Sevastianova, Kotronen, Gastaldelli, Perttilä, Hakkarainen, Lundbom, et al., 2011). This finding underlines the importance of the PNPLA3 I148M variant as a risk factor for liver disease in obese individuals.

In conclusion, excessive caloric intake resulting in obesity is a major determinant of chronic liver disease particularly in carriers of the PNPLA3 148M allele. Importantly, this association is reversible after weight loss.

3. Alcohol intake

Alcohol intake is a well-established risk factor for chronic liver disease. The progression from alcoholic damage to hepatitis and fibrosis is mainly dependent on environmental factors (e.g. age at onset, duration of abuse, quantity of at-risk alcohol intake) and on genetic background (Bedogni et al., 2005; Bellentani, Saccoccio, Costa, Tiribelli, Manenti, Sodde, et al., 1997; Stickel & Hampe, 2012). Alcohol also uncovers the liver disease risk conferred by the PNPLA3 variant. A study in a cohort of Hispanics from Mexico City showed that the 148M allele is associated with an increased risk of developing alcoholic liver disease and cirrhosis (Tian, Stokowski, Kershenobich, Ballinger, & Hinds, 2010). This association has been confirmed in Caucasians (Burza, Molinaro, Attilia, Rotondo, Attilia, Ceccanti, et al., 2013; Seth, Daly, Haber, & Day, 2010; Stickel, Buch, Lau, Zu Schwabedissen, Berg, Ridinger, et al., 2011; Trépo, Gustot, Degré, Lemmers, Verset, Demetter, et al., 2011). PNPLA3 148M allele carriers with alcoholic cirrhosis also show an increased risk of developing HCC (Guyot, Sutton, Rufat, Laguillier, Mansouri, Moreau, et al., 2013; Nischalke, Berger, Luda, Berg, Müller, Grünhage, et al., 2011; Valenti, Dongiovanni, Ginanni Corradini, Burza, & Romeo, 2013), which was recently confirmed by a meta-analysis including a total of 1374 individuals with cirrhosis caused by excessive alcohol intake (Trépo, Nahon, Bontempi, Valenti, Falleti, Nischalke, et al., accepted for publication). In this meta-analysis, the risk of developing HCC was increased over two fold for each PNPLA3 148M allele.

Interestingly, age of onset of the alcohol abuse also seems to change the risk conferred by the PNPLA3 148M mutation. We recently studied a

cohort of 384 Italian at-risk alcohol drinkers and showed that the PNPLA3 148M allele was associated with a three-fold increased risk for alcoholic cirrhosis in subjects who started the harmful alcohol consumption before age 24 compared to those who started after age 24 (Burza et al., 2013).

Moreover in subjects with chronic hepatitis C, the 148M variant exerts a different effect on the liver according to the alcoholic behavior. Specifically research in Caucasians has shown that the PNPLA3 I148M variant influences steatosis susceptibility in individuals with chronic hepatitis C who drink less than 30 g alcohol/day but not in at-risk drinkers (>30 g alcohol/day); however, the PNPLA3 variant associates with cirrhosis risk in both groups (Müller, Buch, Berg, Hampe, & Stickel, 2011; Valenti, Colombo, Fargion, et al., 2011).

In conclusion, excessive alcohol intake is a harmful condition for the liver that should be discouraged in general and especially in individuals carrying the PNPLA3 148M allele.

4. Nutrients

4.1. Macronutrients

Excessive intake of carbohydrates, and in particular simple sugars (e.g. fructose), contributes to fat accumulation in the hepatocytes (Ouyang et al., 2008). In fact, simple sugars are an ideal substrate for the synthesis of short-chain fatty acids, a process known as de novo lipogenesis (Acheson, Flatt, & Jéquier, 1982). Several studies have shown evidence of an interaction between the PNPLA3 I148M variant and carbohydrate intake in determining liver fat accumulation. In a cohort of 153 Hispanic children, those homozygous for the PNPLA3 148M allele showed a positive correlation between carbohydrate intake and hepatic fat fraction measured by magnetic resonance, while there was no association in carriers of the PNPLA3 148I wild-type allele (Davis, Lê, Walker, Vikman, Spruijt-Metz, Weigensberg, et al., 2010). In an interventional study in which 16 Caucasian adults (7 wild-type and 9 homozygous for the PNPLA3 148M allele) received a high-carbohydrate diet for 3 weeks, the wild-type individuals showed an expected positive correlation between lipogenic index (measured by palmitic acid/linoleic acid ratio in plasma very low-density lipoproteins) and liver fat content. However, such a correlation was not present in individuals homozygous for the 148M allele, suggesting that the PNPLA3 I148M variant induces hepatic retention of the de novo synthesized triglycerides (Sevastianova, Santos, Kotronen, Hakkarainen, Makkonen, Silander, et al., 2012). Several studies have shown that the PNPLA3 I148M variant indeed affects hepatic very low-density lipoprotein secretion and consequently plasma triglyceride levels (Krarup, Grarup, Banasik, Friedrichsen, Færch, Sandholt, et al., 2012; Krawczyk, Gruenhage, Mahler, Tirziu, Acalovschi & Lammert, 2011; Palmer et al., 2012; Pirazzi, Adiels, Burza, Mancina, Levin, Ståhlman, et al., 2012). Taken together, these data indicate the presence of an interaction between carbohydrate intake and the PNPLA3 I148M variant on liver fat retention.

Fatty acids are present in the fatty liver as the side chains esterifying the glycerol backbone in triglycerides. Fatty acids are classified as saturated, mono-unsaturated and poly-unsaturated, based on the presence and the number of double bonds in their carbon chain. Moreover, the last carbon atom involved in double bonds represents a marker for the current fatty acid classification (e.g., if the last carbon involved in a double bond is the third last of the chain, the fatty acid will be an ω-3) (Kalish, Fallon, & Puder, 2012). In a recent study, fatty acid composition of circulating triglycerides was measured in 372 Finnish individuals genotyped for the PNPLA3 I148M variant. Carriers of the PNPLA3 148M allele showed reduced amounts of oleic acid (18:1 ω-6, the best PNPLA3 substrate in vitro (Huang, Cohen, & Hobbs, 2011)) compared to wild-type individuals (Hyysalo, Gopalacharyulu, Bian, Hyötyläinen, Leivonen, Jaser, et al., 2014). In another study in Italian children, treatment efficacy of hepatic steatosis using docosahexaenoic acid (22:6 ω-3) was affected by the PNPLA3 I148M genotype. Notably, the presence of the PNPLA3 148M allele worsened the treatment response (Nobili, Bedogni, Donati, Alisi, & Valenti, 2013). Interestingly, in an independent pediatric cohort, ω-6/ω-3 fatty acid intake ratio was positively correlated with liver fat content only in individuals homozygous for the 148M allele (Santoro, Savoye, Kim, Marotto, Shaw, Pierpont, et al., 2012). These results indicate that the PNPLA3 148M variant interacts with unsaturated fatty acids in determining liver fat retention.

4.2. Micronutrients

Iron is a micronutrient with a major role in hematopoiesis (Chung, Chen, & Paw, 2012). However, iron overload is harmful for the liver. Hereditary hemochromatosis is a genetic disease characterized by progressive accumulation of iron mainly in the liver leading to liver fibrosis and cirrhosis (Pietrangelo, 2010). Although the genetic determinant of hemochromatosis is well established, the individual progression of the liver damage is extremely variable. We examined a cohort of 174 subjects affected by hereditary hemochromatosis and showed that the PNPLA3 I148M variant is associated with steatosis and severity of fibrosis also in this genetic disorder (Valenti, Maggioni, Piperno, Rametta, Pelucchi, Mariani, et al., 2012). These results show that a common genetic variant may explain the phenotypic variability in the context of a monogenic disorder.

5. PNPLA3 protein function: a short overview

PNPLA3 function and the effect of the I148M substitution are not yet completely understood. Purified PNPLA3 protein has been shown to have both hydrolase and lysophosphatidic acid transacetylase activities, with the hydrolase activity predominating (Huang et al., 2011; Kumari, Schoiswohl, Chitraju, Paar, Cornaciu, Rangrez, et al., 2012; Pingitore, Pirazzi, Mancina, Motta, Indiveri, Pujia, et al., 2013). The 148M mutation has been associated with a loss of function of hydrolase activity (Huang et al., 2011; Pingitore et al., 2013) and a gain of function of lysophosphatidic acid transacetylase activity (Kumari et al., 2012). Consistent with a hydrolase activity hypothesis, a relative reduction in hepatic very low-density lipoprotein secretion with the 148M mutation has been shown in in vitro studies and in humans (Pirazzi et al., 2012). Furthermore, in a transgenic mouse model, PNPLA3 148M overexpression induced increased formation of fatty acids and triglycerides, impaired hydrolysis of triglycerides, and depletion of long-chain polyunsaturated fatty acids (Li, Huang, Karaman, Ivanova, Brown, Roddy, et al., 2012). On the other hand, wild-type PNPLA3 was also found to have lysophosphatidic acid transacetylase activity and the 148M mutation was found to be a gain of function of the lipogenic activity (Basantani, Sitnick, Cai, Brenner, Gardner, Li, et al., 2011). In agreement, rats on a high-fat diet treated with pnpla3 antisense oligonucleotides showed decreased fatty acid esterification into hepatic triglycerides and reduced insulin resistance (Kumashiro, Yoshimura, Cantley, Majumdar, Guebre-Egziabher, Kursawe, et al., 2013). In a recent study of obese adolescents with hepatic steatosis, the I148M variant has been shown to modulate the association between oxidized metabolites derived from linoleic acid and cytokeratin 18 fragment, a robust biomarker of liver injury (Santoro, Caprio, Giannini, Kim, Kursawe, Pierpont, et al., 2014). However, further studies are needed to fully understand the molecular genetics of the PNPLA3 protein.

6. Conclusions

The interaction between the PNPLA3 I148M variant and excessive intake of food, alcohol or specific nutrients reveals that the effect of the stimuli determining changes in liver fat is enhanced in carriers of the PNPLA3 148M allele. Future studies are warranted to understand the complex interplay between the whole genome variability and nutrients.

In conclusion, the PNPLA3 I148M variant interacts with a wide range of nutrients in determining chronic liver disease. In a future perspective, this may possibly lead to a specific genetic-tailored diet to reduce fatty liver.

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