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Review

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PNPLA3 as a driver of steatotic liver disease: navigating from pathobiology to the clinics via epidemiology

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Abstract

Steatotic liver disease (SLD), particularly metabolic dysfunction-associated SLD, represents a significant public health concern worldwide. Among the various factors implicated in the development and progression of this condition, the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene has emerged as a critical player. Variants of *PNPLA3* are associated with altered lipid metabolism, leading to increased hepatic fat accumulation and subsequent inflammation and fibrosis. Understanding the role of *PNPLA3* not only enhances our comprehension of the pathomechanisms driving SLD but also informs potential therapeutic strategies. The molecular mechanisms through which *PNPLA3* variants contribute to lipid dysregulation and hepatocyte injury in SLD are critically discussed in the present review article. We extensively analyze clinical cohorts and population-based studies underpinning the association between *PNPLA3* polymorphisms and the risk of developing SLD, and its liver-related and protean extrahepatic outcomes, in concert with other risk modifiers, notably including age, sex, and ethnicity in adults and children. We also discuss the increasingly recognized role played by the *PNPLA3* gene in liver transplantation, autoimmune hepatitis, and acquired immunodeficiency syndrome. Finally, we examine the clinical implications of *PNPLA3* diagnostics regarding risk stratification and targeted therapies for patients affected by SLD in the context of precision medicine approaches.

Keywords: Cirrhosis, extrahepatic outcomes, ethnicity, hepatocellular carcinoma, insulin resistance, liver transplantation, MASLD, *PNPLA3* gene, cardio-nephro-metabolic syndrome, precision medicine, sex differences, steatotic liver disease

INTRODUCTION

The global increase in the prevalence of liver diseases, particularly Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD), has become a significant public health issue. MASLD is characterized by an excessive accumulation of fat in the liver. Globally, the adult population has witnessed an increased prevalence of MASLD from 25.3% in 1990-2006 to 38.2% in the years 2016-2019[1]. MASLD identifies steatosis associated with ≥ 1 factor of cardiometabolic risk[2]. Additionally, MASLD is closely associated with either the full metabolic syndrome or its components (e.g., obesity, type 2 diabetes) and often with the complications of the metabolic syndrome (e.g., cardio-nephro-vascular disease)[3]. Given its rising prevalence and potential to progress to more severe forms such as Metabolic Dysfunction-Associated Steatohepatitis (MASH), cirrhosis, and Hepatocellular Carcinoma (HCC), understanding the underlying mechanisms driving MASLD is crucial for the prognosis of patients with MASLD and for developing effective prevention and treatment strategies[4]. Particularly, the Patatin-like phospholipase domain-containing protein 3 (PNPLA3) has garnered significant attention in the pathogenesis of steatotic liver disease due to its strong association with hepatic fat accumulation and progression to MASLD[5]. Genetic variants of *PNPLA3*, particularly the I148M polymorphism, have been associated with increased susceptibility to liver damage and fibrosis[6,7]. This makes *PNPLA3* a key focus for understanding the molecular mechanisms underlying steatosis and potential therapeutic targets for managing liver diseases. Additionally, its role in lipid metabolism further emphasizes its importance in liver health and disease.

Here we try to offer an in-depth overview of *PNPLA3* as a driver of Steatotic Liver Disease (SLD) by exploring both pathobiological mechanisms and epidemiological evidence. By synthesizing current knowledge on how this gene contributes to hepatic fat accumulation and its implications for public health policies aimed at managing rising rates of MASLD globally, we aim to shed light on future research directions that could pave the way for innovative treatments in the setting of precision medicine approaches.

ROLE OF THE *PNPLA3* GENE IN HEALTH AND STEATOTIC LIVER DISEASE

Among the various genetic factors influencing MASLD, the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*, OMIM: 609567) gene has garnered significant attention. The gene, also known as adiponutrin, calcium-independent phospholipase A2ε (iPLA2ε), acylglycerol transacylase, or 1-acylglycerol-3-phosphate O-acyltransferase, was first identified in mouse 3T3 pre-adipocyte cell lines and located on human chromosome 22q13.1 by sequence similarity search[8]. It belongs to the family of patatin-like phospholipase domain-containing proteins (PNPLAs) that in humans consists of nine members with crucial roles in preserving the structure and function of organelle membranes, cell growth, signaling, cell death control, and the general metabolism of lipids, including triacylglycerol, phospholipids, ceramides, and retinyl esters [Table 1][9].

The individual members of this protein family vary in size; the smallest member, PNPLA4, consists of 253 amino acids, while the largest member, PNPLA6, contains 1,327 amino acids [Figure 1][9]. These members are further classified into the adiponutrin group, which includes PNPLA1-5, the neuropathy target esterase group, including PNPLA6 and PNPLA7, and PNPLA8 and PNPLA9 which have specific characteristics and do not belong to either of these two groups[9].

Table 1. Characteristics of the human patatin-like phospholipase domain-containing proteins

Member	OMIM	Alias	Chromosomal localization*	Exons	Transcript length (nt)/acc. no**	Protein size (aa)/acc. no.**	Mw (kDa)***	Selected function
PNPLA1	612121	Omega-hydroxyceramide transacylase; FLJ38755;ARCI10; dJ50J22.1; EC 2.3.1.296	6p21.31	11	2367 NM_173676.2	437 AAI03906.1	48.96	Transfers fatty acyl groups from triacylglycerol to omega-hydroxy ceramides to form acylceramides
PNPLA2	609059	Adipose triglyceride lipase (ATGL); Desnutrin; Transport-secretion protein 2 (TT2); FP17548; Pigment epithelium-derived factor receptor; Phospholipase A_2, calcium-independent ζ; 1110001C14Rik	11p15.5	10	2416 NM_020376.4	504 NP_065109.1	55.32	Triglyceride lipase; catalyzes the first step in the hydrolysis of triglycerides in adipose tissue
PNPLA3	609567	Adiponutrin (ADPN); Phospholipase A_2, calcium-independent ε; C22orf20; 1-Acylglycerol-3-Phosphate O-Acyltransferase; Lysophosphatidic acid acyltransferase; Acylglycerol transacylase	22q13.31	9	2753 NM_025225.3	481 AAH65195.1	52.84	Multifunctional *enzyme* with both triacylglycerol lipase and acylglycerol O-acyltransferase activity
PNPLA4	300102	GS2 Gene (GS2); Phospholipase A_2, calcium-independent η (IPLA2-eta); DXS1283E	Xp22.31	8	3342 NM_004650.3	253 AAH20746.1	27.88	Enzyme that has both triacylglycerol lipase and transacylase activities
PNPLA5	611589	GS2-like protein (GS2L); DJ388M5.4; DJ388M5	22q13.31	9	2540 NM_138814.4	429 AAH31820.1	47.91	Abundant triacylglycerol hydrolase activity
PNPLA6	603197	Neuropathy target esterase (NTE); Neurotoxic esterase; SPG39; Sws; NTEMND; BNHS, LNMS, OMCS	19p13.2	37	4536 NM_001166111.2	1375 NP_001159583.1	146,215.65	Deacetylates intracellular phosphatidylcholine to produce glycerophosphocholine
PNPLA7	612122	NTE-Like1 (NTEL1); Chromosome 9 open reading frame 111 (C9org111); NTE-R1; RP11-48C7.2; FLJ43070; FLJ31318; FLJ44279	9q34.3	37	4675 NM_001098537.3	1342 NP_001092007.2	148,431.39	Endoplasmic reticulum transmembrane protein that specifically promotes hydrolysis of lysophosphatidylcholine
PNPLA8	612123	PNPLA-γ, Phospholipase A_2, calcium-independent, intracellular membrane-associated γ (IPLA2-gamma); IPLA2-2; MMLA	7q31.1	15	4681 NM_015723.5	782 AAH32999.1	88,476.86	Cleavage of fatty acids from phospholipids, thereby regulating membrane physical properties and the release of lipid second messengers and growth factors
PNPLA9/PLA2G6	603604	Phospholipase A_2, group VI (PLA2G6), Phospholipase A_2, calcium-independent (IPLA2); Phospholipase A_2, calcium-independent, group VI, A (IPLA2-VIA); PARK14, Neurodegeneration with brain iron accumulation 2 (NBIA2); 85/88 kDa calcium-independent phospholipase A_2; 2-Lysophosphatidylcholine acylhydrolase; Palmitoyl-CoA hydrolase; CaI-PLA2; GVI PLA2; INAD1; GVI	22q13.1	18	3299 NM_003560.4	806 AAH36742.2	89,903.01	Hydrolyses membrane phospholipids to produce potent lipid second messengers

*The chromosomal locations of *PNPLA* genes were obtained from the online catalog of human genes and genetic disorders (OMIM) at https://www.omim.org; **Transcript lengths and protein sizes were sourced from information provided by the National Library of Medicine at https://www.ncbi.nlm.nih.gov; ***The molecular weight of individual PNPLA proteins was calculated using the expasy tool Compute pI/Mw at https://web.expasy.org/compute_pi. aa: Amino acids; acc. no.: accession number; Mw: molecular weight; nt: nucleotides.

PNPLA3, located on the long arm of chromosome 22, encodes a transmembrane protein with triglyceride hydrolase activity that plays a pivotal role in lipid metabolism within hepatocytes[10]. Variants of this gene, particularly the I148M polymorphism (rs738409), have been linked to increased hepatic steatosis and higher odds of hepatic injury [Figure 2][11]. This single nucleotide polymorphism (SNP) leads to an amino acid substitution that alters the enzymatic function of PNPLA3, impacting triglyceride hydrolysis, lipid droplet-Golgi dynamics, mitochondrial dysfunction, retinol metabolism, antioxidant responses, and increased TGF-β1 signaling, thereby contributing to lipid accumulation in the liver[11-13]. The reasons for these changes that occur in the absence of functional *PNPLA3* will be discussed later (see Section "*PNPLA3*-I148M gene variant: implications for triglyceride hydrolysis"-"*PNPLA3*-I148M and retinol metabolism"), offering a comprehensive understanding of the underlying mechanisms involved.

Research over the past decade has elucidated several mechanisms through which PNPLA3 influences hepatic steatosis. The I148M variant appears to impair lipolysis and promote lipid droplet formation within hepatocytes. This dysfunction not only results in increased triglyceride storage but also triggers inflammatory pathways that can lead to cellular injury and fibrosis over time[12]. Understanding these pathobiological processes is essential for identifying potential therapeutic targets. Studies on human hepatocytes with *PNPLA3*-148I and -148M variants implanted in the livers of immunodeficient chimeric mice have shown that hepatocytes carrying the *PNPLA3*-148M variant, whether from homozygous donors or overexpressed in a heterozygous background, showed more severe microvesicular steatosis and ballooning degeneration compared to those with the 148I variant. This indicates a heightened risk for steatohepatitis[14].

As extensively discussed in Section "ROLE OF THE *PNPLA3* GENE IN HEALTH AND STEATOTIC LIVER DISEASE" of the present review, epidemiological studies have shown a strong link between *PNPLA3* variants and susceptibility to MAFLD in various populations[15-18]. In obese individuals, the expression of *PNPLA3* in the liver was higher in women than in men, correlating with estrogen levels. Estrogen receptor-α (ER-α) agonists increased *PNPLA3* expression in human hepatocytes and liver organoids[18]. Researchers identified an ER-α-binding site within a *PNPLA3* enhancer that drives the upregulation of the p.I148M variant through chromatin immunoprecipitation and luciferase assays, as well as CRISPR-Cas9 genome editing. This ultimately leads to steatogenesis and fibrogenesis in three-dimensional spheroids containing hepatic stellate cells (HSCs), indicating that the interaction between ER-α and the *PNPLA3* p.I148M variant is a key player in the development of SLD in women[18].

Despite considerable advances in our understanding of *PNPLA3*'s role in SLD, there are still several gaps in our knowledge base. For example, while much research has focused on its genetic implications, there is limited insight into how environmental factors, such as diet and lifestyle, interact with genetic predispositions to influence disease outcomes. Additionally, questions about how *PNPLA3*-related pathways can be targeted therapeutically remain largely unanswered. Some of these research questions will be addressed in the next sections of this review, specifically examining how an improved understanding of the role of *PNPLA3* in the initiation and worsening of SLD due to various etiologies has opened avenues for targeted therapeutic interventions and lifestyle modifications aimed at mitigating liver damage.

PNPLA3-I148M gene variant: implications for triglyceride hydrolysis

PNPLA3 is involved in lipid metabolism, facilitating the hydrolysis of triglycerides into free fatty acids and glycerol. The missense mutation at amino acid position 148, which changes isoleucine to methionine in PNPLA3, is located next to the Ser47-Asp166 catalytic dyad and is part of a hydrophobic substrate-binding groove in the active site[19]. When overexpressed in the liver of mice, *PNPLA3*-I148M caused a significant

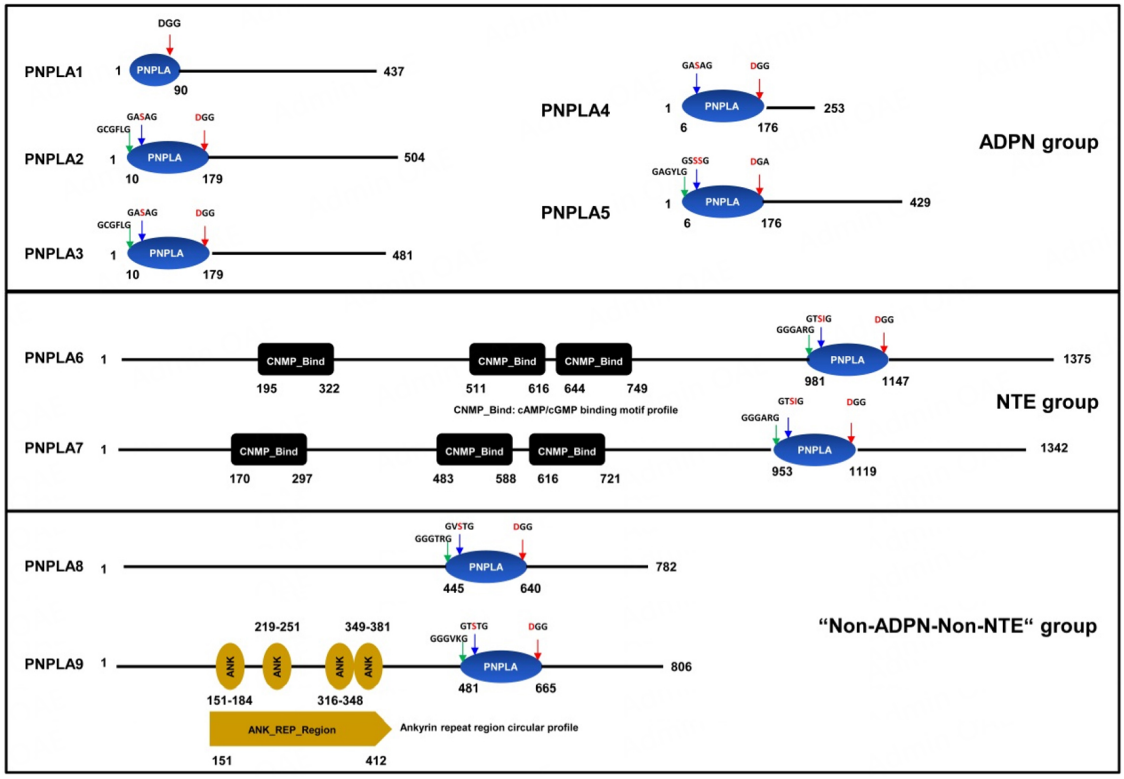

Figure 1. Structure of human patatin-like phospholipases. The family of PNPLAs consists of 9 members (PNPLA1-PNPLA9) characterized by a patatin-like phospholipase domain. The first five members are subclassified into the adiponutrin (ADPN) group, while members PNPLA6-PNPLA7 belong to the neuropathy target esterase (NTE) group, and the last two members do not belong to either group. The PNPLA motif of PNPLA2-PNPLA9 typically contains a GXGXXG motif, a GXSXG motif, and a DGA/G motif. The active nucleophile site in the GXSXG motif and the proton acceptor site in the DGA/G motif are marked in red letters. Members of the NTE groups also have several cAMP/cGMP binding site motifs (CNMP-Bind), while PNPLA9 contains four ankyrin repeats. The numbers correspond to amino acid positions in human proteins. Motif search was done using the Expasy ScanProsite tool (https://prosite.expasy.org/scanprosite/) and protein sequences of individual PNPLA proteins were taken from GenBank sequence (https://www.ncbi.nlm.nih.gov/protein/) entries listed in Table 1.

increase in the number and size of lipid droplets, as well as in the levels of triglycerides and cholesterol esters in the tissue[19]. The authors of the study also showed that the rise in triglycerides is due to a decrease in hydrolysis rather than an increase in fatty acid esterification[19].

Recent findings suggest that PNPLA3 preferentially hydrolyzes polyunsaturated triglycerides in an adipose triglyceride lipase (ATGL)-independent manner, mobilizing polyunsaturated fatty acids for phospholipid desaturation and increasing hepatic secretion of large-sized very low density lipoproteins [Figure 3][20]. However, when mutated, this enzymatic function is compromised, leading to the accumulation of triglycerides within hepatocytes, causing SLD.

In summary, the *PNPLA3*-I148M variant exhibits decreased enzymatic activity in metabolizing triglycerides and is also less active in secreting hepatic triglycerides. Consequently, this variant negatively impacts the balance between hepatic triglyceride metabolism, storage, and secretion.

Effects of *PNPLA3*-I148M on lipid droplet-Golgi dynamics

The Golgi apparatus plays a significant role in processing lipids for secretion or incorporation into membranes[21]. Under normal circumstances, PNPLA3 interacts dynamically with lipid droplets during

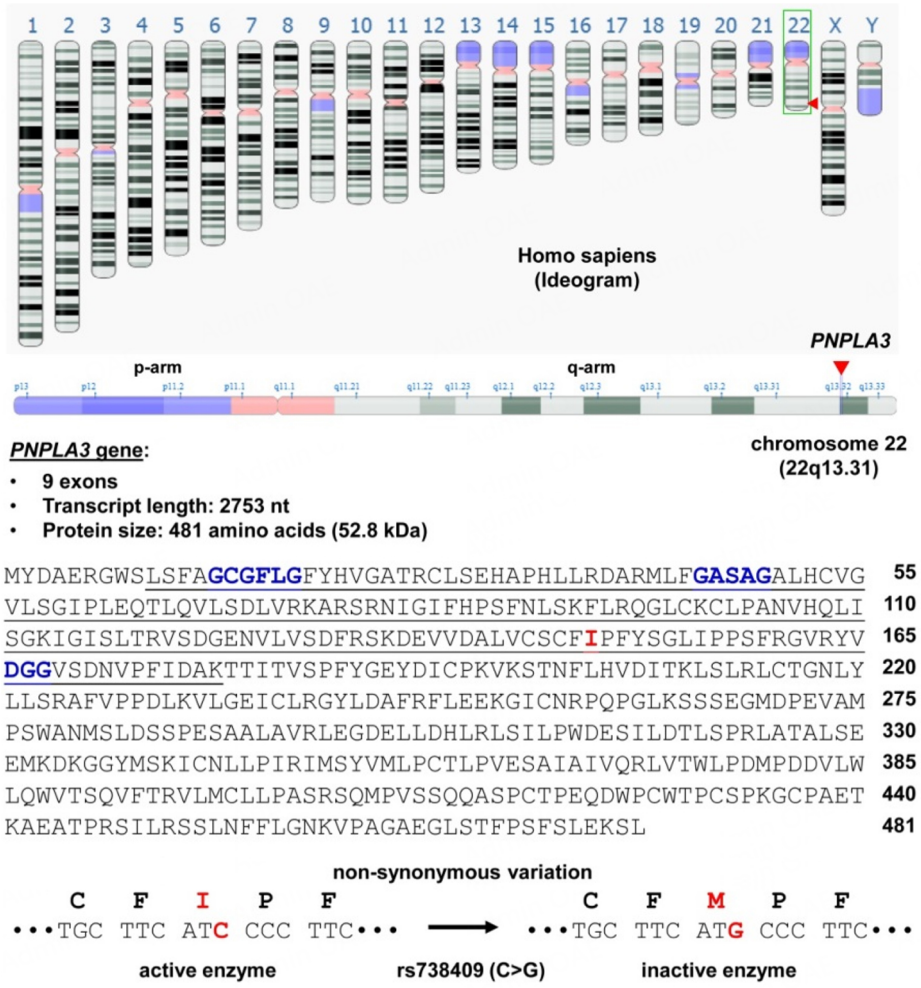

Figure 2. The *PNPLA3* gene. The *PNPLA3* gene is located on the long arm (q-arm) of the human chromosome in region 22q13.31. It encodes a protein of 481 amino acids in size that has a PNPLA motif (underlined), which contains the characteristic GXGXXG, GXSXG, and DGA/G motifs (all in blue). The non-synonymous substitution (rs738409) of isoleucine to methionine at position 148 results in a protein with reduced enzymatic activity. The image of the ideogram was taken from the Genome Data Viewer of the National Library of Medicine (https://www.ncbi.nlm.nih.gov/gdv/).

triglyceride hydrolysis, allowing for efficient transfer of lipids between droplets and other cellular compartments, including the Golgi apparatus. The accumulation of lipids disrupts normal lipid size and composition, affecting their ability to interact efficiently with Golgi membranes. This disruption may also provoke dyslipidemia and trigger stress responses. Recent work demonstrated that the mutated *PNPLA3*-I148M variant induces direct structural alterations in the Golgi apparatus, including increased lipid droplet-Golgi contact sites and enlarged Golgi cisternae[10]. Importantly, these changes were associated with morphological, proteomic, and transcriptional changes within the cell that are compatible with those found in the MASH spectrum. This supports the notion that the I148M mutation alone is capable of driving all stages of MASLD[22]. Another report demonstrated that PNPLA3 can physically interact with the lipid-droplet-associated protein Perilipin 5 (PLIN5) and the adipose triglyceride lipase (ATGL), the rate-limiting enzyme in lipolysis[22]. The binding of either PNPLA3 or PLIN5 to ATGL reduces its lipogenic activity. Interestingly, compared to PNPLA3, the binding of PNPLA3-I148M to ATGL exhibited a stronger inhibitory effect[22]. Since once activated, ATGL facilitates the transfer of lipids to the Golgi apparatus for further processing, reduced activation of ATGL will lead to impaired Golgi dynamics, affecting overall lipid homeostasis[22].

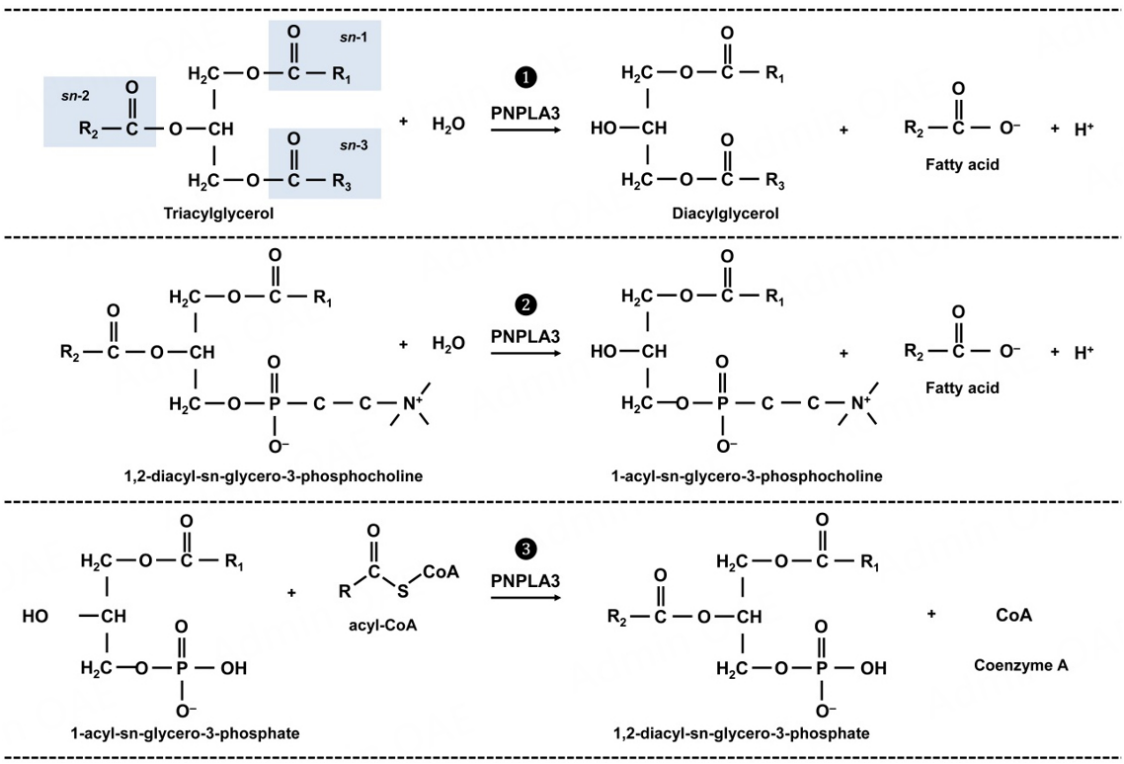

Figure 3. Enzymatic activity of PNPLA3. PNPLA3 is an important protein that catalyzes crucial reactions in lipid metabolism. It primarily catalyzes three reactions: ❶ acting as a triacylglycerol lipase, ❷ functioning as a phospholipase A_2 by removing the fatty acid attached to the 2-position of phosphatidylethanolamine, choline plasmalogen, and phosphatides, and ❸ specifically catalyzing coenzyme A (CoA)-dependent acylation of 1-acyl-sn-glycerol 3-phosphate (2-lysophosphatidic acid) to generate phosphatidic acid.

Role of *PNPLA3*-I148M in mitochondrial dysfunction

In a recent study by Gou *et al.*, it was demonstrated that the non-synonymous *PNPLA3*-I148M substitution provokes free cholesterol accumulation in human hepatic stellate cell (HSC) line LX-2 by reducing the expression of the ATP-binding cassette sub-family G member 1 (ABCG1) and inhibiting cholesterol efflux[12]. The accumulation of cholesterol within cells further impairs mitochondrial structure and functionality, resulting in a reduced expression of proteins associated with mitochondria, including superoxide dismutase (SOD-1) and the mitochondrial protein Mitofusin (MFN2), which plays a central role in regulating mitochondrial fusion and cell metabolism. This process ultimately stimulates the activation of LX-2 cells and contributes to the emergence of a fibrotic phenotype[12]. These results offer fresh insights into how *PNPLA3*-I148M influences lipid metabolism, mitochondrial impairment, and liver fibrosis. In the same line, *PNPLA3*-I148M was linked to respiratory chain complex IV insufficiency, elevated secretion of reactive oxygen species (ROS), and reduced expression of the orphan nuclear receptor NR4A1, which regulates the ROS/endoplasmic reticulum stress pathways[13,23].

Consequently, individuals with homozygous *PNPLA3*-I148M mutations exhibit changes in intrahepatic anabolic and catabolic processes, as well as mitochondrial function. These alterations include a reduction in *de novo* lipogenesis whereas intrahepatic mitochondrial beta-oxidation and ketogenesis are increased[24]. Such changes are associated with an elevated mitochondrial redox state and a decreased flux of hepatic mitochondrial citrate synthase. These findings confirm that SLD caused by the *PNPLA3*-I148M variant

results in dysfunctional liver mitochondria[24].

Modulation of TGF-β1 signaling by *PNPLA3*-I148M

PNPLA3 not only impacts aspects of fat metabolism in the liver but also has a direct profibrogenic activity[25]. The expression of the *PNPLA3* gene and protein rises during the initial stages of activation and remains elevated in fully activated HSCs, while silencing *PNPLA3* notably reduces the levels of the profibrogenic protein α-smooth muscle actin[25]. Primary human HSCs with the I148M variant exhibit significantly increased expression and release of proinflammatory cytokines and show lower retinol levels but a higher accumulation of lipid droplets. Similarly, LX-2 cells that stably overexpress I148M demonstrate enhanced proliferation and migration, reduced retinol levels, diminished transcriptional activities of retinoid X receptor/retinoid A receptor, and increased lipid droplet formation. Silencing *PNPLA3*-I148M leads to decreased expression of collagen1α1, suggesting that *PNPLA3* is essential for HSC activation and that its genetic variant I148M enhances the profibrogenic characteristics of HSCs, elucidating a molecular mechanism underlying the increased risk for progression and severity of liver diseases in patients carrying the I148M variant[25]. In this context, it is worth noting that in primary human HSC, *PNPLA3* is upregulated by TGF-β1 and that the mutant PNPLA3, but not the wild type PNPLA3 protein, reduces the secretion of matrix metalloprotease (MMP) 2 (MMP2) that suppresses collagen type I expression and tissue inhibitor of metalloproteinase 1 and 2 (TIMP1 and TIMP2) that promote fibrosis in the injured liver by inhibiting MMPs[26-28]. Contrastingly, another study has shown that the downregulation of *PNPLA3* resulted in increased expression of profibrogenic factors and exacerbated fibrotic responses in human HSCs in the presence of TGF-β1, regardless of the *PNPLA3* genotype[29].

PNPLA3-I148M and retinol metabolism

Liver extracts from individuals homozygous for the *PNPLA3*-I148M minor allele exhibit increased concentrations of retinyl palmitate but a decreased retinol-to-retinyl-palmitate ratio[30]. These variations were still significant in a multivariate analysis that accounted for the severity of steatosis. Additionally, the levels of minor retinyl-fatty acid esters were also found to be elevated in those carrying two copies of the *PNPLA3*-I148M variant[30]. Excess of retinoids is linked to hepatic fibrosis, and the loss of intracellular retinoid stores is a hallmark of HSC activation[31,32]. This supports a possible association between the *PNPLA3* variant, hepatic retinoid metabolism, SLD, and other types of chronic liver disease in humans. Another report found that the *PNPLA3*-I148M genotype is a strong predictor of circulating retinol-binding protein 4, a reliable surrogate of retinol concentrations in humans[33], underpinning the important role of *PNPLA3*-I148M as a crucial lipase responsible for retinyl-palmitate hydrolysis in HSCs in humans, a crucial factor associated with the initiation and progression of liver disease. Another study reported that carriers of the mutant allele with SLD and obesity or obesity alone had reduced concentrations of fasting retinol[34], again linking *PNPLA3*-I148M to a feature associated with hepatic steatosis.

CLINICAL EPIDEMIOLOGY

PNPLA3 variants have a significant impact on the development, progression, and complications of MASLD, affecting both liver-related and extrahepatic manifestations in adults and children. To support this claim, we will explore the various ways in which the *PNPLA3* gene interacts with ethnicity, population-based epidemiology, and clinical epidemiology. Furthermore, we will discuss how the *PNPLA3* gene interacts with other gene variants, sex, and liver disease among those living with human immunodeficiency virus (HIV) infection, or in the context of liver transplantation, or in autoimmune hepatitis (AIH).

PNPLA3: race/ethnicity, and spectrum of liver disease

Although the concepts of "race" and "ethnicity" are subjective constructs that lack a universally accepted definition, several studies have identified racial/ethnic disparities in MASLD[35]. These differences among

ethnic groups can provide insight into various aspects of SLD, such as access to care and pathogenic determinants. Caldwell was one of the first investigators to note that MASH and cryptogenic cirrhosis (referred to as "burnt-out MASH") were underreported among African Americans. He pointed out that this discovery contradicted the overrepresentation of major risk factors for MASH among this population. Caldwell suggested that this discrepancy could be due to under-recognition, under-referral, or a lower prevalence of these disorders among African Americans[36]. We now understand that, in addition to lifestyle habits and varying access to care, the risk of MASLD in each population tends to align with the frequency of the G allele of the *PNPLA3* gene in that population[35,37]. Supporting this idea, a recent global assessment of disability-adjusted life years and deaths in 2021 identified Mexico as the country with the highest relative MASLD burden worldwide[38]. This finding is consistent with another study showing that Hispanics with Mexican, Central, and South American heritage have a higher prevalence of the *PNPLA3*-G risk allele compared to Hispanics of European or Afro-Caribbean descent[39]. Table 2 summarizes the *PNPLA3*-G risk allele frequency in different countries and geographical areas[6].

The differences in the frequency of the *PNPLA3*-148 M allele [Table 2] likely contribute - along with variations in obesity prevalence, urbanization levels, diet, and other lifestyle habits - to the observed differences in MASLD prevalence in the same countries and geographical areas[6].

Concerningly, the *PNPLA3*-G allele is linked to markers of liver fibrosis[39], cirrhosis[40], liver-related events, and mortality[41], as well as HCC in Caucasians[42]. It is crucial to note that the development of MASLD is complex, and other risk modifiers, including body mass index (BMI), gut microbiota, and mitochondrial genetics, interact with *PNPLA3* gene variants[43,44].

Finally, it should be emphasized that *PNPLA3* risk alleles are strong predictors of disease worsening in individuals with MASLD, as well as in those with alcohol-associated liver disease and HCV[45]. Conversely, *PNPLA3* does not seem to contribute to the risk of HCC in individuals with HBV infection[46], probably because steatosis may hamper HBV replication cycle[47].

PNPLA3 gene variants affect liver-related outcomes in MASLD

A consistent line of research [Table 3][4,18,41,48-65] strongly supports the notion that risk variants of the *PNPLA3* gene significantly contribute to increasing the odds of MASLD development[56] and interact with the host's features (e.g. age, sex, reproductive status, and visceral adipose tissue) to increase the odds of fibrotic progression of MASLD to cirrhosis and its complications[18,52,57,59] accounting for the increased risk of liver-related events[58,59]. Additionally, obesity and excessive alcohol drinking strongly potentiate the risks of liver cirrhosis, hepatocellular carcinoma, and mortality due to hepatic disorders[53].

In contrast to previous studies conducted on polymorphisms of the Apolipoprotein B (apoB) gene[66], individuals with the *PNPLA3* gene variant and MASLD are equally insulin-resistant at multiple levels: liver, muscle, and adipose tissue[60]. This insulin resistance likely drives the worsening of liver fibrosis in a mutual and bi-directional manner[67].

Of clinical interest, PNPLA3 gene variants interact with other gene variants, leading to increased severity of liver disease in cases of SERPINA 1[68], Apolipoprotein B[69,70], and α1 antitrypsin deficiency[71]. Conversely, HSD17B13 mitigates the effects of *PNPLA3* on hepatic fibrosis[72,73]. All of these interactions should be carefully considered in the context of precision medicine approaches.

Table 2. *PNPLA3*-G risk allele frequency in different countries and geographical areas[1]

Country/geographical areas	G allele frequency (%)
Sub-Saharan Africa	12
Europe	23
Dominican Republic	25
South Asia	24-30
East Asia	35-45
Central and South America	~50
Mexico	52
Guatemala	69
Peru	72

[1]All data was taken from[6].

PNPLA3 gene variants affect extrahepatic outcomes in MASLD

Further to its liver-related effects, MASLD is a systemic condition typically associated with extrahepatic outcomes, including cardiovascular events, non-hepatic cancers, and chronic kidney disease (CKD)[2]. Additionally, MASLD may also affect the hepato-dermal axis, predisposing individuals to psoriasis[74]. Interestingly, *PNPLA3* gene variants appear to modulate the entire spectrum of these extrahepatic manifestations [Table 4][75-82] through their effects on insulin resistance, lipid levels, and hepatic fibrosis. In this context, *PNPLA3*-I148M variants are associated with a decreased risk of coronary artery disease[76], an increased risk of extrahepatic cancers[81], and a detrimental effect on renal function in individuals with MASLD[79]. This renal effect seems to occur regardless of liver fibrosis[83].

Association of other lipid genes in the progression and severity of MASLD

Our understanding of gene-to-gene interactions is increasingly being elucidated, with well-characterized examples including 17-beta-hydroxysteroid dehydrogenase 13 (HSD17B13) and transmembrane 6 superfamily 2 (TM6SF2). The *HSD17B3* gene is located on human chromosome 4q22.1 and shows strong liver-specific expression[84]. Associated with lipid droplets, it catalyzes the interconversion between 17-keto and 17-hydroxysteroids, primarily contributing to liver-specific fatty acid metabolism involving lipid droplets. Additionally, it has retinol dehydrogenase (RDH) activity and acts as a binding protein for adipose triglyceride lipase (ATGL), facilitating the interaction between the comparative gene identification-58 (CGI-58), representing a 1-acylglycerol-3-phophate O-acyltransferase, and ATGL on hepatocyte lipid droplets[84]. The expression of HSD17B13 is induced by liver X receptor α via sterol regulatory element-binding protein 1c, which is a crucial transcription factor in the control of lipid metabolism. Importantly, overexpression of *HSD17B13* is associated with increased ATGL- and RDH-mediated lipolysis, resulting in enhanced intrahepatic accumulation of lipid droplets. Conversely, a single nucleotide polymorphism (SNP) in *HSD17B13* termed rs72613567:TA is associated with a prematurely truncated unstable protein with significantly reduced enzymatic activity[84].

Several missense variants of the *TM6SF2* gene, such as E167K, L156P, and P216L, are also associated with higher odds of hepatic steatosis independent of the *PNPLA3* I148M risk allele[85]. This gene is located on chromosome 19 (19p12) and acts as a crucial regulator of the hepatic homeostasis of lipids by influencing the secretion of triglycerides and the intra-hepatic content of lipid droplets[86]. The absence of the *Tm6sf2* gene in mice is causally associated with SLD and raised liver enzymes independent of dietary challenge[87]. The study also revealed that TM6SF2 is required for normal lipidation of triglyceride-rich lipoproteins and is a key player in assembling very low density lipoprotein (VLDL), as evidenced by smaller VLDL particles with reduced triglyceride content. Moreover, disruption of *Tm6sf2* resulted in significantly lowered *Pnpla3*

Table 3. Role of *PNPLA3* gene variants in liver-related outcomes

Author, year [Ref.]	Method	Findings	Conclusions
Lisboa *et al.*, 2020[48]	This study recruited 148 subjects with MASLD, of whom 54 had biopsy-proven MASH and 94 had simple steatosis, as well as 137 HCs	In a fully adjusted multivariable model, the G allele was associated with higher risks of MASLD (OR = 1.69, 95%CI: 1.21-2.36, *P* = 0.002) and MASH (OR = 3.50, 95%CI: 1.84-6.64,*P* < 0.001). The risk of developing MASH was more substantial with GG homozygosis (OR = 5.53, 95%CI: 2.04-14.92, *P* = 0.001). Moreover, *PNPLA3* GG homozygosity was associated with a higher risk of severe MASH activity (OR = 17.11, 95%CI: 1.87-156.25, *P* = 0.01) and fibrosis (OR = 7.42, 95%CI: 1.55-34.47, *P* = 0.01) histologically	The G allele is associated with more severe liver histology
Idilman *et al.*, 2020[49]	174 patients with biopsy-proven MASLD and 151 HCs	After adjustment for confounding variables, the GG genotype was strongly associated with significant hepatic fibrosis (aOR = 3.031, *P* = 0.012)	The *PNPLA3* GG genotype is a risk for more severe MASLD
Grimaudo *et al.*, 2020[41]	471 MASLD subjects were followed for a median time of 64.6 months	After adjusting for confounding factors, *PNPLA3* C>G variant was linked to higher odds of hepatic decompensation, HCC, and liver-related death at multivariate Cox regression analysis [HR], 2.10, 95%CI: 1.03-4.29; *P* = 0.04; HR, 2.68, 95%CI: 1.01-7.26; *P* = 0.04; HR, 3.64, 95%CI: 1.18-11.2; *P* = 0.02, respectively. These results were confirmed in the subset of 162 individuals with AF/cirrhosis	MASLD subjects carrying the *PNPLA3* rs738409 G>C variant are exposed to the risks of liver-related events and death
Pennisi *et al.*, 2020[50]	430 and 342 patients in whom FIB-4 and LSM were available at the baseline and at the last follow-up visit, respectively, were enrolled	Fibrosis progression was observed in 8.1%, 13.2%, and 23.2% of patients with *PNPLA3* CC, CG, and GG genotypes, respectively (*P* = 0.03), regardless of confounding factors (OR, 1.90, 95%CI: 1.05-3.42; *P* = 0.03) Sensitivity analyses confirmed the *PNPLA3* variant as a strong predictor of fibrosis worsening by both FIB-4 (OR, 2.28, 95%CI: 1.22-4.24; *P* = 0.009) and LSM (OR, 2.11, 95%CI: 1.13-4.42; *P* = 0.01) in patients followed for up to 90 months	*PNPLA3* rs738409 C>G variant independently predicts the worsening of
Salari *et al.*, 2021[51]	Meta-analytic review was conducted on 31 published studies, totaling 9,973 cases and 13,048 controls	The CC genotype has a reduced risk of MASLD (OR = 0.48, 95%CI: 0.40-056), while CG (OR 1.19, 95%CI: 1-1.33) and GG genotypes (OR 2.05, 95%CI: 1.64-2.56) were at increased MASLD risk	The CC genotype is 52% less prone to the risk of developing MASLD, while the CG genotype is 19% more exposed to the risk of developing MASLD. Finally, the GG genotype carries a 105% higher odds of MASLD
Li *et al.*, 2022[52]	523 Chinese with biopsy-proven MASLD. VFA was assessed with BEI	For any given level of VFA, the risk of SF was greater among those with the rs738409 G genotype and VFA remained significantly associated with SF only among those with the rs738409 G-allele	*PNPLA3* rs738409 G and VFA interact to increase the risk of SF
Kim *et al.*, 2022[53]	Prospective study of 414, 209 participants who had no previous diagnosis of cirrhosis and HCC at the baseline and were followed for up to 5 years	Compared to non-obese non-excessive drinkers and noncarriers of the *PNPLA3*-I148M variant, individuals with obesity, excessive drinking, and homozygous carriers of the *PNPLA3*-I148M variant exhibited highly increased odds of cirrhosis (aHR 17.52, 95%CI: 12.84-23.90), HCC (aHR, 30.13, 95%CI: 16.51-54.98) and mortality owing to hepatic causes (aHR, 21.82, 95%CI: 13.78-34.56)	The *PNPLA3*-I148M variant status enhances the odds of cirrhosis, HCC, and liver-related mortality among subjects with obesity and high alcohol consumption
Chen *et al.*, 2023[54]	Participants from two unrelated cohorts, MGI (*n* = 7,893) and UK Biobank (*n* = 46,880), were enrolled. In these cohorts, MASLD was defined as raised ALT values after excluding competing etiologies of CLD. Values of 1.3-2.67 defined an "indetermined FIB-4 score", while high-risk FIB4 scores were defined by values > 2.67	Among those who had indeterminate FIB4 scores, individuals with T2D and the *PNPLA3* rs738409-GG genotype had a cirrhosis incidence rate in the same order of magnitude as those with high-risk FIB4 scores and 2.9-4.8 times higher than patients with T2D but CC/CG genotypes Conversely, FIB4 < 1.3 was associated with a significantly lower risk of incident cirrhosis compared to that of those with high-risk FIB4 scores, irrespective of clinical risk factors and PNPLA3 risky genotype	PNPLA3 rs738409 and T2D can be used to identify MASLD subjects who, although currently considered at indeterminate risk, have a cirrhosis risk like those with FIB4 values considered high-risk
Koo *et al.*,	302 individuals with biopsy-proven MASLD were included in the study, with a median follow-up of 54 months. The	The G allele in *PNPLA3* rs738409, along with MASH at baseline, was associated with an increased risk of the primary outcome during follow-up regardless of confounding factors	The G allele in *PNPLA3* rs738409 increases the odds of fibrosis progression in MASLD

2023[55]	primary outcome was a composite of LSM 9.6 kPa during the follow-up (for those subjects exhibiting F0-2 at the baseline), and ΔLSM ≥ 20% compared to baseline (for those individuals exhibiting F3-4 at the baseline)	(HR per 1 risk allele, 2.08; 95%CI 1.45-2.99)	
Zhao *et al.*, 2023[56]	Meta-analytic review of 20 studies totaling 3,240 patients	A significant increase in association was found between rs738409 and MASLD across 5 different models	*PNPLA3* rs738409 plays a major role in increasing the risk of MASLD
Rosso *et al.*, 2023[57]	Retrospective analysis on 756 consecutive biopsy-proven European MASLD patients	After stratifying for age, sex, and BMI, a higher risk of LRE was observed in the subgroup of non-obese women over 50 years with the *PNPLA3* GG risk genotype (log-rank test, $P = 0.0047$)	Compared to the wild-type allele (CC/CG), non-obese postmenopausal women with MASLD and the *PNPLA3* GG risk genotype exhibit an increased risk of LRE
Seko *et al.*, 2023[58]	1,550 Japanese subjects with biopsy-proven MASLD were recruited and followed for a median of 7.1 years	In multivariate analysis, *PNPLA3* CG/GG [HR] 16.04, $P = 0.006$) and FIB-4 index > 2.67 (HR 10.70, $P < 0.01$) independently predicted LRE	The *PNPLA3*-G allele carries an increased risk of LRE
Cherubini *et al.*, 2023[18]	This study is based on three study cohorts: the Liver Biopsy Cohort ($n = 1,861$ European individuals submitted to liver biopsy for suspected MASH); an independent case-control cohort of severe MASLD ($n = 4,374$); and the population-based UK Biobank cohort ($n = 347,127$)	A specific multiplicative interaction occurring in women with *PNPLA3*-I148M determines SLD in at-risk individuals (steatosis and fibrosis, $P < 10^{-10}$ advanced fibrosis/HCC, $P = 0.034$) and in the general population ($P < 10^{-7}$ for ALT values)	A synergistic interaction between the female sex and the *PNPLA3*-I148M variant determines all stages of SLD, which is worse after the decline of estrogens
Chalasani *et al.*, 2024[59]	2,075 adults with biopsy-proven MASLD were followed for a mean time of 4.3 years	The independent predictors of MALO included *PNPLA3*-G, AF, age > 60 years, and T2D Among individuals with AF, those who have the G-allele had the highest cumulative incidence of MALO *PNPLA3* and MALO were significantly more strongly associated among those > 60 years, women, and those with AF or T2D (sHR: 2.1, 95%CI: 1.5-2.8; sHR: 1.4, 95%CI: 1.1-1.9; sHR: 1.9, 95%CI: 1.5-2.4; sHR: 2.1, 95%CI: 1.5-2.8)	AF, age, T2D, and sex interact with *PNPLA3* rs738409, contributing to worsening the risk of MALOs
Bril *et al.*, 2024[60]	204 participants were submitted to *PNPLA3* genotyping, OGTT, MRS, and LB. A subgroup of 55 participants had an EHC with glucose tracer infusion	During MRA, A1c and Adipo-IR were associated with MASLD and advanced liver fibrosis, regardless of *PNPLA3* genotype	Individuals with the *PNPLA3* variant and MASLD were similarly insulin-resistant at various levels (liver, muscle, and AT) compared to non-variant carriers with MASLD
Elmansoury *et al.*, 2024[61]	205 MASLD cases and 187 healthy controls were recruited. Steatosis and fibrosis were assessed using Fibroscan	The *PNPLA3* rs738409 C>G variant was linked to MASLD, fibrosis, steatosis, increased SBP and DBP, as well as elevated ALT (all $P < 0.05$)	This study shows that the *PNPLA3* rs738409 C>G variant is associated with MASLD severity and BP among Egyptians
Kocas-Kilicarslan *et al.*, 2024[62]	123 patients with HC, 145 with MASH, and 72 cases of ESLD owing to MASLD were genotyped	The *PNPLA3* rs738409: G was associated with the healthy state to MASH progression and from MASH to ESLD	The *PNPLA3* alleles play a role in MASLD progression
Lavrado *et al.*, 2024[4]	407 patients with T2D-MASLD were followed for 11 years	Having ≥ 1 G or T allele of *PNPLA3* was strongly linked to a higher risk of cirrhosis. The odds of complications from cirrhosis were higher in *PNPLA3* GG (OR 27.20, 95%CI: 5.26-140.62;$P < 0.001$)	The *PNPLA3* alleles are associated with the progression of MASLD to cirrhosis and its complications
Seko *et al.*, 2024[63]	A longitudinal multicenter cohort study of 1,178 biopsy-proven MASLD cases	During MRA, *PNPLA3* was found to be significantly associated with LREs (HR 1.91, 95%CI: 1.20-3.04)	Genetic variants predict LREs in MASLD among Japanese subjects
Pelusi *et al.*, 2024[64]	A prospective study of 98 probands with advanced MASLD-fibrosis and/or MASLD-HCC, as well as 160 nontwin first-degree relatives who were assessed for MASLD at 4 referral centers in Italy 4	Although the *PNPLA3* risk variant was enriched in probands ($P = 0.003$) and over transmitted to relatives with MASLD ($P = 0.045$), evaluation of genetic risk variants and polygenic risk scores was not useful to direct noninvasive screening of advanced fibrosis among relatives	While these variants do play a role in liver disease within families, they do not improve the risk stratification of fibrosis

Suresh *et al.*, 2024[65]	A retrospective survey conducted on 7,333 MASLD adults who were seen at the University of Michigan Health System. Out of this group, 1,468 individuals (20%) had elevated ferritin values	In a multivariable model, ferritinemia was linked to a higher mortality rate (HR 1.68, CI: 1.35-2.09, $P < 0.001$), incident LRE (HR 1.92, CI: 1.11-3.32, $P = 0.019$), and the *PNPLA3*-rs738409-G cirrhosis-promoting allele ($P = 0.0068$), but not to variants of the *HFE* gene	Metabolic hyperferritinemia is correlated with increased mortality and a higher likelihood of LRE, as well as cirrhosis-promoting alleles rather than *HFE* mutations that promote iron overload

AF: Advanced fibrosis; aHR: adjusted hazard ratio; ALT: alanine aminotransferase; aOR: adjusted odds ratio; AST: aspartate transaminase; AT: adipose tissue; BEI: bioelectrical impedance; BMI: body mass index, BP: blood pressure; CI: confidence intervals, CLD: chronic liver disease; DBP: diastolic blood pressure; EHC: euglycemic hyperinsulinemic clamp; ESLD: end-stage liver disease; FAST: Fibroscan-AST; FIB4: fibrosis 4; HC: healthy controls; HCC: hepatocellular carcinoma; HR: hazard ratio; LB: liver biopsy; LRE: liver-related events; LSM: liver stiffness measurement; MALOs: major adverse liver outcomes; MASH: metabolic dysfunction-associated steatohepatitis; MASLD: metabolic dysfunction-associated steatotic liver disease; MGI: Michigan genomics initiative; MRA: multiple regression analysis; MRS: magnetic resonance spectroscopy; NHB: non-Hispanic Black persons; NHW: non-Hispanic White persons; OGTT: oral glucose tolerance testing; SBP: systolic blood pressure; SF: significant fibrosis (defined as stage F ≥ 2 on histology); sHR: sub-hazard ratio; SLD: steatotic liver disease; T2D: type 2 diabetes; UKBB: United Kingdom Biobank; VFA: visceral fat area.

transcript levels, demonstrating that these genes are functionally linked to one another in regulating hepatic fat content[87].

In addition to variants in the *HSD17B13* and *TM6SF2* genes, there are other genetic variants that exert positive or negative metabolic effects in the pathogenesis of MASLD. These include, for example, the glucokinase regulatory protein (GCKR) variant rs1260326-T (P446L), the single nucleotide polymorphism (SNP) rs12137855-C within the lysophospholipase-like 1 (*LYPLAL1*) gene, and the variant rs641738C>T within the membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*) loci, suggesting that various molecular events contribute to the final outcome of MASLD[88,89]. Most genes associated with the initiation and progression of MASLD are driven either by excessive hepatic glucose levels leading to amplified lipogenesis (e.g., *GCKR* and *LYPLAL1*), reduced VLDL secretion (e.g., TM6SF2), or impaired triglyceride mobilization from hepatic lipid storage (e.g., *PNPLA3*) [Figure 4].

This graphical illustration summarizes how these three proteins interact in hepatocytes in relation to lipid metabolism. PNPLA3 is located on lipid droplets, HSD17B13 is found in the cytoplasm or endoplasmic reticulum (ER), and TM6SF2 is situated on the ER membrane. PNPLA3 catalyzes the hydrolysis of triglycerides into free fatty acids, HSD17B13 modules fatty acid, and steroid hormone metabolism to influence lipid homeostasis, while TM6SF2 facilitates the export of triglycerides from the liver into circulation. There are clear regulatory relationships among these proteins; changes in PNPLA3 activity could impact the size of lipid droplets (budding) and subsequently affect the ability of TM6SF2 to export lipids. Specific genetic variants or single nucleotide polymorphisms, such as *PNPLA3*-I148M or *TM6SF2*-E167K, *TM6SF2*-L156P, and *TM6SF2*-P216L, can influence protein activity, contributing to steatosis in MASLD progression. Additionally, the *HSD17B13*-rs72613567:TA is associated with a prematurely truncated unstable HSD17B13 protein with significantly reduced enzymatic activity.

Moreover, a recent meta-analysis that analyzed 399 eligible studies identified 11 variants in 10 genes that were significantly associated with MASLD, with cumulative epidemiological evidence. The association was graded strong for the homeostatic iron regulator (*HFE*) and the tumor necrosis factor (*TNF*) genes, moderate for four variants located either in TM6SF6, GCKR, or the adipose most abundant gene transcript-1 (*ADIPOQ*), and weak for five variants located in

Table 4. Role of *PNPLA3* gene variants and extrahepatic outcomes

Author, year [Ref.]	Method	Findings	Conclusions
Karamfilova *et al.*, 2019[75]	208 MASLD individuals without (*n* = 125) and with pre-T2D (*n* = 83) were assessed	Compared to the wild CC genotype: - The CG genotype was associated with pre-T2D, IR, dyslipidemia and MetS - The *PNPLA3*-I148M variant exhibits a 9.6-fold higher odds of altered glucose metabolism - (OR 9.649, 95%CI: 2.100-44.328, *P* = 0.004) and a 3-fold higher odds of MetS (OR 2.939, 95%CI: 1.590-5.434, *P* = 0.001) and a 2.1-fold higher odds of IR (OR 2.127, 95%CI: 1.078-4.194, *P* = 0.029)	*PNPLA3*-I148M is associated with an increased risk of pre-T2D, MetS and IR among subjects with obesity and MASLD
Wu *et al.*, 2020[76]	189 subjects with MASLD and CAD, 242 individuals with MASLD and 242 HCs were enrolled	MASLD carriers of the CG + GG genotype exhibited a reduced CAD risk compared to those carrying the CC genotype (OR = 0.6, 95%CI: 0.40-0.90, *P* = 0.01)	*PNPLA3*- I148M variants carry a decreased risk of CAD among MASLD subjects due to reduced lipidemic values
Ajmera *et al.*, 2021[77]	A cross-sectional analysis of 264 middle-aged subjects submitted to genotyping and LSM with MRE. Advanced fibrosis was defined by liver stiffness ≥3.63 kPa	Each *PNPLA3* risk variant copy carried an increase of 0.40 kPa (95%CI: 0.19-0.61, *P* < 0.01) in LS on MRE after adjusting for confounding factors. Moreover, a significant genotype-age interaction was found (*P* < 0.01)	The assessment of the *PNPLA3* genotype may help identify individuals who, due to their high genetic risk, require closer monitoring and aggressive treatment
Moon *et al.*, 2022[78]	A cross-sectional analysis of the Boromae MASLD cohort (*n* = 706) and a longitudinal cohort study of the GENIE study (*n* = 4,998) were conducted	Among MASLD subjects, the G allele was independently associated with a reduced risk of DM in both SLD (OR per 1 allele, 0.66, 95%CI: 0.46-0.97) and MASH (OR per 1 allele, 0.59, 95%CI: 0.38-0.92). This finding was confirmed by the longitudinal GENIE cohort The G allele was linked to a reduced odds of incident DM during the 60-month median follow-up in MASLD subjects (HR 0.65, 95%CI: 0.45-0.93). However, those carrying the G allele without NAFLD had higher risks of T2D (OR, 2.44, 95%CI: 1.00-5.95) in the Boromae cohort	This study supports the notion that "genetic MASLD" may have a lower risk of metabolic dysfunction
Mantovani *et al.*, 2023[79]	A total of 1,144 middle-aged individuals were enrolled. The eGFR was determined using the CKD Epidemiology Collaboration equation. A subgroup of 144 cases were monitored for a median duration of 17 months	The p.I148M variant was associated with lower $eGFR_{CKD-EPI}$ levels (-1.24 mL/min/1.73 m^2 per allele, 95%CI: -2.32 to -0.17; *P* = 0.023), independent of confounders. In the follow-up cohort, the p.I148M variant was independently associated with a faster decline in $eGFR_{CKD-EPI}$ ($\Delta eGFR_{CKD-EPI}$-3.57 mL/min/1.73 m^2 per allele, 95%CI: -6.94 to -0.21; *P* = 0.037)	Among middle-aged subjects with metabolic dysfunction, the *PNPLA3*-I148M variant had a negative impact on renal function, regardless of common risk factors for CKD
Mantovani *et al.*, 2023[80]	Outpatient cohort of 46 postmenopausal women with T2D and preserved kidney function at baseline followed for 5 years	During the 5-year follow-up, the rs738409 CG/GG genotypes were associated with faster eGFR decline (coefficient: -6.55, 95%CI: 11.0 to -2.08; *P* = 0.004 by random-effects panel data analysis) regardless of changes in confounding factors over 5 years	In postmenopausal T2D women, the risk allele (G) of *PNPLA3* rs738409 is linked to a faster decline in eGFR during 5 years, regardless of yearly variations in common factors of risk of renal insufficiency and antidiabetic drugs
Tai *et al.*, 2024[81]	Prospective Taiwanese cohort study of subjects with (*n* = 546) and without (*n* = 580) SLD (controls) conducted in a referral center	A stratified analysis of the data showed that, among SLD subjects with the *PNPLA3*-I148M-rs738409 GG genotype (and also among those with the GC or CC genotype), the FIB-4 score was associated with incident non-hepatic cancers (HR 1.543, 95%CI: 1.195-1.993)	SLD individuals with the *PNPLA3*-I148M-rs738409 GG genotype who have high FIB-4 scores should be closely monitored for early diagnosis of emerging extrahepatic cancers
Agoglia *et al.*, 2024[82]	A cross-sectional analysis of 199 prospectively enrolled subjects with psoriasis	T2D (OR 10.76, 95%CI: 2.42-47.87; *P* = 0.002) and carrying of ≥ 1 *PNPLA3*-G allele (OR 5.66, 95%CI: 1.08-29.52; *P* = 0.039) were linked to advanced liver fibrosis	Metabolic dysfunction and the *PNPLA3*-G allele, rather than TMSF2 variants and MTX therapy, are associated with fibrotic progression in MASLD

CAD: Coronary artery disease; CI: confidence intervals; CKD: chronic kidney disease; CKD^{Epi}: CKD epidemiology Collaboration equation; eGFR: estimated glomerular filtration rate; HCs: healthy controls; HR: hazard ratio; IR: insulin resistance; kPa: kilopascals; LSM: liver stiffness measurement; MASLD: metabolic dysfunction-associated steatotic liver disease; MASH: metabolic dysfunction-associated steatohepatitis; MetS: metabolic syndrome; MTX: methotrexate; OR: odds ratio; pre-T2D: prediabetes; SLD: steatotic liver disease; T2D: type 2 diabetes.

MBOAT7, phosphatidylethanolamine N-methyltransferase (*PEMT*), *PNPLA3*, leptin receptor (*LEPR*), and methylenetetrahydrofolate reductase (*MTHFR*)[90]. For example, *PNPLA3* and *TM6SF2* E167K variants are associated with an increased susceptibility to MASLD development and progression, while HSD17B13 may provide protection against these liver-related outcomes[91]. This suggests that different gene variants could have reinforcing or mitigating effects on pathogenesis, complicating outcome predictions. Therefore, polygenic risk scores are calculated by summing the number of risk alleles (e.g., PNPLA3, TM6SF2) and subtracting the protective variant found in HSD17B13[92].

However, some genetic variants and SNPs may not be associated with the MASLD risk in certain populations or may not significantly impact mortality between lean and non-lean populations with MASLD[93,94]. This indicates that specific gene polymorphisms affecting MASLD development can vary significantly across populations due to genetic, environmental, and lifestyle factors. Variants that pose a risk or offer protection in one population may not have the same effect in another due to differences in allele frequencies, gene-environment interactions, and cultural practices influencing diet and physical activity. It is important to note that the benefits of dietary interventions and probiotics, which are reasonable strategies for managing MASLD, may differ among genotype groups, such as individuals with different *PNPLA3* genotypes[95]. Understanding these population-specific effects and the impact of genotype constellations is crucial for developing tailored prevention strategies and personalized medical interventions that account for genetic diversity.

PNPLA3 gene variants in people living with HIV

Among people living with HIV (PLWH), hepatic disorders account for high morbidity and liver-related causes are among the leading causes of death not related to acquired immunodeficiency syndrome (AIDS)[96]. SLD is common among PLWH[97], including those with normal BMI, in whom it is generally deemed to be associated with antiretroviral treatment (ART)[98]. Moreover, a previous study has indicated that HIV is a steatogenic virus[99].

Based on these findings, it is predicted that mono-infection with HIV amplifies the steatogenic potential of variants of the *PNPLA3* gene. A recent study by Han *et al.* seemingly confirms this prediction[100]. These authors cross-sectionally investigated *PNPLA3* variants and either SLD or MASLD in a Thai patient sample of 764 PLWH, 35% of whom had SLD. In multivariate analysis adjusted for common confounding factors, including ART, *PNPLA3* rs738409 CG/GG genotypes were found to be associated with higher odds of SLD only among lean subjects (aOR: 1.79, 95%CI: 1.18-2.72, $P = 0.006$). This finding remained significant after adjustment for TM6SF2 rs5854292 CT/TT and CC genotypes (2.01, 95%CI: 1.24-3.25, $P = 0.004$) in lean participants[100]. These findings support the notion that *PNPLA3* gene variants may be an independent contributor to MASLD development, suggesting longitudinal follow-up of these subjects[101].

PNPLA3 gene variants in the liver transplant setting

MASH-cirrhosis is the most rapidly growing indication for liver transplantation in the Western world[102]. Metabolic factors increase the likelihood of mortality among those on the waiting list for MASH-cirrhosis and the risks of long-term recurrence of liver disease and cardiometabolic complications after liver transplantation[103]. A recent retrospective study of 55 Japanese SLD recipients and their donors found that donor risk alleles of *PNPLA3*, *TM6SF2*, and *HSD17B13* are implicated in post-transplant SLD, rather than recipient risk alleles[104]. Another study of 83 liver recipients showed that *PNPLA3* gene variants in the recipient genotype impact the post-transplant outcome of individuals transplanted for alcohol-related liver disease, especially in those with heavy alcohol relapse[105].

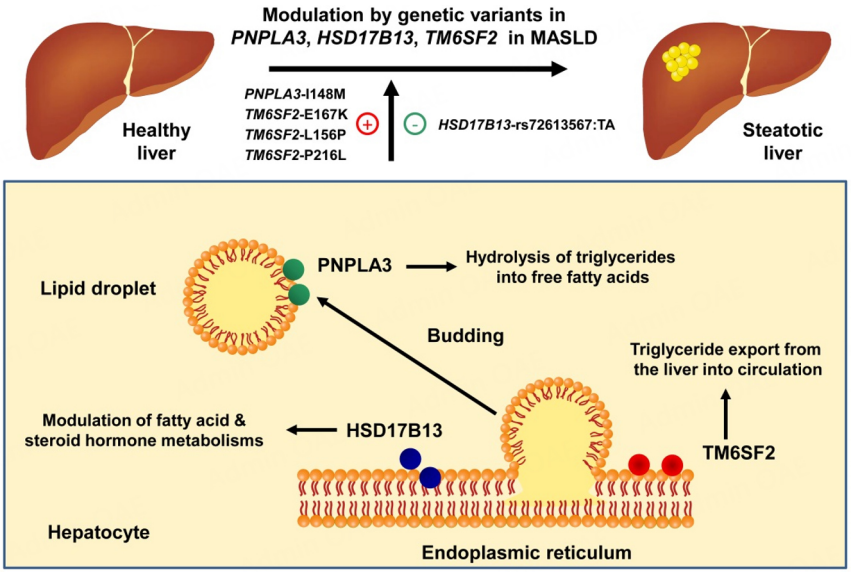

Figure 4. Representation of PNPLA3, HSD17B13, and TM6SF2 proteins in lipid metabolism and the progression of MASLD.

PNPLA3 gene variants in children and adolescents with MASLD

In contrast to adults, MASLD in younger age groups is not expected to be associated with clinically manifest cardiovascular disease or cancer. Nevertheless, data from two meta-analytic reviews suggest that *PNPLA3* variants participate in the development and fibrotic progression of MASLD among children and adolescents[106,107]. Tang *et al.* conducted a study that included nine case-control studies totaling 1,173 children with MASLD and 1,792 healthy controls[106]. The data have demonstrated that *PNPLA3* gene variants were strongly associated with the risk of development and progression of NAFLD in children. Li *et al.* included 27 studies with a total of 10,070 eligible subjects[107]. Their data indicated that the *PNPLA3*-I148M polymorphism is associated with higher odds of early-onset NAFLD, severity, and liver damage, but not with metabolic syndrome.

Ethnicity, dietary habits, obesity, and *PNPLA3* gene variants are likely to interact in the initiation and worsening of MASLD among children and adolescents. In agreement, Mansoor[108] found that, among Hispanic children with obesity, the *PNPLA3* rs738409 C>G polymorphism was associated with an increased risk of MASLD. Schenker *et al.* have reported that, in Latino adolescents with obesity, the PNPLA3 gene GG variant, total sugar, fructose, sucrose, and glucose consumption were all associated with liver stiffness measurement, a noninvasive index of liver fibrosis, in a stronger manner among cases with fibrosing MASLD than among healthy controls and MASLD without fibrosis[109]. Gene-to-gene interactions between the *PNPLA3* gene and other gene variants (such as TM6SF2 and SAMM5) concur in the development and progression of MASLD in children[110]. This finding, together with the observation that children with obesity and *PNPLA3*-MM genotype compared to other genotypes, exhibit reduced renal function, particularly if MASLD coexists[111], pave the way for identifying specific subsets of individuals who, being exposed to the risks of worse outcomes, need more aggressive treatment strategies.

PNPLA3 and autoimmune hepatitis

The *PNPLA3* rs738409 GG variant is associated with prognostic features of AIH, such as time to liver transplantation or death[112]. More recently, Azariadis *et al.* conducted a study involving 200 AIH cases and 100 healthy controls and found that the I148 M variant was present in the same proportion among AIH patients as in healthy controls (47.5% *vs.* 47%, $P = 1.000$)[113]. However, AIH subjects with the GG/CG

genotypes were associated with decompensated cirrhosis at diagnosis (GG/CG 6.3% *vs.* CC 1%, $P = 0.039$), although no correlation was found between the *PNPLA3* genotype, liver histology, and response to treatment. Kaplan Meier analysis showed that G allele homozygosity was associated with reduced decompensation-free survival ($P = 0.006$), cirrhotic events (decompensation, liver transplantation, HCC; $P = 0.001$), and liver-related death or liver transplantation ($P = 0.011$) among patients who received treatment. Collectively, these findings indicate that the *PNPLA3*-I148 M variant may be a novel biomarker of increased risk of AIH progression. Given that steatosis was similarly common across all *PNPLA3*-rs738409 genotypes, mechanisms other than SLD probably play a role in disease progression in AIH patients with the *PNPLA3*-rs738409 GG variant[114].

Utility of PNPLA3 in clinical practice

The incorporation of *PNPLA3* genotyping in clinical practice remains challenging. The European Clinical Practice Guidelines on MASLD management that were recently published clearly state that genotyping should only be performed in the clinical research setting[2]. However, Chen and Vespasiani-Gentilucci have proposed a hierarchy of potential relevance, which is summarized in Table 5[115].

Although well documented, the ranking illustrated in Table 5 remains "Expert Opinion". While specialized centers may consider incorporating the assessment of the genetic risk profiles (comprising *PNPLA3* p.I148M variant and/or polygenic risk scores) to personalize risk stratification, this practice has yet to be validated with large, prospective studies[2].

Relationship between *PNPLA3*, alcohol-related liver disease, and metabolic and alcohol-related/associated liver disease (MetALD)

A seminal meta-analytic review, pooling data from 10 published studies globally involving 4,112 individuals, has reported several important findings on the connection between *PNPLA3* gene variants and alcohol-related liver disease (ALD)[116]. According to this study, the OR for rs738409 CG and GG among alcohol-related cirrhosis (AC) patients was 2.09 (1.79-2.44) and 3.37 (2.49-4.58), respectively *vs.* controls. Among AC patients with HCC, the OR was 2.87 (1.61-5.10) for CG and 12.41 (6.99-22.03) for GG. For ALD patients, the OR of CG and GG genotypes was 2.62 (1.73-3.97) and 8.45 (2.52-28.37), respectively, for AC compared with SLD subjects. The OR for CG and GG genotypes among AC patients for HCC occurrence was 1.43 (0.76-2.72) and 2.81 (1.57-5.01), respectively. These findings collectively support the idea that, among drinkers, the *PNPLA3* rs738409 polymorphism is associated with higher odds for the whole ALD spectrum and a higher likelihood of developing AC and HCC[117].

In the Delphi consensus conducted by Rinella *et al.* in 2023, a new category named MetALD was introduced outside of pure MASLD - MetALD is used to identify individuals with MASLD who also consume 140-350 grams of alcohol weekly for females and 210-420 grams of alcohol weekly for males[116]. Due to the recent introduction of the MetALD nomenclature, we still ignore the existence and the extent of the expected interaction of *PNPLA3* gene variations with MetALD. However, the contribution of *PNPLA3* I148M to the global burden of MetALD may vary depending on the prevalence of dysmetabolic traits among different world regions[6].

CONCLUSION

The spectrum of *PNPLA3*-driven liver disease represents an extraordinary naturally occurring disease model that can be utilized to better understand how precision medicine approaches may be implemented in human medicine. However, it is true that this expectation is still far from reality, although some firm conclusions are at hand.

Table 5. Potential integration of *PNPLA3* genotyping into clinical risk prediction[1]

Hierarchy	Indication	Comment
More relevant	Diagnosis of steatohepatitis	This is best accomplished noninvasively with FAST and MAST scores. However, the role of *PNPLA3* genotyping and PRS remains to be determined
Less relevant	Risk stratification of non-cirrhotic MASLD	The inclusion of *PNPLA3* genotyping in clinical practice would be facilitated by demonstrating that the genotype is associated with LROs independent of established clinical risk scores in both the general population and among those with MASLD
	Risk stratification among those with cirrhosis	Severe LROs include decompensation of cirrhosis and the development of HCC
	Diagnosis of SLD	Various NITs accurately identify SLD, and the addition of *PNPLA3* genotyping minimally improves their diagnostic accuracy
	Staging of fibrosis	Liver histology remains the reference standard and NITs accurately predict liver histology findings

[1]all data were taken from[115]. FAST: Fibroscan AST; HCC: hepatocellular carcinoma; LROs: liver-related outcomes; MAST: Magnetic resonance imaging (MRI)-aspartate aminotransferase (AST); NITs: noninvasive tests; PRS: polygenic risk scores; SLD: severe liver disease.

Regarding diagnostics, clinicians and researchers should consider that the *PNPLA3* genotype reduces the accuracy of noninvasive assessment of MASLD[118]. Additionally, no evidence is presently available to support the use of genetic risk scores for identifying significant fibrosis in MASLD, although the combined use of *PNPLA3* and Fib-4 considerably increases diagnostic accuracy[119].

When it comes to disease stratification, having the *PNPLA3*-I148M variant can increase the odds of the more severe forms of MASLD, particularly in women[120]. This variant could also play a role in pharmacogenetics[121]. It is likely that all types of interventions, whether lifestyle changes or medication, for MASLD are influenced by the *PNPLA3* genotype. Therefore, considering the *PNPLA3*-I148M variant status in therapeutic studies could help prevent inaccurate results due to this potentially confounding factor[122].

Profiling *PNPLA3* variants is of significant value in the field of treatment. Currently, no drug has been approved to target the *PNPLA3*-I148M variant specifically, but precision medicine approaches in this area are anticipated[123]. In elderly Japanese individuals at risk of MASLD, the *PNPLA3* rs738409 genotype may be linked to the positive effects of physical exercise[124]. Another potential option could be vitamin B3 supplementation, as there is an interaction between niacin and *PNPLA3* I148M in MASLD patients[125]. Gene silencing appears to be the most direct approach[126]. However, a recent two-sample, two-step Mendelian randomization analysis investigating the relationship between *PNPLA3* inhibition and cardiovascular diseases (CVDs) found that inhibiting *PNPLA3* gene expression increases the risk of major CVDs[127]. A seminal investigation has suggested that PNPLA3(148M) is a gain-of-function mutation that promotes hepatic steatosis by accumulating on LDs and inhibiting ATGL-mediated lipolysis in an ABHD5-dependent manner[128]. Based on these findings, it is anticipated that reducing (as opposed to increasing) the expression of PNPLA3 would be the most successful strategy to treat PNPLA3(148M)-associated SLD. Collectively, these conflicting results highlight the liver's role as a reservoir of lipid species and a complex regulator of cardiovascular risk. They suggest the need for additional studies following a more holistic, sex-specific, and well-balanced approach to managing hepatic and extrahepatic outcomes simultaneously.

DECLARATIONS

Authors' contributions

Made substantial contributions to conception and design of the review: Weiskirchen R, Lonardo A

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Weiskirchen R is Associate Editors of the *Journal of Translational Genetics and Genomics* and is the Guest Editors for the special issue Genetic and Epigenetic Factors in Liver Disease Pathogenesis. Weiskirchen R was not involved in any steps of edtorial processing, notably including reviewers' selection, manuscript handling and decision making. while the other author have declared that they have no conflicts of interest.

Ethical approval and consent to participate

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Consent for publication

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