



Review Article

Evolution of Non-alcoholic Fatty Liver Disease to Liver Cancer: Insights from Genome-wide Association Studies



Sathish Kumar Mungamuri^{1*} , Yogendra Kumar Gupta², Padaki Nagaraja Rao³ and Bagepally Ravishankar⁴

¹Division of Food Safety, Indian Council of Medical Research-National Institute of Nutrition, Hyderabad, India; ²Department of Pharmacology, All India Institute of Medical Sciences, New Delhi, India; ³Department of Hepatology, Asian Institute of Gastroenterology Hospitals, Hyderabad, India; ⁴Division of Medical Gastroenterology, Yashoda Hospitals, Secunderabad, India

Received: November 11, 2022 | Revised: January 03, 2023 | Accepted: February 22, 2023 | Published online: March 28, 2023

Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease, affecting a quarter of the world's population. The spectrum of NAFLD ranges from simple steatosis, non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and ultimately to hepatocellular carcinoma (HCC). Multiple factors are involved in the pathogenesis and progression of NAFLD, wherein genetic, epigenetic, environmental, gut-microbiome, and dietary factors play a substantial role. Robust genome-wide association studies (GWAS) have recently identified several genetic alterations contributing to NAFLD progression to liver cancer. This review describes the most critical genetic variations identified in various genes that modulate NAFLD pathogenesis. As expected, NAFLD shows gene variations that regulate or influence hepatocyte lipid metabolism. In addition to polymorphisms in genes that regulate insulin signaling, fibrosis, inflammation, and oxidative stress, cytokine/chemokine-expressing genes also participate in NAFLD pathogenesis. GWAS helped us distinguish genetic polymorphisms that modulate steatosis, fibrosis, or both in NAFLD patients. Identifying genetic factors predisposing an individual to NAFLD helps stratify people at high risk and aids in imparting preventive strategies early in life. Such studies should also help in adopting prevention strategies and targeted treatment regimes.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is a non-communicable disease associated with at least a quarter of the world's popula-

tion. The disease is characterized by fat accumulation in >5% of the hepatocytes (steatosis) without significant alcohol intake. The average NAFLD prevalence rate is considerably higher in Asia (27%) compared to the United States (24%) and Europe (23%).^{1–3} NAFLD condition can be seen in both obese individuals as well as people having average weight. “Lean/non-obese NAFLD” is the medical term for NAFLD if seen in normal-weight individuals. Lean/non-obese NAFLD accounts for almost 20% prevalence within the NAFLD population and nearly 5% in the general population.⁴ Pathophysiological conditions such as central obesity, hyperglycemia, dyslipidemia, and hypertension are closely linked to NAFLD.⁵ Adipose tissue lipolysis is the primary source of hepatic lipid accumulation.⁶ During obesity, this basal lipolysis gets enhanced, resulting in an enhanced flow of free fatty acids to the liver (and skeletal muscle).⁷ This, in turn, promotes resistance to insulin.^{7,8} Alternate sources for lipid accumulation in the hepatocytes include *de novo* lipogenesis from excess dietary sugars and dietary fat.⁶ NAFLD patients may have stable disease, progress slowly or rapidly, or even regress,⁹ or some will develop hepatocellular carcinoma (HCC).¹⁰

From the stage of simple steatosis, NAFLD may progress to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and end-stage liver disease, which ultimately necessitates liver transplantation.¹¹ NASH is characterized by simple steatosis plus inflammation and hepatocyte ballooning. NAFLD progresses to NASH when the liver exceeds its capacity to metabolize free fatty acids and is character-

Keywords: Non-alcoholic fatty liver disease; Liver cancer genome-wide association studies; Single nucleotide polymorphisms; Risk analysis.

Abbreviations: ALT, alanine aminotransferase; APOB, apolipoprotein B; APOH, apolipoprotein H; AST, aspartate aminotransferase; GSKR, glucokinase regulatory protein; GK, glucokinase; GWAS, genome-wide association studies; GG, guanine guanine; GUSB, Glucuronidase beta; HCC, hepatocellular carcinoma; TIMP, tissue inhibitor of metalloproteinase; HFE, hemochromatosis; IR, insulin resistance; MBOAT7, membrane-bound O-acyltransferase domain containing protein 7; MTP, microsomal triglyceride transfer protein; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NCAN, neurocanin; PEMT, phosphatidylethanolamine methyl transferase; PGC-1 α , peroxisome proliferator-activated receptor gamma-coactivator 1 alpha; PI3K, phosphatidylinositol 3-kinase; PNPLA3, patatin-like phospholipase 3; PPAR, peroxisome proliferator-activated receptor; PRS, polygenic risk scores; SNP, single nucleotide polymorphism; STAT3, signal transducer and activator of transcription 3; TA, thymine and adenine; TG, triglyceride; TM6SF2, transmembrane 6 superfamily member 2; TNF- α , tumor necrosis factor-alpha; VLDL, very-low-density lipoprotein; TCF7L2, Transcription factor 7 like 2.

*Correspondence to: Sathish Kumar Mungamuri, Division of Food Safety, Indian Council of Medical Research-National Institute of Nutrition, Jamai-Osmania PO, Tarnaka, Hyderabad 500007, India. ORCID: <https://orcid.org/0000-0002-0012-5298>. Tel: +91-40-27197281 (Office), +91-8247336934 (Cell), Fax: +91-40-27019074, E-mail: mungamurisk@gmail.com; sathish.mungamuri@mssm.edu

How to cite this article: Mungamuri SK, Gupta YK, Rao PN, Ravishankar B. Evolution of Non-alcoholic Fatty Liver Disease to Liver Cancer: Insights from Genome-wide Association Studies. *Gene Expr* 2023;22(1):47–61. doi: 10.14218/GE.2022.00014.

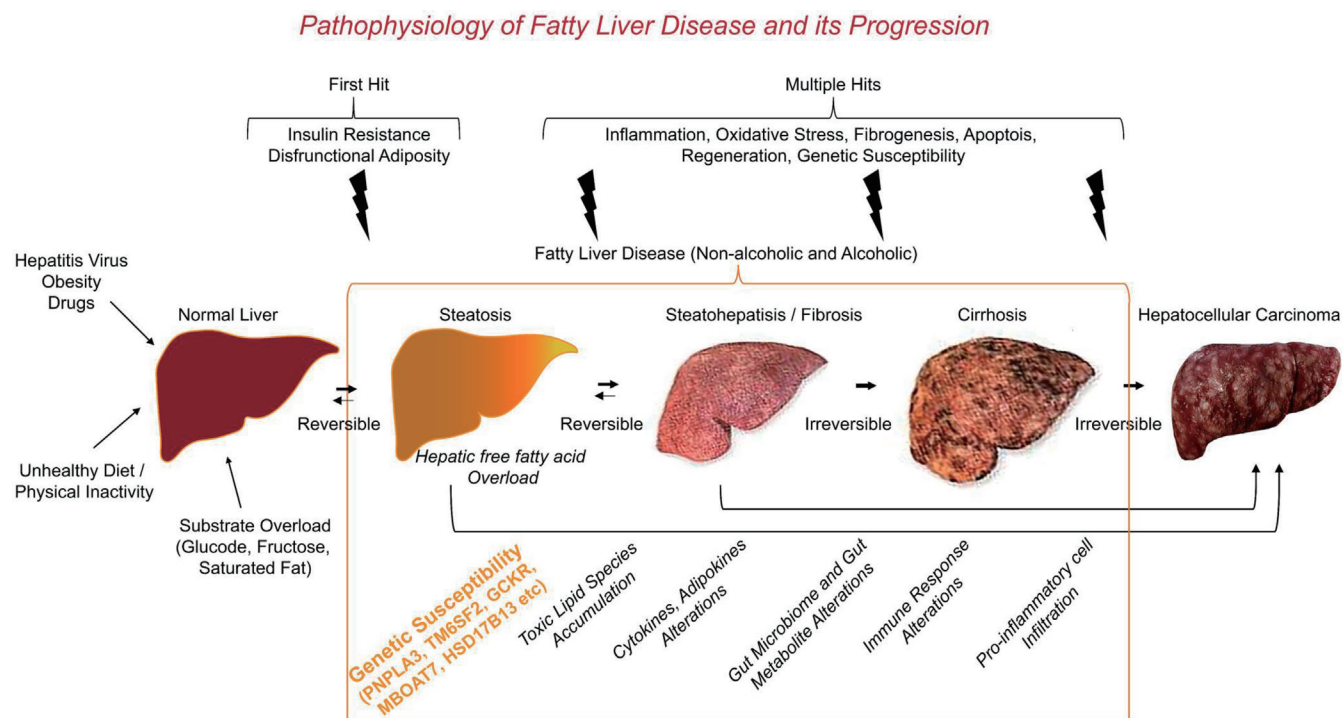


Fig. 1. Schematic representation showing the spectrum of the different stages of FLD and its progression toward HCC. Healthy liver encounters several insults in daily life, including unhealthy diet, physical inactivity, drugs, and hepatitis virus infection. Insulin resistance and dysfunctional adiposity mainly represent the first hit, wherein the healthy liver gets converted FLD state. Steatosis represents FLD's first stage, wherein there is an abnormal accumulation of lipids in the hepatocytes in the form of cytoplasmic lipid droplets. Steatohepatitis represents FLD's next stage, where lipid accumulation is accompanied by chronic inflammation. During steatohepatitis, fibrotic scars are developed, and the parenchymal hepatic tissue is also seen. Undifferentiated regenerating hepatocyte nodules with extended fibrosis characterize steatohepatitis and fibrosis progression to cirrhosis. The FLD is reversible until the cirrhosis stage (from the healthy liver). This cirrhosis is a significant risk factor for FLD progression into HCC. Note that HCC may also occur in non-cirrhotic livers showing the characteristics of only fibrosis and/or inflammation. FLD, fatty liver disease; HCC, hepatocellular carcinoma.

ized by the formation of harmful lipid species and activation of lipotoxic pathways, leading to hepatocellular injury, as observed both in humans and mice models.^{12–15} Repeated hepatocellular injuries lead to irregular wound healing, ultimately leading to fibrosis or scarring of liver tissue. During fibrosis, inflammatory molecules activate hepatic stellate cells to become myofibroblasts. These myofibroblasts migrate into the parenchyma of the liver and secrete the collagen-rich extracellular matrix that characterizes fibrosis or scarring.¹⁶ Scarring also gets increased due to inhibition in fibrolysis.¹⁶ Scarring leads to cirrhosis, characterized by thick, fibrous septae and architectural distortion. While steatosis and fibrosis can be reversed, it becomes irreversible once the cirrhosis stage is reached. Once cirrhosis is developed, it predisposes to portal hypertension and organ failure complications and progresses into HCC (Fig. 1).¹¹

Consumption of high fructose, food rich in calories, and less physical activity undoubtedly increases the risk of NAFLD.¹⁷ NAFLD is positively associated with insulin resistance (IR) and other metabolic syndromes and is highly prevalent in adults and children.¹⁸ In addition to these environmental and metabolic dysregulations, both epigenetic changes (histone modifications, non-coding RNAs, *etc.*), and genetic (inherited genetic variants) factors are identified, which are responsible and/or leading risk factors for NAFLD progression into NASH, cirrhosis, and/or HCC.¹⁹ The role of epigenetics and gut microbiota in NAFLD progression is beyond the scope of this review, and the readers are advised to go through some of the recent reviews on this topic.^{20–23} Several genome-wide association studies (GWAS) were carried out over the past decade, which led to the

identification of multiple genetic variants which predispose people to NAFLD. Aberrations in lipid processing play a central role in the pathogenesis of steatosis and progressive liver disease. It is not surprising that the variants that affect the function of genes, which are involved in the triglyceride (TG) synthesis, storage, and export, are known to modulate the individual's susceptibility to NAFLD (Fig. 1).²⁴ The other genetic variants that have been associated with NAFLD include the genes regulating fibrogenesis, oxidative stress, and insulin signaling (Table 1).^{25–71} Besides, aberrant expression of several adipokines and cytokines are also associated with NAFLD onset and progression. In this review, we discuss various classes of genetic variants that predispose individuals to NAFLD.

Major genetic risk alleles identified by GWAS studies modulate the progression of non-alcoholic fatty liver disease

The heritable nature of NAFLD was proven in many studies.^{72,73} GWAS, Exome sequencing, and candidate gene studies have unearthed NAFLD susceptibility genes. Below, we explain the five significant gene products in detail, whose alteration in their genomic sequence majorly affects NAFLD's development and progression.

PNPLA3 (Patatin-like phospholipase 3; adiponutrin, or calcium-independent phospholipase A2-epsilon, or acylglycerol O-acyltransferase)

Patatin-like phospholipase domain-containing protein 3 is a sin-

Table 1. : Candidate genes discussed in this review that are involved in the non-alcoholic fatty liver disease progression

S. No	Gene Name	Pathway Involved	References
1	Adiponectin	Determine the Insulin Sensitivity	25
2	Angiotensin II	Involved in Fibrosis	26
3	Angiotensin II type 1 receptor	Involved in Fibrosis	27
4	Apolipoprotein C3	Involved in Lipid Metabolism	28
5	Apolipoprotein E	Involved in Lipid Metabolism	29
6	CD14	Regulates cytokine release	30
7	CYP2E1	Involved in Oxidative Stress	31
8	ENPP1	Involved in Lipid Metabolism	32
9	HFE	Regulates Iron Absorption	33,34
10	Glutathione-S-Transferase	Involved in Oxidative Stress	35
11	GCKR	Involved in Lipid Metabolism	36–38
12	HSD17B13	Involved in Lipid Metabolism	39–41
13	IL-6	Regulates Inflammation	42,43
14	IRS-1	Involved in Insulin Signaling	32
15	KLF6	Involved in Fibrosis	44
16	Leptin Receptor	Regulates Inflammation	45
17	MTP	Involved in Lipid Metabolism	46,47
18	MBOAT7	Involved in Lipid Metabolism	48–50
19	NCAN	Remodeling of the extracellular matrix	51
20	PEMT	Involved in Lipid Metabolism	52
21	PIK3AP1	Involved in PI3K Signaling	53
22	PNPLA3	Controls Lipid Accumulation	54–56
23	PPARGC1A	Controls Lipid Accumulation	57
24	PPAR α	Controls Lipid Accumulation	58
25	PPAR γ	Controls Lipid Accumulation	59
26	PXR	Involved in Lipid Metabolism	60
27	SOD2	Involved in Oxidative Stress	61
28	STAT3	Regulates Inflammation	62
29	TCF7L2	Determine the Insulin Sensitivity	63
30	TNF- α	Regulates Inflammation	64
31	TGF- β 1	Regulates Inflammation	26
32	TM6SF2	Involved in Lipid Metabolism	65–67
33	TRAIL	Regulates Inflammation	68
34	TRIB1	Involved in Lipid Metabolism	69,70
35	UGT1A1	Involved in Oxidative Stress	71

CD14, cluster of differentiation 14; CYP2E1, cytochrome P450; ENPP1, ectoenzyme nucleotide pyrophosphate phosphodiesterase 1/PC-1; GCKR, glucokinase regulatory protein; HFE, hemochromatosis; KLF6, kruppel-like factor 6; IL-6, interleukin-6; IRS-1, insulin receptor substrate-1; MBOAT7, membrane-bound O-acyltransferase domain containing protein 7; MTP, microsomal triglyceride transfer protein; NCAN, neurocanin; PEMT, phosphatidyl ethanolamine methyl transferase; PIK3AP1, phosphoinositide-3-kinase adaptor protein 1; PI3K, phosphatidylinositol 3-kinase; PNPLA3, patatin-like phospholipase 3; PPARGC1A, peroxisome proliferator-activated receptor gamma, coactivator 1 alpha, PPAR α , peroxisome proliferator-activated receptor-alpha; PPAR γ , peroxisome proliferator-activated receptor gamma; PXR, pregnane X receptor; STAT3, signal transducer and activator of transcription 3; SOD2, super oxide dismutase-2; TCF7L2, transcription factor 7 like 2; TM6SF2, transmembrane 6 superfamily member 2; TNF- α , tumor necrosis factor-alpha; TGF- β 1, transforming growth factor, beta 1. TRAIL, TNF-related apoptosis-inducing ligand; TRIB1, tribbles homolog 1; UGT1A1, UDP glucuronosyltransferase 1 family, polypeptide A1.

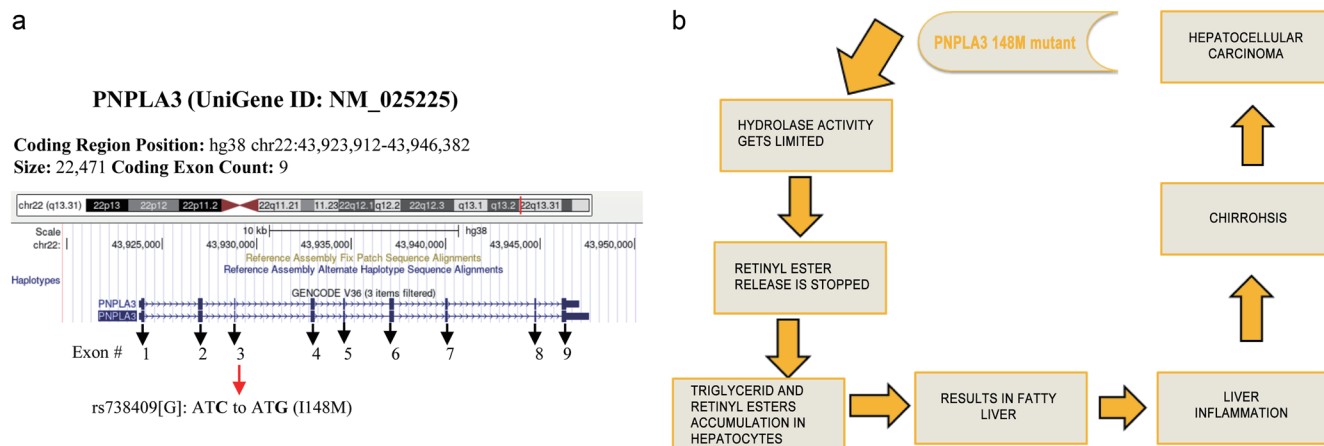


Fig. 2. PNPLA3 gene locus showing the rs738409 polymorphism and its role in HCC progression. A) Schematic diagram showing the presence of the PNPLA3 gene at chromosome 22q13.31. The gene spans the length of 22471 nucleotides with 9 exons. rs738409 variant in PNPLA3 is a point mutation, wherein 'cytosine' nucleotide gets converted into 'guanine' nucleotide in exon 3 (C→G). B) Schematic representation showing how I148M variant in PNPLA3 gene locus leads to HCC. HCC, hepatocellular carcinoma; PNPLA3, patatin-like phospholipase 3.

gle-pass type II membrane protein. This enzyme is multifunctional and is shown to have at least three enzymatic activities, including triacylglycerol lipase, acylglycerol O-acyltransferase, and retinyl esterase.^{74–76} PNPLA3 is present mainly on the endoplasmic reticulum membrane and on the lipid droplets' surface in hepatocytes, hepatic stellate cells, and adipocytes.^{77–78} Pingitore *et al.* demonstrated that overexpression of wild-type PNPLA3 protects against fibrosis by decreasing the secretion of tissue inhibitor of metalloproteinases 1 and 2 (TIMP1 and 2) and secretion of matrix metalloproteinase 2.⁷⁷

To date, the C>G transversion polymorphism (rs738409[G]) encoding isoleucine to methionine (I148M) substitution in PNPLA3 has been correlated the best with susceptibility to non-alcoholic,⁵⁴ as well as alcoholic⁷⁹ liver disease (Fig. 2a). This transversion is observed at a high frequency in Hispanics, which subjects the liver to damage from fatty liver to NASH, fibrosis, and HCC.⁸⁰ The presence of a homozygous minor allele (I148M; non-synonymous variant) in the PNPLA3 gene generally results in high hepatic fat content^{54,55} and high hepatic inflammation,⁸¹ as indicated by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST) concentrations.⁵⁶ PNPLA3 I148M variant has limited hydrolase activity, therefore halts retinyl ester release, and finally accumulating of TGs and retinyl esters in the hepatocytes and stellate cells, leading to fatty liver.^{76,82,83} Besides, PNPLA3 I148M variant protein accumulates on lipid droplets' surface and obstructs lipid modifications in hepatocytes. Furthermore, it also inhibits other lipases' activity (*i.e.*, PNPLA2), and finally, reduces the turnover and release of TGs.⁸⁴ This may be due to less availability of the I148M protein to ubiquitin ligases, leading to an impairment in 26S proteasomal degradation.⁸³ It is important to note that environmental factors trigger this protein accumulation. A meta-analytic study of 24 studies with 9915 patients from diverse backgrounds identified that the PNPLA3 I148M variant was extensively associated with fibrosis (Fig. 2b).⁸⁵ Another meta-analysis report of 16 studies showed that the presence of the PNPLA3 I148M variant had significantly increased steatosis and fibrosis. Guanine guanine (GG) homozygous genotype individuals demonstrated 77% more fat content⁸⁶ and a 5-fold increase in disease development than the cytosine and cytosine (CC) nucleotides genotype.⁸⁷ In contrast, another allele (rs6006460[T]) encoding

serine to isoleucine (S453I) substitution in the same gene is most prevalent in the African-American population and is associated with diminished fat content in the liver.⁵⁴

The PNPLA3 I148M variant has been associated with the clinical presentation of HCC, particularly with more advanced disease and a poorer prognosis.^{88,89} Allelic frequency calculations showed that rs738409 C>G shows more risk of HCC progression than the progression to cirrhosis.^{87,90} The unadjusted risk for HCC with the homozygote GG genotype was 12.19 (95% CI 6.89–21.58) over CC.⁸⁷ Homozygous PNPLA3 148M individuals (along with long-standing advanced liver disease and cirrhosis) have a 2 to 16-fold increased risk of developing HCC.⁹¹ Similarly, a 16-fold higher HCC risk was observed in obese subjects with rs738409 C>G homozygosity.⁹²

Transmembrane 6 superfamily member 2

Transmembrane 6 superfamily member 2 (TM6SF2) is mainly expressed in the liver and small intestine and regulates cholesterol synthesis and secretion of lipoproteins.^{65,93,94} The protein coded by TM6SF2 gets concentrated at the endoplasmic reticulum and golgi in the hepatocytes and aids in TG-rich lipoproteins secretion. A decreased expression of TM6SF2 upsurges hepatic TG content in cell culture models,⁹³ suggesting that loss of TM6SF2 may be priming HCC development and progression.

Single nucleotide polymorphism (SNP) rs58542926 (c.449 C>T; causing E167K missense mutation) in TM6SF2 is strongly associated with NAFLD⁶⁵ in both adults^{65,95–98} and pediatric^{99,100} cohorts. This non-synonymous polymorphism is second to that of PNPLA3 and seen in Europeans (~7%), Hispanics (~4%), African Americans (~2–3%),^{65,101} as well as Indians.¹⁰² This SNP is associated with advanced hepatic fibrosis/cirrhosis.⁹⁶ Due to the loss-of-function mutation of this variant protein, the reduced TM6SF2 mRNA and protein expression result in withholding lipids by impairing very-low-density lipoprotein (VLDL)-mediated lipid secretion (Fig. 3). Therefore, the predisposition of the TM6SF2 variant increases the susceptibility to liver damage.^{65,95,96,103,104} NAFLD with TM6SF2 E167K polymorphism comes under a distinct category. Even though these patients show increased liver fat content, they preserve the insulin sensitivity regarding lipolysis and hepatic glucose.⁶⁶ Moreover, persons with TM6SF2 E167K polymorphism

TM6SF2 (UniGene ID: NM_001001524)

Coding Region Position: hg38 chr19:19,264,664-19,273,215

Size: 8,552 **Coding Exon Count:** 10

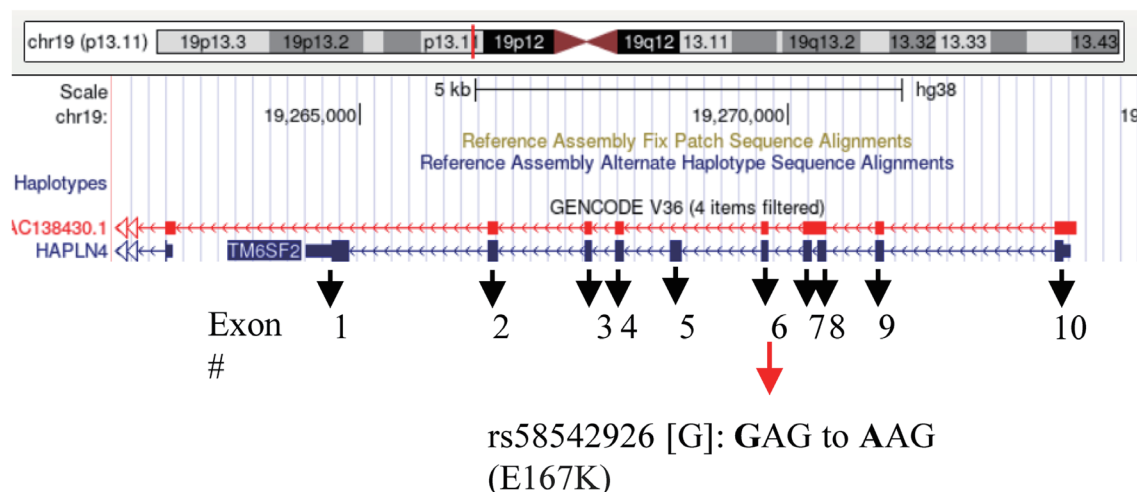


Fig. 3. TM6SF2 gene locus showing the rs58542926 polymorphism. Schematic diagram showing the presence of TM6SF2 gene at chromosome 19p13.11. The gene spans the length of 8552 nucleotides with 10 exons. rs58542926 variant in TM6SF2 is a point mutation, wherein the 'Guanine' nucleotide gets converted into 'adenine' nucleotide in exon 6 (G→A). TM6SF2, transmembrane 6 superfamily member 2.

do not show hypertriglyceridemia.⁶⁶ The TM6SF2 function is not well established; however, it is thought to be involved in VLDL secretion.⁶⁵ Subjects carrying TM6SF2 rs58542926 C>T E167K variant had reduced circulating lipids and improved lipid profiles, and had reduced cardiovascular disease risk. Substitution of lysine with glutamic acid at residue 167 of TM6SF2, which reduces the protein production by at least 2-fold, decreased the VLDL secretion by 50%.⁶⁵ TM6SF2 loss of function using Caco-2 cells *in vitro* and the zebrafish model *in vivo* demonstrated diminished lipid clearance and increased ER stress and TG accumulation in enterocytes.⁶⁷ TM6SF2 is required for apolipoprotein B (APOB) protein stability.¹⁰⁵ The E167K mutation in TM6SF2 cannot stabilize the APOB protein, resulting in hepatic lipid accumulation and low serum lipid levels.¹⁰⁵

Liver-specific deletion of TM6SF2 in mice leads to impairment in VLDL secretion, resulting in hepatic steatosis and fibrosis, ultimately causing accelerated HCC development.¹⁰⁶ Further, in these mice models, the tumor burden is inversely correlated with the TM6SF2 protein levels.¹⁰⁶

Glucokinase regulatory protein

The glucokinase regulatory protein (*GCKR*) gene encodes glucokinase regulatory protein and is expressed mainly in the liver, pancreas, and colon. In the hepatocytes, GCKR shuttles between the cytoplasm and nucleus and regulates *de novo* lipolysis by controlling the glucose influx. GCKR is a potent glucokinase (GK) enzyme inhibitor and prevents the liver's glucose storage and disposal. GCKR binds to the enzyme and forms an inactive complex. Fructose metabolites regulate the binding affinity of GCKR for GK.

The rs780094 (C>T) variant is present in an intronic region of GCKR. Still, it shows a high percentage of linkage disequilibrium with rs1260326, a functional non-synonymous substitution C>T

encoding the GCKR protein with P446L substitution (Fig. 4).¹⁰⁷ This SNP in the GCKR contributes to the risk of type-2 diabetes and dyslipidemia in multiple populations.³⁶ The association between the rs1260326 C>T polymorphism and the NAFLD progression is demonstrated in various GWAS studies from East Asia,^{37,38,108} South Asia,¹⁰⁹ and Europe.^{110,111} African American and Hispanic NAFLD patients also show concordant but nonsignificant trends in rs1260326 C>T polymorphism.¹⁰¹ The P446L substitution is closely associated with liver fat deposition and is one of NAFLD's inheritable risk factors.^{112,113} GCKR rs1260326 P446L variant reduces the inhibitory activity of GCKR over glucokinase, which results in an enhancement in glucose flux and an increase in *de novo* lipogenesis.^{114,115} GCKR rs1260326 P446L variant negatively regulates the GK activity in response to fructose-6-phosphate. This means it increases glucose uptake by the liver and decreases the circulating glucose and insulin levels. In addition, the GCKR rs1260326 P446L variant also directs increased glycolysis and malonyl-CoA production, which is the substrate for lipogenesis and blocks fatty acid oxidation, thus, favoring hepatic fat accumulation.¹¹⁵ A meta-analysis of five studies revealed that the rs780094 variant in GCKR is positively associated with hepatic steatosis.¹¹⁶ Thus, not surprisingly, the same variant is also associated with a modest risk of having a fatty liver, whereas homozygous people are at a 1.2-fold higher risk of developing NAFLD in their lifetime.³⁷

Membrane-bound O-acyltransferase domain-containing protein 7

The available data suggest that the *TCM4* gene is not abundantly expressed in the human liver (Fig. 5).⁴⁸ However, the available eQTL (Expression Quantitative Trait Loci) analysis suggests that rs641738 SNP present in the first exon of *TCM4* gene leads to C>T missense mutation, and leads to reduced expression and activity of MBOAT7, and perhaps participates in the progression of liver

GCKR (UniGene ID: NM_001486)

Coding Region Position: hg38 chr2:27,496,905-27,523,439
Size: 26,535 Coding Exon Count: 19

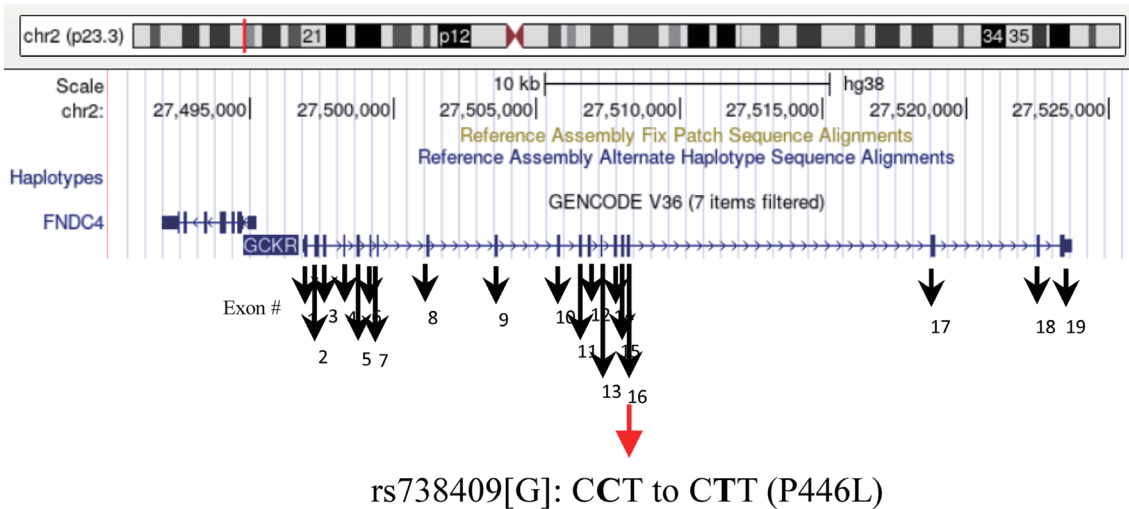


Fig. 4. GCKR gene locus showing the rs738409 polymorphism. Schematic diagram showing the presence of GCKR gene at chromosome 2p23.3. The gene spans the length of 26,535 nucleotides with 19 exons. rs738409 variant in GCKR is a point mutation, wherein the ‘cytosine’ nucleotide gets converted into the “thymine” nucleotide in exon 16 (C→T). GCKR, glucokinase regulatory protein.

disease.^{48,117–119}

Membrane-bound O-acyltransferase domain-containing protein 7 (MBOAT7) is an integral membrane protein with LPIAT1 (lysophosphatidyl acyl-transferase1) enzymatic activity. MBOAT7 is mainly expressed in the hepatocytes, hepatic sinusoidal, and stellate cells and localized in the mitochondria-associated membrane.

The mitochondria-associated membrane connects the endoplasmic reticulum and mitochondria, where biosynthesis of fats and formation of lipid droplets occurs.^{120,121} LPIAT1 is a critical enzyme of the phospholipid acyl-chain remodeling pathway called the “Lands’ Cycle”.¹²² LPIAT1 adds acyl-CoA to the second acyl chain of lysophospholipids, using arachidonoyl-CoA as an acyl

TMC4 (UniGene ID: NM_001145303)

Coding Region Position: hg38 chr19:54,160,306-54,173,117
Size: 12,812 Coding Exon Count: 15

MBOAT7 (UniGene ID: NM_024298)

Coding Region Position: hg38 chr19:54,174,044-54,188,508
Size: 14,465 Coding Exon Count: 7

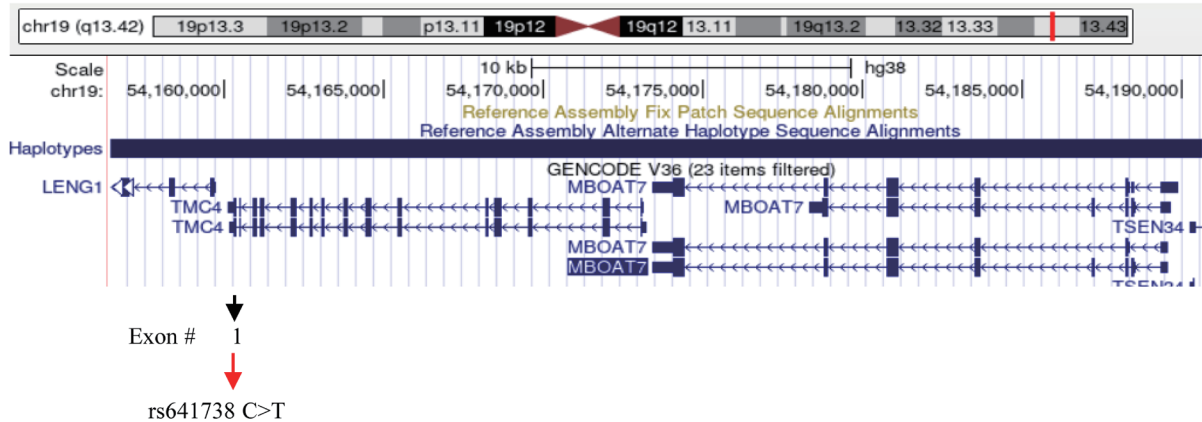


Fig. 5. TMC4 and MBOAT7 genes locus showing the rs641738 polymorphism. Schematic diagram showing the presence of TMC4 and MBOAT7 genes at chromosome 19q13.42. The TMC4 gene spans 12,812 nucleotides with 15 exons, while the MBOAT7 gene spans 14,465 nucleotides with 7 exons. rs641738 single nucleotide polymorphism is a point mutation wherein the ‘cytosine’ nucleotide gets converted into “thymine” nucleotide (C→T) in exon 1 of TMC4. Note that the expression of TMC4 in hepatocytes is minimal, and rs641738 SNP in TMC4 affects the MBOAT7 expression. MBOAT7, Membrane-Bound O-acyltransferase Domain Containing Protein 7; SNP, single nucleotide polymorphism.

HSD17B13 (UniGene ID: NM_178135)

Coding Region Position: hg38 chr4:87,305,218-87,322,841

Size: 17,624 Coding Exon Count: 7

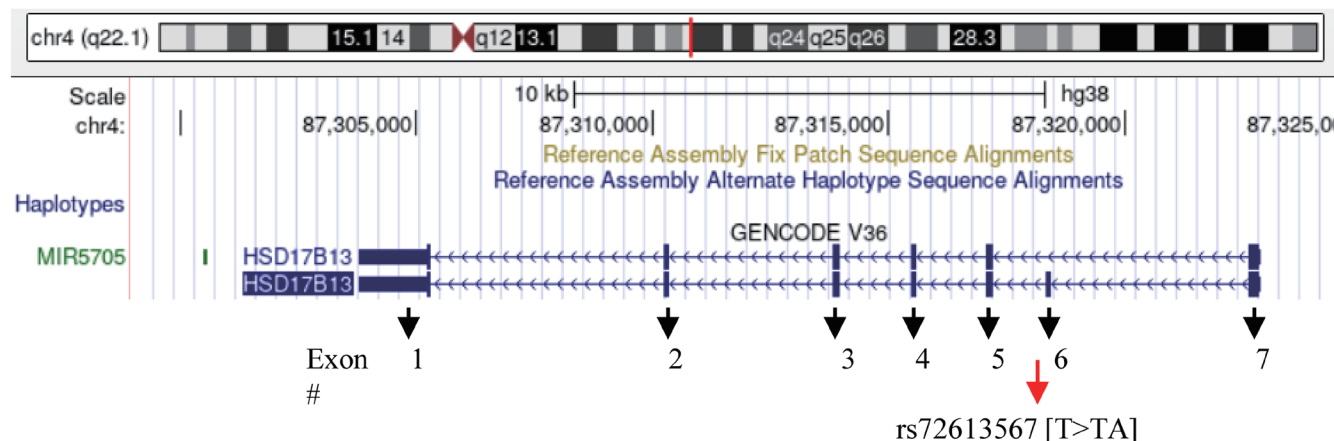


Fig. 6. HSD17B13 gene locus showing the rs72613567 polymorphism. Schematic diagram showing the presence of the HSD17B13 gene at chromosome 4q21.1. The gene spans the length of 17,624 nucleotides with 7 exons. rs72613567 variant in HSD17B13 is an insertion mutation, wherein the 'Adenine' nucleotide gets incorporated next to "thymine" present at the donor splice site of exon 6 (T→TA).

donor. This way, it controls phospholipids' desaturation and the quantity of free arachidonic acid, a predecessor of proinflammatory mediators.¹²³

SNP rs641738 C>T results in diminished MBOAT7 protein expression and reduced desaturation of phosphatidyl-inositol in the hepatocytes. rs641738T SNP favors hepatic fat accumulation and contributes to various liver diseases, including NAFLD, fibrosis, and HCC.⁴⁸⁻⁵⁰ SNP rs641738 was initially associated with alcoholic cirrhosis.¹²⁴ However, immediately after, it was also linked with steatosis and progressive NAFLD,^{48,98,125} as well as with markers of liver fibrosis.¹²⁶ MBOAT7 rs641738 C>T polymorphism also correlates with high hepatic fat content and elevated serum ALT levels,¹¹⁹ and perhaps may also regulate IR and glucose metabolism in NAFLD patients.¹²⁷

HSD17B13

The gene HSD17B13 encodes for 17 β -HSD type 13 (17 β -hydroxysteroid dehydrogenase type 13) enzyme with retinol dehydrogenase activity. It is involved in metabolizing retinoids, fatty acids, and sex steroids.¹²⁸ The liver is the predominant site of HSD17B13 protein expression¹²⁹ and is localized to hepatocytes lipid droplets.¹³⁰

The rs72613567 variant in HSD17B13 is an insertion, wherein the 'adenine' nucleotide gets incorporated next to "thymine" (T) present at the donor splice site of exon 6 (T> Thymine and Adenine (TA)). This insertion leads to truncated and unstable protein with diminished enzymatic activity.³⁹ The 'TA' minor allele is commonly seen in Europeans and East Asians, while it is seen to a lesser extent in Africans and Hispanic/Latino individuals. HSD17B13 rs72613567: TA is generally associated with lower serum AST and ALT levels and has an overall reduced risk of chronic liver disease (Fig. 6).^{39,40} Further, the rs72613567: TA is significantly associated with reduced occurrence of chronic liver disease, non-alcoholic cirrhosis, alcoholic liver disease, as well as alcoholic cirrhosis. Surprisingly, data from bariatric surgery patients showed that the HSD17B13 rs72613567: TA allele is associated

with liver fibrosis but not with steatosis.^{41,131} The data from this study confirm that this SNP is probably not involved in steatosis development, but it regulates the disease progression. Although the HSD17B13 expression is high in NAFLD subjects, the precise biological function of HSD17B13 and the pathway/mechanism through which the rs72613567: TA variant contributes to fat accumulation in the liver is still not known.¹³² Overexpression of HSD17B13 in mouse liver increases lipogenesis, leading to hepatic steatosis.¹³³ This data is consistent because NAFLD patients show enhanced HSD17B13 expression in their livers.^{132,133} However, HSD17B13 knock-out mice also show increased hepatic steatosis and inflammation phenotype, mainly due to an increase in *de novo* lipogenesis.¹³⁴ This observation contrasts with what is observed in humans; HSD17B13 loss-of-function protects from liver disease.³⁹ A recent study in Chinese ethnicity suggests that this variant in the HSD17B13 gene confers disease susceptibility rather than disease progression.¹³⁵ In highlighting these discrepancies, further studies are warranted to define the role of wt. HSD17B13 and its variants in liver metabolism, both under physiological and pathological conditions.

Recently, another protein-truncating variant in HSD17B13, *i.e.*, rs143404524 and c.573delC, was identified. This deletion leads to a frameshift at codon 192, resulting in prematurely truncated protein.¹³⁶ This variant is common in African Americans and rare in Whites and Hispanic/Latino people.¹³⁶ Similar to rs72613567: TA, people with rs143404524 show reduced liver disease. Similarly, rs62305723 G>A encoding missense variant p.P260S, which shows lower enzymatic activity, is also associated with decreased disease severity.¹³² All these data support the idea that reducing the HSD17B13 expression may halt liver disease progression.

Polymorphisms/mutations in genes involved in insulin signaling/insulin resistance

121Gln mutation in ENPP1 (ectoenzyme nucleotide pyrophosphate phosphodiesterase 1/PC-1) and several polymorphisms of

IRS-1 (insulin receptor substrate-1) correlate with liver damage and fibrosis.³² These genetic determinants are associated with IR, which more often predisposes people to NAFLD. Transcription factor 7 like 2 (TCF7L2) is a known regulator of glucose homeostasis. Polymorphisms in this gene that increase the type 2 diabetes risk are correlated with IR and NAFLD severity.^{63,137} Polymorphisms observed in apolipoprotein E (APOE),²⁹ and nuclear receptor pregnane X receptor (PXR)⁶⁰ also correlate well with either NAFLD appearance or severity.

Polymorphisms/mutations in genes involved in lipid metabolism

The 493 G>T SNP in the 5' promoter region of the microsomal triglyceride transfer protein (MTP) is associated with increased steatosis and histological NASH grade compared to either with high-activity homozygous allele or with heterozygous allele-containing patients.^{46,47} MTP encodes protein-disulfide isomerase and mediates neutral lipid transfer between the membranes.¹³⁸ MTP is essential for VLDL synthesis and secretion in the liver and the intestine. The G>T SNP in MTP decreases the transcription rate, reducing protein levels that are insufficient to excrete all the TGs from liver cells.

The presence of C-482T, T-455C, or both polymorphisms in the apolipoprotein C3 (APOC3) gene increase the NAFLD prevalence by 38% compared to wild-type homozygotes.¹³⁹ These same authors showed that people with these polymorphisms also exhibit a whopping 60% increase in plasma TG levels.¹³⁹

Phosphatidyl Ethanolamine MethylTransferase (PEMT) gene product participates in the phosphatidylcholine synthesis, which is required to synthesize VLDL. Compared to controls, NASH patients show a higher frequency of PEMT loss-of-function (V175M) allele, and these patients mainly show low body mass index.⁵²

Polymorphisms/mutations in cytokine/adipokines

Genetic studies have shown a clear correlation between the severity of liver diseases, such as steatosis, inflammation, IR, and fibrosis, with the SNPs of genes involved in fibrogenesis, inflammation, and oxidative stress-responsive pathways.^{42,140–143} TNF- α (Tumor Necrosis Factor- α) is the first cytokine whose SNPs are linked to susceptibility for NAFLD, steatohepatitis, and IR.⁶⁴ Mutations/polymorphisms at -238G, -863A, and -1031C in TNF- α are identified in NASH patients.^{64,144,145} Several other SNPs in genes such as TNF-related apoptosis-inducing ligand (TRAIL),⁶⁸ Interleukin-6,^{42,43} Signal Transducer and Activator of Transcription 3 (STAT3),⁶² Adiponectin,²⁵ Leptin receptor,⁴⁵ peroxisome proliferator-activated receptor- α (PPAR α),⁵⁸ PPAR γ ,⁵⁹ peroxisome proliferator-activated receptor gamma-coactivator 1 α (PGC-1 α)⁵⁷ have also been correlated with the NAFLD disease progression (Table 1).

Miscellaneous Polymorphisms

The role of phosphatidylinositol 3-kinase (PI3K) signaling as a significant pathway in human cancer development and progression, primarily HCC, is well known.^{146–148} Moreover, IR abrogation is invariably linked to NAFLD progression.^{149,150} A recent high-throughput whole exome gene sequencing had identified a familial c.512C>T variation in the phosphoinositide-3-kinase adaptor protein 1 gene.⁵³ However, this single-family study requires a more extensive population-based genomic analysis before understanding its role in NAFLD progression. Neurocan (NCAN),

also known as chondroitin sulfate proteoglycan 3 is a proteoglycan mainly involved in remodeling the extracellular matrix of the central nervous system¹⁵¹ but also shows its expression in the liver.¹⁵² Gordon *et al.* in 2013 demonstrated that NCAN rs2228603 (T variant; P92S) is positively linked with steatosis, lobular inflammation, and fibrosis.⁵¹ Mammalian tribbles homolog 1 (TRIB1) regulates the liver's lipogenesis by modulating the plasma's TG and Cholesterol levels. SNP rs6982502 present in the enhancer sequence of TRIB1 genomic sequence was shown to be significantly associated with NAFLD.⁶⁹ SNP rs17321515 and rs2954029 gene polymorphisms found in TRIB1 also increase NAFLD.⁷⁰ Furthermore, TRIB1 rs17321515 gene polymorphism increased coronary heart disease risk in both the general population and NAFLD patients.¹⁵³ A recent Multiomics study of NAFLD had identified 18 independent sequence variants at 17 loci, of which 4 loci, *i.e.*, rs140201358 in *PNPLA2*, rs7029757 in *TOR1B*, rs1801689 in apolipoprotein H (*APOH*), and rs6955582 in Glucuronidase beta (*GUSB*) are novel variants.¹⁵⁴ *PNPLA2* homozygous mutations are associated with neutral lipid storage disease and fatty liver.¹⁵⁵ *PNPLA2* p.Asn252Lys variant leads to an increase in the levels of high-density lipoprotein-cholesterol^{156,157} and is associated with a high waist: hip ratio. The rs7029757[A] polymorphism in *TOR1B* has been associated with ALT levels and cirrhosis but not NAFLD.¹⁵⁸ *APOH* is highly and almost exclusively expressed in the liver.^{159,160} The trait associations of p.Cys325Gly in *APOH* strongly suggest its role in lipid metabolism.^{157,161–163} *GUSB* encodes β -glucuronidase, a lysosomal enzyme involved in the breakdown of glycosaminoglycans.¹⁶⁴ The exact role of how p.Leu649Pro in *GUSB* associates with NAFL disease progression needs to be established.

Other than these polymorphisms in genes that influence the severity of fibrosis like transforming growth factor, beta 1 (TGF- β 1) and Angiotensin II,²⁶ angiotensin II type 1 receptor,²⁷ Kruppel-like factor 6,⁴⁴ in genes influencing the release and/or response to endotoxin or cytokines like cluster of differentiation 14 (CD14),³⁰ and in genes that regulate oxidative stress like hemochromatosis (*HFE*),^{33,34} superoxide dismutase-2,⁶¹ cytochrome P450,³¹ UDP glucuronosyl-transferase 1 family, polypeptide A1,⁷¹ and glutathione-S-transferase genes³⁵ are also reported. However, most of these studies were conducted with a small cohort of patients and requires further validation using a larger sample size.

GWAS analysis largely failed to discriminate the polymorphisms present in the conditions of high-fat liver accumulation cases vs. the onset of NAFL and/or cirrhosis. Magnetic resonance imaging (MRI)-derived proton density fat fraction provides accurate liver fat quantification, but liver biopsy is essential for NASH diagnosis and staging. In most cases, the gene polymorphisms that account for high fatty deposition in liver cells correlate well with the onset of cirrhosis. The exception is p.His48Arg in alcohol dehydrogenase 1b¹⁶⁵ and p.Cys282Tyr in homeostatic iron regulator.¹⁶⁶ These two variants are associated with cirrhosis through alcohol consumption 1b and *HFE* rather than solely through hepatic fat.¹⁵⁴ A recent multi-omics approach using 4,809 all-cause cirrhosis and 861 HCC cases led to the identification of 4 gene variants, *i.e.*, rs738409 in *PNPLA3*, rs28929474 in *SERPINA1*, rs58542926 in *TM6SF2*, and rs72613567 in *HSD17B13* that are significantly associated with cirrhosis.¹⁵⁴ It is worth noting that only two of these four variants (rs738409 in *PNPLA3* and rs58542926 in *TM6SF2*) are associated with lipid accumulation in the liver cells, while the other two variants, rs72613567 in *HSD17B13* and rs28929474 in *SERPINA1*, are associated with NASH³⁹ and α 1-antitrypsin deficiency,¹⁶⁷ respectively.

Polygenic risk scores and prediction panels and their clinical relevance

Polygenic risk scores (PRS; also known as genetic risk scores) help assess the heritable risk of developing a particular disease in an individual's lifetime based on the combined number of genetic variations associated with that particular individual.¹⁶⁸ GWAS studies had identified robust and reproducible associations for the NAFLD progression with variants in the genes such as *PNPLA3*, *TM6SF2*, *HSD17B13*, *MBOAT7*, and *GCKR*. However, PRS forms the forefront frontier in the studies involving NAFLD heritability. PRS helps aggregate the most appropriate genetic variants associated with NAFLD progression with biochemical parameters to identify the patients at greater risk of developing severe NAFLD.¹⁶⁹ These PRS and metabolic factors could be utilized to identify severe liver diseases in patients with gene regulatory networks. PRS helps guarantee the most highly predictive value, the best diagnostic accuracy, and the more precise individualized therapy.¹⁷⁰

Conclusions and future perspectives

Genetic studies over the past decade, particularly family studies and inter-ethnic susceptibility variations, have significantly advanced our understanding of NAFLD's molecular basis. The genome-wide scans continue to provide compelling new insights into NAFLD biology and highlight desirable pharmaceutical targets. Identifying genetic factors predisposing an individual to NAFLD also helps to stratify the people at high risk and gives them a chance to follow preventive strategies early in their life. GWAS analysis helped distinguish the genetic polymorphisms that modulate either the steatosis, fibrosis, or both in NAFLD patients. For example, the *TM6SF2* E167K genotype associates with steatosis but not fibrosis, whereas *MBOAT7* rs641738 is solely associated with increased fibrosis.⁹⁸ On the other hand, for example, the *PNPLA3* genotype is generally correlated with S2-S3 grades of steatosis and F2-F4 stages of fibrosis.⁹⁸

NAFLD pathogenesis shares several genes involved in more general processes, such as inflammation and fibrosis. NAFLD generally occurs along with systemic metabolic dysfunction leading to a concomitant increase in cardiovascular and diabetes risk.¹⁷¹ Characterizing this shared genetic basis of NAFLD and other related disorders should enable the development of precision medicine approaches and the identification of preventive and therapeutic strategies.¹⁷¹ The data generated from studies involving either candidate-gene associations or GWAS is also exploited to discover drugs. Based on the candidate gene approach and GWAS studies, therapeutic targeting of *PPAR α* , *PPAR γ* , *PGC-1 α* , *STAT3*, and *TNF* should benefit NAFLD/NASH patients. Elafibranor is a dual *PPAR α* and *PPAR δ* ligand that alleviates histological outcomes associated with NASH without worsening fibrosis.¹⁷²

Despite numerous GWAS studies, the identified variants can explain only a fraction of calculated NAFLD heritability.^{173–176} It may be easy to feel that the previous generation GWAS studies, mainly array-based, are not powerful enough to identify the minute fraction of the genetic variants responsible for NAFLD heritability. However, the more important cause could be that none of the GWAS studies probably considered gene-gene interaction and/or gene-environment interaction to predict the progression of NAFLD. Strengthening this idea, it was observed that the relative risk associated with the NAFLD progression is modified by obesity, which leads to inter-individual risk variability.¹⁷⁷ However, all individuals with this jeopardy do not develop the disease, signify-

ing that these inter-individual differences are products of interactions between genes and the environment.¹⁷⁸ It is also possible that various genetic factors also could work interactively. For example, *APOC3* and *PNPLA3* gene variants account for nearly 11% and 6.5% of NAFLD, respectively, while both account for 13.1% of the variance, suggesting the interactive effects of these two polymorphisms.¹³⁹ GWAS's vital contribution is identifying polygenic risk scores, which combine the effects of known genetic risk variants. These polygenic risk scores should help predict the patients at the highest risk of developing/progressing the disease.^{108,179} Although such a risk scoring assessment is highly statistically significant, this process is highly limited by their classification protocol and accuracy in prediction.¹⁰⁸

Future Perspectives

GWAS and Omics platforms have identified the gene variants that increase the risk of NAFLD and HCC. However, these variants are comparatively rare and cannot completely explain the increasing prevalence of NAFLD worldwide. It is important to note that diet, lifestyle, and metabolic co-morbidities are the most critical environmental risks.^{17,180} Lifestyle modifications, essentially involving dietary energy restriction and regular physical activity, are fundamental to the treatment of NAFLD.^{181,182} American Association for the Study of Liver Diseases recommends at least 3–5% total weight reduction to ameliorate steatosis, while 7–10% weight loss improves most pathophysiological features.¹⁸³ The European Association for the Study of the Liver, European Association for the Study of Diabetes, and European Association for the Study of Obesity also advise 7–10% of total weight loss target.¹⁸⁴

The interaction between genes and nutrients is called *Nutrigenomics*,^{185,186} including 'nutrigenetics' and 'nutriepigenetics'. It gives insight into how nutrients influence the expression of a person's genetic makeup that swaps from healthiness to disease. *PNPLA3* is the most widely studied gene, which shows interaction with the diet patterns in NAFLD patients.¹⁸⁷ An Italian cohort study showed that high carbohydrate and sugar intake in *PNPLA3* homozygous mutated people, but not wild type group led to high TG accumulation.¹⁸⁸ *PNPLA3* is also influenced by dietary fatty acids, particularly n-6/n-3 polyunsaturated fatty acids.^{189,190} When challenged with a high-fat diet, the *TM6SF2* mutated allele carriers showed better fasting and postprandial lipid profiles. There was also a consequent reduction of circulating atherogenic lipoproteins in them.^{67,191} *MBOAT7* expression was shown to be also downregulated by diet-induced hyperinsulinemia.⁵⁰ Independent of other metabolic risk factors and dietary restrictions, whole-grain supplementation to the *GCKR* mutated allele carriers shows a reduction in fasting glucose levels associated with reduced insulin concentrations.¹⁹² In addition to these gene variants, hepatic transcription factors, which regulate the metabolism, are known to be perturbed in metabolic syndromes. These include cAMP-responsive element binding protein like 3 (*CREB3L3*), *PPAR α* , and forkhead box O1 (*FoxO1*), *etc.* Proteins like these can sense nutrient availability and thus can bridge the NAFLD genetics with the environment. *i.e.*, these transcription factors appear to be amenable to dietary and/or nutrient-based therapies, being potential targets of nutritional therapy.¹⁹³ No drugs are certified to treat NAFLD,¹⁹⁴ thus, a better understanding of *Nutrigenomics* and the development of genome-based dietary guidelines and personalized nutrition therapy for NAFL disease management will help develop effective treatments.

Acknowledgments

The authors would like to acknowledge the contributions of the scientists for their excellent research studies that we have cited in this review and apologize that, due to space constraints, we had to omit some of the studies. We also thank Dr. Ravikanth Vishnubhotla, Group Leader-Genetics, Asian Healthcare Foundation/AIG Hospitals, for the critical reading of the manuscript and valuable suggestions.

Funding

This work is supported by ICMR-NIN intramural grants (20-FT04, 21-FS01, and 21-FS03).

Conflict of interest

SKM has been an editorial board member of *Gene Expression* since October 2022. The authors declare that they have no other conflict of interests.

Author contributions

SKM searched the literature, collected the data, and wrote and finalized the manuscript. YKG reviewed the literature and improved the manuscript. PNR and BRS are clinical doctors who reviewed the literature and helped ensure that all critical data about this topic is represented in this review. All authors had reviewed, finalized, and approved the manuscript for publication.

References

- [1] Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, *et al*. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nat Rev Gastroenterol Hepatol* 2018;15(1):11–20. doi:10.1038/nrgastro.2017.109, PMID:28930295.
- [2] Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. *J Hepatol* 2017;67(4):862–873. doi:10.1016/j.jhep.2017.06.003, PMID:28642059.
- [3] Rinella M, Charlton M. The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health. *Hepatology* 2016;64(1):19–22. doi:10.1002/hep.28524, PMID:26926530.
- [4] Kuchay MS, Martínez-Montoro JJ, Choudhary NS, Fernández-García JC, Ramos-Molina B. Non-alcoholic fatty liver disease in lean and non-obese individuals: Current and future challenges. *Biomedicine* 2021;9(10):1346. doi:10.3390/biomedicine9101346, PMID:34680463.
- [5] Grundy SM, Hansen B, Smith SC Jr, Cleeman JI, Kahn RA, American Heart Association, National Heart, Lung, and Blood Institute, American Diabetes Association. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management. *Arterioscler Thromb Vasc Biol* 2004;24(2):e19–e24. doi:10.1161/01.ATV.0000112379.88385.67, PMID:14766740.
- [6] Donnelly KL, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005;115(5):1343–1351. doi:10.1172/jci23621, PMID:15864352.
- [7] Arner P, Langin D. Lipolysis in lipid turnover, cancer cachexia, and obesity-induced insulin resistance. *Trends Endocrinol Metab* 2014;25(5):255–262. doi:10.1016/j.tem.2014.03.002, PMID:24731595.
- [8] Boden G, Shulman GI. Free fatty acids in obesity and type 2 diabetes: defining their role in the development of insulin resistance and beta-cell dysfunction. *Eur J Clin Invest* 2002;32(Suppl 3):14–23. doi:10.1046/j.1365-2362.32.s3.3.x, PMID:12028371.
- [9] Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015;13(4):643–654.e1–9. doi:10.1016/j.cgh.2014.04.014, PMID:24768810.
- [10] Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol* 2019;16(7):411–428. doi:10.1038/s41575-019-0145-7, PMID:31028350.
- [11] Martin K, Hatab A, Athwal VS, Jokl E, Piper Hanley K. Genetic Contribution to Non-alcoholic Fatty Liver Disease and Prognostic Implications. *Curr Diab Rep* 2021;21(3):8. doi:10.1007/s11892-021-01377-5, PMID:33544287.
- [12] Friedman SL, Neuschwander-Tetri BA, Rinella M, Sanyal AJ. Mechanisms of NAFLD development and therapeutic strategies. *Nat Med* 2018;24(7):908–922. doi:10.1038/s41591-018-0104-9, PMID:29967350.
- [13] Yamaguchi K, Yang L, McCall S, Huang J, Yu XX, Pandey SK, *et al*. Inhibiting triglyceride synthesis improves hepatic steatosis but exacerbates liver damage and fibrosis in obese mice with nonalcoholic steatohepatitis. *Hepatology* 2007;45(6):1366–1374. doi:10.1002/hep.21655, PMID:17476695.
- [14] Marra F, Svegliati-Baroni G. Lipotoxicity and the gut-liver axis in NASH pathogenesis. *J Hepatol* 2018;68(2):280–295. doi:10.1016/j.jhep.2017.11.014, PMID:29154964.
- [15] Machado MV, Diehl AM. Pathogenesis of Nonalcoholic Steatohepatitis. *Gastroenterology* 2016;150(8):1769–1777. doi:10.1053/j.gastro.2016.02.066, PMID:26928243.
- [16] Friedman SL. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008;134(6):1655–1669. doi:10.1053/j.gastro.2008.03.003, PMID:18471545.
- [17] Dongiovanni P, Valenti L. A nutrigenomic approach to non-alcoholic fatty liver disease. *Int J Mol Sci* 2017;18(7):1534. doi:10.3390/ijms18071534, PMID:28714900.
- [18] Younossi ZM. Non-alcoholic fatty liver disease - A global public health perspective. *J Hepatol* 2019;70(3):531–544. doi:10.1016/j.jhep.2018.10.033, PMID:30414863.
- [19] Mungamuri SK, Nagasuryaprasad K. Chapter 11. Epigenetic mechanisms of hepatocellular carcinoma progression: Potential therapeutic opportunities. In: Agrawala PK, Rana P (eds). *Epigenetics and Metabolomics*. New York: Academic Press; 2021:279–296.
- [20] Vachher M, Bansal S, Kumar B, Yadav S, Burman A. Deciphering the role of aberrant DNA methylation in NAFLD and NASH. *Heliyon* 2022;8(10):e11119. doi:10.1016/j.heliyon.2022.e11119, PMID:36299516.
- [21] Guha S, Sesili S, Mir IH, Thirunavukkarasu C. Epigenetics and mitochondrial dysfunction insights into the impact of the progression of non-alcoholic fatty liver disease. *Cell Biochem Funct* 2023;41(1):4–19. doi:10.1002/cbf.3763, PMID:36330539.
- [22] Zhu X, Xia M, Gao X. Update on genetics and epigenetics in metabolic associated fatty liver disease. *Ther Adv Endocrinol Metab* 2022;13:20420188221132138. doi:10.1177/20420188221132138, PMID:36325500.
- [23] Mungamuri SK, Vijayarath K. Role of the Gut Microbiome in Nonalcoholic Fatty Liver Disease Progression. *Crit Rev Oncog* 2020;25(1):57–70. doi:10.1615/CritRevOncog.2020035667, PMID:32865911.
- [24] Mungamuri SK, Sinha SN, Javvadi Y. Understanding the alterations in lipid metabolism in NAFLD progression: Current trends and future directions. *Crit Rev Oncog* 2021;26(1):35–49. doi:10.1615/CritRevOncog.2020035839, PMID:33641283.
- [25] Musso G, Gambino R, De Micheli F, Durazzo M, Pagano G, Cassader M. Adiponectin gene polymorphisms modulate acute adiponectin response to dietary fat: Possible pathogenetic role in NASH. *Hepatology* 2008;47(4):1167–1177. doi:10.1002/hep.22142, PMID:18311774.
- [26] Dixon JB, Bhathal PS, Jonsson JR, Dixon AF, Powell EE, O'Brien PE. Pro-fibrotic polymorphisms predictive of advanced liver fibrosis in the severely obese. *J Hepatol* 2003;39(6):967–971. doi:10.1016/s0168-8278(03)00459-8, PMID:14642613.
- [27] Yoneda M, Hotta K, Nozaki Y, Endo H, Uchiyama T, Mawatari H, *et al*. Association between angiotensin II type 1 receptor polymorphisms and the occurrence of nonalcoholic fatty liver disease. *Liver Int* 2009;29(7):1078–1085. doi:10.1111/j.1478-3231.2009.01988.x, PMID:19302184.

- [28] Petersen KF, Dufour S, Harii A, Nelson-Williams C, Foo JN, Zhang XM, *et al.* Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010;362(12):1082–1089. doi:10.1056/NEJMoa0907295, PMID:20335584.
- [29] Sazci A, Akpınar G, Aygun C, Ergül E, Sentürk O, Hulagu S. Association of apolipoprotein E polymorphisms in patients with non-alcoholic steatohepatitis. *Dig Dis Sci* 2008;53(12):3218–3224. doi:10.1007/s10620-008-0271-5, PMID:18465245.
- [30] Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A Polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999;20(5):976–983. doi:10.1165/ajrcmb.20.5.3494, PMID:10226067.
- [31] Daly AK. Relevance of CYP2E1 to non-alcoholic fatty liver disease. *Subcell Biochem* 2013;67:165–175. doi:10.1007/978-94-007-5881-0_5, PMID:23400921.
- [32] Dongiovanni P, Valenti L, Rametta R, Daly AK, Nobili V, Mozzi E, *et al.* Genetic variants regulating insulin receptor signalling are associated with the severity of liver damage in patients with non-alcoholic fatty liver disease. *Gut* 2010;59(2):267–273. doi:10.1136/gut.2009.190801, PMID:20176643.
- [33] George DK, Goldwurm S, MacDonald GA, Cowley LL, Walker NI, Ward PJ, *et al.* Increased hepatic iron concentration in nonalcoholic steatohepatitis is associated with increased fibrosis. *Gastroenterology* 1998;114(2):311–318. doi:10.1016/s0016-5085(98)70482-2, PMID:9453491.
- [34] Nelson JE, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, *et al.* HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. *Hepatology* 2007;46(3):723–729. doi:10.1002/hep.21742, PMID:17680648.
- [35] Hashemi M, Eskandari-Nasab E, Fazaeli A, Bahari A, Hashemzahi NA, Shafieipour S, *et al.* Association of genetic polymorphisms of glutathione-S-transferase genes (GSTT1, GSTM1, and GSTP1) and susceptibility to nonalcoholic fatty liver disease in Zahedan, Southeast Iran. *DNA Cell Biol* 2012;31(5):672–677. doi:10.1089/dna.2011.1343, PMID:22011249.
- [36] Qi Q, Wu Y, Li H, Loos RJ, Hu FB, Sun L, *et al.* Association of GCKR rs780094, alone or in combination with GCK rs1799884, with type 2 diabetes and related traits in a Han Chinese population. *Diabetologia* 2009;52(5):834–843. doi:10.1007/s00125-009-1290-2, PMID:19241058.
- [37] Yang Z, Wen J, Tao X, Lu B, Du Y, Wang M, *et al.* Genetic variation in the GCKR gene is associated with non-alcoholic fatty liver disease in Chinese people. *Mol Biol Rep* 2011;38(2):1145–1150. doi:10.1007/s11033-010-0212-1, PMID:20625834.
- [38] Kitamoto A, Kitamoto T, Nakamura T, Ogawa Y, Yoneda M, Hyogo H, *et al.* Association of polymorphisms in GCKR and TRIB1 with nonalcoholic fatty liver disease and metabolic syndrome traits. *Endocr J* 2014;61(7):683–689. doi:10.1507/endocrj.ej14-0052, PMID:24785259.
- [39] Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, *et al.* A protein-truncating HSD17B13 variant and protection from chronic liver disease. *N Engl J Med* 2018;378(12):1096–1106. doi:10.1056/NEJMoa1712191, PMID:29562163.
- [40] Gellert-Kristensen H, Nordestgaard BG, Tybjaerg-Hansen A, Stender S. High Risk of Fatty Liver Disease Amplifies the Alanine Transaminase-Lowering Effect of a HSD17B13 Variant. *Hepatology* 2020;71(1):56–66. doi:10.1002/hep.30799, PMID:31155741.
- [41] Kallwitz E, Tayo BO, Kuniholm MH, Daviglus M, Zeng D, Isasi CR, *et al.* Association of HSD17B13 rs72613567:TA with non-alcoholic fatty liver disease in Hispanics/Latinos. *Liver Int* 2020;40(4):889–893. doi:10.1111/liv.14387, PMID:31965669.
- [42] Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6(10):772–783. doi:10.1038/nri1937, PMID:16998510.
- [43] Carulli L, Canedi I, Rondinella S, Lombardini S, Ganazzi D, Fargion S, *et al.* Genetic polymorphisms in non-alcoholic fatty liver disease: interleukin-6-174G/C polymorphism is associated with non-alcoholic steatohepatitis. *Dig Liver Dis* 2009;41(11):823–828. doi:10.1016/j.dld.2009.03.005, PMID:19403348.
- [44] Miele L, Beale G, Patman G, Nobili V, Leathart J, Grieco A, *et al.* The Kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. *Gastroenterology* 2008;135(1):282–291.e1. doi:10.1053/j.gastro.2008.04.004, PMID:18515091.
- [45] Lu H, Sun J, Sun L, Shu X, Xu Y, Xie D. Polymorphism of human leptin receptor gene is associated with type 2 diabetic patients complicated with non-alcoholic fatty liver disease in China. *J Gastroenterol Hepatol* 2009;24(2):228–232. doi:10.1111/j.1440-1746.2008.05544.x, PMID:18713300.
- [46] Namikawa C, Shu-Ping Z, Vyselaar JR, Nozaki Y, Nemoto Y, Ono M, *et al.* Polymorphisms of microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in non-alcoholic steatohepatitis. *J Hepatol* 2004;40(5):781–786. doi:10.1016/j.jhep.2004.01.028, PMID:15094225.
- [47] Bernard S, Touzet S, Personne I, Lapras V, Bondon PJ, Berthezène F, *et al.* Association between microsomal triglyceride transfer protein gene polymorphism and the biological features of liver steatosis in patients with type II diabetes. *Diabetologia* 2000;43(8):995–999. doi:10.1007/s001250051481, PMID:10990076.
- [48] Mancina RM, Dongiovanni P, Petta S, Pingitore P, Meroni M, Rametta R, *et al.* The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. *Gastroenterology* 2016;150(5):1219–1230.e6. doi:10.1053/j.gastro.2016.01.032, PMID:26850495.
- [49] Donati B, Dongiovanni P, Romeo S, Meroni M, McCain M, Miele L, *et al.* MBOAT7 rs641738 variant and hepatocellular carcinoma in non-cirrhotic individuals. *Sci Rep* 2017;7(1):4492. doi:10.1038/s41598-017-04991-0, PMID:28674415.
- [50] Meroni M, Dongiovanni P, Longo M, Carli F, Baselli G, Rametta R, *et al.* Mboat7 down-regulation by hyper-insulinemia induces fat accumulation in hepatocytes. *EBioMedicine* 2020;52:102658. doi:10.1016/j.ebiom.2020.102658, PMID:32058943.
- [51] Gordon A, Yang R, Yerges-Armstrong LM, Ryan KA, Spiliotes E, Borecki IB, *et al.* Genetic variation at NCAN locus is associated with inflammation and fibrosis in non-alcoholic fatty liver disease in morbid obesity. *Hum Hered* 2013;75(1):34–43. doi:10.1159/000346195, PMID:23594525.
- [52] Dong H, Wang J, Li C, Hirose A, Nozaki Y, Takahashi M, *et al.* The phosphatidylethanolamine N-methyltransferase gene V175M single nucleotide polymorphism confers the susceptibility to NASH in Japanese population. *J Hepatol* 2007;46(5):915–920. doi:10.1016/j.jhep.2006.12.012, PMID:17391797.
- [53] Abby Phillips C, Agarwal M, Phadke N, Rajesh S, Padsalgi G, Ahamed R, *et al.* A novel Phosphoinositide-3-kinase adapter protein 1 gene missense mutation in familial cirrhosis. *J Clin Exp Hepatol* 2019;9(5):652–656. doi:10.1016/j.jceh.2019.02.002, PMID:31695254.
- [54] Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, *et al.* Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2008;40(12):1461–1465. doi:10.1038/ng.257, PMID:18820647.
- [55] Kotronen A, Johansson LE, Johansson LM, Roos C, Westerbacka J, Hamsten A, *et al.* A common variant in PNPLA3, which encodes adiponutrin, is associated with liver fat content in humans. *Diabetologia* 2009;52(6):1056–1060. doi:10.1007/s00125-009-1285-z, PMID:19224197.
- [56] Kollerits B, Coassin S, Kiechl S, Hunt SC, Paulweber B, Willeit J, *et al.* A common variant in the adiponutrin gene influences liver enzyme values. *J Med Genet* 2010;47(2):116–119. doi:10.1136/jmg.2009.066597, PMID:19542081.
- [57] Yoneda M, Hotta K, Nozaki Y, Endo H, Uchiyama T, Mawatari HI, *et al.* Association between PPARGC1A polymorphisms and the occurrence of nonalcoholic fatty liver disease (NAFLD). *BMC Gastroenterol* 2008;8:27. doi:10.1186/1471-230X-8-27, PMID:18588668.
- [58] Chen S, Li Y, Li S, Yu C. A Val227Ala substitution in the peroxisome proliferator activated receptor alpha (PPAR alpha) gene associated with non-alcoholic fatty liver disease and decreased waist circumference and waist-to-hip ratio. *J Gastroenterol Hepatol* 2008;23(9):1415–1418. doi:10.1111/j.1440-1746.2008.05523.x, PMID:18853997.
- [59] Hui Y, Yu-Yuan L, Yu-Qiang N, Wei-Hong S, Yan-Lei D, Xiao-Bo L, *et al.* Effect of peroxisome proliferator-activated receptors-gamma and co-activator-1alpha genetic polymorphisms on plasma adiponectin levels and susceptibility of non-alcoholic fatty liver disease in Chinese people. *Liver Int* 2008;28(3):385–392. doi:10.1111/j.1478-3231.2007.01623.x, PMID:17999673.

- [60] Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. The nuclear receptor PXR gene variants are associated with liver injury in nonalcoholic fatty liver disease. *Pharmacogenet Genomics* 2010;20(1):1–8. doi:10.1097/FPC.0b013e328333a1dd, PMID:19940802.
- [61] Al-Serri A, Anstee QM, Valenti L, Nobili V, Leathart JB, Dongiovanni P, *et al*. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. *J Hepatol* 2012;56(2):448–454. doi:10.1016/j.jhep.2011.05.029, PMID:21756849.
- [62] Sookoian S, Castaño G, Gianotti TF, Gemma C, Rosselli MS, Pirola CJ. Genetic variants in STAT3 are associated with nonalcoholic fatty liver disease. *Cytokine* 2008;44(1):201–206. doi:10.1016/j.cyt.2008.08.001, PMID:18789715.
- [63] Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, *et al*. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006;38(3):320–323. doi:10.1038/ng1732, PMID:16415884.
- [64] Valenti L, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A, Taioli E, *et al*. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. *Gastroenterology* 2002;122(2):274–280. doi:10.1053/gast.2002.31065, PMID:11832442.
- [65] Kozlitina J, Smagris E, Stender S, Nordestgaard BG, Zhou HH, Tybjaerg-Hansen A, *et al*. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet* 2014;46(4):352–356. doi:10.1038/ng.2901, PMID:24531328.
- [66] Zhou Y, Llauradó G, Orešić M, Hyötyläinen T, Orho-Melander M, Yki-Järvinen H. Circulating triacylglycerol signatures and insulin sensitivity in NAFLD associated with the E167K variant in TM6SF2. *J Hepatol* 2015;62(3):657–663. doi:10.1016/j.jhep.2014.10.010, PMID:25457209.
- [67] O'Hare EA, Yang R, Yerges-Armstrong LM, Sreenivasan U, McFarland R, Leitch CC, *et al*. TM6SF2 rs58542926 impacts lipid processing in liver and small intestine. *Hepatology* 2017;65(5):1526–1542. doi:10.1002/hep.29021, PMID:28027591.
- [68] Yan X, Xu L, Qi J, Liang X, Ma C, Guo C, *et al*. sTRAIL levels and TRAIL gene polymorphisms in Chinese patients with fatty liver disease. *Immunogenetics* 2009;61(8):551–556. doi:10.1007/s00251-009-0389-4, PMID:19629467.
- [69] Ishizuka Y, Nakayama K, Ogawa A, Makishima S, Boonvisut S, Hirao A, *et al*. TRIB1 downregulates hepatic lipogenesis and glycogenesis via multiple molecular interactions. *J Mol Endocrinol* 2014;52(2):145–158. doi:10.1530/JME-13-0243, PMID:24389359.
- [70] Liu Q, Xue F, Meng J, Liu SS, Chen LZ, Gao H, *et al*. TRIB1 rs17321515 and rs2954029 gene polymorphisms increase the risk of non-alcoholic fatty liver disease in Chinese Han population. *Lipids Health Dis* 2019;18(1):61. doi:10.1186/s12944-019-1001-z, PMID:30851741.
- [71] Lin YC, Chang PF, Hu FC, Chang MH, Ni YH. Variants in the UGT1A1 gene and the risk of pediatric nonalcoholic fatty liver disease. *Pediatrics* 2009;124(6):e1221–e1227. doi:10.1542/peds.2008-3087, PMID:19948621.
- [72] Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* 2018;67(1):123–133. doi:10.1002/hep.29466, PMID:28802062.
- [73] Taliento AE, Dallio M, Federico A, Prati D, Valenti L. Novel Insights into the Genetic Landscape of Nonalcoholic Fatty Liver Disease. *Int J Environ Res Public Health* 2019;16(15):2755. doi:10.3390/ijerph16152755, PMID:31375010.
- [74] Jenkins CM, Mancuso DJ, Yan W, Sims HF, Gibson B, Gross RW. Identification, cloning, expression, and purification of three novel human calcium-independent phospholipase A2 family members possessing triacylglycerol lipase and acylglycerol transacylase activities. *J Biol Chem* 2004;279(47):48968–48975. doi:10.1074/jbc.M407841200, PMID:15364929.
- [75] Bruschi FV, Claudel T, Tardelli M, Caligiuri A, Stulnig TM, Marra F, *et al*. The PNPLA3 I148M variant modulates the fibrogenic phenotype of human hepatic stellate cells. *Hepatology* 2017;65(6):1875–1890. doi:10.1002/hep.29041, PMID:28073161.
- [76] Pirazzi C, Valenti L, Motta BM, Pingitore P, Hedfalk K, Mancina RM, *et al*. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. *Hum Mol Genet* 2014;23(15):4077–4085. doi:10.1093/hmg/ddu121, PMID:24670599.
- [77] Pingitore P, Dongiovanni P, Motta BM, Meroni M, Lepore SM, Mancina RM, *et al*. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. *Hum Mol Genet* 2016;25(23):5212–5222. doi:10.1093/hmg/ddw341, PMID:27742777.
- [78] Mondul A, Mancina RM, Merlo A, Dongiovanni P, Rametta R, Montalcini T, *et al*. PNPLA3 I148M Variant influences circulating retinol in adults with nonalcoholic fatty liver disease or obesity. *J Nutr* 2015;145(8):1687–1691. doi:10.3945/jn.115.210633, PMID:26136587.
- [79] Tian C, Stokowski RP, Kershenovich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. *Nat Genet* 2010;42(1):21–23. doi:10.1038/ng.488, PMID:19946271.
- [80] Dongiovanni P, Romeo S, Valenti L. Hepatocellular carcinoma in non-alcoholic fatty liver: role of environmental and genetic factors. *World J Gastroenterol* 2014;20(36):12945–12955. doi:10.3748/wjg.v20.i36.12945, PMID:25278690.
- [81] Sookoian S, Castaño GO, Burgueño AL, Gianotti TF, Rosselli MS, Pirola CJ. A nonsynonymous gene variant in the adiponutrin gene is associated with nonalcoholic fatty liver disease severity. *J Lipid Res* 2009;50(10):2111–2116. doi:10.1194/jlr.P900013-JLR200, PMID:19738004.
- [82] Pingitore P, Pirazzi C, Mancina RM, Motta BM, Indiveri C, Pujia A, *et al*. Recombinant PNPLA3 protein shows triglyceride hydrolase activity and its I148M mutation results in loss of function. *Biochim Biophys Acta* 2014;1841(4):574–580. doi:10.1016/j.bbali.2013.12.006, PMID:24369119.
- [83] BasuRay S, Smagris E, Cohen JC, Hobbs HH. The PNPLA3 variant associated with fatty liver disease (I148M) accumulates on lipid droplets by evading ubiquitylation. *Hepatology* 2017;66(4):1111–1124. doi:10.1002/hep.29273, PMID:28520213.
- [84] Donati B, Motta BM, Pingitore P, Meroni M, Pietrelli A, Alisi A, *et al*. The rs2294918 E434K variant modulates patatin-like phospholipase domain-containing 3 expression and liver damage. *Hepatology* 2016;63(3):787–798. doi:10.1002/hep.28370, PMID:26605757.
- [85] Singal AG, Manjunath H, Yopp AC, Beg MS, Marrero JA, Gopal P, *et al*. The effect of PNPLA3 on fibrosis progression and development of hepatocellular carcinoma: a meta-analysis. *Am J Gastroenterol* 2014;109(3):325–334. doi:10.1038/ajg.2013.476, PMID:24445574.
- [86] Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. *Hepatology* 2011;53(6):1883–1894. doi:10.1002/hep.24283, PMID:21381068.
- [87] Liu YL, Patman GL, Leathart JB, Piguet AC, Burt AD, Dufour JF, *et al*. Carriage of the PNPLA3 rs738409 C > G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. *J Hepatol* 2014;61(1):75–81. doi:10.1016/j.jhep.2014.02.030, PMID:24607626.
- [88] Takeuchi Y, Ikeda F, Moritou Y, Hagihara H, Yasunaka T, Kuwaki K, *et al*. The impact of patatin-like phospholipase domain-containing protein 3 polymorphism on hepatocellular carcinoma prognosis. *J Gastroenterol* 2013;48(3):405–412. doi:10.1007/s00535-012-0647-3, PMID:22869157.
- [89] Valenti L, Motta BM, Soardo G, Iavarone M, Donati B, Sangiovanni A, *et al*. PNPLA3 I148M polymorphism, clinical presentation, and survival in patients with hepatocellular carcinoma. *PLoS One* 2013;8(10):e75982. doi:10.1371/journal.pone.0075982, PMID:24155878.
- [90] Huang Z, Guo X, Zhang G, Liang L, Nong B. Correlation between PNPLA3 rs738409 polymorphism and hepatocellular carcinoma: a meta-analysis of 10,330 subjects. *Int J Biol Markers* 2019;34(2):117–122. doi:10.1177/1724600818812471, PMID:30852978.
- [91] Valenti L, Dongiovanni P, Ginanni Corradini S, Burza MA, Romeo S. PNPLA3 I148M variant and hepatocellular carcinoma: a common genetic variant for a rare disease. *Dig Liver Dis* 2013;45(8):619–624. doi:10.1016/j.dld.2012.12.006, PMID:23333103.
- [92] Burza MA, Pirazzi C, Maglio C, Sjöholm K, Mancina RM, Svensson PA, *et al*. PNPLA3 I148M (rs738409) genetic variant is associated with hepatocellular carcinoma in obese individuals. *Dig Liver Dis* 2012;44(12):1037–1041. doi:10.1016/j.dld.2012.05.006, PMID:22704398.
- [93] Mahdessian H, Taxiarchis A, Popov S, Silveira A, Franco-Cereceda

- A, Hamsten A, *et al.* TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. *Proc Natl Acad Sci USA* 2014;111(24):8913–8918. doi:10.1073/pnas.1323785111, PMID:24927523.
- [94] Li TT, Li TH, Peng J, He B, Liu LS, Wei DH, *et al.* TM6SF2: A novel target for plasma lipid regulation. *Atherosclerosis* 2018;268:170–176. doi:10.1016/j.atherosclerosis.2017.11.033, PMID:29232562.
- [95] Dongiovanni P, Petta S, Maglio C, Fracanzani AL, Pipitone R, Mozzi E, *et al.* Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. *Hepatology* 2015;61(2):506–514. doi:10.1002/hep.27490, PMID:25251399.
- [96] Liu YL, Reeves HL, Burt AD, Tiniakos D, McPherson S, Leathart JB, *et al.* TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. *Nat Commun* 2014;5:4309. doi:10.1038/ncomms5309, PMID:24978903.
- [97] Wang X, Liu Z, Peng Z, Liu W. The TM6SF2 rs58542926 T allele is significantly associated with non-alcoholic fatty liver disease in Chinese. *J Hepatol* 2015;62(6):1438–1439. doi:10.1016/j.jhep.2015.01.040, PMID:25687425.
- [98] Krawczyk M, Rau M, Schattenberg JM, Bantel H, Pathil A, Demir M, *et al.* Combined effects of the PNPLA3 rs738409, TM6SF2 rs58542926, and MBOAT7 rs641738 variants on NAFLD severity: A multicenter biopsy-based study. *J Lipid Res* 2017;58(1):247–255. doi:10.1194/jlr.P067454, PMID:27836992.
- [99] Goffredo M, Caprio S, Feldstein AE, D'Adamo E, Shaw MM, Pierpont B, *et al.* Role of TM6SF2 rs58542926 in the pathogenesis of nonalcoholic pediatric fatty liver disease: A multiethnic study. *Hepatology* 2016;63(1):117–125. doi:10.1002/hep.28283, PMID:26457389.
- [100] Grandone A, Cozzolino D, Marzuillo P, Cirillo G, Di Sessa A, Ruggiero L, *et al.* TM6SF2 Glu167Lys polymorphism is associated with low levels of LDL-cholesterol and increased liver injury in obese children. *Pediatr Obes* 2016;11(2):115–119. doi:10.1111/ijpo.12032, PMID:25893821.
- [101] Palmer ND, Musani SK, Yerges-Armstrong LM, Feitosa MF, Bielak LF, Hernaez R, *et al.* Characterization of European ancestry nonalcoholic fatty liver disease-associated variants in individuals of African and Hispanic descent. *Hepatology* 2013;58(3):966–975. doi:10.1002/hep.26440, PMID:23564467.
- [102] Bale G, Steffie AU, Ravi Kanth VV, Rao PN, Sharma M, Sasikala M, *et al.* Regional differences in genetic susceptibility to non-alcoholic liver disease in two distinct Indian ethnicities. *World J Hepatol* 2017;9(26):1101–1107. doi:10.4254/wjh.v9.i26.1101, PMID:28989566.
- [103] Eslam M, Mangia A, Berg T, Chan HL, Irving WL, Dore GJ, *et al.* Diverse impacts of the rs58542926 E167K variant in TM6SF2 on viral and metabolic liver disease phenotypes. *Hepatology* 2016;64(1):34–46. doi:10.1002/hep.28475, PMID:26822322.
- [104] Holmen OL, Zhang H, Fan Y, Hovelson DH, Schmidt EM, Zhou W, *et al.* Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. *Nat Genet* 2014;46(4):345–351. doi:10.1038/ng.2926, PMID:24633158.
- [105] Li BT, Sun M, Li YF, Wang JQ, Zhou ZM, Song BL, *et al.* Disruption of the ERLIN-TM6SF2-APOB complex destabilizes APOB and contributes to non-alcoholic fatty liver disease. *PLoS Genet* 2020;16(8):e1008955. doi:10.1371/journal.pgen.1008955, PMID:32776921.
- [106] Newberry EP, Hall Z, Xie Y, Molitor EA, Bayguinov PO, Strout GW, *et al.* Liver-specific deletion of mouse Tm6sf2 promotes steatosis, fibrosis, and hepatocellular cancer. *Hepatology* 2021;74(3):1203–1219. doi:10.1002/hep.31771, PMID:33638902.
- [107] Kozlitina J. Genetic Risk Factors and Disease Modifiers of Nonalcoholic Steatohepatitis. *Gastroenterol Clin North Am* 2020;49(1):25–44. doi:10.1016/j.gtc.2019.09.001, PMID:32033763.
- [108] Kawaguchi T, Shima T, Mizuno M, Mitsumoto Y, Umemura A, Kanbara Y, *et al.* Risk estimation model for nonalcoholic fatty liver disease in the Japanese using multiple genetic markers. *PLoS One* 2018;13(1):e0185490. doi:10.1371/journal.pone.0185490, PMID:29385134.
- [109] Tan HL, Zain SM, Mohamed R, Rampal S, Chin KF, Basu RC, *et al.* Association of glucokinase regulatory gene polymorphisms with risk and severity of non-alcoholic fatty liver disease: An interaction study with adiponutrin gene. *J Gastroenterol* 2014;49(6):1056–1064. doi:10.1007/s00535-013-0850-x, PMID:23800943.
- [110] Hernaez R, McLean J, Lazo M, Brancati FL, Hirschhorn JN, Borecki IB, *et al.* Association between variants in or near PNPLA3, GCKR, and PPP1R3B with ultrasound-defined steatosis based on data from the third National Health and Nutrition Examination Survey. *Clin Gastroenterol Hepatol* 2013;11(9):1183–1190.e2. doi:10.1016/j.cgh.2013.02.011, PMID:23416328.
- [111] Di Costanzo A, Belardinelli F, Bailetti D, Sponziello M, D'Erasmo L, Polimeni L, *et al.* Evaluation of polygenic determinants of non-alcoholic fatty liver disease (NAFLD) by a candidate genes resequencing strategy. *Sci Rep* 2018;8(1):3702. doi:10.1038/s41598-018-21939-0, PMID:29487372.
- [112] Dongiovanni P, Romeo S, Valenti L. Genetic Factors in the Pathogenesis of Nonalcoholic Fatty Liver and Steatohepatitis. *Biomed Res Int* 2015;2015:460190. doi:10.1155/2015/460190, PMID:26273621.
- [113] Dongiovanni P, Anstee QM, Valenti L. Genetic predisposition in NAFLD and NASH: impact on severity of liver disease and response to treatment. *Curr Pharm Des* 2013;19(29):5219–5238. doi:10.2174/13816128113199990381, PMID:23394097.
- [114] Rees MG, Wincovitch S, Schultz J, Waterstradt R, Beer NL, Baltrusch S, *et al.* Cellular characterisation of the GCKR P446L variant associated with type 2 diabetes risk. *Diabetologia* 2012;55(1):114–122. doi:10.1007/s00125-011-2348-5, PMID:22038520.
- [115] Beer NL, Tribble ND, McCulloch LJ, Roos C, Johnson PR, Orho-Melander M, *et al.* The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. *Hum Mol Genet* 2009;18(21):4081–4088. doi:10.1093/hmg/ddp357, PMID:19643913.
- [116] Zain SM, Mohamed Z, Mohamed R. Common variant in the glucokinase regulatory gene rs780094 and risk of nonalcoholic fatty liver disease: A meta-analysis. *J Gastroenterol Hepatol* 2015;30(1):21–27. doi:10.1111/jgh.12714, PMID:25167786.
- [117] Luukkonen PK, Zhou Y, Sädevirta S, Leivonen M, Arola J, Orešič M, *et al.* Hepatic ceramides dissociate steatosis and insulin resistance in patients with non-alcoholic fatty liver disease. *J Hepatol* 2016;64(5):1167–1175. doi:10.1016/j.jhep.2016.01.002, PMID:26780287.
- [118] Eslam M, Hashem AM, Romero-Gomez M, Berg T, Dore GJ, Mangia A, *et al.* FibroGENE: A gene-based model for staging liver fibrosis. *J Hepatol* 2016;64(2):390–398. doi:10.1016/j.jhep.2015.11.008, PMID:26592354.
- [119] Viitasalo A, Eloranta AM, Atalay M, Romeo S, Pihlajamäki J, Lakka TA. Association of MBOAT7 gene variant with plasma ALT levels in children: the PANIC study. *Pediatr Res* 2016;80(5):651–655. doi:10.1038/pr.2016.139, PMID:27411039.
- [120] Barbosa AD, Siniossoglou S. Function of lipid droplet-organelle interactions in lipid homeostasis. *Biochim Biophys Acta Mol Cell Res* 2017;1864(9):1459–1468. doi:10.1016/j.bbamer.2017.04.001, PMID:28390906.
- [121] Jacquemyn J, Cascalho A, Goodchild RE. The ins and outs of endoplasmic reticulum-controlled lipid biosynthesis. *EMBO Rep* 2017;18(11):1905–1921. doi:10.15252/embr.201643426, PMID:29074503.
- [122] Wang B, Tontonoz P. Phospholipid Remodeling in Physiology and Disease. *Annu Rev Physiol* 2019;81:165–188. doi:10.1146/annurev-physiol-020518-114444, PMID:30379616.
- [123] Zarini S, Hankin JA, Murphy RC, Gijón MA. Lysophospholipid acyltransferases and eicosanoid biosynthesis in zebrafish myeloid cells. *Prostaglandins Other Lipid Mediat* 2014;113-115:52–61. doi:10.1016/j.prostaglandins.2014.08.003, PMID:25175316.
- [124] Buch S, Stickel F, Trépo E, Way M, Herrmann A, Nischalke HD, *et al.* A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet* 2015;47(12):1443–1448. doi:10.1038/ng.3417, PMID:26482880.
- [125] Luukkonen PK, Zhou Y, Hyötyläinen T, Leivonen M, Arola J, Orho-Melander M, *et al.* The MBOAT7 variant rs641738 alters hepatic phosphatidylinositols and increases severity of non-alcoholic fatty liver disease in humans. *J Hepatol* 2016;65(6):1263–1265. doi:10.1016/j.jhep.2016.07.045, PMID:27520876.
- [126] Di Sessa A, Umamo GR, Cirillo G, Del Prete A, Iacomino R, Marzuillo P, *et al.* The membrane-bound o-acyltransferase7 rs641738 variant in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2018;67(1):69–74. doi:10.1097/MPG.0000000000001979, PMID:29601441.

- [127] Umano GR, Caprio S, Di Sessa A, Chalasani N, Dykas DJ, Pierpont B, *et al*. The rs626283 variant in the MBOAT7 gene is associated with insulin resistance and fatty liver in caucasian obese youth. *Am J Gastroenterol* 2018;113(3):376–383. doi:10.1038/ajg.2018.1, PMID:29485130.
- [128] Moeller G, Adamski J. Integrated view on 17 β -hydroxysteroid dehydrogenases. *Mol Cell Endocrinol* 2009;301(1-2):7–19. doi:10.1016/j.mce.2008.10.040, PMID:19027824.
- [129] Liu S, Huang C, Li D, Ren W, Zhang H, Qi M, *et al*. Molecular cloning and expression analysis of a new gene for short-chain dehydrogenase/reductase 9. *Acta Biochim Pol* 2007;54(1):213–218. PMID:17311113.
- [130] Horiguchi Y, Araki OV, Motojima K. 17 β -Hydroxysteroid dehydrogenase type 13 is a liver-specific lipid droplet-associated protein. *Biochem Biophys Res Commun* 2008;370(2):235–238. doi:10.1016/j.bbrc.2008.03.063, PMID:18359291.
- [131] Carlsson B, Lindén D, Brölén G, Liljeblad M, Bjursell M, Romeo S, *et al*. Review article: the emerging role of genetics in precision medicine for patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2020;51(12):1305–1320. doi:10.1111/apt.15738, PMID:32383295.
- [132] Ma Y, Belyaeva OV, Brown PM, Fujita K, Valles K, Karki S, *et al*. 17-Beta hydroxysteroid dehydrogenase 13 is a hepatic retinol dehydrogenase associated with histological features of nonalcoholic fatty liver disease. *Hepatology* 2019;69(4):1504–1519. doi:10.1002/hep.30350, PMID:30415504.
- [133] Su W, Wang Y, Jia X, Wu W, Li L, Tian X, *et al*. Comparative proteomic study reveals 17 β -HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. *Proc Natl Acad Sci USA* 2014;111(31):11437–11442. doi:10.1073/pnas.1410741111, PMID:25028495.
- [134] Adam M, Heikelä H, Sobolewski C, Portius D, Mäki-Jouppila J, Mehmood A, *et al*. Hydroxysteroid (17 β) dehydrogenase 13 deficiency triggers hepatic steatosis and inflammation in mice. *FASEB J* 2018;32(6):3434–3447. doi:10.1096/fj.201700914R, PMID:29401633.
- [135] Liu WY, Eslam M, Zheng KI, Ma HL, Rios RS, Lv MZ, *et al*. Associations of hydroxysteroid 17-beta dehydrogenase 13 variants with liver histology in chinese patients with metabolic-associated fatty liver disease. *J Clin Transl Hepatol* 2021;9(2):194–202. doi:10.14218/JCTH.2020.00151, PMID:34007801.
- [136] Kozlitina J, Stender S, Hobbs HH, Cohen JC. HSD17B13 and Chronic Liver Disease in Blacks and Hispanics. *N Engl J Med* 2018;379(19):1876–1877. doi:10.1056/NEJMc1804027, PMID:30403941.
- [137] Musso G, Gambino R, Pacini G, Pagano G, Durazzo M, Cassader M. Transcription factor 7-like 2 polymorphism modulates glucose and lipid homeostasis, adipokine profile, and hepatocyte apoptosis in NASH. *Hepatology* 2009;49(2):426–435. doi:10.1002/hep.22659, PMID:19105201.
- [138] Shelness GS, Sellers JA. Very-low-density lipoprotein assembly and secretion. *Curr Opin Lipidol* 2001;12(2):151–157. doi:10.1097/00041433-200104000-00008, PMID:11264986.
- [139] Salamone F, Galvano F, Li Volti G. Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease. *N Engl J Med* 2010;363(2):194–195. PMID:20653078.
- [140] Wilfred de Alwis NM, Day CP. Genetics of alcoholic liver disease and nonalcoholic fatty liver disease. *Semin Liver Dis* 2007;27(1):44–54. doi:10.1055/s-2006-960170, PMID:17295176.
- [141] Tilg H, Diehl AM. Cytokines in alcoholic and nonalcoholic steatohepatitis. *N Engl J Med* 2000;343(20):1467–1476. doi:10.1056/NEJM200011163432007, PMID:11078773.
- [142] Syn WK, Choi SS, Diehl AM. Apoptosis and cytokines in non-alcoholic steatohepatitis. *Clin Liver Dis* 2009;13(4):565–580. doi:10.1016/j.cld.2009.07.003, PMID:19818305.
- [143] Diehl AM, Li ZP, Lin HZ, Yang SQ. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. *Gut* 2005;54(2):303–306. doi:10.1136/gut.2003.024935, PMID:15647199.
- [144] Hu ZW, Luo HB, Xu YM, Guo JW, Deng XL, Tong YW, *et al*. Tumor necrosis factor- α gene promoter polymorphisms in Chinese patients with nonalcoholic fatty liver diseases. *Acta Gastroenterol Belg* 2009;72(2):215–221. PMID:19637776.
- [145] Tokushige K, Takakura M, Tsuchiya-Matsushita N, Tani M, Hashimoto E, Shiratori K. Influence of TNF gene polymorphisms in Japanese patients with NASH and simple steatosis. *J Hepatol* 2007;46(6):1104–1110. doi:10.1016/j.jhep.2007.01.028, PMID:17395331.
- [146] Akula SM, Abrams SL, Steelman LS, Emma MR, Augello G, Cusimano A, *et al*. RAS/RAF/MEK/ERK, PI3K/PTEEN/AKT/mTORC1 and TP53 pathways and regulatory miRs as therapeutic targets in hepatocellular carcinoma. *Expert Opin Ther Targets* 2019;23(11):915–929. doi:10.1080/14728222.2019.1685501, PMID:31657972.
- [147] Fruman DA, Chiu H, Hopkins BD, Bagrodia S, Cantley LC, Abraham RT. The PI3K Pathway in Human Disease. *Cell* 2017;170(4):605–635. doi:10.1016/j.cell.2017.07.029, PMID:28802037.
- [148] Janku F, Yap TA, Meric-Bernstam F. Targeting the PI3K pathway in cancer: are we making headway? *Nat Rev Clin Oncol* 2018;15(5):273–291. doi:10.1038/nrclinonc.2018.28, PMID:29508857.
- [149] Pafili K, Roden M. Nonalcoholic fatty liver disease (NAFLD) from pathogenesis to treatment concepts in humans. *Mol Metab* 2021;50:101122. doi:10.1016/j.molmet.2020.101122, PMID:33220492.
- [150] Chakravarthy MV, Neuschwander-Tetri BA. The metabolic basis of nonalcoholic steatohepatitis. *Endocrinol Diabetes Metab* 2020;3(4):e00112. doi:10.1002/edm2.112, PMID:33102794.
- [151] Rauch U, Feng K, Zhou XH. Neurocan: a brain chondroitin sulfate proteoglycan. *Cell Mol Life Sci* 2001;58(12-13):1842–1856. doi:10.1007/PL00000822, PMID:11766883.
- [152] Nischalke HD, Lutz P, Krämer B, Söhne J, Müller T, Rosendahl J, *et al*. A common polymorphism in the NCAN gene is associated with hepatocellular carcinoma in alcoholic liver disease. *J Hepatol* 2014;61(5):1073–1079. doi:10.1016/j.jhep.2014.06.006, PMID:24946282.
- [153] Liu Q, Liu SS, Zhao ZZ, Zhao BT, Du SX, Jin WW, *et al*. TRIB1 rs17321515 gene polymorphism increases the risk of coronary heart disease in general population and non-alcoholic fatty liver disease patients in Chinese Han population. *Lipids Health Dis* 2019;18(1):165. doi:10.1186/s12944-019-1108-2, PMID:31470861.
- [154] Sveinbjornsson G, Ulfarsson MO, Thorolfsdottir RB, Jonsson BA, Einarsson E, Gunnlaugsson G, *et al*. Multicomics study of nonalcoholic fatty liver disease. *Nat Genet* 2022;54(11):1652–1663. doi:10.1038/s41588-022-01199-5, PMID:36280732.
- [155] Schweiger M, Lass A, Zimmermann R, Eichmann TO, Zechner R. Neutral lipid storage disease: genetic disorders caused by mutations in adipose triglyceride lipase/PNPLA2 or CGI-58/ABHD5. *Am J Physiol Endocrinol Metab* 2009;297(2):E289–E296. doi:10.1152/ajpendo.00099.2009, PMID:19401457.
- [156] Pulit SL, Stoneman C, Morris AP, Wood AR, Glastonbury CA, Tyrrell J, *et al*. Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Hum Mol Genet* 2019;28(1):166–174. doi:10.1093/hmg/ddy327, PMID:30239722.
- [157] Richardson TG, Sanderson E, Palmer TM, Ala-Korpela M, Ference BA, Davey Smith G, *et al*. Evaluating the relationship between circulating lipoprotein lipids and apolipoproteins with risk of coronary heart disease: A multivariable Mendelian randomisation analysis. *PLoS Med* 2020;17(3):e1003062. doi:10.1371/journal.pmed.1003062, PMID:32203549.
- [158] Emdin CA, Haas M, Ajmera V, Simon TG, Homburger J, Neben C, *et al*. Association of genetic variation with cirrhosis: A multi-trait genome-wide association and gene-environment interaction study. *Gastroenterology* 2021;160(5):1620–1633.e13. doi:10.1053/j.gastro.2020.12.011, PMID:33310085.
- [159] Polz E, Kostner GM. The binding of beta 2-glycoprotein-I to human serum lipoproteins: distribution among density fractions. *FEBS Lett* 1979;102(1):183–186. doi:10.1016/0014-5793(79)80955-2, PMID:222615.
- [160] Battle A, Brown CD, Engelhardt BE, Montgomery SB. Genetic effects on gene expression across human tissues. *Nature* 2017;550(7675):204–213. doi:10.1038/nature24277, PMID:29022597.
- [161] Jamialahmadi O, Mancina RM, Ciociola E, Tavaglione F, Luukkonen PK, Baselli G, *et al*. Exome-wide association study on alanine aminotransferase identifies sequence variants in the GPAM and APOE associated with fatty liver disease. *Gastroenterology* 2021;160(5):1634–1646.e7. doi:10.1053/j.gastro.2020.12.023, PMID:33347879.
- [162] Hoffmann TJ, Theusch E, Haldar T, Ranatunga DK, Jorgenson E, Medina MW, *et al*. A large electronic-health-record-based genome-wide study of serum lipids. *Nat Genet* 2018;50(3):401–413. doi:10.1038/s41588-018-0064-5, PMID:29507422.
- [163] Hoekstra M, Chen HY, Rong J, Dufresne L, Yao J, Guo X, *et al*. Genome-Wide Association Study Highlights *APOH* as a Novel Locus for

- Lipoprotein(a) Levels-Brief Report. *Arterioscler Thromb Vasc Biol* 2021; 41(1):458–464. doi:10.1161/ATVBAHA.120.314965, PMID:33115273.
- [164] Oshima A, Kyle JW, Miller RD, Hoffmann JW, Powell PP, Grubb JH, *et al.* Cloning, sequencing, and expression of cDNA for human beta-glucuronidase. *Proc Natl Acad Sci USA* 1987;84(3):685–9. doi:10.1073/pnas.84.3.685, PMID:3468507.
- [165] Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S, Fox L, *et al.* ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. *Mol Psychiatry* 2012;17(4):445–450. doi:10.1038/mp.2011.124, PMID:21968928.
- [166] Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, *et al.* A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13(4):399–408. doi:10.1038/ng0896-399, PMID:8696333.
- [167] Yoshida A, Lieberman J, Gaidulis L, Ewing C. Molecular abnormality of human alpha1-antitrypsin variant (Pi-ZZ) associated with plasma activity deficiency. *Proc Natl Acad Sci U S A* 1976;73(4):1324–1328. doi:10.1073/pnas.73.4.1324, PMID:1083527.
- [168] Lewis CM, Vassos E. Polygenic risk scores: from research tools to clinical instruments. *Genome Med* 2020;12(1):44. doi:10.1186/s13073-020-00742-5, PMID:32423490.
- [169] Sharma D, Mandal P. NAFLD: genetics and its clinical implications. *Clin Res Hepatol Gastroenterol* 2022;46(9):102003. doi:10.1016/j.clinre.2022.102003, PMID:35963605.
- [170] Meroni M, Longo M, Tria G, Dongiovanni P. Genetics Is of the Essence to Face NAFLD. *Biomedicines* 2021;9(10):1359. doi:10.3390/biomedicines9101359, PMID:34680476.
- [171] Eslam M, George J. Genetic contributions to NAFLD: leveraging shared genetics to uncover systems biology. *Nat Rev Gastroenterol Hepatol* 2020;17(1):40–52. doi:10.1038/s41575-019-0212-0, PMID:31641249.
- [172] Ratzliff V, Harrison SA, Francque S, Bedossa P, Leheret P, Serfaty L, *et al.* Elafibranor, an agonist of the peroxisome proliferator-activated receptor- α and - δ , induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. *Gastroenterology* 2016;150(5):1147–1159.e5. doi:10.1053/j.gastro.2016.01.038, PMID:26874076.
- [173] Brouwers MC, van Greevenbroek MM, Cantor RM. Heritability of nonalcoholic fatty liver disease. *Gastroenterology* 2009;137(4):1536. doi:10.1053/j.gastro.2009.03.065, PMID:19717129.
- [174] Loomba R, Schork N, Chen CH, Bettencourt R, Bhatt A, Ang B, *et al.* Heritability of hepatic fibrosis and steatosis based on a prospective twin study. *Gastroenterology* 2015;149(7):1784–1793. doi:10.1053/j.gastro.2015.08.011, PMID:26299412.
- [175] Speliotes EK, Yerges-Armstrong LM, Wu J, Hernaez R, Kim LJ, Palmer CD, *et al.* Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. *PLoS Genet* 2011;7(3):e1001324. doi:10.1371/journal.pgen.1001324, PMID:21423719.
- [176] Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ, *et al.* Finding the missing heritability of complex diseases. *Nature* 2009; 461(7265):747–753. doi:10.1038/nature08494, PMID:19812666.
- [177] Stender S, Kozlitina J, Nordestgaard BG, Tybjaerg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. *Nat Genet* 2017;49(6):842–847. doi:10.1038/ng.3855, PMID:28436986.
- [178] Panera N, Barbaro B, Della Corte C, Mosca A, Nobili V, Alisi A. A review of the pathogenic and therapeutic role of nutrition in pediatric nonalcoholic fatty liver disease. *Nutr Res* 2018;58:1–16. doi:10.1016/j.nutres.2018.05.002, PMID:30340810.
- [179] León-Mimila P, Vega-Badillo J, Gutiérrez-Vidal R, Villamil-Ramírez H, Villareal-Molina T, Larrieta-Carrasco E, *et al.* A genetic risk score is associated with hepatic triglyceride content and non-alcoholic steatohepatitis in Mexicans with morbid obesity. *Exp Mol Pathol* 2015;98(2):178–183. doi:10.1016/j.yexmp.2015.01.012, PMID:25597287.
- [180] Dongiovanni P, Valenti L. Genetics of nonalcoholic fatty liver disease. *Metabolism* 2016;65(8):1026–1037. doi:10.1016/j.metabol.2015.08.018, PMID:26409295.
- [181] Parra-Vargas M, Rodríguez-Echevarría R, Jimenez-Chillaron JC. Nutritional Approaches for the Management of Nonalcoholic Fatty Liver Disease: An Evidence-Based Review. *Nutrients* 2020;12(12):E3860. doi:10.3390/nu12123860, PMID:33348700.
- [182] Lombardi R, Iuculano F, Pallini G, Fargion S, Fracanzani AL. Nutrients, Genetic Factors, and Their Interaction in Non-Alcoholic Fatty Liver Disease and Cardiovascular Disease. *Int J Mol Sci* 2020;21(22):8761. doi:10.3390/ijms21228761, PMID:33228237.
- [183] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, *et al.* The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology* 2018;67(1):328–357. doi:10.1002/hep.29367, PMID:28714183.
- [184] European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol* 2016;64(6):1388–1402. doi:10.1016/j.jhep.2015.11.004, PMID:27062661.
- [185] Hesketh J. Personalised nutrition: how far has nutrigenomics progressed? *Eur J Clin Nutr* 2013;67(5):430–435. doi:10.1038/ejcn.2012.145, PMID:23093344.
- [186] Stover PJ, Caudill MA. Genetic and epigenetic contributions to human nutrition and health: managing genome-diet interactions. *J Am Diet Assoc* 2008;108(9):1480–1487. doi:10.1016/j.jada.2008.06.430, PMID:18755320.
- [187] Davis JN, Lê KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, *et al.* Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *Am J Clin Nutr* 2010;92(6):1522–1527. doi:10.3945/ajcn.2010.30185, PMID:20962157.
- [188] Nobili V, Liccardo D, Bedogni G, Salvadori G, Gnani D, Bersani I, *et al.* Influence of dietary pattern, physical activity, and PNPLA3 on steatosis severity in at-risk adolescents. *Genes Nutr* 2014;9(3):392. doi:10.1007/s12263-014-0392-8, PMID:24627307.
- [189] Santoro N, Savoye M, Kim G, Marotto K, Shaw MM, Pierpont B, *et al.* Hepatic fat accumulation is modulated by the interaction between the rs738409 variant in the PNPLA3 gene and the dietary omega6/omega3 PUFA intake. *PLoS One* 2012;7(5):e37827. doi:10.1371/journal.pone.0037827, PMID:22629460.
- [190] Scorletti E, West AL, Bhatia L, Hoile SP, McCormick KG, Burdge GC, *et al.* Treating liver fat and serum triglyceride levels in NAFLD, effects of PNPLA3 and TM6SF2 genotypes: Results from the WELCOME trial. *J Hepatol* 2015;63(6):1476–1483. doi:10.1016/j.jhep.2015.07.036, PMID:26272871.
- [191] Musso G, Cipolla U, Cassader M, Pinach S, Saba F, De Michieli F, *et al.* TM6SF2 rs58542926 variant affects postprandial lipoprotein metabolism and glucose homeostasis in NAFLD. *J Lipid Res* 2017;58(6):1221–1229. doi:10.1194/jlr.M075028, PMID:28242789.
- [192] Nettleton JA, McKeown NM, Kanoni S, Lemaitre RN, Hivert MF, Ngwa J, *et al.* Interactions of dietary whole-grain intake with fasting glucose- and insulin-related genetic loci in individuals of European descent: a meta-analysis of 14 cohort studies. *Diabetes Care* 2010;33(12):2684–2691. doi:10.2337/dc10-1150, PMID:20693352.
- [193] Yang Z, Roth K, Agarwal M, Liu W, Petriello MC. The transcription factors CREBH, PPAR α , and FOXO1 as critical hepatic mediators of diet-induced metabolic dysregulation. *J Nutr Biochem* 2021;95:108633. doi:10.1016/j.jnutbio.2021.108633, PMID:33789150.
- [194] Perdomo CM, Frühbeck G, Escalada J. Impact of Nutritional Changes on Nonalcoholic Fatty Liver Disease. *Nutrients* 2019;11(3):677. doi:10.3390/nu11030677, PMID:30901929.