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Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis

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Summary

Background: Non-alcoholic steatohepatitis (NASH) is a severe form of non-alcoholic fatty liver disease (NAFLD) characterised by liver fat accumulation, inflammation and progressive fibrosis. Emerging data indicate that genetic susceptibility increases risks of NAFLD, NASH and NASH-related cirrhosis.

Aims: To review NASH genetics and discuss the potential for precision medicine approaches to treatment.

Method: PubMed search and inclusion of relevant literature.

Results: Single-nucleotide polymorphisms in *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7* and *HSD17B13* are clearly associated with NASH development or progression. These genetic variants are common and have moderate-to-large effect sizes for development of NAFLD, NASH and hepatocellular carcinoma (HCC). The genes play roles in lipid remodelling in lipid droplets, hepatic very low-density lipoprotein (VLDL) secretion and de novo lipogenesis. The *PNPLA3* I148M variant (rs738409) has large effects, with approximately twofold increased odds of NAFLD and threefold increased odds of NASH and HCC per allele. Obesity interacts with *PNPLA3* I148M to elevate liver fat content and increase rates of NASH. Although the isoleucine-to-methionine substitution at amino acid position 148 of the PNPLA3 enzyme inactivates its lipid remodelling activity, the effect of *PNPLA3* I148M results from trans-repression of another lipase (ATGL/PNPLA2) by sequestration of a shared cofactor (CGI-58/ABHD5), leading to decreased hepatic lipolysis and VLDL secretion. In homozygous *Pnpla3* I148M knock-in rodent models of NAFLD, targeted PNPLA3 mRNA knockdown reduces hepatic steatosis, inflammation and fibrosis.

Conclusion: The emerging genetic and molecular understanding of NASH paves the way for novel interventions, including precision medicines that can modulate the activity of specific genes associated with NASH.

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1 | INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) affects about 25% of the global population.[1] NAFLD includes non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH). Patients whose NAFL develops into NASH have increased overall and liver-specific mortality[1,2] and increased risks of cirrhosis, liver failure and hepatocellular carcinoma (HCC).[3-5] The burden of NASH is expected to increase in line with the global epidemic of obesity, type 2 diabetes and metabolic syndrome.[6] NASH is fast becoming the leading cause of chronic liver disease, and is set to overtake hepatitis C as the leading cause of liver transplantation in the US.[7,8]

Hepatic steatosis in people with NAFL is characterised by substantial accumulation of lipid droplets within hepatocytes. The progression to NASH is marked by hepatic inflammation and hepatocellular injury, with or without hepatic fibrosis, in histological examinations of liver biopsies.[3-5] Limited evidence suggests that NAFL progresses to NASH in up to 44% of patients undergoing random or voluntary biopsy,[1,9] with higher rates in those referred for biopsy because NASH is suspected.[1] Progressive fibrosis drives poor liver-related clinical outcomes, and develops in 35%-41% of patients with NASH, according to meta-analyses of paired biopsy studies.[1,2] About 20% of patients with NASH develop end-stage cirrhosis or HCC.[10]

Advances in human genetics present new opportunities to address the unmet need for NASH therapeutics, based on improved understanding of the multifactorial pathogenesis of NASH and the interaction between genetic and environmental risk factors. These new findings have opened up the possibility of precision medicine for patients with NASH based on inherited genetic variants. In this approach, the identification of people who carry a specific genetic variant predisposing them to NASH allows targeting of the same specific gene or molecular pathway to halt or reverse their hepatic steatosis, inflammation and fibrosis.

Here, we review the associations of five key genetic variants with NASH and discuss the potential for targeted interventions in particular disease pathways (Section 3). We then focus on a variant in the gene patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), a common and strong genetic risk factor for NASH (Section 4). We assess the extensive genetic, molecular and cell biological evidence that therapies able to modulate *PNPLA3* expression levels may represent precision medicine approaches in patients with NASH.

2 | METHODS

This was not a formal systematic review, but a comprehensive search strategy was used to identify and prioritise published findings for inclusion. A PubMed search was performed without language or date restrictions, aiming to identify publications on known genetic variants associated with NASH including genetic, epidemiological, clinical, pre-clinical and molecular cell biological studies. Search terms included 'non alcoholic fatty liver disease' or 'non alcoholic steatohepatitis' and 'genetic' or 'genetics' or 'PNPLA3' or 'TM6SF2' or 'MBOAT7' or 'HSD17B13' or 'GCKR' or 'epidemiology' or 'prevalence' or 'treatment' or 'insulin resistance' or 'risk factors'. Hits were refined based on the relevance of the title and abstract to the aims of the review. Following peer-review, 24 articles were added, of which four were suggested directly by the peer-reviewers. A total of 163 articles were included in the final review.

3 | IDENTIFYING TARGETABLE PATHWAYS FOR PRECISION MEDICINE

3.1 | Environmental risk factors

Modern sedentary lifestyles and the overconsumption of food drive a consistent positive energy balance and fuel the obesity epidemic, with subsequent increases in incidence of type 2 diabetes, metabolic syndrome and NAFLD. In the US, the estimated prevalence of NASH rises from 12% in middle-aged adults to 22% among those with diabetes and 33% among those with obesity.[11,12] Worldwide, NASH prevalence increases from 15% to 30% in people with obesity to up to 70% in those with morbid obesity.[10] Among patients with NASH worldwide, a reported 31%-89% have obesity and 33%-56% have diabetes.[10] Lifestyle interventions are the cornerstone of NAFLD management, and are discussed in Section 4.3. Not all obese individuals with fatty liver develop NASH, however, and some lean individuals do develop NASH,[1] indicating interaction with heritable risk factors.

3.2 | Genetic risk factors

Hepatic steatosis and fibrosis cluster in families, with a heritability value of about 0.5 in a twin study, after adjustment for age, sex and ethnicity.[13] In a familial cohort study, the risk of advanced fibrosis was 12 times higher in first-degree relatives of people with NAFLD and cirrhosis than in population controls, even after adjustment for other risk factors.[14] Of over 100 loci examined in genome-wide association studies and candidate gene studies,[15] genetic variations in five genes have emerged as reproducibly and robustly predisposing individuals to development of NASH (*PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7* and *HSD17B13*), as previously reviewed.[16] Unexplained variance remains despite these discoveries, indicating that future genome-wide studies may reveal additional associations.[13]

Table 1 shows published allele frequencies for the five principal genetic variants associated with NAFLD, NASH and HCC, together with allelic odds ratios for each disease. Odds ratios from different cohorts are not comparable with one another, and those from populations with liver disease may represent overestimates (eg liver biopsy cohorts). Nevertheless, published allelic odds ratios provide a useful indication of the approximate magnitude of the effects of the five principal variants on liver disease.

The genetic variants associated with NASH are common, with minor allele frequencies of 7%-37%, but nevertheless have moderate to large effect sizes for NAFLD, NASH and HCC (Table 1). This observation contrasts with the evolutionary theory that decreased reproductive fitness should select against genetic variants that confer disease risk.[17] Present-day reproductive fitness may not, however, reflect the pressures that have shaped genetic variation throughout evolution.[17] The five genes known to be associated with NASH (Table 1) are all involved in glucose and fat homeostasis regulatory pathways. The modern obesogenic environment may expose a disease risk associated with genetic variants that are advantageous when food supplies are erratic. Increased liver fat levels in people with the genetic variation in *PNPLA3* that predisposes to NASH may represent just such an example.

3.2.1 | *PNPLA3*

The first genetic variant found to be associated with NASH is a nonsynonymous single-nucleotide polymorphism (SNP) in *PNPLA3* known as rs738409 c.444 C > G p.I148M.[18] The C to G substitution at nucleotide position 444 of *PNPLA3* encodes an isoleucine

TABLE 1 Published allele frequencies and odds ratios for the principal genetic variants associated with NAFLD, NASH and HCC

Gene	SNP	Minor allele frequency, 1000G (max)	NAFLD allelic odds ratio[a] (95% CI)	NASH allelic odds ratio[a] (95% CI)	HCC allelic odds ratio[a] (95% CI)
PNPLA3	rs738409 c.444 C > G p.I148M	0.26 (0.72)[33]	1.91 (1.64, 2.21)[24]	2.54 (2.03, 3.16)[24]	5.9 (1.5, 23.8)[b,26] 2.68 (1.01, 7.26)[c,25]
TM6SF2	rs58542926 c.499 G > A p.E167K	0.07 (0.16)[41]	1.82 (1.59, 2.08)[d,39]	1.37 (1.11, 1.72)[d,e,39]	1.72 (1.27, 2.38)[d,f,39]
GCKR	rs1260326 c.1337 C > T p.P446L	0.29 (0.59)[46]	1.38 (1.25, 1.53)[44] 1.49 (1.09, 2.05)[45]	1.55 (1.10, 2.17)[45]	1.84 (1.23, 2.75)[f,44]
MBOAT7/ TMC4	rs641738 g.54173068 C > T/ c.50 G > A p.G17E	0.37 (0.63)[51]	1.42 (1.07, 1.91)[g,47]	1.18 (1.00, 1.40)[g,47]	2.10 (1.33, 3.31)[h,48]
HSD17B13	rs72613567[i] c.704/812 + 2dup (usually referred to as T to TA insertion)[j]	0.18 (0.40)[58]	0.84 (0.78, 0.91)[k,52]	0.86 (0.72, 1.02)[k,52]	0.67 (0.45, 1.00)[k,l,52] 0.77 (0.64, 0.93)[m,56]

Abbreviations: 1000G, 1000 Genomes Project phase 3; CI, confidence interval; HCC, hepatocellular carcinoma; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NR, not reported; SNP, single-nucleotide polymorphism.

[a]Note that odds ratios are from different cohorts and are not comparable across variants or diseases.

[b]In people with obesity; liver fibrosis not assessed.

[c]In patients with NAFLD (76.2% with NASH and 34.4% with fibrosis stage 3 and 4).

[d]Calculated as reciprocal of published odds ratio for the protective allele.[39]

[e]NASH cirrhosis.

[f]Severity of liver fibrosis not assessed.

[g]Liver biopsy cohort.

[h]UK/Italian cohort without advanced fibrosis/cirrhosis.

[i]Other liver disease-associated SNPs in *HSD17B13* are rs72613567 (linkage with rs72613567), rs143404524 and rs62305723.

[j]TA to TAA insertion on the chromosomal forward strand is a TA to TTA duplication in *HDS17B13* on the opposite strand, affecting introns of two transcripts.

[k]Data shown from Geisinger Health System cohorts; generally similar results in Dallas Heart Study cohort.

[l]Severity of liver fibrosis not assessed.

[m]Adjusted for age, sex, fibrosis stage and aetiology.

to methionine substitution at amino acid position 148 of PNPLA3 protein (also known as adiponutrin). This genetic variant (herein referred to as *PNPLA3* I148M) is associated with hepatic steatosis, steatohepatitis, elevated plasma liver enzyme levels, hepatic fibrosis and cirrhosis.[18-22] Associations between *PNPLA3* I148M and NAFLD have been demonstrated in multiple different geographical regions and ethnicities[22] and in populations of all ages including children and adolescents.[23]

PNPLA3 I148M has allelic odds ratios of approximately two to three for risks of NAFLD, NASH and HCC (Table 1).[24-26] Compared with allelic odds ratios, genotypic odds ratios are lower in *PNPLA3* 148IM heterozygotes and higher in *PNPLA3* 148MM homozygous risk allele carriers.[25-27] In a meta-analysis of 13 817 patients, the allelic odds ratio for NASH was 2.54 (2.03, 3.16) and the genotypic odds ratios were 1.75 (1.24, 2.46) for heterozygotes and 4.44 (2.92, 6.76) for homozygotes.[24]

Elevated risks of hepatic decompensation and liver-related death were also associated with *PNPLA3* I148M in a recent Italian prospective study.[25] *PNPLA3* I148M was strongly associated with liver-related death in an analysis of the US National Health and Nutrition Examination Survey with a median follow-up of 23 years.[28] Genetic association studies have established *PNPLA3* I148M as a strong genetic determinant of NAFLD in multiple populations worldwide.[29-31] The penetrance of *PNPLA3* I148M in 148MM homozygotes in Europeans is similar to that of mutations causing canonical monogenic liver disorders.[32] The *PNPLA3* I148M allele is common, with a frequency of 26% in the 1000 Genomes Project phase 3 combined population.[33] The minor allele frequency rises to 50% or higher in Latin American populations, contributing to explanation of the high incidence of NASH in this ethnic group (Table 1).[33]

3.2.2 | *TM6SF2*

A SNP in transmembrane 6 superfamily member 2 (*TM6SF2*) is associated with increased liver fat content,[34] NASH, advanced hepatic fibrosis and cirrhosis.[35,36] Rs58542926 c.499 G > A p.E167K is a G to A substitution encoding a glutamate to lysine substitution at amino acid position 167 of TM6SF2 protein (E167K), leading to a loss of its function in the hepatic very low-density lipoprotein (VLDL) secretion pathway.[34,37]

TM6SF2 variants have a moderate to large effect on the risk of NAFLD,[36,38] with the 167K allele having an allelic odds ratio of 1.82 for steatosis (Table 1).[39] Consistent with these findings, the 167EE homozygous ancestral genotype was associated with a significantly reduced risk of NAFLD in recent meta-analyses.[39,40] The *TM6SF2* E167K allele has a frequency of 7% in the 1000 Genomes Project phase 3 combined population (Table 1).[36,41]

3.2.3 | *GCKR*

Variations in the glucokinase regulator (*GCKR*) gene are associated with histological NAFLD[30] and have a modest effect on the risk of NAFLD.[38] A SNP in *GCKR*, rs1260326 c.1337 C > T p.P446L, is a C to T substitution encoding a proline to leucine substitution at amino acid position 446 of GCKR protein (P446L).[42] *GCKR* P446L is a loss-of-function variant that increases de novo lipogenesis by inducing glycolysis.[43] This variant is associated with increased susceptibility to NAFLD, NASH and NASH-derived HCC (Table 1).[44,45] The minor allele frequency of *GCKR* P446L in the 1000 Genomes Project phase 3 combined population is 29%.[46] *GCKR* P446L interacts with *PNPLA3* I148M in elevating susceptibility to NASH in people with both risk alleles.[45]

3.2.4 | *MBOAT7*

A SNP downstream of the gene encoding membrane bound O-acyltransferase domain-containing 7 (*MBOAT7*) has been linked with an increased risk of NAFLD, inflammation and fibrosis, and may predispose to HCC (Table 1).[38,47,48] The rs641738 g.54173068 C > T variant may be associated with downregulation of *MBOAT7* at an mRNA and protein level.[38] The same SNP also affects another gene, *TMC4* (rs641738 c.50 G > A p.G17E), with a resulting glycine to glutamate substitution in transmembrane channel-like protein 4. Unlike *MBOAT7*, *TMC4* lacks any known function related to lipid metabolism.[49] Rs641738 was identified first as a susceptibility locus for cirrhosis in alcohol abusers,[50] then for NAFLD[47] and HCC.[48] The minor allele frequency of rs641738 in the 1000 Genomes Project phase 3 combined population is 37% (Table 1).[51]

3.2.5 | *HSD17B13*

Inactivating variants in the *HSD17B13* gene, which encodes the hepatic lipid droplet protein hydroxysteroid 17-β dehydrogenase 13, have recently been linked with a reduced risk of chronic liver disease.[52-54] The rs72613567 T to TA insertion variant adjacent to the donor splice site downstream of exon 6 of *HSD17B13* may affect mRNA splicing and lead to the production of a truncated protein.[52] Rs72613567 is in strong linkage disequilibrium with rs6834314, which is associated with decreased steatohepatitis and serum liver enzyme levels.[54] Another variant in *HSD17B13* (rs143404524) is a deletion and frameshift that also leads to production of a truncated protein, and is associated with a reduced risk of chronic liver disease.[53] Finally, rs62305723 is a missense variant (c.778 C > T p.P260S) that also confers loss of enzymatic activity and is associated with decreased steatohepatitis.[54]

Compared with ancestral allele homozygotes (T/T), *HSD17B13* rs72613567 insertion variant allele heterozygotes (T/TA) have an 83% odds ratio and homozygotes (TA/TA) have a 70% odds ratio for developing non-alcoholic liver disease.[52] In a bariatric surgery cohort, the prevalence of NASH decreased with each TA allele, and there was evidence of reduced progression from simple steatosis to NASH or fibrosis in patients carrying the insertion variant.[52] Associations between the variant and reduced odds of HCC have been reported

in three different populations (Table 1).[55,56] Rs72613567 TA has also been reported to protect against histological steatohepatitis and fibrosis and to reduce plasma alanine aminotransferase (ALT) levels in people with NAFLD.[54,55,57] Rs72613567 interacted with *PNPLA3* I148M in one cohort studied, such that additional *HSD17B13* TA alleles reduced the effect of additional *PNPLA3* I148M alleles on serum ALT levels.[52]

The minor allele frequency of rs72613567 in the 1000 Genomes Project phase 3 combined population is 18% (Table 1).[58] The rs143404524 frameshift variant in *HSD17B13* has a minor allele frequency of 6% in the 1000 Genomes Project phase 3 combined population, rising to 2.9% in Latin America and up to 33% in Africa.[59] Reported allelic odds ratios for chronic liver disease are 0.24 (95% confidence interval [CI]: 0.07, 0.76) in black populations and 0.10 (0.01, 0.79) in Hispanic children in the US.[53] *HSD17B13* P260S (rs62305723) has a frequency of 2% in the 1000 Genomes Project phase 3 combined population.[60]

3.3 | Interaction of genetic and environmental risk factors

3.3.1 | Interaction of obesity with genetic risk factors

Obesity exposes the association of *PNPLA3* I148M with increased liver fat levels and risk of NASH, as revealed by studies in children, adolescents and adults[61-63] soon after its discovery.[18] *PNPLA3* I148M has a more extreme effect on liver injury in people with obesity than in lean individuals, and confers genetic susceptibility from a young age.[61-63] Odds ratios for elevated ALT levels in *PNPLA3* I148M homozygous children with obesity increased with abdominal fat levels in an Italian cohort study, from 1.2 (95% CI: 0.7, 2.4) to 4.9 (3.2, 7.8) in subgroups of low and high waist-to-height ratio, respectively.[64]

Liver fat levels increased dramatically with each additional *PNPLA3* I148M allele in people with high visceral abdominal fat levels, but there was no association in those with low visceral abdominal fat levels, in a European-American cohort study.[65] The effect of *PNPLA3* I148M on liver fat significantly increased with body mass index (BMI) in the Dallas Heart Study cohort.[66] In lean individuals (BMI <25 kg/m^2), liver fat content was only about 50% higher in *PNPLA3* 148MM than in 148II homozygotes, but in those with obesity (BMI >35 kg/m^2), liver fat content was 300% higher in *PNPLA3* 148MM than in 148II homozygotes.[66] The effect of high BMI in amplifying the risk of steatosis in carriers of PNPLA3 148M may be mediated by insulin resistance.[67] The prevalence of NASH ranged from 9% in lean 148II homozygotes to 84% in 148MM homozygotes with obesity.[66] Adiposity also amplified the interaction of *PNPLA3* I148M with ALT levels and cirrhosis in other cohorts.[66] Interactions of obesity with *TM6SF2* E167K and *GCKR* P446L have also been reported.[66,68]

Taken together, published evidence indicates that the common *PNPLA3* I148M variant is usually benign in lean individuals, perhaps reflecting a selective advantage of increased liver fat storage in our evolutionary past.[69,70] In people with lipodystrophy, however, impaired expansion of adipose tissue may lead to lipid accumulation at ectopic sites (including the liver).[71,72] A polygenic risk score for lipodystrophy has been associated with increased hepatic steatosis and fibrosis.[73] Whether *PNPLA3* I148M plays any pathophysiological role in a subset of lean patients with NAFLD requires further investigation.

In contrast, an obesogenic environment transforms *PNPLA3* I148M into a major factor in NAFLD and NASH pathophysiology. Furthermore, evidence presented in Section 4.3.1 suggests that *PNPLA3* I148M may modify the response to treatments that can lower body weight and liver fat levels in patients with NAFLD (omega-3 fatty acids, lifestyle modification and bariatric surgery).

3.3.2 | Insulin resistance and NAFLD genetic risk factors

Insulin resistance and type 2 diabetes are both significant risk factors for development of NASH. The identified genetic risk factors for elevated liver fat and NASH do not associate with insulin resistance, except in individuals with severe obesity.[18,61,74] In liver lipidomic analyses, NAFLD associated with *PNPLA3* I148M was characterised by high levels of hepatic polyunsaturated triacylglycerols,[75,76] but NAFLD associated with insulin resistance was characterised by high levels of saturated and mono-unsaturated triacylglycerols, free fatty acids and ceramides.[76] The altered lipid composition in the liver in carriers of *PNPLA3* 148M is reflected in reduced polyunsaturated triglyceride levels in very low-density lipoprotein particles.[77] Furthermore, hepatic diacylglycerols are implicated in the development of insulin resistance, and were elevated in *PNPLA3* ancestral allele carriers but not I148M carriers in another lipidomic study of people with hepatic steatosis.[78]

PNPLA3 I148M was, however, associated with a small increase in the risk of type 2 diabetes in a very large genome-wide association study of type 2 diabetes (allelic odds ratio 1.04 [95% CI: 1.01, 1.07]).[79,80] A phenome-wide analysis confirmed the association of *PNPLA3* I148M with increased risk of type 2 diabetes (odds ratio 1.08)[81] reported in a fine-mapping meta-analysis (odds ratio 1.05 [95% CI: 1.03, 1.07]).[82] In a Mendelian randomisation study, the genetic risk score for hepatic fat accumulation showed a causal relationship with insulin resistance, but this relationship disappeared when the model was adjusted for liver fibrosis.[79] This suggests that insulin resistance is not caused by genetically determined high liver fat levels per se, but rather that it develops as subsequent liver disease progresses and may be related to the inflammatory and pro-fibrotic environment. A limitation of Mendelian randomisation approaches is that they do not provide insights on the underlying mechanisms.[79]

The causes and consequences of liver steatosis and inflammation in patients with NASH may differ between *PNPLA3* I148M carriers and those lacking the variant. The effect of *PNPLA3* I148M on retinol metabolism in hepatic stellate cells (detailed in Section 4.2) may trigger or exacerbate hepatic inflammation in carriers with other risk factors for NASH. Further research into NASH pathophysiology in the presence and absence of specific genetic risk factors is needed

to understand the relationships between NAFLD, fibrosis and insulin resistance in different patients.

3.3.3 | Interaction of cardiovascular disease and NAFLD

Although advanced forms of NAFLD associate with increased risk of coronary artery disease, there is no evidence proving that accumulation of liver fat causes atherosclerosis, as recently reviewed in depth by one of the authors.[83] Indeed, *PNPLA3* I148M may be associated with a very small reduction in the risk of ischaemic heart disease, with odds ratios of 0.98 (95% CI: 0.96, 1.00; P = 0.79) in a recent large meta-analysis (N = 279 013)[84] and 0.96 (0.94, 0.97; $P = 4 \times 10^{-8}$) in a recent large exome-wide lipidomic study (N > 300 000).[85] *PNPLA3* I148M was associated with liver-related and all-cause mortality but not with cardiovascular mortality, in a retrospective US general population survey with median follow-up of 23 years.[28] Circulating triglyceride and LDL cholesterol levels are reduced or unchanged in *PNPLA3* I148M carriers compared with noncarriers in multiple studies.[85-88] Furthermore, NAFLD itself increases mortality, at least in populations with high rates of obesity.[89] The evidence therefore indicates that the association of NAFLD with coronary artery disease is mainly due to shared underlying risk factors, depending on the pathophysiology of NAFLD.

3.4 | Potential pathways for targeted therapeutic manipulation

The genetic variants most robustly associated with development of NAFLD (Table 1) highlight pathophysiological processes that may represent new targets for therapeutic intervention in patients with NASH. These include lipid remodelling in lipid droplets, hepatic VLDL secretion and de novo lipogenesis.

3.4.1 | PNPLA3 and lipid droplet remodelling

The molecular mechanisms underpinning the strong association of the common *PNPLA3* I148M variant with NAFLD are the most well characterised among the genetic associations identified to date. Section 4 is entirely devoted to *PNPLA3* I148M and the rationale for a precision medicine that can modulate *PNPLA3* expression in patients with NASH carrying the variant. PNPLA3 148M protein acts as a trans-repressor of hepatocyte lipid droplet lipase activity by competing for a shared co-activator, leading to lipid accumulation in hepatocytes.[90,91] *PNPLA3* I148M also impairs retinol production by hepatic stellate cells.[90] Section 4.2 and Figure 1 present current understanding of how *PNPLA3* I148M drives the pathogenesis of NASH in people with overweight or obesity.

Evidence that reduced expression of *PNPLA3* attenuates the effect of the I148M variant on liver fat levels is also provided by genetic association studies. Another SNP in *PNPLA3* associated with NAFLD (rs2294918 c.1300 G > A p.E434K) encodes a glutamate to lysine substitution in PNPLA3 protein (E434K).[92] In vitro, E434K has no effect on the enzymatic activity of PNPLA3, but it is associated with reduced levels of *PNPLA3* mRNA. People who inherit E434K together with I148M variant are partially protected from the NASH-promoting effect of I148M, as seen in a genetic study of associations with ALT levels.[92]

3.4.2 | TM6SF2 and VLDL secretion

TM6SF2 plays a role in the pathway for hepatic VLDL secretion.[34,38] Selective knockdown of TM6SF2 protein expression in mice led to a threefold increase in liver triglyceride content and a 50% decrease in VLDL secretion, indicating that TM6SF2 normally promotes VLDL secretion.[34] In people with the *TM6SF2* E167K variant, loss of function of TM6SF2 may lead to increased hepatic triglyceride content.[34] TM6SF2 E167K protein was associated with increased de novo lipogenesis and reduced secretion of apolipoprotein B particles in a recent study using 3D spheroid cultures of primary human hepatocytes.[37] A precision medicine able to restore deficient TM6SF2 activity in E167K carriers with NASH might also increase hepatic VLDL secretion and reduce triglyceride levels in the liver. This approach might, however, elevate the risk of adverse cardiovascular events, which is reduced in E167K carriers.[35] The modest odds ratios for the association of *TM6SF2* E167K with NASH suggest that any potential therapeutic benefit associated with restored function may also be modest. Furthermore, *TM6SF2* E167K is rare, so only a small population of patients would be targetable (Table 1).

3.4.3 | GCKR and de novo lipogenesis

GCKR is a fructose-6-phosphate-dependent inhibitor of glucokinase involved in regulating de novo lipogenesis.[42,93] The *GCKR* P446L variant disrupts negative regulation of glucokinase by GCKR in response to fructose-6-phosphate, leading to constitutive glucokinase activation.[38,42] This increases hepatic glucose uptake, glucose metabolism and malonyl CoA production.[38,42] Malonyl CoA is a substrate for de novo lipogenesis and blocks fatty acid oxidation (via inhibition of carnitine-palmitoyltransferase), and thereby favours hepatic fat accumulation.[38,42] These findings may explain the association of *GCKR* P446L with hepatic steatosis and increased susceptibility to NASH.[45] In vitro and animal model data on whether this pathway may be amenable to modulation with a precision medicine are currently lacking.

3.4.4 | MBOAT7 and phospholipid remodelling

MBOAT7 is a membrane-anchored enzyme with six transmembrane domains and is involved in remodelling endomembrane phospholipid acyl chains.[47,94] MBOAT7 expression levels are reduced in people with obesity and in rodent models of obesity compared with controls.[95]

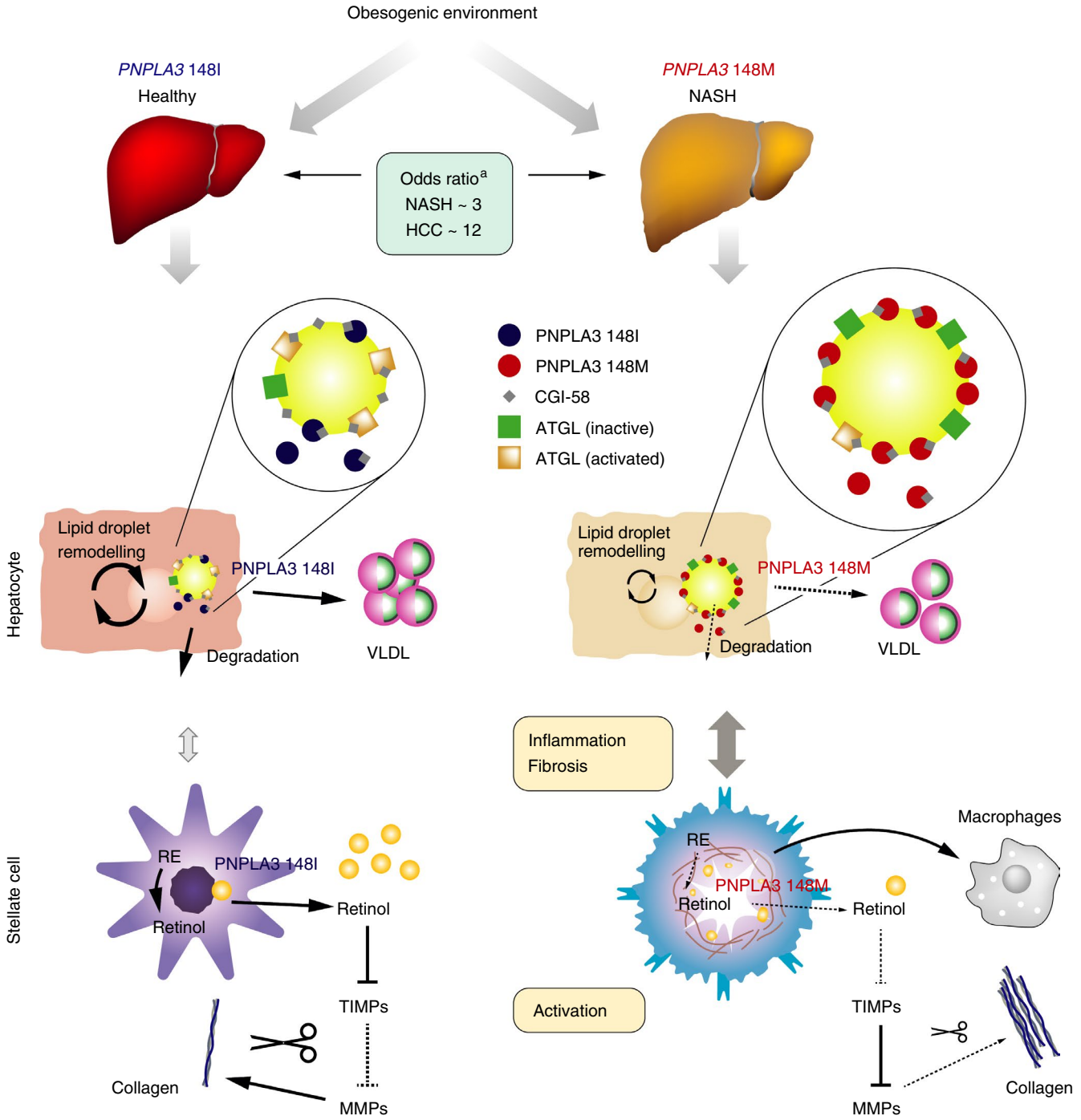

FIGURE 1 Role of PNPLA3 in the pathophysiology of NASH. Abbreviations: ATGL, adipose triglyceride lipase; HCC, hepatocellular carcinoma; MMP, matrix metalloproteinase; NASH, non-alcoholic steatohepatitis; RE, retinol esters; TIMP, tissue inhibitor of metalloproteinase; VLDL, very low-density lipoprotein. [a]Liu et al[27]

The rs641738 variant of *MBOAT7* may predispose to NAFLD and NASH by changing the acyl remodelling of phospholipids in the liver.[47] Some genetic studies have failed to detect an association of this variant with NAFLD, most likely because they were underpowered to detect the small effect size.[40] The details of the enzymatic activity of MBOAT7, including the preferential acyl donor and phospholipid substrate, are subjects of research.[94] Recent findings in rodent models suggest that acetylation of lysophosphatidylinositol lipids by MBOAT7 may play a protective role against development of liver steatosis in an obesogenic environment.[95]

3.4.5 | HSD17B13 and lipid droplets

HSD17B13 has been identified as a hepatic lipid droplet-associated protein with retinol dehydrogenase activity, and the variants that

protect against NAFLD confer loss of this enzymatic activity.[52,54] The physiological function of HSD17B13 is not well characterised, but other members of the hydroxysteroid 17-β dehydrogenase family are involved in steroid and fatty acid metabolism.[52,96] A role for HSD17B13 in oestradiol metabolism has been proposed, and it has enzymatic activity against bioactive lipid mediators, such as leukotriene B_4, which are involved in lipid-mediated inflammation.[52] Whether loss of HSD17B13 retinol dehydrogenase activity directly affects retinoic acid homeostasis within hepatocytes to modulate retinol levels is the subject of ongoing research.[54] Section 4.2 provides further detail on the role of retinol in modulating hepatic stellate cell fibrogenesis.

Significant upregulation of HSD17B13 protein expression has been observed in the livers of patients with NAFLD, and hepatic overexpression of human HSD17B13 led to a fatty liver phenotype in C57BL/6 mice.[97] The rs72613567 TA loss-of-function variant of *HSD17B13* was associated with a reduced risk of NASH in human liver samples.[52] It also mitigated liver injury in people genetically predisposed to hepatic steatosis by *PNPLA3* I148M and was associated with reduced *PNPLA3* mRNA expression.[52] In patients with functional variants of *HSD17B13*, carriers of *PNPLA3* I148M might be a relevant subpopulation for potential therapeutic inhibition of the activity or expression of HSD17B13.[52]

4 | PNPLA3 PRECISION MEDICINE IN PATIENTS WITH NASH

4.1 | Global prevalence of NASH and *PNPLA3* I148M

An increasingly large number of people could benefit from precision medicine approaches to treating NASH in carriers of *PNPLA3* I148M. NASH prevalence is predicted to rise from 2.4% in China, 3.6%–4.4% in five European countries and 5.3% in the US in 2016 to 3.4% in China, 5.0%–6.3% in five European countries and 7.6% in the US in 2030,[6] with a similar rise predicted in Saudi Arabia (4.2% to 6.8%

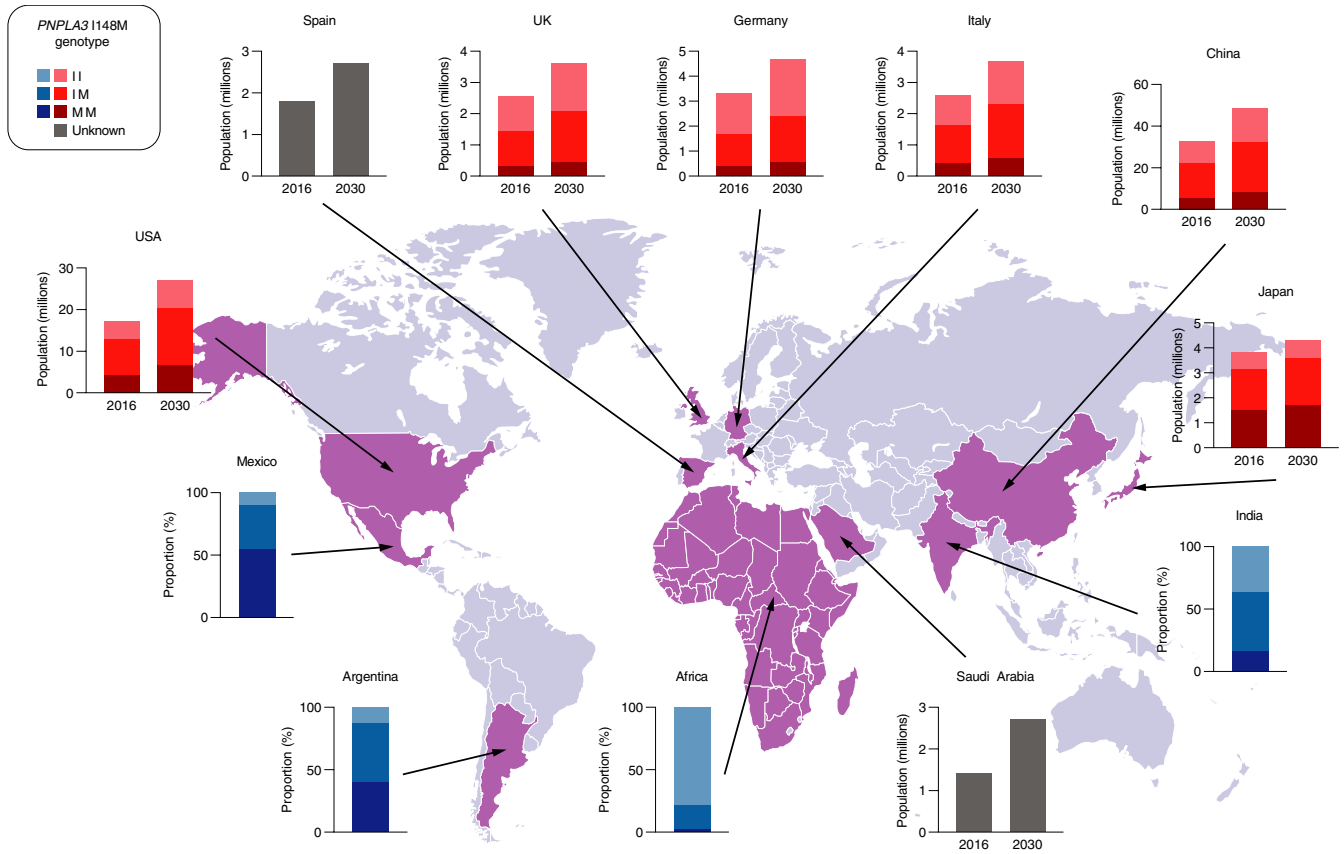

FIGURE 2 Worldwide prevalence of NASH and *PNPLA3* I148M genotypes approximated from their frequencies in patients with NAFLD. Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; *PNPLA3* I148M, rs738409 c.444 C > G p.I148M. Green: only NASH prevalence data available. Blue: only *PNPLA3* genotype data available. Red: NASH prevalence and *PNPLA3* genotype data available. Data are approximations based on published NASH prevalence estimates and genotype frequencies in patients with NAFLD as follows: Africa, 1000 Genomes Project (general population)[33]; Argentina, calculated from allele frequency[21,99]; China, data from Peng[6,99]; France[6]; Germany, data from Kantartzis[99,100]; India, calculated from allele frequency[101]; Italy, data from Valenti[6,99]; Japan, data from Kitamoto[6,99]; Mexico[102]; Saudi Arabia, data from 2017[98]; Spain[6]; UK[103]; US, data from Rotman and Speliotes and calculated from allele frequency.[6,99] Africa includes Esan in Nigeria (II, 78%; IM, 19%; MM, 3%), Gambia (II, 81%; IM, 18%; MM, 2%), Luhya in Kenya (II, 85%; IM, 13%; MM, 2%), Mende in Sierra Leone (II, 80%; IM, 18%; MM, 2%), Yoruba in Nigeria (II, 78%; IM, 21%; MM, 1%), Afro-Caribbean in Barbados (II, 76%; IM, 22%; MM, 2%) and African ancestry in south-west US (II, 72%; IM, 21%; MM, 7%)[33]

from 2017 to 2030).[98] Figure 2 combines these data with current *PNPLA3* genotype frequencies, based on the 1000 Genomes Project and other published data.[6,21,33,98-103] In China, Japan, Germany, Italy, the UK and the US, the total population of *PNPLA3* 148MM homozygotes with NASH will increase from about 12.5 million to over 18 million by 2030 (Figure 2).

South America and the Middle East have the highest prevalence of NAFLD globally (>30%).[1,104] The frequency of *PNPLA3* I148M genotypes is particularly high in Mexico and Latin America (Figure 2), and in populations of Latin American origin in the US (48% MM, 42% IM, 10% II).[99] Further epidemiological and genetic studies are needed to understand the increasing prevalence of NASH and the contribution of genetic variability in the many different heritage groups in South America[105] and elsewhere.

4.2 | PNPLA3 structure and function in health and disease

PNPLA3 is a member of a family of patatin-domain containing lipid hydrolases with specificity for an array of different substrates, including triacylglycerols, phospholipids and retinol esters.[106] *PNPLA3* expression levels are highest in hepatocytes and hepatic stellate cells in humans and in adipocytes in mice, followed by the retina and other tissues in both species.[22] High carbohydrate levels upregulate PNPLA3 levels in mice[107,108] and in human hepatocytes[109,110] by increasing transcription and reducing protein turnover. In contrast, fasting downregulates PNPLA3 levels.[111] Knockdown of wild-type *Pnpla3* expression in rats on a high-fat diet reduced liver fat content by diminishing fatty acid esterification,[112] consistent with data showing that *Pnpla3* overexpression can promote lipogenesis in mammalian cells in vitro.[113]

In vitro studies indicate that PNPLA3 localises to the surface of lipid droplets,[114] has triglyceride lipase activity,[115-117] and is involved in lipid remodelling and hepatic retention of polyunsaturated fatty acids (Figure 1).[77,118] Recent data indicate that the enzymatic activity of PNPLA3 also mediates the transfer of polyunsaturated fatty acids from triglycerides to phospholipids in hepatocytes,[119] with potentially broad effects on hepatic lipid metabolism. Furthermore, PNPLA3 has retinyl-palmitate lipase activity in vitro and is involved in retinol release by hepatic stellate cells.[120]

Evidence indicates that the NASH-associated I148M substitution abolishes the enzymatic activity of PNPLA3. This includes both the in vitro triglyceride lipase activity[114,117,121] and the ex vivo lipid remodelling activity.[119] Molecular dynamics simulations show that the substitution of methionine for isoleucine at position 148 prevents access of fatty acid substrates to the catalytic dyad (serine 47 and aspartate 166).[122]

Although lipolysis and VLDL secretion are reduced in *PNPLA3* I148M hepatocytes,[110,123] hepatic fat accumulation cannot be explained by an absence of PNPLA3 protein. Instead, impairment of hepatic triglyceride mobilisation results from build-up of PNPLA3 on lipid droplets (Figure 1). Genetic deletion of the mouse *Pnpla3* gene from conception does not influence hepatic fat accumulation.[124,125] Hepatic steatosis develops in mice overexpressing exogenous human PNPLA3 148M, but not those overexpressing the ancestral 148I protein.[126] Hepatic steatosis also develops in knock-in mice carrying the I148M variant in endogenous mouse *Pnpla3* when they are fed a high-sucrose diet,[127] and this can be ameliorated by silencing *Pnpla3* expression with antisense oligonculeotides.[128] Furthermore, PNPLA3 protein levels on the surface of lipid droplets are higher for 148M than 148I in both the *PNPLA3*-overexpressing and knock-in mouse models (Figure 1). This difference results from decreased ubiquitination and proteasomal degradation of PNPLA3 148M,[129] not from increased mRNA levels.[126,127]

Increased PNPLA3 levels on lipid droplets appear to reduce hepatic lipolysis via sequestration of a lipase cofactor, CGI-58 (also known as 1-acylglycerol-3-phosphate O-acyltransferase, or ABHD5). Enzymatically inactive PNPLA3 148M is still able to bind CGI-58 and prevent it from activating other lipases present on lipid droplets.[91] Competition for CGI-58 between PNPLA3 and adipose triglyceride lipase (ATGL, encoded by *PNPLA2*) has been reported in brown adipocytes.[130] Furthermore, a loss-of-function variant in ABHD5 (encoding CGI-58) is associated with a rare autosomal dominant form of inherited NAFLD.[131] A ubiquitination-resistant but fully enzymatically active variant of PNPLA3 has recently been shown to accumulate on lipid droplets and increase hepatic triglyceride levels in transgenic mice.[132] Increased de novo lipogenesis does not appear to be involved in the effect of PNPLA3 148M.[133,134] Reduced degradation of lipid droplets via autophagy is one potential mechanism for the increase in hepatic triglyceride levels in the presence of PNPLA3 148M.[135]

Current understanding is therefore that the association of *PNPLA3* I148M with hepatic steatosis may result less from direct loss of PNPLA3 lipase/lipid remodelling activity than from indirect reduction of CGI58-mediated ATGL activity. PNPLA3 148M acts as a trans-repressor of lipid droplet lipase activity by competing for a shared co-activator, and this trans-repression is what causes hepatic lipid accumulation (Figure 1), rather than the loss of PNPLA3 enzymatic activity itself.[91]

PNPLA3 I148M may also disrupt retinol release by hepatic stellate cells, potentially leading to fibrosis (Figure 1). PNPLA3 promotes release of retinol by hepatic stellate cells in response to insulin and transforming growth factor β in vitro.[120,136] The I148M variant is associated with a loss of retinyl-palmitate lipase activity and a resulting impairment in retinol production by hepatic stellate cells.[120,136] Impaired retinoid production may lead to reduced secretion of matrix metalloproteinases and tissue inhibitors of metalloproteinase, resulting in extracellular matrix deposition (Figure 1).[136,137] Hepatic stellate cells expressing PNPLA3 148M also secrete elevated levels of pro-inflammatory cytokines, which may potentiate their fibrogenic potential compared with ancestral PNPLA3 148I.[137] In agreement with these in vitro findings, *PNPLA3* I148M was associated with reduced circulating retinol and increased intrahepatic retinol levels in individuals with NAFLD or obesity.[138,139] Furthermore, reduced levels of retinoic acid metabolites in the liver may promote activation of hepatic stellate cells by macrophages in response to internalisation of apoptotic cells (mediated by c-mer tyrosine kinase).[140]

The effects of *PNPLA3* I148M on lipid droplet remodelling in hepatocytes and retinol production by hepatic stellate cells (Figure 1) suggest that a precision medicine able to reduce PNPLA3 levels could provide therapeutic benefits to I148M-carrying patients with NASH. Silencing of *Pnpla3* expression with hepatocyte-targeted *N*-acetylgalactosamine-conjugated antisense oligonucleotides ameliorated steatohepatitis and liver fibrosis in homozygous *Pnpla3* 148MM knock-in mice, but not in wild-type *Pnpla3* 148II littermates fed a NASH-inducing diet.[128] Furthermore, liver steatosis was reduced in *Pnpla3* 148MM knock-in mice fed a high-fructose diet following either knockdown of *Pnpla3* expression with short hairpin RNA (expressed from an adeno-associated virus vector), or lowering of PNPLA3 protein levels with a proteolysis-targeting chimera (PROTAC).[132,141]

Modulating levels of PNPLA3 or its interaction with lipase cofactors with these or other approaches could provide routes to therapeutic intervention in *PNPLA3* I148M-carrying patients with NASH, as previously suggested.[142] Reduced levels of PNPLA3 148M protein may, however, have broad effects on lipid metabolism or may be compensated for by multiple mechanisms. Improved understanding of downstream mediators could also provide potential routes to therapeutic intervention in patients with NASH, including those not carrying *PNPLA3* I148M.

4.3 | Impact of *PNPLA3* I148M on treatment of NASH

4.3.1 | *PNPLA3* I148M and response to treatment

No pharmacological therapies are approved for the treatment of NASH, and liver transplantation is the only available treatment for cirrhosis.[143,144] Guidelines recommend reducing body weight through lifestyle interventions, dietary restriction and physical activity,[145] but the effectiveness of lifestyle interventions is often limited and short term.[146,147] Bariatric surgery can significantly reduce hepatic steatosis, steatohepatitis and fibrosis in patients with obesity, as detailed in two recent meta-analyses,[148,149] but it carries a significant risk of complications.[145]

Very limited evidence suggests that *PNPLA3* I148M may modulate the response to treatment in patients with NASH. Lifestyle modification and bariatric surgery have been reported to be more effective in reducing liver fat levels in *PNPLA3* I148M carriers than in noncarriers (Table 2).[61,150-152] In contrast, omega-3 fatty acid supplementation may be less effective in decreasing liver fat levels in *PNPLA3* I148M carriers than in noncarriers in randomised-controlled trials (Table 3).[153-156] An increased effect of high dietary omega-6 to omega-3 polyunsaturated fatty acid ratio on liver fat levels has also been reported in homozygotes (Table 3).[157] The modest effect size of omega-3 fatty acids on liver fat levels in clinical trials and the small number of homozygous participants make the magnitude of the genotypic effect difficult to quantify.

Associations of *PNPLA3* I148M with reduced protective effects of statins on steatosis and NASH in clinical trials have been reported.[156,158] *PNPLA3* I148M has also been associated with an increased risk of ALT elevation in patients receiving potentially hepatotoxic medications, such as asparaginase for acute lymphoblastic leukaemia,[159,160] or diabetes medications that can increase liver fat levels.[161,162]

Although patients with NASH carrying *PNPLA3* I148M may lose more liver fat than noncarriers after a successful intervention, they are also likely to start from a worse pre-treatment baseline. Patients may benefit most from personalised therapies that act upstream to reduce the liver fat accumulation associated with the variant.

4.3.2 | Risk stratification based on *PNPLA3* I148M genotype

Guidelines recommend that people with important risk factors such as type 2 diabetes, insulin resistance, obesity and metabolic syndrome should be screened for NASH because of its prognostic

TABLE 2 Interaction of weight-loss interventions with *PNPLA3* genotype in NAFLD or obesity

Reference	Participants	Interventions	Study design	Main liver-related outcome	*PNPLA3* I148M association
Shen et al[150]	Adults with NAFLD (N = 154)	12-mo dietician-led programme or standard care	Parallel-group	Greater decrease in liver fat[a] than standard care	Increased reduction in liver fat[a] in the intervention group
Krawczyk et al[151]	Adults with suspected NAFLD (N = 143)	4-mo dietician-led programme	Prospective, observational	Significant decrease in liver fat[b] from baseline	None
Krawczyk et al[152]	Adults with obesity (N = 84)	Bariatric surgery	Prospective, observational	Decrease from baseline in liver fat[c]	Increased reduction in liver fat[c]
Palmer et al[61]	Adults with obesity (N = 3473)	Bariatric surgery	Parallel-group	Reduced BMI and serum triglycerides in surgery group only[d]	Increased reduction in BMI and serum triglycerides

Abbreviations: BMI, body mass index; NAFLD, non-alcoholic fatty liver disease.

[a]Magnetic resonance spectroscopy.

[b]Ultrasonography.

[c]Magnetic resonance imaging proton density fat fraction.

[d]Liver fat not assessed.

TABLE 3 Interaction of omega-3 fatty acid intake with *PNPLA3* genotype in NAFLD

Reference	Participants	Interventions	Study design	Main liver-related outcome	*PNPLA3* I148M association
Oscarsson et al[155]	Adults with NAFLD and lipidaemia (N = 78)	ω-3 carboxylic acids, fenofibrate or placebo	Randomised, double-blind, parallel-group	No significant effect on liver fat[a] versus placebo	No effect on response to either treatment
Eriksson et al[156]	Adults with NAFLD and T2DM (N = 84)	Dapagliflozin, ω-3 carboxylic acids, both, or placebo	Randomised, double-blind, parallel-group	Decreased liver fat[a] with all active treatments versus placebo	Trend towards reduced response to treatment with ω-3 carboxylic acids
Scorletti et al[154]	Adults with NAFLD (N = 103)	ω-3 ethyl esters (EPA + DHA) or placebo	Randomised, double-blind, parallel-group	Association between DHA enrichment and decreased liver fat[b]	Reduced response to treatment
Nobili et al[153]	Children with NAFLD (N = 60)	DHA or placebo	Randomised, double-blind, parallel-group	Decreased liver fat[c] versus placebo	Reduced response to treatment
Santoro et al[157]	Children and adolescents with obesity (N = 127)	None	Genetic and dietary association	Dietary ω-6/ω-3 PUFA ratio associated with liver fat[a]	Increased effect of high dietary ω-6/ω-3 PUFA ratio in homozygotes

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NAFLD, non-alcoholic fatty liver disease; PUFA, polyunsaturated fatty acid; ω, omega.

[a]Magnetic resonance imaging proton density fat fraction.

[b]Magnetic resonance spectroscopy.

[c]Ultrasonography.

implications.[145] While recognising that *PNPLA3* I148M may allow risk stratification for tailored HCC surveillance, guidelines do not recommend routine genotyping of any variant.[145] The recent association of *PNPLA3* I148M with both liver-related and all-cause mortality in a general US population sample highlights the unmet need for effective targeted therapies to prevent disease progression and death in carriers.[28,89]

Polygenic risk scores adjusted for conventional risk factors may, in the future, have the potential to guide care of patients with NAFLD. Age, sex, BMI, fasting glucose levels and risk variants in *PNPLA3*, *TM6SF2* and *MBOAT7* all emerged as independent predictors of liver damage in a logistic regression analysis, with an additive effect of the genetic variants on hepatic triglyceride content.[47] Polygenic risk scores for coronary artery disease, however, are the furthest advanced but have still not yet entered routine clinical practice.[163] Additional studies and evidence are needed before speculating on whether genotyping could be used to guide treatment decisions in patients at risk of NASH.

5 | CONCLUSION

The identification of genes associated with development and progression of NASH provides important insights into the pathophysiology that may in time provide novel opportunities for therapeutic intervention. Genetic discoveries provided the impetus for cell and molecular biological studies aiming to elucidate the mechanism responsible for the association between genetic variants and liver disease progression. *PNPLA3* 148M acts as a trans-repressor of lipid droplet lipase activity by competing for a shared co-activator. This indicates that reducing *PNPLA3* expression levels could potentially attenuate its negative effect on hepatic lipolysis. Consistent with this possibility, people with a genetic variant that reduces *PNPLA3* expression levels are less susceptible to the effect of I148M on liver fat than those without the expression-reducing variant. Knowledge of the underlying mechanisms remains incomplete, with current research focusing on cofactor recruitment to lipid droplets and lipidomic analyses of alterations to lipid metabolism in hepatocytes and hepatic stellate cells. Despite these uncertainties, an aetiological distinction can be drawn between NASH associated with *PNPLA3* I148M and other forms of NASH that are primarily driven by insulin resistance. This possibility presents opportunities for the development of a precision medicine that can modulate the activity of a specific gene (*PNPLA3*) in a specific organ (the liver) of a specific group of patients (I148M carriers with NASH). Other genes associated with NASH, including *HSD17B13*, may provide future targets for intervention strategies. All novel therapies require extensive assessment of safety and efficacy in clinical trials. Progress towards proof-of-concept studies of a precision medicine for patients with NASH is ultimately driven by the robust human genetic and molecular and cell biological evidence base.

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AUTHORSHIP

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