Review

MOLECULAR METABOLISM

Genetic and epigenetic factors determining NAFLD risk

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ABSTRACT

Background: Hepatic steatosis is a common chronic liver disease that can progress into more severe stages of NAFLD or promote the development of life-threatening secondary diseases for some of those affected. These include the liver itself (nonalcoholic steatohepatitis or NASH; fibrosis and cirrhosis, and hepatocellular carcinoma) or other organs such as the vessels and the heart (cardiovascular disease) or the islets of Langerhans (type 2 diabetes). In addition to elevated caloric intake and a sedentary lifestyle, genetic and epigenetic predisposition contribute to the development of NAFLD and the secondary diseases.

Scope of review: We present data from genome-wide association studies (GWAS) and functional studies in rodents which describe polymorphisms identified in genes relevant for the disease as well as changes caused by altered DNA methylation and gene regulation via specific miRNAs. The review also provides information on the current status of the use of genetic and epigenetic factors as risk markers.

Major conclusion: With our overview we provide an insight into the genetic and epigenetic landscape of NAFLD and argue about the applicability of currently defined risk scores for risk stratification and conclude that further efforts are needed to make the scores more usable and meaningful.
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Keywords NAFLD; Genetic variants; Epigenetics; Risk score

1. INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) represents a disease spectrum ranging from simple benign steatosis, which can develop further, to steatohepatitis characterized by inflammation and fibrosis. Disease progression eventually leads to cirrhosis or liver cell carcinoma. Epidemiological and clinical studies imply that NAFLD is strongly associated with other metabolic disorders such as obesity [1], insulin resistance [2], and type 2 diabetes (T2D) [3]. In fact, NAFLD is diagnosed in >70% of T2D patients [4]. Furthermore, NAFLD increases the risk of cardiovascular disease, including heart failure [5].

Genetic and environmental factors such as nutrition and physical activity interact and modulate individual risk of NAFLD development and the severity of progression. Several genetic variants associated with NAFLD and/or NASH were identified by genome-wide association studies (GWAS) and candidate gene approaches. Among these, a few genetic variants were proposed in a genetic risk score (GRS) for predicting individual risks [6] and might contribute to the early diagnosis and development of precision treatments. Genetics also helps to understand the NAFLD prevalence in different ethnic groups. For example, as shown by a meta-analysis, the prevalence in Hispanics is particularly high, whereas that in Blacks is the lowest [7]. This can partly be explained by the different occurrence of single nucleotide polymorphisms (SNPs) in risk variants in these cohorts.

In addition to genetic predisposition, epigenetic changes that occur in response to environmental factors such as nutrition contribute to disease risk. Epigenetic changes include modifications that alter gene expression and ultimately the phenotype. In various mouse [8–10] and human studies [11–13], epigenetic modifications have been associated with pathomechanisms of NAFLD. These include altered DNA methylation patterns [11], expression of miRNAs [14], and histone modifications [15]. Epigenetic alterations can be transferred to the next generation and thus transgenerationally modify disease risk of offspring [16]. Development of metabolic diseases later in life partly depends on the metabolic phenotype of the mother and the intrauterine environment [17–19]. Establishing an epigenetic profile that reflects disease status could improve individual NAFLD risk assessment. As epigenetic alterations are not only inheritable but also reversible, this could offer new approaches for individualized prevention and therapy. Our review presents major genetic and epigenetic alterations on the level of DNA methylation and miRNA changes that have been observed in relation to the risk of NAFLD and provides information on their potential for risk assessment.

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2. GENETIC CONTRIBUTION TO NAFLD

2.1. Frequent genetic variants that mediate NAFLD risk

A GWAS by Romeo et al. [20] was the first to identify the most prominent fatty liver gene patatin-like phospholipase domain-containing 3 (*PNPLA3*), also known as adiponutrin. The association of the SNP *rs738409* (C > G) in *PNPLA3* with NAFLD was replicated in several subsequent GWAS [21,22]. *PNPLA3*, which exhibits a 46% sequence homology with the lipase PNPLA2, also designated adipose triglyceride lipase (ATGL), acts as triglyceride lipase, exhibits acylglycerol transacylase activities, and appears to play a role in lipid remodeling of hepatic triglycerides [23,24]. The SNP *rs738409* causes the missense sequence variation I148M that disrupts the enzyme's phospholipase activity, thereby interfering with lipid catabolism (Figure 1). *PNPLA3*^{I148M} is associated with increased hepatic fat content, elevated liver enzymes, hepatic fibrosis, and cirrhosis [20,25,26]. Lipidomic analyses of five lipid fractions from liver tissue samples of control and *PNPLA3*^{I148M} carriers revealed unaltered levels of palmitic acid, oleic acid, and linoleic acid in the triglyceride fraction. However, the concentration of trans-palmitoleic acid was increased and that of stearic, arachidic, and lignoceric acid saturated fatty acid was decreased in this fraction. The fatty acid composition of the other lipid fractions (phospholipids, diacylglycerols, and cholesteryl esters) was not affected [27]. Similar effects were detected in mice over-expressing the *PNPLA3*^{I148M} variant in the liver [28]. The effect on hepatic fat accumulation was strongest in Hispanics, who also displayed the highest allele frequency (0.49) compared to that in European Americans (0.23) and African Americans (0.17) [20]. Therefore, the SNP *rs738409* may partly explain the variable prevalence of hepatic steatosis among different ethnic groups in the US population [7], with individuals of Hispanic descent displaying more NAFLD (45%) than those of European descent (33%), who have a higher prevalence of NAFLD than those of African descent (24%) [29].

Most interesting, the effect of *PNPLA3* was independent of insulin resistance and lipid concentration, because *PNPLA3*^{I148M} allele carriers had significantly higher levels of liver fat but no difference in glucose tolerance, C-reactive protein, lipids, and liver enzymes compared to controls [20,30]. The *PNPLA3*^{I148M} effect is modulated by dietary conditions in mice [11,31] and humans, as its expression is regulated by the transcription factors sterol regulatory binding protein 1c (SREBP1c) [32,33] and carbohydrate response element binding protein (ChREBP) [34]. High carbohydrate levels cause transcriptional upregulation of *Pnpla3* and indirect inhibition of protein degradation [33], whereas *Pnpla3* expression was reduced in mice by fasting [33,35]. In a human study, Davis et al. showed that in Hispanic children carrying the risk allele, the effect on hepatic fat storage was amplified by carbohydrate-rich diets. The authors proposed that specific dietary interventions based on genetic predisposition may lead to more effective therapeutic outcomes for fatty liver [36].

Although the importance of *PNPLA3* for NAFLD based on GWAS has been repeatedly confirmed, the underlying pathogenic mechanism is still not fully understood. As *PNPLA3* acts as a lipase intracellularly in

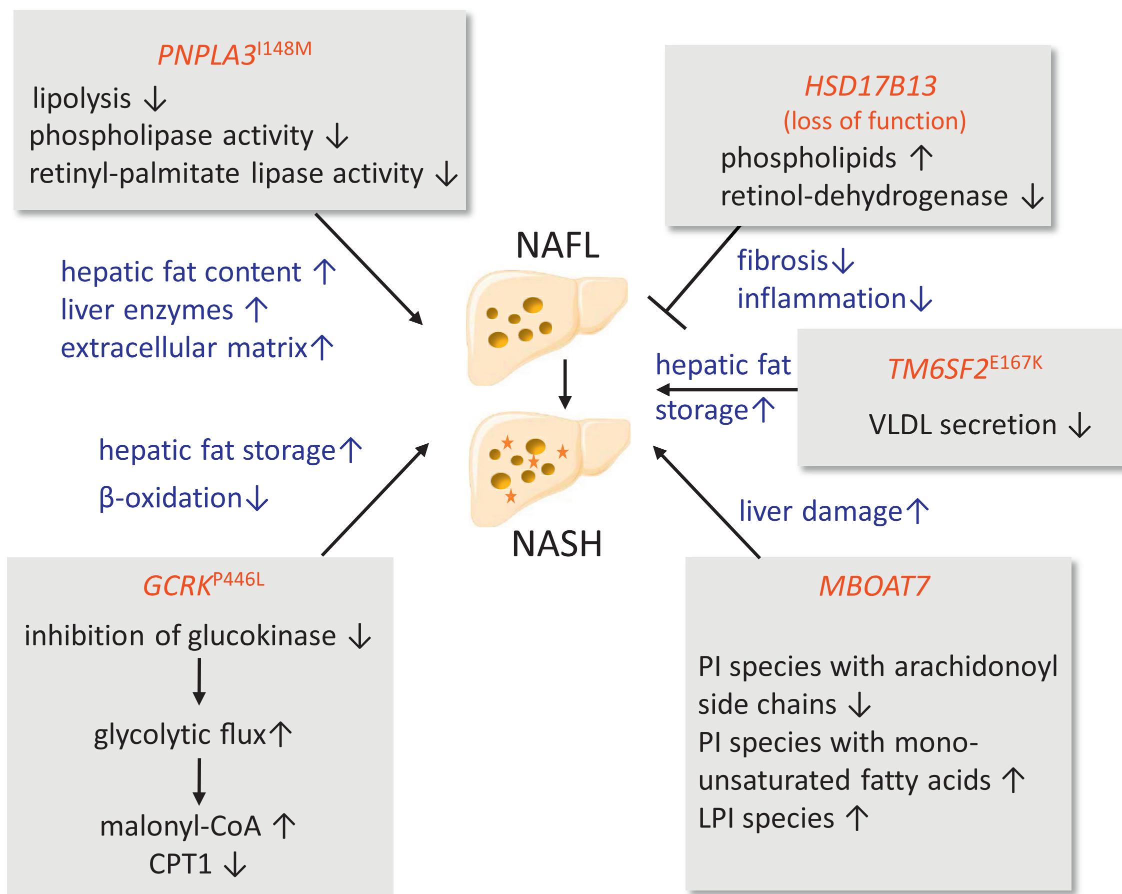

Figure 1: Frequent gene variants associated with NAFL and/or NASH and their major effects. CPT1, carnitine palmitoyl transferase-1; LPI, lysophosphatidylinositol; PI, phosphatidylinositol.

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Table 1 — Expression patterns (mRNA and/or protein) and function of genes associated with NAFLD risk. The information is based on the Human Protein Atlas (http://www.proteinatlas.org [145,146]; shown in italics) or the indicated references.

Gene	Tissue expression [145,146]	Liver cell type	Function
PNPLA3	*Liver and kidney*	Hepatocytes [43] Stellate cells [43]	Lipid droplet remodeling [40,42] Modulation of retinol production and release [43,147]
GCKR	*Liver, smooth muscle, and stomach*	Hepatocytes	Increasing glycolytic flux [51], regulation of *de novo* lipogenesis [48,148]
TM6SF2	*RNA enriched in intestine and liver* [57]	Hepatocytes [60]	VLDL secretion [53,57,58]
HSD17B13	*Ubiquitous (RNA enriched in liver)* and liver [64]	Hepatocytes [149]	Lipid droplet remodeling [63,64,149], involved in retinol metabolism [64]
MBOAT7	*Ubiquitous and RNA low tissue specificity* [69]	Hepatocytes, hepatic sinusoidal cells, and stellate cells [69]	Remodeling of phosphatidylinositol [69,150]
PPP1R3B	*Ubiquitous and low tissue specificity*	Hepatocytes [79]	Hepatic glycogen storage [77,79]
IRGM	Liver [82]	Hepatocytes [82]	Modulation of lipophagy [39] via interaction with lipase ATGL [82]
LPIN1	*Ubiquitous, adipose tissue,* and liver [84,151]	Hepatocytes [152]	Regulation of fatty acid metabolism [153,154]

ATGL, adipose triglyceride lipase; VLDL, very low-density lipoproteins.

hepatocytes and hepatic stellate cells (Table 1) [37], the lack of triglyceride hydrolase activity was postulated to cause hepatic triglyceride accumulation. However, the deletion of *Pnpla3* in mice did not cause hepatic steatosis [38]. Thus, not a loss of function of *PNPLA3* but probably a change in its function could lead to elevated hepatic fat storage induced by $PNLPA3^{I148M}$. Two independent studies demonstrated that overexpression of $PNPLA3^{I148M}$ in the liver of mice induced hepatic steatosis [31,39], whereas mice overexpressing the wild-type *PNPLA3* had normal hepatic triglyceride levels [40]. Basuray et al. showed in a series of *in vivo* experiments that the degradation of the $PNPLA3^{I148M}$ variant was prevented by inhibiting autophagy or proteasomal degradation. As a result, the protein accumulated in the lipid droplets, which limited their mobilization and promoted hepatic steatosis [41]. In another set of *in vitro* and *in vivo* studies, Wang et al. [42] demonstrated that *PNPLA3* recruitment to lipid droplets depends on cofactor comparative gene identification-58 (CGI-58), a cofactor for ATGL. Co-expression of ATGL and PNPLA3 (either wild-type or I148M) in hepatoma cells prevented the depletion of lipid droplets, suggesting the inhibition of ATGL-mediated lipid hydrolysis by PNPLA3 (Table 1). The authors hypothesized that $PNPLA3^{I148M}$ sequestrates CGI-58 to the surface of lipid droplets, which limits its availability for activation of ATGL [42]. In addition, $PNPLA3^{I148M}$ appears to interfere with retinol production and release of hepatic stellate cells by affecting retinyl-palmitate lipase activity (Figure 1 and Table 1), thereby promoting fibrosis development [43]. The lack of enzymatic activity leads to a reduced secretion of matrix-modulating enzymes, resulting in the deposition of extracellular matrix [44]. This potential mechanism is supported by data on NAFLD patients, who have reduced circulating retinol concentrations and concurrent intrahepatic retinol increases [43].

In contrast to *Pnpla3* knockout mice, which did not store ectopic fat in the liver, a shRNA-mediated reduction in *Pnpla3* expression in mice after development of high-fructose diet-induced steatosis resulted in decreased hepatic triglyceride levels, supporting the assumption that PNPLA3 accumulation *per se* causes steatosis [41]. Overall, different mouse studies showed that PNPLA3-associated hepatic steatosis requires the presence of the catalytically inactive protein and not simply the absence PNPLA3 activity [31,41]. The action of PNPLA3 is not restricted to the liver. It was recently shown that *PNPLA3* is also expressed in adipose tissue where the protein itself is more abundant than in the liver. $PNPLA3^{I148M}$ carriers exhibited increased levels of PUFA triglycerides than controls. However, adipocyte lipolysis was not altered [45].

A second robust NAFLD gene is glucokinase regulatory protein (*GCKR*), which is involved in the control of glucose metabolism by regulating hepatic glucose uptake and hepatic glucokinase activity. The intronic SNP rs780094 (G $>$ A), which is associated with hepatic lipid content, has been identified in various GWAS [22,46]. A meta-analysis by Zain et al. confirmed the association of rs780094 with increased NAFLD risk and demonstrated different allele frequencies for Africans (0.13), Europeans (0.41), and East Asians (0.48) [47]. In addition, association of rs780094 with other metabolic traits such as decreased fasting blood glucose [48] and decreased risk of T2D [49,50] were reported. The variant rs780094 is in strong linkage disequilibrium with the non-synonymous SNP rs1260326 (C $>$ T; P446L). $GCKR^{P446L}$ is supposed to exhibit a reduced ability to inhibit GCK (glucokinase) and thereby increasing glycolytic flux and glucose uptake by the liver (Figure 1 and Table 1). GCK serves as a metabolic switch that controls glucose metabolism. In the postprandial state when more glucose is taken up by the liver, GCK phosphorylates glucose to glucose-6-phosphate, which is converted into glycogen for storage. However, excess dietary glucose that cannot be stored as glycogen is converted into fat by *de novo* lipogenesis using acetyl-CoA that is generated from glycolysis-driven pyruvate and NADPH [51]. Fructose-6-phosphate (F6P) enhances the GCKR-mediated inhibition and this effect was shown to be significantly attenuated in the $GCKR^{P446L}$ variant, which indirectly enhances GCK activity and glycolysis. Consequently, the production of metabolites such as malonyl-CoA increases and elevates triglyceride storage in the liver theoretically via two mechanisms [48]. On the one hand, malonyl-CoA serves as a substrate for *de novo* lipogenesis; on the other hand, it inhibits the import of fatty acids into the mitochondria by blocking carnitine palmitoyl transferase-1 (CPT1), thus disrupting fatty acid oxidation (Figure 1).

Santoro et al. explored the combined effect of the two genetic risk variants $PNPLA3^{I148M}$ and $GCKR^{P446L}$. In a study cohort of 455 obese children and adolescents, an additive effect of both variants on liver fat content was reported. Furthermore, this additive effect explained approximately 32% of the liver fat variance in Caucasians, 39% in African Americans, and 15% in Hispanics [52].

In a human exome-wide association study [53], the rs58542926 (G $>$ A; E167K) variant transmembrane 6 superfamily member 2 (*TM6SF2*) was associated with increased hepatic triglyceride content and higher risk of advanced fibrosis in NAFLD patients [53—55], but paradoxically associated with a lower concentration of hepatic-derived triglyceride-rich lipoproteins [54,56]. Therefore, despite the increased risk of NAFLD, carriers of $TM6SF2^{E167K}$ have a lower risk of cardiovascular disease

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[54]. *TM6SF2^{E167K}* is the causal gene variant that explains the association of the *NCAN* locus with hepatic triglyceride content and lipid levels already described in a GWAS by Speliotes et al. [22]. TM6SF2 is a transmembrane protein located in the endoplasmic reticulum (ER) and the ER-Golgi intermediate compartment. *In vitro* silencing of *TM6FS2* in hepatocytes reduced the secretion of VLDL and thus promoted the retention of triglycerides [57] (Figure 1 and Table 1). *In vivo* knockdown of *Tm6sf2* or transient overexpression of human *TM6SF2* in mice altered serum lipids and hepatic fat content [56], which again illustrates that TM6SF2 is relevant for aberrant hepatic lipid storage. To ascertain the effect of TM6SF2 on hepatic lipids and subsequent NAFLD risk, Luukkonen et al. analyzed hepatic lipid profiles between carriers and non-carriers of the *TM6SF2^{E167K}* gene variant [58]. *TM6SF2^{E167K}* carriers were characterized by impaired incorporation of polyunsaturated fatty acids into hepatic triglycerides, phospholipids, and cholesterol esters. The deficiency of polyunsaturated phosphatidylcholines that are required for VLDL assembly was described to cause increased degradation of intrahepatic VLDL, thereby reducing their secretion [59].

The previously described observations were verified using an *in vitro* approach. Prill et al. generated and characterized a 3D spheroid model from primary human hepatocytes obtained from individual donors, either wild-type or heterozygous for the *TM6SF2^{E167K}* allele, and demonstrated that the genetic variant induced elevated fat storage in hepatocytes by reducing secretion of APOB particles. In addition, mRNA expression of genes related to cholesterol biosynthesis (*FDPS*, *HMGCS1*, *FDFT1*, *DHCR7*, and *SC5D*), *de novo* lipogenesis (*FASN* and *ACSS2*), phospholipid dephosphorylation (*PLPP3*), and gluconeogenesis (*FBP1*) was higher in hepatic *TM6SF2^{E167K}* spheroids than in those of wild-type donors [60].

A further gene variant that describes a link between hepatic phospholipids and the risk of advanced NAFLD is the splicing variant rs72613567 (T > TA) with an adenine insertion in *HSD17B1*3 that encodes for the hepatic lipid droplet protein hydroxysteroid 17-beta dehydrogenase 13. The *HSD17B13* rs72613567 variant leads to the synthesis of a truncated loss-of-function enzyme [61] that protects against advanced NAFLD, NASH, ballooning degeneration, lobular inflammation, and fibrosis [62] (Figure 1). Surprisingly, the gene variant does not influence the development of steatosis, as several studies showed no difference in the degree of steatosis between rs72613567 carriers and noncarriers; however, it decreases the risk of chronic liver damage in NAFLD patients [61–63]. Interestingly, the loss-of-function *HSD17B13* rs72613567 allele is sufficient to mitigate the risk of liver injury in *PNPLA3^{I148M}* allele carriers who are genetically predisposed to NAFLD. This effect was associated with a decrease in *PNPLA3* mRNA in an allele dose-dependent manner [61]. Ma et al. investigated two other SNPs of *HSD17B13*, rs683413 (T > G/C), which links with the splice variant rs72613567, and rs62305723 (G > A; P260S), which encodes an *HSD17B13^{P260S}* variant. Both were associated with increased steatosis but decreased ballooning and inflammation [64]. Discrepant results exist regarding the protein levels of *HSD17B13* in liver samples from NAFLD and controls. Ma et al. detected a higher expression of *HSD17B13* in NASH compared to controls, but with no differences between wild-type, rs683413, or rs72613567 allele carriers [64]. In contrast, Pirola et al. reported lower or absent *HSD17B13* levels in NAFLD patients hetero- or homozygous for rs72613567 in an allele-dependent manner [62]. Overexpression or deletion of *HSD17B13* in HepG2 cells did not affect lipid content, demonstrating an indirect function [64]. Luukkonen et al. suggested that the splicing variant rs72613567 might protect from progressive liver disease by increasing the synthesis and/or decreasing the degradation of phospholipids. Lipidomics revealed a general increase in hepatic phospholipids in rs72613567 carriers, and transcriptomics showed a downregulation of inflammation-related genes. These effects were accompanied by lower plasma concentrations of the proinflammatory cytokine interleukin-6 [63].

The family of 17β-hydroxysteroid dehydrogenases (HSD17Bs) consists of 15 members that are mainly involved in sex hormone metabolism. Some members also play key roles in cholesterol and fatty acid metabolism. The substrate and enzymatic function of HSD17B13 is not entirely known [65]. However, there is indication that similar to PNPLA3, it also acts as a retinyl-palmitate lipase, and the loss-of-function variant *HSD17B13* rs72613567 affects retinol metabolism (Figure 1). Ma et al. discovered a retinol-dehydrogenase enzymatic activity of HSD17B13 that requires its binding to lipid droplets (Table 1). The enzymatic activity to catalyze the oxidation of retinol is reduced or absent in gene variants mediating anti-fibrotic/anti-inflammatory effects [64]. Based on several studies, a number of proteins involved in retinol metabolism, including retinol-binding protein 4 (RBP4) and aldehyde dehydrogenase 1A1 (ALDH1A1), have also been implicated in metabolic diseases including NAFLD and NASH [66].

A recent study combining animal models and human data challenged the dogma that lobular inflammation precedes hepatic fibrosis by mechanistically linking membrane-bound O-acyltransferase domain-containing 7 (*MBOAT7*) to lipid-driven inflammation-independent development of fibrosis [67]. A genetic variant rs641738 (C > T) located near two genes encoding *MBOAT7* and the transmembrane channel-like 4 (*TMC4*) was first reported to increase the risk of alcoholic cirrhosis [68], but a subsequent candidate gene study also linked rs641738 to NAFLD and disease progression [69]. In particular, eQTL analysis and the characterization of *Mboat7* and *Tmc4* knockout mice demonstrated that *Mboat7* loss of function mediates the progression of NAFLD [70]. Interestingly, *MBOAT7* expression is reduced in livers of obese mice and humans, independent of the rs641738 risk allele [70]. In the Liver Biopsy Cross-Sectional Cohort of Individuals of European descent, the *MBOAT7* rs641738 SNP was associated with the spectrum of liver damage related to NAFLD (Figure 1), including a higher degree of steatosis, more severe necroinflammation, and more advanced fibrosis. MBOAT7 belongs to the family of lysophospholipid acyltransferases with a specificity for arachidonoyl-CoA (Table1) [71]. Multiple phosphatidylinositol species showed differences in the plasma of *MBOAT7* rs641738 allele carriers who also have lower hepatic MBOAT7 protein levels. Other lipid classes such as triglycerides, ceramides, or phospholipids were not affected by the genotype [69]. This again highlights the role of phospholipid remodeling in NAFLD pathogenesis. Mice with an hepatocyte-specific knockout of *Mboat7* had increased hepatic fibrosis on a NASH-inducing diet without induction of inflammation as shown by a decrease in monocytes and unchanged levels of inflammatory mediators [67]. Thus, fibrosis development might occur independent of the inflammatory state. Similarly, in those with a BMI ≤35 in a cross-sectional NAFLD liver biopsy cohort, the *MBOAT7* rs641738 allele was significantly associated with the presence of fibrosis in the absence of lobular inflammation. Helsley et al. also detected a general decrease in M2 macrophages in *Mboat7* knockout mice. However, the increase in M1 macrophages and higher hepatic expression of the pro-inflammatory markers *Tnfα* and *Il1b* are an indication of inflammation in this model of *Mboat7* deletion mediated by antisense nucleotides that was not restricted to the liver but also affected the fat tissue [70]. In accordance with Mancina et al. [69], remodeling of the lipidomic pattern was detected that was similar between humans carrying the rs641738 risk genotype and mice with

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hepatocyte-specific deletion of *Mboat7* [67]. In particular, phosphatidylinositol (PI) species with arachidonoyl side chains were reduced and PI species with monounsaturated fatty acids were increased. Lysophosphatidylinositol (LPI) species serving as MBOAT7 substrates were also elevated (Figure 1), which may cause the development of fibrosis, as plasma LPI levels are elevated in fibrosis patients. Furthermore, treating *Mboat7* loss-of-function mice with LPI resulted in the induction of pro-fibrotic genes. Based on these findings, the authors concluded that healthy subjects are protected from obesity-linked progression of NAFLD by the MBOAT7-mediated acylation of LPI lipids [70]. Thus, the risk genotype *MBOAT7* rs641738 appears to mediate its pro-fibrotic effect in patients via LPI remodeling without significant induction of liver inflammation.

Polymorphisms of two fatty liver genes (*TM6SF2* and *PNPLA3*) have been shown to associate with one specific diabetes cluster. Based on several pathophysiological parameters, patients with adult-onset diabetes are allocated to five clusters [72,73]. Patients in the severe autoimmune diabetes (SAID) cluster exhibit a T1D/LADA-like phenotype, severe insulin-deficient diabetes (SIDD) and severe insulin-resistant (SIRD) patients display the most severe T2D forms with a high risk of developing secondary complications [73], whereas mild age-related diabetes (MARD) and mild obese diabetes (MOD) patients exhibited only minor metabolic abnormalities. Among these groups, SIRD patients exhibit the highest hepatic fat content and the lowest whole-body insulin sensitivity and this cluster showed a significant association with the rs10401969 (T $>$ C) variant of *TM6SF2* [73]. Furthermore, patients in the SIRD cluster were shown to more frequently carry the risk variants rs738409 (CG and GG) of *PNPLA3* and exhibit higher circulating free fatty acid concentrations and a more pronounced adipose tissue insulin resistance than non-carriers [74].

2.2. Rare genetic determinants of NAFLD and NASH

In addition to the most reliable fatty liver genes *PNPLA3*, *TM6SF2*, *HSD17B13*, and *MBOAT7*, several other genetic determinants of NAFLD and NASH have been identified that appear to be specific for only one ethnic population or have been confirmed by few studies, presumably due to their small effect size. In this section, we will introduce some of these candidates.

The contribution of protein phosphatase 1 regulatory subunit 3B (*PPP1R3B*) to the genetic risk of NAFLD is rather controversial. The noncoding SNP rs4240624 (G $>$ A/C) near *PPP1R3B* that was associated with hepatic steatosis diagnosed by computed tomography but not histologically defined NAFLD was identified in the same GWAS that linked *PNPLA3* and *TM6SF2* (the *NCAN* locus) to NAFLD [22]. Interestingly, the risk allele was also associated with altered serum lipids, increased HDL and LDL cholesterol, and decreased fasting glucose. Histological assessment of hepatic steatosis in bariatric patients could not confirm the initial association of *PPP1R3B* rs4240624 with steatosis [75]. Speliotes et al. already questioned this association, which could instead be linked to hepatic glycogen storage (Table 1) than increased hepatic fat content [22]. Accordingly, PPP1R3B promotes hepatic glycogen storage by dephosphorylation and activation of glycogen synthase and decreases glycogen breakdown by inactivation of glycogen phosphorylase, which is the rate-limiting enzyme in glycogenolysis [76]. Thus, Stender et al. [77] and Seidelin et al. [78] attempted to clarify if the higher computed tomography attenuation associated with *PPP1R3B* rs4240624 is caused by differences in hepatic glycogen or hepatic triglyceride content. The minor allele was shown to promote hepatic glycogen synthesis in the postprandial state [78] and was linked to a mild form of liver glycogenosis leading to hepatic injury [77]. The SNP rs4841132 (A $>$ G) is in complete linkage disequilibrium with rs4240624 and is associated with increased hepatic X-ray attenuation and serum liver enzyme alanine aminotransaminase (ALT) but not with hepatic triglycerides [22]. Interestingly, rs4841132 was associated with an increased hepatic expression of *PPP1R3B* [77]. Experiments in mice confirmed this relationship, as *Ppp1r3b* knockout mice displayed lower hepatic glycogen [77,79] and liver-specific overexpression of PPP1R3B and increased glycogen content [77]; however, hepatic triglycerides were not altered in either model.

In a genome-wide approach conducted in obese children and adolescents from a predominantly Han Chinese population, Lin et al. detected a variant in the immunity-related GTPase M (*IRGM*) gene (rs10065172, C $>$ T) in addition to *PNPLA3*, *GCKR*, and *TM6SF2* polymorphisms associated with NAFLD detected by ultrasonography [39]. Similar results were reported in an Italian study showing that the risk allele of *IRGM* rs10065172 was significantly associated with elevated plasma aminotransferase levels and mild to severe steatosis in children. However, in adults, no link with the *IRGM* risk allele was observed in liver disease progression diagnosed by histological evaluation of ballooning, inflammation, and fibrosis [80]. The role of *IRGM* in the development of hepatosteatosis became questionable when another variant of *IRGM* rs13361189 (C $>$ T), which is in linkage disequilibrium with the SNP rs1006517, was analyzed in Framingham Heart Study participants who underwent computed tomography scans. No association of rs13361189 with NAFLD was established [81]. Nevertheless, there were indications that IRGM plays a role in the regulation of autophagy [39] and hepatic lipid storage. We have discovered that the mouse orthologues of *IRGM* are the immunity-related GTPases 2 and 4 (*Ifgga2* and *Ifgga4*), which are located in close proximity on mouse chromosome 18. Their expression was markedly reduced in mice with NAFLD, and accordingly, suppression of their expression in hepatocytes or mouse liver increased fat accumulation, whereas the overexpression of *Ifgga2* in hepatocytes decreased fat storage. IFGGA2 appears to induce lipophagy via interacting with the lipase ATGL and increasing the association of the autophagy protein LC3B with lipid droplets. Interestingly, we also showed that the human IRGM protein interacts with ATGL (Table 1) and that the expression of *IRGM* was significantly reduced in livers of NAFLD patients [82].

Similar to *Ifgga2* and *Ifgga4*, *Lpin1* (Lipin 1) mRNA levels are reduced in a rat model of NAFLD [83]. As *LPIN1* expression in the liver and adipose tissue is inversely correlated with adiposity and positively associated with insulin resistance, Valenti et al. evaluated the association of an *LPIN1* SNP (rs13412852, C $>$ T) with the susceptibility to and progression of NAFLD [84]. The authors conducted a study of Italian children and adults and tested the rs13412852 SNP that was earlier linked to lower body weight [85]. Only in children but not in adults was the homozygous rs13412852-T allele associated with protection from NAFLD. However, pediatric and adult patients homozygous for the minor allele of *LPIN1* exhibited a significantly reduced risk of histological fibrosis and less severe liver damage [84]. Lipin 1 plays a major role in adipose tissue and influences its development and function. Deletion of *Lpin1* in mice results in a marked reduction in adipose tissue depots (lipodystrophic phenotype) and insulin resistance, whereas the adipocyte-specific overexpression of *Lpin1* causes diet-induced obesity [86].

2.3. Genetic risk scores for predicting steatohepatitis

As approximately 10—30% of patients with a simple hepatosteatosis develop NASH [87,88] that is associated with liver-related morbidity and mortality, a specific focus has been placed on developing a genetic

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risk score that allows the prediction or early diagnosis when fibrosis is still at an early stage.

Nobili et al. developed a genetic risk score that in combination with clinical risk markers such as aminotransferases significantly predicts NASH in obese children and adolescents [89]. In 152 study participants with biopsy-proven NAFLD and increased liver enzyme, polymorphisms of *PNPLA3* rs738409 (C $>$ G), *SOD2* rs4880 (C $>$ T), *KLF6* rs3750861 (G $>$ A), and *LPIN1* rs13412852 (C $>$ T) were tested. Polymorphisms of *SOD2* and *KLF6* were included in the analysis because they were associated with progressed liver disease in pediatric and adult NAFLD patients, whereby *SOD2* is supposed to be involved in the induction of oxidative stress and *KLF6* in determining fibrogenesis [90,91]. The prediction of fibrosis based on the four genetic determinants was less accurate (ROC-AUC of 0.60) than by combining this information with three clinical risk factors (age, diastolic blood pressure, and AST [aspartate aminotransferase]; ROC-AUC of 0.80) [89]. Similarly, a NASH risk score was established in a Korean cohort. The scoring system (NASH *PNPLA3-TM6SF2* score, NASH-PT) was based on risk alleles of *PNPLA3* rs738409 (C $>$ G) and *TM6SF2* rs58542926 (G $>$ A), diabetes status, insulin resistance, and levels of AST and C-reactive protein. NASH-PT scores identified patients with NASH with a ROC-AUC between 0.787 and 0.859 [92]. In a cohort of 514 obese children and adolescents in which almost 70% of the participants were diagnosed with NAFLD by ultrasonography, Zusi et al. tested 11 genes and detected highly significant associations with risk alleles of *PNPLA3* rs738409 (C $>$ G), *TM6SF2* rs58542926 (G $>$ A), and *GCKR* rs1260326 (C $>$ T) and a weaker association with a polymorphism of *ELOVL2* rs2236212 (G $>$ C) with a higher risk of NAFLD [93].

Di Costanzo et al. conducted exon sequencing of fatty liver genes discovered by GWAS and determined a polygenic risk score for NAFLD by applying logistic regression analysis. The authors confirmed *PNPLA3* rs738409 (C $>$ G), *GCKR* rs1260326 (C $>$ T), *TM6SF2* rs58542926 (G $>$ A), and *MBOAT7* rs641738 (C $>$ T) as genetic contributors of hepatosteatosis, whereby the *PNPLA3* SNP exhibited the strongest association, followed by *GCKR*, *TM6SF2*, and *MBOAT7*. The probability of NAFLD was highest (5-fold), when a risk score of all four SNPs was used [6]. EASL-EASD-EASO Clinical Practice Guidelines [94] already suggest genotyping for *PNPLA3* rs738409 and *TM6SF2* rs58542926 to identify individuals with a higher risk of hepatic steatosis.

In summary, a genetic risk score should include SNPs with three to four genes that showed the strongest and most robust association with hepatosteatosis and hepatosteatitis. These are *PNPLA3*, *TM6SF2*, *GCKR* plus *MBOAT7*, *SOD2*, and *KLF6*. Risk scores that combine the detection of these genes' genotypes with clinical parameters might help clinicians to more effectively identify NAFLD patients at risk of NASH without taking liver biopsies.

3. ALTERED DNA METHYLATION AND MIRNA EXPRESSION IN NAFLD

In general, there is increasing interest in elucidating disease-relevant epigenetic alterations, as they also have potential for therapeutic approaches. Lifestyle changes such as calorie restriction [95] and exercise [96] as well as more invasive interventions such as bariatric surgery [97,98] have already proven to cause changes in DNA methylation that positively affect the metabolic status of obese and diabetic patients or mice. Whether this also applies to NAFLD is subject of current and future research. Much less is known about specific histone modifications that lead to NAFLD and liver fibrosis. A review by Moran-Savador and Mann [15] provided information on the modifications of the histone code in liver disease.

3.1. Alterations of DNA methylation linked to NAFLD

The causal relationship between differential DNA methylation and disease has been extensively studied for various types of cancer [99]. The amount of data on the effects of aberrant DNA methylation on the development of metabolic diseases such as NAFLD has also increased enormously over the past decade. The relationship of methylome-transcriptome was analyzed in a histologically characterized NAFLD cohort to investigate whether differences between mild and advanced NAFLD are detectable [100]. Overall, hypomethylation occurred in NAFLD compared to controls regardless of disease severity. Furthermore, the genes whose transcription correlated with DNA methylation status were different in mild and advanced NAFLD. In advanced NAFLD, genes involved in wound-healing responses such as fibrogenesis were hypomethylated and their expression was upregulated, which distinguished them from the mild form. Murphy et al. suggested that the data might help to establish non-invasive markers to identify NAFLD patients at a high risk of liver disease progression [100]. Other genes with an altered DNA methylation pattern were identified in liver biopsies from mild and severe NAFLD cohorts using a candidate gene approach analyzing pro- and anti-fibrogenic genes. Zeybel et al. detected a higher methylation of specific CpGs within *TGFβ1* and *PDGFα*, whereas specific CpGs exhibited a lower degree of methylation in the anti-fibrogenic *PPARα* and *PPARδ* genes in patients with mild fibrosis [101]. The same group later detected hypermethylation at the *PPARγ* promoter by analyzing plasma cell-free circulating DNA methylation [102].

In 2010, Sookoian et al. showed in a case-control study that epigenetic changes occur in hepatic insulin resistance in NAFLD patients [103]. Specifically, the decreased expression of *PGC-1α*, a key regulator of mitochondrial biogenesis and fatty acid oxidation in NAFLD, compared to controls was inversely correlated with its promotor methylation. Moreover, *PGC1-α* methylation positively correlated with peripheral insulin resistance and negatively correlated with mitochondrial biogenesis, two features that contribute to the pathogenesis of fatty liver. Further data describing a positive correlation between maternal BMI on methylation of the *PGC1-α* promoter in neonatal cord blood support the concept of intrauterine overnutrition and fetal epigenetic programming [104].

To investigate whether methylation changes can be detected even before disease onset, Kammel et al. conducted an experiment with the inbred mouse strain C57BL/6J, whereby a genetic effect on the phenotype could be precluded [9]. The experimental design allowed the identification of obesity-prone animals at a very young age when they were still in a metabolically stable state and did not show ectopic fat accumulation in the liver. As impaired hepatic insulin sensitivity contributes to the development of metabolic diseases, insulin growth factor 2 (IGF-2) and thus the IGF axis are crucial for regulating body weight. Disorders such as reduced plasma levels of IGF-1-binding protein 2 (IGFBP-2), which controls the bioavailability of IGF-1 and contributes to the development of obesity, have been observed in obese adults [105,106]. In young obesity-prone mice, *Igfbp2* was hypermethylated at specific CpG sites and transcriptionally repressed even before the development of fatty liver and impaired glucose homeostasis in adolescence [9]. The results were not restricted to the mouse model. Interestingly, hypermethylation occurred at CpG sites that are homologous to humans with NAFLD [11], and *IGFBP2* promoter hypermethylation correlates with type-2 diabetes risk [107]. IGFBP-2 levels are sensitive to weight changes as weight loss induced

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by bariatric surgery normalized IGFBP-2 levels and reduced liver fat content [108]. Whether this could partly be attributed to changes in methylation was not reported.

Hyperglycemia precedes ectopic fat accumulation in the liver, thus laying the grounds for further progression of fatty liver disease. Dipeptidyl peptidase 4 (DPP4), an exopeptidase that cleaves and inactivates numerous peptides including the incretin hormones glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP) [109], has been studied as a novel adipokine elevated in obesity [110]. Because of these facts, DPP4 inhibitors are widely used in clinical practice to improve glycemic control. *DPP4* expression was elevated in NAFLD livers compared to healthy controls. In addition, expression levels negatively correlated with HOMA-IR, suggesting an association with DPP4 and insulin resistance and further supporting the relationship for disease progression and poor glycemic control [111]. Our own studies support the role of DPP4 in hepatosteatosis as well as broaden the information on this enzyme. *In vitro* studies have shown that DPP4 circulating in the blood is secreted primarily from the liver, so DPP4 can be referred to as a hepatokine [8]. Furthermore, the expression of *Dpp4* in mice and humans negatively correlated with the methylation status, and similar to *Igfbp2*, these changes preceded the manifestation of the phenotype in mice [8]. A detailed analysis of human *DPP4* expression in the ABOS cohort in combination with histology showed the inverse correlation of *DPP4* expression and degree of steatosis, which was also valid for a second cohort (KOBS) comparing NASH patients and controls [8]. To determine whether the hepatic expression and methylation of *Dpp4* could be influenced by diet, we used the New Zealand obese (NZO) mouse model, which is known for its early onset of hyperglycemia and T2D that can be postponed by low-protein diets [112]. Indeed, methylation of the *Dpp4* gene was higher in mice fed a low-protein diet, which was associated with lower expression and reduced circulating DPP4 concentration. Furthermore, we reported a positive correlation of liver triglyceride content and DPP4 activity in the previously described setting [113].

Taking liver biopsies to reliably confirm NASH and measuring gene expression and the corresponding DNA methylation is highly invasive and poses risks to patients. Therefore, several groups are attempting to use less invasive procedures to screen for changes in DNA methylation in peripheral blood cells in epigenome-wide association studies (EWAS) to test whether specific changes allow a stratification of NAFLD patients with a higher risk of liver fibrosis. This approach with a total of more than 4,500 participants from four population-based cohort studies including European, Hispanic, and African participants was used to link elevated liver fat content measured by computed tomography or ultrasound imaging to altered DNA methylation levels. In the European participants, 22 CpGs were identified as associated with hepatic fat; of these, 19 CpGs were annotated to 18 unique genes such as *DHCR24*, *SLC43A1*, *CPT1A*, *SREBF1*, *SC4MOL*, and *SLC9A3R1*, which are involved in liver function. Epigenetic changes in *ABCG1* and *SREBF1* were linked to cholesterol biosynthesis. Thus, most affected CpGs were located in genes regulating key biological processes relevant to developing steatosis and explained approximately 10% of interindividual variations [114]. A smaller study including 18 histologically confirmed NAFLD and 17 NASH patients from a Han Chinese population identified 6 CpG sites located in the *ACSL4*, *CRLS1*, *CTP1A*, *SIGIRR*, *SSBP1*, and *ZNF622* genes, which are differentially methylated in peripheral blood leukocytes of patients with NASH compared with those exhibiting simple steatosis. However, only differences in DNA methylation of *ACSL4* were confirmed by pyrosequencing [115]. Thus, in particular for an early distinction between people at risk of simple hepatosteatosis and NASH will require further and larger studies to identify and verify robust epigenetic biomarkers.

3.2. Role of miRNAs in the development of NAFLD

Micro-RNAs (miRNAs) have been implicated in a number of diseases including metabolic diseases such as T2D and obesity [116]. Each miRNA acts post-transcriptionally to control the expression of multiple genes by translational repression or interference with RNA stability [117,118]. Thus, miRNAs are involved in regulating liver development, metabolic functions, and regeneration. Some alterations in intrahepatic miRNA networks have been associated with hepatosteatosis and NASH [119]. In addition, as miRNAs are also released into the circulation, specific miRNA signatures in blood can serve as noninvasive biomarkers for disease state and progression [14] and might perform similar or slightly superior compared to established biomarkers such as cytokeratin 18 (CK-18) or ALT and aspartate aminotransferase (AST). A review by Torres et al. provided a list of most miRNAs and their main targets identified in animal models, hepatocytes, human liver biopsies, and plasma [120]. We will focus on those candidates (miR-122, miR-33a/b, miR-34a, and miR-192) that are important for the pathogenesis of NAFLD.

MiR-122 stands out as it accounts for approximately 70% of all miRNAs expressed in the liver [121]. By targeting important transcription factors (for example, *HNF6*), miR-122 is implicated in liver development and physiology [122] and plays a fundamental role in lipid metabolism by targeting *ACC2* [123] and *SREBP* [124]. In liver biopsies of obese patients with or without NAFLD, a reduced miR-122 expression was shown to be associated with fatty liver due to decreased fatty acid metabolism and altered expression of the transcription factors *ChREBP*, *PPAR*γ, *PPAR*α, and *LXR*α [124]. Whereas in serum of NAFLD patients, miR-122 levels were higher than in healthy controls and further increased in the state of NASH [125]. In contrast to humans, who exhibit a reduced hepatic miR-122 expression under circumstances of liver disease, high-fat diet-fed mice in which miR-122 was transiently inhibited by an antisense approach were protected from hepatosteatosis. They displayed reduced plasma cholesterol levels, increased hepatic fatty acid oxidation, and decreased rates of fatty acids and cholesterol synthesis in the liver [123]. Conversely, whole-body and liver-specific miR-122 knockout mice showed the expected phenotype; they developed steatohepatitis, fibrosis, and hepatocellular carcinoma [10,126]. Interestingly, fibrosis appeared to be mediated via targeting *Klf6* [10], a known liver disease gene (see [89]).

MiR-192 is mainly expressed in the liver, especially in hepatocytes; lower amounts are detected in most other tissues [127]. Several members of the hepatocyte nuclear factor family play important roles in liver metabolism. One, HNF4α, regulates miR-122 and the expression of miR-192 (Figure 2). The deletion of this transcription factor in mice resulted in a marked reduction in miR-192 expression and a subsequent upregulation of miR-192 targets (activated leukocyte cell adhesion molecule [*Alcam*], epiregulin [*Ereg*], and moesin [*Msn*]) [128]. In contrast, TGF-β1 downregulates the expression of miR-192 by decreasing the binding of HNF family members to the promoter [129]. MiR-192 is specifically downregulated in hepatocytes upon liver injury in response to the induction of ischemic liver damage. As miR-192 knockdown experiments in Hepa1-6 cells resulted in an increased cell survival, it was speculated that the downregulation of miR-192 caused by liver damage might represent a protective mechanism against hepatocyte cell death. This effect appears to be mediated via the miR-192 target zinc finger E-box binding homeobox 2 (*Zeb2*), as the effect of miR-192 inhibition was reverted by co-transfecting *Zeb2*-siRNA

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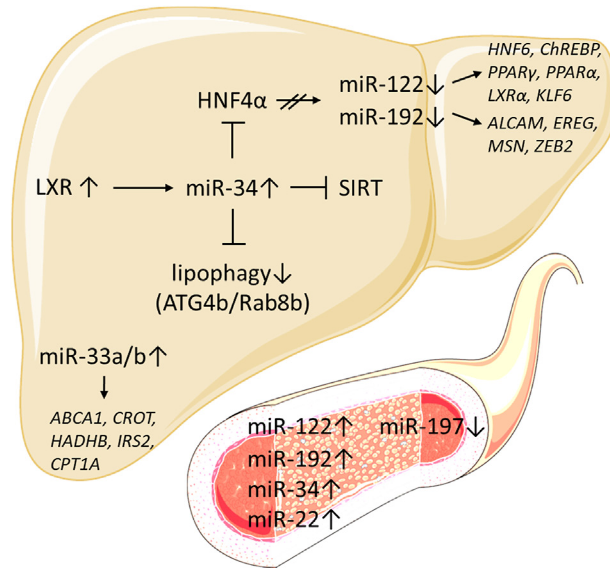

Figure 2: Summary of the most relevant miRNAs and their targets in the liver and those detected in the plasma of NAFL and NASH patients.

[127]. Zeb2 is best known for its impact on epithelial to mesenchymal transitions [130] and its role as an anti-apoptotic protein that was mainly investigated in cancer. Through its interaction with SMAD, it mediates survival of tumor cells, particularly by enhancing cell proliferation and promoting cell migration and invasion [131].

In isolated exosomes of diet-induced obese (DIO) mice, Castano et al. detected elevated levels of miR-122 and miR-192 compared to those of lean mice. In livers of DIO mice, the amount of both miRNAs was also increased, whereas opposite effects were observed in white adipose tissue, and miR-122 and miR-192 were decreased compared to lean mice [132]. Similar to mice, miR-122 and miR-192 were upregulated 2-fold in serum of people with simple steatosis compared to controls. The difference was higher (7.2- and 4.4-fold, respectively) between control and NASH [14].

MiR-33a and miR-33b are two additional important miRNAs involved in fatty liver disease, lipid metabolism, and energy homeostasis of mice and humans. They differ only in two nucleotides in their mature form but are identical in their seed sequence. Both miRNAs are located in introns of SREBP-2 and SREBP-1, respectively, and are co-transcribed with these genes [133]. SEREBP-1 regulates genes required for fatty acid biosynthesis and SREBP-2 is involved in cholesterol metabolism [134]. Silencing miR-33a in mice increased the hepatic expression of the cholesterol transporter ABCA1 and increased HDL synthesis and circulating HDL levels [135]. Interesting results were obtained by inhibiting both miR-33 forms in non-human primates (African green monkeys). Again, *ABCA1* was the most affected target and showed an elevated expression after miR-33 inhibition. Other upregulated genes were carnitine O-octanoyltransferase (*CROT*) and hydroxyacyl-coenzyme A-dehydrogenase (*HADHB*) encoding two enzymes involved in fatty acid oxidation, and insulin receptor substrate 2 (*IRS2*), which plays a key role in insulin signaling. When monkeys were fed a carbohydrate-enriched, moderate cholesterol diet, the authors detected an increased expression of carnitine palmitoyltransferase 1A (*CPT1A*), which is involved in fatty acid oxidation (Figure 2). Presumably via upregulating genes active in fatty acid synthesis (*SREBF1*, *FASN*, *ACLY*, and *ACACA*), miR-33 inhibition reduced the monkeys' plasma VLDL triglyceride levels. Therefore, it was speculated that therapeutic inhibition of miR-33 could be a sufficient strategy for treating dyslipidemias [133]. Koyama et al. discovered that, at least in mice, miR-33b exhibits a higher atherogenic potential than miR-33a. Characterizing specific mouse lines that either carry a knockout of miR-33a or miR-33b on the genetic background of *ApoE*-deficient mice, an appropriate model for atherosclerosis progression, revealed that those mice that lacked miR-33a but expressed miR-33b developed increased atherosclerotic plaques. These results correlated with the expression levels of both miR-33 in wild-type mice, wherein miR-33b was much higher in the liver than miR-33a [136]. Martino et al. detected elevated circulating miR-33a and miR-33b levels in familial hypercholesterolemic children compared to aged-matched controls. For both miRNAs, the authors observed a positive correlation with total cholesterol, LDL cholesterol, the LDL cholesterol/HDL cholesterol ratio, APOB, C-reactive protein, and glycemia [137].

MiR-34 plays a fundamental role in the dysregulation of lipid metabolism associated with NAFLD [120]. The expression of miR-34 was induced by liver X receptor-α (LXRα), which itself was 4 and 7 times higher in NAFLD and NASH than in controls, respectively [138]. A recent study provided evidence that miR-34 plays a role in regulating autophagy/lipophagy by targeting *ATG4B* and *Rab-8B*, which are responsible for autophagosome and autolysosome formation [139]. Thus, after activation of LXRα, lipids accumulate in the liver due to the induction of miR-34, which suppresses autophagy (Figure 2). Another important target of miR-34 is sirtuin 1 (*SIRT1*), which is downregulated in the liver of NAFLD patients. SIRT1 is a regulator of energy homeostasis, which itself activates *PPARα* and *LXR* and inhibits *PGC1-α* expression [140]. Accordingly, Ding et al. observed a suppression of *PPARα* and *SIRT1* in hepatocytes and livers in response to an upregulation of miR-34a, whereas silencing of miR-34 lead to an elevated expression of both regulatory proteins [141].

The impact of HNF4α on the liver function was mentioned previously; it exhibits a 2-fold higher expression in livers of NASH patients. Mice lacking this central transcription factor develop fatty liver. In humans it was shown that the expression of *HNF4α* is markedly reduced in NAFLD and NASH. This appears to be mediated by miR-34 binding to the 3′-UTR of *HNF4α*. The adenovirus-mediated application of miR-34a in mice reduced *HNF4α* expression by 40%, increased ectopic fat storage in the liver, and reduced plasma triglyceride concentrations. Opposite effects were observed in miR-34$^{-/-}$ mice, which showed a more than 3-fold increased HNF4α protein level [142].

3.3. MiRNAs detected in serum as diagnostic biomarkers

Several miRNAs are released by the cells packed in exosomes or circulating in a complex with argonaute2, which protects miRNAs from degradation via plasma RNases [116]. A review by Newman et al. summarized the miRNAs that were shown to exhibit significant alterations in liver disease and indicated the potential of their individual or combined levels as noninvasive diagnostic biomarkers [143]. Lopez-Rira et al. re-evaluated all 18 previously described serum miRNAs detected in clinical studies. Among these miR-122, -192, -34a, -16, and -21 were recognized in more than one study and Lopez-Rira et al. confirmed that they were affected in NAFLD. In addition, miR-27b, miR-22, miR-197, and miR-30c were significantly altered in more severe NAFLD patients. It was also confirmed that serum levels of miR-192, miR-34a, and miR-22 increased and miR-197 decreased in NASH patients (Figure 2). The authors also tested the diagnostic potential of miRNAs and observed similar classification performances of miRNAs vs conventional serum markers such as transaminases. However, when different ratios between induced and repressed miRNAs were considered (for miR-34a, miR-122, miR-192, miR-375, and miR-21), NASH

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prediction was better than using serum markers with ROC area under the curve values between 0.68 and 0.81 [144]. Pirola et al. previously reported that the detection of miR-122, miR-192, and miR-375 in serum has the potential to distinguish NASH from simple steatosis [14].

4. CONCLUSION

Predicting the individual risk of NAFLD and determining the probability of disease progression is the basis for further developing prevention and treatment strategies. Among other parameters, this requires knowledge of the genetic and epigenetic modifiers of NAFLD for genotype-guided risk stratification. In the near future, the current gold standard of liver biopsy could be circumvented by using the specified non-invasive risk scores that include plasma parameters, relevant clinical variables and a list of genetic and epigenetic changes. In the next step, risk scores need to be translated into clinical settings to benefit patients.

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CONFLICT OF INTEREST

None declared.

REFERENCES

[1] Loomis, K.A., Kabadi, S., Preiss, D., Hyde, C., Bonato, V., Louis, M.S., et al., 2016. Body mass index and risk of nonalcoholic fatty liver disease: two electronic health record prospective studies. Journal of Clinical Endocrinology & Metabolism 101(3):945—952. https://doi.org/10.1210/jc.2015-3444.

[2] Tilg, H., Moschen, A.R., Roden, M., 2017. NAFLD and diabetes mellitus. Nature Reviews Gastroenterology & Hepatology 14(1):32—42. https://doi.org/10.1038/nrgastro.2016.147.

[3] Mantovani, A., Byrne, C.D., Bonora, E., Targher, G., 2018. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care 41(2):372—382. https://doi.org/10.2337/dc17-1902.

[4] Williams, C.D., Stengel, J., Asike, M.I., Torres, D.M., Shaw, J., Contreras, M., et al., 2011. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. Gastroenterology 140(1):124—131. https://doi.org/10.1053/j.gastro.2010.09.038.

[5] Chiu, L.S., Pedley, A., Massaro, J.M., Benjamin, E.J., Mitchell, G.F., McManus, D.D., et al., 2020. The association of non-alcoholic fatty liver disease and cardiac structure and function—Framingham Heart Study. Liver International. https://doi.org/10.1111/liv.14600.

[6] Di Costanzo, A., Belardinilli, F., Bailetti, D., Sponziello, M., D'Erasmo, L., Polimeni, L., et al., 2018. Evaluation of polygenic determinants of non-alcoholic fatty liver disease (NAFLD) by a candidate genes resequencing strategy. Scientific Reports 8(1):1—10. https://doi.org/10.1038/s41598-018-21939-0.

[7] Rich, N.E., Oji, S., Mufti, A.R., Browning, J.D., Parikh, N.D., Odewole, M., et al., 2018. Racial and ethnic disparities in nonalcoholic fatty liver disease prevalence, severity, and outcomes in the United States: a systematic review and meta-analysis. Clinical Gastroenterology and Hepatology 16(2):198—210. https://doi.org/10.1016/j.cgh.2017.09.041 e2.

[8] Baumeier, C., Saussenthaler, S., Kammel, A., Jähnert, M., Schlüter, L., Hesse, D., et al., 2017. Hepatic DPP4 DNA methylation associates with fatty liver. Diabetes 66(1):25—35. https://doi.org/10.2337/db15-1716.

[9] Kammel, A., Saussenthaler, S., Jähnert, M., Jonas, W., Stirm, L., Hoeflich, A., et al., 2016. Early hypermethylation of hepatic Igfbp2 results in its reduced expression preceding fatty liver in mice. Human Molecular Genetics 25(12): 2588—2599. https://doi.org/10.1093/hmg/ddw121.

[10] Tsai, W.C., Hsu, S. Da., Hsu, C.S., Lai, T.C., Chen, S.J., Shen, R., et al., 2012. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. Journal of Clinical Investigation 122(8):2884—2897. https://doi.org/10.1172/JCI63455.

[11] Ahrens, M., Ammerpohl, O., Von Schönfels, W., Kolarova, J., Bens, S., Itzel, T., et al., 2013. DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. Cell Metabolism 18(2):296—302. https://doi.org/10.1016/j.cmet.2013.07.004.

[12] Pirola, C.J., Fernández Gianotti, T., Burgueño, A.L., Rey-Funes, M., Loidl, C.F., Mallardi, P., et al., 2013. Epigenetic modification of liver mitochondrial DNA is associated with histological severity of nonalcoholic fatty liver disease. Gut 62(9):1356—1363. https://doi.org/10.1136/gutjnl-2012-302962.

[13] Pogribny, I.P., Tryndyak, V.P., Bagnyukova, T.V., Melnyk, S., Montgomery, B., Ross, S.A., et al., 2009. Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. Journal of Hepatology 51(1):176—186. https://doi.org/10.1016/j.jhep.2009.03.021.

[14] Pirola, C.J., Gianotti, T.F., Castaño, G.O., Mallardi, P., Martino, J.S., Ledesma, M.M.G.L., et al., 2015. Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. Gut 64(5):800—812. https://doi.org/10.1136/gutjnl-2014-306996.

[15] Moran-Salvador, E., Mann, J., 2017. Epigenetics and liver fibrosis. Cellular and Molecular Gastroenterology and Hepatology 4(1):125—134. https://doi.org/10.1016/j.jcmgh.2017.04.007.

[16] de Jesus, D.F., Orime, K., Kaminska, D., Kimura, T., Basile, G., Wang, C.H., et al., 2020. Parental metabolic syndrome epigenetically reprograms offspring hepatic lipid metabolism in mice. Journal of Clinical Investigation 130(5):2391—2404. https://doi.org/10.1172/JCI127502.

[17] Desai, M., Jellyman, J.K., Ross, M.G., 2015. Epigenomics, gestational programming and risk of metabolic syndrome. International Journal of Obesity 39(4):633—641. https://doi.org/10.1038/ijo.2015.13.

[18] Dudley, K.J., Sloboda, D.M., Connor, K.L., Beltrand, J., Vickers, M.H., 2011. Offspring of mothers fed a high fat diet display hepatic cell cycle inhibition and associated changes in gene expression and DNA methylation. PloS One 6(7). https://doi.org/10.1371/journal.pone.0021662.

[19] Bruce, K.D., Cagampang, F.R., Argenton, M., Zhang, J., Ethirajan, P.L., Burdge, G.C., et al., 2009. Maternal high-fat feeding primes steatohepatitis in adult mice offspring, involving mitochondrial dysfunction and altered lipogenesis gene expression. Hepatology 50(6):1796—1808. https://doi.org/10.1002/hep.23205.

[20] Romeo, S., Kozlitina, J., Xing, C., Pertsemlidis, A., Cox, D., Pennacchio, L.A., et al., 2008. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nature Genetics 40(12):1461—1465. https://doi.org/10.1038/ng.257.

[21] Kitamoto, T., Kitamoto, A., Yoneda, M., Hyogo, H., Ochi, H., Nakamura, T., et al., 2013. Genome-wide scan revealed that polymorphisms in the PNPLA3, SAMM50, and PARVB genes are associated with development and progression of nonalcoholic fatty liver disease in Japan. Human Genetics 132(7): 783—792. https://doi.org/10.1007/s00439-013-1294-3.

[22] Speliotes, E.K., Yerges-Armstrong, L.M., Wu, J., Hernaez, R., Kim, L.J., Palmer, C.D., et al., 2011. Genome-wide association analysis identifies variants associated with nonalcoholic fatty liver disease that have distinct effects on metabolic traits. PLoS Genetics 7(3). https://doi.org/10.1371/journal.pgen.1001324.

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[23] Basuray, S., 2019. PNPLA3-I148M: a problem of plenty in non-alcoholic fatty liver disease. Adipocyte 8(1):201—208. https://doi.org/10.1080/21623945.2019.1607423.

[24] Pingitore, P., Romeo, S., 2019. The role of PNPLA3 in health and disease. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 900—906. https://doi.org/10.1016/j.bbalip.2018.06.018.

[25] Dongiovanni, P., Donati, B., Fares, R., Lombardi, R., Mancina, R.M., Romeo, S., et al., 2013. PNPLA3 I148M polymorphism and progressive liver disease. World Journal of Gastroenterology 19(41):6969—6978. https://doi.org/10.3748/wjg.v19.i41.6969.

[26] Sookoian, S., Pirola, C.J., 2011. 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 53(6):1883—1894. https://doi.org/10.1002/hep.24283.

[27] Peter, A., Kovarova, M., Nadalin, S., Cermak, T., Königsrainer, A., Machicao, F., et al., 2014. PNPLA3 variant I148M is associated with altered hepatic lipid composition in humans. Diabetologia 57(10):2103—2107. https://doi.org/10.1007/s00125-014-3310-0.

[28] Li, J.Z., Huang, Y., Karaman, R., Ivanova, P.T., Brown, H.A., Roddy, T., et al., 2012. Chronic overexpression of PNPLA3I148M in mouse liver causes hepatic steatosis. Journal of Clinical Investigation 122(11):4130—4144. https://doi.org/10.1172/JCI65179.

[29] Browning, J.D., Szczepaniak, L.S., Dobbins, R., Nuremberg, P., Horton, J.D., Cohen, J.C., et al., 2004. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 40(6):1387—1395. https://doi.org/10.1002/hep.20466.

[30] Kantartzis, K., Peter, A., Machicao, F., Machann, J., Wagner, S., Königsrainer, I., et al., 2009. Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. Diabetes 58(11):2616—2623. https://doi.org/10.2337/db09-0279.

[31] Smagris, E., Basuray, S., Li, J., Huang, Y., Lai, K.M.V., Gromada, J., et al., 2015. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. Hepatology 61(1):108—118. https://doi.org/10.1002/hep.27242.

[32] Dubuquoy, C., Robichon, C., Lasnier, F., Langlois, C., Dugail, I., Foufelle, F., et al., 2011. Distinct regulation of adiponutrin/PNPLA3 gene expression by the transcription factors ChREBP and SREBP1c in mouse and human hepatocytes. Journal of Hepatology 55(1):145—153. https://doi.org/10.1016/j.jhep.2010.10.024.

[33] Huang, Y., He, S., Li, J.Z., Seo, Y.K., Osborne, T.F., Cohen, J.C., et al., 2010. A feed-forward loop amplifies nutritional regulation of PNPLA3. Proceedings of the National Academy of Sciences of the United States of America 107(17):7892—7897. https://doi.org/10.1073/pnas.1003585107.

[34] Perttilä, J., Huaman-Samanez, C., Caron, S., Tanhuanpää, K., Staels, B., Yki-Järvinen, H., et al., 2012. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. American Journal of Physiology - Endocrinology And Metabolism 302(9). https://doi.org/10.1152/ajpendo.00125.2011.

[35] Hoekstra, M., Li, Z., Kruijt, J.K., Eck, M. Van., Berkel, T.J.C.V., Kuiper, J., 2010. The expression level of non-alcoholic fatty liver disease-related gene PNPLA3 in hepatocytes is highly influenced by hepatic lipid status. Journal of Hepatology 52(2):244—251. https://doi.org/10.1016/j.jhep.2009.11.004.

[36] Davis, J.N., Lê, K.A., Walker, R.W., Vikman, S., Spruijt-Metz, D., Weigensberg, M.J., et al., 2010. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. American Journal of Clinical Nutrition 92(6):1522—1527. https://doi.org/10.3945/ajcn.2010.30185.

[37] Dong, X.C., 2019. PNPLA3—a potential therapeutic target for personalized treatment of chronic liver disease. Frontiers of Medicine 6:304. https://doi.org/10.3389/fmed.2019.00304.

[38] Basantani, M.K., Sitnick, M.T., Cai, L., Brenner, D.S., Gardner, N.P., Li, J.Z., et al., 2011. Pnpla3/adiponutrin deficiency in mice does not contribute to fatty liver disease or metabolic syndrome. Journal of Lipid Research 52(2):318—329. https://doi.org/10.1194/jlr.M011205.

[39] Lin, Y.-C., Chang, P.-F., Lin, H.-F., Liu, K., Chang, M.-H., Ni, Y.-H., 2016. Variants in the autophagy-related gene IRGM confer susceptibility to non-alcoholic fatty liver disease by modulating lipophagy. Journal of Hepatology 65(6):1209—1216. https://doi.org/10.1016/j.jhep.2016.06.029.

[40] Li, J.Z., Huang, Y., Karaman, R., Ivanova, P.T., Brown, H.A., Roddy, T., et al., 2012. Chronic overexpression of PNPLA3 I148M in mouse liver causes hepatic steatosis. Journal of Clinical Investigation 122(11):4130—4144. https://doi.org/10.1172/JCI65179.

[41] Basuray, S., Wang, Y., Smagris, E., Cohen, J.C., Hobbs, H.H., 2019. Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. Proceedings of the National Academy of Sciences of the United States of America 116(19):9521—9526. https://doi.org/10.1073/pnas.1901974116.

[42] Wang, Y., Kory, N., Basuray, S., Cohen, J.C., Hobbs, H.H., 2019. PNPLA3, CGI-58, and inhibition of hepatic triglyceride hydrolysis in mice. Hepatology 69(6):2427—2441. https://doi.org/10.1002/hep.30583.

[43] Pirazzi, C., Valenti, L., Motta, B.M., Pingitore, P., Hedfalk, K., Mancina, R.M., et al., 2014. PNPLA3 has retinyl-palmitate lipase activity in human hepatic stellate cells. Human Molecular Genetics 23(15):4077—4085. https://doi.org/10.1093/hmg/ddu121.

[44] Pingitore, P., Dongiovanni, P., Motta, B.M., Meroni, M., Lepore, S.M., Mancina, R.M., et al., 2016. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. Human Molecular Genetics 25(23):5212—5222. https://doi.org/10.1093/hmg/ddw341.

[45] Qadri, S., Lallukka-Brück, S., Luukkonen, P.K., Zhou, Y., Gastaldelli, A., Orho-Melander, M., et al., 2020. The PNPLA3-I148M variant increases polyunsaturated triglycerides in human adipose tissue. Liver International: Official Journal of the International Association for the Study of the Liver 40(9):2128—2138. https://doi.org/10.1111/liv.14507.

[46] Chambers, J.C., Zhang, W., Sehmi, J.S., Li, X., Wass, M.N., Van der Harst, P., et al., 2011. Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. Nature Genetics 43(11):1131—1138. https://doi.org/10.1038/ng.970.

[47] Zain, S.M., Mohamed, Z., Mohamed, R., 2015. A common variant in the glucokinase regulatory gene rs780094 and risk of nonalcoholic fatty liver disease: a meta-analysis. Journal of Gastroenterology and Hepatology 30(1):21—27. https://doi.org/10.1111/jgh.12714.

[48] Beer, N.L., Tribble, N.D., McCulloch, L.J., Roos, C., Johnson, P.R.V., Orho-Melander, M., et al., 2009. The P446L variant in GCKR associated with fasting plasma glucose and triglyceride levels exerts its effect through increased glucokinase activity in liver. Human Molecular Genetics 18(21):4081—4088. https://doi.org/10.1093/hmg/ddp357.

[49] Sparsø, T., Andersen, G., Nielsen, T., Burgdorf, K.S., Gjesing, A.P., Nielsen, A.L., et al., 2008. The GCKR rs780094 polymorphism is associated with elevated fasting serum triacylglycerol, reduced fasting and OGTT-related insulinaemia, and reduced risk of type 2 diabetes. Diabetologia 51(1):70—75. https://doi.org/10.1007/s00125-007-0865-z.

[50] Saxena, R., Voight, B.F., Lyssenko, V., Burtt, N.P., De Bakker, P.I.W., Chen, H., et al., 2007. Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316(5829):1331—1336. https://doi.org/10.1126/science.1142358.

[51] Rajas, F., Gautier-Stein, A., Mithieux, G., 2019. Glucose-6 phosphate, A central hub for liver carbohydrate metabolism. Metabolites 9(12). https://doi.org/10.3390/metabo9120282.

[52] Santoro, N., Zhang, C.K., Zhao, H., Pakstis, A.J., Kim, G., Kursawe, R., et al., 2012. Variant in the glucokinase regulatory protein (GCKR) gene is associated with fatty liver in obese children and adolescents. Hepatology 55(3):781—789. https://doi.org/10.1002/hep.24806.

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[53] Kozlitina, J., Smagris, E., Stender, S., Nordestgaard, B.G., Zhou, H.H., Tybjærg-Hansen, A., et al., 2014. Exome-wide association study identifies a TM6SF2 variant that confers susceptibility to nonalcoholic fatty liver disease. Nature Genetics 46(4):352—356. https://doi.org/10.1038/ng.2901.

[54] Dongiovanni, P., Petta, S., Maglio, C., Fracanzani, A.L., Pipitone, R., Mozzi, E., et al., 2015. Transmembrane 6 superfamily member 2 gene variant disentangles nonalcoholic steatohepatitis from cardiovascular disease. Hepatology 61(2):506—514. https://doi.org/10.1002/hep.27490.

[55] Liu, Y.L., Reeves, H.L., Burt, A.D., Tiniakos, D., McPherson, S., Leathart, J.B.S., et al., 2014. TM6SF2 rs58542926 influences hepatic fibrosis progression in patients with non-alcoholic fatty liver disease. Nature Communications 5(1):1—6. https://doi.org/10.1038/ncomms5309.

[56] Holmen, O.L., Zhang, H., Fan, Y., Hovelson, D.H., Schmidt, E.M., Zhou, W., et al., 2014. Systematic evaluation of coding variation identifies a candidate causal variant in TM6SF2 influencing total cholesterol and myocardial infarction risk. Nature Genetics 46(4):345—351. https://doi.org/10.1038/ng.2926.

[57] Mahdessian, H., Taxiarchis, A., Popov, S., Silveira, A., Franco-Cereceda, A., Hamsten, A., et al., 2014. TM6SF2 is a regulator of liver fat metabolism influencing triglyceride secretion and hepatic lipid droplet content. Proceedings of the National Academy of Sciences of the United States of America 111(24):8913—8918. https://doi.org/10.1073/pnas.1323785111.

[58] Luukkonen, P.K., Zhou, Y., Nidhina Haridas, P.A., Dwivedi, O.P., Hyötyläinen, T., Ali, A., et al., 2017. Impaired hepatic lipid synthesis from polyunsaturated fatty acids in TM6SF2 E167K variant carriers with NAFLD. Journal of Hepatology 67(1):128—136. https://doi.org/10.1016/j.jhep.2017.02.014.

[59] Cole, L.K., Vance, J.E., Vance, D.E., 2012. Phosphatidylcholine biosynthesis and lipoprotein metabolism. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1821(5):754—761. https://doi.org/10.1016/j.bbalip.2011.09.009.

[60] Prill, S., Caddeo, A., Baselli, G., Jamialahmadi, O., Dongiovanni, P., Rametta, R., et al., 2019. The TM6SF2 E167K genetic variant induces lipid biosynthesis and reduces apolipoprotein B secretion in human hepatic 3D spheroids. Scientific Reports 9(1):11585. https://doi.org/10.1038/s41598-019-47737-w.

[61] Abul-Husn, N.S., Cheng, X., Li, A.H., Xin, Y., Schurmann, C., Stevis, P., et al., 2018. A protein-truncating HSD17B13 variant and protection from chronic liver disease. New England Journal of Medicine 378(12):1096—1106. https://doi.org/10.1056/NEJMoa1712191.

[62] Pirola, C.J., Garaycoechea, M., Flichman, D., Arrese, M., Martino, J.S., Gazzi, C., et al., 2019. Splice variant rs72613567 prevents worst histologic outcomes in patients with nonalcoholic fatty liver disease. Journal of Lipid Research 60(1):176—185. https://doi.org/10.1194/jlr.P089953.

[63] Luukkonen, P.K., Tukiainen, T., Juuti, A., Sammalkorpi, H., Nidhina Haridas, P.A., Niemelä, O., et al., 2020. Hydroxysteroid 17-β dehydrogenase 13 variant increases phospholipids and protects against fibrosis in nonalcoholic fatty liver disease. JCI Insight 5(5). https://doi.org/10.1172/jci.insight.132158.

[64] Ma, Y., Belyaeva, O.V., Brown, P.M., Fujita, K., Valles, K., Karki, S., et al., 2019. 17-Beta hydroxysteroid dehydrogenase 13 is a hepatic retinol dehydrogenase associated with histological features of nonalcoholic fatty liver disease. Hepatology 69(4):1504—1519. https://doi.org/10.1002/hep.30350.

[65] Su, W., Mao, Z., Liu, Y., Zhang, X., Zhang, W., Gustafsson, J.A., et al., 2019. Role of HSD17B13 in the liver physiology and pathophysiology. Molecular and Cellular Endocrinology 489:119—125. https://doi.org/10.1016/j.mce.2018.10.014.

[66] Blaner, W.S., 2019. Vitamin A signaling and homeostasis in obesity, diabetes, and metabolic disorders. Pharmacology & Therapeutics 197:153—178. https://doi.org/10.1016/j.pharmthera.2019.01.006.

[67] Thangapandi, V.R., Knittelfelder, O., Brosch, M., Patsenker, E., Vvedenskaya, O., Buch, S., et al., 2020. Loss of hepatic Mboat7 leads to liver fibrosis. Gut: Gutjnl, 320853. https://doi.org/10.1136/gutjnl-2020-320853, 2020.

[68] Buch, S., Stickel, F., Trépo, E., Way, M., Herrmann, A., Nischalke, H.D., et al., 2015. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nature Genetics 47(12):1443—1448. https://doi.org/10.1038/ng.3417.

[69] Mancina, R.M., Dongiovanni, P., Petta, S., Pingitore, P., Meroni, M., Rametta, R., et al., 2016. The MBOAT7-TMC4 variant rs641738 increases risk of nonalcoholic fatty liver disease in individuals of European descent. Gastroenterology 150(5):1219—1230. https://doi.org/10.1053/j.gastro.2016.01.032 e6.

[70] Helsley, R.N., Varadharajan, V., Brown, A.L., Gromovsky, A.D., Schugar, R.C., Ramachandiran, I., et al., 2019. Obesity-linked suppression of membrane-bound o-acyltransferase 7 (MBOAT7) drives non-alcoholic fatty liver disease. ELife 8. https://doi.org/10.7554/eLife.49882.

[71] Gijón, M.A., Riekhof, W.R., Zarini, S., Murphy, R.C., Voelker, D.R., 2008. Lysophospholipid acyltransferases and arachidonate recycling in human neutrophils. Journal of Biological Chemistry 283(44):30235—30245. https://doi.org/10.1074/jbc.M806194200.

[72] Zaharia, O.P., Strassburger, K., Strom, A., Bönhof, G.J., Karusheva, Y., Antoniou, S., et al., 2019. Risk of diabetes-associated diseases in subgroups of patients with recent-onset diabetes: a 5-year follow-up study. The Lancet Diabetes and Endocrinology 7(9):684—694. https://doi.org/10.1016/S2213-8587(19)30187-1.

[73] Ahlqvist, E., Storm, P., Käräjämäki, A., Martinell, M., Dorkhan, M., Carlsson, A., et al., 2018. Novel subgroups of adult-onset diabetes and their association with outcomes: a data-driven cluster analysis of six variables. The Lancet Diabetes and Endocrinology 6(5):361—369. https://doi.org/10.1016/S2213-8587(18)30051-2.

[74] Zaharia, O.P., Strassburger, K., Knebel, B., Kupriyanova, Y., Karusheva, Y., Wolkersdorfer, M., et al., 2020. Role of patatin-like phospholipase domain—containing 3 gene for hepatic lipid content and insulin resistance in diabetes. Diabetes Care dc200329. https://doi.org/10.2337/dc20-0329.

[75] Gorden, A., Yang, R., Yerges-Armstrong, L.M., Ryan, K.A., Speliotes, E., Borecki, I.B., et al., 2013. Genetic variation at NCAN locus is associated with inflammation and fibrosis in non-alcoholic fatty liver disease in morbid obesity. Human Heredity 75(1):34—43. https://doi.org/10.1159/000346195.

[76] Agius, L., 2015. Role of glycogen phosphorylase in liver glycogen metabolism. Molecular Aspects of Medicine 46:34—45. https://doi.org/10.1016/j.mam.2015.09.002.

[77] Stender, S., Smagris, E., Lauridsen, B.K., Kofoed, K.F., Nordestgaard, B.G., Tybjaerg-Hansen, A., et al., 2018. Relationship between genetic variation at *PPP1R3B* and levels of liver glycogen and triglyceride. Hepatology 67(6):2182—2195. https://doi.org/10.1002/hep.29751.

[78] Seidelin, A.-S., Nordestgaard, B.G., Tybjærg-Hansen, A., Stender, S., 2020. Genetic variation at PPP1R3B increases hepatic CT attenuation and interacts with prandial status on plasma glucose. Journal of Clinical Endocrinology & Metabolism 105(6). https://doi.org/10.1210/clinem/dgaa151.

[79] Mehta, M.B., Shewale, S.V., Sequeira, R.N., Millar, J.S., Hand, N.J., Rader, D.J., 2017. Hepatic protein phosphatase 1 regulatory subunit 3B (Ppp1r3b) promotes hepatic glycogen synthesis and thereby regulates fasting energy homeostasis. Journal of Biological Chemistry 292(25):10444—10454. https://doi.org/10.1074/jbc.M116.766329.

[80] Bellini, G., Miraglia del Giudice, E., Nobili, V., Rossi, F., 2017. The IRGM rs10065172 variant increases the risk for steatosis but not for liver damage progression in Italian obese children. Journal of Hepatology 67(3):653—655. https://doi.org/10.1016/j.jhep.2017.02.037.

[81] Simon, T.G., Deng, X., Liu, C.T., Chung, R.T., Long, M.T., 2019. The immunity-related GTPase M rs13361189 variant does not increase the risk

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for prevalent or incident steatosis; results from the Framingham Heart Study. Liver International 39(6):1022—1026. https://doi.org/10.1111/liv.14039.

[82] Schwerbel, K., Kamitz, A., Krahmer, N., Hallahan, N., Jähnert, M., Gottmann, P., et al., 2020. Immunity-related GTPase induces lipophagy to prevent excess hepatic lipid accumulation. Journal of Hepatology. https://doi.org/10.1016/j.jhep.2020.04.031.

[83] Alisi, A., Da Sacco, L., Bruscalupi, G., Piemonte, F., Panera, N., De Vito, R., et al., 2011. Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. Laboratory Investigation 91(2):283—293. https://doi.org/10.1038/labinvest.2010.166.

[84] Valenti, L., Motta, B.M., Alisi, A., Sartorelli, R., Buonaiuto, G., Dongiovanni, P., et al., 2012. LPIN1 rs13412852 polymorphism in pediatric nonalcoholic fatty liver disease. Journal of Pediatric Gastroenterology and Nutrition 54(5):588—593. https://doi.org/10.1097/MPG.0b013e3182442a55.

[85] Fawcett, K.A., Grimsey, N., Loos, R.J.F., Wheeler, E., Daly, A., Soos, M., et al., 2008. Evaluating the role of LPIN1 variation in insulin resistance, body weight, and human lipodystrophy in U.K. populations. Diabetes 57(9):2527—2533. https://doi.org/10.2337/db08-0422.

[86] Phan, J., Reue, K., 2005. Lipin, a lipodystrophy and obesity gene. Cell Metabolism 1(1):73—83. https://doi.org/10.1016/j.cmet.2004.12.002.

[87] Estes, C., Razavi, H., Loomba, R., Younossi, Z., Sanyal, A.J., 2018. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 67(1):123—133. https://doi.org/10.1002/hep.29466.

[88] Younossi, Z.M., 2018. The epidemiology of nonalcoholic steatohepatitis. Clinical Liver Disease 11(4):92—94. https://doi.org/10.1002/cld.710.

[89] Nobili, V., Donati, B., Panera, N., Vongsakulyanon, A., Alisi, A., Dallapiccola, B., et al., 2014. A 4-polymorphism risk score predicts steatohepatitis in children with nonalcoholic fatty liver disease. Journal of Pediatric Gastroenterology and Nutrition 58(5):632—636. https://doi.org/10.1097/MPG.0000000000000279.

[90] Al-Serri, A., Anstee, Q.M., Valenti, L., Nobili, V., Leathart, J.B.S., Dongiovanni, P., et al., 2012. The SOD2 C47T polymorphism influences NAFLD fibrosis severity: evidence from case-control and intra-familial allele association studies. Journal of Hepatology 56(2):448—454. https://doi.org/10.1016/j.jhep.2011.05.029.

[91] Miele, L., Beale, G., Patman, G., Nobili, V., Leathart, J., Grieco, A., et al., 2008. The kruppel-like factor 6 genotype is associated with fibrosis in nonalcoholic fatty liver disease. Gastroenterology 135(1). https://doi.org/10.1053/j.gastro.2008.04.004.

[92] Koo, B.K., Joo, S.K., Kim, D., Lee, S., Bae, J.M., Park, J.H., et al., 2020. Development and validation of a scoring system, based on genetic and clinical factors, to determine risk of steatohepatitis in asian patients with nonalcoholic fatty liver disease. Clinical Gastroenterology and Hepatology. https://doi.org/10.1016/j.cgh.2020.02.011.

[93] Zusi, C., Mantovani, A., Olivieri, F., Morandi, A., Corradi, M., Miraglia Del Giudice, E., et al., 2019. Contribution of a genetic risk score to clinical prediction of hepatic steatosis in obese children and adolescents. Digestive and Liver Disease 51(11):1586—1592. https://doi.org/10.1016/j.dld.2019.05.029.

[94] Marchesini, G., Day, C.P., Dufour, J.F., Canbay, A., Nobili, V., Ratziu, V., et al., 2016. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. Journal of Hepatology 64(6):1388—1402. https://doi.org/10.1016/j.jhep.2015.11.004.

[95] Milagro, F.I., Campión, J., Cordero, P., Goyenechea, E., GGómez-Uriz, A.M., Abete, I., et al., 2011. A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss. The FASEB Journal 25(4):1378—1389. https://doi.org/10.1096/fj.10-170365.

[96] Kanzleiter, T., Jähnert, M., Schulze, G., Selbig, J., Hallahan, N., Schwenk, R.W., et al., 2015. Exercise training alters DNA methylation patterns in genes related to muscle growth and differentiation in mice. American Journal of Physiology - Endocrinology And Metabolism 308(10):E912—E920. https://doi.org/10.1152/ajpendo.00289.2014.

[97] Barres, R., Kirchner, H., Rasmussen, M., Yan, J., Kantor, F.R., Krook, A., et al., 2013. Weight loss after gastric bypass surgery in human obesity remodels promoter methylation. Cell Reports 3(4):1020—1027. https://doi.org/10.1016/j.celrep.2013.03.018.

[98] Gancheva, S., Ouni, M., Jelenik, T., Koliaki, C., Szendroedi, J., Toledo, F.G.S., et al., 2019. Dynamic changes of muscle insulin sensitivity after metabolic surgery. Nature Communications 10(1). https://doi.org/10.1038/s41467-019-12081-0.

[99] Weisenberger, D.J., 2014. Characterizing DNA methylation alterations from the cancer genome atlas. Journal of Clinical Investigation 124(1):17—23. https://doi.org/10.1172/JCI69740.

[100] Murphy, S.K., Yang, H., Moylan, C.A., Pang, H., Dellinger, A., Abdelmalek, M.F., et al., 2013. Relationship between methylome and transcriptome in patients with nonalcoholic fatty liver disease. Gastroenterology 145(5):1076—1087. https://doi.org/10.1053/j.gastro.2013.07.047.

[101] Zeybel, M., Hardy, T., Robinson, S.M., Fox, C., Anstee, Q.M., Ness, T., et al., 2015. Differential DNA methylation of genes involved in fibrosis progression in non-alcoholic fatty liver disease and alcoholic liver disease. Clinical Epigenetics 7(1):25. https://doi.org/10.1186/s13148-015-0056-6.

[102] Hardy, T., Zeybel, M., Day, C.P., Dipper, C., Masson, S., McPherson, S., et al., 2017. Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease. Gut 66(7):1321—1328. https://doi.org/10.1136/gutjnl-2016-311526.

[103] Sookoian, S., Rosselli, M.S., Gemma, C., Burgueño, A.L., Fernández Gianotti, T., Castaño, G.O., et al., 2010. Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: impact of liver methylation of the peroxisome proliferator-activated receptor γ coactivator 1α promoter. Hepatology 52(6):1992—2000. https://doi.org/10.1002/hep.23927.

[104] Gemma, C., Sookoian, S., Alvarõas, J., García, S.I., Quintana, L., Kanevsky, D., et al., 2009. Maternal pregestational BMI is associated with methylation of the PPARGC1A promoter in newborns. Obesity 17(5):1032—1039. https://doi.org/10.1038/oby.2008.605.

[105] Allen, N.E., Appleby, P.N., Kaaks, R., Rinaldi, S., Davey, G.K., Key, T.J., 2003. Lifestyle determinants of serum insulin-like growth-factor-I (IGF-I), C-peptide and hormone binding protein levels in British women. Cancer Causes & Control 14(1):65—74. https://doi.org/10.1023/A:1022518321634.

[106] Nam, S.Y., Lee, E.J., Kim, K.R., Cha, B.S., Song, Y.D., Lim, S.K., et al., 1997. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. International Journal of Obesity 21(5):355—359. https://doi.org/10.1038/sj.ijo.0800412.

[107] Wittenbecher, C., Ouni, M., Kuxhaus, O., Jähnert, M., Gottmann, P., Teichmann, A., et al., 2019. Insulin-like growth factor binding protein 2 (IGFBP-2) and the risk of developing type 2 diabetes. Diabetes 68(1):188—197. https://doi.org/10.2337/db18-0620.

[108] Fahlbusch, P., Knebel, B., Hörbelt, T., Barbosa, D.M., Nikolic, A., Jacob, S., et al., 2020. Physiological disturbance in fatty liver energy metabolism converges on IGFBP2 abundance and regulation in mice and men. International Journal of Molecular Sciences 21(11):4144. https://doi.org/10.3390/ijms21114144.

[109] Drucker, D.J., 2007. The role of gut hormones in glucose homeostasis. Journal of Clinical Investigation 117(1):24—32. https://doi.org/10.1172/JCI30076.

[110] Sell, H., Blüher, M., Klöting, N., Schlich, R., Willems, M., Ruppe, F., et al., 2013. Adipose dipeptidyl peptidase-4 and obesity: correlation with insulin resistance and depot-specific release from adipose tissue in vivo and in vitro. Diabetes Care 36(12):4083—4090. https://doi.org/10.2337/dc13-0496.

[111] Miyazaki, M., Kato, M., Tanaka, K., Tanaka, M., Kohjima, M., Nakamura, K., et al., 2012. Increased hepatic expression of dipeptidyl peptidase-4 in non-

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alcoholic fatty liver disease and its association with insulin resistance and glucose metabolism. Molecular Medicine Reports 5(3):729—733. https://doi.org/10.3892/mmr.2011.707.

[112] Laeger, T., Castaño-Martinez, T., Werno, M.W., Japtok, L., Baumeier, C., Jonas, W., et al., 2018. Dietary carbohydrates impair the protective effect of protein restriction against diabetes in NZO mice used as a model of type 2 diabetes. Diabetologia 61(6):1459—1469. https://doi.org/10.1007/s00125-018-4595-1.

[113] Saussenthaler, S., Ouni, M., Baumeier, C., Schwerbel, K., Gottmann, P., Christmann, S., et al., 2019. Epigenetic regulation of hepatic Dpp4 expression in response to dietary protein. Journal of Nutritional Biochemistry 63: 109—116. https://doi.org/10.1016/j.jnutbio.2018.09.025.

[114] Ma, J., Nano, J., Ding, J., Zheng, Y., Hennein, R., Liu, C., et al., 2019. A peripheral blood DNA methylation signature of hepatic fat reveals a potential causal pathway for nonalcoholic fatty liver disease. Diabetes 68(5): 1073—1083. https://doi.org/10.2337/DB18-1193.

[115] Wu, J., Zhang, R., Shen, F., Yang, R., Zhou, D., Cao, H., et al., 2018. Altered DNA methylation sites in peripheral blood leukocytes from patients with simple steatosis and nonalcoholic steatohepatitis (NASH). Medical Science Monitor 24:6946—6967. https://doi.org/10.12659/MSM.909747.

[116] Ouni, M., Schürmann, A., 2020. Epigenetic contribution to obesity. Mammalian Genome 31(5—6):134—145. https://doi.org/10.1007/s00335-020-09835-3.

[117] Bartel, D.P., 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281—297. https://doi.org/10.1016/S0092-8674(04)00045-5.

[118] Guo, H., Ingolia, N.T., Weissman, J.S., Bartel, D.P., 2010. Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466(7308):835—840. https://doi.org/10.1038/nature09267.

[119] Bandiera, S., Pfeffer, S., Baumert, T.F., Zeisel, M.B., 2015. MiR-122 - a key factor and therapeutic target in liver disease. Journal of Hepatology 62(2): 448—457. https://doi.org/10.1016/j.jhep.2014.10.004.

[120] Torres, J.L., Novo-Veleiro, I., Manzanedo, L., Suárez, L.A., MacÍas, R., Laso, F.J., et al., 2018. Role of microRNAs in alcohol-induced liver disorders and non-alcoholic fatty liver disease. World Journal of Gastroenterology 24(36):4104—4118. https://doi.org/10.3748/wjg.v24.i36.4104.

[121] Lagos-Quintana, M., Rauhut, R., Yalcin, A., Meyer, J., Lendeckel, W., Tuschl, T., 2002. Identification of tissue-specific MicroRNAs from mouse. Current Biology 12(9):735—739. https://doi.org/10.1016/S0960-9822(02)00809-6.

[122] Laudadio, I., Manfroid, I., Achouri, Y., Schmidt, D., Wilson, M.D., Cordi, S., et al., 2012. A feedback loop between the liver-enriched transcription factor network and miR-122 controls hepatocyte differentiation. Gastroenterology 142(1):119—129. https://doi.org/10.1053/j.gastro.2011.09.001.

[123] Esau, C., Davis, S., Murray, S.F., Yu, X.X., Pandey, S.K., Pear, M., et al., 2006. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metabolism 3(2):87—98. https://doi.org/10.1016/j.cmet.2006.01.005.

[124] Latorre, J., Moreno-Navarrete, J.M., Mercader, J.M., Sabater, M., Rovira, Gironès, J., et al., 2017. Decreased lipid metabolism but increased FA biosynthesis are coupled with changes in liver microRNAs in obese subjects with NAFLD. International Journal of Obesity 41(4):620—630. https://doi.org/10.1038/ijo.2017.21.

[125] Becker, P.P., Rau, M., Schmitt, J., Malsch, C., Hammer, C., Bantel, H., et al., 2015. Performance of serum microRNAs-122,-192 and-21 as biomarkers in patients with non-Alcoholic steatohepatitis. PloS One 10(11). https://doi.org/10.1371/journal.pone.0142661.

[126] Hsu, S.H., Wang, B., Kota, J., Yu, J., Costinean, S., Kutay, H., et al., 2012. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. Journal of Clinical Investigation 122(8):2871—2883. https://doi.org/10.1172/JCI63539.

[127] Roy, S., Benz, F., Alder, J., Bantel, H., Janssen, J., Vucur, M., et al., 2016. Down-regulation of miR-192-5p protects from oxidative stress-induced acute liver injury. Clinical Science 130(14):1197—1207. https://doi.org/10.1042/CS20160216.

[128] Morimoto, A., Kannari, M., Tsuchida, Y., Sasaki, S., Saito, C., Matsuta, T., et al., 2017. An HNF4α—microRNA-194/192 signaling axis maintains hepatic cell function. Journal of Biological Chemistry 292(25):10574—10585. https://doi.org/10.1074/jbc.M117.785592.

[129] Jenkins, R.H., Martin, J., Phillips, A.O., Bowen, T., Fraser, D.J., 2012. Transforming growth factor β1 represses proximal tubular cell microRNA-192 expression through decreased hepatocyte nuclear factor DNA binding. Biochemical Journal 443(2):407—416. https://doi.org/10.1042/BJ20111861.

[130] Brabletz, S., Brabletz, T., 2010. The ZEB/miR-200 feedback loop-a motor of cellular plasticity in development and cancer? EMBO Reports 11(9):670—677 https://doi.org/10.1038/embor.2010.117.

[131] Ashrafizadeh, M., Ang, H.L., Moghadam, E.R., Mohammadi, S., Zarrin, V., Hushmandi, K., et al., 2020. MicroRNAs and their influence on the zeb family: mechanistic aspects and therapeutic applications in cancer therapy. Biomolecules 10(7):1—45. https://doi.org/10.3390/biom10071040.

[132] Castaño, C., Kalko, S., Novials, A., Párrizas, M., 2018. Obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice. Proceedings of the National Academy of Sciences of the United States of America 115(48):12158—12163. https://doi.org/10.1073/pnas.1808855115.

[133] Rayner, K.J., Esau, C.C., Hussain, F.N., McDaniel, A.L., Marshall, S.M., Van Gils, J.M., et al., 2011. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature 478(7369):404—407. https://doi.org/10.1038/nature10486.

[134] Horton, J.D., 2002. Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochemical Society Transactions 30(6):1091—1095. https://doi.org/10.1042/BST0301091.

[135] Horie, T., Ono, K., Horiguchi, M., Nishi, H., Nakamura, T., Nagao, K., et al., 2010. MicroRNA-33 encoded by an intron of sterol regulatory element-binding protein 2 (Srebp2) regulates HDL in vivo. Proceedings of the National Academy of Sciences of the United States of America 107(40):17321—17326. https://doi.org/10.1073/pnas.1008499107.

[136] Koyama, S., Horie, T., Nishino, T., Baba, O., Sowa, N., Miyasaka, Y., et al., 2019. Identification of differential roles of microRNA-33a and -33b during atherosclerosis progression with genetically modified mice. Journal of the American Heart Association 8(13). https://doi.org/10.1161/JAHA.119.012609.

[137] Martino, F., Carlomosti, F., Avitabile, D., Persico, L., Picozza, M., Barillà, F., et al., 2015. Circulating miR-33a and miR-33b are up-regulated in familial hypercholesterolaemia in paediatric age. Clinical Science 129(11):963—972. https://doi.org/10.1042/CS20150235.

[138] Ahn, S.B., Jang, K., Jun, D.W., Lee, B.H., Shin, K.J., 2014. Expression of liver X receptor correlates with intrahepatic inflammation and fibrosis in patients with nonalcoholic fatty liver disease. Digestive Diseases and Sciences 59(12): 2975—2982. https://doi.org/10.1007/s10620-014-3289-x.

[139] Kim, Y.S., Nam, H.J., Han, C.Y., Joo, M.S., Jang, K., Jun, D.W., et al., 2020. LXRα activation inhibits autophagy and lipophagy in hepatocytes by dysregulating ATG4B and Rab-8B, reducing mitochondrial fuel oxidation. Hepatology. https://doi.org/10.1002/hep.31423.

[140] Wu, T., Liu, Y.H., Fu, Y.C., Liu, X.M., Zhou, X.H., 2014. Direct evidence of sirtuin downregulation in the liver of non-alcoholic fatty liver disease patients. Annals of Clinical and Laboratory Science 44(4):410—418.

[141] Ding, J., Li, M., Wan, X., Jin, X., Chen, S., Yu, C., et al., 2015. Effect of MIR-34a in regulating steatosis by targeting PPARα expression in nonalcoholic fatty liver disease. Scientific Reports 5. https://doi.org/10.1038/srep13729.

[142] Xu, Y., Zalzala, M., Xu, J., Li, Y., Yin, L., Zhang, Y., 2015. A metabolic stress-inducible miR-34a-HNF4α pathway regulates lipid and lipoprotein metabolism. Nature Communications 6. https://doi.org/10.1038/ncomms8466.

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[143] Newman, L.A., Sorich, M.J., Rowland, A., 2020. Role of extracellular vesicles in the pathophysiology, diagnosis and tracking of non-alcoholic fatty liver disease. Journal of Clinical Medicine 9(7):2032. https://doi.org/10.3390/jcm9072032.

[144] López-Riera, M., Conde, I., Quintas, G., Pedrola, L., Zaragoza, Á., Perez-Rojas, J., et al., 2018. Non-invasive prediction of NAFLD severity: a comprehensive, independent validation of previously postulated serum microRNA biomarkers. Scientific Reports 8(1). https://doi.org/10.1038/s41598-018-28854-4.

[145] Thul, P.J., Akesson, L., Wiking, M., Mahdessian, D., Geladaki, A., Ait Blal, H., et al., 2017. A subcellular map of the human proteome. Science 356(6340). https://doi.org/10.1126/science.aal3321.

[146] Uhlen, M., Fagerberg, L., Hallstrom, B.M., Lindskog, C., Oksvold, P., Mardinoglu, A., et al., 2015. Tissue-based map of the human proteome. Science 347(6220). https://doi.org/10.1126/science.1260419, 1260419–1260419.

[147] Mondul, A., Mancina, R.M., Merlo, A., Dongiovanni, P., Rametta, R., Montalcini, T., et al., 2015. PNPLA3 I148M variant influences circulating retinol in adults with nonalcoholic fatty liver disease or obesity. Journal of Nutrition 145(8):1687–1691. https://doi.org/10.3945/jn.115.210633.

[148] Santoro, N., Caprio, S., Pierpont, B., Van Name, M., Savoye, M., Parks, E.J., 2015. Hepatic de novo lipogenesis in obese youth is modulated by a common variant in the GCKR gene. Journal of Clinical Endocrinology & Metabolism 100(8):E1125–E1132. https://doi.org/10.1210/jc.2015-1587.

[149] Su, W., Wang, Y., Jia, X., Wu, W., Li, N., Tian, X., et al., 2014. Comparative proteomic study reveals 17β-HSD13 as a pathogenic protein in nonalcoholic fatty liver disease. Proceedings of the National Academy of Sciences of the United States of America 111(31):11437–11442. https://doi.org/10.1073/pnas.1410741111.

[150] Meroni, M., Dongiovanni, P., Longo, M., Carli, F., Baselli, G., Rametta, R., et al., 2020. Mboat7 down-regulation by hyper-insulinemia induces fat accumulation in hepatocytes: Mboat7 reduction and hepatic steatosis. EBioMedicine 52. https://doi.org/10.1016/j.ebiom.2020.102658.

[151] Donkor, J., Sariahmetoglu, M., Dewald, J., Brindley, D.N., Reue, K., 2007. Three mammalian lipins act as phosphatidate phosphatases with distinct tissue expression patterns. Journal of Biological Chemistry 282(6):3450–3457. https://doi.org/10.1074/jbc.M610745200.

[152] Reue, K., Dwyer, J.R., 2009. Lipin proteins and metabolic homeostasis. Journal of Lipid Research, S109. https://doi.org/10.1194/jlr.R800052-JLR200.

[153] Finck, B.N., Gropler, M.C., Chen, Z., Leone, T.C., Croce, M.A., Harris, T.E., et al., 2006. Lipin 1 is an inducible amplifier of the hepatic PGC-1α/PPARα regulatory pathway. Cell Metabolism 4(3):199–210. https://doi.org/10.1016/j.cmet.2006.08.005.

[154] Chen, Z., Gropler, M.C., Mitra, M.S., Finck, B.N., 2012. Complex interplay between the lipin 1 and the hepatocyte nuclear factor 4 α (HNF4α) pathways to regulate liver lipid metabolism. PloS One 7(12). https://doi.org/10.1371/journal.pone.0051320.

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