**PNPLA3-Associated Steatohepatitis: Toward a Gene-Based Classification of Fatty Liver Disease**

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**Abstract**

Nonalcoholic fatty liver disease is one of the most common hepatic disorders worldwide. Given the high-calorie nutrition of children and adults, nonalcoholic fatty liver disease (NAFLD) is expected to become a major cause of cirrhosis and eventually liver transplantation. Familial clustering and ethnic differences indicate that genetic factors contribute to NAFLD. Recently, the common variant p.I148M of the enzyme adiponutrin (PNPLA3) has emerged as a major genetic determinant of hepatic steatosis and nonalcoholic steatohepatitis as well as its pathobiological sequelae fibrosis, cirrhosis, and hepatocellular cancer. PNPLA3 encodes a lipid droplet-associated, carbohydrate-regulated lipogenic and/or lipolytic enzyme. Homozygous carriers of the PNPLA3 variant are prone to develop cirrhosis in the absence of other risk factors such as alcohol or viral hepatitis. Here we review the plethora of studies that unraveled the association between PNPLA3 and NAFLD in children and adults, discuss its distinct effects on liver and metabolic traits, and introduce the term PNPLA3-associated steatohepatitis (PASH) as a novel gene-based liver disease. Given the prevalence of the risk allele in 40 to 50% of Europeans, the authors conclude that PNPLA3 should be considered in the diagnostic workup of fatty liver disease and that homozygous risk allele carriers might benefit from careful cancer surveillance.

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To date, fatty liver disease is one of the most common diseases worldwide. According to the latest surveys as much as 21% of adults in the United States may suffer from fatty liver disease—more than 30 million patients nationwide. The most recent data on the burden of liver disease by the European Association for the Study of the Liver (EASL) indicate that nonalcoholic fatty liver disease (NAFLD) is emerging as the most common hepatic disorder and is likely to become a major cause for liver transplantation in Europe. Depending on the survey, the prevalence of NAFLD ranges from 2% to 44%, but the prevalence of this condition among patients with type 2 diabetes can be as high as 70%. Often detected by abdominal ultrasonography in individuals without any apparent liver disease who do not consume excessive amounts of alcohol, fatty liver is often considered to be simply one of the “fellow travelers” of the obesity pandemics, given its epidemiological associations with metabolic syndrome and diabetes. However, an estimated 3 to 12% of the population suffer from the progressive form of NAFLD known as nonalcoholic steatohepatitis (NASH)...

According to the latest guidelines, the term nonalcoholic fatty liver disease covers several types of disorders, which may be benign or more severe in nature. In brief, NAFLD subdivides into nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH), and NASH-cirrhosis. Nonalcoholic fatty liver disease comprises simple hepatic steatosis without any evidence of hepatic damage. According to current paradigms, patients with NAFL have only a small risk of liver disease progression. Nonalcoholic steatohepatitis in turn represents a progressive form of NAFLD characterized by chronic hepatocellular injury and inflammatory responses, which might...
progress to liver fibrosis, cirrhosis, and hepatocellular cancer (HCC). If a patient with NASH develops liver cirrhosis, then this condition is termed NASH-cirrhosis. This classification implies that there are two major types of NAFLD: the most common benign NASH type and a defined subgroup of patients with more severe lipid accumulation and inflammation, namely NASH. Indeed, patients with NASH are characterized by reduced overall life expectancy due to liver-related mortality, diabetes, and cardiovascular deaths, which is not generally observed in patients with steatosis. Hence, the early detection of individuals who are at increased risk of developing NASH is pivotal to prevent the progression of liver disease, and also to dissect the subgroup of patients at increased cardiovascular risk. A major problem, however, is that NASH remains asymptomatic for a long period in most patients. Given the lack of adequate imaging techniques and reliable noninvasive markers, liver biopsy is still formally required to detect or exclude the presence of NASH and to adequately grade and stage the liver condition in NAFLD patients.

Lately, a genetic predisposition caused by the frequent variant p.I148M of adiponutrin (PNPLA3) has been established as a strong genetic (i.e., noninvasive) hallmark of fatty liver disease even in the absence of environmental prosteatotic triggers. Moreover this variant has been associated not only with liver injury both in children and adults who suffer from hepatic steatosis, but also with steatosis and fibrosis in other chronic liver diseases. Here we present studies that established PNPLA3 as a genetic determinant of fatty liver, and we introduce the term PNPLA3-associated steatohepatitis (PASH) as a novel gene-based term for this type of fatty liver disease.

**PNPLA3 (Adiponutrin) Variant and Fatty Liver Disease**

Adiponutrin, the enzyme encoded by the PNPLA3 gene, is a 481-amino acid member of the patatin-like phospholipase domain-containing family (PNPLA). This domain was originally discovered in lipid hydrolases of potato and named after the most abundant protein of the potato tuber, patatin. However, because several family members are not phospholipases, a more appropriate gene symbol has been called for. PNPLA3 is expressed predominantly in the liver, skin, and adipose tissue. In a series of seminal genetic studies, the common nonsynonymous variant p.I148M (rs738409 (G > C)) of the PNPLA3 gene has emerged as the key genetic determinant of NAFLD in adults and pediatric patients. The first genome-wide association study (GWAS) in a large U.S.-based population comprising 2,111 individuals from different ethnic backgrounds demonstrated the p.I148M variant to be associated (p = 5.9 × 10^{-10}) with increased liver fat content on a genome-wide significance level, as determined by 1H-magnetic resonance spectrometry, irrespective of alcohol consumption, body mass index (BMI), and diabetes. This association was most prominent among patients of Hispanic descent, who are in general at a greater risk of developing fatty liver as compared with Caucasians and African Americans. A subgroup analysis restricted to African Americans identified a second PNPLA3 variant (p.S453I, rs6006460, G > T), which is strongly associated with lower hepatic fat contents in this ethnic group.

Interestingly, the common polymorphism p.I148M had been shown previously to correlate with serum activities of liver enzymes. In particular, the analyses of two large population-based cohorts with 12,419 and 61,089 participants, respectively, demonstrated that PNPLA3 polymorphisms are associated with serum alanine aminotransferase (ALT) and γ-glutamyl transpeptidase activities in healthy individuals. The genetic association between the PNPLA3 mutation p.I148M and fatty liver disease was subsequently replicated in many studies. Kotronen et al. investigated the effects of this variant on hepatic fat accumulation in Finnish individuals. In line with the results of the first GWAS, Finnish carriers of the risk allele were characterized by increased hepatic lipid contents (also quantified with proton magnetic resonance spectroscopy) irrespective of age, gender, or BMI.

The PNPLA3 variant was also found to be strongly (p = 4.3 × 10^{-44}) associated with hepatic lipid levels in a GWAS genotyping 2.4 million single-nucleotide polymorphisms (SNPs) in 7,176 individuals whose lipid contents in liver were quantified by another noninvasive method (computed tomography [CT]). Further studies showed that the PNPLA3 variant not only increases the odds of developing fatty liver itself, but it also determines the degree of hepatic injury and all histopathological aspects of NAFLD. A study of the histopathological hallmarks of NAFLD in 103 subjects from Argentina provided evidence that the PNPLA3 risk allele determines NAFLD severity. The comparison of genotype frequencies between individuals with simple steatosis (N = 40) and patients with NASH (N = 63) demonstrated an association between the risk genotype and histopathological disease severity, as assessed by liver biopsy (odds ratio, [OR] = 1.9). A similar analysis was performed in a cohort encompassing 574 Italian and English patients with NAFLD and 179 controls without fatty liver disease. Also in this study the frequencies of the PNPLA3 variant were associated with NAFLD severity as determined by liver biopsy. Here the authors identified not only a higher prevalence of the risk genotype among cases as compared with controls, but the PNPLA3 mutation was strongly associated with the presence of NASH, steatosis grade >1, and fibrosis stage >1 independent of age, BMI, or diabetes. The detailed analysis of histopathological markers of NAFLD was performed by Rotman et al., demonstrating that the PNPLA3 variant is associated with portal (p = 2.5 × 10^{-4}) and lobular (p = 0.005) inflammation, Mallory-Denk bodies (p = 0.02), and fibrosis (p = 7.7 × 10^{-6}). A meta-analysis concluded that the PNPLA3 p.I148M variant is associated with increased risks for fatty liver (OR for homozygous carriers = 3.3; OR for heterozygous carriers = 1.9), NASH (OR for homozygous carriers = 3.1–3.3; OR for heterozygous carriers = 2.7), and fibrosis (OR for homozygous carriers = 3.3; OR for heterozygous carriers = 2.1–2.4) and most remarkably, the association with inflammation and fibrosis is independent of the severity of steatosis.

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Recently, Anstee and coworkers from the European FLIP (Fatty Liver: Inhibition of Progression) Consortium have elegantly confirmed, genotyping candidate genes from association studies in a large cohort with histopathologically characterized liver disease, that the PNPLA3 variant is strongly associated with all histopathological features of NAFLD at genome-wide significance levels. Albeit other polymorphisms in the glucokinase regulator (GCKR) and tribbles homolog 1 (TRIB1) genes represented additional risk factors, only variant PNPLA3 conferred substantial and clinically relevant ORs (3.0–3.1) for the development of steatosis, its transition to NASH, and the progression of fibrosis. In contrast, the other genes identified represent only modifiers of subphenotypes with smaller effect sizes. According to our previous study, the risk allele is present in as many as 40 to 50% of European patients carry at least one copy of the rare allele (M) that is associated with progressive liver disease.

### Association of the PNPLA3 Variant with Progressive Chronic Liver Diseases

The PNPLA3 variant is associated not only with NAFLD, but also increases the odds of severe hepatic phenotypes in patients with other chronic liver diseases. In particular, the PNPLA3 risk allele p.148M is associated with increased inflammation and severe fibrosis as well as cirrhosis in patients with alcoholic liver disease. Similarly, carriers of this allele are also at risk for enhanced increased hepatic steatosis and fibrosis in the setting of chronic hepatitis C virus (HCV) infection, which can be further modulated by the amounts of the intraabdominal fat, and increased steatosis in chronic hepatitis B virus (HBV) infection. Finally, in our previous analysis using transient elastography to quantify liver fibrosis in 899 patients with chronic liver diseases, we identified a prominent association between the PNPLA3 mutation and enhanced liver stiffness in a wide spectrum viral and nonviral chronic liver diseases. Sensitivity analysis showed that the association was present across a broad range of stiffness values (12–40 kPa), indicating that the variant affects not only fibrogenesis, but also cirrhosis severity. In this line, once cirrhosis is present, carriers of the PNPLA3 mutation also have a 2- to 16-fold increased risk of developing HCC (\( \Delta \)). The homozygous p.148M genotype was found to predominantly increase the HCC risk in patients with alcoholic liver disease. In a series of newly diagnosed HCC cases, homozygosity for the genotype p.148M was even an independent risk factor for death. According to Hassan et al, patients with this PNPLA3 genotype displayed reduced median survival (16.8 mo) in comparison to carriers of the wild-type allele (25.9 mo). Furthermore, a recent report by the European FLIP consortium showed that the cancer risk of homozygous PNPLA3 mutation carriers is 15-fold higher in comparison to the general population in the United Kingdom.

The latest reports investigated the association between PNPLA3 and liver status after transplantation. An early analysis of 176 subjects who were transplanted for HCV-cirrhosis did not identify any association between the recipient PNPLA3 genotype and the presence of advanced (F3) fibrosis (as determined by liver biopsy) 1, 3, and 5 years after transplantation. However, in this study the donor PNPLA3 genotypes were not determined. Finkenstedt et al in turn analyzed PNPLA3 genotype frequencies in both transplant recipients \((N = 237)\) and donors \((N = 255)\). Interestingly, homozygous carriers of the risk genotype were 14 times more likely to develop graft steatosis independently of other prosteatotic triggers (age, underlying disease, weight gain). Here, the donor genotype did not affect the development of graft steatosis, but further studies are required to dissect the specific effects of variant PNPLA3 after liver transplantation.

### Studies in Pediatric Cohorts

Liver steatosis is emerging as an additional health problem in children as well. In line with the studies in adult patients, significant association between fatty liver and the PNPLA3 mutation was observed in children with NAFLD, too. In the study by Valenti et al, 149 children with biopsy-proven NAFLD were included. The analysis of PNPLA3 genotypes in this cohort showed that the variant was not associated with adiposity, BMI, insulin resistance, lipid profile, or serum AST activities. However, the PNPLA3 variant determined the degree of hepatic steatosis. Moreover, children homozygous for the mutated allele were at highest risk of developing NASH: In the investigated cohort all children \((N = 23)\) who were homozygous carriers of the PNPLA3 mutation were diagnosed with NASH. The association between the PNPLA3 mutation and pediatric NAFLD has also been detected in Caucasian and African American, Taiwanese, and to a
Table 1 Selected studies investigating association of the PNPLA3 variant with hepatic and metabolic phenotypes (in chronological order)

<table>
<thead>
<tr>
<th>Phenotypic parameter</th>
<th>Number of individuals</th>
<th>Population / ethnicity</th>
<th>OR (95% CI)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum levels of liver enzymes&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>12,419</td>
<td>Indian Asian, European</td>
<td>OR = 8.4 × 10&lt;sup&gt;-16&lt;/sup&gt;</td>
<td>16</td>
</tr>
<tr>
<td>NAFLD&lt;sup&gt;a&lt;/sup&gt; (1H-MRS)</td>
<td>2,111</td>
<td>Hispanic, African American, and European American</td>
<td>Steatosis OR = 5.9 × 10&lt;sup&gt;-10&lt;/sup&gt; ALT OR = 3.7 × 10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>12</td>
</tr>
<tr>
<td>NAFLD (1H-MRS)</td>
<td>291</td>
<td>Finland</td>
<td>Liver fat content OR = 0.011 OR = 0.002</td>
<td>18</td>
</tr>
<tr>
<td>NAFLD (1H-MRS)</td>
<td>330</td>
<td>Germany</td>
<td>Increased steatosis OR = 0.0001粋</td>
<td>58</td>
</tr>
<tr>
<td>NAFLD (abdominal ultrasonography, liver biopsy)</td>
<td>172 NAFLD 94 cases</td>
<td>County hospital of the city of Buenos Aires</td>
<td>NAFLD OR = 2.8 (1.5–5.2)</td>
<td>20</td>
</tr>
<tr>
<td>Alcoholic liver disease (biochemical, clinical assessment and imaging)</td>
<td>434 ALD 482 alcoholic cirrhosis 305 controls</td>
<td>Mestizos from Mexico City</td>
<td>Alcoholic cirrhosis OR = 1.7 × 10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>28</td>
</tr>
<tr>
<td>NAFLD (liver biopsy)</td>
<td>574 cases 179 controls</td>
<td>UK, Italy</td>
<td>Steatosis grade &gt; 1 OR = 1.35 (1.04–1.76) NASH OR = 1.50 (1.12–2.04) Fibrosis stage &gt; 1 OR = 1.50 (1.09–2.12)</td>
<td>21</td>
</tr>
<tr>
<td>Serum aminotransferase activities</td>
<td>475 overweight children</td>
<td>Italy</td>
<td>ALT OR = 0.001 OR = 1.46 (1.07–2.01) AST OR = 1.57 (1.24–1.99)</td>
<td>51</td>
</tr>
<tr>
<td>NAFLD (liver biopsy)</td>
<td>592 cases 1405 controls</td>
<td>European non-Hispanic ancestry</td>
<td>NAFLD OR = 3.6 × 10&lt;sup&gt;-43&lt;/sup&gt;</td>
<td>59</td>
</tr>
<tr>
<td>NAFLD (liver biopsy)</td>
<td>149 children and adolescents</td>
<td>Italy</td>
<td>Severity of steatosis OR &lt; 0.0001 NASH OR &lt; 0.0001 Hepatocellular ballooning OR &lt; 0.0001 Lobular inflammation OR &lt; 0.0001 Fibrosis OR = 0.01</td>
<td>45</td>
</tr>
<tr>
<td>NAFLD (liver biopsy)</td>
<td>1,117 cases (894 adults, 223 children)</td>
<td>USA</td>
<td>Steatosis OR = 1.46 (1.07–2.01) Portal inflammation OR = 1.57 (1.24–1.99) Lobular inflammation OR = 1.84 (1.33–2.55) Mallory-Denk bodies OR = 1.60 (1.46–3.07) Fibrosis OR = 0.004</td>
<td>22</td>
</tr>
<tr>
<td>NAFLD (MRI, liver biopsy)</td>
<td>85 obese children and adolescents</td>
<td>Caucasian, African American, Hispanic</td>
<td>NAFLD Caucasians OR = 3.6 × 10&lt;sup&gt;-4&lt;/sup&gt; NAFLD African Americans OR = 0.012 NAFLD Hispanics OR = 0.52</td>
<td>46</td>
</tr>
<tr>
<td>NAFLD (1H-MRS)</td>
<td>218 diabetic patients</td>
<td>France</td>
<td>Steatosis OR = 0.04</td>
<td>77</td>
</tr>
<tr>
<td>Phenotypic parameter</td>
<td>Number of individuals</td>
<td>Population / ethnicity</td>
<td>P value</td>
<td>OR (95% CI)</td>
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<tr>
<td>NAFLD (MRI)</td>
<td>327</td>
<td>Hispanics</td>
<td>Steatosis $p &lt; 0.0001$ Lower HDL $p = 0.03$</td>
<td></td>
</tr>
<tr>
<td>NAFLD (abdominal sonography)</td>
<td>520 obese children</td>
<td>Taiwan</td>
<td>Steatosis $p &lt; 0.0001$ Increased ALT $p &lt; 0.0001$</td>
<td>2.96 (1.57–5.59) 5.84 (2.59–13.16)</td>
</tr>
<tr>
<td>Liver stiffness (transient elastography)</td>
<td>899</td>
<td>Germany</td>
<td>TE $&gt; 13.0$ [kPa] $p = 0.005$</td>
<td>1.56 (1.14–2.14)</td>
</tr>
<tr>
<td>Liver steatosis in patients with non-3 HCV (liver biopsy)</td>
<td>626</td>
<td>Caucasian</td>
<td>$p &lt; 0.001$</td>
<td>1.9 (1.6–2.3)</td>
</tr>
<tr>
<td>Alcoholic liver disease (liver biopsy)</td>
<td>1,043</td>
<td>Germany</td>
<td>Alcoholic cirrhosis $p = 1.2 \times 10^{-5}$ Increased ALT $p = 0.0042$</td>
<td>2.79 (1.55–5.04) 2.33 (1.27–4.26)</td>
</tr>
<tr>
<td>Steatosis, liver fibrosis and HCC in HCV (liver biopsy)</td>
<td>819 HCV 261 NAFLD 179 controls</td>
<td>Italy</td>
<td>Steatosis $p &lt; 0.001$ Cirrhosis $p = 0.002$ Treatment response $p = 0.006$ HCC occurrence $p = 0.002$</td>
<td>1.90 (1.4–2.7) 1.47 (1.2–1.9) 0.63 (0.4–0.8) 2.16 (1.3–3.6)</td>
</tr>
<tr>
<td>Alcoholic liver disease (liver biopsy)</td>
<td>330 cases 328 controls</td>
<td>Caucasian Europeans</td>
<td>ALD $p = 0.008$ Steatosis $p = 0.048$ Fibrosis $p = 0.001$ Cirrhosis $p = 0.02$</td>
<td>1.54 (1.12–2.11) 2.08 (1.15–3.77)</td>
</tr>
<tr>
<td>NAFLD* (CT, liver biopsy)</td>
<td>7,176 - CT 592 - biopsied cases 1,405 - Controls</td>
<td>Iceland, USA</td>
<td>CT-assessed steatosis $p = 4.3 \times 10^{-34}$ Biopsy-proven NAFLD $p = 3.6 \times 10^{-43}$</td>
<td></td>
</tr>
<tr>
<td>Steatosis and fibrosis in HCV (liver biopsy)</td>
<td>537</td>
<td>Caucasian: Belgium, Germany, France</td>
<td>Steatosis $p = 0.034$ Fibrosis $p = 0.002$ Fibrosis progression $p = 0.013$</td>
<td>2.55 (1.08–6.03) 3.13 (1.50–6.51) 2.64 (1.22–5.67)</td>
</tr>
<tr>
<td>Serum glucose levels</td>
<td>487</td>
<td>Romania</td>
<td>Increased serum glucose $p = 0.0001$</td>
<td></td>
</tr>
<tr>
<td>Liver density (CT)</td>
<td>422 cases with T2D</td>
<td>African American</td>
<td>Liver density $p = 0.0075$ Steatosis $p = 0.035$</td>
<td></td>
</tr>
<tr>
<td>Concentrations of liver enzymes in plasma*</td>
<td>61,089</td>
<td>Caucasian, Indian Asian</td>
<td>ALT $p = 1.2 \times 10^{-45}$</td>
<td></td>
</tr>
<tr>
<td>NAFLD* (liver biopsy)</td>
<td>529 cases 932 controls</td>
<td>Japan</td>
<td>NAFLD $p = 1.4 \times 10^{-10}$ NASH $p = 1.7 \times 10^{-16}$</td>
<td>1.66 (1.43–1.94) 2.18 (1.81–2.63)</td>
</tr>
<tr>
<td>Serum triglyceride levels</td>
<td>18,921</td>
<td>Sweden, Scotland</td>
<td>T2D risk $p = 0.04$ T2D risk $p = 0.001$ (severely obese)</td>
<td>1.09 (1.01–1.39) 1.37 (1.13–1.66)</td>
</tr>
<tr>
<td>Serum cholesterol and triglycerides</td>
<td>5,847</td>
<td>Denmark</td>
<td>Decreased TG in IGR $p = 5.1 \times 10^{-5}$ Decreased cholesterol in IGR $p = 1.5 \times 10^{-4}$</td>
<td></td>
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</tbody>
</table>

(Continued)
limited extent, Hispanic obese children. Interestingly, children carrying the PNPLA3 risk allele seem to be predisposed to an early development of NAFLD. The latest analysis of 6 to 12-year-old Mexican children showed that already at this age the PNPLA3 mutation may be associated with increased serum ALT activities, and we detected the same association in a cohort of German children aged 5 to 9 years. A practical consequence is that weight loss might substantially improve the liver status in children carrying the risk variant and rescue the deleterious PNPLA3-associated liver phenotype. This possibility points to the need for early detection of pediatric patients carrying this mutation who require more careful follow-up and tailored therapies aiming at weight loss and physical activity.

Table 2 Key examples of liver diseases associated with variant PNPLA3

<table>
<thead>
<tr>
<th>Disease</th>
<th>Study</th>
<th>Year</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonalcoholic fatty liver disease</td>
<td>Romeo et al&lt;sup&gt;12&lt;/sup&gt;</td>
<td>2008</td>
<td></td>
</tr>
<tr>
<td>Alcoholic liver cirrhosis</td>
<td>Tian et al&lt;sup&gt;28&lt;/sup&gt;</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Stickel&lt;sup&gt;29&lt;/sup&gt;</td>
<td>2011</td>
<td></td>
</tr>
<tr>
<td>Liver fibrosis</td>
<td>Krawczyk et al&lt;sup&gt;25&lt;/sup&gt;</td>
<td>2011</td>
<td></td>
</tr>
<tr>
<td>HBV steatosis</td>
<td>Vigano et al&lt;sup&gt;36&lt;/sup&gt;</td>
<td>2013</td>
<td></td>
</tr>
<tr>
<td>HCV steatosis</td>
<td>Cai et al&lt;sup&gt;32&lt;/sup&gt;</td>
<td>2011</td>
<td></td>
</tr>
<tr>
<td>Alcohol and HCV cirrhosis</td>
<td>Müller et al&lt;sup&gt;31&lt;/sup&gt;</td>
<td>2011</td>
<td></td>
</tr>
<tr>
<td>HCV cirrhosis</td>
<td>Valenti et al&lt;sup&gt;33&lt;/sup&gt;</td>
<td>2011</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular cancer</td>
<td>Nischalke et al&lt;sup&gt;30&lt;/sup&gt;</td>
<td>2011</td>
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</tr>
</tbody>
</table>

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.
been reported that the *PNPLA3* variant results in decreased hepatic very-low-density lipoprotein (VLDL) secretion, which would further contribute to increased lipid accumulation in liver.\(^5\) However, several studies did not show any relationship between this mutation and HOMA index, serum glucose, or lipid levels.\(^19,45,58,59\) These results contradict the prevailing paradigm that insulin resistance represents the main driver of common NAFLD.\(^60\) Indeed, a dissociation between the presence of fatty liver and insulin resistance appears to be present among carriers of the *PNPLA3* risk variant.\(^58\) Moreover, carriers of the rare allele develop fatty liver irrespective of their BMI.\(^21,22,59\) Hence, *PNPLA3*-associated fatty liver represents an example that excessive deposition of fat in the liver is not the cause of hepatic insulin resistance, pointing to the more complex relationships between steatosis and insulin resistance.\(^61\) Vice versa, the effect of *PNPLA3* variation on metabolic traits does not seem to be the driving force of hepatic fat accumulation, and carriers of the risk variant may develop severe hepatic steatosis even in the absence of disrupted systemic glucose and/or lipid homeostasis.

**Functional Analyses of the PNPLA3 Variant**

All the above studies established the *PNPLA3* mutation p.1148M as a common genetic marker of NAFLD and triggered studies to unravel the functional consequences of the variant. The close similarity to adipose triglyceride lipase and the presence of typical structural motifs (α − β − α sandwich structure, GXSGXG motif within a catalytic dyad) suggested a lipase function for PNPLA3.\(^16\) Chen et al\(^62\) generated a *Pnpla3*-knockout mouse and investigated metabolic traits and lipid contents of livers from these mice. Interestingly, loss of *Pnpla3* in mice neither affected hepatic lipid composition nor serum activities of liver enzymes under normal or high-fat diets.\(^62\) Moreover, knocking-out *Pnpla3* apparently did not have any effects on body fat composition and metabolic markers, in particular insulin sensitivity or glucose levels.\(^62\) Comparable results were provided by Basantani et al\(^63\) who also investigated metabolic and hepatic phenotypes in *Pnpla3*\(^{−/−}\) mice and did not detect any specific phenotypes that could be related to *PNPLA3* deficiency.

Because it became apparent that loss of the PNPLA3 function is not a driver of the hepatic phenotype in individuals carrying the p.1148M variant and that knockout mice do not provide related phenotypes, the variant was studied further in vitro.\(^64,65\) The expression of PNPLA3 was shown to be regulated by carbohydrates, via sterol regulatory element binding protein-1c (SREBP1c) as well as specific fatty acids.\(^31\) Initially, the p.1148M substitution was demonstrated to reduce the lipolytic activity (but only modestly the substrate affinity) of recombinant PNPLA3.\(^65\) Kumari et al\(^64\) demonstrated that overexpression of PNPLA3 in various cell lines enhances intracellular diacylglycerol and phospholipid synthesis. Subsequently, the p.148M mutant was generated by site-directed mutagenesis, purified in *Escherichia coli*, and incubated with radiolabeled substrates.\(^64\) Analysis by thin-layer chromatography demonstrated that the purified enzyme enhances the acyl-CoA-dependent acylation of lysophosphatic acid (LPA) to generate phosphatidic acid, i.e. possesses LPA acyltransferase activity.\(^64\) This activity of the mutated enzyme was in turn 2.0-fold higher as compared with the wild-type form, indicating that the amino acid substitution p.1148M leads to a gain of function of the enzyme.\(^64\) These findings suggested that PNPLA3 might represent an enzyme that metabolizes LPA to phosphatidic acid, which can subsequently be used in the synthesis of triglycerides. Further studies\(^66\) performed in transgenic mice overexpressing *Pnpla3* in liver demonstrated that these animals develop fatty liver due to triacylglycerol accumulation as well as several alterations of hepatic lipid metabolism (i.e., increased synthesis of fatty acids and triacylglycerol, impaired hydrolysis of triglycerides, depletion of long-chain polyunsaturated fatty acids).\(^66\) Indeed in hepatocytes, most of the lipids are stored within lipid droplets, and livers from NAFLD patients are characterized by the increased number and size of lipid droplets within hepatocytes. Of note, PNPLA3

**Table 3** Studies reporting an association between variant *PNPLA3* and HCC

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Setting</th>
<th>Etiology</th>
<th>N (HCC: cirrhosis)</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valenti et al(^33)</td>
<td>Retrospective</td>
<td>Cirrhotic patients</td>
<td>HCV</td>
<td>50: 275</td>
<td>2.2</td>
<td>1.3–2.6</td>
</tr>
<tr>
<td>Ginanni Corradini et al(^82)</td>
<td>Retrospective</td>
<td>Cirrhotic patients</td>
<td>HCV</td>
<td>90: 131</td>
<td>2.2</td>
<td>1.4–3.5</td>
</tr>
<tr>
<td>Nischale et al(^62)</td>
<td>Retrospective</td>
<td>Cirrhotic patients</td>
<td>HCV, Alcohol</td>
<td>80: 80, 81: 81</td>
<td>1.7</td>
<td>0.5–5.3</td>
</tr>
<tr>
<td>Falletti et al(^83)</td>
<td>Retrospective</td>
<td>Cirrhotic patients</td>
<td>Mixed</td>
<td>141: 342</td>
<td>1.8</td>
<td>1.1–2.9</td>
</tr>
<tr>
<td>Trepo et al(^37)</td>
<td>Retrospective</td>
<td>Cirrhotic patients</td>
<td>Alcohol</td>
<td>145: 426</td>
<td>4.7</td>
<td>2.6–8.4</td>
</tr>
<tr>
<td>Burza et al(^38)</td>
<td>Prospective</td>
<td>Swedish Obese Subjects Study</td>
<td>Obesity</td>
<td>407</td>
<td>16.0</td>
<td>2.3–111</td>
</tr>
<tr>
<td>Guyot et al(^84)</td>
<td>Prospective</td>
<td>Cirrhotic patients</td>
<td>HCV</td>
<td>93: 160</td>
<td>1.0</td>
<td>0.6–1.9</td>
</tr>
<tr>
<td>Hassan et al(^39)</td>
<td>Case-control</td>
<td>Cirrhotic patients</td>
<td>Mixed</td>
<td>257: 494 controls</td>
<td>3.2</td>
<td>1.7–6.4</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OR, odds ratio.

Source: Adapted and modified from Valenti et al.\(^74\)

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PASH: PNPLA3-Associated Steatohepatitis and Future Directions

Nonalcoholic fatty liver disease gains growing acceptance as a potentially severe chronic liver disease among hepatologists in particular and physicians in general. On the other hand, the diagnosis of NAFLD and its subtypes is still challenging in clinical practice. Fatty liver disease is usually diagnosed in individuals who present with a typical “bright liver” image on abdominal ultrasonography do not consume excessive amounts of alcohol (i.e. less than 20–30 g/d), and do not suffer from other specific liver diseases. In addition to ultrasound, which can be used as a screening tool, other noninvasive methods for quantifying hepatic fat contents have been developed; however, there is still a need for further evaluation.

Given the studies reviewed here (Table 1), PNPLA3 genotyping may be used as a novel noninvasive marker for an increased risk of progressive fatty liver disease and could be included in the clinical decision making in patients with chronic liver diseases. So far, several subtypes of steatohepatitis are known by familiar acronyms, in particular NASH and alcoholic steatohepatitis (ASH). More recently, BASH (both alcoholic and nonalcoholic steatohepatitis), CASH (chemotherapy-associated steatohepatitis), and DASH (drug-associated steatohepatitis) have been suggested to be added to the etiology-oriented inventory of steatohepatitis subtypes. Of note, such conditions are caused by definable environmental prosteatotic triggers. Because in patients who carry the PNPLA3 risk variant increased lipid contents and inflammation in liver can be driven exclusively by PNPLA3 in the absence of environmental risk factors, we propose “PASH” (i.e., PNPLA3-associated steatohepatitis) as a novel gene-based liver disease entity (Fig. 2). PNPLA3-associated steatohepatitis represents an example how to (re)classify disease according to molecular pathways and pathophysiological changes in the era of personalized medicine.

As of now, we suggest to diagnose PASH in the subgroup of patients with steatohepatitis who are homozygous carriers of the risk allele and who do not present other risk factors for fatty liver disease such as alcohol abuse and metabolic syndrome, or chronic viral hepatitis. Fig. 2 summarizes the spectrum of PNPLA3-related liver phenotypes.

The new gene-based disease classification might have clinical implications that need to be tested in prospective clinical studies. Indeed, carriers of the PNPLA3 risk allele could benefit from a more systematic, early and careful surveillance of complications of progressive fatty liver disease, including HCC in the absence of cirrhosis. Although to date prospective studies concerning long-term effects of this variant on liver status are lacking, investigations in small groups of patients underscore the notion that weight loss may have beneficial effects on the liver status in the carriers of the rare allele. Although fatty liver disease is often associated with the metabolic syndrome, the PASH subtype does not seem to be substantially associated with the nonhepatic manifestations of this syndrome (body mass index, insulin resistance, dyslipidemia). As delineated above, the presence of the PNPLA3 risk allele is predominantly associated with liver phenotypes. As a result, we speculate that patients with this prosteatotic variant might easily be overlooked using our conventional connotations in clinical practice. On the other hand, because the frequency of the PNPLA3 risk allele is ~ 20% of Europeans it might even be incorporated in the routine workup of patients with chronic liver diseases of unknown etiology. The variant could also be also used in future intervention studies. Indeed, at the moment we are still in need of evidence-based therapeutic strategies that lower hepatic fat content. Although several compounds have been tested, the latest American Association for the Study of Liver Diseases and American Gastroenterological Association guidelines recommend, as before, lifestyle interventions and vitamin E in nondiabetic adults with biopsy-proven NASH. Future interventions with randomization of patients according to the PNPLA3 genotype might result in precise therapy for carriers of the prosteatotic genotype. In patients with PNPLA3-associated steatohepatitis the design and evaluation of gene-based preventive and therapeutic approaches to a common liver disease, once
thought to be a visionary promise, is closer to clinical practice than ever before.

Acknowledgments
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