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Unraveling the pathogenesis of non-alcoholic fatty liver diseases through genome-wide association studies

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a significant health burden around the world, affecting approximately 25% of the population. Recent advances in human genetic databases have allowed for the identification of various single nucleotide polymorphisms associated with NAFLD-related traits. Investigating the functions of these genetic factors provides insight into the pathogenesis of NAFLD and potentially identifies novel therapeutic targets for NAFLD. In this review, we summarized current research on genes with NAFLD-associated mutations, highlighting phospholipid remodeling and spatially clustered loci as common pathological and genetic features of these mutations. These features suggest a complex yet intriguing mechanism of dissociated steatosis and insulin resistance, which is observed in a subset of patients and may lead to more precise therapy against NAFLD in the future.

Keywords

GWAS; NAFLD; NASH; phospholipid remodeling

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term that encompasses a series of chronic liver conditions, including steatosis or non-alcoholic fatty liver and non-alcoholic steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). The global prevalence of NAFLD is increasing at a rate of approximately 1% every year, affecting 30% of the population. However, there are currently no FDA

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approved drugs for its treatment.[1] More importantly, NAFLD has become the fastest growing cause of HCC in the USA, and it is estimated that the incidence rate of NAFLD-related HCC will increase by 122% in 2030.[2] As a result, the annual medical cost associated with NAFLD has exceeded $100 billion in the USA. Therefore, extensive research into the pathogenesis and progression of NAFLD is urgently needed to address its global health burden.

Patients with NAFLD exhibit significant variations in phenotypes, which are determined by a combination of genetic and environmental factors, as is the case with other complex traits. Previous research has revealed the important roles of genetic factors in the susceptibility and progression of NAFLD, showing the heritability of NAFLD between 22% and 50%.[3] In recent years, an explosion of genome-wide association studies (GWAS) has identified single nucleotide polymorphisms (SNPs) that are closely correlated with NAFLD or its progression based on data from hundreds of thousands of human patients. These SNPs have been mapped to genes that are summarized in Table 1. Although the genes identified from GWAS shed lights on the genetics of NAFLD, understanding the pathogenesis of NAFLD requires investigating the function of these genes and the mechanisms that connect them to NAFLD progression. In this review, we summarize the physiological and pathological functions of genes identified from GWAS that are associated with NAFLD or its severity, focusing on recent advances in novel SNPs and proposing potential implication on understanding of NAFLD development.

PNPLA3

Mutations in patatin-like phospholipase domain containing 3 (*PNPLA3*) have been associated with NAFLD and its severity by multiple GWAS since 2008.[5,25] PNPLA3 is highly expressed in the liver, with higher expression in hepatic stellate cells (HSCs) than in hepatocytes.[26] The rs738409[G] variant, which encodes an I148M nonsynonymous mutation in the patatin-like domain of PNPLA3, has been shown to correlate with liver fat content and fibrosis,[27] cirrhosis,[28] and serum liver enzyme levels.[29] Such significant correlation with the spectrum of liver diseases urges thorough understanding of the pathophysiological function of PNPLA3.

In hepatocytes, PNPLA3 exhibits hydrolase and acyltransferase activity towards triglyceride (TG),[9] with preferential substrates of TG containing monounsaturated and polyunsaturated fatty acids (PUFA).[30] Interestingly, knockout of *Pnpla3* in hepatocytes did not lead to steatosis or dysregulation of lipid homeostasis.[4] In contrast, overexpression of I148M mutant induced lipid accumulation due to increased fatty acid and TG biosynthesis and impaired hydrolysis of TG,[6] suggesting that in addition to defective TG hydrolase activity, mutant PNPLA3 likely gains other functions that contribute to the development of steatosis. *In vitro* study from Kumari *et al.* showed that the TG hydrolase activity of PNPLA3 is low compared with its lysophosphatidic acid acyltransferase activity with a preference of PUFA as substrates.[9] They further showed that I148M mutation promotes its lysophosphatidic acid acyltransferase activity and lipid synthesis leading to increased cellular lipid accumulation. A recent lipidomic analysis in human livers revealed similar result showing retention of PUFA in TG in I148M carriers.[31] These results indicate that augmented enzymatic activity

towards lipid synthesis likely contributes to increased susceptibility of NAFLD in I148M carriers. Another study demonstrated that PNPLA3 with I148M mutation was resistant to ubiquitin-mediated degradation, which resulted in the accumulation of PNPLA3 on hepatic lipid droplets and impaired the utilization of TG stored in lipid droplets.[32]

Given the gain-of-function nature of I148M mutation, strategies targeting PNPLA3 have been tested to treat NAFLD in mouse models. Inhibition of PNPLA3 by shRNA or proteolysis-targeting chimera-mediated degradation dramatically decreased liver TG content in I148M knock-in mice.[8] Knockdown of *Pnpla3* in high-fat diet fed rats using antisense oligonucleotide also significantly reduced hepatic lipid content and improved insulin signaling,[7] indicating that PNPLA3 could be a potential therapeutic target for NAFLD in human patients.

Although the roles of PNPLA3 in hepatocytes have been extensively investigated, its functions in HSCs remain poorly understood. PNPLA3 has been shown to regulate retinol pool in HSCs and I148M mutation leads to reduced release of retinol from HSCs.[26] Previous studies have shown that the expression of *PNPLA3* in HSCs is regulated by retinol availability and transforming growth factor-β signaling in response to liver damage.[33] Further study showed that upon HSC activation, I148M mutation resulted in lipid accumulation and fatty acid imbalance in HSCs, thereby inducing cytokine release and promoting inflammation.[33] Thus, PNPLA3 likely mediates fibrogenic and proinflammatory responses upon liver damage and HSC activation. A recent *in vitro* study using human HSCs showed that I148M variant impaired liver X receptor signaling and cholesterol homeostasis in HSCs, leading to HSC activation and collagen synthesis.[34] Given that PNPLA3 is expressed in both HSCs and hepatocytes, it is important to explore the interactions between mutant PNPLA3 in different cells to fully understand its pathological function. Park *et al.* utilized human pluripotent stem cell-derived multicellular liver culture and revealed a critical role of activated nuclear factor-κB–interleukin-6/signal transducer and activator of transcription 3 axis in I148M-induced NAFLD progression.[35]

TM6SF2

In 2014, three studies independently found that SNPs mapped to transmembrane 6 superfamily member 2 (*TM6SF2*) gene are associated with NAFLD.[10,11,36] These early findings demonstrated that TM6SF2 is localized in the endoplasmic reticulum (ER) and ER–Golgi intermediate compartment of human liver cells[36] and that downregulation of *Tm6sf2* resulted in less very low-density lipoprotein (VLDL) secretion and lipid accumulation in the liver.[11] Following studies confirmed the adverse effects of *TM6SF2* mutation E167K on liver TG content and fibrosis.[37] Surprisingly, E167K carriers in humans showed improved insulin sensitivity, despite increased lipid accumulation in the liver.[38] Furthermore, serum TG levels were also reduced in these patients. Gene expression analysis showed that E167K mutation results in loss of function of TM6SF2. However, overexpression of *Tm6sf2* in mice exhibited similar phenotypes as mutants, including lower circulating lipids and steatosis and liver damages.[12] Despite the conflicting results, these studies indicate a potential deficiency in VLDL secretion in mutants and a subtype of NAFLD characterized by dissociation between hepatic lipid accumulation and insulin responses.

TM6SF2 is predominantly expressed in the liver and intestine, with ~10-fold higher in small intestine compared with liver in mice.[13] Depletion of *Tm6sf2* in the intestine has been shown to impair lipid absorption and cause lipid accumulation in the enterocytes. However, the contribution of TM6SF2 in the intestine to serum and hepatic lipid levels requires further investigation.[13] In the liver, *Tm6sf2* deficiency induced hepatic steatosis, hypocholesterolemia, and transaminitis without dietary challenge. This phenotype was attributed to dramatic decrease in the lipidation of apolipoprotein B without any effect on apolipoprotein B secretion.[13] Further studies revealed impaired TG-rich lipoprotein production and subsequent hepatic lipid accumulation in E167K mutant carriers or *Tm6sf2* null mice and rats, with slight downregulation of de novo lipogenesis likely due to compensation.[39,40]

In human patients carrying E167K mutation and mice lacking *Tm6sf2*, PUFA were found to be depleted from TG and phosphatidylcholine (PC) in the liver and TG in the serum, which was further confirmed by *in vitro* study showing that the incorporation of PUFA is deficient in E167K carriers.[13,41] It has been shown that increasing phospholipid saturation blocks VLDL secretion in the liver[42] and lipid absorption in the intestine.[43] Therefore, the shift of lipid composition in the absence of TM6SF2 may contribute to the impaired VLDL secretion and lipid absorption in the liver and intestine. Therefore, it is possible that the pathological consequence of *TM6SF2* deficiency may be attributed to imbalanced phospholipid composition.

SUGP1

SURP and G-patch domain containing 1 (*SUGP1*) is ubiquitously expressed in a wide range of tissues and located closely to *TM6SF2* on human chromosome 19.[44] It was first described in 2003 as a pre-mRNA splicing factor containing SURP motif and G-patch domain at C-termini, indicating a potential RNA binding function.[45] *SUGP1* and *TM6SF2* are located both in *NCAN* locus, where many SNPs have been found to be significantly associated with plasma lipid concentrations,[46] hepatic steatosis, lobular inflammation, and fibrosis.[47] Specifically, rs10401969, an SNP in the intron 8 of *SUGP1*, was strongly associated with liver lipid content in patients with obesity and NAFLD.[46] How this intronic SNP may contribute to hepatic lipid accumulation remains elusive.

SUGP1 depletion has been shown to involve in splicing defects caused by a cancer-related mutation of the spliceosomal gene *SF3B1*,[48] indicating that the pathological roles of SUGP1 are likely related to dysregulation of alternative splicing. Kim *et al.* found that rs10401969 led to non-sense-mediated *SUGP1* mRNA decay and that overexpression of *Sugp1* in mouse liver increased plasma cholesterol levels.[15] On the contrary, knockdown of *SUGP1* promoted alternative splicing of 3-hydroxy-3-methylglutaryl-CoA reductase, a rate-limiting enzyme of cholesterol synthesis, and reduced cholesterol synthesis and increased low-density lipoprotein uptake.[15] While these results are in line with alternative splicing function of SUGP1 and its roles in lipid metabolism, this does not explain how rs10401969 leads to liver lipid accumulation as rs10401969 decreases *SUGP1* expression, which is supposed to reduce lipid synthesis. Given that this SNP is located in an intron, efforts have been made to identify genes around this locus whose expression is indeed altered in

patients with steatosis. Among ~20 genes in this locus, only lysophosphatidic acid receptor 2 (*LPAR2*) was significantly upregulated in fatty livers.[49] It is possible that lysophospholipid signaling in the liver may be involved in the underlying pathological mechanisms of SNPs found in *NCAN* locus. However, we cannot rule out the possibility that these mutations, especially synonymous SNPs, may affect other neighboring genes through transcriptional or splicing regulation.

MBOAT7

The membrane-bound *O*-acyltransferase 7 (MBOAT7) is an ER membrane protein that belongs to MBOAT family. MBOAT7 has been shown to have lysophospholipid acyltransferase activity with the substrate preference of PUFA such as arachidonic acid.[50] In 2015, GWAS identified risk loci in *MBOAT7* for alcohol-related cirrhosis, drawing attention of this enzyme to liver diseases.[51] Studies in human NAFLD cohorts showed close association between hepatic fat content and fibrosis and rs641738T in *MBOAT7*, which leads to decreased protein levels in the liver.[52] A meta-analysis with more than 1 million participants confirmed these correlations and revealed additional links to higher serum alanine aminotransferase and lower serum TG levels.[53] Given the enzymatic activity of MBOAT7, the mutation carriers showed severer fibrosis and increased hepatic phosphatidylinositol (PI) saturation.[52] These findings suggested that the pathophysiological role of MBOAT7 could be attributed to its phospholipid remodeling activity.

Consistent with human cohort studies, liver *Mboat7* expression was also significantly downregulated in genetic or diet-induced obesity murine models.[16] To investigate the mechanisms of MBOAT7 in the pathogenesis of NAFLD, several groups have generated *Mboat7* liver-specific knockout mice and revealed spontaneous steatosis and exacerbated fibrosis under diet challenge in the knockout mice.[17–19] In agreement with its enzyme activity, hepatic *Mboat7* deficiency resulted in significant changes in PI composition in the liver and hepatocyte ER, with selective depletion of arachidonoyl-containing PIs and increased lysophospholipids.[52] Although PI only comprises about 5% of total cellular phospholipids, the change in hepatic membrane composition was shown to promote de novo lipogenesis mediated by sterol regulatory element binding protein-1c (SREBP-1c) and upregulate fibrogenic gene expression independent of hepatic inflammation.[16,19] However, there are still discrepancies with regard to insulin signaling and liver inflammation in these models,[16,18,19] which requires further investigation. Nevertheless, *Mboat7* deficiency alters the liver lipidome in all models, which likely contributes to NAFLD phenotypes. Therefore, these data further emphasize the important roles of lipid composition in NAFLD development.

HSD17B13

17β-Hydroxysteroid dehydrogenase 13 (HSD17B13) is a member of hydroxysteroid dehydrogenase family with catabolism activities for estrogens and androgens. Human *HSD17B13* gene was first cloned in 2007 and has been shown to be exclusively expressed in the liver.[54] In 2018, it was first reported that a loss-of-function splice variant rs72613567:TA in *HSD17B13* was significantly associated with reduced risk of NASH and fibrosis, but not steatosis, in human cohorts.[55] rs72613567 variant is located at a splice site outside of exon

6 of *HSD17B13*, and the T>TA mutation leads to the expression of a truncated form of this enzyme, which is unstable with less enzymatic activity.[55] Further GWAS have identified other SNPs mapped to this gene that produce different loss-of-function variants, which also showed protective effects on NAFLD.[56]

The pathological effect of HSD17B13 dissociates inflammation and fibrosis from steatosis in patients with NASH. Although variants of *HSD17B13* were highly correlated with reduced fibrosis, inflammation, and risk of NASH, they had no effect on steatosis.[55,56] To decipher the mechanisms underlying its protective role against fibrosis, studies have focused on examining its enzyme activity and identifying substrates. The loss-of-function variants of *HSD17B13* exhibited a reduced ability to catalyze estradiol[55] and less retinol dehydrogenase activity.[56] As a consequence, mutation carriers were found to have increased hepatic sex steroids, including pregnenolone and androstenediol.[20] Moreover, lipidomic and metabolomic analyses revealed increased hepatic phospholipid levels[57] and suppressed pyrimidine catabolism.[20] However, how these altered metabolic pathways contribute to fibrosis remains to be determined.

It was reported that the expression of *HSD17B13* is upregulated in patients with NASH through liver X receptor α–SREBP-1c axis, resulting in steatosis.[56,58] Previous studies have shown that HSD17B13 is associated with lipid droplets[56]; however, the specific roles of HSD17B13 in regulating lipid droplet metabolism and its potential contribution to steatosis remain unclear. Interestingly, studies using mouse models have revealed discrepancies with human data. For example, loss of *Hsd17b13* in the liver has been shown to increase hepatic fatty acid synthesis, leading to steatosis and inflammation without diet challenge.[22] Mice with whole-body deletion of *Hsd17b13* gained more body weight under chow diet, and no protective effect was observed in the knockout mice under diet-induced NAFLD conditions.[23] In contrast, protection against fibrosis was observed in adeno-associated virus-mediated *Hsd17b13* shRNA knockdown mouse model on choline-deficient diet.[20] Therefore, extra caution is needed when translating mechanisms established in mouse models to humans. This species variation may also draw our attention to the unique metabolic pathways that distinguish mouse and human, where HSD17B13 acts differently.

MARC1

Mitochondrial amidoxime-reducing component 1 (MARC1) is a mammalian molybdenum-containing enzyme located on the outer mitochondrial membrane first discovered in 2008.[59] The activity of this enzyme family (including MARC1 and MARC2 in humans) is to reduce N-hydroxylated compounds by forming a complex with cytochrome b_5 and its reductase.[60] The physiological function of this enzyme is largely unknown, except for its involvement in drug metabolism and detoxification.[61] In 2010, a meta-analysis in European population identified an SNP (rs2642442) in the intron of *MARC1* to be significantly associated with decreased plasma total cholesterol levels,[62] suggesting its potential roles in lipid metabolism. More recently, two GWAS demonstrated that rs2642438, another SNP in exon 3 of *MARC1* that encodes a p.A165T variant, is associated with less severity of NAFLD, lower hepatic fat, serum aspartate aminotransferase/alanine aminotransferase

levels, and all-cause cirrhosis.[63,64] These studies suggest significant protective effects of MARC1 against NAFLD. However, the underlying mechanisms are poorly understood.

Lipidomic data from human p.A165T carrier have shown increased hepatic PC levels, especially polyunsaturated PC species,[64] indicating a potential role in phospholipid remodeling. This alteration in PC composition is opposite to what was observed in patients carrying mutations in *TM6SF2*, which promote NAFLD, raising the possibility that their pathological roles may converge in phospholipid remodeling. A recent study on primary human hepatocytes and mouse knockdown model confirmed the protection effect of *Marc1* deficiency on diet-induced steatosis and fibrosis. Interestingly, this study suggested that the physiological effect of *Marc1* knockdown may act through Kennedy pathway, a phospholipid de novo synthesis pathway, and fatty acid metabolism.[24] These results consistently implied the potential roles of phospholipids in NAFLD pathogenesis and connected it to mitochondria function. Further studies on mouse models are still required to uncover the physiological role of MARC1. Moreover, the high expression of *MARC1* in both adipose tissue and liver of humans adds more complexity to deciphering the pathological function of MARC1 in NAFLD progression.

GCKR

Glucokinase regulator (*GCKR*), also known as glucokinase regulatory protein (GKRP), is primarily expressed in the liver and plays a crucial role in glucose metabolism. It regulates glucose metabolism by competitively binding to glucokinase, which cannot be regulated by substrate saturation or product inhibition as other hexokinases.[65] GWAS have identified SNPs in *GCKR* that are associated with NAFLD-related traits.[25,47] In fact, SNPs in *GCKR* are associated with over 130 metabolic traits and diseases, making it one of the most pleiotropic GWAS loci.[66] Specifically, variants at rs1260326, resulting in a missense mutation, have been linked to increased liver fat, fibrosis, and inflammation,[25,47] but also with lower fasting glucose and lower insulin levels.[67] The dissociation between glucose homeostasis and NAFLD observed in GWAS is likely due to its glycolysis regulation function. We note here that although the reference allele of rs1260326 in GRCh38 human genome is T, rs1260326C appears to be more frequent in various human cohorts, and rs1260326T is the minor allele associated with multiple disease traits. Therefore, the mutant variant is considered as rs1260326C>T P446L.

It has been reported that GKRP is involved in hepatic glucose sensing and uptake,[68] thereby contributing to improved glucose handling. The P446L variant of GKRP shows deficient binding ability with glucokinase, leading to its release from the nucleus, which increases hepatic glucose uptake and promotes glycogen synthesis and glycolysis. This, in turn, results in excessive acetyl-CoA production and fuels the SREBP-1c-mediated de novo lipogenesis.[69] This is consistent with the GWAS data showing protective effect of *GCKR* mutation on insulin resistance, with lower fasting glucose and insulin levels.[67] However, studies using human liver organoids derived from induced pluripotent stem cells from different mutant carriers showed impaired hepatic insulin response in P446L carriers when treated with fatty acids. The conflicting results may be attributed to the elevated “reductive stress” caused by increased NADH/NAD^+ ratio during glycolysis. It was proposed that

although the increased glycolysis and glucose uptake lowers blood glucose levels, the increase in free cytosolic NADH leads to higher hepatic glucose production with insulin, causing hepatic glucose intolerance.[66] Overall, the "double-edged sword" effect of GKRP could limit its potential as a therapeutic target for metabolic disorders.

The effect of GKRP in the pathogenesis of NAFLD is complex. A recent cohort study found that the P446L variant had opposite effect with different diabetic status. P446L carriers with HbA1c < 5.7% (nondiabetic) showed lower lobular inflammation and NAFLD activity score, whereas patients with HbA1c > 6.4% showed higher inflammation and NAFLD activity score.[69] In addition, patients with diabetes carrying P446L were resistant to metformin treatment, while nondiabetic carriers responded to it. These results suggest a complex interplay between insulin resistance and fatty liver in carriers of GCKR mutation. Nevertheless, deciphering the pathogenesis of this mutation may reveal the role of NADH/NAD^+ regulation in NAFLD development, which could uncover a novel pathway involving "reductive stress" and improve our understanding of NAFLD.

Impact of combined polymorphisms on non-alcoholic steatohepatitis

As discussed earlier, polymorphisms in these risk genes can affect different aspects of lipid metabolism (Fig. 1). For instance, I148M mutation in *PNPLA3* gene enhances lipogenesis and impairs lipolysis of lipid droplets,[6] leading to steatosis. *TM6SF2* E167K mutation causes defective VLDL secretion,[36] contributing to NASH pathogenesis. Notably, the combined effects of multiple genetic polymorphisms in different genes can result in a synergistic effect on NASH development in patients. Xu *et al.* reported that patients carrying both *PNPLA3* I148M and *TM6SF2* E167K mutations exhibit the highest odds ratio for developing NASH, compared with those with only one of these mutations.[70] Another study by Longo and colleagues demonstrated that the copresence of *PNPLA3* I148M, *TM6SF2* E167K, and rs641738 *MBOAT7* genetic variants leads to a synergistic effect on liver damage, which is evidenced by more severe steatosis, lobular inflammation, ballooning, fibrosis, and NAFLD activity score.[71] Understanding these genetic interactions is crucial for better risk assessment and potentially personalized therapeutic strategies in managing NASH.

Interactions between genetic polymorphisms and other metabolic factors

In addition to genetic predisposition, various metabolic conditions are linked to an increased risk of NASH, including obesity and insulin resistance. Studies have demonstrated that adiposity greatly promotes the full spectrum of NAFLD in patients carrying certain genetic variants, such as *PNPLA3* I148M, *TM6SF2* E167K, or *GCKR* P446L, leading to more severe steatosis, inflammation, and cirrhosis.[72] Furthermore, insulin resistance, either directly or through elevated insulin levels, has been shown to interact with the *PNPLA3* I148M variant and exacerbate the genetic risk for hepatic steatosis in nondiabetic individuals.[73] These findings underscore the intricate interplay between genetic variants and metabolic diseases and suggest that such interactions can substantially enhance an individual's susceptibility to developing NASH and increase the severity of the disease.

Conclusion

Since the first assembly of whole human genome sequence, our understanding of the functions of genes in health and diseases has significantly advanced, especially with a plethora of knowledge learned from GWAS, which have identified loci associated with various traits. In recent years, studies have been focused on investigating the functions of genes surrounding these identified loci and their potential contribution to the pathogenesis of NAFLD.

Some NAFLD-related mutations tend to cluster around certain regions of the genome, which are sometimes considered as large loci instead of specific genes in some GWAS. For example, the *TM6SF2* and *SUGP1* are located less than 3000 bp apart, and the region containing these two genes, together with ~20 other nearby genes, is referred to as *NCAN* locus. The clustering of SNPs linked to similar traits may suggest aberrant expression regulation in this region, rather than dysfunction of a particular gene. A large proportion of discovered SNPs are in the intron regions, most of which are still functionally unknown. Moreover, the genes that have been mapped, such as *TM6SF6* and *SUGP1*, lack mechanistic links between the function of their encoded proteins and the associated traits. Interestingly, one study found that among the 20 genes in the *NCAN* locus, only *LPAR2* was significantly upregulated in patients with steatosis, even though SNPs were mapped to *SUGP1* from the same cohort. Therefore, focusing on the mapped genes from GWAS may mislead us away from the true genetic factors underlying NAFLD.

The lipid composition of TG and PC has been found to be significantly altered in carriers of many NAFLD-related mutations. Interestingly, lipidomic studies have shown that mutations in *TM6SF2*, *MARC1*, and *HSD17B13* significantly alter the composition of liver phospholipids and/or TG in human carriers, and *PNPLA3* and *MBOAT7* themselves possess the enzymatic activity to remodel TG and PI, respectively.[52,57,64] One common feature of these lipid remodeling processes is that saturation tends to increase, whereas PUFA incorporation is hindered, which is associated with increased risk or severity of NAFLD. Interestingly, lipidomic analyses of patients with NASH have revealed significant decrease in PUFA content in PC and TG in non-alcoholic fatty livers, with no further change as progression to NASH.[74] Furthermore, deficiency in *Lpcat3* (*Mboat5*), an enzyme involved in PC remodeling, results in decreased PUFA in PC and increased steatosis and inflammation in the liver.[42] Our recent studies demonstrated that increasing membrane phospholipid saturation per se is sufficient to drive the initiation and progression of NASH and HCC.[75] These findings suggest that lipid remodeling may play a causal role in the pathogenesis of NAFLD.

In many mutant carriers, serum lipids, insulin resistance, and NAFLD can be dissociated. Although type II diabetes and obesity are considered as significant risk factors for NAFLD, it is important to note that not all patients with NAFLD have these disorders. For example, SNP in *TM6SF2* shows protection against serum lipid and insulin resistance while promoting steatosis,[11,40] while GCKR mutant exhibits opposite effect on NAFLD severity depending on diabetic background.[69] Interestingly, increasing membrane PC saturation has been shown to improve insulin sensitivity despite promoting NASH progression.[75]

Therefore, it is possible that altered PC composition could be the underlying mechanism that explains the disconnect between NAFLD and insulin resistance in lean NAFLD individuals.

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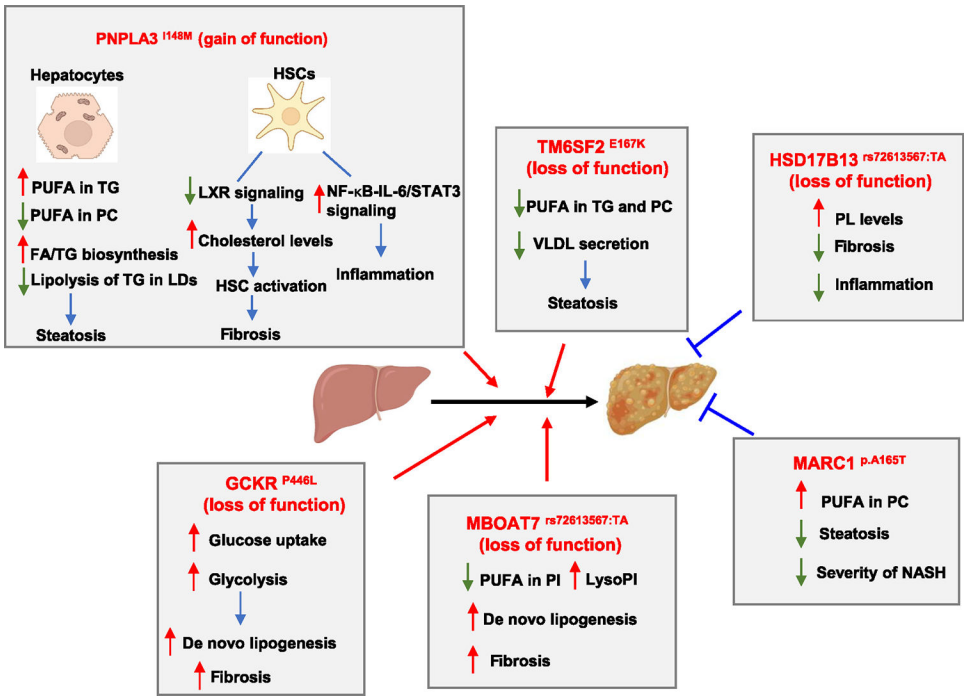

Figure 1.

Most common genetic variants associated with non-alcoholic fatty liver disease and their roles in its progression. FA, fatty acids; GCKR, glucokinase regulator; HSCs, hepatic stellate cells; HSD17B13, 17β-hydroxysteroid dehydrogenase 13; IL-6, interleukin-6; LDs, lipid droplets; LXR, liver X receptor; MARC1, mitochondrial amidoxime-reducing component 1; MBOAT7, membrane-bound *O*-acyltransferase 7; NASH, non-alcoholic steatohepatitis; NF-κB, nuclear factor-κB; PC, phosphatidylcholine; PI, phosphatidylinositol; PL, phospholipids; PNPLA3, patatin-like phospholipase domain containing 3; PUFA, polyunsaturated fatty acids; STAT3, signal transducer and activator of transcription 3; TG, triglyceride; TM6SF2, transmembrane 6 superfamily member 2; VLDL, very low-density lipoprotein.

Table 1

Genetics and experimentalmodels of GWAS identified genes related to NAFLD

Gene	SNPs	Correlation with NAFLD	Function	Mouse model	Phenotypes	Ref.
PNPLA3	rs738409 rs2896019 rs3747207	Promote	Retinyl ester hydrolase, TG acyltransferase	*Pnpla3* KO	No difference in energy, glucose, and lipid homoeostasis or hepatic steatosis or injury	4
				OE of I148M in liver	Increased formation of fatty acids and TAG, impaired hydrolysis of TAG, and relative depletion of TAG long-chain polyunsaturated fatty acids	5, 6
				KD of *Pnpla3* by ASO	Reduced hepatic steatosis, decreased fatty acid esterification, and increased hepatic insulin sensitivity	7
				shRNA KD of *Pnpla3* in I148M OE mice	Reduced liver TG level	8
				OE of *PNPLA3* in cell lines	Increased cellular lipid synthesis	9
TM6SF2	rs58542926	Promote (withNot clear improved insulin sensitivity)		*TM6SF2* siRNA inhibition in human cell lines	Reduced secretion of TG-rich lipoproteins (TRLs) and increased cellular TG concentration and lipid droplet content	10
				AAV-shRNA KD of *Tm6sf2* in mouse liver	Lower plasma cholesterol and TG levels and increased liver TG and cholesterol ester	11
				Liver-specific *Tm6sf2* OE and KO	OE of *Tm6sf2* increases liver TG and cholesterol on chow or HFD No change in liver TG and cholesterol levels in *Tm6sf2* KO	12
				Tm6sf2 whole-body KO	Blocked VLDL secretion and higher liver lipids	13
				AAV-mediated OE of wild-type or E167K *TM6SF2*	Lower liver and serum lipids with reduced fibrosis	14
SUGP1	rs8107974	Promote	RNA splicing factor	Liver *Sugp1* OE	Increased plasma TG and cholesterol	15
MBOAT7	rs626283 rs641738	Promote	Lysophospholipid acyltransferase	Global *Mboat7* KD by ASO	Promoted NAFLD development on HFD and impaired insulin signaling	16
				Liver-specific *Mboat7* KO	Increased liver inflammation and fibrosis on HFCDD	17
				Liver-specific *Mboat7* KO	Develop steatosis on chow diet and fibrosis on HFD	18, 19
HSD17B13	rs72613567 rs13118664 rs9992651	Protect	Hydroxysteroid dehydrogenase for estrogens and androgens	*Hsd17b13* global KD by AAV-shRNA	Protects from CD-induced fibrosis	20
				Transgenic *HSD17B13* S33A mutation (HSD17B13 33A/A) KI mouse	Develop spontaneous hepatic steatosis and increased inflammation	21
				Hsd17b13 global KO	Hepatic steatosis and increased inflammation	22
				Hsd17b13 whole-body KO	Higher body weight on chow diet No difference on body weight, liver TG, inflammation, and fibrosis on HFD and WD	23

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Gene	SNPs	Correlation with NAFLD	Function	Mouse model	Phenotypes	Ref.
MARC1	rs2642438	Protect	Molybdenum-containing enzyme catabolizing N-hydroxylated compounds	Liver-specific *Marc1* KD	Protects against diet-induced NASH with lower liver lipids and fibrosis	24
GCKR	rs780094 rs1260326	Promote	Competitively binding and regulating glucokinase	No mouse model specific for NASH	—	—

AAV, adeno-associated virus; ASO, antisense oligonucleotide; CD, choline-deficient; GCKR, glucokinase regulator; GWAS, genome-wide association studies; HFCDD, high-fat, methionine-low, choline-deficient diet; HFD, high-fat diet; HSD17B13, 17β-hydroxysteroid dehydrogenase 13; KD, knockdown; KI, knock-in; KO, knockout; MARC1, mitochondrial amidoxime-reducing component 1; MBOAT7, membrane-bound *O*-acyltransferase 7; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; OE, overexpression; PNPLA3, patatin-like phospholipase domain containing 3; SNPs, single nucleotide polymorphisms; SUGP1, SURP and G-patch domain containing 1; TAG, triacylglycerol; TG, triglyceride; TM6SF2, transmembrane 6 superfamily member 2; VLDL, very low-density lipoprotein; WD, western diet.