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

Marcin Krawczyk, MD[1] Piero Portincasa, MD, PhD[2] Frank Lammert, MD, PhD[1]

[1] Department of Medicine II, Saarland University Medical Center, Homburg, Germany
[2] Department of Biomedical Sciences and Human Oncology, Clinica Medica "A. Murri," University of Bari Medical School, Bari, Italy

Address for correspondence Frank Lammert, MD, PhD, Department of Medicine II, Saarland University Hospital, Kirrberger Str. 100, 66421 Homburg, Germany (e-mail: frank.lammert@uks.eu).

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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.

Keywords
- adiponutrin
- genomics
- genetic susceptibility
- nonalcoholic steatohepatitis
- steatosis

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.[1] 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%.[2] 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).[3,4]

According to the latest guidelines,[5] 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

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progress to liver fibrosis, cirrhosis, and hepatocellular cancer (HCC). If a patient with NASH develops liver cirrhosis, then this condition is termed NASH-cirrhosis.[5] This classification implies that there are two major types of NAFLD: the most common benign NAFL 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,[6] 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,[7,8] 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.[9] 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.[10] *PNPLA3* is expressed predominantly in the liver, skin, and adipose tissue.[11] In a series of seminal genetic studies, the common nonsynonymous variant p.I148M (rs738409 (> G)) of the *PNPLA3* gene has emerged as the key genetic determinant of NAFLD in adults and pediatric patients.[9] 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 ($p = 5.9 \times 10^{-10}$) with increased liver fat content on a genome-wide significance level, as determined by ^{1}H-magnetic resonance spectrometry, irrespective of alcohol consumption, body mass index (BMI), and diabetes.[12] This association was most prominent among patients of Hispanic descent,[12] who are in general at a greater risk of developing fatty liver as compared with Caucasians and African Americans.[13–15] 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.[12]

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.[16,17] The genetic association between the *PNPLA3* mutation p.I148M and fatty liver disease was subsequently replicated in many studies. Kotronen et al[18] 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.[18] The *PNPLA3* variant was also found to be strongly ($p = 4.3 \times 10^{-34}$) 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]).[19] 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.[20] 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.[21] Also in this study the frequencies of the *PNPLA3* variant were associated with NAFLD severity as determined by liver biopsy.[21] 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.[21] The detailed analysis of histopathological markers of NAFLD was performed by Rotman et al,[22] demonstrating that the *PNPLA3* variant is associated with portal ($p = 2.5 \times 10^{-4}$) and lobular ($p = 0.005$) inflammation, Mallory-Denk bodies ($p = 0.02$), and fibrosis ($p = 7.7 \times 10^{-6}$). A meta-analysis[9] 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.

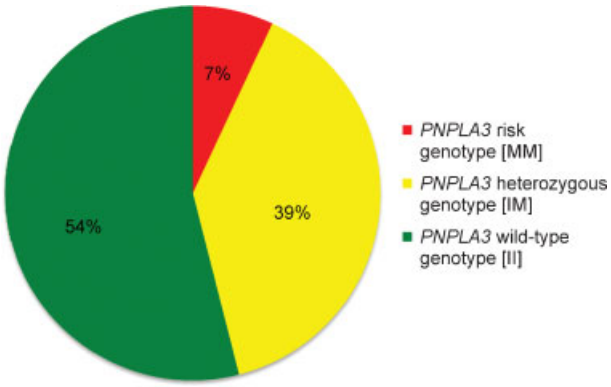

Fig. 1 Frequencies of the *PNPLA3* p.I148M genotypes. According to our previous study in 899 individuals with chronic liver diseases,[25] as many as 40 to 50% of European patients carry at least one copy of the rare allele (M) that is associated with progressive liver diseases.

Recently, Anstee and coworkers from the European FLIP (Fatty Liver: Inhibition of Progression) Consortium[23] 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.[23] In contrast, the other genes identified represent only modifiers of subphenotypes with smaller effect sizes.[24] According to our previous study, the risk allele is present in as many as 40 to 50% of Europeans (►**Fig. 1**).[25] These findings have also been extended to other ethnic groups: Two recent Japanese histopathologically-based GWASs replicated the association with NAFLD severity and identified no other major susceptibility loci.[26,27] Overall, the above studies summarized in ►**Table 1** document that the *PNPLA3* mutation increases the risk of developing severe hepatic fat accumulation, progressive inflammation, and advanced fibrosis not only in Caucasian patients with NAFLD, but across different ethnicities.

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 (►**Table 2**). 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.[28–31] 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,[32–34] which can be further modulated by the amounts of the intraabdominal fat,[35] and increased steatosis in chronic hepatitis B virus (HBV) infection.[36] 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.[25] Sensitivity analysis showed that the association was present across a broad range of stiffness values (12–40 kPa),[25] 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 (►**Table 3**).[37–40] The homozygous p.148M genotype was found to predominantly increase the HCC risk in patients with alcoholic liver disease.[40,41] 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,[39] HCC 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.[42]

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.[43] However, in this study the donor *PNPLA3* genotypes were not determined. Finkenstedt et al[44] 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).[44] 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,[45] 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.[45] However, the *PNPLA3* variant determined the degree of hepatic steatosis.[45] 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.[45] The association between the *PNPLA3* mutation and pediatric NAFLD has also been detected in Caucasian and African American,[46] Taiwanese,[47] and to a

Table 1 Selected studies investigating association of the *PNPLA3* variant with hepatic and metabolic phenotypes (in chronological order)

Phenotypic parameter	Number of individuals	Population / ethnicity	*P* value	OR (95% CI)	Reference
Serum levels of liver enzymes[a,b]	12,419	Indian Asian, European	ALT $p = 8.4 \times 10^{-16}$		16
NAFLD[a] (^{1}H-MRS)	2,111	Hispanic, African American, and European American	Steatosis $p = 5.9 \times 10^{-10}$ ALT $p = 3.7 \times 10^{-4}$		12
NAFLD (^{1}H-MRS)	291	Finland	Liver fat content $p = 0.011$ AST $p = 0.002$		18
NAFLD (^{1}H-MRS)	330	Germany	Increased steatosis $p = 0.0001$	2.38 (1.37–4.20)	58
NAFLD (abdominal ultrasonography, liver biopsy)	172 NAFLD 94 cases	County hospital of the city of Buenos Aires	NAFLD $p < 0.001$	2.8 (1.5–5.2)	20
Alcoholic liver disease (biochemical, clinical assessment and imaging)	434 ALD 482 alcoholic cirrhosis 305 controls	Mestizos from Mexico City	Alcoholic cirrhosis $p = 1.7 \times 10^{-10}$	2.25 (1.74–2.90)	28
NAFLD (liver biopsy)	574 cases 179 controls	UK, Italy	Steatosis grade > 1 $p = 0.02$ NASH $p = 0.007$ Fibrosis stage > 1 $p = 0.01$	1.35 (1.04–1.76) 1.50 (1.12–2.04) 1.50 (1.09–2.12)	21
Serum aminotransferase activities	475 overweight children	Italy	ALT $p = 0.001$ AST $p = 0.022$		51
NAFLD (liver biopsy)	592 cases 1405 controls	European non-Hispanic ancestry	NAFLD $p = 3.6 \times 10^{-43}$	3.26 (2.11–7.21)	59
NAFLD (liver biopsy)	149 children and adolescents	Italy	Severity of steatosis $p < 0.0001$ NASH $p < 0.0001$ Hepatocellular ballooning $p < 0.0001$ Lobular inflammation $p < 0.0001$ Fibrosis $p = 0.01$		45
NAFLD (liver biopsy)	1,117 cases (894 adults, 223 children)	USA	Steatosis $p = 0.03$ Portal inflammation $p = 2.5 \times 10^{-4}$ Lobular inflammation $p = 0.005$ Mallory-Denk bodies $p = 0.015$ Fibrosis $p = 7.7 \times 10^{-6}$ NAS $p = 0.004$	1.46 (1.07–2.01) 1.57 (1.24–1.99) 1.84 (1.33–2.55) 1.60 (1.46–3.07)	22
NAFLD (MRI, liver biopsy)	85 obese children and adolescents	Caucasian, African American, Hispanic	NAFLD Caucasians $p = 3.6 \times 10^{-4}$ NAFLD African Americans $p = 0.012$ NAFLD Hispanics $p = 0.52$		46
NAFLD (^{1}H-MRS)	218 diabetic patients	France	Steatosis $p = 0.04$		77

Table 1 (*Continued*)

Phenotypic parameter	Number of individuals	Population / ethnicity	*P* value	OR (95% CI)	Reference
NAFLD (MRI)	327	Hispanics	Steatosis $p < 0.0001$ Lower HDL $p = 0.03$		78
NAFLD (abdominal sonography)	520 obese children	Taiwan	Steatosis $p < 0.0001$ Increased ALT $p < 0.0001$	2.96 (1.57–5.59) 5.84 (2.59–13.16)	47
Liver stiffness (transient elastography)	899	Germany	TE > 13.0 [kPa] $p = 0.005$	1.56 (1.14–2.14)	25
Liver steatosis in patients with non-3 HCV (liver biopsy)	626	Caucasian	$p < 0.001$	1.9 (1.6–2.3)	32
Alcoholic liver disease (liver biopsy)	1,043	Germany	Alcoholic cirrhosis $p = 1.2 \times 10^{-5}$ Increased ALT $p = 0.0042$	2.79 (1.55–5.04) 2.33 (1.27–4.26)	29
Steatosis, liver fibrosis and HCC in HCV (liver biopsy)	819 HCV 261 NAFLD 179 controls	Italy	Steatosis $p < 0.001$ Cirrhosis $p = 0.002$ Treatment response $p = 0.006$ HCC occurrence $p = 0.002$	1.90 (1.4–2.7) 1.47 (1.2–1.9) 0.63 (0.4–0.8) 2.16 (1.3–3.6)	33
Alcoholic liver disease (liver biopsy)	330 cases 328 controls	Caucasian Europeans	ALD $p = 0.008$ Steatosis $p = 0.048$ Fibrosis $p = 0.001$ Cirrhosis $p = 0.02$	1.54 (1.12–2.11) 2.08 (1.15–3.77)	30
NAFLD[a] (CT, liver biopsy)	7,176 - CT 592 - biopsied cases 1,405 - Controls	Iceland, USA	CT-assessed steatosis $p = 4.3 \times 10^{-34}$ Biopsy-proven NAFLD $p = 3.6 \times 10^{-43}$		19
Steatosis and fibrosis in HCV (liver biopsy)	537	Caucasian: Belgium, Germany, France	Steatosis $p = 0.034$ Fibrosis $p = 0.002$ Fibrosis progression $p = 0.013$	2.55 (1.08–6.03) 3.13 (1.50–6.51) 2.64 (1.22–5.67)	34
Serum glucose levels	487	Romania	Increased serum glucose $p = 0.0001$		54
Liver density (CT)	422 cases with T2D	African American	Liver density $p = 0.0075$ Steatosis $p = 0.035$		79
Concentrations of liver enzymes in plasma[a]	61,089	Caucasian, Indian Asian	ALT $p = 1.2 \times 10^{-45}$		17
NAFLD[a] (liver biopsy)	529 cases 932 controls	Japan	NAFLD $p = 1.4 \times 10^{-10}$ NASH $p = 1.7 \times 10^{-16}$	1.66 (1.43–1.94) 2.18 (1.81–2.63)	26
Serum triglyceride levels	18,921	Sweden, Scotland	T2D risk $p = 0.04$ T2D risk $p = 0.001$ (severely obese)	1.09 (1.01–1.39) 1.37 (1.13–1.66)	56
Serum cholesterol and triglycerides	5,847	Denmark	Decreased TG in IGR $p = 5.1 \times 10^{-5}$ Decreased cholesterol in IGR $p = 1.5 \times 10^{-4}$		53

(*Continued*)

Table 1 (*Continued*)

Phenotypic parameter	Number of individuals	Population / ethnicity	*P* value	OR (95% CI)	Reference
HCC prognosis	638	Japan	Poor prognosis in ALD patients with low BMI and HCC $p = 0.028$		41
Insulin resistance and viral load in HCV (genotype 2) patients	308	Denmark, Finland, Norway, Sweden	Insulin resistance $p = 0.023$ Lower viral load $p = 0.005$		55
NAFLD (abdominal sonography)	203 cases 202 controls	China	NAFLD $p = 7.6 \times 10^{-8}$		80
ALT levels in obese children	1,037	Mexico	Elevated ALT $p = 3.7\ 10^{-8}$	3.7 (2.3–5.9)	48
NAFLD[a] (liver biopsy, CT, MRI)	564 cases 1,946 controls	Japan	NAFLD $p = 6.8 \times 10^{-14}$	2.05	27
Steatosis in HBV (liver biopsy)	235	Italy	Steatosis $p = 0.05$ Severe steatosis $p = 0.011$ NAS > 2 $p = 0.023$	1.62 (1.00–7.0) 6.03 (1.23–5.0) 1.70 (1.07–2.74)	81
HCC risk	257 cases 494 controls	Caucasian	HCC $p < 0.001$ Cirrhosis P 0.039 Death P 0.004	3.21 (1.68–6.41) 2.48 (1.05–5.87) HR 2.11 (1.26–3.52)	39

Abbreviations: ALD, alcoholic liver disease; ALT, alanine aminotrasferase; AST, aspartate transaminase; CT, computed tomography; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IGR, impaired glucose regulation; kPa, kilopascal; MRI, magnetic resonance imaging, MRS; magnetic resonance spectroscopy; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; T2D, type 2 diabetes; TE, transient elastography.
[a]GWAS design.
[b]Study investigating the *PNPLA3* SNP rs2281135.

limited extent, Hispanic[46] obese children. Interestingly, children carrying the *PNPLA3* risk allele seem to be predisposed to an early development of NAFLD.[22] 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,[48] and we detected the same association in a cohort of German children aged 5 to 9 years.[49] 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.[50] 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.[51]

Table 2 Key examples of liver diseases associated with variant *PNPLA3*

Disease	Study	Year
Nonalcoholic fatty liver disease	Romeo et al[12]	2008
Alcoholic liver cirrhosis	Tian et al[28]	2010
	Stickel[29]	2011
Liver fibrosis	Krawczyk et al[25]	2011
HBV steatosis	Vigano et al[36]	2013
HCV steatosis	Cai et al[32]	2011
Alcohol and HCV cirrhosis	Müller et al[31]	2011
HCV cirrhosis	Valenti et al[33]	2011
Hepatocellular cancer	Nischalke et al[40]	2011

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus.

Variant *PNPLA3* and Metabolic Traits

In general, patients with NAFLD often present with pathological lipid and glucose homeostasis.[52] However, an association of *PNPLA3* with metabolic traits in humans remains controversial. Interestingly, the effects of variant *PNPLA3* on metabolic characteristics might be different in individuals with normal and impaired glucose tolerance. A Danish analysis of more than 4,000 individuals with normal glucose tolerance demonstrated a potential association of the risk allele with increased fasting glucose levels ($p = 0.04$), but the same allele was associated with lower serum levels of triglycerides and cholesterol in patients with impaired glucose intolerance ($N = 1,357$).[53] In line with these results, we[54] and other groups[55,56] identified a possible association between glucose metabolism and the *PNPLA3* p.I148M mutation. It has also

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

Study	Design	Setting	Etiology	*N* (HCC: cirrhosis)	OR	95% CI
Valenti et al[33]	Retrospective	HCV patients	HCV	50: 275	2.2	1.3–2.6
Ginanni Corradini et al[82]	Retrospective	Cirrhotic patients	HCV	90: 131	2.2	1.4–3.5
Nischalke et al[40]	Retrospective	Cirrhotic patients	HCV Alcohol	80: 80 81: 81	1.7 2.8	0.5–5.3 1.6–6.4
Falleti et al[83]	Retrospective	Cirrhotic patients	Mixed	141: 342	1.8	1.1–2.9
Trepo et al[37]	Retrospective	Cirrhotic patients	Alcohol	145: 426	4.7	2.6–8.4
Burza et al[38]	Prospective	Swedish Obese Subjects Study	Obesity	407	16.0	2.3–111
Guyot et al[84]	Prospective	Cirrhotic patients	HCV Alcohol	93: 160 66: 213	1.0 1.9	0.6–1.9 1.3–2.8
Hassan et al[39]	Case-control	Cirrhotic patients	Mixed	257: 494 controls	3.2	1.7–6.4

Abbreviations: CI, confidence interval; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OR, odds ratio.
Source: Adapted and modified from Valenti et al.[74]

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.[57] 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.I148M 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 ($\alpha - \beta - \alpha$ sandwich structure, GXSXG motif within a catalytic dyad) suggested a lipase function for PNPLA3.[10] 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.I148M 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.[11] Initially, the p.I148M 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 PNPAL3 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 lysophosphatidic 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.I148M 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

is predominantly localized to the membrane- and lipid droplet-associated cellular fractions.[67] Apparently, in carriers of the p.148M mutation, modulation of PNPLA3-associated pathways might increase the size of the lipid droplets.[68] The mice overexpressing *Pnpla3* were characterized by enhanced hydrolysis of triglycerides and increased formation of fatty acids and triglycerides, which all point to the fact that the PNPLA3 variant leads to numerous aberrations in intrahepatic lipid homeostasis and remodeling of hepatic lipid droplets.[66]

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.[69] 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.[70] 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.[71] 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[72] 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.[73] 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.[74] 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.[50,75] 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 (►**Fig. 1**),[21,27,30] 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.[7,76] Although several compounds have been tested, the latest American Association for the Study of Liver Diseases and American Gastroenterological Association guidelines[5] recommend, as before, lifestyle interventions and vitamin E in nondiabetic adults with biopsy-proven NASH.[5] 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

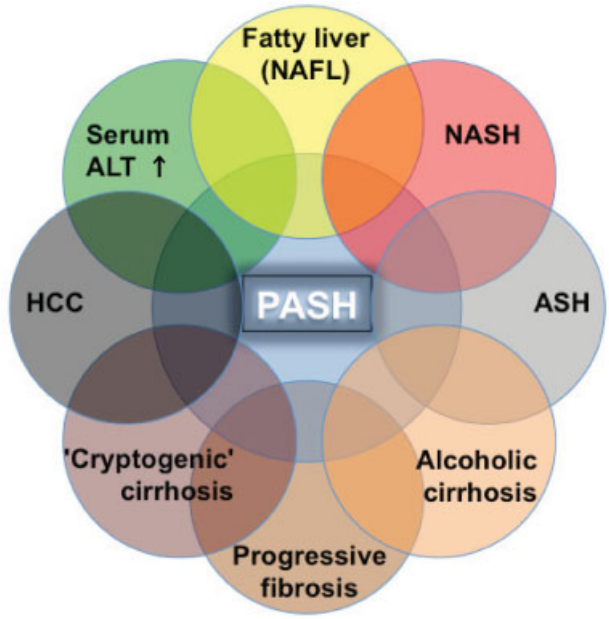

Fig. 2 *PNPLA3*-associated steatohepatitis (PASH) phenotypes associated with variant *PNPLA3*. Carriers of the p.148M allele are at increased risk of severe hepatic phenotypes, ranging from excessive hepatic fat accumulation and steatohepatitis to progressive fibrosis and HCC. ALT, alanine aminotransferase; ASH, alcoholic steatohepatitis, HCC, hepatocellular carcinoma; NAFL, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.

thought to be a visionary promise, is closer to clinical practice than ever before.

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